METAL MINING AND THE NATURAL CYCLING OF MERCURY IN FRESHWATER LAKES

How Legacy Mining Pollution is Affecting the Toxicity of Mercury

MIJA AZDAJIC

Thesis Submitted to The University of Ottawa
In Partial Fulfillment of the Requirements For

Ph.D. of Science
in
Biology

DEPARTMENT OF BIOLOGY
FACULTY OF SCIENCE
UNIVERSITY OF OTTAWA
OTTAWA, ONTARIO, CANADA

ABSTRACT

Methylmercury (MeHg) is a global pollutant and potent neurotoxin that bioaccumulates in aquatic and terrestrial foodwebs. As such, predicting the fate of MeHg in the environment is important in addressing ecosystem and human health concerns. Pollution that results from mining activities (i.e. ore roasting and smelting) is one way in which geochemistry of lakes can be altered, in turn altering the cycling of mercury (Hg) overall and the kinetics of MeHg production and bioaccumulation. My thesis is focused on examining the effect of different pollution gradients on the cycling of Hg at two different sites impacted by legacy mining in Canada. In Yellowknife (Northwest Territories, Canada) I assessed the effect of sulfate and arsenic (As) emissions on the production of MeHg in lake sediments, while in Sudbury (Ontario, Canada) I examined the effect of selenium (Se) emissions on the bioaccumulation of Hg and MeHg in freshwater biota.

In Yellowknife, an environment polluted with both sulfate and As from mining activities, lakes were sampled using a factorial design which controlled for environmental variables known to affect MeHg production (i.e. sulfate, iron, productivity, pH, and dissolved organic matter). We used stable Hg isotope tracers to quantify Hg methylation and demethylation rate constants in sediments. Results showed that %MeHg in the water is best correlated with sulfate concentrations, while the rate at which Hg is methylated (K_m) in sediments is negatively correlated with total As, and positively correlated with dissolved organic carbon, total phosphorus, and %MeHg in the water. Furthermore, a detailed examination of a lake with representative

limnological characteristics of the area showed that addition of sulfate and organic carbon does increase the production of MeHg in the sediments, while addition of arsenate (0 to 10 mM) showed significant decrease in MeHg production, regardless of sulfate concentrations.

Next, Se emissions in Sudbury (Ontario, Canada) correlated with lower total Hg and MeHg in tissues of zooplankton, amphipods (*Hyalella azteca),* mayflies (*Stenonema femoratum*), and young-of-the-year perch (*Perca flavescens*). However, despite ten years of emission reductions, results show that total Se concentrations in the majority of lakes have increased, most likely due to the long residence time of Se in the watershed and the water column. Consequently, Se continues to exhibit a protective effect on total Hg and MeHg bioaccumulation in biota, even a decade after emissions have greatly decreased.

Canada's numerous mining operations have left a legacy of pollution and it is important to understand the effects of these pollutants on the biogeochemistry of surrounding lakes. The results from my thesis demonstrate how mining emissions can alter the kinetics and bioaccumulation of MeHg in freshwater lakes, highlighting the complexity of Hg cycling in response to mining activities. My thesis is an important step in identifying and modeling the controls of MeHg production and bioaccumulation in environments impacted by emissions from mining operations.

RÉSUMÉ

Le méthylmercure (MeHg) est un polluant mondial et une puissante neurotoxine qui se bioaccumule dans les réseaux trophiques aquatiques et terrestres. En tant que tel, il est important de prédire le devenir du MeHg dans l'environnement pour répondre aux préoccupations vis à vis des écosystèmes et de la santé humaine. La pollution résultante des activités minières (c.-à-d. smeltage ou grillage) peut modifier la géochimie des lacs, modifiant par conséquent le cycle du mercure (Hg) dans son ensemble ainsi que la cinétique de production et de bioaccumulation du MeHg. Ma thèse porte sur l'examination de l'effet de différents gradients de pollution sur le cycle du Hg sur deux sites différents touchés par l'exploitation minière au Canada. À Yellowknife (Territoires du Nord-Ouest, Canada), j'ai évalué l'effet des émissions de sulfate et d'arsenic (As) sur la production de MeHg dans les sédiments lacustres, tandis qu'à Sudbury (Ontario, Canada) j'ai examiné l'effet des émissions de sélénium (Se) sur la bioaccumulation de Hg et MeHg dans le biote d'eau douce.

À Yellowknife, un environnement pollué par le sulfate et As provenant des activités minières, les lacs ont été échantillonnés à l'aide d'un plan factoriel contrôlant les variables environnementales connues pour affecter la production de MeHg (c.-à-d. sulfate, fer, productivité du lac, pH, et matière organique dissoute). Nous avons utilisé des traceurs isotopiques stables du Hg pour quantifier les constantes de taux de méthylation et de déméthylation du Hg dans les sédiments. Les résultats montrent que le %MeHg dans l'eau est significativement corrélé avec les concentrations de sulfate, tandis que le taux auquel le Hg est

méthylé (K_m) dans les sédiments est négativement corrélé avec l'As total et positivement corrélé avec le carbone organique dissous, le phosphore total et le %MeHg dans l'eau. De plus, un examen détaillé d'un lac présentant des caractéristiques limnologiques représentatives de la région a montré que l'ajout du sulfate et de carbone organique augmente la production de MeHg dans les sédiments, tandis que l'ajout d'arséniate (0 à 10 mM) a montré une diminution significative de la production de MeHg, indépendamment des concentrations de sulfate.

Ensuite, les émissions de Se à Sudbury (Ontario, Canada) étaient significativement corrélées à des concentrations plus faibles de Hg et de MeHg totaux dans les tissus du zooplancton, des amphipodes (*Hyalella azteca*), des éphémères (*Stenonema femoratum*) et des jeunes perches de l'année (*Perca flavescens*). Cependant, malgré dix années de réduction des émissions, les résultats montrent que les concentrations totales de Se dans la majorité des lacs ont augmenté, potentiellement en raison du long temps de résidence du Se dans le bassin versant et la colonne d'eau. Par conséquent, Se continue d'exercer un effet protecteur sur la bioaccumulation totale du Hg et MeHg dans le biote, malgré une décennie de diminution vigoureuse d'émissions.

Les nombreuses exploitations minières du Canada laissent un héritage de pollution après le déclassement, donc il est important de comprendre les effets de ces polluants sur la biogéochimie des lacs environnants. Les résultats de ma thèse démontrent comment les émissions minières peuvent modifier la cinétique et la bioaccumulation du MeHg dans les lacs d'eau douce, soulignant la complexité du cycle du Hg en réponse aux activités minières. Ma

thèse est une étape importante dans l'identification et la modélisation des contrôles de la production et de la bioaccumulation de MeHg dans les environnements impactés par les émissions provenant des opérations minières.

ACKNOWLEDGEMENTS

Finishing my thesis in the midst of a global pandemic was not the easiest, however, I am extremely fortunate to be surrounded by awesome people that have helped me complete this journey. First, I'd like to thank my supervisors, Jules Blais and Alexandre Poulain, for all of their support. Jules, thank you for all the opportunities you have given me and all of the guidance that you have provided through my graduate studies. I am thankful that you have always been available and enthusiastic to discusses my research challenges. Alex, thank you for always being supportive and for pushing me to do more than I thought I was capable of accomplishing. But most of all, thank you for being such a great mentor and for making me a better scientist. To my committee members, Drs Danielle Fortin, Frances Pick, and John Chételat, thank you for sharing your expertise and insights during my graduate studies.

To all Blais and Poulain lab members, past and present, thank you for your help, your encouragement, and company in the office. I was very lucky to be surrounded by such smart, passionate, and friendly lab mates. Linda, thank you for your help with field work and thank you for always being there for my 1001 questions. Dave, thank you for being the best field buddy, for helping me finish sangrias, and for all the coffee walks. Phil, thank you for your support and your friendship. I came with zero molecular lab knowledge and you were (very!) patient, kind, and understanding while teaching me. Emmanuel, I can confidently say that my thesis would have not be possible without your expertise and your calm supportive presence. You have been there for every failed experiment and your encouragement always helped me

to forge ahead. To my OG lab mates, M-C, Alexandre, Pepe, Kirsten, Dan, Galen, Ben, Graham, and Martin, thank you for all always being eager to talk science, for the long life discussions, and karaoke sessions. To the Guardians of Sanity Discord Gang; Lauren, Cynthia, Jen, and Madison, thank you for your friendships and all the work sessions that have kept me sane (and focused!) during this last year. To Jess, Michelle, and Noémie; thank you for all the support, the laughs, the bad movies, and especially all the deserts.

Most of all, thank you to my family. I'd like to thank each and every one of you for all for your unwavering support during both my graduate career and the years before, which laid the foundation for all my accomplishments. To my parents, although a simple thank you will never be enough for all that you have done for me, thank you for always supporting me in all my academic pursuits (I promise that this my last degree!). Mama, thank you for your fierce encouragement, the consent support (especially bringing me baked goods when I was writing), and most of all thank you for showing me how to persevere when faced with challenges. Tata, thank you for instilling in me a sense of curiosity, for answering all the (very simple!) math questions I had during my grad studies, and for always being there with a hug when I needed it the most. Most of all, thank you both for always being my biggest cheerleaders. To my siblings, Ivana and Petar, thank you for the laughs along the way and for all the teasing that distracted me from my thesis worries. Thank you to my grandmas, Mira and Janja, and to my aunts and uncles, for always being full of positivity and support. To my uncle Marko and Branka, thank you for you the encouragement and (most of all?) the wine. To Dominik and Luka (aka the chicken butts), thank you for you for distracting me with Beyblade battles when

things got too stressful. To Pamela, Nayla, and Kayla; your friendships have been invaluable to me during these last six years, thank you for your support and your positivity (and for reading my articles!). I am so blessed to have friends like you.

LIST OF ABBREVIATIONS

[x]	Concentration of specific compound x
AIC	Akaike information criterion
ANOVA	Analysis of variance
As	Arsenic
As(III)	Arsenite
As(V)	Arsenate
ATP	Adenosine triphosphate
CALA	Canadian Association of Laboratory Accreditation
CV-AFS	Cold-vapor atomic fluorescence spectrometry
db-RDA	Distance based redundancy analysis
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EPA	Environmental Protection Agency (US)
GC-AFS	Gas chromatography-atom fluorescence spectrometry
GDP	Gross domestic product
Hg	Mercury
ICP-MS	Inductively coupled plasma mass spectrometry
K_d	Methylmercury demethylation rate constant (day^{-1})
K_m	Mercury methylation rate constant (day^{-1})
L	Litre
LC-ICP-MS	Liquid chromatography inductively coupled plasma mass
ln	Natural logarithm
LOQ	Limit of quantification
MeHg	Methylmercury
mM	micro molar
NWT	Northwest Territories
ON	Ontario
PCA	Principal component analysis
PCNM	Principal coordinates of neighbour matrices
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
pH	Concentration of protons ($-\log[H^+]$)
SD	Standard deviation
Se	Selenium

sqrt	Square root
SRMs	Sulfate reducing microbes
SUVA	Specific ultraviolet absorbance
t	Time
TOC	Total organic carbon
µL	micro litre
µm	micro metre

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

STATEMENT OF CONTRIBUTIONS

CHAPTER 2: THE EFFECT OF LEGACY GOLD MINING ON METHYLMERCURY CYCLING AND MICROBIAL COMMUNITY STRUCTURE IN NORTHERN FRESHWATER LAKES

Authors:

Aždajić, Mija[1]; Yumvihoze, Emmanuel[1]; Blais, Jules M[1]; Poulain, Alexandre J[1]

Affiliations :

[1] Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, Ontario K1N 6N5, Canada; email: apoulain@uottawa.ca

Study Design …….. Mija Azdajic
Jules Blais
Alexandre Poulain

Sample Collection …… Mija Azdajic
Jules Blais
David Eikckmeyer
Cynthia Cheney
Martin Pothier
Branaavan Sivarajah
Claudia Tanamal
Emily Stewart
Mike Palmer
Jennifer Korosi

Experimental Setup and Execution …… Mija Azdajic
Emmanuel Yumvihoze

Isotope Extraction and Analysis …….. Mija Azdajic
Emmanuel Yumvihoze
Javanté Godbout-Mascoll
Raphaël Siegel

Mercury analysis in water …… Mija Azdajic
Emmanuel Yumvihoze
Laurie Nadon Sabourin

Chemical Analysis ..... Mija Azdajic
Emmanuel Yumvihoze
Taiga Laboratory

DNA Extraction ..... Mija Azdajic
Philip Pelletier

Bioinformatics Pipeline ..... Mija Azdajic
Galen Guo
Matti Ruuskanen
Graham Colby

Data Analysis ..... Mija Azdajic
Jose Luis Rodriguez Gil
Julien Martin

Results Interpretation ..... Mija Azdajic
Alexandre Poulain

Manuscript Preparation ..... Mija Azdajic

Review and Editing ..... Alexandre Poulain
Jules Blais
Emmanuel Yumvihoze

CHAPTER 3: ARSENATE AMENDMENTS DECREASE PRODUCTION OF METHYLMERCURY ACROSS SULFATE CONCENTRATION GRADIENT IN FRESHWATER LAKE SEDIMENTS

Authors:

Ažđajić, Mija[1]; Blais, Jules M[1]; Poulain, Alexandre J[1]

Affiliations :

[1] Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, Ontario K1N 6N5, Canada; email: jules.blais@uottawa.ca, apoulain@uottawa.ca

Study Design ……. Mija Azdajic
Jules Blais
Alexandre Poulain

Sample Collection ……. Mija Azdajic
David Eikckmeyer
Martin Pothier
Claudia Tanamal

Experimental Setup and Execution …… Mija Azdajic
Emmanuel Yumvihoze

Isotope Extraction and Analysis …….. Mija Azdajic
Emmanuel Yumvihoze

Chemical Analysis ……. Mija Azdajic
Emmanuel Yumvihoze

Data Analysis …….. Mija Azdajic

Results Interpretation ……..…. Mija Azdajic
Alexandre Poulain

Manuscript Preparation …… Mija Azdajic

Review and editing …….. Alexandre Poulain
Jules Blais

CHAPTER 4: EFFECTS OF A DECADE OF SELENIUM EMISSION REDUCTIONS ON MERCURY ACCUMULATION IN AQUATIC BIOTA IN THE SUDBURY REGION OF ONTARIO

Authors:

Ažđajić, Mija[1]; Belzile, Nelson[2]; Gunn, John M[3]; Blais, Jules M[1]; Poulain, Alexandre J[1]

Affiliations :

[1] Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, Ontario K1N 6N5, Canada; email: jules.blais@uottawa.ca, apoulain@uottawa.ca

[2] Cooperative Freshwater Ecology Unit, and Department of Chemistry and Biochemistry, Laurentian University, Sudbury, ON P3E 2C6, Canada; email: nbelzile@laurentian.ca

[3] Cooperative Freshwater Ecology Unit, and Department of Biology, Laurentian University, Sudbury, ON P3E 2C6, Canada; email: jgunn@laurentian.ca

Study Design ..... Mija Azdajic
Jules Blais
Alexandre Poulain
Nelson Belzile
John Gunn

Sample Collection ..... Emily Smenderovac
Jasmine Louste-Fillion
Jordon Courchesne

Mercury Extraction and Analysis ..... Mija Azdajic
Emmanuel Yumvihoze

Water Chemistry Analysis ..... Mija Azdajic
Emmanuel Yumvihoze

Data Analysis ..... Mija Azdajic

Results Interpretation ..... Mija Azdajic
Alexandre Poulain
Jules Blais

Manuscript Preparation ..... Mija Azdajic

Review and editing ..... Alexandre Poulain
Jules Blais
Nelson Belzile
John Gunn

CHAPTER 1: INTRODUCTION

The history of the western world is ultimately one of metal exploration, with each new era - from the Bronze Age to the Iron Age to the 20th Century Gold rush - ushering a higher demand for metals. In the current age of technological innovation, our need for metals is the highest it has ever been and metal extraction contributes significantly to many countries' economies[1,2]. In fact, Canadian economic prosperity is due in large part to its active mining sector, with CAN$28 billion worth of metals extracted in 2019[3]. However, the delicate ecological balance of Canadian landscapes exists at the mercy this metal exploration. The legacy of pollution left by this industry affects the lakes, rivers, and forests in the surrounding landscape, long after the last mineral is pulled from the ground[4–6].

The longevity of pollution from the metal industry raises many questions, ones that scientists are trying to answer so that future environmental damage can be mitigated. How long do pollutants stay in the surrounding lands? How do these legacy pollutants affect natural ecosystem functions? And what lessons can we learn from past mining projects to better manage present and future metal extraction?

1.1 METALS, MICROBES, AND MINING

Microbes first evolved billions of years ago; they are the oldest living organisms. Due to microbes' tremendous metabolic capabilities and their incredible capacity for adaptation, the

cycling of most elements on Earth involves microbial transformations. Consequently, microbes play a large role in the biogeochemistry of many metals, including the cycling of Hg[7,8].

Elements can neither be created nor destroyed, but they can be redistributed naturally by geological and biological cycles. Trace metals especially can exist in different redox states, allowing for a wide array of metallic species that can move through geological compartments easily. Metal species differ in their toxicity and ease of transportation through the environment and into cells, and certain microbes can directly alter metal speciation. Therefore, the interactions between microbes and the metals in their environment can directly influence the toxicity of that environment to other resident species.

1.1.1 Why It Matters

Presently, metal extraction is the backbone of Canada's economy[9]. However, mining operations leave behind a legacy of contamination such as mine tailings and roasting emissions. This legacy of contamination can have serious consequences on the environment and the surrounding communities. Whereas the consequences of legacy contamination on the socio-economic fabric is not fully understood, we know that contaminants in the environment can have negative health consequences on the communities inhabiting these impacted areas[10]. By researching how natural resource extractions affect the cycling of metals (i.e. Hg), we can be better equipped to assess the risk of such operations on ecosystem and public health.

Understanding how Hg cycling is impacted by anthropogenic stressors is also important in the context of international environmental policy. The Minamata Convention is a unilateral environmental agreement between 133 governments (128 governments have ratified the treaty as of Aug 2021) which addresses the global problem of Hg pollution. Within this agreement under Article 19, the parties agree to cooperate to develop and improve "[m]odelling and geographically representative monitoring of levels of mercury and mercury compounds in vulnerable populations and in environmental media, including biotic media such as fish…" and "[i]nformation on the environmental cycle […] transformation and fate of mercury and mercury compounds in a range of ecosystems"[11]. Therefore, understanding how mining activities affect MeHg cycling can help to better model Hg and MeHg levels in environmental media and will help to further our understanding of the environmental cycle of Hg.

1.2 MINING POLLUTION IN CANADA'S NORTH

Canada's north has a long history of natural resource extraction. While the first mining operation opened in 1782 in New Brunswick, mining steadily spread through the country and reached the region of Yellowknife (Northwest Territories) by the mid 1930's[12]. This industry has arguably improved the quality of life to northern residents and has been the driving force in the development of towns in most provinces and territories. However, this mining activity also has negative consequences, both sociological and environmental[4,13,14]. Mining can have long lasting environmental consequences; for example, impacting aquatic systems such as lakes and rivers[10]. With the associated increase in development resulting from mining operations, we can expect

the Canadian landscape to be further affected in the years to come[15]. It is therefore important to study how the pollution from historical mining practices has affected the environment in order to better mitigate and avoid damages that might arise from future mining activities.

1.2.1 Rationale for Thesis

Mining operations often release contaminants to the environment, and this can potentially impact the biogeochemical cycling of metals in freshwater lakes, particularly mercury (Hg) that is already present in the water. The fate of Hg in the environment is very important to understand as its speciation is related to its toxicity. The dominant form of organic Hg, methylmercury (MeHg), is more neurotoxic and bioaccumulative than inorganic Hg forms[16]. Many environmental variables can impact the production of MeHg in freshwater systems, such as sulfate[17], dissolved organic carbon, temperature, and pH[18,19] (see Section 1.3 for a more details about Hg geochemistry). As such, understanding the impact that mining operations have on the speciation of Hg found naturally in lakes can greatly help to assess the risk of such disturbances.

The overarching objective of this thesis is to investigate the impact of mining on the fate of Hg in freshwater lakes in Canada's boreal forest. A complete understanding of the environmental parameters controlling Hg speciation, and ultimately its toxicity, can help to develop mitigation strategies to implement in the mining life cycle.

1.3 MERCURY

1.3.1 General

The many different species of Hg found in the environment can be divided into two major categories: organic and inorganic. Organic species are composed of Hg covalently bound to one or more carbon atoms; the most notable organic species is MeHg, which is the primary concern in aquatic environments. MeHg is highly toxic to organisms and has a high bioaccumulation factor, much higher than inorganic forms of Hg. Consequently, MeHg can reach high concentrations in fish, which is problematic for other species, or mining community members, who depend on fish for food. As a result, it is vital to understand Hg cycling in the environment to protect residents from MeHg exposure[16].

1.3.2 Mercury, Mining, and Pollution

In most boreal ecosystems, MeHg is usually 5% of the overall total Hg in water (Table S1.1)[20–25]. However, it is important to consider environments in which Hg is at natural concentrations but the proportion of bioavailable Hg (i.e. MeHg) is high, as MeHg can biomagnify in foodwebs. As such, changes in certain environmental variables (e.g. lake productivity, pH, dissolved organic matter concentrations), can alter the ratio of MeHg to total Hg[26]. Therefore, Hg toxicity in a system can be increased in two ways in the environment: 1) by adding Hg to the system or 2) by changing the environmental conditions (e.g. adding mining pollutants) causing the natural cycling of Hg to favour production of MeHg.

Most studies that have investigated mining and Hg focused on sites that are contaminated by high Hg from mining itself[26]. However, few studies have investigated how mining pollution can perturb the natural cycling of Hg in a freshwater system. In my thesis, I examined how roasting operations at mining sites can affect the cycling of Hg that is already present in the ecosystem.

The ores most commonly associated with metal mining are sulfide bearing minerals[27]. Within these sulfide minerals are two types of ores: non-refractory and refractory. Metals are easily extracted from the former type, whereas extraction is much more difficult from the latter type; refractory ores include economically viable material (e.g. copper, gold, silver) that are usually "locked" into the mineral matrix and are resistant to normal extraction techniques, such as cyanidation. Therefore, the ore needs to first be roasted to release the metal particle before extraction can take place[28]. These mining practices, especially those done in the early 20th century, released significant amounts of pollutants to the environment, including sulfate, arsenic (As), and selenium (Se)[4,29,30]. Even though this pollution is usually present as a mixture in the environment, most studies concentrate on a single pollutant, mostly Hg[31–35] as the increase of Hg in the environment presents a clear increase of possible toxicity in the environment. While these single pollutant studies provide foundational knowledge of Hg behaviour, they do not investigate how pollutant mixtures or variable additions of pollutants affect the natural cycling of

Hg in freshwater systems, and thus we have a limited understanding of the link between natural Hg cycling and pollution gradients of other metals.

1.3.3 Effect of Sulfate on Hg Cycling

Sulfur is an essential element to many living organisms as it is one of the fundamental components of protein reactivity[36]. Due to its many oxidation states (-2, 0, +2, +4,+6) it can form various species, each with different chemical characteristics[37,38]. Its fate in the environment is controlled by chemical and biotic reactions that occur in oxic and anoxic conditions, which constitutes its biogeochemical cycle[37,39]. Unfortunately, the sulfur cycle is heavily perturbed by human activity[38] as anthropogenic emissions of sulfur into the atmosphere have far exceeded natural sulfur emissions[36,38]. Given the impact that human activities have on the sulfur cycle, it is important to understand this relationship and determine how changes to the sulfur cycle can affect the health of the environment.

Pyrite (FeS) smelting and roasting are one of the primary sources of sulfur emissions. FeS is a host for many metals, and thus FeS is commonly roasted to remove the metals of interest. During this process, great amounts of sulfur dioxide (SO_2) are released into the atmosphere[40]. The SO_2 subsequently goes through a series of reactions, reacting with either hydroxyl radicals or hydrogen peroxide, to form sulfuric acid. This sulfuric acid is then deposited into lakes, where it dissociates and becomes sulfate[41]. In lakes surrounding mining sites, there are often increased sulfate concentrations which can have widespread environmental effects[42].

Both reduction and oxidation of sulfur species in the environment can be done through microbial metabolism[37]. Sulfate reducing microbes (SRMs) are anaerobic organisms that use sulfate as their terminal electron acceptor (TEA). Most SRMs have adapted to many different environments, they use a wide array of electron donors (e.g. acetate, fumarate, pyruvate, H_2)[37,43] and can also use less common alternative compounds as terminal electron acceptors (e.g. As(V))[44–47].

In addition, microbes that utilize sulfate as a TEA are one of the main microbial guilds responsible for Hg cycling[48], possessing the capacity to both methylate and demethylate Hg species[49]. Despite that, not all species of SRMs are capable of Hg methylation[50], however their presence has been found to correlate with Hg methylation[51]. SRMs were shown to be one of the main methylators of Hg in freshwater systems[48]. Studies have shown that net MeHg production in water increases with the addition of sulfate[17] and that Hg methylation is significantly correlated with sulfate reducing rates[50].

1.3.4 Effect of Arsenic on Hg Cycling

The toxic nature of As has been common knowledge for centuries as it has been used as a poison and pesticide. Most toxic elements on Earth exist in small quantities in the upper mantle of the crust; however, As is an exception to this rule as it is a relatively abundant trace element. As exists under four oxidation states: As[-3], As(0), As[+3], and As[+5][52]. The two most abundant

states are arsenite (+3) and arsenate (+5), which are found in reducing and oxidizing environments respectively[53].

Pentavalent (As(V)) and trivalent (As(III)) As species have different proposed mechanisms of toxicity, however trivalent species are considered the more toxic form. Pentavalent As can mimic phosphate (PO_4^{-3}) in the environment, and this can have detrimental effects on several biochemical reactions in microbial cells. However, trivalent species exhibit more potent toxicity than their pentavalent counterparts, as they inhibit the activity of thiols in proteins of microbial cells[52]. Trivalent species are also less strongly adsorbed onto minerals compared to pentavalent species, meaning that reduction of As(V) species can increase the mobility and the bioavailability of As in the aqueous environment[54].

Although As is a relatively abundant metalloid in the Earth's crust, it is locked into minerals and not bioavailable to organisms. Naturally, As is released into the environment by volcanic activity. However, due to the fact that geological formations containing As also contain valuable metals (e.g. gold, iron, copper), the most important modern source of As release is the burning of coal and ore roasting[4,55–57]. Notably, sulfide bearing minerals are commonly enriched in As (e.g. arsenopyrite), as is the case in the bedrock of Yellowknife (NWT)[58].

Despite its microbial toxicity, there is growing evidence that shows the role of microbes in As geochemical cycling[59]. Various microbes are capable of oxidizing[60,61], methylating[62], demethylating, and reducing[63,64] As species. Additionally, microbes have been shown to have

As resistance genes in plasmids, which code for reductive detoxification (*ars* operon) of As(V)[65]. In the case of oxidation and reduction transformations, these transformations have been identified as a detoxification method[66], and in some cases, microbes are capable of reducing or oxidizing As as an energy source. For example, Ahmann et al. (1994) first reported a microbe isolated from an As contaminated site was able to gain energy for growth from As(V) reduction[67]. Since then, multiple studies have demonstrated that certain bacterial strains reduce and oxidize As as a source of energy, usually referred to as As metabolizing microbes[44,46,47,60,68–70].

Most studies examining dissimilatory As(V) reduction show that As(V) is used as an alternative TEAs[44,66,68,69,71–74]. Sulfate reducing microbes (SRMs) in particular are known to use compounds other than sulfate, such as As(V)[45,72,73,75,76], as TEA under anoxic conditions[77]. As previously mentioned in section 1.3.3, SRMs are also primary producers of MeHg in many freshwater environments[51,78,79]. Therefore, SRMs could possibly affect the speciation of both Hg and As. However, presently there is no scientific studies, in the field or laboratory, that have examined the effect of As on Hg cycling.

1.3.5 Effect of Selenium on Hg Cycling

Se is an essential element for various organisms and is a nutrient commonly found in fish[80]. Of all environmental components, the Earth's crust contains the majority of the Se and many geological formations tend to be rich in this element. Because of this phenomenon, weathering of geological formations (i.e. water and wind action) is a natural source of Se to in-land waters. Still,

anthropogenic activity is also an important source of Se to the environment. In particular, ore roasting displaces large quantities of Se from geological formations to other environmental compartments such as lakes and rivers[29,81]. As a consequence, during the metal extraction process, the environment surrounding mining sites can become enriched with Se[29,81–83].

The range over which Se is beneficial to organisms is narrow and it is easy for environments to become polluted with this metal; many studies have demonstrated the toxic effects of Se on lakes[84,85] and biota[86–88]. However, some studies have shown favourable effects of Se on the accumulation and production of MeHg in a system[86,87,89–91]. In particular, Se has been shown to reduce the mobility, bioavailability, and toxicity of Hg in aquatic ecosystems[86,87,89,92,93]. Because of this, Se addition has been proposed as a means of mitigating Hg contamination[92,94,95]. However, there remains a lack of scientific evidence for the long-term effects of Se additions on Hg cycling in a system[93]. Understanding how both Se and Hg respond, long term, in lake systems will provide a better understanding of the feasibility of such remediation actions and how legacy Se pollution in the environment affects Hg cycling long term.

1.4 STUDY SITES

Considering that most environmental regulations pertaining to mining activities were not put into effect until the mid 1900's[96], it is reasonable to assume that the vast majority of historical mining operations created some sort of pollution and that this legacy of pollution affects the surrounding landscape. In fact, many sites near smelters show some legacy contamination[5,30,42,97].

In addition, mining sites are complex; many have an intricate mixture of contaminants and a considerable temporal variability in the volume of pollutants emitted in the environment. With that in mind, it is important to understand how this complexity can affect the natural cycling of Hg as this will inform how particular sites are remediated.

In this thesis, I studied two different historical mining sites, both located in Canada: Yellowknife in the Northwest Territories and Sudbury in Ontario. Both sites have an extensive history of mining pollution, which has resulted in varying pollution gradients (i.e. distributions) surrounding the sites. Yellowknife presents an ideal opportunity to study the effect of a mixture of pollutants, As and sulfate, on the cycling of Hg in the lakes. Sudbury, a site that has been extensively studied by environmental scientists during peak Se emissions, offers an ideal opportunity to understand how current decreases in Se loading to the environment can affect Hg bioaccumulation in lake biota.

1.4.1 Yellowknife

The City of Yellowknife sits on the shore of Great Slave Lake, in the southern part of the Northwest Territories. For more than 7 000 years, Yellowknife and the territory surrounding it has been home to the Yellowknives Dene First Nations; whose name originates from the copper tools they fabricated out of rock found in the area. Yellowknife sits on ancient volcanic bedrock composed of a greenstone belt that is rich in many minerals and metals, including gold. Because of its rich underlying geology, it was prime territory for mineral and metal exploration[13].

Giant Mine, located on the outskirts of Yellowknife, was one of Canada's largest mining operations. The mine opened in 1948 and closed in 2004, during which time it extracted gold from arsenopyrite deposits[98]. The arsenopyrite deposits found in the Yellowknife area consist of a refractory ore that is resistant to normal extraction techniques like cyanidation due to the presence of sulfides. Thus the ore was first roasted to release sulfur, followed by cyanidation to collect the gold[99]. The roasting of arsenopyrite minerals increased the release of sulfur, As, and antimony to the atmosphere. These contaminants were ultimately deposited in the environment surrounding the mine and the city of Yellowknife[55] along a gradient, with deposition decreasing with distance away from the roasting stack[100].

In 1999, the previous owner of the mine, Royal Oak Mines, went into receivership. At that point, the ownership of the mine was transferred to Indigenous and Northern Affairs Canada (INAC), who initiated remediation efforts of the Giant Mine property[101]. At the moment, six parties have signed the Giant Mine Remediation Project Environmental Agreement, including: INAC, Yellowknives Dene First Nation, and the City of Yellowknife. The objective of this agreement includes, but is not limited to: "…the remediation of the Giant Mine site in a manner that eliminates or substantially mitigates the environmental risks posed by the site"[102]. However, despite numerous studies showing that contamination from Giant Mine operations extends beyond the boundaries of the mining lease property[4,30,100,103,104], the above agreement does not mention any plans to remediate the surrounding land. Presently no government body has taken

responsibility to assess and potentially remediate the off-site contamination from the roasting at Giant Mine[105].

Addition of mining pollutants to the surrounding environment can have significant effects on the biogeochemical processes, which in turn affects the wildlife and residents of nearby communities. The environment surrounding Giant Mine includes many rivers, lakes, wetlands, and forests[98]. Residents of local communities interact and depend on these ecosystems for food (i.e. fishing) and leisure (e.g. swimming), which directly exposes them to environmental changes caused by the roasting emissions from Giant Mine. Additionally, as the lakes in Yellowknife have background levels of Hg in the water column, it presents a great opportunity to observe how these pollutants can affect the natural cycling of Hg in freshwater lakes. The results from my thesis play a small part in understanding the full effect of legacy mining pollution on the lakes surrounding the city.

1.4.2 Sudbury

Similar to Yellowknife, the city Sudbury is shaped by its geological foundation. Sudbury is located in Ontario, Canada and is the largest city in northern Ontario, with an approximate population of 160 000 people[106]. The Greater Sudbury area is part the traditional territory of the Atikameksheng Anishinaabek, which are decedents of the Ojibwe, Algonquin, and Oddawa Nations[107,108]. It is proposed by academics that these nations used the mineral deposits for tools and possibility assisted the first colonial prospectors in the area[107,109]. However, although it was

known that the area of Sudbury was rich in mineral deposits, commercial mining did not start until the late 1880's when the first mining operations were set in the region, one of them being the Copper Cliff mining site that opened in May 1886. From then until the early 2000's, Sudbury was one of Canada's most productive mining cities with two companies occupying most of the production: Inco (presently Vale) and Falconbridge[110].

Intense mining and smelting activities in the area created a pollution "halo" around the city, with high concentrations of sulfur, Se, nickel, and copper in the surrounding lakes[29,42,111]. In particular, smelting activities in the early to mid 20th century emitted great amounts of SO_2, which acidified the landscape around the smelter sites and created one of the most contaminated environments in Canada[110]. Fortunately, Sudbury is also an example of a successful remediation effort, in which the acidification caused by smelting activity was counteracted with liming and reduced release of pollutants[14,110,112,113].

Despite these remediation efforts, other pollutants were still emitted from the smelter sites until the early 2000's. One of these pollutants was Se[29,114], which was released in great amounts to the atmosphere and deposited in the surrounding environment[14,115]. Due to the interesting interplay between Se and Hg (see "Effects of Selenium on Hg Cycling" section above), Belzile et al (2006)[86] have shown that the addition of Se in Sudbury was decreasing the accumulation of Hg and MeHg in the biota of the surrounding lakes.

In the past few years, there have been stricter emission regulations, more efficient smelting techniques, and periods of reduced production that have resulted in reduced atmospheric Se emissions from the smelter stacks in the Sudbury area[14,116]. Consequently, due to these restrictions and previous studies[86], this site is an ideal opportunity to study the effects of varying Se deposition on supressing Hg accumulation in lake biota.

1.5 THESIS STRUCTURE

The goal of this thesis is to model how historical mining pollution affects the cycling of natural levels of Hg in boreal freshwater lakes. Each research chapter of the thesis focuses on a type of contaminant that is commonly associated with mining activities: sulfate, As, and Se.

The second chapter of this thesis investigated the effect of sulfate and As gradients on the cycling of Hg in lakes surrounding in Yellowknife, NWT, Canada. We hypothesized that enhanced sulfate deposition from smelting affected MeHg dynamics in sediments of lakes surrounding Giant Mine. Assuming that sulfate reducing metabolism was the main variable limiting MeHg in the system and not the amount or delivery rate of bioavailable Hg, we predicted that sulfate would be positively correlated with both rates of MeHg production and %MeHg.

The third chapter was a laboratory study which examined the effect of As(V) on microbial Hg methylation under controlled conditions using freshwater lake sediments. Considering that SRMs are the main Hg methylators in freshwater systems and that As(V) can be used by SRMs as

a TEA, we postulated that presence of As(V) in the system could influence the production of MeHg. This study was exploratory by nature; our goal was to understand how As(V) addition to sediment slurries would affect the rate of production of MeHg in lake sediments.

The fourth chapter investigated the effect of a long-term variable dosing of Se in freshwater lakes on the accumulation of Hg and MeHg in the foodweb. We hypothesized that changes in Se emissions in Sudbury will alter Hg accumulation in biota, and predicted that a decrease in emissions would lead to an increase in Hg and MeHg in biota.

Finally, the fifth chapter is the conclusion to the thesis, which will provide the main conclusions, the potential directions of future research, and the contribution of my findings to the present knowledge of Hg cycling in freshwater environments.

1.6 REFERENCES

1 Harry. Chandler, *Metallurgy for the non-metallurgist*, ASM Int'l., Materials Park, OH, 1998.

2 F. P. Carvalho, Mining industry and sustainable development: time for change, *Food and Energy Security*, 2017, **6**, 61–77.

3 Government of Canada, Minerals and the economy, https://www.nrcan.gc.ca/our-natural-resources/minerals-mining/minerals-metals-facts/minerals-and-the-economy/20529#revenue.

4 H. E. Jamieson, *Reviews in Mineralogy and Geochemistry*, 2014, 79, 533–551.

5 D. D. Sprague and J. C. Vermaire, *Water, Air, & Soil Pollution*, 2018, 229, 87.

6 W. S. Gratton, K. K. Nkongolo and G. A. Spiers, Heavy Metal Accumulation in Soil and Jack Pine (Pinus banksiana) Needles in Sudbury, Ontario, Canada, *Bull. Environ. Contam. Toxicol*, 2000, **64**, 550–557.

7 P. G. Falkowski, T. Fenchel and E. F. Delong, *Science (New York, N.Y.)*, 2008, 320, 1034.

8 C. Cravo-Laureau, C. Cagnon, B. Lauga and R. Duran, 2017.

9 Anonymous, *Canadian Mining Journal*, 2005, 126, 10–13.

10 M. J. Hird, *Theory, Culture & Society*, 2017, 34, 187–209.

11 UNEP, *Minamata Convention on Mercury*, New York, 2013.

12 D. A. Cranstone and C. N. R. Canada, 2002.

13 J. Sandlos and A. Keeling, *Giant Mine : historical summary*, Memorial University, St. John's, N.L, 2015.

14 M. Boerchers, P. Fitzpatrick, C. Storie and G. Hostetler, *The Extractive Industries and Society*, 2016, 3, 793–801.

15 Jeffrey Jones, *The Globe and Mail*, 2014.

16 E. M. Sunderland and M. Tumpney, in *Persistent Organic Pollutants and Toxic Metals in Foods*, eds. M. Rose and A. Fernandes, Woodhead Publishing, 2013, pp. 392–413.

17 C. C. Gilmour, E. A. Henry and R. Mitchell, *Environ Sci Technol*, 1992, 26, 6.

18 S. M. Ullrich, T. W. Tanton and S. A. Abdrashitova, *Critical Reviews in Environmental Science and Technology*, 2001, 31, 241–293.

19 A. R. Paranjape and B. D. Hall, *FACETS*, 2017, 2, 85–119.

20 H. F. V. Braaten, H. A. de Wit, E. Fjeld, S. Rognerud, E. Lydersen and T. Larssen, Environmental factors influencing mercury speciation in Subarctic and Boreal lakes, *Science of The Total Environment*, 2014, **476–477**, 336–345.

21 C. E. Moreno, E. Fjeld and E. Lydersen, The effects of wildfire on mercury and stable isotopes (δ15N, δ13C) in water and biota of small boreal, acidic lakes in southern Norway, *Environmental Monitoring and Assessment*, 2016, **188**, 178.

22 M. S. Evans, W. L. Lockhart, L. Doetzel, G. Low, D. Muir, K. Kidd, G. Stephens and J. Delaronde, Elevated mercury concentrations in fish in lakes in the Mackenzie River Basin: the role of physical, chemical, and biological factors., *Sci Total Environ*, 2005, **351–352**, 479–500.
23 J. Chételat, M. Amyot and E. Garcia, Habitat-specific bioaccumulation of methylmercury in invertebrates of small mid-latitude lakes in North America., *Environ Pollut*, 2011, **159**, 10–17.
24 A. E. Poste, H. F. V. Braaten, H. A. de Wit, K. Sørensen and T. Larssen, Effects of photodemethylation on the methylmercury budget of boreal Norwegian lakes, *Environmental Toxicology and Chemistry*, 2015, **34**, 1213–1223.
25 C. A. Emmerton, C. A. Cooke, G. R. Wentworth, J. A. Graydon, A. Ryjkov and A. Dastoor, Total Mercury and Methylmercury in Lake Water of Canada's Oil Sands Region, *Environ. Sci. Technol.*, 2018, **52**, 10946–10955.
26 A. R. Paranjape and B. D. Hall, Recent advances in the study of mercury methylation in aquatic systems, *FACETS*, 2017, **2**, 85–119.
27 G. A. Palyanova, Gold and Silver Minerals in Sulfide Ore, *Geology of Ore Deposits*, 2020, **62**, 383–406.
28 J. P. Vaughan, The process mineralogy of gold: The classification of ore types, *JOM*, 2004, **56**, 46–48.
29 J. O. Nriagu and H. K. T. Wong, *Nature*, 1983, 301, 55.
30 A. J. Houben, R. D'Onofrio, S. V. Kokelj and J. M. Blais, *PLoS ONE*, 2016, 11.
31 R.-Q. Yu, J. R. Flanders, E. E. Mack, R. Turner, M. B. Mirza and T. Barkay, Contribution of Coexisting Sulfate and Iron Reducing Bacteria to Methylmercury Production in Freshwater River Sediments, *Environ. Sci. Technol.*, 2012, **46**, 2684–2691.
32 J. Stamenkovic, M. S. Gustin, M. C. Marvin-DiPasquale, B. A. Thomas and J. L. Agee, Distribution of total and methyl mercury in sediments along Steamboat Creek (Nevada, USA), *Science of the Total Environment*, 2004, **322**, 167–177.
33 J. E. Gray, M. E. Hines, D. P. Krabbenhoft and B. Thoms, *Applied Geochemistry*, 2012, 27, 106–114.
34 M.-L. Avramescu, E. Yumvihoze, H. Hintelmann, J. Ridal, D. Fortin and D. R. S. Lean, Biogeochemical factors influencing net mercury methylation in contaminated freshwater sediments from the St. Lawrence River in Cornwall, Ontario, Canada., *Sci Total Environ*, 2011, **409**, 968–978.
35 N. W. Johnson, C. P. J. Mitchell, D. R. Engstrom, L. T. Bailey, J. K. Coleman Wasik and M. E. Berndt, Methylmercury production in a chronically sulfate-impacted sub-boreal wetland, *Environ. Sci.: Processes Impacts*, 2016, **18**, 725–734.
36 R. J. Charlson, T. L. Anderson and R. E. McDuff, *International Geophysics*, 2000, 72, 343–359.
37 H. L. Ehrlich and D. K. Newman, 2008.

38 W. W. Kellogg, R. D. Cadle, E. R. Allen, A. L. Lazrus and E. A. Martell, *Science*, 1972, 175, 587–596.
39 J.-C. editor Bertrand, 2015.
40 P. Rasch, M. Barth, J. Kiehl, S. Schwartz and C. Benkovitz, *Journal of Geophysical Research: Atmospheres*, 2000, 105, 1367–1385.
41 V. A. Mohnen, *Scientific American*, 1988, 259, 30–38.
42 K. Winterhalder, Environmental degradation and rehabilitation of the landscape around Sudbury, a major mining and smelting area, *Environ. Rev.*, 1996, **4**, 185–224.
43 V. N. de Jonge, M. Elliott and E. Orive, Causes, historical development, effects and future challenges of a common environmental problem: eutrophication, *Hydrobiologia*, 2002, **475**, 1–19.
44 B. Planer-Friedrich, C. Härtig, R. Lohmayer, E. Suess, S. H. McCann and R. Oremland, *Environmental science & technology*, 2015, 49, 6554.
45 J. R. Pérez-Jiménez, C. Defraia and L. Y. Young, *Biochemical and Biophysical Research Communications*, 2005, 338, 825–829.
46 D. K. Newman, E. K. Kennedy, J. D. Coates, D. Ahmann, D. J. Ellis, D. R. Lovley and F. M. M. Morel, *Archives of Microbiology*, 1997, 168, 380–388.
47 J. M. Macy, J. M. Santini, B. V. Pauling, A. H. O'Neill and L. I. Sly, *Archives of Microbiology*, 2000, 173, 49–57.
48 A. Kerry, P. Welbourn, B. Prucha and G. Mi, *Water Air & Soil Pollution*, 1991, 56, 565–575.
49 Y. Li and Y. Cai, *Chinese Science Bulletin*, 2013, 58, 177–185.
50 J. K. King, F. M. Saunders, R. F. Lee and R. A. Jahnke, *Environmental Toxicology and Chemistry*, 1999, 18, 1362–1369.
51 R. Devereux, M. R. Winfrey, J. Winfrey and D. A. Stahl, *FEMS Microbiology Ecology*, 1996, 20, 23–31.
52 M. F. Hughes, *Toxicology Letters*, 2002, 133, 1–16.
53 R. Briones-Gallardo, V. M. Escot-Espinoza and E. Cervantes-González, *International Journal of Environmental Science and Technology*, 2017, 14, 609–622.
54 J. Gulens, D. R. Champ and R. E. Jackson, *Chemical Modeling in Aqueous Systems*, 1979, 93, 81–95.
55 M. J. Bromstad, Thesis, Queen's University, 2011.
56 D. J. Vaughan, *Elements*, 2006, 2, 71–75.
57 D. J. Hoffman, 2003.
58 C. D. Anglin, *Gold in the Yellowknife greenstone belt, Northwest Territories : results of the EXTECH III multidisciplinary research project : collected papers and DVD of data reporting on results of the Yellowknife EXTECH III multidisciplinary research project*, Geological Association of Canada, Mineral Deposits Division, St. John's, Nfld, 2006.

59 C. Cervantes, G. Ji, J. Ramirez and S. Silver, *FEMS Microbiology Reviews*, 1994, 15, 355–367.
60 F. H. Osborne and H. L. Enrlich, *The Journal of applied bacteriology*, 1976, 41, 295.
61 F. Battaglia-Brunet, M. -C Dictor, F. Garrido, C. Crouzet, D. Morin, K. Dekeyser, M. Clarens and P. Baranger, *Journal of Applied Microbiology*, 2002, 93, 656–667.
62 R. Turpeinen, M. Pantsar-Kallio, M. Häggblom and T. Kairesalo, Influence of microbes on the mobilization, toxicity and biomethylation of arsenic in soil., *Sci Total Environ*, 1999, **236**, 173–180.
63 W. Cullen and K. Reimer, 1989.
64 C. N. Cheng and D. D. Focht, *Applied and Environmental Microbiology*, 1979, 38, 494.
65 S.-L. Tsai, S. Singh and W. Chen, *Current Opinion in Biotechnology*, 2009, 20, 659–667.
66 G. Ji and S. Silver, *Proceedings of the National Academy of Sciences of the United States of America*, 1992, 89, 9474–9478.
67 D. Ahmann, A. L. Roberts, L. R. Krumholz and F. M. M. Morel, *Nature*, 1994, 371, 750–750.
68 A. Niggemyer, S. Spring, E. Stackebrandt and R. F. Rosenzweig, *Applied and Environmental Microbiology*, 2001, 67, 5568.
69 Y. Chang, A. Nawata, K. Jung and S. Kikuchi, *Official Journal of the Society for Industrial Microbiology and Biotechnology*, 2012, 39, 37–44.
70 T. H. Osborne, J. M. McArthur, P. K. Sikdar and J. M. Santini, *Environmental science & technology*, 2015, 49, 4193.
71 N. Kumari and S. Jagadevan, *Chemosphere*, 2016, 163, 400–412.
72 D. Y. Sorokin, T. P. Tourova, M. V. Sukhacheva and G. Muyzer, *Extremophiles : life under extreme conditions*, 2012, 16, 597–605.
73 J. S. Blum, T. R. Kulp, S. Han, B. Lanoil, C. W. Saltikov, J. F. Stolz, L. G. Miller and R. S. Oremland, *Extremophiles : life under extreme conditions*, 2012, 16, 727–742.
74 Z. Qian, H. Tianwei, H. R. Mackey, M. C. M. van Loosdrecht and C. Guanghao, *Water research (Oxford)*, 2019, 150, 162–181.
75 D. K. Newman, T. J. Beveridge and F. M. M. Morel, *Applied and Environmental Microbiology*, 1997, 63, 2022.
76 R. S. Oremland, P. R. Dowdle, S. Hoeft, J. O. Sharp, J. K. Schaefer, L. G. Miller, J. Switzer Blum, R. L. Smith, N. S. Bloom and D. Wallschlaeger, *Geochimica et Cosmochimica Acta*, 2000, 64, 3073–3084.
77 G. Muyzer and A. J. M. Stams, The ecology and biotechnology of sulphate-reducing bacteria, *Nature Reviews Microbiology*, 2008, **6**, 441–454.

78 R.-Q. Yu, J. R. Flanders, E. E. Mack, R. Turner, M. B. Mirza and T. Barkay, *Environmental Science & Technology*, 2012, 46, 2684–2691.
79 G. C. Compeau and R. Bartha, *Applied and Environmental Microbiology*, 1985, 50, 498.
80 P. M. Chapman, W. J. Adams, M. Brooks, and Setac, 2010.
81 A. Fernández-Martínez and L. Charlet, *Reviews in Environmental Science and Bio/Technology*, 2009, 8, 81–110.
82 J. R. Muscatello, A. M. Belknap and D. M. Janz, Accumulation of selenium in aquatic systems downstream of a uranium mining operation in northern Saskatchewan, Canada, *Environmental Pollution*, 2008, **156**, 387–393.
83 C. C. Wellen, N. J. Shatilla and S. K. Carey, Regional scale selenium loading associated with surface coal mining, Elk Valley, British Columbia, Canada, *Science of The Total Environment*, 2015, **532**, 791–802.
84 M. Barwick and W. Maher, *Marine Environmental Research*, 2003, 56, 471–502.
85 N. Belzile, Y. W. Chen and R. Xu, *Applied Geochemistry*, 2000, 15, 1439–1454.
86 N. Belzile, Y.-W. Chen, J. M. Gunn, J. Tong, Y. Alarie, T. Delonchamp and C.-Y. Lang, *Canadian Journal of Fisheries and Aquatic Sciences*, 2006, 63, 1–10.
87 P. Bjerregaard, S. Fjordside, M. G. Hansen and M. B. Petrova, *Environmental Science & Technology*, 2011, 45, 9793–9798.
88 M. Mailman, R. A. Bodaly, M. J. Paterson, S. Thompson and R. J. Flett, *Archives of Environmental Contamination and Toxicology*, 2014, 66, 32–40.
89 S. Nuutinen and J. Kukkonen, *Biogeochemistry*, 1998, 40, 267–278.
90 L. Jin, P. Guo and X. Xu, *Toxicological & Environmental Chemistry*, 1999, 69, 255–261.
91 L. J. Jin, P. Guo and X. Q. Xu, *Bulletin of Environmental Contamination and Toxicology*, 1997, 59, 994–999.
92 E. E. V. Chapman, J. Robinson, J. Berry and L. M. Campbell, *Water, Air, & Soil Pollution*, 2016, 227, 216.
93 J. R. Gerson, D. M. Walters, C. A. Eagles-Smith, E. S. Bernhardt and J. E. Brandt, Do Two Wrongs Make a Right? Persistent Uncertainties Regarding Environmental Selenium–Mercury Interactions, *Environ. Sci. Technol.*, 2020, **54**, 9228–9234.
94 G. R. Southworth, M. J. Peterson and R. R. Turner, *Chemosphere*, 1994, 29, 71–79.
95 K. Paulsson and K. Lundbergh, *Water Air & Soil Pollution*, 1991, 56, 833–841.
96 M. Laforce, U. Lapointe and V. Lebuis, Mining Sector Regulation in Quebec and Canada: is a Redefinition of Asymmetrical Relations Possible?, *null*, 2009, **84**, 47–78.
97 A. S. Dixit, S. Alpay, S. S. Dixit and J. P. Smol, Paleolimnological reconstructions of Rouyn-Noranda lakes within the zone of influence of the Horne Smelter, Québec, Canada, *Journal of Paleolimnology*, 2007, **38**, 209.

98 I. Canada and N. Affairs, *Giant Mine Remediation Project: Developer's Assessment Report*, Indian and Northern Affairs Canada, 2010.
99 C. D. Anglin, I. Yellowknife Extech, D. Geological Association of Canada. Mineral Deposits, C. Geological Survey of and O. Northwest Territories Geoscience, 2006.
100 M. J. Palmer, J. M. Galloway, H. E. Jamieson, R. T. Patterson, H. Falck and S. V. Kokelj, 2016.
101 Stantec Consulting Ltd, *Aquatic Data Collection in Lower Martin Lake, Upper Baker Creek and Trapper Creek*, Stantec Consulting Ltd., 2014.
102 participant in a treaty Canada, participant in a treaty Dene Nation, participant in a treaty Northwest Territories, participant in a treaty Yellowknife, participant in a treaty Alternatives North, A. Canada. Aboriginal, issuing body Northern Development Canada and participant in a treaty North Slave Métis Alliance, *Giant Mine Remediation Project Environmental Agreement*, 2015.
103 M. J. Bromstad, L. A. Wrye and H. E. Jamieson, *Applied Geochemistry*, 2017, 82, 102–118.
104 R. Dirszowsky and K. Wilson, *Environmental Earth Sciences*, 2016, 75, 1–13.
105 *Giant Mine Oversight Board establishment report July 2015 - December 2016*, Giant Mine Oversight Board, 2017.
106 Statistics Canada, *2016 Census. Statistics Canada Catalogue no. 98-316-X2016001*, Statistics Canada, Ottawa.
107 D. Munton and O. Temby, Smelter Fumes, Local Interests, and Political Contestation in Sudbury, Ontario, during the 1910s, *Urban History Review / Revue d'histoire urbaine*, 2015, **44**, 24–36.
108 S. Manitowabi, *Historical and Contemporary Realities: Movement Towards Reconciliation*, Press Books, Montreal, 2018.
109 R. M. Telford, *The Sound of the Rustling of the Gold is Under My Feet where I Stand, We Have a Rich Country [microform] : a History of Aboriginal Mineral Resources in Ontario*, Thesis (Ph.D.)–University of Toronto, 1996.
110 O. W. Saarinen, *From meteorite impact to constellation city: A historical geography of greater Sudbury*, Wilfrid Laurier University Press, 2013.
111 E. Gorham and A. G. Gordon, *Canadian Journal of Botany*, 1960, 38, 477–487.
112 J. M. Gunn, 1995.
113 W. Keller and T. Roger Pitblado, Water quality changes in sudbury area lakes: A comparison of synoptic surveys in 1974–1976 and 1981–1983, *Water, Air, and Soil Pollution*, 1986, **29**, 285–296.
114 Y.-W. Chen, N. Belzile and J. Gunn, 2001, 46.
115 Cem, *Metal Levels in the Soils of the Sudbury Smelter Footprint*, 2004.
116 K. Winterhaider, *Environmental Technology Letters*, 1983, 4, 209–216.

1.7 SUPPLEMENTARY INFORMATION

Order occurring in the text:

Table S1.1: Relative concentrations of methylmercury (MeHg) in water columns of freshwater boreal lakes as a percent (%) of total mercury in various peer-reviewed

Table S 1.1: Relative concentrations of methylmercury (MeHg) in water columns of freshwater boreal lakes as a percent (%) of total mercury in various peer-reviewed studies

Reference	MeHg in Water (%)
Evans et al. (2005)[22]	4.2 (±2.1)
Chetelat et al. (2011)[23]	8 (±5)
Moreno et al. (2016)[21]	5.8 (± 3)
Poste et al. (2015)[24]	2.3 (±0.3)
Emmerton et al. (2018)[25]	5.3 (±0.7)

CHAPTER 2: THE EFFECT OF LEGACY GOLD MINING ON METHYLMERCURY CYCLING AND MICROBIAL COMMUNITY STRUCTURE IN NORTHERN FRESHWATER LAKES

Ažđajić, Mija[1]; Yumvihoze, Emmanuel[1]; Blais, Jules M[1]; Poulain, Alexandre J[1]

[1] Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, Ontario K1N 6N5, Canada

A modified version of this manuscript was published: July 16th, 2021
Environmental Science: Processes and Impacts | DOI: 10.1039/D1EM00129A

Main Conclusions:

1) Fraction of methylated total Hg (%MeHg) found in surface water is positively correlated to the sulfate gradient (0 – 115 mg/L)

2) Rate at which Hg is methylated (K_m) in sediments is negatively correlated with total arsenic, and positively correlated with dissolved organic carbon, total phosphorous, and %MeHg in the water

3) Six of the 28 lakes that had detectable demethylation rate constants (K_d) also had significantly lower DOC concentrations than lakes with non-detectable K_d

4) Arsenic and sulfate concentrations are significantly correlated with changes in microbial communities of sediments, along with other environmental variables (total phosphorous, and dissolved organic matter composition).

Contribution to Field:

The majority of studies that investigate Hg cycling in the environment usually concentrate on a few lakes or sampling sites, mostly along Hg pollution gradients. This limits the environmental variables that can be controlled for, and might ultimately affect the conclusions that are drawn from these results. Our results, collected over 3 years and covering 2800 km^2 highlight the importance of conducting larger scale

landscape studies in which we can investigate the effect of coincident pollution gradients (e.g. arsenic and sulfate) affecting Hg cycling

2.1 ABSTRACT

Smelting activities at Giant Mine (Yellowknife, NWT, Canada) have resulted in high sulfate and arsenic concentrations in nearby lakes. Here we tested whether historic smelting affects current mercury (Hg) cycling in 35 freshwater lakes over a 2800 km^2 area around the former gold mine. We sampled lake water and sediment over three consecutive years (2015-2017) using a factorial sampling design that accounted for different environmental variables known to affect the net methylmercury (MeHg) levels in water. Stable Hg(II) and MeHg isotope tracers were used to quantify Hg methylation and demethylation rate constants in sediments, and 16S rRNA gene amplicon sequencing was used to characterize microbial community structure. This study reveals that the fraction of methylated total Hg (%MeHg) found in surface water is positively correlated to the sulfate gradient, while the rate at which Hg is methylated (K_m) in sediments is negatively correlated with total arsenic, and positively correlated with dissolved organic carbon, total phosphorous, and %MeHg in the water. Furthermore, 6 of the 28 lakes that had detectable demethylation rate constants (K_d) also had significantly lower DOC concentrations than lakes with non-detectable K_d. Our results also show legacy pollution from smelting activities is affecting the structure of the microbial communities in lake sediments. This study revealed the complex dynamics of Hg cycling in this northern environment, highlighting the importance of large-scale

studies in which the effect of multiple pollution gradients (e.g. arsenic and sulfate) must be taken into consideration.

2.2 ENVIRONMENTAL SIGNIFICANCE

Studies that have investigated the effect of mining activities on mercury cycling are mostly done on a small subset of lakes that cannot fully capture the range of limnological characteristics in a landscape. Our study assessed the effect of multiple variables associated with gold mining and arsenopyrite roasting (e.g. sulfate and arsenic concentrations) on mercury cycling on such a diverse set of lakes. Using stable mercury isotopes to measure methylation and demethylation rate constants, our results show that the sulfate concentration is a predictor of the fraction of total mercury that is present as methylmercury in the water column (%MeHg), while the rate at which microbes methylate mercury in sediments is correlated with total arsenic, dissolved organic carbon, total phosphorous, and %MeHg in water. We also show that arsenic and sulfate concentrations are significantly correlated with changes in microbial communities of sediments, along with other environmental variables (total phosphorous, and dissolved organic matter composition). Our results highlight the complex interaction between signature variables of mining activities and mercury transformations in heterogeneous landscapes.

2.3 INTRODUCTION

Mercury (Hg) is a toxic trace metal whose methylated form (i.e. MeHg) is a primary concern due to its neurotoxicity and ability to bioaccumulate and biomagnify in higher trophic level organisms, resulting in high [MeHg] in some predatory fish. Once in the body, MeHg can easily cross the blood-brain or placenta barriers and affect either the neurological system of a person or disrupt the normal development of a fetus[1]. As a result, understanding MeHg production and degradation in the environment is vital to protecting wildlife and humans whose diet exposes them to high levels of Hg[2].

Whereas abiotic Hg methylation in natural environments has been shown to be possible[3,4], Hg methylation by microorganisms is the most probable pathway to produce MeHg[5]. The identification of genes (*hgc*AB) essential for microbial Hg methylation[6], has shown that different guilds of microbes possess the genetic potential to produce MeHg[7–9]. However, sulfate reducing microbes, which use sulfate as a terminal electron acceptor, remain important Hg methylators in freshwater environments[10].

Although [MeHg] and [total Hg] in environmental matrices can provide information on how much Hg is methylated (e.g. %MeHg), these values provide only a snapshot at a given moment in time and do not address the dynamic cycling of Hg. In particular, %MeHg does not reveal how fast the reactions proceed in a given system. It

is now well established that complementing %MeHg data with methylation/demethylation experiments can offer a more complete view of MeHg dynamics[11,12], particularly to understand how perturbations to systems (e.g. addition of sulfate) affect the fate of Hg.

For Hg(II) to be methylated, not only must microbes capable of Hg methylation be metabolically active, but the environmental conditions must also allow for Hg to be bioavailable to microbial cells responsible for its transformation[13,14]. Variables that affect Hg methylation, either by affecting Hg bioavailability or the activity of Hg methylating microbes, include factors such as quantity and quality of dissolved organic matter (DOM), pH, sulfate concentrations, lake productivity, iron concentrations, and temperature[5]. Multiple studies have investigated the effect of various environmental variables on Hg methylation rate constants[15–17], with some concentrating on sites contaminated with Hg[18,19]. However, no studies have investigated the production of MeHg in a vast landscape that is greatly affected by pollution (excluding Hg) resulting from smelting activity.

Canada's North has a long history of natural resource extraction that contributes significantly to its economic sustainability[20] and development[21]. In recent years, a concerted effort was made in expanding the natural extraction industry in Canada's

North, possibly affecting the landscape during and after operations, both directly (e.g. emission of pollutants) and indirectly (e.g. increased nutrient loading to local waters due to population increase)[22,23].

Here we examined factors affecting MeHg concentrations in lake waters near Giant Mine; a gold mine located on the outskirts of Yellowknife and one of Canada's largest mining operations. The mine operated from 1948 to 1999, during which time it extracted gold from arsenopyrite deposits[24]. The arsenopyrite deposits found in the Yellowknife area consist of a refractory ore that is resistant to normal extraction techniques like cyanidation due to the presence of sulfides. Thus the ore was first smelted to release sulfur, followed by cyanidation to collect the gold[25]. The smelting of metal ores released great quantities of pollutants into the atmosphere that were later deposited on the surrounding landscape, making this area one the most polluted sites in Canada[26]. Many studies in the last decade have shown the extent of pollution far beyond the Giant Mine lease boundaries[27–30]. In particular, Houben et al. (2016) showed that arsenic (2.0 – 136 μg/L), antimony (0.1 – 2.0 μg/L), and sulfate (0.5 – 48 mg/L) concentrations in lake water decreased with distance from the roaster stack. These anthropogenic stressors can potentially affect the biogeochemical cycling of metals in the environment, particularly Hg.

Smelting operations have resulted in a 'halo' of high [sulfate] (up to 115 mg/L) in lakes near Giant Mine (<25 km). Although [total Hg] in the lakes remain within concentrations typical of these sub-Arctic environments (1-6 ng/L)[31], the ratio of MeHg relative to total Hg (%MeHg) is higher close to the mine and decreases with distance[27]. These aquatic systems presented a great opportunity to study the effect of sulfate loading on Hg methylation in northern freshwater lakes. We hypothesized that enhanced sulfate deposition from smelting affected MeHg dynamics in sediments of lakes surrounding Giant Mine. Assuming that sulfate reducing metabolism was the main variable limiting [MeHg] in the system and not the amount or delivery rate of bioavailable mercury, we predicted that [sulfate] would be positively correlated with both rates of MeHg production and %MeHg.

We tested our hypothesis by i) identifying which chemical variables correlated with MeHg production in lakes around Giant Mine, and ii) quantifying Hg methylation/demethylation rate potentials in lake sediments along the sulfate gradient around Giant Mine using stable isotopes of Hg. We also measured environmental variables that likely influence microbial Hg cycling. To further assess the response of the microbial community, we characterized changes in microbial community structure along the pollution gradient. We used these changes as one integrative response to the geochemical variation present across the spatial gradient sampled.

2.4 MATERIALS AND METHODS

2.4.1 Study Sites

Sampling took place within a 30 km radius around the Giant Mine roaster stack (Figure 2.1). This radius of sampling was chosen to include reference sites unaffected by the mining activity based on studies that have shown the full extent of the roasting emissions being within a 25 km radius[27]. Lakes in the Yellowknife area were sampled during three years (2015, 2016, and 2017) using a factorial sampling design while attempting to account for variables that may be important in directly or indirectly affecting [MeHg] in freshwater ecosystems such as aqueous [sulfate], [DOC], [total iron], [total phosphorus], and pH. Figure 2.2, a principal component analysis (PCA) of the water chemical variables, shows an orthogonal distribution of the concentration gradients for the environmental variables mentioned above.

Both surface lake water and the top 5 cm of sediment were sampled at each lake. The sampled surface water was acidified and stored at 4°C. Sediment was sampled in triplicate using a Uwitec corer. The top 5 cm of each core was subsampled, using an extruder, into a sterile bag and a small amount of sediment was put into cryotubes using a sterile spatula. The cryotubes were flash-frozen with dry ice and preserved at -80°C

until further analyses. Due to logistical constrains, the rest of the sediment was frozen and preserved at -20°C until analysis.

2.4.2 Water Chemistry

Triplicate surface water samples were analysed for total Hg as described in EPA Method 1631 using cold-vapor atomic fluorescence spectrometry (CV-AFS, Tekran Instrument Corp. Series 2600 spectrophotometer)[32] (average recovery of laboratory control sample: 97.66%). MeHg in water was extracted using the method described in Cai et al. (1997) and analysed with capillary gas chromatography-atomic fluorescence spectrometry (GC-AFS, Ai Cambridge Model 94 GC with a CTC Autosampler and PSA Merlin Detector)[33] (average recovery of laboratory control sample: 88.51%).

Total arsenic and total phosphorous concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS) using EPA method 200.8[34] (average recovery of laboratory control sample: 102.30%).

$SUVA_{254}$ (L/mg-M) is the ratio of absorbance at UV254 and [DOC] in the water, this ratio can be used as a general quantitative estimate of aromaticity of DOC in the sample[35]. The absorbance UV_{254} was measured with a spectrophotometer

(Cary 300 BIO UV–Vis Spectrophotometer) and [DOC] were measured using a modified OI Analytical model 1030 wet TOC analyser, with a model 1051 autosampler[36].

All other chemical analyses (i.e. total iron, pH, sulfate, and total nitrogen) with water samples were done at Taiga Environmental Laboratory (Yellowknife, NWT, Canada), which is certified by the Canadian Association for Laboratory Accreditation (CALA) (ISO/IEC 17025).

2.4.3 Incubation Experiments

The lake sediments were thawed overnight at 4°C. In each vial, 18 g of homogenized sediment was mixed with 54 mL of anaerobic miliQ (1:3 slurry ratio) in the anaerobic chamber. The slurry was prepared in a serum vial with a rubber stopper to ensure no oxygen would penetrate into the slurry samples. The slurry was first left at 4°C for 3 days, then overnight at room temperature in the anaerobic chamber.

Two treatments, each in triplicate, were applied to each lake sediment sample. The first treatment was spiked with $Me^{198}Hg$ and ^{199}Hg solutions (prepared in miliQ and left to equilibrate for 2 hours in a cool dark space), the second was left unspiked to track the natural variation in isotope concentrations. The spiked treatments had a final concentration of 10 ng/g of ^{199}Hg (inorganic) and 5 ng/g of $Me^{198}Hg$, respectively

representing on average 114% and 278% of the Hg and MeHg already present in the system. 2.5 g of sediment were subsampled at each time point (0h, 6h, 12h, 24h, 48h) and flash frozen in liquid nitrogen. Samples were stored at -20°C, then freeze-dried and stored in a cool dark place until analysis.

Several MeHg extraction methods were tested due the large number of lakes with different chemical and geological properties. Ultimately, a modified Cai et al. (1997)[37] extraction method was found to yield the best results, as demonstrated by an average 92.50% recovery rate of $Me^{198}Hg$ isotope. Freeze-dried samples were weighted out and placed into a glass vial, 5 mL of 4 M nitric acid (HNO_3) were added. An internal isotope standard ($Me^{201}Hg$) was added and samples were left in a dark cool place for 30 minutes. Following this, samples were placed in an oven at 55°C for 16 hours. After, 0.5 mL of 1.0 M copper sulfate ($CuSO_4$) was added to cooled samples along with 7 mL of dichloromethane (DCM). The mixture was shaken for 3 hours, then centrifuged for 10 minutes at 3500 rpm. The overlying DCM was then extracted into a second glass vial and 2 mL of 1 mM sodium thiosulfate solution ($Na_2S_2O_3$) was added. The mixture was then shaken for 1 hour and centrifuged for 10 minutes at 3500 rpm. Finally, the overlying sodium thiosulfate was removed and placed into an amber GC vial for analysis. Hg isotopes were then analyzed using liquid chromatography inductively coupled plasma

mass spectrometry (LC-ICP-MS) following the method described in Batista et al. (2011)[38] (Table S2.1, Figure S2.4).

First, for each lake, a linear segment of the methylation and demethylation figure (Figure S2.4) was visually assessed to find the best time point for maximum methylation/demethylation rates. Next, a pseudo first-order relationship for mercury species was assumed, and both the first-order methylation rate constant (K_m, Equation 2.1) and the first-order demethylation rate constant (K_d, Equation 2.2) were calculated based on Hintelmann et al (2000)[39–41]. Considering that the Hg isotopes that are added to sediments have been shown to be more bioavailable that ambient Hg[41], the calculations henceforth will refer to the potential methylation/demethylation rate constants of each lake sediment. Most of the lakes for which we determined K_d (22 out of 28) did not show clear demethylation with kd values either around zero or even slightly positive (Figure S2.4). We did not setup "killed" experiments for every lake sediment types to obtain control K_d values; this was not logistically possible considering our field constraints. In absence of dataset and to provide an estimate of the variance associated with K_d, we used 3 times the standard deviations around the average K_d for lakes that did not show demethylation; we used this value as our limit of quantification (LOQ = 0.304 day^{-1}) to include lakes in our statistical analyses. Note that raw data are provided in supporting information.

Equation 2.1:

$$K_m = \frac{[Me^{199}Hg]_{t24} - [Me^{199}Hg]_{t0}}{[^{199}Hg]_{spiked} * t}$$

Equation 2.2:

$$K_d = -1 * \frac{ln[Me^{198}Hg]_{t24} - ln[Me^{198}Hg]_{t0}}{t}$$

2.4.4 Microbial Community Structure

Sediment DNA was extracted using the MoBio PowerSoil DNA Extraction Kit following the manufacturer's instructions. The quality of DNA extracted was tested ensuring that PCR targeting 16S rRNA and *gln*A genes yielded amplicons of the correct size visible by gel electrophoresis. 16S rRNA gene amplicons were sequenced using Illumina MiSeq with primers 341F (CCTACGGGNGGCWGCAG) and 785R (GACTACHVGGGTATCTAATCC) targeting the V3-V4 region at the MR DNA sequencing facility (Sallowater, Texas, USA).

16S rRNA gene amplicon sequencing results were analyzed with a QIIME2 pipeline using DADA2[42] to infer amplicon sequences and the SILVA 138 database to assign taxonomy[43]. All statistical analyses found in this study pertaining to the microbial community of the lakes were done in R with the following packages: *phyloseq*[44], *vegan*[45],

and *ggvegan*[46]. The details pertaining to the pipeline analysis can be found in a github repository (mijaazdajic/ykn_QIIME_analysis).

2.4.5 Statistical Analyses

Three main statistical techniques were used to analyze the results: general linear regression analyses, unconstrained ordinations, and constrained ordinations. All statistical analyses were done in R with the following packages: *vegan*[45], *MASS*[47], *lmtest*[48], and *car*[49]. The full analyses can be found in a github repository (mijaazdajic/ykn_R_statistical_anlayses).

To analyze which environmental variable best correlated with %MeHg and Hg kinetics (K_m and K_d), we used a stepwise regression analysis to build the best regression model. All residuals of models were tested for model assumptions (i.e. linearity, homoscedasticity, and normality).

An unconstrained multivariate ordination was used to assess the differences in chemical composition of water samples. Each sampling site had four different environmental variables (i.e. sulfate, pH, DOC, total iron) that were normalized and used to make a Euclidean distance matrix. Then, this distance matrix was used in a PCA.

Spatial distances between sampling sites were transformed into rectangular vectors by principal coordinates of neighbour matrices (PCNM)[50]. This transformation reflects the spatial relationships among sampling sites using vectored variables, which are then directly included as covariates in constrained ordinations, allowing to test if the geographic placement of sites explained the changes in microbial community composition.

A UniFrac dissimilarity matrix was calculated to assess the differences in microbial community structures of the sampled lake sediments. UniFrac metrics rely on the phylogenetic distances of microbial communities between samples. Here, we report on the weighted UniFrac metric which considers both phylogenetic relationship and differences in abundance between sampling sites. Using the Unifrac distance matrix, we performed distance based redundancy analysis (db-RDA) to test variability of microbial communities against spatial, environmental, and kinetic (K_m/K_d) data. A db-RDA analysis implements classical multidimensional scaling on a dissimilarity matrix and performs a redundancy analysis on the ordination results to measure the variation explained by a given set of explanatory variables. A db-RDA was chosen because i) it is a constrained analysis complementary to a principal coordinate analysis (PCoA), therefore appropriate to use with non-Euclidean distance matrices such as UniFrac, ii) it has been shown to be more powerful than the commonly used Mantel correlation test[51], and iii) it does not

assume multi-normality in the response variables[52]. We performed a stepwise model selection in order to minimize the number of variables in the analyses and used ANOVA-like permutation tests to assess the significance of coefficients. All analyses were handled with the *vegan* package in R[45].

2.5 RESULTS AND DISCUSSION

Giant Mine smelting operations created a gradient of sulfate (0 – 115 mg/L) in a wide area for which the levels of Hg in lakes remain within natural concentrations (1-6 ng/L)[31]. Based on our current knowledge of MeHg production, we predicted that increasing [sulfate] would stimulate sulfate-reducing microbial metabolism which would result in more MeHg (%MeHg) and higher rate of MeHg production (K_m).

We first set out to choose lakes that were broadly representative of the geochemistry of the region surrounding Giant Mine by sampling lakes in a large geographic area (Figure 2.1, Table 2.1), which we sampled over three years (2015-2017). This spatial variability allowed us to capture the effect of multiple environmental variables on MeHg cycling namely in terms of pH, [sulfate], [DOC], [total phosphorous], and [iron], which are important variables known to affect MeHg production[5] (Figure 2.2). We also explored the role of arsenic, a toxic metalloid, that could possibly confound the results due to the strong gradient that is present in the lakes studied (0.42 – 1327 µg/L).

Our first objective was to identify which environmental variables (sulfate, arsenic, iron, DOC, pH, phosphorous) were correlated with %MeHg observed in lakes. Using a step-wise regression model, [sulfate] in water was the only environmental variable that was significantly correlated with %MeHg in the water column (p-value < 0.05). The relationship between %MeHg and [sulfate] was nonlinear ($\log(y) = \log(x) + a$) and reached a plateau at ca. [sulfate] = 40 mg/L (Figure 2.3a). Most studies show significant non-linear relationships between [sulfate] and [MeHg][53,54] or methylation rate constants[17], and most systems do reach a level at which addition of sulfate does not stimulate production of MeHg[11,55]. This phenomenon is mostly attributed to the relationship between Hg and sulfide, a by-product of sulfate reduction. At low [sulfate], the additions of sulfate stimulate sulfate-reducing microbial metabolism, leading to an increase in the production of MeHg[10]. As sulfide concentrations increase, mercury sulfide complexes form aggregates that are less bioavailable. Therefore, less Hg is methylated in the system due to decreased bioavailability to Hg methylating microbes[56,57].

However, the bioavailability of Hg species is not the only variable that can affect the final concentrations of MeHg in the surface water. Limnologic characteristics, such as lake and catchment size, have been shown to affect the concentrations of total Hg and MeHg in water[58]. The relationship between the production (K_m) and degradation

(K_d) of MeHg in the system is also important to consider. Net [MeHg] (and %MeHg) is the result of both MeHg production and MeHg degradation[59]; hence identifying the environmental variables that affect the rate of MeHg transformations will help better identify how emissions from Giant Mine affected the final %MeHg in the systems.

The potential rate constants of Hg methylation in our study ranged from 0.010 day^{-1} to 0.743 day^{-1} with an average of 0.163 day^{-1}, which is comparable to rate constants found in other lake sediments[40,60,61] wetland systems[11,17,62–65]. We used stepwise regression to identify which of the environmental variables collected in the water column (sulfate, arsenic, pH, DOC, SUVA, total iron, total phosphorus, and %MeHg) best explained changes in K_m in the studied lakes sediments. Our statistical analysis (Table 2.2, Figure S2.1) revealed that four environmental variables were significantly correlated with K_m; total arsenic (p-value = 0.0129), DOC (p-value = 0.0467), total phosphorous (p-value = 0.0301), and %MeHg (p-value = 0.0039).

Total arsenic concentration was negatively correlated with K_m. The literature seldom refers to the interplay between arsenic and Hg, and we could not find any studies that addressed this possible relationship over large spatial scales. Our results show that the historical arsenic pollution gradient does have an effect on the production of MeHg

in lakes around Giant Mine, possibly limiting methylation due to the toxic nature of arsenic[66,67].

DOC had a positive and significant relationship with K_m. The role of dissolved organic matter (DOM), for which we measured [DOC] as a proxy, is complex. DOM can affect Hg bioavailability, both increasing and decreasing it[60,68–70]. First, DOM can help stabilize Hg-sulfide complexes[71,72] and can increase the bioavailability of these complexes to Hg methylating microbes[73], however aged DOM can decrease the bioavailability of Hg[68]. Secondly, DOC can also stimulate microbial metabolism acting as carbon and energy sources[69,74]. Additionally, studies have shown that the origin (i.e. composition) of the DOM can affect the final production of MeHg in freshwater systems[60]. In fact, SUVA was also identified during the model selection; however, in the selected model, the relationship between SUVA and K_m fell marginally short of significance (p-value = 0.088).

Total phosphorous, which we used as a proxy for lake productivity [75,76], was also positively and significantly correlated with K_m. It is reasonable to expect methylation rate constants to increase with increasing productivity of the lakes, as shown by other studies, possibly providing labile carbon sources to microbes[16,77].

Finally, our analysis revealed that %MeHg in water was positively correlated with K_m measured in sediments, which is in agreement with other studies[12,62]. Indeed, MeHg that is produced in the sediments is one of the largest contributors of MeHg in the water column[40].

Although sulfate was significantly correlated with %MeHg, our analysis did not show sulfate in water as being a significant predictor of K_m in sediments (Figure 2.3b). This is not unexpected as sulfate levels in water do not necessarily reflect sediment porewater sulfate levels, due to the rapid cycling of sulfate in lacustrine sediments[78]. However, examination of the relationship between K_m and [sulfate] (Figure 2.3b) clearly shows that four lakes with the highest sulfate concentrations clustered together, possibly standing as outliers. These lakes all have [sulfate] > 40 mg/L, which is also the threshold above which we observed a plateau in the relationship between %MeHg and [sulfate] (Figure 2.3a). We decided to run an analysis without these four lakes on the basis of their high [sulfate], above the threshold at which evidence suggested methylation is hampered (here, ca. 40 $mg.L^{-1}$). Two regression models were found to best explain the changes in K_m in sediments (Figure S2.2). The first regression model identified sulfate as significantly and positively correlated with K_m (p-value = 0.03, R^2 = 0.15), while the second regression model identified %MeHg as being significantly and positively correlated with K_m (p-value = 0.04, R^2 = 0.15). Considering that the Akaike information

criterions (AIC) for both regression models were very similar (71.74 and 71.95 respectively) and that there was no statistically significant difference between the two models (p-value > 0.05), we conclude that both variables are equally good predictors of K_m in the system. Therefore, for this subset of lakes (with only 4 lakes with very high [sulfate] that were excluded), we can infer that K_m is significantly correlated to the pollution gradient (i.e. [sulfate] in the water column) as well as %MeHg in the water column. This suggests that the activity of Hg methylators (e.g., sulfate reducers) is likely affected by the pollution gradient and that this effect could result in the gradient of %MeHg we have observed. However, it should be highlighted that 4/28 lakes (ca.15% of samples) were not included in this subset of lakes, suggesting that this predictive model may only be applicable to lakes with [sulfate] ≤ 40 $mg.L^{-1}$, a threshold above which additional variables are needed to explain k_m=f([sulfate]).

Mercury methylation is a microbially mediated process. We used 16S rRNA gene amplicon sequencing as a means to test whether the pollution gradient affected the microbial community structure, and explored how these changes might be associated with variations in MeHg transformation rate constants.

Variables that could possibly be related to microbial community structure were divided into three categories: spatial variation (spatial relationships among sampling

sites using vectored variables, PCNM), water chemistry (i.e. [DOC], pH, [sulfate], [total Fe], [total phosphorous], [total arsenic], and SUVA), and Hg transformation rate constants (i.e., K_m and K_d). Following a stepwise regression, our results indicated that two of the major pollutants emitted from Giant Mine smelting, namely sulfate (p-value 0.004) and arsenic (p-value = 0.001), were significantly correlated with changes in microbial community structure together with total phosphorous (p-value = 0.045) and SUVA (p-value = 0.040). The constrained ordination (db-RDA) showed that 28.42% of the total variation of microbial community structure was explained by these variables with each axis explaining 42.67%, 28.75%, 16.98%, and 11.60% of the fitted variation. Using a partial db-RDA analysis we showed that 10.50% of variation could be attributed to [total arsenic], 6.65% to [sulfate], 5.68% to SUVA, and 5.59% to [total phosphorous] (Figure 2.4, Table 2.3). Admittedly, the variability explained within microbial community structure can be considered small. This reflects the complex interplay of variables affecting microbial community structure in environmental samples and to the fact that we likely have not measured all possible explanatory variables.

Interestingly, the two variables that significantly correlated with K_m (Table 2.2) were also significantly correlated with changes in microbial community: arsenic and phosphorous concentrations. Some studies have found that arsenic can alter microbial community structure and diversity[79], while other studies have found no significant

effect[67]. Total phosphorous has also been found to influence microbial community structure[80] and phosphate has specifically been shown to increase the activity of heterotrophic microbes[81]. The correlation between arsenic and total phosphorous with both changes in microbial community structure and K_m indicate that variation in the structure of the microbial community due to changes in nutrients or toxicant concentrations possibly affect Hg methylation. When a db-RDA was performed for the subset of lakes for which incubation data was available (Table 2.4, Figure 2.5), only [total arsenic] was significantly correlated with changes in microbial community (p-value = 0.002) explaining 16.20% of the fitted variability. Obtaining additional information on the genetic potential of these microbial communities to methylate Hg, such as *hgc*AB genes, diversity, and abundance would allow to better constrain this variability[6,9].

Our results also indicate that DOM origin (characterized by SUVA) was a significant contributor to the microbial community structure of these northern lakes. SUVA can be used as a proxy for DOC origins, samples with high SUVA (≥4 L/mg•m) tend to have more complex and heterogenous organic compounds that are rich in aromatics, while samples with lower SUVA (<3 L/mg•m) tend to have more homogenous and low-molecular weight molecules that are low in aromatics[82]. This value provides information on the source of the DOM in the water column; water samples with high SUVA indicate a large presence of allochthonous DOM in the water column, while lower values are

indicative of autochthonous sources of DOM[83]. SUVA in the lakes ranged from 0.029 L/mg•m to 2.400 L/mg•m, with an average of 0.846 L/mg•m which is comparable to other northern Canadian lakes[84]. The SUVA between the lakes spans two orders of magnitude indicating that certain lakes were possibly receiving more allochthonous DOM than others, although all samples seem to indicate a larger presence of homogenous and low-molecular weight molecules. Autochtonous organic matter has been shown to enhance methylation rates in boreal lake sediments by increasing overall microbial activity[60], however SUVA was not identified as an significant predictor of %MeHg nor K_m in the set of lake studied here. Therefore, our results show that carbon substrate (i.e. DOC) availability is associated with both the assemblage of microbes in sediment and the activity of Hg methylating microbes, although only the microbial community structure seems to be significantly affected the by the nature of this carbon substrate.

MeHg demethylation rate constants (K_d) ranged between below the limit of quantification (LOQ=0.304) to 1.166 day^{-1} which is comparable to other studies both in lakes[60,61] and wetlands[55,62,65]. Note that 22 out of 28 lakes sampled had K_d below LOQ during incubations (Figure S2.4). Considering the large number of values below LOQ in our dataset, we could not meet normality nor linearity assumptions of residuals for a general linear regression model. However, we separated the lakes into two groups:

lakes for which K_d > LOQ and those for which k_d < LOQ, and tested whether environmental variables were significantly different between the two groups using a non-parametric test (Figure S2.3). DOC was the only variable that was significantly different between the two groups of lakes (p-value< 0.01) with the lakes that exhibited demethylation having significantly lower [DOC], which could be linked to decreasing bioavailability of MeHg[85]. This contrasts with studies that have shown that demethylation increases with increasing organic carbon concentrations in the system[86], and others that have not found any significant correlation between K_d and DOC[19,87].

To conclude, Hg cycling in lakes around Giant Mine is affected by several variables associated with historical mining activities. The fraction of total Hg found as MeHg (%MeHg) in these Yellowknife lakes is significantly correlated with the historical deposition of sulfate from Giant Mine. The rate at which MeHg is formed in the sediments (i.e. K_m) is negatively correlated with total arsenic, and positively correlated with DOC, total phosphorous, and %MeHg in the water. However, only a subset of lakes has a significant correlation between the Hg cycling kinetics (i.e. K_m) and the sulfate concentration gradient in this landscape. These results speak to the complexity of Hg cycling in a heterogeneous landscape. The majority of studies that investigate Hg cycling in the environment usually concentrate on a few lakes or sampling sites, mostly along Hg pollution gradients[12,17,19,62,63,88]. This limits the environmental variables that can

be controlled for, and might ultimately affect the conclusions that are drawn from these results. Our results, collected over 3 years and covering 2800 km^2 highlight the importance of conducting larger scale landscape studies in which we can investigate the effect of coincident pollution gradients (e.g. arsenic and sulfate) affecting Hg cycling.

2.6 ACKNOWLEDGEMENTS

The authors would like to thank David Eickmeyer, Cynthia Cheney, Martin Pothier, Branaavan Sivarajah, Claudia Tanamal, Emily Stewart, Mike Palmer, and Jennifer Korosi for their assistance in the field and Linda Kimpe for the logistical planning of all three field seasons. This work was funded by an NSERC DG to AJP, a Strategic Grant from the Natural Science and Engineering Research Council of Canada (NSERC) (Grant # STPGP 462955-14 (to JMB, AJP, and JPS)) and the Northern Scientific Training Program (CLC).

Table 2.1: Information of lakes sampled including sampling spatial coordinates, aqueous Hg and MeHg concentrations, and Hg transformation rate constants. (LOQ = limit of quantification)

Lake	Latitude	Longitude	Area (m^2)	MeHg (ng/L)	THg (ng/L)	K_m (/day)	K_d (/day)
BC14	62° 31' 30.57'' N	114° 25' 07.48'' W	193 952	0.79	2.92		
BC17	62° 29' 59.45'' N	114° 25' 14.90'' W	156 331	0.29	1.97	0.194	<LOQ
BC18	62° 31' 04.57'' N	114° 23' 40.92'' W	155 789	0.15	1.41	0.038	<LOQ
BC20	62° 30' 19.50'' N	114° 23' 16.03'' W	437 109	1.15	2.32	0.263	<LOQ
BC21	62° 29' 16.61'' N	114° 26' 23.85'' W	92 916	0.07	0.96	0.085	<LOQ
BC22	62° 32' 27.31'' N	114° 50' 24.64'' W	8 301	0.28	1.63		
BC24	62° 32' 48.05'' N	114° 44' 43.27'' W	11 384	0.08	0.99	0.013	<LOQ
BC30	62° 31' 08.11'' N	114° 36' 43.51'' W	622 416	0.04	0.33	0.167	<LOQ
BC31	62° 32' 50.16'' N	114° 34' 57.98'' W	349 068	0.09	1.35		
BC32	62° 30' 26.04'' N	114° 32' 07.44'' W	118 138	0.30	1.26		
BC36	62° 32' 27.12'' N	113° 55' 51.05'' W	132 511	0.37	5.80	0.137	<LOQ
BC43	62° 30' 30.65'' N	114° 10' 30.04'' W	358 987	0.09	0.55	0.147	<LOQ
BCR07	62° 32' 34.62'' N	114° 21' 09.83'' W	14 069	0.21	0.93	0.237	<LOQ
David	62° 32' 36.46'' N	114° 22' 37.31'' W	132 815	0.15	0.69	0.084	<LOQ
Frame	62° 27' 38.19'' N	114° 23' 02.53'' W	881 244	0.18	0.70	0.021	0.483
Icing	62° 39' 18.75" N	114° 23' 08.23" W	1 210 000	0.10	0.77	0.010	1.166
Martin	62° 31' 49.49" N	114° 26' 26.49" W	2 929 991	0.13	0.42		
BCR-Mija	62° 31' 55.45'' N	114° 21' 21.62'' W	15 016	0.18	0.80	0.398	<LOQ
Niven	62° 27' 41.36'' N	114° 22' 04.52'' W	107 463	0.98	2.19	0.743	<LOQ
Pocket	62° 30' 32.30'' N	114° 22' 25.60'' W	48 000	0.75	2.50		
Pontoon	62° 32' 46.31" N	114° 01' 28.79" W	3 534 190	0.07	0.26	0.313	<LOQ
Prosperous	62° 32' 47.92" N	114° 11' 10.84" W	160 930 000	0.02	0.22	0.073	0.318
Rat	62° 26' 45.22'' N	114° 21' 48.95'' W	38 213	0.60	1.08	0.192	<LOQ
Vee	62° 33' 04.01'' N	114° 21' 12.94'' W	697 807	0.22	0.52		
YK11	62° 29' 05.43'' N	114° 25' 03.53'' W	548 597	0.27	0.71	0.088	<LOQ

Lake	Latitude	Longitude	Area (m^2)	MeHg (ng/L)	THg (ng/L)	K_m (/day)	K_d (/day)
YK12	62° 29' 09.19'' N	114° 25' 30.33'' W	64 225	0.30	3.03	0.325	<LOQ
YK40	62° 21' 59.63'' N	114° 08' 01.50'' W	340 232	0.05	0.74	0.170	<LOQ
YK42	62° 29' 31.57'' N	114° 23' 46.52'' W	212 361	0.14	0.95	0.058	0.522
YK60	62° 30' 18.15'' N	114° 27' 11.94'' W	393 444	0.03	0.28	0.013	0.972
YK67	62° 29' 31.24'' N	114° 18' 06.58'' W	87 674	0.10	2.16	0.068	<LOQ
YKE1	62° 31' 54.77'' N	113° 22' 23.76'' W	227 031	0.10	0.52	0.083	<LOQ
YKN1	62° 48' 48.50'' N	114° 22' 13.95'' W	260 633	0.04	0.41	0.086	<LOQ
YKS1	62° 17' 37.92" N	113° 57' 06.36" W	202 589	0.12	0.65	0.171	0.432
YKS2	62° 18' 14.64" N	113° 57' 04.74" W	2 347 017	0.02	0.32	0.156	<LOQ
YKW1	62° 38' 06.87'' N	115° 01' 08.62'' W	928 750	0.13	0.93	0.250	<LOQ

3

4

Table 2.2: Results from the step-wise regression model selection. Regression model (R^2 = 0.329, F(5, 22) = 3.653, p-value = 0.0148) below was chosen to best explain the changes in Hg methylation rate constants (K_m) in sediments.

Variable	Estimate	St Dev	t	p-value
(intercept)	-7.1855	1.3081	-5.493	< 0.0001 **
ln(SUVA)	0.7195	0.4030	1.785	0.0880
ln(Total Arsenic)	-0.2915	0.1077	-2.706	0.0129 *
ln(DOC)	0.7049	0.3344	2.108	0.0467 .
ln(Phosphorous +1)	0.4263	0.1839	2.318	0.0301 *
ln(%MeHg)	0.9230	0.2862	3.225	0.0039 **

Table 2.3: Results from stepwise model (ordistep) for the constrained ordination method (db-RDA). This analysis was done on all lakes sampled during the study.

Variable	Sum of squares	Pseudo F	p-value
ln(Total Arsenic)	0.4482	3.1209	0.001 **
ln(Sulfate)	0.2954	2.0566	0.004 **
sqrt(Total Phospohorus)	0.2320	1.6153	0.045 .
SUVA	0.2405	1.6748	0.040 *

Table 2.4: Results from stepwise model (ordistep) for the constrained ordination method (db-RDA) for the subset of lakes for which incubation data was available.

Variable	Sum of squares	Pseudo F	p-value
ln(Total Arsenic)	0.3710	2.3432	0.002 *
ln(Sulfate)	0.2371	1.5571	0.050 .

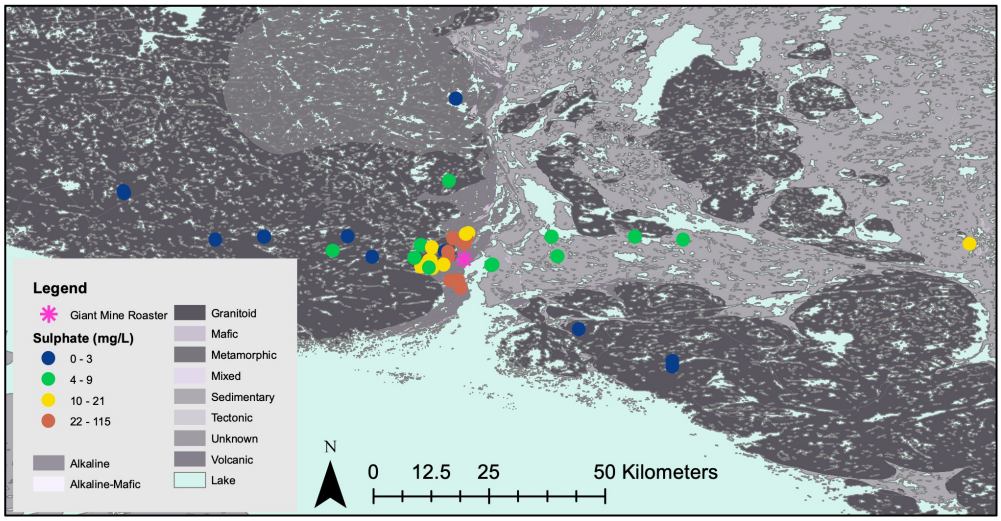


Figure 2.1: Map of sampling sites in Yellowknife, NWT, Canada. Each point represent a lake sampled during the study in 2015, 2016, and 2017.

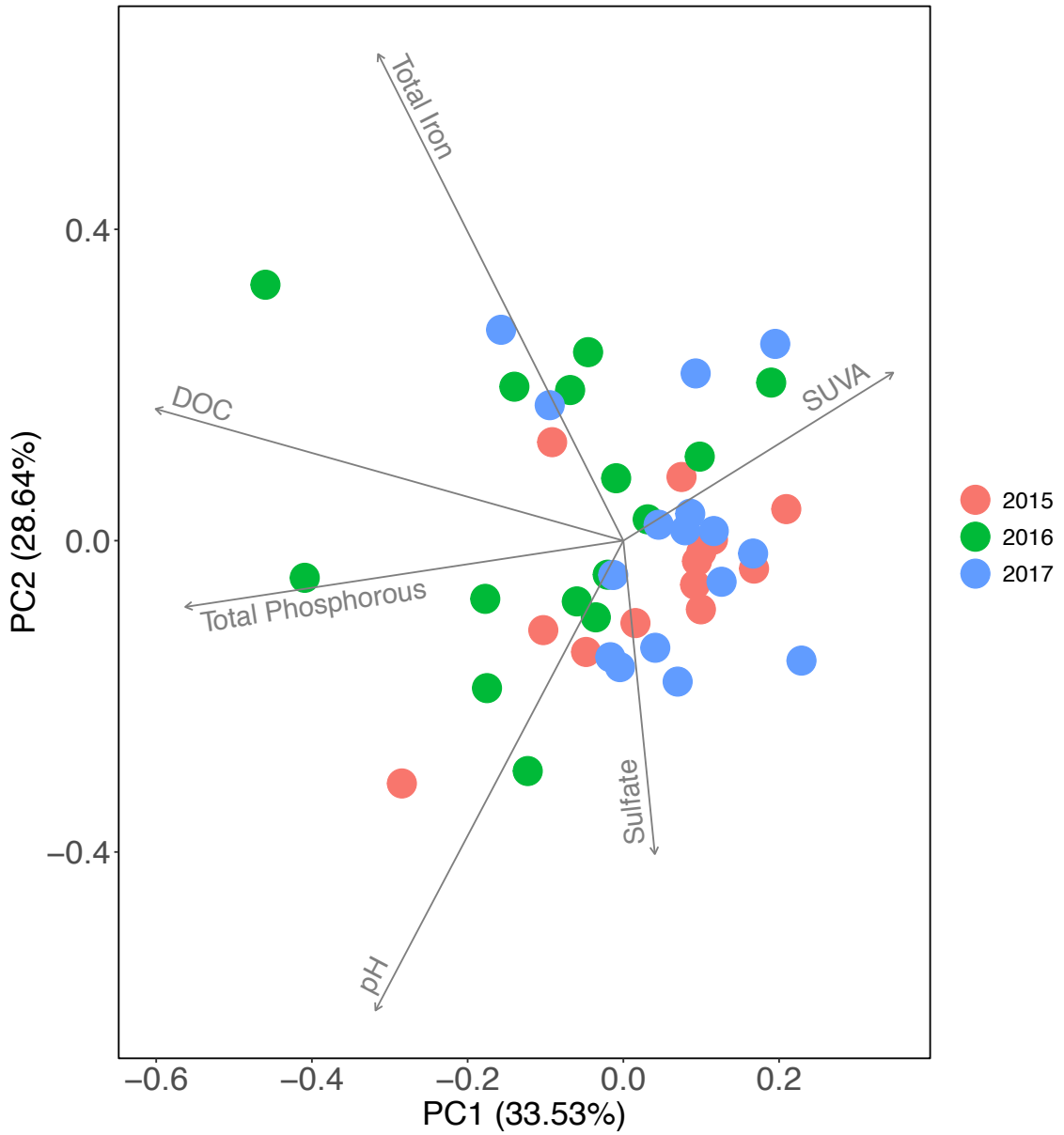


Figure 2.2: A principal coordinate analysis (PCA) plot showing the differences in water chemistry of each lakes sampled. Points represent the sampled lakes and the arrows of water chemistry for each lake during the three year

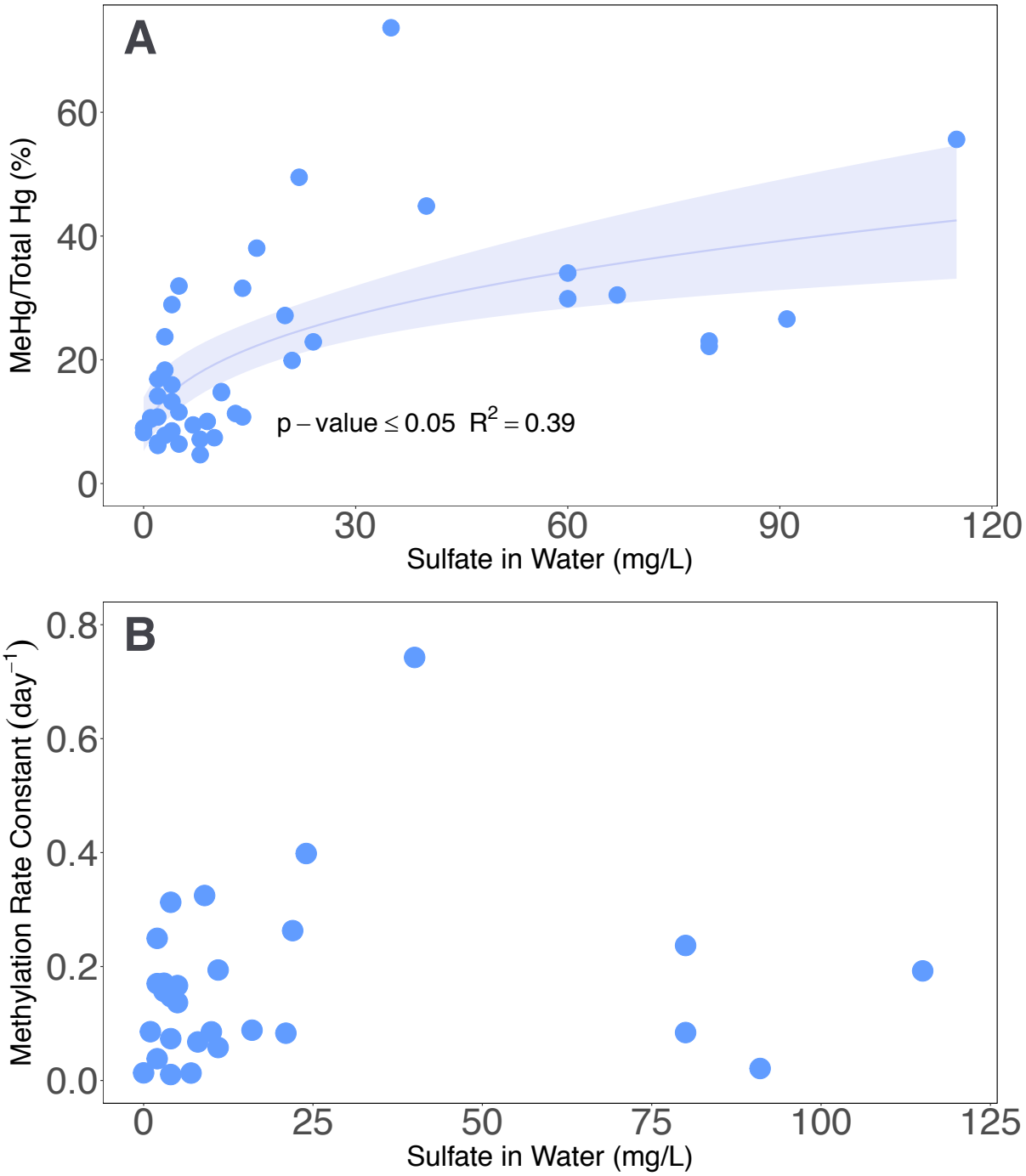


Figure 2.3: Results from chemical and incubation experiments. Panel A shows %MeHg with respect to sulfate concentrations in the water column of all lakes from the study. Panel B shows the methylation rate constant (day^{-1}) in the sediments with respect to sulfate concentration in the water column.

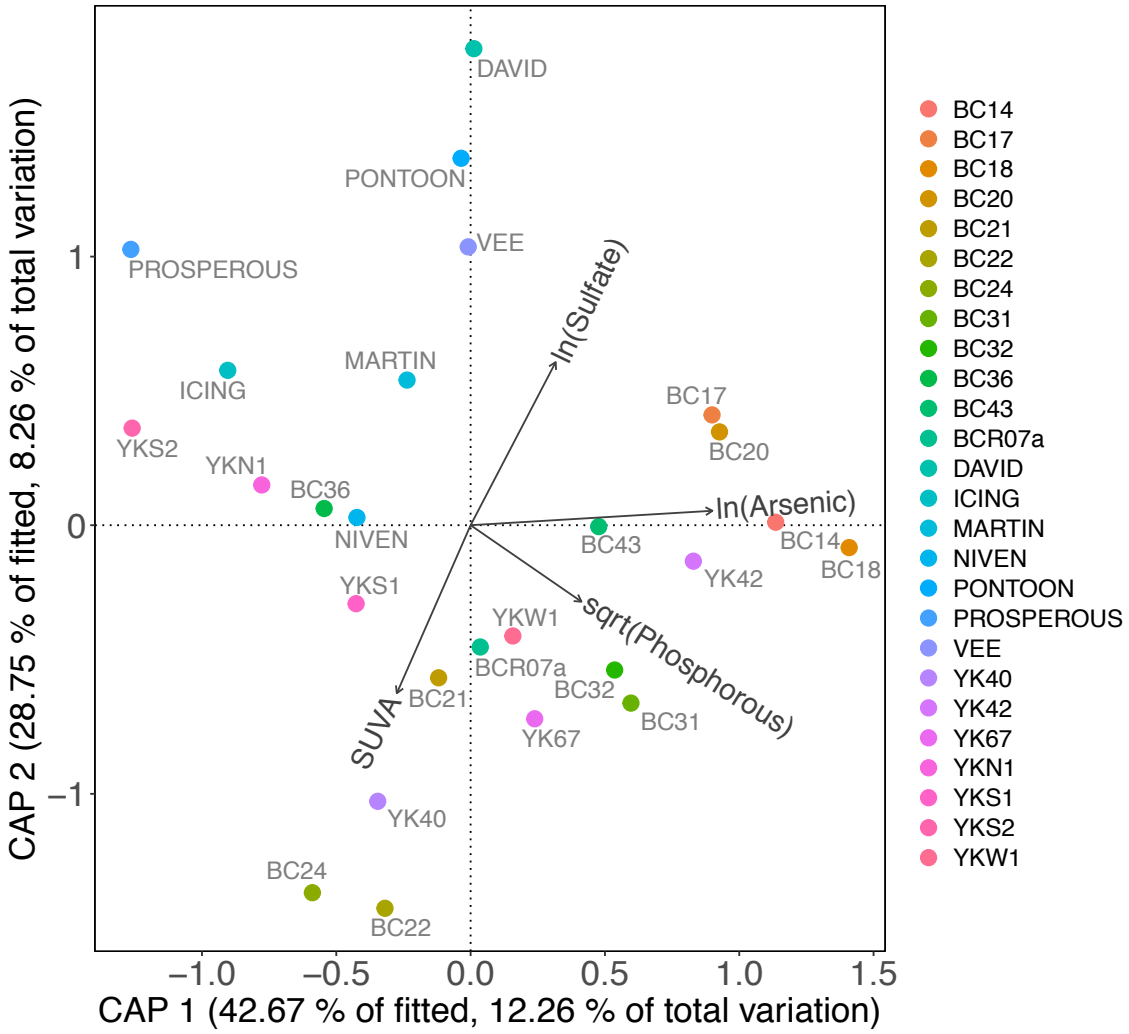


Figure 2.4: Distance based redundancy analysis (db-RDA) plot of microbial community based on the chemical and spatial variables. This analysis was done on all lakes sampled during the study.

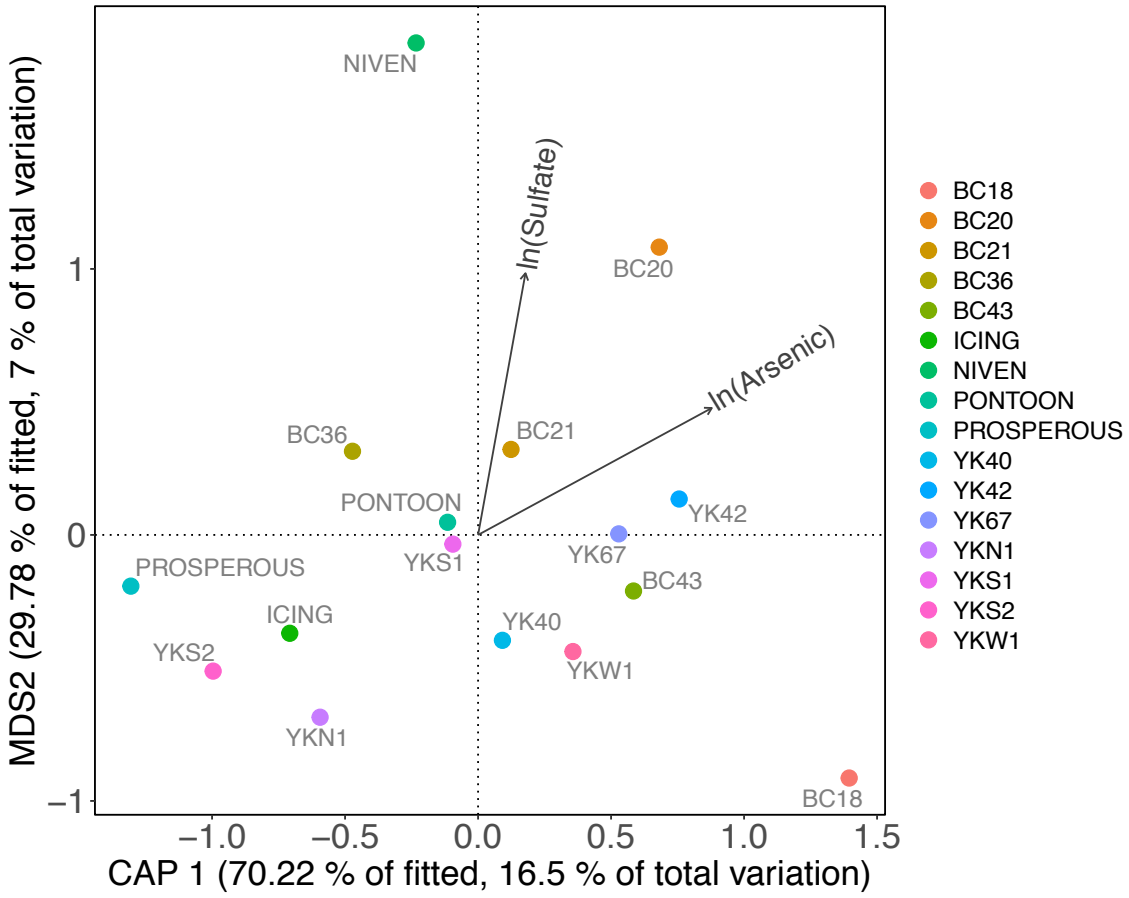


Figure 2.5: db-RDA of microbial community of incubation lakes Distance based redundancy analysis (db-RDA) plot of microbial community based on the chemical, spatial, and kinetic variables. This analysis was done on all lakes for which incubation data was available.

2.7 REFERENCES

1 E. M. Sunderland and M. Tumpney, in *Persistent Organic Pollutants and Toxic Metals in Foods*, eds. M. Rose and A. Fernandes, Woodhead Publishing, 2013, pp. 392–413.

2 C.-C. Lin, N. Yee and T. Barkay, in *Environmental Chemistry and Toxicology of Mercury*, John Wiley & Sons, Ltd, 2011, pp. 155–191.

3 V. Celo, D. R. S. Lean and S. L. Scott, Abiotic methylation of mercury in the aquatic environment, *Science of The Total Environment*, 2006, 368, 126–137.

4 J. H. Weber, Review of possible paths for abiotic methylation of mercury(II) in the aquatic environment, *Chemosphere*, 1993, 26, 2063–2077.

5 A. R. Paranjape and B. D. Hall, Recent advances in the study of mercury methylation in aquatic systems, *FACETS*, 2017, 2, 85–119.

6 J. M. Parks, A. Johs, M. Podar, R. Bridou, R. A. Hurt, S. D. Smith, S. J. Tomanicek, Y. Qian, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. C. Smith, J. D. Wall, D. A. Elias and L. Liang, The Genetic Basis for Bacterial Mercury Methylation, *Science*, 2013, 339, 1332–1335.

7 G. A. Christensen, A. M. Wymore, A. J. King, M. Podar, R. A. Hurt, E. U. Santillan, A. Soren, C. C. Brandt, S. D. Brown, A. V. Palumbo, J. D. Wall, C. C. Gilmour, D. A. Elias and G. Voordouw, Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment, *Applied and Environmental Microbiology*, 2016, 82, 6068–6078.

8 M. Podar, C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A. V. Palumbo, A. C. Somenahally and D. A. Elias, Global prevalence and distribution of genes and microorganisms involved in mercury methylation, *Science Advances*, , DOI:10.1126/sciadv.1500675.

9 C. C. Gilmour, M. Podar, A. L. Bullock, A. M. Graham, S. D. Brown, A. C. Somenahally, A. Johs, R. A. Hurt, K. L. Bailey and D. A. Elias, Mercury Methylation by Novel Microorganisms from New Environments, *Environ. Sci. Technol.*, 2013, 47, 11810–11820.

10 L. Zeng, G. Luo, T. He, Y. Guo and X. Qian, Effects of sulfate-reducing bacteria on methylmercury at the sediment-water interface., *J Environ Sci (China)*, 2016, 46, 214–219.

11 N. W. Johnson, C. P. J. Mitchell, D. R. Engstrom, L. T. Bailey, J. K. Coleman Wasik and M. E. Berndt, Methylmercury production in a chronically sulfate-impacted sub-boreal wetland, *Environ. Sci.: Processes Impacts*, 2016, 18, 725–734.

12 A. Drott, L. Lambertsson, E. Björn and U. Skyllberg, Do Potential Methylation Rates Reflect Accumulated Methyl Mercury in Contaminated Sediments?, *Environ. Sci. Technol.*, 2008, 42, 153–158.

13 H. Hsu-Kim, K. H. Kucharzyk, T. Zhang and M. A. Deshusses, Mechanisms Regulating Mercury Bioavailability for Methylating Microorganisms in the Aquatic Environment: A Critical Review, *Environ. Sci. Technol.*, 2013, 47, 2441–2456.

14 G. Liu, Y. Cai and N. O'Driscoll, *Environmental Chemistry and Toxicology of Mercury*, Wiley, Hoboken, 1. Aufl., 2011.

15 A. G. Bravo, S. Bouchet, S. Guédron, D. Amouroux, J. Dominik and J. Zopfi, High methylmercury production under ferruginous conditions in sediments impacted by sewage treatment plant discharges, *Water Research*, 2015, 80, 245–255.
16 J. E. Gray and M. E. Hines, Biogeochemical mercury methylation influenced by reservoir eutrophication, Salmon Falls Creek Reservoir, Idaho, USA, *Chemical Geology*, 2009, 258, 157–167.
17 C. G. J. Hoggarth, B. D. Hall and C. P. J. Mitchell, Mercury methylation in high and low-sulphate impacted wetland ponds within the prairie pothole region of North America., *Environ Pollut*, 2015, 205, 269–277.
18 R.-Q. Yu, J. R. Flanders, E. E. Mack, R. Turner, M. B. Mirza and T. Barkay, Contribution of Coexisting Sulfate and Iron Reducing Bacteria to Methylmercury Production in Freshwater River Sediments, *Environ. Sci. Technol.*, 2012, 46, 2684–2691.
19 J. Stamenkovic, M. S. Gustin, M. C. Marvin-DiPasquale, B. A. Thomas and J. L. Agee, Distribution of total and methyl mercury in sediments along Steamboat Creek (Nevada, USA), *Science of the Total Environment*, 2004, 322, 167–177.
20 S. Haley, M. Klick, N. Szymoniak and A. Crow, Observing trends and assessing data for Arctic mining, *Polar Geography*, 2011, 34, 37–61.
21 G. McMahon and F. Remy, *Large Mines and the Community: Socioeconomic and Environmental Effects in Latin America, Canada and Spain*, The World Bank, 2001.
22 V. N. de Jonge, M. Elliott and E. Orive, Causes, historical development, effects and future challenges of a common environmental problem: eutrophication, *Hydrobiologia*, 2002, 475, 1–19.
23 M. J. Hird, Waste, Environmental Politics and Dis/Engaged Publics, *Theory, Culture & Society*, 2017, 34, 187–209.
24 INAC, *Giant Mine Remediation Project: Developer's Assessment Report*, 2010.
25 C. D. Anglin, *Gold in the Yellowknife greenstone belt, Northwest Territories : results of the EXTECH III multidisciplinary research project : collected papers and DVD of data reporting on results of the Yellowknife EXTECH III multidisciplinary research project*, Geological Association of Canada, Mineral Deposits Division, St. John's, Nfld, 2006.
26 J. Sandlos and A. Keeling, *Giant Mine : historical summary*, Memorial University, St. John's, N.L, 2015.
27 A. J. Houben, R. D'Onofrio, S. V. Kokelj and J. M. Blais, Factors Affecting Elevated Arsenic and Methyl Mercury Concentrations in Small Shield Lakes Surrounding Gold Mines near the Yellowknife, NT, (Canada) Region, *PloS one*, 2016, 11, e0150960.
28 M. J. Bromstad, L. A. Wrye and H. E. Jamieson, The characterization, mobility, and persistence of roaster-derived arsenic in soils at Giant Mine, NWT, *Applied geochemistry*, 2017, 82, 102–118.
29 H. E. Jamieson, The Legacy of Arsenic Contamination from Mining and Processing Refractory Gold Ore at Giant Mine, Yellowknife, Northwest Territories, Canada, *Reviews in Mineralogy and Geochemistry*, 2014, 79, 533–551.
30 M. J. Bromstad, Thesis, Queen's University, 2011.
31 S. M. Ullrich, T. W. Tanton and S. A. Abdrashitova, Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation, *null*, 2001, 31, 241–293.

32 EPA, *Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*, U.S. Environmental Protection Agency, Washington, DC, 2002.
33 Y. Cai, R. Jaffé, A. Alli and R. D. Jones, Determination of organomercury compounds in aqueous samples by capillary gas chromatography-atomic fluorescence spectrometry following solid-phase extraction, *Analytica Chimica Acta*, 1996, 334, 251–259.
34 US EPA, *Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry*, U.S. EPA, Cincinnati, OH, 1994.
35 J. L. Weishaar, G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii and K. Mopper, Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon, *Environ. Sci. Technol.*, 2003, 37, 4702–4708.
36 G. St-Jean, Automated quantitative and isotopic (13C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyser, *Rapid Communications in Mass Spectrometry*, 2003, 17, 419–428.
37 Y. Cai, R. Jaffé and R. Jones, Ethylmercury in the Soils and Sediments of the Florida Everglades, *Environ. Sci. Technol.*, 1997, 31, 302–305.
38 B. L. Batista, J. L. Rodrigues, S. S. de Souza, V. C. O. Souza and F. Barbosa, Mercury speciation in seafood samples by LC–ICP-MS with a rapid ultrasound-assisted extraction procedure: Application to the determination of mercury in Brazilian seafood samples, *Food Chemistry*, 2011, 126, 2000–2004.
39 W. Zhu, Y. Song, G. A. Adediran, T. Jiang, A. T. Reis, E. Pereira, U. Skyllberg and E. Björn, Mercury transformations in resuspended contaminated sediment controlled by redox conditions, chemical speciation and sources of organic matter, *Geochimica et Cosmochimica Acta*, 2018, 220, 158–179.
40 I. Lehnherr, V. L. St. Louis, C. A. Emmerton, J. D. Barker and J. L. Kirk, Methylmercury Cycling in High Arctic Wetland Ponds: Sources and Sinks, *Environ. Sci. Technol.*, 2012, 46, 10514–10522.
41 M.-L. Avramescu, E. Yumvihoze, H. Hintelmann, J. Ridal, D. Fortin and D. R. S. Lean, Biogeochemical factors influencing net mercury methylation in contaminated freshwater sediments from the St. Lawrence River in Cornwall, Ontario, Canada., *Sci Total Environ*, 2011, 409, 968–978.
42 B. J. Callahan, P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson and S. P. Holmes, DADA2: High-resolution sample inference from Illumina amplicon data, *Nature Methods*, 2016, 13, 581–583.
43 C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies and F. O. Glöckner, The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, *Nucleic Acids Res*, 2013, 41, D590–D596.
44 P. J. McMurdie and S. Holmes, phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data, *PLOS ONE*, 2013, 8, 1–11.
45 Oksanen J, Blanchet F G, Kindt R, Legendre P, Minchin P R, O'Hara R B, Simpson G L, Solymos P, Stevens M H H, and Wagner H, *Vegan: community ecology package. R package vegan, vers. 2.2-1*, 2015.
46 G. L. Simpson, 2019.

47 W. N. Venables, B. D. Ripley and W. N. (William N.). Venables, *Modern applied statistics with S*, Springer, New York, 4th ed., 2002.
48 A. Zeileis and T. Hothorn, Diagnostic checking in regression relationships, *R News*.
49 J. Fox, S. Weisberg and J. Fox, *An R companion to applied regression.*, SAGE Publications, Thousand Oaks, Calif, 2nd ed. / John Fox, Sanford Weisberg., 2011.
50 D. Borcard and P. Legendre, All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices, *Ecological Modelling*, 2002, 153, 51–68.
51 P. Legendre, M. Fortin, D. Borcard and P. Peres-Neto, Should the Mantel test be used in spatial analysis?, *Methods in ecology and evolution*, 2015, 6, 1239–1247.
52 P. Legendre and M. J. Anderson, Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments, *Ecological Monographs*, 1999, 69, 1–24.
53 A. Canredon, P. Anschutz, D. Buquet, C. Charbonnier, D. Amouroux, E. Tessier, D. Poirier, S. Bujan, L. Devaux, B. Gouillieux, S. Gentès, A. Legeay, A. Feurtet-Mazel, S. Galaup and R. Maury-Brachet, Lake sediment mercury biogeochemistry controlled by sulphate input from drainage basin, *Applied Geochemistry*, 2019, 104, 135–145.
54 C. C. Gilmour, G. S. Riedel, M. C. Ederington, J. T. Bell, G. A. Gill and M. C. Stordal, Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades, *Biogeochemistry*, 1998, 40, 327–345.
55 L. T. Bailey, C. P. J. Mitchell, D. R. Engstrom, M. E. Berndt, J. K. Coleman Wasik and N. W. Johnson, Influence of porewater sulfide on methylmercury production and partitioning in sulfate-impacted lake sediments., *Sci Total Environ*, 2017, 580, 1197–1204.
56 T. Zhang, K. H. Kucharzyk, B. Kim, M. A. Deshusses and H. Hsu-Kim, Net Methylation of Mercury in Estuarine Sediment Microcosms Amended with Dissolved, Nanoparticulate, and Microparticulate Mercuric Sulfides, *Environ. Sci. Technol.*, 2014, 48, 9133–9141.
57 C. S. Eckley and H. Hintelmann, Determination of mercury methylation potentials in the water column of lakes across Canada., *Sci Total Environ*, 2006, 368, 111–125.
58 H. F. V. Braaten, H. A. de Wit, E. Fjeld, S. Rognerud, E. Lydersen and T. Larssen, Environmental factors influencing mercury speciation in Subarctic and Boreal lakes, *Science of The Total Environment*, 2014, 476–477, 336–345.
59 K. H. Kucharzyk, M. A. Deshusses, K. A. Porter and H. Hsu-Kim, Relative contributions of mercury bioavailability and microbial growth rate on net methylmercury production by anaerobic mixed cultures, *Environ Sci Process Impacts*, 2015, 17, 1568–1577.
60 A. G. Bravo, S. Bouchet, J. Tolu, E. Björn, A. Mateos-Rivera and S. Bertilsson, Molecular composition of organic matter controls methylmercury formation in boreal lakes, *Nature Communications*, 2017, 8, 14255.
61 H. Hintelmann, K. Keppel-Jones and R. D. Evans, Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury availability, *Environmental Toxicology and Chemistry*, 2000, 19, 2204–2211.
62 I. Tjerngren, M. Meili, E. Björn and U. Skyllberg, Eight Boreal Wetlands as Sources and Sinks for Methyl Mercury in Relation to Soil Acidity, C/N Ratio, and Small-Scale Flooding, *Environ. Sci. Technol.*, 2012, 46, 8052–8060.

63 L. Windham-Myers, J. A. Fleck, J. T. Ackerman, M. Marvin-DiPasquale, C. A. Stricker, W. A. Heim, P. A. M. Bachand, C. A. Eagles-Smith, G. Gill, M. Stephenson and C. N. Alpers, Mercury cycling in agricultural and managed wetlands: a synthesis of methylmercury production, hydrologic export, and bioaccumulation from an integrated field study., *Sci Total Environ*, 2014, 484, 221–231.
64 R. J. Strickman and C. P. J. Mitchell, Methylmercury production and accumulation in urban stormwater ponds and habitat wetlands, *Environmental Pollution*, 2017, 221, 326–334.
65 D. D. Bussan, R. F. Sessums and J. V. Cizdziel, Activated Carbon and Biochar Reduce Mercury Methylation Potentials in Aquatic Sediments., *Bull Environ Contam Toxicol*, 2016, 96, 536–539.
66 R. Turpeinen, M. Pantsar-Kallio, M. Häggblom and T. Kairesalo, Influence of microbes on the mobilization, toxicity and biomethylation of arsenic in soil., *Sci Total Environ*, 1999, 236, 173–180.
67 A. Mahamoud Ahmed, E. Lyautey, C. Bonnineau, A. Dabrin and S. Pesce, Environmental Concentrations of Copper, Alone or in Mixture With Arsenic, Can Impact River Sediment Microbial Community Structure and Functions, *Frontiers in Microbiology*, 2018, 9, 1852.
68 S. A. Chiasson-Gould, J. M. Blais and A. J. Poulain, Dissolved Organic Matter Kinetically Controls Mercury Bioavailability to Bacteria, *Environ. Sci. Technol.*, 2014, 48, 3153–3161.
69 E. B. Graham, J. E. Knelman, R. S. Gabor, S. Schooler, D. M. McKnight and D. R. Nemergut, Dissolved organic matter and inorganic mercury loadings favor novel methylators and fermentation metabolisms in oligotrophic sediments, *bioRxiv*, 2016, 072017.
70 T. D. French, A. J. Houben, J.-P. W. Desforges, L. E. Kimpe, S. V. Kokelj, A. J. Poulain, J. P. Smol, X. Wang and J. M. Blais, Dissolved Organic Carbon Thresholds Affect Mercury Bioaccumulation in Arctic Lakes, *Environ. Sci. Technol.*, 2014, 48, 3162–3168.
71 C. A. Gerbig, C. S. Kim, J. P. Stegemeier, J. N. Ryan and G. R. Aiken, Formation of Nanocolloidal Metacinnabar in Mercury-DOM-Sulfide Systems, *Environ. Sci. Technol.*, 2011, 45, 9180–9187.
72 A. Deonarine and H. Hsu-Kim, Precipitation of Mercuric Sulfide Nanoparticles in NOM-Containing Water: Implications for the Natural Environment, *Environ. Sci. Technol.*, 2009, 43, 2368–2373.
73 A. M. Graham, G. R. Aiken and C. C. Gilmour, Dissolved organic matter enhances microbial mercury methylation under sulfidic conditions., *Environ Sci Technol*, 2012, 46, 2715–2723.
74 K. M. A. Paulson, C. J. Ptacek, D. W. Blowes, W. D. Gould, J. Ma, R. C. Landis and J. A. Dyer, Role of Organic Carbon Sources and Sulfate in Controlling Net Methylmercury Production in Riverbank Sediments of the South River, VA (USA), *null*, 2018, 35, 1–14.
75 E. M. Smith and Y. T. Prairie, Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability, *Limnology and Oceanography*, 2004, 49, 137–147.
76 V. H. Smith, S. B. Joye and R. W. Howarth, Eutrophication of freshwater and marine ecosystems, *Limnology and Oceanography*, 2006, 51, 351–355.

77 B. Meng, X. B. Feng, C. X. Chen, G. L. Qiu, J. Sommar, Y. N. Guo, P. Liang and Q. Wan, Influence of eutrophication on the distribution of total mercury and methylmercury in hydroelectric reservoirs., *J Environ Qual*, 2010, 39, 1624–1635.
78 M. Holmer and P. Storkholm, Sulphate reduction and sulphur cycling in lake sediments: a review, *Freshwater Biology*, 2001, 46, 431–451.
79 J. Zhao, X. Zhao, L. Chao, W. Zhang, T. You and J. Zhang, Diversity change of microbial communities responding to zinc and arsenic pollution in a river of northeastern China, *J Zhejiang Univ Sci B*, 2014, 15, 670–680.
80 Y. Li, J. Zhang, J. Zhang, W. Xu and Z. Mou, Microbial Community Structure in the Sediments and Its Relation to Environmental Factors in Eutrophicated Sancha Lake, *Int J Environ Res Public Health*, 2019, 16, 1931.
81 Y. Zhao, M. Chen, J. Bai, X. Li, F. Zulfiqar and Q. Wang, Response of microbial community to petroleum stress and phosphate dosage in sediments of Jiaozhou Bay, China, *Journal of Ocean University of China*, 2014, 13, 249–256.
82 J. Peuravuori and K. Pihlaja, in *Handbook of Water Analysis*, eds. L. M. L. Nollet and L. S. P. De Gelder, CRC Press, Boca Raton, 2nd edn., 2007, pp. 449–462.
83 K. K. Mueller, C. Fortin and P. G. C. Campbell, Spatial Variation in the Optical Properties of Dissolved Organic Matter (DOM) in Lakes on the Canadian Precambrian Shield and Links to Watershed Characteristics, *Aquatic Geochemistry*, 2012, 18, 21–44.
84 S. E. Johnston, R. G. Striegl, M. J. Bogard, M. M. Dornblaser, D. E. Butman, A. M. Kellerman, K. P. Wickland, D. C. Podgorski and R. G. M. Spencer, Hydrologic connectivity determines dissolved organic matter biogeochemistry in northern high-latitude lakes, *Limnology and Oceanography*, 2020, 65, 1764–1780.
85 Ndu Udonna, Mason Robert P., Zhang Huan, Lin Senjie, and Visscher Pieter T., Effect of Inorganic and Organic Ligands on the Bioavailability of Methylmercury as Determined by Using a mer-lux Bioreporter, *Applied and Environmental Microbiology*, 2012, 78, 7276–7282.
86 M. Marvin-DiPasquale, J. Agee, C. McGowan, R. S. Oremland, M. Thomas, D. Krabbenhoft and C. C. Gilmour, Methyl-Mercury Degradation Pathways: A Comparison among Three Mercury-Impacted Ecosystems, *Environ. Sci. Technol.*, 2000, 34, 4908–4916.
87 B. M. Miskimmin, J. W. M. Rudd and C. A. Kelly, Influence of Dissolved Organic Carbon, pH, and Microbial Respiration Rates on Mercury Methylation and Demethylation in Lake Water, *Can. J. Fish. Aquat. Sci.*, 1992, 49, 17–22.
88 B. Ouddane, M. Monperrus, M. Kadlecova, M. Daye and D. Amouroux, Mercury methylation and demethylation in highly contaminated sediments from the Deûle River in Northern France using species-specific enriched stable isotopes., *Environ Sci Process Impacts*, 2015, 17, 145–155.

2.8 SUPPLEMENTARY INFORMATION

Order occurring in the text:

Figure S2.1: Partial regression plots for each variable from the regression model in Table 2. Each plots shows the estimated relationship between the response and the explanatory variable after adjusting for the other variables in the model.

Figure S2.2: Regression plots showing relationship between K_m with respect to sulfate concentrations (A) and %MeHg (B) in water, plot only contains samples that have sulfate concentrations < 40 mg/L.

Figure S2.3: Boxplots of the two groups of demethylation rate constants (K_d) in lake sediments with respect to the different water chemistry measured. Lakes were categorized as following: lakes with detectable demethylation (>LOQ) and lakes with demethylation below detection limit (<LOQ). (LOQ = limit of quantification)

Figure S2.4: Methylation and demethylation figures for all lakes. The top panel (pink points) for each lake represents the increase in $Me^{199}Hg$ (i.e. methylation) and the bottom panel (green points) for each lake represents the decrease in $Me^{198}Hg$ (i.e. demethylation). The horizontal red line is the concentration of spiked MeHg at the start (t=0) of each incubation.

Figure S2.5: Relative abundance of microbial Phyla in each lake sediment sample

Table S2.1: Raw results from ICP-MS analysis of methylmercury isotopes in incubation sediments. For each lake two types of treatment are shown, one spiked with mercury isotopes (spiked) and one that is left unspiked (unspiked).

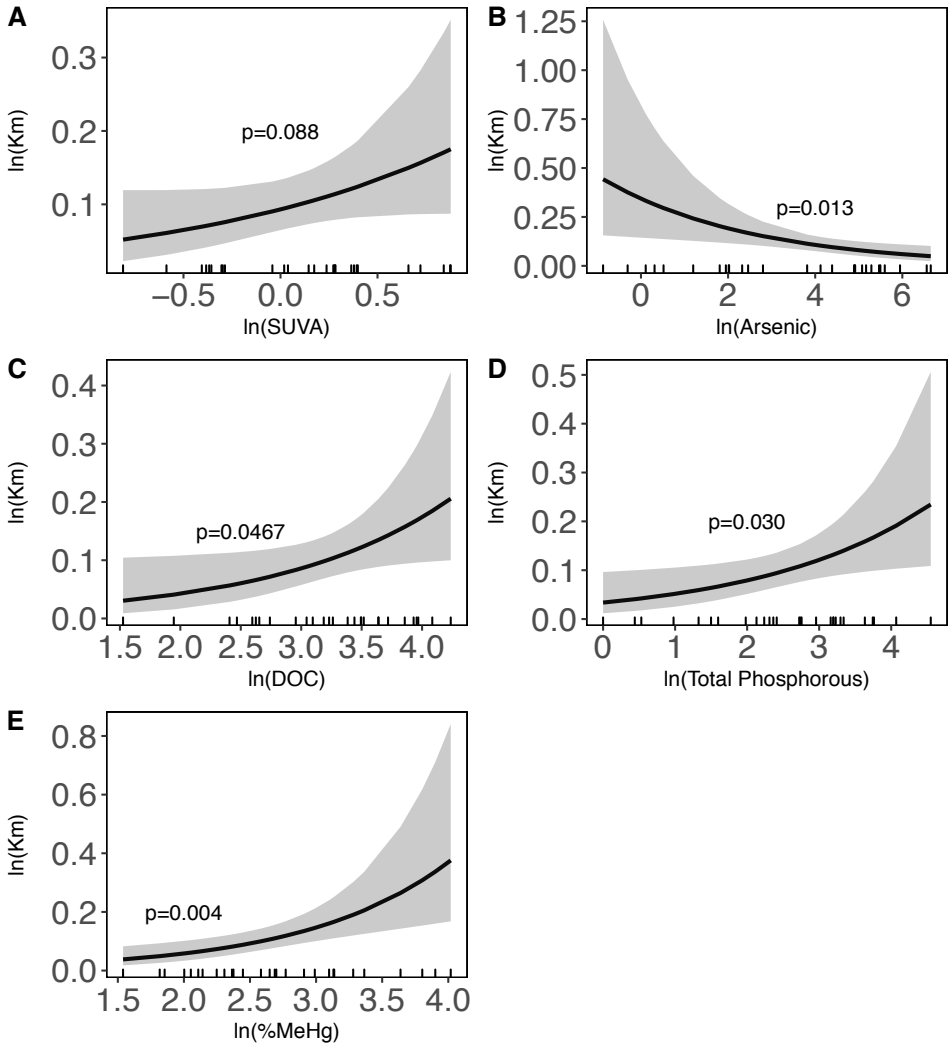


Figure S2.1: Partial regression plots for each variable from the regression model in Table 2.2. Each plots shows the estimated relationship between the response and the explanatory variable after adjusting for the other variables in the model.

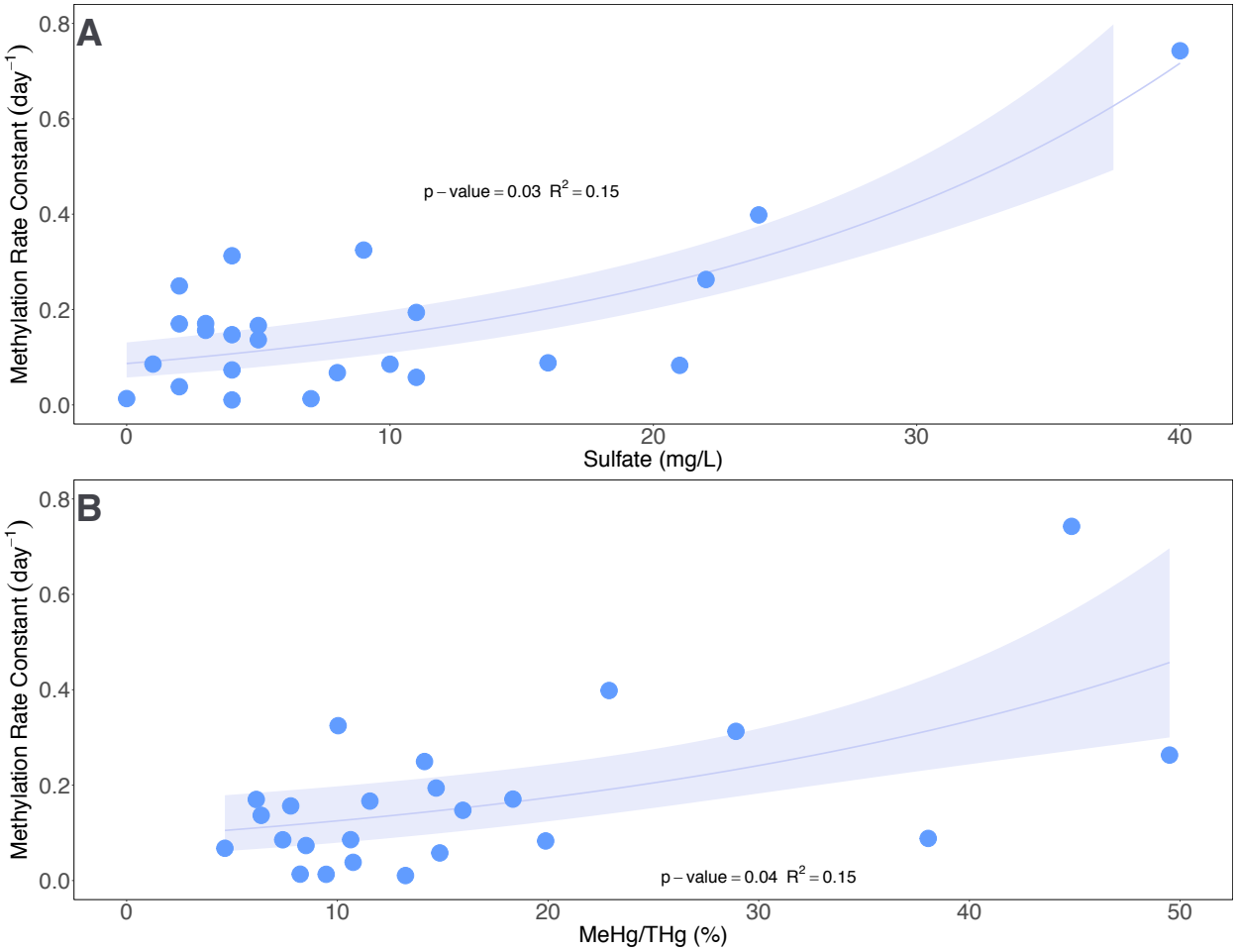


Figure S2.2: Regression plots showing relationship between K_m with respect to sulfate concentrations (A) and %MeHg (B) in water, plot only contains samples that have sulfate concentrations < 40 mg/L.

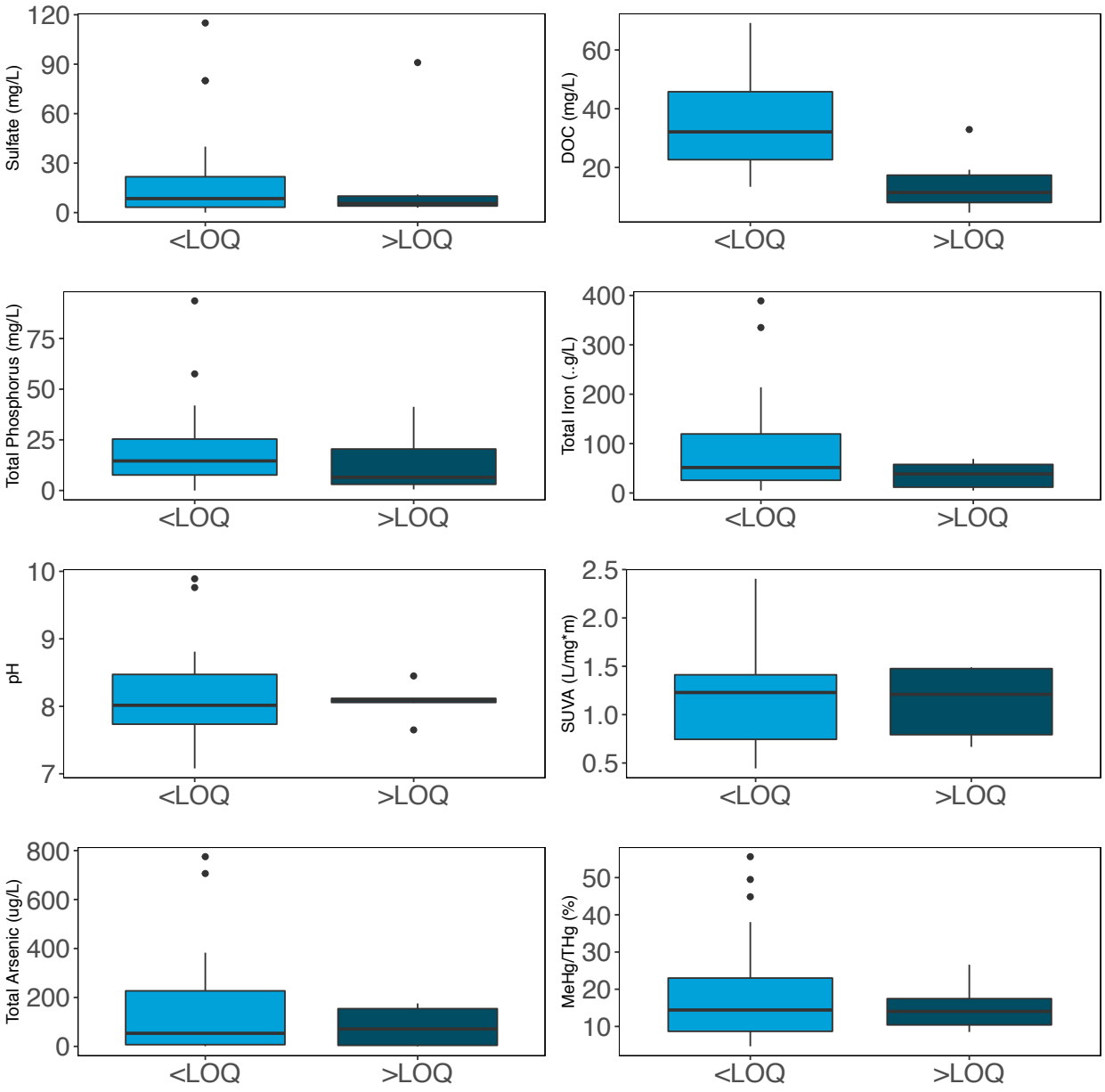
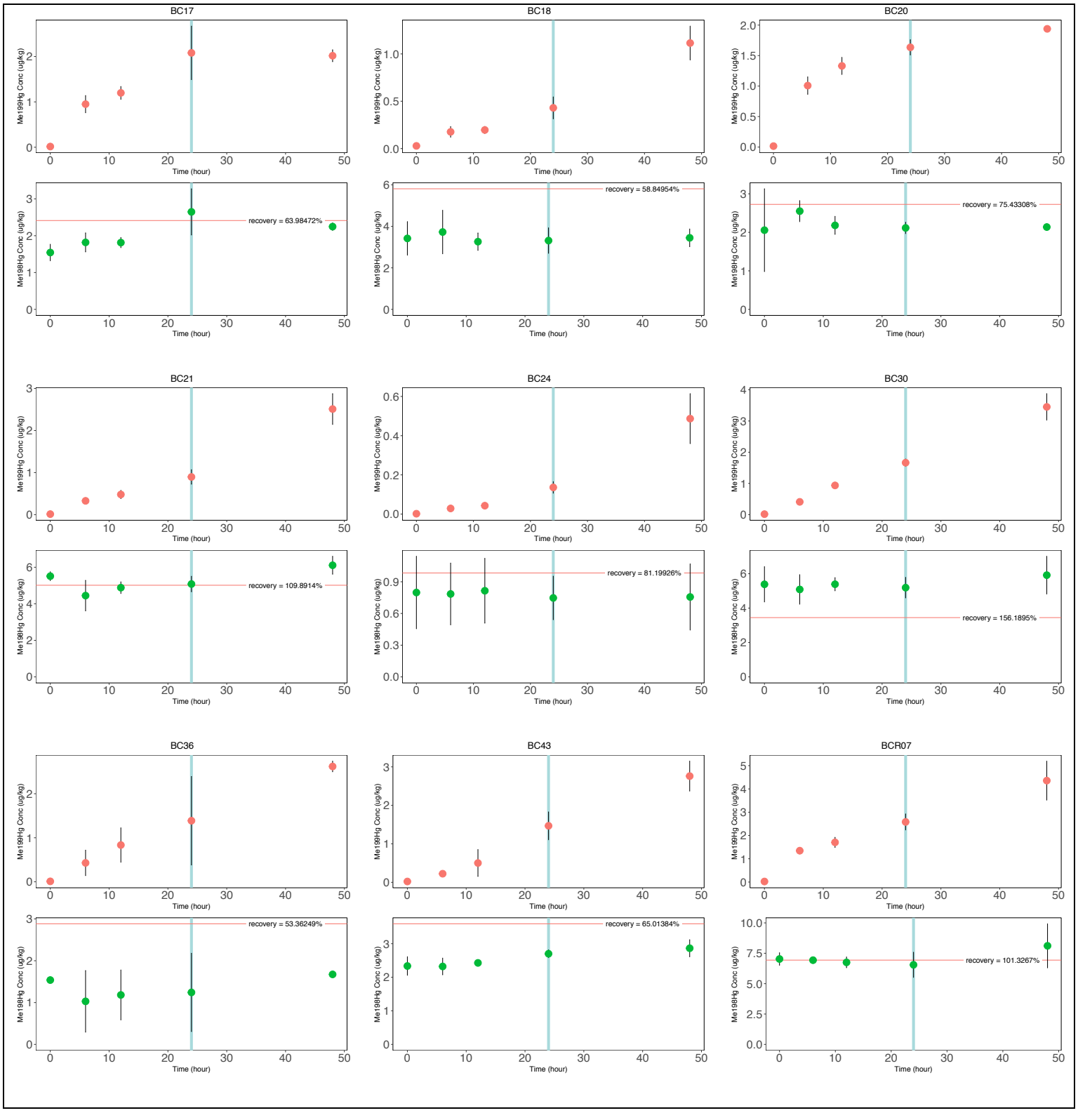
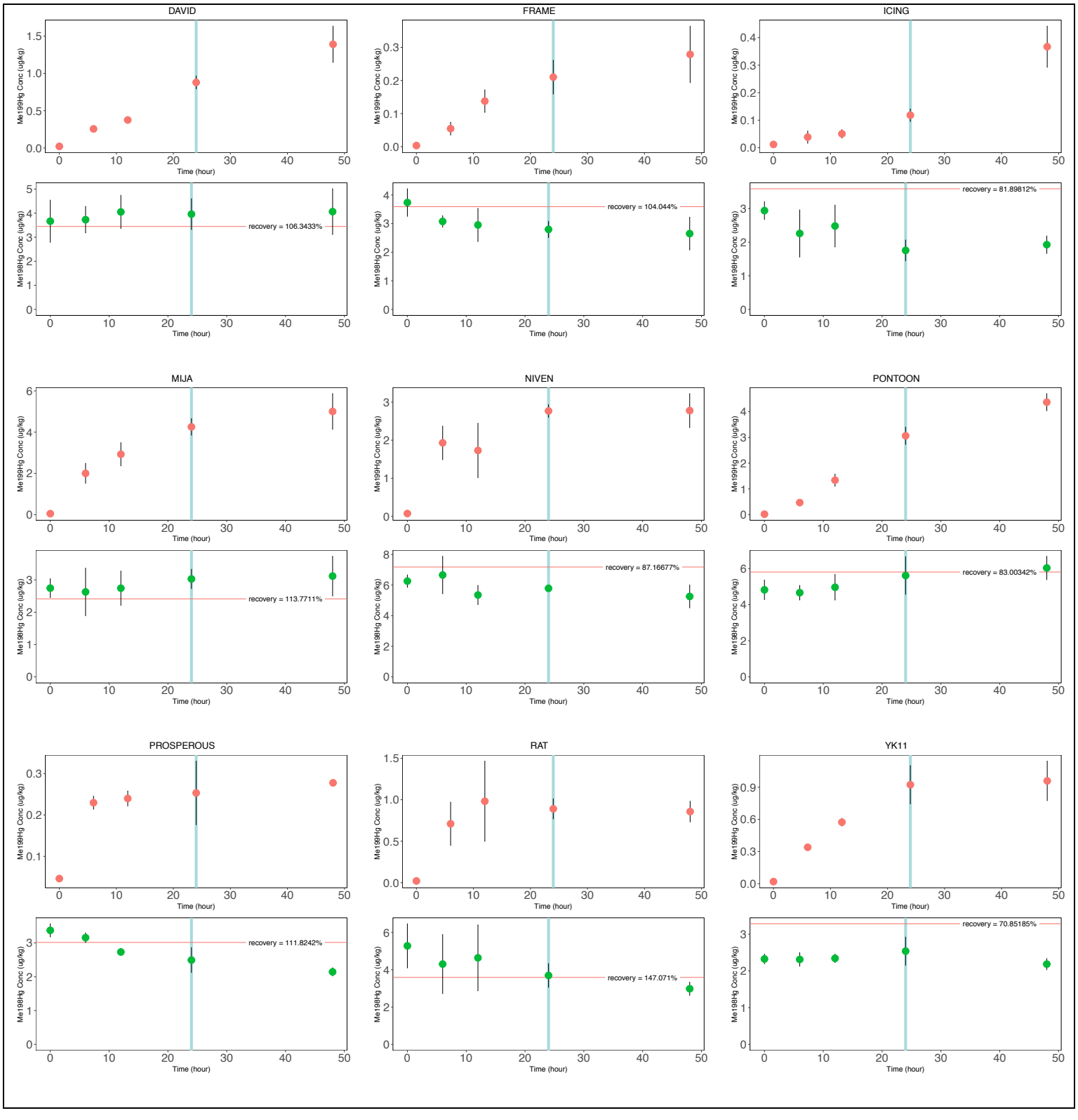
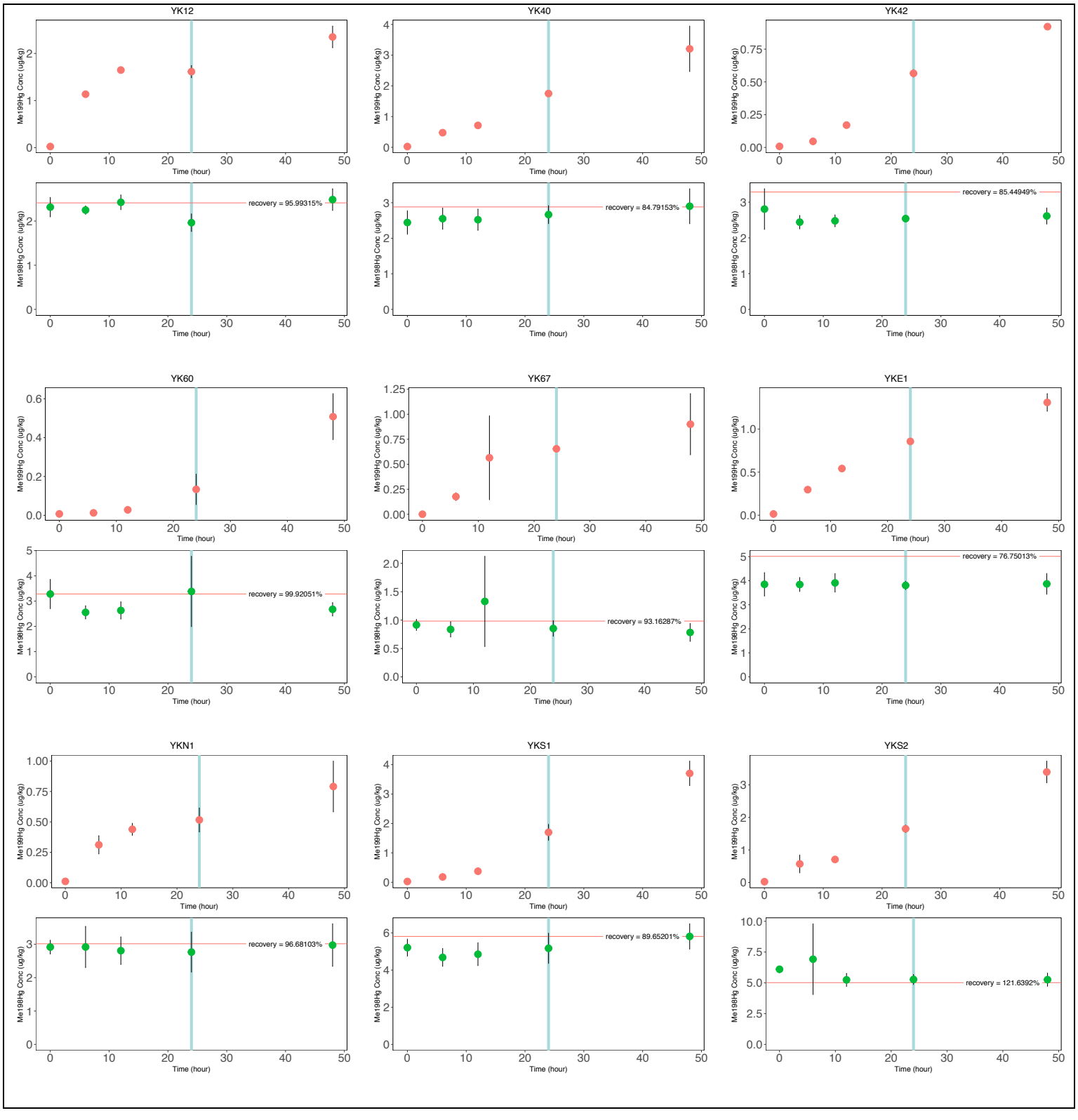


Figure S2.3: Boxplots of the two groups of demethylation rate constants (K_d) in lake sediments with respect to the different water chemistry measured. Lakes were categorized as following: lakes with detectable demethylation (>LOQ) and lakes with demethylation below detection limit (<LOQ). (LOQ = limit of quantification)


BC17
Me199Hg Conc (ug/kg)
Time (hour)
Me198Hg Conc (ug/kg)
recovery = 63.98472%
BC18
recovery = 58.84954%
BC20
recovery = 75.43308%
BC21
recovery = 109.8914%
BC24
recovery = 81.19926%
BC30
recovery = 156.1895%
BC36
recovery = 53.36249%
BC43
recovery = 65.01384%
BCR07
recovery = 101.3267%


DAVID
FRAME
ICING
MIJA
NIVEN
PONTOON
PROSPEROUS
RAT
YK11
Me199Hg Conc (ug/kg)
Me198Hg Conc (ug/kg)
Time (hour)
recovery = 106.3433%
recovery = 104.044%
recovery = 81.89812%
recovery = 113.7711%
recovery = 87.16677%
recovery = 83.00342%
recovery = 111.8242%
recovery = 147.071%
recovery = 70.85185%


YK12
Me199Hg Conc (ug/kg)
Time (hour)
Me198Hg Conc (ug/kg)
recovery = 95.99315%
YK40
recovery = 84.79153%
YK42
recovery = 85.44949%
YK60
recovery = 99.92051%
YK67
recovery = 93.16287%
YKE1
recovery = 76.75013%
YKN1
recovery = 96.68103%
YKS1
recovery = 89.65201%
YKS2
recovery = 121.6392%

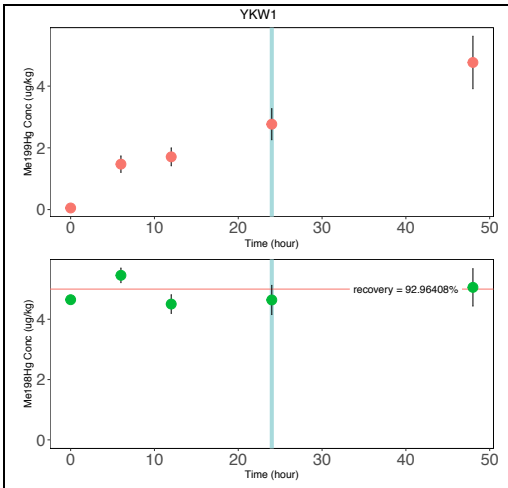


Figure S2.4: Methylation and demethylation figures for all lakes. The top panel (pink points) for each lake represents the increase in $Me^{199}Hg$ (i.e. methylation) and the bottom panel (green points) for each lake represents the decrease in $Me^{198}Hg$ (i.e. demethylation). The horizontal red line is the concentration of spiked MeHg at the start (t=0) of each incubation.

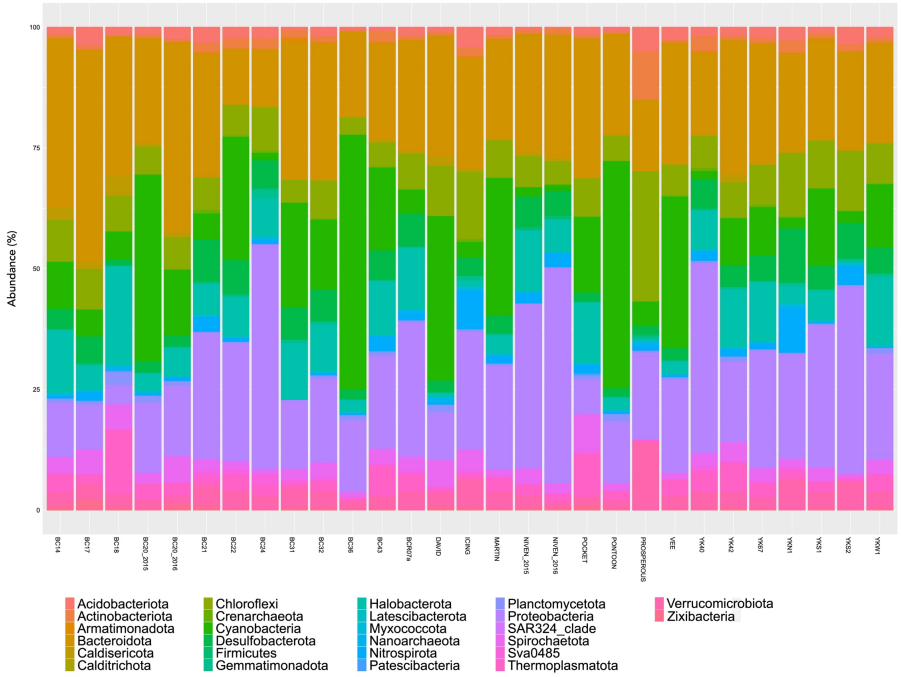


Figure S2.5: Relative abundance of microbial Phyla in each lake sediment sample

Table S2.1: Raw results from ICP-MS analysis of methylmercury isotopes in incubation sediments. For each lake two types of treatment are shown, one spiked with mercury isotopes (spiked) and one that is left unspiked (unspiked).

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
BC17	spiked	0	3.0717	0.2074	24.9149	1
BC17	spiked	6	6.3792	21.7901	39.7944	1
BC17	spiked	12	6.0907	26.364	38.1802	1
BC17	spiked	24	8.5983	38.7424	48.4085	1
BC17	spiked	48	10.6998	39.6034	42.2161	1
BC17	spiked	0	3.6246	0.3069	29.9407	2
BC17	spiked	6	3.7897	13.2288	27.2396	2
BC17	spiked	12	4.5107	19.6373	30.269	2
BC17	spiked	24	6.254	27.1395	37.2342	2
BC17	spiked	48	7.4011	33.114	38.82	2
BC17	spiked	0	3.5228	0.3159	30.1425	3
BC17	spiked	6	5.0985	17.8619	33.9861	3
BC17	spiked	12	5.062	20.5241	32.0964	3
BC17	spiked	24	11.9725	49.4971	60.9777	3
BC17	spiked	48	9.1764	39.014	43.2173	3
BC17	unspiked	0	3.4314	0.132	0.1015	1
BC17	unspiked	6	4.2659	0.0384	0.0724	1
BC17	unspiked	12	4.4374	0.0088	-0.0136	1
BC17	unspiked	24	4.5721	-0.0404	0.0003	1
BC17	unspiked	48	4.7669	0.1798	0.0856	1
BC17	unspiked	0	3.2875	-0.0595	-0.0536	2
BC17	unspiked	6	4.4301	0.0676	0.0746	2
BC17	unspiked	12	3.2009	0.0075	-0.0054	2
BC17	unspiked	24	3.7116	0.0495	-0.0302	2
BC17	unspiked	48	3.6798	0.0281	0.0327	2
BC17	unspiked	0	4.2669	0.0283	0.0742	3
BC17	unspiked	6	3.8993	-0.0898	0.0117	3
BC17	unspiked	12	4.3654	0.0874	-0.0145	3
BC17	unspiked	24	4.4456	0.0463	0.1031	3
BC17	unspiked	48	4.544	-0.0903	0.0706	3
BC18	spiked	0	3.812	1.2872	147.8713	1
BC18	spiked	6	6.0025	8.2224	179.4489	1
BC18	spiked	12	2.7248	7.869	129.7219	1
BC18	spiked	24	4.6234	17.941	139.3452	1
BC18	spiked	48	6.8828	48.2847	135.3772	1
BC18	spiked	0	2.4684	0.7751	106.1976	2

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
BC18	spiked	6	2.2877	4.8024	111.8177	2
BC18	spiked	12	2.8853	6.6555	119.2556	2
BC18	spiked	24	2.8751	12.8144	111.3671	2
BC18	spiked	48	6.2533	39.1658	127.3155	2
BC18	spiked	0	2.2447	1.0545	125.6992	3
BC18	spiked	6	2.6981	6.4913	124.5676	3
BC18	spiked	12	2.822	7.3232	115.9701	3
BC18	spiked	24	3.5605	16.7866	118.8209	3
BC18	spiked	48	5.6932	37.5433	122.9637	3
BC18	unspiked	0	2.8009	-0.0616	0.0204	1
BC18	unspiked	6	4.348	0.0081	0.1317	1
BC18	unspiked	12	2.7425	0.0222	0.0066	1
BC18	unspiked	24	2.3074	0.2273	0.2715	1
BC18	unspiked	48	3.4622	0.2288	0.3704	1
BC18	unspiked	0	2.2391	0.0154	0.0599	2
BC18	unspiked	6	2.1343	0.0291	0.0621	2
BC18	unspiked	12	2.1767	-0.0029	0.0385	2
BC18	unspiked	24	2.1511	0.0115	0.0308	2
BC18	unspiked	48	2.4372	0.008	-0.0269	2
BC18	unspiked	0	2.0006	0.0122	0.0662	3
BC18	unspiked	6	2.2517	-0.0491	0.0404	3
BC18	unspiked	12	2.273	-0.0417	0.0635	3
BC18	unspiked	24	1.904	0.0631	0.0108	3
BC18	unspiked	48	2.5019	-0.0201	0.0081	3
BC20	spiked	0	2.3595	0.0888	26.3018	1
BC20	spiked	6	2.8552	9.0143	23.3248	1
BC20	spiked	12	3.5195	12.7289	21.8393	1
BC20	spiked	24	4.0922	15.5297	20.8155	1
BC20	spiked	48	4.5987	17.9541	20.5951	1
BC20	spiked	0	1.4695	0.2975	9.2195	2
BC20	spiked	6	3.001	9.8054	25.4171	2
BC20	spiked	12	4.4031	15.986	25.1113	2
BC20	spiked	24	4.3107	16.572	21.7086	2
BC20	spiked	48	5.1803	21.9645	23.4439	2
BC20	spiked	0	2.4531	0.0895	23.8477	3
BC20	spiked	6	4.0582	11.1299	27.1247	3
BC20	spiked	12	3.3969	11.2149	18.3347	3
BC20	spiked	24	4.2083	16.5614	20.4216	3
BC20	spiked	48	4.4624	18.0957	19.8007	3

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
BC20	unspiked	0	2.2322	0.0021	0.0139	1
BC20	unspiked	6	2.5018	0.0198	-0.0089	1
BC20	unspiked	12	2.5906	0.0184	0.0126	1
BC20	unspiked	24	2.9891	-0.0094	-0.0186	1
BC20	unspiked	48	2.8149	0.0209	0.0015	1
BC20	unspiked	0	3.6263	-0.0799	-0.0455	2
BC20	unspiked	6	3.7894	-0.2054	-0.005	2
BC20	unspiked	12	3.5539	-0.0924	0.0035	2
BC20	unspiked	24	3.3987	0.0116	0.0037	2
BC20	unspiked	48	3.7936	-0.0302	-0.0012	2
BC20	unspiked	0	2.3065	0.0195	0.0233	3
BC20	unspiked	6	2.7812	-0.0199	0.0154	3
BC20	unspiked	12	2.8016	0.0056	0.0094	3
BC20	unspiked	24	3.1434	-0.0662	0.0094	3
BC20	unspiked	48	2.7027	0.0281	0.0033	3
BC21	spiked	0	2.4578	0.1394	62.8655	1
BC21	spiked	6	1.7561	3.2594	55.0451	1
BC21	spiked	12	1.5265	4.4021	50.171	1
BC21	spiked	24	2.4838	7.7204	51.6253	1
BC21	spiked	48	4.7501	23.0345	63.3129	1
BC21	spiked	0	1.7442	0.1901	60.7771	2
BC21	spiked	6	2.0486	4.5812	55.4915	2
BC21	spiked	12	2.4124	6.789	55.7148	2
BC21	spiked	24	2.5622	11.9902	57.3169	2
BC21	spiked	48	5.3597	29.9785	67.5363	2
BC21	spiked	0	1.6331	0.1118	58.4466	3
BC21	spiked	6	1.2959	3.068	37.0529	3
BC21	spiked	12	2.7005	4.7564	55.4127	3
BC21	spiked	24	3.2525	10.0062	58.8641	3
BC21	spiked	48	6.6549	29.9539	70.813	3
BC21	unspiked	0	1.5716	0.0186	0.0597	1
BC21	unspiked	6	1.8245	0.0569	0.0304	1
BC21	unspiked	12	1.9965	0.0185	0.059	1
BC21	unspiked	24	2.5371	0.0109	0.0293	1
BC21	unspiked	48	3.4232	0.0473	0.0624	1
BC21	unspiked	0	1.8043	0.1292	0.064	2
BC21	unspiked	6	2.0012	-0.0022	0.0215	2
BC21	unspiked	12	2.7775	0.0197	0.1072	2
BC21	unspiked	24	3.6967	0.0208	0.0085	2

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
BC21	unspiked	48	5.1412	0.0241	-0.0425	2
BC21	unspiked	0	2.0721	0.0662	0.1074	3
BC21	unspiked	6	1.8067	0.0063	0.0396	3
BC21	unspiked	12	2.2585	0.0374	0.0484	3
BC21	unspiked	24	2.1759	-0.0293	0.0404	3
BC21	unspiked	48	2.8987	-0.022	0.0331	3
BC24	spiked	0	2.531	-0.2622	25.2629	1
BC24	spiked	6	3.0901	1.5099	30.469	1
BC24	spiked	12	1.9523	2.0846	34.5865	1
BC24	spiked	24	30.1573	4.0484	21.5299	1
BC24	spiked	48	8.4741	-0.3626	35.0236	1
BC24	spiked	0	1.5162	0.1426	21.4056	2
BC24	spiked	6	1.023	0.8457	22.2524	2
BC24	spiked	12	1.2805	1.4234	23.0273	2
BC24	spiked	24	1.8247	4.4385	23.1556	2
BC24	spiked	48	3.9255	15.2746	20.5835	2
BC24	unspiked	0	4.3245	-0.3409	-0.2652	1
BC24	unspiked	6	2.5569	0.1533	-0.0175	1
BC24	unspiked	12	4.5125	0.074	0.0301	1
BC24	unspiked	24	3.4768	5.2535	1.8501	1
BC24	unspiked	48	9.7462	24.8651	1.072	1
BC24	unspiked	0	1.4281	-0.0136	0.0192	2
BC24	unspiked	6	1.3053	0.0352	0.0209	2
BC24	unspiked	12	1.4984	0.0573	0.1648	2
BC24	unspiked	24	2.9967	0.0676	0.1527	2
BC24	unspiked	48	3.5457	0.017	0.0553	2
BC30	spiked	0	1.5027	0.0826	37.061	1
BC30	spiked	6	1.8896	2.7843	34.5447	1
BC30	spiked	12	2.2983	6.0966	36.2361	1
BC30	spiked	24	3.0435	10.5291	34.6018	1
BC30	spiked	48	6.0327	25.1417	45.8037	1
BC30	spiked	0	1.305	0.1003	38.2806	2
BC30	spiked	6	1.5927	2.7785	35.7794	2
BC30	spiked	12	2.092	5.8225	34.7516	2
BC30	spiked	24	3.055	11.0889	35.1807	2
BC30	spiked	48	4.8094	21.2809	35.8524	2
BC30	spiked	0	1.4458	0.1459	33.4093	3
BC30	spiked	6	1.6682	2.7548	32.59	3
BC30	spiked	12	2.5651	7.2946	39.0248	3

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
BC30	spiked	24	3.2262	12.3924	35.7648	3
BC30	spiked	48	5.7827	23.8748	38.4573	3
BC30	unspiked	0	1.6185	-0.0377	0.0358	1
BC30	unspiked	6	1.5594	-0.0715	0.0322	1
BC30	unspiked	12	2.1114	-0.003	0.0209	1
BC30	unspiked	24	2.3239	-0.0393	-0.0041	1
BC30	unspiked	48	2.7508	0	0.0123	1
BC30	unspiked	0	1.3641	0.0217	0.0357	2
BC30	unspiked	6	1.5485	0.0006	0.0436	2
BC30	unspiked	12	1.7917	0.0001	0.0112	2
BC30	unspiked	24	1.7514	0.0088	0.0253	2
BC30	unspiked	48	2.3104	0.0036	0.0062	2
BC30	unspiked	0	1.3379	0.0092	0.0839	3
BC30	unspiked	6	1.618	-0.0058	0.0459	3
BC30	unspiked	12	1.7763	0.0238	0.021	3
BC30	unspiked	24	2.2334	-0.0176	-0.0234	3
BC30	unspiked	48	2.1064	0.0482	0.0043	3
BC36	spiked	0	26.0214	0.9374	88.8387	1
BC36	spiked	6	9.5578	12.648	28.5681	1
BC36	spiked	12	21.7187	31.3919	42.7226	1
BC36	spiked	24	18.8925	38.011	32.6563	1
BC36	spiked	48	52.6405	151.9022	93.9749	1
BC36	spiked	0	5.8965	0.169	68.1329	2
BC36	spiked	6	8.97	28.8685	70.6534	2
BC36	spiked	12	12.2796	50.6201	73.1146	2
BC36	spiked	24	18.1025	95.5853	86.7959	2
BC36	spiked	48	22.0511	115.5051	76.3366	2
BC36	unspiked	0	7.4766	-0.2491	2.3886	1
BC36	unspiked	6	5.8629	0.2735	0.0173	1
BC36	unspiked	12	12.0639	-0.5708	2.1255	1
BC36	unspiked	24	7.7471	0.5302	0.0551	1
BC36	unspiked	48	5.7997	0.7122	0.1632	1
BC36	unspiked	0	6.4745	-0.0616	-0.0119	2
BC36	unspiked	6	6.5516	-0.0272	0.0528	2
BC36	unspiked	12	7.2978	0.053	0.0753	2
BC36	unspiked	24	9.0896	0.0911	0.0309	2
BC36	unspiked	48	12.6168	0.0849	-0.0168	2
BC43	spiked	0	1.2176	0.4188	31.3012	1
BC43	spiked	6	1.6789	2.359	28.6387	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
BC43	spiked	12	1.8435	4.8211	32.1466	1
BC43	spiked	24	4.3005	20.5109	38.935	1
BC43	spiked	48	5.7175	34.5564	35.6669	1
BC43	spiked	0	1.3053	0.2292	33.1278	2
BC43	spiked	6	1.5476	2.7778	32.2423	2
BC43	spiked	12	1.5484	3.2181	30.7286	2
BC43	spiked	24	2.886	13.7871	32.4784	2
BC43	spiked	48	6.1734	32.283	38.5375	2
BC43	spiked	0	1.3835	0.2445	28.0159	3
BC43	spiked	6	1.8666	3.7413	30.9913	3
BC43	spiked	12	6.6166	12.0282	33.4511	3
BC43	spiked	24	4.4462	24.2937	35.9565	3
BC43	spiked	48	7.7805	42.7672	39.2306	3
BC43	unspiked	0	1.5846	0.0172	0.0854	1
BC43	unspiked	6	1.5937	0.0999	0.0349	1
BC43	unspiked	12	2.0257	-0.0388	0.0268	1
BC43	unspiked	24	2.9566	-0.0484	-0.0745	1
BC43	unspiked	48	3.515	0.0539	0.0246	1
BC43	unspiked	0	0.8331	0.0443	0.0249	2
BC43	unspiked	6	0.777	0.0132	0.041	2
BC43	unspiked	12	1.1364	-0.0101	0.0081	2
BC43	unspiked	24	1.2626	-0.0328	0.0363	2
BC43	unspiked	48	1.2594	0.055	0.023	2
BC43	unspiked	0	1.3775	0.0589	0.0554	3
BC43	unspiked	6	1.5849	0.0567	-0.0066	3
BC43	unspiked	12	1.8546	0.0236	0.0467	3
BC43	unspiked	24	2.8173	-0.0113	-0.0145	3
BC43	unspiked	48	3.4398	-0.0107	0.0283	3
BCR07	spiked	0	3.9007	0.1352	60.4913	1
BCR07	spiked	6	3.79	13.6428	61.0381	1
BCR07	spiked	12	4.4781	13.411	58.4199	1
BCR07	spiked	24	4.7322	20.2021	53.0756	1
BCR07	spiked	48	15.2037	47.8232	92.5831	1
BCR07	spiked	0	1.9663	0.1737	64.2019	2
BCR07	spiked	6	3.468	10.8551	59.0439	2
BCR07	spiked	12	3.5446	14.6161	55.3835	2
BCR07	spiked	24	4.5969	22.0878	51.2249	2
BCR07	spiked	48	7.5179	35.3719	61.6215	2
BCR07	spiked	0	2.3669	0.3231	60.8403	3

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
BCR07	spiked	6	3.6754	11.1915	63.1696	3
BCR07	spiked	12	4.1873	17.0107	64.7639	3
BCR07	spiked	24	5.8615	25.9305	69.0502	3
BCR07	spiked	48	6.9756	32.2556	61.0753	3
BCR07	unspiked	0	3.0232	0.0265	0.0954	1
BCR07	unspiked	6	1.7056	0.0984	0.1813	1
BCR07	unspiked	12	2.9037	-0.0668	0.0754	1
BCR07	unspiked	24	3.422	0.0493	0.0135	1
BCR07	unspiked	48	4.975	-0.0323	0.0135	1
BCR07	unspiked	0	1.8308	-0.0027	0.0681	2
BCR07	unspiked	6	2.4762	0.0289	0.1124	2
BCR07	unspiked	12	2.2861	0.0533	0.0414	2
BCR07	unspiked	24	3.604	-0.0059	-0.0113	2
BCR07	unspiked	48	3.4709	0.0137	0.0221	2
BCR07	unspiked	0	2.3932	0.0122	0.0216	3
BCR07	unspiked	6	2.3292	0.0326	0.015	3
BCR07	unspiked	12	3.1766	-0.0544	-0.0007	3
BCR07	unspiked	24	3.8011	0.0105	0.0364	3
BCR07	unspiked	48	3.82	0.0623	0.0438	3
DAVID	spiked	0	2.3255	0.7606	121.6125	1
DAVID	spiked	6	2.8618	7.8334	123.0387	1
DAVID	spiked	12	3.5684	10.3004	134.2383	1
DAVID	spiked	24	4.6468	27.6063	131.6842	1
DAVID	spiked	48	7.2869	46.5075	144.527	1
DAVID	spiked	0	2.6589	0.6884	132.6369	2
DAVID	spiked	6	2.8225	7.1369	115.5881	2
DAVID	spiked	12	4.1752	12.394	132.9034	2
DAVID	spiked	24	4.3664	27.7536	125.323	2
DAVID	spiked	48	6.2312	44.8087	125.5306	2
DAVID	spiked	0	2.7946	0.7523	114.025	3
DAVID	spiked	6	3.4755	11.7105	142.0834	3
DAVID	spiked	12	3.9882	16.4308	144.0287	3
DAVID	spiked	24	5.5763	34.8488	145.9191	3
DAVID	spiked	48	6.8223	49.6522	139.6102	3
DAVID	unspiked	0	2.3954	0.0798	0.142	1
DAVID	unspiked	6	2.4336	-0.0128	0.0387	1
DAVID	unspiked	12	2.7482	0.0298	0.0339	1
DAVID	unspiked	24	3.0602	0.1174	0.2509	1
DAVID	unspiked	48	3.5401	-0.0335	0.0243	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
DAVID	unspiked	0	2.3392	0.0223	0.0886	2
DAVID	unspiked	6	2.1899	0.0362	0.0609	2
DAVID	unspiked	12	2.413	0.0185	0.0644	2
DAVID	unspiked	24	2.9311	0.0273	0.085	2
DAVID	unspiked	48	3.2352	0.0209	0.0421	2
DAVID	unspiked	0	3.2469	-0.016	0.0603	3
DAVID	unspiked	6	3.6616	-0.0329	0.0633	3
DAVID	unspiked	12	3.2965	-0.0205	0.0482	3
DAVID	unspiked	24	3.6167	-0.0426	0.0267	3
DAVID	unspiked	48	4.1564	-0.0292	0.0858	3
FRAME	spiked	0	3.1607	0.0838	37.5918	1
FRAME	spiked	6	3.3755	0.7583	35.5193	1
FRAME	spiked	12	2.9614	1.4231	29.5361	1
FRAME	spiked	24	3.4121	2.3662	31.3406	1
FRAME	spiked	48	3.7868	3.2309	30.7892	1
FRAME	spiked	0	4.9514	-0.1585	48.1015	2
FRAME	spiked	6	4.1207	0.3827	39.06	2
FRAME	spiked	12	3.4374	1.3673	32.6133	2
FRAME	spiked	24	3.0918	1.9523	30.931	2
FRAME	spiked	48	2.8095	2.3232	24.8823	2
FRAME	spiked	0	4.0651	0.0329	45.5133	3
FRAME	spiked	6	3.2103	0.7676	33.5213	3
FRAME	spiked	12	4.3628	2.0293	41.3721	3
FRAME	spiked	24	4.6834	3.0463	35.8544	3
FRAME	spiked	48	4.4602	4.2114	37.2285	3
FRAME	unspiked	0	2.9596	0.0037	-0.0018	1
FRAME	unspiked	6	3.0594	-0.0198	-0.0078	1
FRAME	unspiked	12	3.1524	-0.0092	-0.0194	1
FRAME	unspiked	24	3.0595	0.0015	0.0028	1
FRAME	unspiked	48	2.8413	0.0583	0.0038	1
FRAME	unspiked	0	2.3633	0.0832	0.0603	2
FRAME	unspiked	6	2.9478	-0.0026	0.0397	2
FRAME	unspiked	12	2.6861	0.0065	0.0179	2
FRAME	unspiked	24	2.518	-0.0411	0.0415	2
FRAME	unspiked	48	2.7375	-0.0044	0.0015	2
FRAME	unspiked	0	4.1824	-0.0424	0.18	3
FRAME	unspiked	6	2.2483	0.0048	0.1427	3
FRAME	unspiked	12	3.2094	-0.0887	-0.0196	3
FRAME	unspiked	24	2.4835	0.0036	0.015	3

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
FRAME	unspiked	48	4.0067	-0.0461	0.0606	3
ICING	spiked	0	1.7281	0.1164	29.549	1
ICING	spiked	6	0.918	0.252	18.51	1
ICING	spiked	12	1.7677	0.7164	34.6833	1
ICING	spiked	24	1.6926	1.5935	23.054	1
ICING	spiked	48	2.6628	4.7764	23.3449	1
ICING	spiked	0	1.9669	0.0486	34.3088	2
ICING	spiked	6	1.1265	0.7351	35.0108	2
ICING	spiked	12	1.4414	0.5996	28.0805	2
ICING	spiked	24	1.5144	1.1606	19.9129	2
ICING	spiked	48	2.0792	3.2812	18.7601	2
ICING	spiked	0	1.2796	0.2181	33.7707	3
ICING	spiked	6	1.0722	0.3047	22.0265	3
ICING	spiked	12	1.1385	0.3652	19.9943	3
ICING	spiked	24	1.4078	1.165	15.4994	3
ICING	spiked	48	2.5621	4.0958	21.7437	3
ICING	unspiked	0	1.044	0.0206	0.1513	1
ICING	unspiked	6	1.5985	0.0449	0.1467	1
ICING	unspiked	12	1.0202	0.0711	0.0565	1
ICING	unspiked	24	1.2471	0.0331	0.0134	1
ICING	unspiked	48	1.4942	0.0153	0.025	1
ICING	unspiked	0	1.1738	0.0215	0.05	2
ICING	unspiked	6	1.0968	-0.0621	0.0932	2
ICING	unspiked	12	1.3244	0.0024	0.0589	2
ICING	unspiked	24	1.6968	-0.0225	0.0933	2
ICING	unspiked	48	2.4183	-0.0096	0.0129	2
ICING	unspiked	0	1.0373	0.0406	0.0407	3
ICING	unspiked	6	1.4396	-0.0012	0.0235	3
ICING	unspiked	12	1.8278	-0.1167	0.0389	3
ICING	unspiked	24	1.2638	-0.0044	0.0606	3
ICING	unspiked	48	1.8857	-0.0122	0.0081	3
MIJA	spiked	0	2.4241	0.6004	26.2111	1
MIJA	spiked	6	3.7661	16.7156	21.6058	1
MIJA	spiked	12	7.3614	31.9358	28.9929	1
MIJA	spiked	24	9.1093	41.4161	28.2201	1
MIJA	spiked	48	11.3625	51.1567	31.7612	1
MIJA	spiked	0	2.4595	0.1636	22.4012	2
MIJA	spiked	6	3.6804	14.9647	18.863	2
MIJA	spiked	12	5.0783	23.0727	20.2601	2

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
MIJA	spiked	24	8.6316	35.5835	25.0429	2
MIJA	spiked	48	8.9867	37.5633	22.4631	2
MIJA	spiked	0	2.7848	0.5326	22.4871	3
MIJA	spiked	6	5.0272	19.6695	26.7032	3
MIJA	spiked	12	4.6988	21.2899	21.8017	3
MIJA	spiked	24	6.4479	33.5196	25.1788	3
MIJA	spiked	48	9.0375	40.8541	26.3084	3
MIJA	unspiked	0	2.9239	0.0363	0.0623	1
MIJA	unspiked	6	3.7497	0.0144	-0.0109	1
MIJA	unspiked	12	4.5672	0.0135	0.0597	1
MIJA	unspiked	24	4.1546	0.0521	0.0351	1
MIJA	unspiked	48	5.7694	-0.0848	-0.0511	1
MIJA	unspiked	0	2.6286	-0.0273	0.0318	2
MIJA	unspiked	6	2.7548	0.0078	0.1018	2
MIJA	unspiked	12	3.731	0.0515	-0.052	2
MIJA	unspiked	24	4.2665	-0.0381	0.0594	2
MIJA	unspiked	48	5.3865	-0.1136	-0.0484	2
MIJA	unspiked	0	2.5115	0.0029	0.2008	3
MIJA	unspiked	6	3.2396	0.046	0.0192	3
MIJA	unspiked	12	4.0505	-0.0465	0.0124	3
MIJA	unspiked	24	3.6579	0.0464	0.0888	3
MIJA	unspiked	48	4.2318	0.0433	-0.0262	3
NIVEN	spiked	0	3.5885	0.2246	25.1542	1
NIVEN	spiked	6	4.8603	7.5346	26.7158	1
NIVEN	spiked	12	3.6039	7.939	20.2828	1
NIVEN	spiked	24	5.835	12.5153	25.4045	1
NIVEN	spiked	48	4.6537	10.3928	19.3187	1
NIVEN	spiked	0	3.9655	0.3425	28.8028	2
NIVEN	spiked	6	4.5834	6.9696	24.8517	2
NIVEN	spiked	12	5.4898	10.3064	25.7898	2
NIVEN	spiked	24	5.6423	12.1721	24.896	2
NIVEN	spiked	48	6.5468	14.1835	25.8761	2
NIVEN	spiked	0	3.9513	0.3819	27.5101	3
NIVEN	spiked	6	5.9185	10.6158	35.15	3
NIVEN	spiked	12	1.5744	4.2209	23.6122	3
NIVEN	spiked	24	5.7832	11.2863	25.0192	3
NIVEN	spiked	48	5.9074	11.5424	23.25	3
NIVEN	unspiked	0	3.7932	-0.007	0.1391	1
NIVEN	unspiked	6	3.4915	0.0849	0.1845	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
NIVEN	unspiked	12	3.6993	0.0419	0.114	1
NIVEN	unspiked	24	3.6726	0.0616	0.0759	1
NIVEN	unspiked	48	4.4596	-0.1083	0.1215	1
NIVEN	unspiked	0	4.395	-0.0888	-0.0099	2
NIVEN	unspiked	6	3.6497	0.0077	0.0318	2
NIVEN	unspiked	12	4.3181	-0.0577	0.1124	2
NIVEN	unspiked	24	3.5876	-0.0288	0.0896	2
NIVEN	unspiked	48	4.3897	-0.0219	0.1428	2
NIVEN	unspiked	0	3.9414	0.0368	0.0613	3
NIVEN	unspiked	6	4.3156	0.0061	-0.0434	3
NIVEN	unspiked	12	3.6434	0.024	0.0409	3
NIVEN	unspiked	24	4.3027	0.0311	-0.0102	3
NIVEN	unspiked	48	4.2647	-0.074	0.0121	3
PONTOON	spiked	0	1.3991	0.2554	71.2267	1
PONTOON	spiked	6	2.1586	6.6151	66.8093	1
PONTOON	spiked	12	4.1613	21.0512	75.4337	1
PONTOON	spiked	24	8.5135	45.1978	89.0953	1
PONTOON	spiked	48	10.6987	59.7416	88.2486	1
PONTOON	spiked	0	1.035	0.2381	60.489	2
PONTOON	spiked	6	1.6176	5.8006	58.7449	2
PONTOON	spiked	12	2.6014	15.9995	59.9719	2
PONTOON	spiked	24	6.0771	39.0488	66.0579	2
PONTOON	spiked	48	10.1778	62.4369	80.9421	2
PONTOON	spiked	0	0.9812	0.2524	57.3808	3
PONTOON	spiked	6	1.463	6.0122	57.2482	3
PONTOON	spiked	12	3.0013	15.4502	59.2127	3
PONTOON	spiked	24	6.0571	35.9728	64.9689	3
PONTOON	spiked	48	8.2961	49.5837	67.8644	3
PONTOON	unspiked	0	1.4106	0.0324	0.1402	1
PONTOON	unspiked	6	1.067	0.0401	0.067	1
PONTOON	unspiked	12	1.8151	0.0514	0.2563	1
PONTOON	unspiked	24	2.9558	0.1849	0.1572	1
PONTOON	unspiked	48	3.7347	0.1219	-0.0073	1
PONTOON	unspiked	0	1.054	-0.0121	0.002	2
PONTOON	unspiked	6	0.922	0.0091	0.0111	2
PONTOON	unspiked	12	1.332	-0.0101	0.0073	2
PONTOON	unspiked	24	2.2225	0.0066	0.0296	2
PONTOON	unspiked	48	3.2983	0	0.0685	2
PONTOON	unspiked	0	0.9856	0.024	0.0221	3

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
PONTOON	unspiked	6	0.8834	0.0458	0.0481	3
PONTOON	unspiked	12	1.2476	0.0362	0.0499	3
PONTOON	unspiked	24	2.1918	0.0329	-0.008	3
PONTOON	unspiked	48	3.0503	0.047	0.0185	3
PROSPEROUS	spiked	0	0.0466	0.0848	7.1507	1
PROSPEROUS	spiked	6	0.0618	0.5441	6.5808	1
PROSPEROUS	spiked	12	0.0813	0.5648	5.7481	1
PROSPEROUS	spiked	24	0.0908	0.7518	6.3756	1
PROSPEROUS	spiked	48	0.1014	0.592	4.4301	1
PROSPEROUS	spiked	0	0.0756	0.1196	7.9686	3
PROSPEROUS	spiked	6	0.1051	0.4963	7.1468	3
PROSPEROUS	spiked	12	0.0945	0.5363	6.1774	3
PROSPEROUS	spiked	24	0.0928	0.456	4.8659	3
PROSPEROUS	spiked	48	0.0998	0.6268	4.8092	3
PROSPEROUS	spiked	0	0.0545	0.1004	7.1011	2
PROSPEROUS	spiked	6	0.0702	0.4731	7.0596	2
PROSPEROUS	spiked	12	0.0771	0.48	6.0693	2
PROSPEROUS	spiked	24	0.0771	0.4606	5.1788	2
PROSPEROUS	spiked	48	0.0943	0.6106	4.8886	2
PROSPEROUS	unspiked	0	0.0401	0.0002	0.0014	1
PROSPEROUS	unspiked	6	0.044	0.0008	0.0019	1
PROSPEROUS	unspiked	12	0.0302	0.0038	0.004	1
PROSPEROUS	unspiked	24	0.0446	0.0019	0.0015	1
PROSPEROUS	unspiked	48	0.0628	0.0018	0.0006	1
PROSPEROUS	unspiked	0	0.0428	0.0028	0.0016	3
PROSPEROUS	unspiked	6	0.0592	0.0047	0.006	3
PROSPEROUS	unspiked	12	0.0771	0.0012	0.0112	3
PROSPEROUS	unspiked	24	0.0683	0.0082	0.0069	3
PROSPEROUS	unspiked	48	0.0729	0.0023	0.007	3
PROSPEROUS	unspiked	0	0.0431	0.0002	0.0038	2
PROSPEROUS	unspiked	6	0.0442	0.0003	0.0026	2
PROSPEROUS	unspiked	12	0.0493	0.0018	0.003	2
PROSPEROUS	unspiked	24	0.0475	0.0015	0.0032	2
PROSPEROUS	unspiked	48	0.0605	0.003	0.0018	2
RAT	spiked	0	5.2582	0.17	41.6669	1
RAT	spiked	6	3.8637	4.7261	29.0312	1
RAT	spiked	12	4.5675	5.7036	30.0434	1
RAT	spiked	24	6.4872	8.6846	37.3597	1
RAT	spiked	48	4.4586	7.6845	26.3721	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
RAT	spiked	0	6.5695	0.0997	60.523	2
RAT	spiked	6	7.1661	9.2724	56.2594	2
RAT	spiked	12	8.0702	14.1262	61.2736	2
RAT	spiked	24	4.6981	7.6075	30.4497	2
RAT	spiked	48	3.2624	6.5627	23.6804	2
RAT	spiked	0	4.5756	0.2756	39.2188	3
RAT	spiked	6	3.7065	5.0865	30.4025	3
RAT	spiked	12	4.26	6.6499	33.4971	3
RAT	spiked	24	4.4536	7.3927	30.3953	3
RAT	spiked	48	4.4258	8.6093	29.4742	3
RAT	unspiked	0	3.6728	-0.0518	0.0384	1
RAT	unspiked	6	4.3176	-0.0381	-0.0441	1
RAT	unspiked	12	3.9261	-0.0687	0.0075	1
RAT	unspiked	24	3.8642	0.0127	0.0345	1
RAT	unspiked	48	4.5703	-0.0643	0.0328	1
RAT	unspiked	0	2.6388	0.1785	0.0211	2
RAT	unspiked	6	2.5813	0.1085	0.0542	2
RAT	unspiked	12	2.86	0.0922	0.0186	2
RAT	unspiked	24	2.2307	0.0663	0.1264	2
RAT	unspiked	48	3.5321	0.0273	0.1052	2
RAT	unspiked	0	5.7352	0.052	-0.0501	3
RAT	unspiked	6	3.1188	0.1215	0.0534	3
RAT	unspiked	12	2.6403	0.0254	0.0278	3
RAT	unspiked	24	3.8041	0.0494	-0.0358	3
RAT	unspiked	48	3.3053	0.0994	0.1167	3
YK11	spiked	0	4.9327	0.423	46.2103	1
YK11	spiked	6	6.1717	7.2963	50.5304	1
YK11	spiked	12	6.4266	12.3886	50.1299	1
YK11	spiked	24	8.9711	21.6986	58.1124	1
YK11	spiked	48	7.94	22.5237	47.421	1
YK11	spiked	0	4.8471	0.3245	42.6356	3
YK11	spiked	6	4.7994	5.7522	38.4943	3
YK11	spiked	12	5.6277	9.6656	39.8999	3
YK11	spiked	24	6.1851	14.0522	40.0095	3
YK11	spiked	48	6.2335	14.6154	36.6455	3
YK11	unspiked	0	5.2283	-0.1158	0.0211	1
YK11	unspiked	6	4.8225	0.0486	0.0489	1
YK11	unspiked	12	5.7133	0.0959	0.061	1
YK11	unspiked	24	6.1633	0.0013	0.0487	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
YK11	unspiked	48	6.0097	0.0501	-0.0722	1
YK11	unspiked	0	4.2814	0.0443	0.0796	3
YK11	unspiked	6	4.7917	0.074	0.0555	3
YK11	unspiked	24	5.4353	0.0647	0.0178	3
YK11	unspiked	48	5.5292	0.049	-0.0132	3
YK12	spiked	0	2.7667	0.3312	26.9588	1
YK12	spiked	6	3.9804	13.7481	27.6459	1
YK12	spiked	12	4.5145	19.2916	27.5862	1
YK12	spiked	24	4.3122	19.3767	22.8624	1
YK12	spiked	48	7.2023	29.748	31.479	1
YK12	spiked	0	3.4214	0.281	29.4706	2
YK12	spiked	6	3.8006	13.5479	26.1179	2
YK12	spiked	12	4.664	18.5275	26.8702	2
YK12	spiked	24	3.495	17.0412	20.5957	2
YK12	spiked	48	6.6412	25.2738	26.7119	2
YK12	spiked	0	2.4729	0.2297	24.6107	3
YK12	spiked	6	3.7729	12.4458	24.99	3
YK12	spiked	12	5.3561	19.873	30.4343	3
YK12	spiked	24	4.5093	20.1105	25.3039	3
YK12	unspiked	0	3.2389	-0.018	0.1143	1
YK12	unspiked	6	2.6399	-0.0104	0.0376	1
YK12	unspiked	12	3.7636	-0.0243	0.0069	1
YK12	unspiked	24	3.6442	0.0775	0.0582	1
YK12	unspiked	48	4.2675	0.0124	0.0247	1
YK12	unspiked	0	1.9864	-0.0038	0.0632	2
YK12	unspiked	6	2.9939	-0.0142	-0.0114	2
YK12	unspiked	12	2.7636	0.0336	-0.0106	2
YK12	unspiked	24	2.9696	0.1431	0.0732	2
YK12	unspiked	48	3.0003	0.0471	0.0969	2
YK12	unspiked	0	2.8775	-0.0186	0.0176	3
YK12	unspiked	6	2.6324	0.0336	0.1872	3
YK12	unspiked	12	3.5052	-0.0204	-0.0036	3
YK12	unspiked	24	2.9437	0.0481	0.0281	3
YK12	unspiked	48	4.1604	-0.0335	0.1062	3
YK40	spiked	0	3.204	0.2365	22.2157	1
YK40	spiked	6	3.7231	3.6258	21.6822	1
YK40	spiked	12	4.0477	5.6753	22.0946	1
YK40	spiked	24	6.8306	15.1644	24.6805	1
YK40	spiked	48	9.3918	23.6339	22.7921	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
YK40	spiked	0	3.2394	0.1904	20.9287	2
YK40	spiked	6	3.8976	4.2462	22.6549	2
YK40	spiked	12	4.1027	6.3042	21.9698	2
YK40	spiked	24	5.7298	13.6796	20.4474	2
YK40	spiked	48	11.998	32.5391	27.7116	2
YK40	spiked	0	2.9298	0.1575	18.0706	3
YK40	spiked	6	3.4541	4.0549	19.5744	3
YK40	spiked	12	3.6944	5.877	19.1255	3
YK40	spiked	24	6.5532	15.1603	21.8458	3
YK40	spiked	48	9.8486	23.9122	22.088	3
YK40	unspiked	0	3.109	0.0666	0.0125	1
YK40	unspiked	6	3.7666	0.1014	0.037	1
YK40	unspiked	12	4.7378	0.0864	0.0273	1
YK40	unspiked	24	5.7361	0.1462	0.0261	1
YK40	unspiked	48	6.7543	0.161	0.0484	1
YK40	unspiked	0	3.554	0.0072	-0.008	2
YK40	unspiked	6	4.5135	0.0664	0.0038	2
YK40	unspiked	12	5.2069	0.0353	0.0037	2
YK40	unspiked	24	7.701	0	0.0073	2
YK40	unspiked	48	8.0123	0.101	-0.0032	2
YK40	unspiked	0	2.9203	-0.0162	0.011	3
YK40	unspiked	6	3.7178	-0.0252	0.0217	3
YK40	unspiked	12	4.8951	-0.0173	-0.0198	3
YK40	unspiked	24	6.5523	-0.0009	0.0115	3
YK40	unspiked	48	3.5183	-0.0026	0.0007	3
YK42	spiked	0	4.6124	0.1541	49.8057	1
YK42	spiked	6	3.3762	0.6408	35.8401	1
YK42	spiked	12	3.9876	2.4473	36.6572	1
YK42	spiked	24	5.7351	8.4705	39.8258	1
YK42	spiked	48	6.9967	14.3556	43.0504	1
YK42	spiked	0	3.3321	0.0903	34.7171	3
YK42	spiked	6	3.0707	0.7435	37.1564	3
YK42	spiked	12	3.7708	2.6407	37.4647	3
YK42	spiked	24	4.3388	8.4551	36.2967	3
YK42	spiked	48	5.6081	13.2693	35.4214	3
YK42	unspiked	0	3.8505	0.1614	0.192	1
YK42	unspiked	6	3.7797	0.0261	0.1351	1
YK42	unspiked	12	3.8338	-0.0218	0.0732	1
YK42	unspiked	24	4.1846	-0.0784	0.1023	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
YK42	unspiked	48	4.8135	0.0923	0.0791	1
YK42	unspiked	0	3.0378	0.0618	0.0687	3
YK42	unspiked	6	3.2844	-0.0147	0.0102	3
YK42	unspiked	12	3.1142	-0.0144	0.0095	3
YK42	unspiked	24	3.6467	0.0165	-0.0102	3
YK42	unspiked	48	3.961	0.0558	0.0618	3
YK60	spiked	0	2.6739	0.0905	58.2153	1
YK60	spiked	6	1.5967	0.2245	34.8845	1
YK60	spiked	12	1.4826	0.5001	41.8514	1
YK60	spiked	24	1.8292	1.4766	41.9177	1
YK60	spiked	48	2.5699	5.9113	40.2931	1
YK60	spiked	0	0.9184	0.098	39.8202	3
YK60	spiked	6	0.8001	0.2187	35.8663	3
YK60	spiked	12	0.7604	0.4281	32.3202	3
YK60	spiked	24	0.8542	1.1399	34.2039	3
YK60	spiked	48	2.2213	7.151	34.6173	3
YK60	spiked	0	1.1322	0.1354	45.477	2
YK60	spiked	6	1.0692	0.1107	40.6327	2
YK60	spiked	12	0.9151	0.3064	40.7434	2
YK60	spiked	24	2.0073	3.1928	71.0382	2
YK60	spiked	48	2.6496	9.0367	41.8349	2
YK60	unspiked	0	1.8658	-0.1182	-0.0156	1
YK60	unspiked	6	0.8709	0.0261	0.0287	1
YK60	unspiked	12	1.019	-0.0036	0.0307	1
YK60	unspiked	24	1.8531	0.0385	0.1216	1
YK60	unspiked	48	3.9755	-0.0097	0.1777	1
YK60	unspiked	0	0.6421	0.042	0.0404	3
YK60	unspiked	6	0.7618	0.0455	0.0303	3
YK60	unspiked	12	0.7378	0.0232	0.0434	3
YK60	unspiked	24	1.1903	0.0403	0.0331	3
YK60	unspiked	48	3.3912	0.0477	0.0389	3
YK60	unspiked	0	0.809	0.0037	0.033	2
YK60	unspiked	6	0.9292	-0.0056	0.0445	2
YK60	unspiked	12	0.8732	-0.0069	0.0239	2
YK60	unspiked	24	1.2757	0.0281	0.0267	2
YK60	unspiked	48	3.0423	-0.0668	0.0006	2
YK67	spiked	0	1.6942	-0.0128	13.9125	1
YK67	spiked	6	1.6593	2.1081	10.4774	1
YK67	spiked	12	8.053	12.1629	26.7269	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
YK67	spiked	24	9.3289	9.1212	10.6863	1
YK67	spiked	48	8.2833	9.6596	9.4892	1
YK67	spiked	0	0.8089	-0.0221	12.1005	2
YK67	spiked	6	0.9035	2.9033	13.3103	2
YK67	spiked	12	1.6923	3.8253	10.9631	2
YK67	spiked	24	1.4708	9.4618	13.566	2
YK67	spiked	48	4.297	15.9426	12.7826	2
YK67	unspiked	0	2.6881	-0.0173	0.5645	1
YK67	unspiked	6	0.9416	-0.0352	0.1203	1
YK67	unspiked	12	19.3781	-1.6544	0.8737	1
YK67	unspiked	24	2.479	-0.0505	0.4026	1
YK67	unspiked	48	2.4571	0.3261	0.009	1
YK67	unspiked	0	1.2181	-0.027	0.1461	2
YK67	unspiked	6	0.5168	0.0618	0.0936	2
YK67	unspiked	12	0.8183	-0.0067	-0.0168	2
YK67	unspiked	24	1.3553	0.0216	-0.0267	2
YK67	unspiked	48	-5.1033	0.0431	-1.1823	2
YKE1	spiked	0	1.8411	0.1626	46.0778	1
YKE1	spiked	6	2.4147	3.881	48.5595	1
YKE1	spiked	12	2.877	6.9596	48.4298	1
YKE1	spiked	24	3.4991	11.7959	49.866	1
YKE1	spiked	48	4.3495	16.9107	46.9775	1
YKE1	spiked	0	2.0354	0.2213	53.9666	2
YKE1	spiked	6	2.493	3.6751	51.111	2
YKE1	spiked	12	3.0758	7.1659	53.5686	2
YKE1	spiked	24	3.2441	10.3463	48.6321	2
YKE1	spiked	48	4.4413	16.0816	49.6416	2
YKE1	spiked	0	2.2564	0.1744	52.2237	3
YKE1	spiked	6	2.6157	4.2137	52.5618	3
YKE1	spiked	12	2.973	7.344	52.8008	3
YKE1	spiked	24	3.7898	11.8965	52.47	3
YKE1	spiked	48	5.5592	18.9703	56.7218	3
YKE1	unspiked	0	1.6746	0.0135	0.0525	1
YKE1	unspiked	6	1.803	0.027	0.0319	1
YKE1	unspiked	12	1.984	-0.0269	0.0273	1
YKE1	unspiked	24	2.3646	0.0295	0.0219	1
YKE1	unspiked	48	2.8392	-0.0039	0.0052	1
YKE1	unspiked	0	2.0528	0.0153	0.0921	2
YKE1	unspiked	12	2.5395	0.0201	0.0073	2

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
YKE1	unspiked	24	2.5265	0.0143	0.0132	2
YKE1	unspiked	48	3.1228	0.0071	0.0101	2
YKE1	unspiked	0	1.8316	0.001	0.0364	3
YKE1	unspiked	6	1.9313	-0.0088	0.0139	3
YKE1	unspiked	12	2.1636	-0.026	-0.012	3
YKE1	unspiked	24	2.4781	-0.0089	0.0087	3
YKE1	unspiked	48	2.8227	0.0051	-0.0121	3
YKN1	spiked	0	0.5767	0.0659	24.0855	1
YKN1	spiked	6	0.7296	3.426	30.9133	1
YKN1	spiked	12	0.4138	4.0326	26.5204	1
YKN1	spiked	24	0.7541	5.1315	27.2149	1
YKN1	spiked	48	1.3169	8.8639	30.2255	1
YKN1	spiked	0	0.5089	0.1107	25.8925	2
YKN1	spiked	6	0.5916	2.1812	22.9708	2
YKN1	spiked	12	1.2169	3.8366	24.7338	2
YKN1	spiked	24	0.7199	4.4864	25.1268	2
YKN1	spiked	48	0.8781	5.7654	25.8501	2
YKN1	spiked	0	0.4899	0.1194	28.7992	3
YKN1	spiked	6	0.7205	2.7937	24.4144	3
YKN1	spiked	12	0.8311	3.9354	24.0271	3
YKN1	spiked	24	0.642	4.186	21.3991	3
YKN1	spiked	48	0.8946	6.6066	23.3811	3
YKN1	unspiked	0	0.5544	-0.0331	-0.0036	1
YKN1	unspiked	6	0.3635	0.0399	-0.004	1
YKN1	unspiked	12	0.5961	-0.073	0.0525	1
YKN1	unspiked	48	0.7449	-0.0071	-0.0035	1
YKN1	unspiked	0	0.7048	0.0252	-0.0435	2
YKN1	unspiked	6	0.8167	-0.0657	0.037	2
YKN1	unspiked	12	0.4611	0.0314	0.0215	2
YKN1	unspiked	24	0.472	0.0048	-0.0101	2
YKN1	unspiked	48	0.7571	-0.0091	-0.0149	2
YKN1	unspiked	0	0.9762	-0.0216	0.0111	3
YKN1	unspiked	6	0.7204	-0.0157	-0.0334	3
YKN1	unspiked	12	0.4915	0.0231	0.0699	3
YKN1	unspiked	24	0.7084	-0.0062	0.0514	3
YKN1	unspiked	48	0.6459	-0.0241	0.0669	3
YKS1	spiked	0	3.2224	0.2778	70.758	1
YKS1	spiked	6	2.8877	2.2358	64.1997	1
YKS1	spiked	12	3.5397	5.3253	69.4191	1

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
YKS1	spiked	24	7.1137	24.8583	75.0636	1
YKS1	spiked	48	12.1984	51.5408	81.3442	1
YKS1	spiked	0	2.324	0.2889	59.6433	2
YKS1	spiked	6	2.0966	2.076	52.5929	2
YKS1	spiked	12	2.897	4.5957	60.5769	2
YKS1	spiked	24	5.6162	21.5781	66.4408	2
YKS1	spiked	48	10.5625	41.2799	64.492	2
YKS1	spiked	0	2.6339	0.4669	65.032	3
YKS1	spiked	6	2.6366	2.5139	58.805	3
YKS1	spiked	12	2.4203	4.2075	52.3155	3
YKS1	spiked	24	4.5775	17.4901	52.9099	3
YKS1	spiked	48	11.4425	46.1699	72.1254	3
YKS1	unspiked	0	2.8087	0.0473	0.1409	1
YKS1	unspiked	6	2.4628	0.0475	0.1755	1
YKS1	unspiked	12	3.5778	0.0987	0.0827	1
YKS1	unspiked	24	5.8957	0.0081	0.0369	1
YKS1	unspiked	48	9.7745	-0.098	-0.0734	1
YKS1	unspiked	0	1.899	0.0057	0.1009	2
YKS1	unspiked	6	2.1843	0.0158	0.0284	2
YKS1	unspiked	12	2.4021	0.0197	0.0904	2
YKS1	unspiked	24	3.6911	-0.0178	0.0593	2
YKS1	unspiked	48	6.6122	-0.1422	-0.07	2
YKS1	unspiked	0	1.791	0.0838	0.105	3
YKS1	unspiked	6	1.9982	0.0422	0.0282	3
YKS1	unspiked	12	2.2769	0.0232	0.0231	3
YKS1	unspiked	24	3.9698	0.0134	0.0326	3
YKS1	unspiked	48	6.0301	0.1183	0.045	3
YKS2	spiked	0	0.6625	0.1045	27.6326	1
YKS2	spiked	6	0.8037	1.818	23.222	1
YKS2	spiked	12	1.0477	2.6681	19.9464	1
YKS2	spiked	24	2.4968	7.4829	23.2884	1
YKS2	spiked	48	4.7883	13.0213	20.2299	1
YKS2	spiked	0	0.6718	0.0958	25.6818	2
YKS2	spiked	6	1.2007	3.8384	44.3477	2
YKS2	spiked	12	1.1876	3.2687	23.8223	2
YKS2	spiked	24	2.3698	6.599	20.8739	2
YKS2	spiked	48	5.6405	15.1632	23.0219	2
YKS2	spiked	0	0.816	0.0929	26.0497	3
YKS2	spiked	6	0.9918	1.7451	22.4493	3

Lake Name	Treatment	Time (hour)	^{202}Hg (ug/kg)	^{199}Hg (ug/Kg)	^{198}Hg (ug/kg)	Replicate
YKS2	spiked	12	1.3018	3.2464	24.4372	3
YKS2	spiked	24	2.5271	7.3762	24.4392	3
YKS2	spiked	48	5.7819	16.0051	25.1547	3
YKS2	unspiked	0	0.596	0.0288	0.0238	1
YKS2	unspiked	6	1.3571	-0.0033	-0.0029	1
YKS2	unspiked	12	2.1187	-0.0041	0.0038	1
YKS2	unspiked	24	3.4543	0.0394	0.0209	1
YKS2	unspiked	48	4.8373	0.0243	0.003	1
YKS2	unspiked	0	0.6557	0.011	0.0242	2
YKS2	unspiked	6	1.4544	-0.0031	0.0056	2
YKS2	unspiked	12	2.2987	0.0028	-0.0017	2
YKS2	unspiked	24	3.8694	-0.0082	-0.0065	2
YKS2	unspiked	48	4.9506	0.0085	-0.006	2
YKS2	unspiked	0	0.6042	0.0028	0.0453	3
YKS2	unspiked	6	1.1755	0.0038	0.0223	3
YKS2	unspiked	12	2.3044	-0.0306	-0.008	3
YKS2	unspiked	24	3.4584	-0.0014	0.0086	3
YKS2	unspiked	48	5.5105	-0.0402	-0.0486	3
YKW1	spiked	0	0.8688	0.2445	23.8057	1
YKW1	spiked	6	1.3066	5.9134	26.3821	1
YKW1	spiked	12	1.7592	10.3484	24.3648	1
YKW1	spiked	24	2.7399	16.8634	26.2689	1
YKW1	spiked	48	4.743	29.0851	29.2181	1
YKW1	spiked	0	0.803	0.2689	24.3404	2
YKW1	spiked	6	1.5814	8.2277	29.6984	2
YKW1	spiked	12	1.5386	8.1234	23.4984	2
YKW1	spiked	24	2.1508	12.3168	22.294	2
YKW1	spiked	48	3.6672	21.9047	24.1688	2
YKW1	spiked	0	0.7532	0.2962	25.3152	3
YKW1	spiked	6	1.7758	9.2399	30.2399	3
YKW1	spiked	12	1.5409	8.4313	23.1943	3
YKW1	spiked	24	2.4282	14.4233	24.6546	3
YKW1	spiked	48	4.131	24.0865	26.3741	3
YKW1	unspiked	0	0.7477	-0.0057	0.016	1
YKW1	unspiked	6	1.0423	0.0039	0.0181	1
YKW1	unspiked	12	1.0303	-0.0008	0.0081	1
YKW1	unspiked	24	1.4505	-0.0144	0.0077	1
YKW1	unspiked	48	1.8113	-0.0285	-0.0023	1
YKW1	unspiked	0	0.7351	0.0225	0.0092	2

Lake Name	*Treatment*	*Time (hour)*	*^{202}Hg (ug/kg)*	*^{199}Hg (ug/Kg)*	*^{198}Hg (ug/kg)*	*Replicate*
YKW1	unspiked	6	0.9451	0.0202	0.0247	2
YKW1	unspiked	12	1.0652	0.0091	0.0195	2
YKW1	unspiked	24	1.2564	0.0138	0.017	2
YKW1	unspiked	48	1.6297	0.0269	0.0132	2
YKW1	unspiked	0	0.6862	0.0139	0.0199	3
YKW1	unspiked	6	0.8866	0.0198	0.0059	3
YKW1	unspiked	12	1.0065	0.0257	0.0073	3
YKW1	unspiked	24	1.6259	0.0169	0.0148	3
YKW1	unspiked	48	1.956	0.0222	0.0079	3

CHAPTER 3: ARSENATE AMENDMENTS DECREASE PRODUCTION OF METHYLMERCURY ACROSS SULFATE CONCENTRATION GRADIENT IN FRESHWATER LAKE SEDIMENTS

Aždajić, Mija[1]; Blais, Jules M[1]; Poulain, Alexandre J[1]

[1] Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, Ontario K1N 6N5, Canada

Main Conclusions:

1) Hg methylation in lake sediments was primarily limited by carbon substrate availability

2) Once a carbon source was amended to sediments, addition of sulfate significantly increased MeHg production

3) Addition of As(V) significantly hampered MeHg production regardless of sulfate concentrations

Contribution to the Field:

Arsenic (As) and sulfate pollution are often found in the same environments as a result of smelting metal ores. Previous studies showed that sulfate reducing microbes can use As(V) as a terminal electron acceptor, while others reported that sulfate reducing microbes (SRMs) are the main Hg methylators in freshwater systems. Although interactions between As and sulfate cycling has been explored by a few studies, most of these studies are *in-vitro* and the biogeochemistry of SRMs and As in environmental settings remains largely unexplored. Furthermore, the interplay between As, Hg and sulfur biogeochemical cycles, to the best of our knowledge, has not been studied at all. As such, this study is the first to investigate the effect of As(V) on the production of MeHg in freshwater lake sediments.

3.1 ABSTRACT

Arsenic (As) and sulfate pollution are often found co-occurring as a result of smelting metal ores. Previous studies showed that sulfate reducing microbes (SRMs) can use As(V) as a terminal electron acceptor, while others reported that SRMs are the main mercury (Hg) methylators in freshwater systems. However, the scientific community has yet to fully explore how As(V) can affect methylmercury (MeHg) production. In this study, we examined whether additions of As(V) and sulfate in freshwater sediments near a major gold mine with a history of sulfur and As emissions affect Hg methylation. First, we show that Hg methylation in lake sediments was primarily limited by carbon substrate availability rather by that of sulfate as terminal electron acceptors. Then, under conditions where carbon is not limiting, sulfate addition to the system significantly increased Hg methylation rate constants. Finally, we also show that MeHg production rate in sediments significantly decreased with increasing As(V) concentrations, regardless of sulfate concentration amended to the sediments. This work underscores the apparent antagonistic effects of As(V) on the one hand, and the stimulatory effects of carbon and sulfate on the other hand on the kinetics of Hg methylation.

3.2 INTRODUCTION

Arsenic (As) can exist in many different species under four oxidation states: As^{-3}, As^{0}, As^{+3}, and As^{+5} [1]. The two most abundant states are arsenite, (As(III)) and arsenate (As(V))[2], have different proposed mechanisms of toxicity. As(V) can mimic phosphate and detrimentally affect several biochemical reactions in microbial cells. As(III) is typically more toxic than As(V), as it inhibits protein activity by interacting with thiol moities[1]. These trivalent species are also less strongly adsorbed onto minerals compared to pentavalent species, meaning that reduction of As(V) can increases the mobility and the bioavailability of As in the aqueous environment[3].

One of the main anthropogenic sources of As in the environment is from mining metals complexed in As bearing minerals, such as arsenopyrite (FeAsS), realgar (AsS), and orpiment (As_2O_3)[4,5]. Mining of such minerals can create legacy pollution[6,7] which changes the geochemistry[8], biota assemblages[9], and microbial community composition[10–13] of lakes in the surrounding landscape. Some studies show that As pollution from mining can affect microbial community in sediments[14–16], such as lowering microbial biodiversity[17] and selecting microbial communities that are resistant to contaminants like As[18]. However, other studies show that As contamination does not always change the microbial community composition of sediments[19].

As is pervasive in the environment and microbes have various pathways to transform As species[20,21] including oxidation[22,23], reduction[24,25], methylation[26], and demethylation[27,28]. Notably, As(V) reduction has been identified as a detoxification pathway for some microbes. As(V)

detoxification is an energy-dependent process, by which the *ars* operon encodes for proteins to reduce As(V) and actively transport it outside of the cell[29,30]. However, some microbes are also capable of dissimilatory As(V) reduction[31]; a process by which a microbial cell will use As(V) as a terminal electron acceptor (TEA), reducing As(V) to As(III) and ultimately leading to the production of adenosine triphosphate (ATP)[29,32–35]. Regardless of the pathway used by microbes, detoxification or dissimilatory reduction, the final product is the more toxic form of As: As(III)[36].

Although As transforming microbes have been identified as early as 1917[37], it was in 1944 that Ahmann et al. first reported a microbe isolated from an As contaminated site that was able to gain energy for growth from As(V) reduction[24]. While the *deltaproteobacteria* MLMS-1 strain is the only obligate As(V) reducer reported so far[38], most studies examining dissimilatory As(V) reduction show that As(V) is used as an alternative TEA[29,33–35,38–41]. Sulfate reducing microbes (SRMs) are known to use compounds other than sulfate, such as As(V)[25,34,35,42,43], as TEAs under anoxic conditions[44]. In fact, As rich environments have been shown to contain bacteria (e.g. *Desulfotomaculum auripigeneutum)* known to reduce both As and sulfate[45,46].

SRMs also play a large role in the cycling of mercury (Hg), as they are the primary producers of methylmercury (MeHg) in many freshwater environments[47–49]. Hg is a naturally occurring element that is of concern because it is highly toxic to organisms and has a high bioaccumulation factor in its methylated form (i.e. MeHg). The production of MeHg by microbes is coded by the *hgc*AB[50] gene cluster which has been identified in all SRMs capable of Hg methylation and a diversity of other microbes found in the environment[51]. MeHg can biomagnify

in higher trophic level organisms, resulting in high concentrations of MeHg in certain piscivorous fish. Once it has entered organisms, MeHg can easily cross the blood-brain or placenta barriers and affect the neurological system of individuals or disrupt the normal development of a fetus[52]. As a result, understanding MeHg production in the environment is vital to protecting wildlife and human communities from MeHg exposure[53].

Arsenic reducing microbes and Hg methylating microbes are both found in the large guild that are SRMs and some genera known to reduce As(V) also contain species that methylate Hg (Table S3.1). Both genes responsible for Hg methylation (i.e. *hgc*AB) and As(V) reduction (e.g. *ars*) have been exchanged among microbes via horizontal gene transfer[54–56]; however this is likely not the case for dissimilatory As(V) reduction (i.e., *arr* genes)[42,57]. Nonetheless, microbes with either Hg methylation or As(V) reduction genes are phylogenetically diverse[51,51] but occupy similar anoxic niches[44], therefore SRMs could possibly affect the speciation of both Hg and As, leading to the production of the potent toxins MeHg and As(III).

In a previous large scale field study (Chapter 2), we have shown that microbial community structure was significantly affected by both As and sulfate pollution gradients while Hg methylation rate constants were significantly and negatively affected by the As pollution gradient[13]. In the context of these findings and of the current available literature on As reduction by SRMs outlined above, we designed a series of laboratory experiments to understand the role of As(V) on Hg methylation in freshwater sediments. Considering that several mining environments are simultaneously affected by both sulfate and As contamination[7,58], these

findings will help to fill an important gap in our knowledge essential to improve models aimed at predicting the production and fate of MeHg in freshwater ecosystems.

3.3 METHODS

3.3.1 Study Site

The top ~5 cm of sediment was sampled at lake YK-E1 in Yellowknife, NWT (Canada) (62°31'56.96"N, 113°22'19.73"W). This lake is ~25 km east of Giant Mine, which is outside of the pollution gradient created by historic smelting activity at this gold mine[7]. YK-E1 is representative of the limnologic characteristics and methylation rate constants of lakes in the Yellowknife area[15]. Considering the large amount of material needed for replicating our experiments, sampling with a corer was not feasible. Therefore, sediment was sampled using a sterile trowel from three different spots in the littoral zone. The sediment was frozen and preserved at -20°C until analysis.

3.3.2 Experimental Design

An orthogonal experimental design was created to test the effect of carbon and sulfate, and of sulfate and As(V) amendments on the production of MeHg in lake sediments.

A prior large scale field study conducted on 36 lakes and led by our group (see Chapter 2) showed that Hg methylation was positively correlated with [dissolved organic carbon] and

[sulfate], and negatively affected by [total As]. In this study, we designed controlled laboratory experiments to test for the role of carbon, sulfate and As(V) using freshwater sediments.

Sediments were amended with lactate and acetate at 1 mM and 10 mM. We used lactate and acetate because they were shown to be the best carbon sources to stimulate SRMs[59].

We performed three different sulfate treatments: 0 mM (or no sulfate added), 0.2 mM (effectively doubling sulfate concentration), and 2 mM sulfate added (increasing sulfate concentrations by one order of magnitude).

As(V) was added to the following final concentrations: 0.01 mM, 0.1 mM, 1 mM, and 10 mM, corresponding to a 1,000 – 1,000,000 times increase of the expected concentration of the overlying water in this lake and 1 – 1,000 times increase of the concentrations of As found in the most polluted lakes in the area[9]. These amendments were chosen to be similar to other studies that have examined As(V) reduction in laboratory and environmental samples for which the amendments ranged from 1 mM to 10 mM[25,39,40,60].

We also performed two different controls: an abiotic treatment (adding 1% formaldehyde)[61], to test whether the transformations were microbially mediated, and a treatment for which SRMs were inhibited (adding 0.2 mM sodium molybdate)[62], to test if the transformations were mediated by SRM activity.

3.3.3 Amendment Preparation

Carbon treatments were prepared by dissolving lactate ($C_3H_6O_3$) and sodium acetate ($C_2H_3NaO_2$) into miliQ water and sediments were supplied with 1 mM and 10 mM of this mixture, 16 hours before isotope spiking. Sulfate spiking solution was made by dissolving anhydrous sodium sulfate (Na_2SO_4) in miliQ water. As(V) spiking solution was made by dissolving sodium As(V) dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) into miliQ water and acidifying (0.1%) the solution with HNO_3 for stability.

3.3.4 Incubation Experiments

Lake sediments were thawed overnight at 4 °C, then 3 g of sediment was mixed with 9 mL of anaerobic miliQ (1:3 slurry ratio) in a falcon tube. The slurry was prepared and stored at room temperature for 5 days in a dark anaerobic chamber. 16 hours before the Hg and MeHg isotopes were spiked, each treatment was spiked with 1 mM of lactate and 1 mM of acetate to stimulate microbial activity and to insure that carbon was not the limiting factor in MeHg production in the system. Each treatment was done twice in triplicate, the first triplicate was spiked with isotopes and the second was left unspiked to track the "background" variation in isotope concentrations. The spiked treatments had a final concentration of 10 $ng \cdot g^{-1}$ of ^{199}Hg (inorganic) and 5 $ng \cdot g^{-1}$ of $Me^{198}Hg$. One 50ml centrifuge tube of spiked and one tube of unspiked slurry was frozen at each time point (0h, 24h, 48h). Samples were frozen at -20°C, then freeze-dried and stored in a cool dark place until analysis.

Next, MeHg isotopes in sediments were extracted using a modified version of the Cai et al. (1997) method[63] as described in Ažđajić et al. (2021)[13]. Hg isotopes were then analyzed using liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS) following the method described in Batista et al. (2010)[64]. The recovery of the isotope was calculated to ensure that our extraction, analysis, and calculations were accurate; the average isotope recovery for all experiments was 90.95% (± 18.93%).

A linear segment of the methylation and demethylation figure was visually assessed to find the best time point for maximum methylation rates (Figures S3.1 and S3.2). A pseudo first-order relationship for Hg species was assumed and calculations were based on Hintelmann et al (2000) to measure the first-order methylation rate constant (K_m, Equation 3.1) and the first-order demethylation rate constant (K_d, Equation 3.2)[65–67].

Equation 3.1:

$$K_m = \frac{[Me^{199}Hg]_{t24} - [Me^{199}Hg]_{t0}}{[^{199}Hg]_{spiked} * t}$$

Equation 3.2:

$$K_d = -1 * \frac{ln[Me^{198}Hg]_{t24} - ln[Me^{198}Hg]_{t0}}{t}$$

3.3.5 Chemical Analysis of Overlying Water

Sulfide and As species in the overlying water in the slurry were analyzed at the beginning and end of the experiment for each treatment. Sulfide was analyzed using a portable meter (DR2800 Spectrophotometere, Hach Lange, Germany). As(V) were analyzed on filtered samples (0.2 µm) with ICP-MS following method described in Pothier et al. (2018)[70].

3.3.5 Statistical Analyses

Simple linear regression models and analyses of covariance (ANCOVA) were used to test the relationships between variables in the program R. Model assumptions (i.e., linearity, heteroscedasticity, and normality of residuals) were tested using formal tests, and dependent variables were transformed to meet assumptions. In certain instances, nonlinear relationships were deemed more suitable after visual examination. When assumption for parametric models could not be met, a complementary non-parametric statistical model was used; in such cases, the final model selected is specified in the text.

3.4 RESULTS AND DISCUSSION

3.4.1 Control Experiments

In a previous field study, we showed that the ratio of total Hg as MeHg (i.e. %MeHg) was positively correlated with sulfate and that the rate of MeHg production (K_m) was positively correlated with [dissolved organic carbon] and negatively correlated with [As][13]. In a series of controlled laboratory experiments, we aimed to test the effect of each of these variables.

First, we observed that the Hg methylation rate constant, K_m, did not significantly increase with increasing [sulfate] (Figure 3.1), which acts as a TEA for SRM[71]. During preliminary experiments, control sediments (no sulfate nor carbon added) had a K_m of 0.170 day^{-1} (± 0.013), whereas K_m in sediments with 0.2 mM and 2 mM of added sulfate (no carbon added) was 0.080 day^{-1} (± 0.047) and 0.029 day^{-1} (± 0.014) respectively (Figure 3.1). Thus, we suspected that the activity of SRMs could be limited by other factors, one of them being carbon substrate. Carbon substrate is necessary for microbial activity as it is used by microbes for energy and carbon sources. In some environments, carbon can be the limiting factor in microbial activity and growth[72,73].

3.4.2 Effect of Carbon and Sulfate

The addition of carbon (amended as a lactate and acetate solution) led to a significant increase in K_m (p-value < 0.01) (Figure S3.3). Furthermore, after amending the sediments with 1 mM carbon, Hg methylation rates were higher in treatments with added sulfate (0.2 mM and 2 mM) (p-value < 0.01) (Figure 3.1) compared to samples with no added carbon and sulfate (0.2 mM and 2mM). This was also corroborated with the higher production of sulfide (Figure S3.3), which is a by-product of sulfate reduction by microbial activity[74]. Therefore, experiments showed that Hg methylation in sediments from YK-E1 in Yellowknife was limited by carbon substrate availability across all concentrations of sulfate (0 mM, 0.2 mM, and 2 mM). These results support our previous field study[13], in which we found Hg methylation rate constants were significantly

and positively correlated with dissolved organic carbon (i.e. substrate availability) but not with sulfate concentrations.

However, in the presence of sulfate (2 mM), increasing carbon concentrations from 1 mM to 10 mM did not result in a significant increase in K_m (Figure S3.3, p-value > 0.05). This could be because the microbial biomass is not large enough to be stimulated by additional carbon substrate or that other variables are limiting the microbial activity at very high carbon concentration (10 mM)[72]. Therefore, a 1 mM carbon amendment was chosen as a concentration high enough to stimulate microbial activity. All carbon amendments in subsequent experiments will refer to the 1 mM amendment, unless otherwise specified.

3.4.3 Effect of Arsenate

Next, our goal for this study was to understand the effect of As(V) addition on the rate constant of Hg methylation. Addition of As(V) to lake sediments (0.01 mM, 0.1 mM, 1 mM, and 10 mM), led to an order of magnitude decrease in methylation rate constants, K_m, ranging from 0.157 day^{-1} (± 0.020), 0.097 day^{-1} (± 0.01), 0.013 day^{-1} (± 0.001), and 0.013 day^{-1} (± 0.01) respectively (Figure 3.2a-c). A bootstrapped regression model showed that Hg methylation is inhibited by As(V) in a concentration-dependent manner (Figure 3.22a-c, p-value < 0.01, R^2 = 0.28).

Additionally, the presence of As(V) negatively affected sulfate reduction. In the absence of As(V), sulfide production increased with increasing sulfate amendments, increasing 6.7, 1.3,

and 5.2 fold for samples with 0 mM, 0.2 mM, and 2 mM sulfate amendments respectively (Figure 3.2g-i). However, the production of sulfide decreased with increasing As(V) amendment regardless of sulfate amendment concentrations (p-value = 0.001) (Figure S3.4).

SRMs are active in a large range of [As], however, when taking into account the effect of sulfate amendments (0, 0.2, and 2 mM) along with the As(V) amendment gradient, there was no significant difference in the change of K_m (p-value > 0.05) between the different sulfate amendments (Table 3.1, Figure 3.3). When sediments were treated to inhibit all microbial activity (using formaldehyde, B1), there was no production of As(III) and non-detectable Hg methylation rate constants (0.004 (±0.005) day^{-1}) (Figure S3.5), confirming that both are microbially-mediated processes. However, when sediments were amended with 0.1 mM As(V) and when SRM activity was inhibited (using sodium molybdate), there was production of As(III) in sediments while the methylation rate remained non-detectable 0.002 (±0.003) day^{-1} (Figure S3.5). These results suggest that SRMs involved in mercury methylation were not responsible for the majority of the As(V) reduction observed in these sediments. In other studies, amendments of molybdate reduced the production of As(III)[25], and the abundance of *dsr*B genes, which are used to identify SRMs in the environment[75], have been positively correlated with As concentrations[76], showing that SRMs can be involved in the reduction of As(V), but likely not in YK-E1.

The range of As concentrations tested here is not only representative of what can be found in mining-affected, but also corresponds to a concentration range over which microbial As(V) reduction was tested in previous studies. For example, Macy et al (2000) incubated two

different SRMs *(Desulfomicrobium* strain Ben-RB and *Desulfovibrio* strain Ben-RA) in 10 mM and 30 mM concentrations of As(V) growth media. These two strains have different As(V) reduction pathways; *Desulfomicrobium* strain Ben-RB can use As(V) as the TEA, whereas *Desulfovibrio* strain Ben-RA reduces As(V) as part of an As resistance system[77]. Other studies have shown that microbial As(V) reduction can occur at much lower As concentrations (e.g. < 0.007 mM)[78].

Together, our results indicate that the inhibition of Hg methylation by As(V) addition is stronger than the stimulation of Hg methylation by sulfate addition. Reasons that may explain this include: (1) some microbes may be inhibited by As(V), reducing biotic Hg methylation rates; and/or (2) As(V) may alter microbial community structure to favour species that do not methylate Hg.

Note that this study did not explicitly differentiate between As(V) reduction that is used for catabolic activity (i.e. respiration pathway) and the reduction supporting detoxification. The respiration pathway can either occur in microbes that are strict As(V) reducers, or secondary As(V) reducers (i.e. SRMs that use As(V) an alternative TEA)[45,79], which has been shown in several microbial guilds[80]. On the other hand, As(V) reduction is equally widely distributed[81] as a detoxification mechanism and is controlled by the *ars* operon, during which the As(V) is transported through the cell, reduced into As(III), and transported out of the cell[29]. Both of these could potentially affect the production of MeHg in our sediments.

Interestingly, As(III) production rates exhibited a bell-shaped curve, suggesting a stimulating effect followed by an inhibiting effect as As(V) concentrations further increased; however, the changes were not significantly correlated with As(V) nor sulfate concentrations increases (Figure S3.4). In the case of microbes that reduce As(V) through detoxification (*ars* operon), As(III) produced can be actively transported outside of the microbial cell reducing intracellular As(III) damages[79]. Consequently, microbes that express the *ars* operon could potentially tolerate environments with higher As(III) concentrations, while dissimilatory As(V) reducing microbes do not necessarily have a mechanism to actively transport As(III) out of the cell[79], potentially causing damages to cells respiring As(V), at high As(V) concentrations. Therefore, because of this bell-shaped curve and the range of concentrations involved (with a peak in As(III) production at 100 μM; Figure 3.2d-f), we suspect that the majority of As(V) reduction observed in these sediments was performed by dissimilatory As(V) reducers. However, results from studies that have looked at As transformation genes along [As] gradients do not offer a clear consensus on this matter. Some studies show that *ars* genes are inhibited at high [As(V)][8282], which could potentially indicate that dissimilatory arsenate reduction would be the dominant reduction type in high As concentrations, while other show an increase[83,84] in *ars* genes. Therefore, additional genetic screening would be necessary to identify the pathway(s) involved.

3.5 CONCLUSION

Interactions between As and sulfate cycling has been explored by a few studies, however most of these studies are *in-vitro* and experiments in environmental settings remain largely

unexplored[59]. Furthermore, the role of Hg within those two cycles, to the best of our knowledge, has not been studied at all. Working towards a better understanding of the interplay between As, Hg, and sulfur biogeochemical cycles is important because sulfur and As pollution often coincide[7,85–87]. Iron-sulfide minerals (e.g. pyrite, marcasite, and arsenopyrite) tend to be associated with economically viable ore deposits (e.g. iron, gold, nickel, copper) and certain minerals in this mineralogical group also have As as part of their mineral structure (e.g. arsenopyrite). As such, when these ore deposits are mined for metals, the extraction process tends to create sulfate and As pollution in the same area[7,88]. Furthermore, the global demand for metals is increasing, leading to more metal extraction and mining[89], and we can expect more environments that are affected by sulfate and As pollution. The work presented here is an important step in understanding the potential ramifications of such mixed pollution gradients on the cycling of Hg.

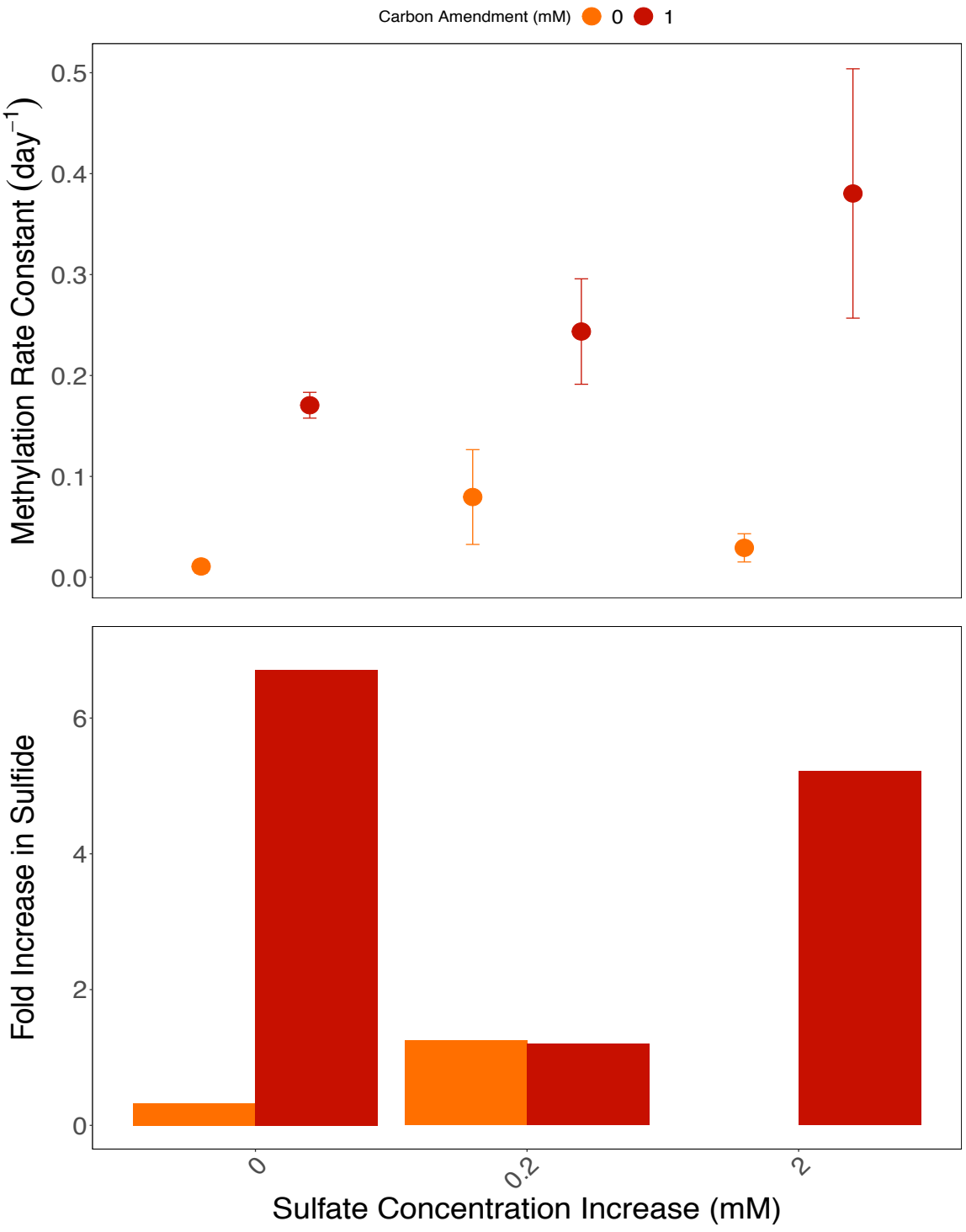


Figure 3.1: Methylation rates of treatments without carbon, only with carbon, and with carbon and sulfate.

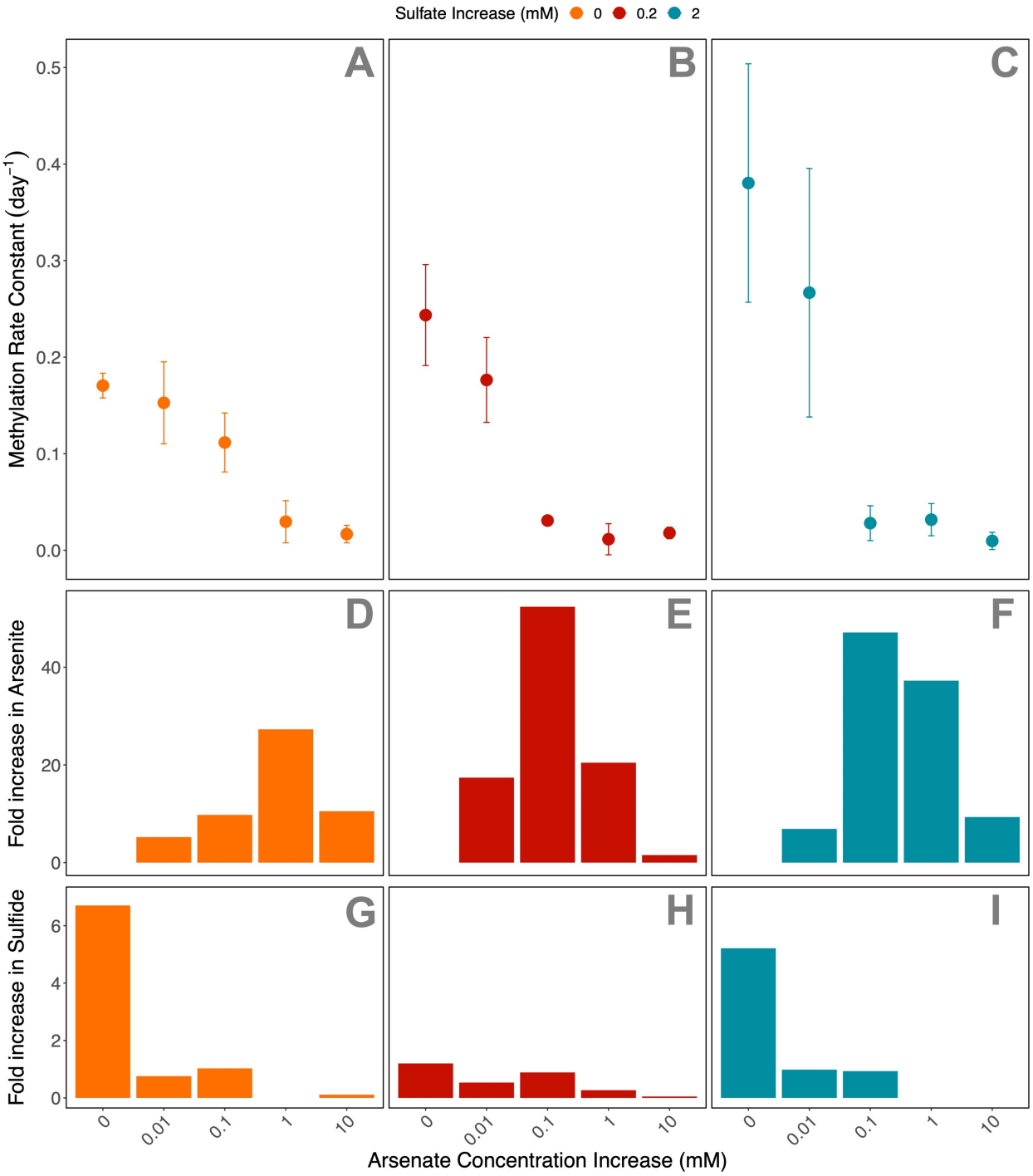


Figure 3.2: Chemical results from experimental incubations. The bottom figures show the fold change in sulfide concentration in overlying water. The second row of figures show the change in As(III) concentrations in overlying water. Lastly, the top row of figures show the methylation rate constant (day^{-1}) of sediments. All treatments have added carbon to stimulate microbial activity.

Table 3.1: Methylation and demethylation dynamics along with the different treatments of As(V) and sulfate amendments. Each methylation and demethylation represents the mean calculated along with the standard deviation of the mean. K_m = methylation rate constant, K_d = demethylation rate constant, SD = standard deviation

Amendment					
Sulfate (mM)	As(V) (mM)	K_m (day^{-1})	SD	K_d (day^{-1})	SD
0.0	0.00	0.171	0.013	-0.119	0.099
	0.01	0.153	0.042	-0.011	0.040
	0.10	0.112	0.031	1.005	1.595
	1.00	0.030	0.022	0.021	0.092
	10.00	0.017	0.001	-0.040	0.037
0.2	0.00	0.244	0.052	-0.668	0.790
	0.01	0.176	0.044	-0.118	0.038
	0.10	0.031	0.001	-0.057	0.025
	1.00	0.011	0.016	0.156	0.481
	10.00	0.018	0.006	-0.024	0.154
2.0	0.00	0.380	0.124	-0.185	0.123
	0.01	0.267	0.129	-0.203	0.068
	0.10	0.028	0.018	0.018	0.131
	1.00	0.032	0.017	-0.014	0.124
	10.00	0.010	0.009	-0.030	0.036

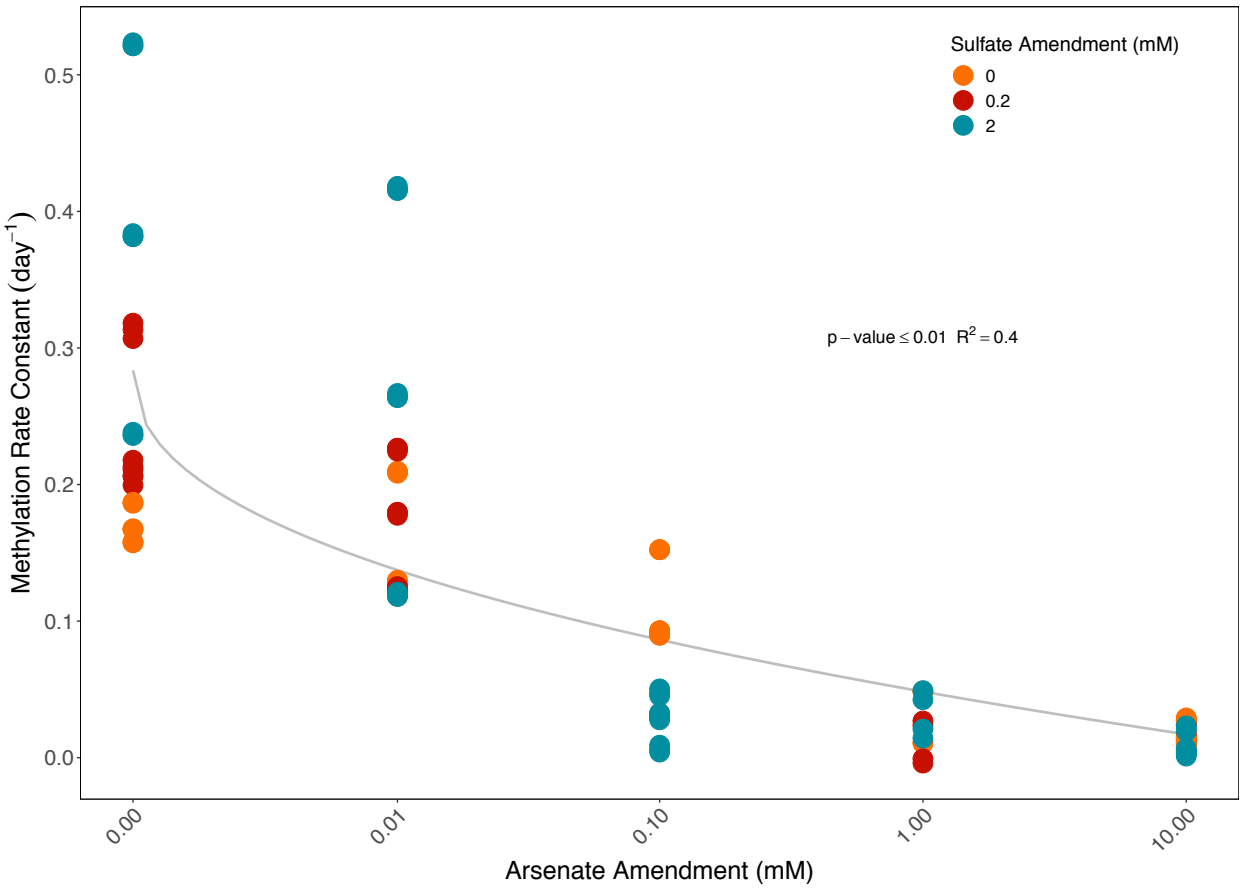


Figure 3.3: Calculated methylation rate constants (day^{-1}) in sediments with respect to different amendments of As(V) (mM). The different colours represent the different amendments of sulfate (mM). A bootstrapped regression indicated no significant difference of the slopes for different sulfate amendments, however there is a significant decrease in methylation rate constant with increasing concentrations of As(V) amendments.

3.6 REFERENCES

1 M. F. Hughes, *Toxicology Letters*, 2002, 133, 1–16.

2 R. Briones-Gallardo, V. M. Escot-Espinoza and E. Cervantes-González, *International Journal of Environmental Science and Technology*, 2017, 14, 609–622.

3 J. Gulens, D. R. Champ and R. E. Jackson, *Chemical Modeling in Aqueous Systems*, 1979, 93, 81–95.

4 P. L. Smedley and D. G. Kinniburgh, *Applied Geochemistry*, 2002, 17, 517–568.

5 A. Acquavita, F. Floreani and S. Covelli, Occurrence and speciation of arsenic and mercury in alluvial and coastal sediments, *Current Opinion in Environmental Science & Health*, 2021, 22, 100272.

6 D. D. Sprague and J. C. Vermaire, *Water, Air, & Soil Pollution*, 2018, 229, 87.

7 A. J. Houben, R. D'Onofrio, S. V. Kokelj and J. M. Blais, *PLoS ONE*, 2016, 11.

8 C. L. Cheney, K. M. Eccles, L. E. Kimpe, J. R. Thienpont, J. B. Korosi and J. M. Blais, Determining the effects of past gold mining using a sediment palaeotoxicity model, *Science of The Total Environment*, 2020, 718, 137308.

9 J. R. Thienpont, J. B. Korosi, K. E. Hargan, T. Williams, D. C. Eickmeyer, L. E. Kimpe, M. J. Palmer, J. P. Smol and J. M. Blais, *Proceedings of the Royal Society B: Biological Sciences*, 2016, 283, 20161125.

10 S. Kang, J. D. Van Nostrand, H. L. Gough, Z. He, T. C. Hazen, D. A. Stahl and J. Zhou, Functional gene array–based analysis of microbial communities in heavy metals-contaminated lake sediments, *FEMS Microbiology Ecology*, 2013, 86, 200–214.

11 T. A. Jackson, *Heavy metals in the environment*, 1991, 191–217.

12 T. A. Jackson, N. Nguyen and W.-C. Li, Effects of Copper, Nickel, and Sulfate from the Smelters at Sudbury, Ontario (Canada) on Microbial Communities in Lakes, *null*, 2017, 34, 400–420.

13 M. Aždajić, E. Yumvihoze, J. M. Blais and A. J. Poulain, The effect of legacy gold mining on methylmercury cycling and microbial community structure in northern freshwater lakes, *Environ. Sci.: Processes Impacts*, 2021, 23, 1220–1230.

14 W. Li, J. Liu and K. A. Hudson-Edwards, *Science of The Total Environment*, 2020, 749, 142353.

15 M. Aždajić, N. Belzile, J. M. Gunn, J. M. Blais and A. J. Poulain, Effects of a decade of selenium emission reductions on mercury accumulation in aquatic biota in the Sudbury region of Ontario, *Can. J. Fish. Aquat. Sci.*, 2020, 77, 848–856.

16 Y. Zhao, M. Chen, J. Bai, X. Li, F. Zulfiqar and Q. Wang, Response of microbial community to petroleum stress and phosphate dosage in sediments of Jiaozhou Bay, China, *Journal of Ocean University of China*, 2014, 13, 249–256.

17 T. A. Jackson, S. Vlaar, N. Nguyen, G. G. Leppard and T. M. Finan, Effects of Bioavailable Heavy Metal Species, Arsenic, and Acid Drainage from Mine Tailings on a Microbial Community Sampled Along a Pollution Gradient in a Freshwater Ecosystem, *null*, 2015, 32, 724–750.

18 Y. Li, M. Zhang, R. Xu, H. Lin, X. Sun, F. Xu, P. Gao, T. Kong, E. Xiao, N. Yang and W. Sun, Arsenic and antimony co-contamination influences on soil microbial community composition and functions: Relevance to arsenic resistance and carbon, nitrogen, and sulfur cycling, *Environment International*, 2021, 153, 106522.
19 A. Mahamoud Ahmed, E. Lyautey, C. Bonnineau, A. Dabrin and S. Pesce, Environmental Concentrations of Copper, Alone or in Mixture With Arsenic, Can Impact River Sediment Microbial Community Structure and Functions, *Frontiers in Microbiology*, 2018, 9, 1852.
20 C. Cervantes, G. Ji, J. Ramirez and S. Silver, *FEMS Microbiology Reviews*, 1994, 15, 355–367.
21 J. F. Stolz, P. Basu, J. M. Santini and R. S. Oremland, *Annual Review of Microbiology*, 2006, 60, 107–130.
22 F. H. Osborne and H. L. Enrlich, *The Journal of applied bacteriology*, 1976, 41, 295.
23 F. Battaglia-Brunet, M. -C Dictor, F. Garrido, C. Crouzet, D. Morin, K. Dekeyser, M. Clarens and P. Baranger, *Journal of Applied Microbiology*, 2002, 93, 656–667.
24 D. Ahmann, A. L. Roberts, L. R. Krumholz and F. M. M. Morel, *Nature*, 1994, 371, 750–750.
25 D. K. Newman, T. J. Beveridge and F. M. M. Morel, *Applied and Environmental Microbiology*, 1997, 63, 2022.
26 R. Turpeinen, M. Pantsar-Kallio, M. Häggblom and T. Kairesalo, *Science of the Total Environment*, 1999, 236, 173–180.
27 W. Cullen and K. Reimer, 1989.
28 C. N. Cheng and D. D. Focht, *Applied and Environmental Microbiology*, 1979, 38, 494.
29 N. Kumari and S. Jagadevan, *Chemosphere*, 2016, 163, 400–412.
30 S.-L. Tsai, S. Singh and W. Chen, *Current Opinion in Biotechnology*, 2009, 20, 659–667.
31 J. Andres and P. N. Bertin, *FEMS Microbiology Reviews*, 2016, 40, 299–322.
32 Y. K. Kawa, J. Wang, X. Chen, X. Zhu, X.-C. Zeng and Y. Wang, Reductive dissolution and release of arsenic from arsenopyrite by a novel arsenate-respiring bacterium from the arsenic-contaminated soils, *International Biodeterioration & Biodegradation*, 2019, 143, 104712.
33 G. Ji and S. Silver, *Proceedings of the National Academy of Sciences of the United States of America*, 1992, 89, 9474–9478.
34 D. Y. Sorokin, T. P. Tourova, M. V. Sukhacheva and G. Muyzer, *Extremophiles : life under extreme conditions*, 2012, 16, 597–605.
35 J. S. Blum, T. R. Kulp, S. Han, B. Lanoil, C. W. Saltikov, J. F. Stolz, L. G. Miller and R. S. Oremland, *Extremophiles : life under extreme conditions*, 2012, 16, 727–742.
36 M. Bissen and F. H. Frimmel, *Acta hydrochimica et hydrobiologica*, 2003, 31, 9–18.
37 H. H. Green, Description of a bacterium which oxidizes arsenite to arsenate, and of one which reduces arsenate to arsenite, isolated from a cattle-dipping tank, *S. Afr. J. Sci.*, 1917, 14, 465–467.
38 B. Planer-Friedrich, C. Härtig, R. Lohmayer, E. Suess, S. H. McCann and R. Oremland, *Environmental science & technology*, 2015, 49, 6554.

39 A. Niggemyer, S. Spring, E. Stackebrandt and R. F. Rosenzweig, *Applied and Environmental Microbiology*, 2001, 67, 5568.
40 Y. Chang, A. Nawata, K. Jung and S. Kikuchi, *Official Journal of the Society for Industrial Microbiology and Biotechnology*, 2012, 39, 37–44.
41 Z. Qian, H. Tianwei, H. R. Mackey, M. C. M. van Loosdrecht and C. Guanghao, *Water research (Oxford)*, 2019, 150, 162–181.
42 J. R. Pérez-Jiménez, C. Defraia and L. Y. Young, *Biochemical and Biophysical Research Communications*, 2005, 338, 825–829.
43 R. S. Oremland, P. R. Dowdle, S. Hoeft, J. O. Sharp, J. K. Schaefer, L. G. Miller, J. Switzer Blum, R. L. Smith, N. S. Bloom and D. Wallschlaeger, *Geochimica et Cosmochimica Acta*, 2000, 64, 3073–3084.
44 G. Muyzer and A. J. M. Stams, The ecology and biotechnology of sulphate-reducing bacteria, *Nature Reviews Microbiology*, 2008, 6, 441–454.
45 J. F. Stolz and R. S. Oremland, *FEMS Microbiology Reviews*, 1999, 23, 615–627.
46 Z. Zhang, H. S. Moon, S. C. B. Myneni and P. R. Jaffé, *Journal of Hazardous Materials*, 2017, 321, 382–389.
47 R.-Q. Yu, J. R. Flanders, E. E. Mack, R. Turner, M. B. Mirza and T. Barkay, *Environmental Science & Technology*, 2012, 46, 2684–2691.
48 G. C. Compeau and R. Bartha, *Applied and Environmental Microbiology*, 1985, 50, 498.
49 R. Devereux, M. R. Winfrey, J. Winfrey and D. A. Stahl, *FEMS Microbiology Ecology*, 1996, 20, 23–31.
50 J. M. Parks, A. Johs, M. Podar, R. Bridou, Jr. Hurt R. A., S. D. Smith, S. J. Tomanicek, Y. Qian, S. D. Brown, C. C. Brandt, A. V. Palumbo, J. C. Smith, J. D. Wall, D. A. Elias and L. Liang, *Science*, 2013, 339, 1332–5.
51 C. Gilmour, M. Podar, A. L. Bullock, A. M. Graham, S. D. Brown, A. Somenahally, A. Johs, R. A. Hurt, K. L. Bailey and D. A. Elias, *Environmental Science & Technology*, 2013, 47, 11810–11820.
52 E. M. Sunderland and M. Tumpney, *Persistent Organic Pollutants and Toxic Metals in Foods*, 2013, 392–413.
53 C.-C. Lin, N. Yee and T. Barkay, *Environmental Chemistry and Toxicology of Mercury*, 2011, 155–191.
54 M. Podar, C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A. V. Palumbo, A. C. Somenahally and D. A. Elias, *Science Advances*, 2015, 1.
55 C. R. Jackson and S. L. Dugas, *BMC evolutionary biology*, 2003, 3, 18–18.
56 T. K. Dunivin, S. Y. Yeh and A. Shade, *BMC Biology*, 2019, 17, 45.
57 C. W. Saltikov and D. K. Newman, *Proceedings of the National Academy of Sciences*, 2003, 100, 10983–10988.
58 G. Morin and G. Calas, *Elements*, 2006, 2, 97–101.
59 R. Alam and K. McPhedran, *Chemosphere*, 2019, 222, 932–944.
60 S. E. Hoeft, F. Lucas, J. T. Hollibaugh and R. S. Oremland, *Geomicrobiology Journal*, 2002, 19, 23–40.
61 L. Tuominen, T. Kairesalo and H. Hartikainen, *Applied and environmental microbiology*, 1994, 60, 3454–3457.

62 E. J. Fleming, E. E. Mack, P. G. Green and D. C. Nelson, *Applied and Environmental Microbiology*, 2006, 72, 457–464.
63 Y. Cai, G. Tang, R. Jaffé and R. Jones, *International Journal of Environmental Analytical Chemistry*, 1997, 68, 331–345.
64 B. L. Batista, J. L. Rodrigues, S. S. de Souza, V. C. Oliveira Souza and F. Barbosa, *Food Chemistry*, 2011, 126, 2000–2004.
65 W. Zhu, Y. Song, G. A. Adediran, T. Jiang, A. T. Reis, E. Pereira, U. Skyllberg and E. Björn, *Geochimica et Cosmochimica Acta*, 2018, 220, 158–179.
66 I. Lehnherr, V. L. St. Louis, C. A. Emmerton, J. D. Barker and J. L. Kirk, *Environmental Science & Technology*, 2012, 46, 10514–10522.
67 M.-L. Avramescu, E. Yumvihoze, H. Hintelmann, J. Ridal, D. Fortin and D. R.S. Lean, *Science of The Total Environment*, 2011, 409, 968–978.
68 C. Laskov, C. Herzog, J. Lewandowski and M. Hupfer, *Limnology and Oceanography: Methods*, 2007, 5, 63–71.
69 M. A. Tabatabai, *Environmental letters*, 1974, 7, 237–243.
70 M. P. Pothier, A. J. Hinz and A. J. Poulain, Insights Into Arsenite and Arsenate Uptake Pathways Using a Whole Cell Biosensor, *Frontiers in Microbiology*, 2018, 9, 2310.
71 P. A. Loka Bharathi, in *Encyclopedia of Ecology*, eds. S. E. Jørgensen and B. D. Fath, Academic Press, Oxford, 2008, pp. 3424–3431.
72 I. Torres, K. Inglett and K. Reddy, *An International Journal*, 2011, 104, 165–181.
73 W. S. Gardner, J. F. Chandler and G. A. Laird, Organic nitrogen mineralization and substrate limitation of bacteria in Lake Michigan, *Limnology and Oceanography*, 1989, 34, 478–485.
74 G. M. Gadd, *Encyclopedia of Microbiology (Third Edition)*, 2009, 321–334.
75 J. R. Pérez-Jiménez, L. Y. Young and L. J. Kerkhof, *FEMS Microbiology Ecology*, 2001, 35, 145–150.
76 P. Li, B. Li, G. Webster, Y. Wang, D. Jiang, X. Dai, Z. Jiang, H. Dong and Y. Wang, *Geomicrobiology Journal*, 2014, 31, 802–812.
77 J. M. Macy, J. M. Santini, B. V. Pauling, A. H. O'Neill and L. I. Sly, *Archives of Microbiology*, 2000, 173, 49–57.
78 M. F. Kirk, E. E. Roden, L. J. Crossey, A. J. Brealey and M. N. Spilde, *Geochimica et Cosmochimica Acta*, 2010, 74, 2538–2555.
79 R. S. Oremland and J. F. Stolz, *Science*, 2003, 300, 939.
80 M. L. S. Suhadolnik, A. P. C. Salgado, L. L. S. Scholte, L. Bleicher, P. S. Costa, M. P. Reis, M. F. Dias, M. P. Ávila, F. A. R. Barbosa, E. Chartone-Souza and A. M. A. Nascimento, *Scientific Reports*, 2017, 7, 11231.
81 D. Páez-Espino, J. Tamames, V. de Lorenzo and D. Cánovas, *BioMetals*, 2009, 22, 117–130.
82 V. H.-C. Liao, Y.-J. Chu, Y.-C. Su, S.-Y. Hsiao, C.-C. Wei, C.-W. Liu, C.-M. Liao, W.-C. Shen and F.-J. Chang, Arsenite-oxidizing and arsenate-reducing bacteria associated with arsenic-rich groundwater in Taiwan, *Journal of Contaminant Hydrology*, 2011, 123, 20–29.

83 S.-Y. Zhang, F.-J. Zhao, G.-X. Sun, J.-Q. Su, X.-R. Yang, H. Li and Y.-G. Zhu, Diversity and Abundance of Arsenic Biotransformation Genes in Paddy Soils from Southern China, *Environ. Sci. Technol.*, 2015, 49, 4138–4146.
84 P. S. Costa, L. L. S. Scholte, M. P. Reis, A. V. Chaves, P. L. Oliveira, L. B. Itabayana, M. L. S. Suhadolnik, F. A. R. Barbosa, E. Chartone-Souza and A. M. A. Nascimento, Bacteria and Genes Involved in Arsenic Speciation in Sediment Impacted by Long-Term Gold Mining, *PLOS ONE*, 2014, 9, e95655.
85 P. M. Ashley and B. G. Lottermoser, Arsenic contamination at the Mole River mine, northern New South Wales, *Australian Journal of Earth Sciences*, 1999, 46, 861–874.
86 H. E. Jamieson, *Reviews in Mineralogy and Geochemistry*, 2014, 79, 533–551.
87 R. N. Tempel, L. A. Shevenell, P. Lechler and J. Price, Geochemical modeling approach to predicting arsenic concentrations in a mine pit lake, *Applied Geochemistry*, 2000, 15, 475–492.
88 D. J. Vaughan and A. R. Lennie, *Science Progress (1933-)*, 1991, 75, 371–388.
89 A. Elshkaki, T. E. Graedel, L. Ciacci and B. K. Reck, *Environmental Science & Technology*, 2018, 52, 2491–2497.

3.7 SUPPLEMENTARY INFORMATION

Supporting information (order occurring in the text)

Table S3.1: List of microbial genera that have been shown to reduce arsenate and methylate mercury. Genus names marked with an asterisk contain iron reducing microbes, all others contain sulfate reducing bacteria

Figure S3.1: Methylation and demethylation figures for experiments testing the effect of added carbon (1 mM and 10 mM). The top panel (pink points) for each lake represents the increase in $Me^{199}Hg$ (i.e. methylation) and the bottom panel (green points) for each lake represents the decrease in $Me^{198}Hg$ (i.e. demethylation). The horizontal red line is the concentration of spiked MeHg at the start (t=0) of each incubation.

Figure S3.2: Methylation and demethylation figures for all experiments testing the effect of sulfate and arsenate amendments, all samples also have a 1 mM carbon amendment. The top panel (pink points) for each lake represents the increase in $Me^{199}Hg$ (i.e. methylation) and the bottom panel (green points) for each lake represents the decrease in $Me^{198}Hg$ (i.e. demethylation). The horizontal red line is the concentration of spiked MeHg at the start (t=0) of each incubation.

Figure S3.3: Methylation rates, arsenite increase, and sulfide increase of treatments without carbon, only with carbon, and with carbon and sulfate. These results are from preliminary experiments in which we tested the effect of 1 mM and 10 mM carbon amendments.

Figure S3.4: Fold change in sulfide (A) and arsenite (B) produced in overlying water during the incubation experiments.

Figure S3.5: Results from control experiments. Top figure show the fold change in sulfide produced in overlying water, middle figure shows the fold change in arsenite produced in overlying water and the bottom figure show methylation rate constants (with standard deviations) for control experiments. Biotic controls (biotic) were tested when all microbial activity was suppressed while sulfate reduction (SRB) controls were tested when all microbial activity from sulfate reducing microbes were suppressed.

Table S3.1: List of microbial genera that have been shown to reduce arsenate and methylate mercury. Genus names marked with an asterisk (*) contain iron reducing microbes, all others contain sulfate reducing bacteria

Genus	Mercury Methylating Species	Arsenate Reducing Species
Bacillus	Hamdy and Noyes (1975)	Yamamura et al. (2003)
Chrysiogenes	Gionfriddo et al. (2016)	Krafft et al. (1998)
Citrobacter	Chang et al. (2012)	Chang et al. (2012)
Clostridium	Christensen et al. (2016)	Stolz et al. (2007)
Desulfitobacterium	Gilmour et al. (2013)	Niggemyer et al. (2001)
Desulfosporosinus	Liu et al. (2004)	Pérez-Jiménez et al. (2005)
Desuflovibrio	Choi et Bartha (1993)	Macy et al. (2000)
*Desulfuromonas**	Kerin et al. (2006)	Osborne et al. (2015)
*Shewanella**	Si et al. (2015)	Cummings et al. (1999)

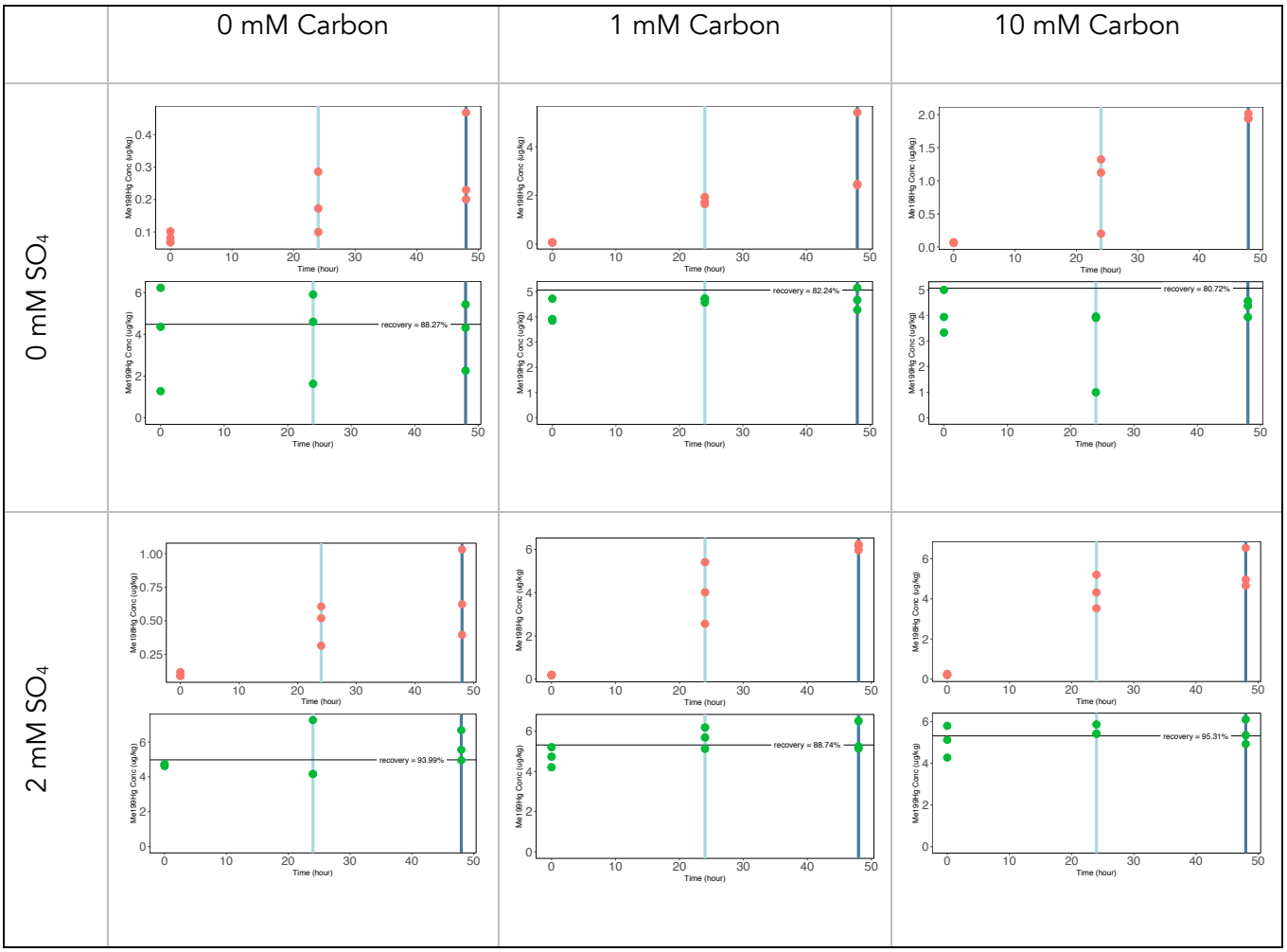
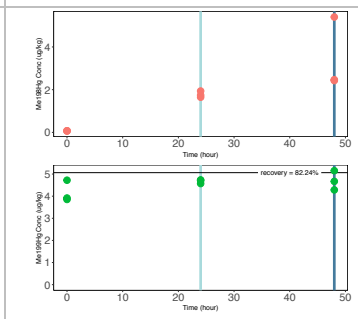
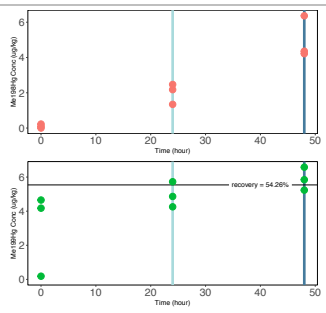
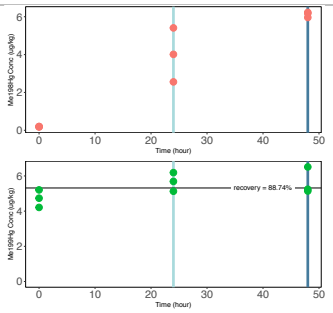
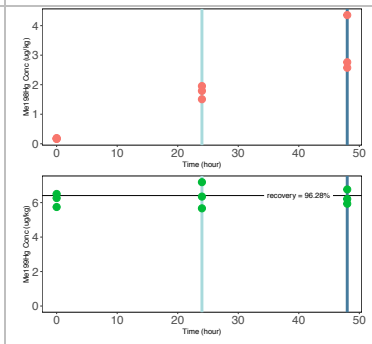
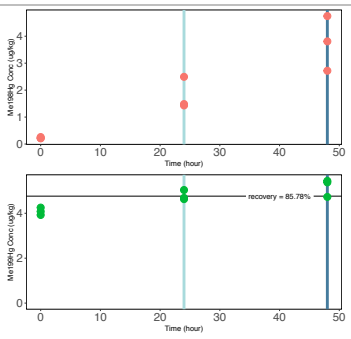
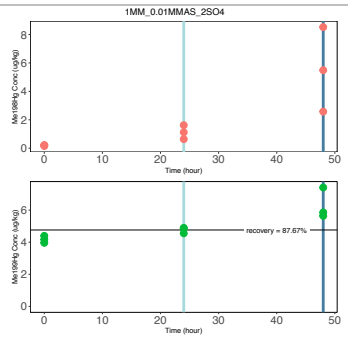
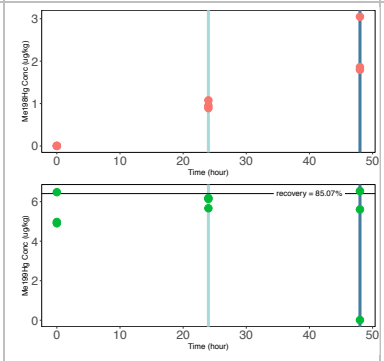
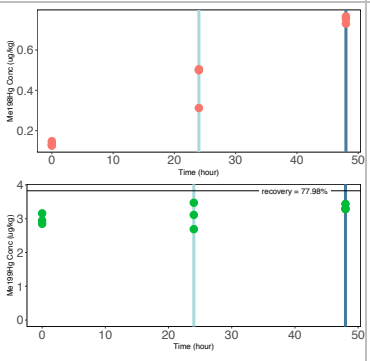
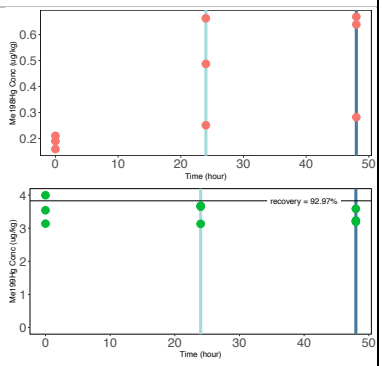
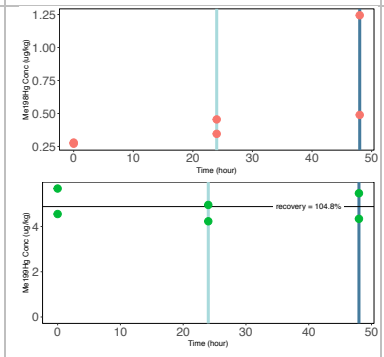
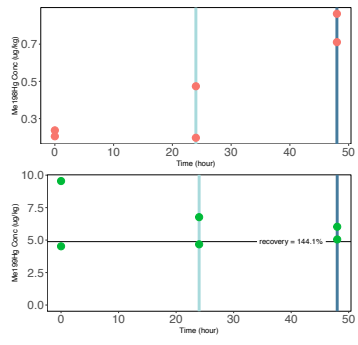
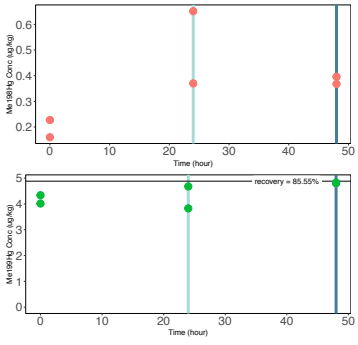


Figure S3.1: Methylation and demethylation figures for experiments testing the effect of added carbon (1 mM and 10 mM). The top panel (pink points) for each lake represents the increase in $Me^{199}Hg$ (i.e. methylation) and the bottom panel (green points) for each lake represents the decrease in $Me^{198}Hg$ (i.e. demethylation). The horizontal red line is the concentration of spiked MeHg at the start (t=0) of each incubation.

	0 mM SO_4	0.2 mM SO_4	2 mM SO_4
0 mM As(V)			
0.01 mM As(V)			
0.1 mM As(V)			
1 mM As(V)			

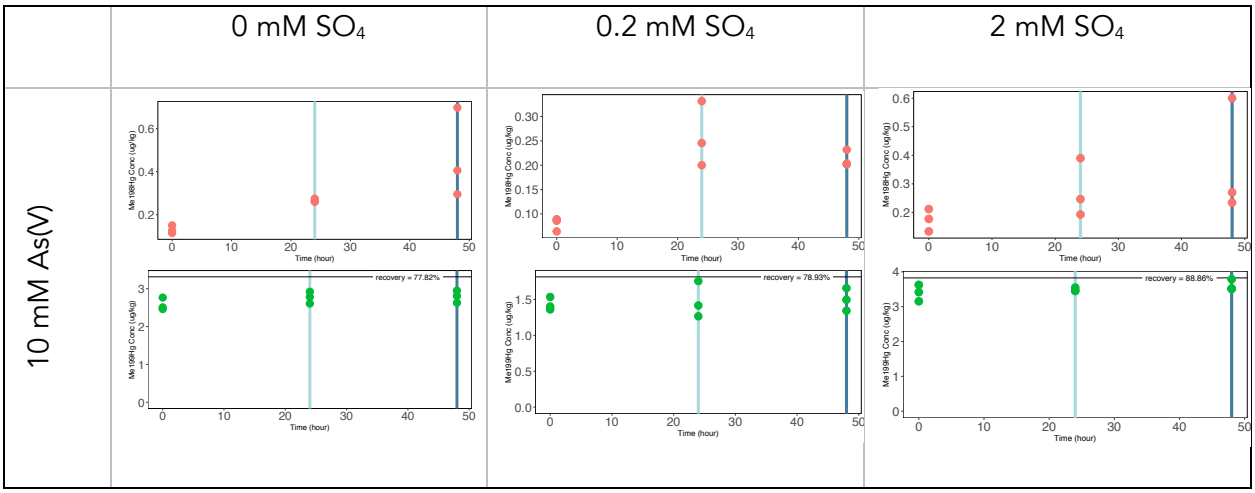


Figure S3.2: Methylation and demethylation figures for all experiments testing the effect of sulfate and arsenate amendments, all samples also have a 1 mM carbon amendment. The top panel (pink points) for each lake represents the increase in $Me^{199}Hg$ (i.e. methylation) and the bottom panel (green points) for each lake represents the decrease in $Me^{198}Hg$ (i.e. demethylation). The horizontal red line is the concentration of spiked MeHg at the start (t=0) of each incubation.

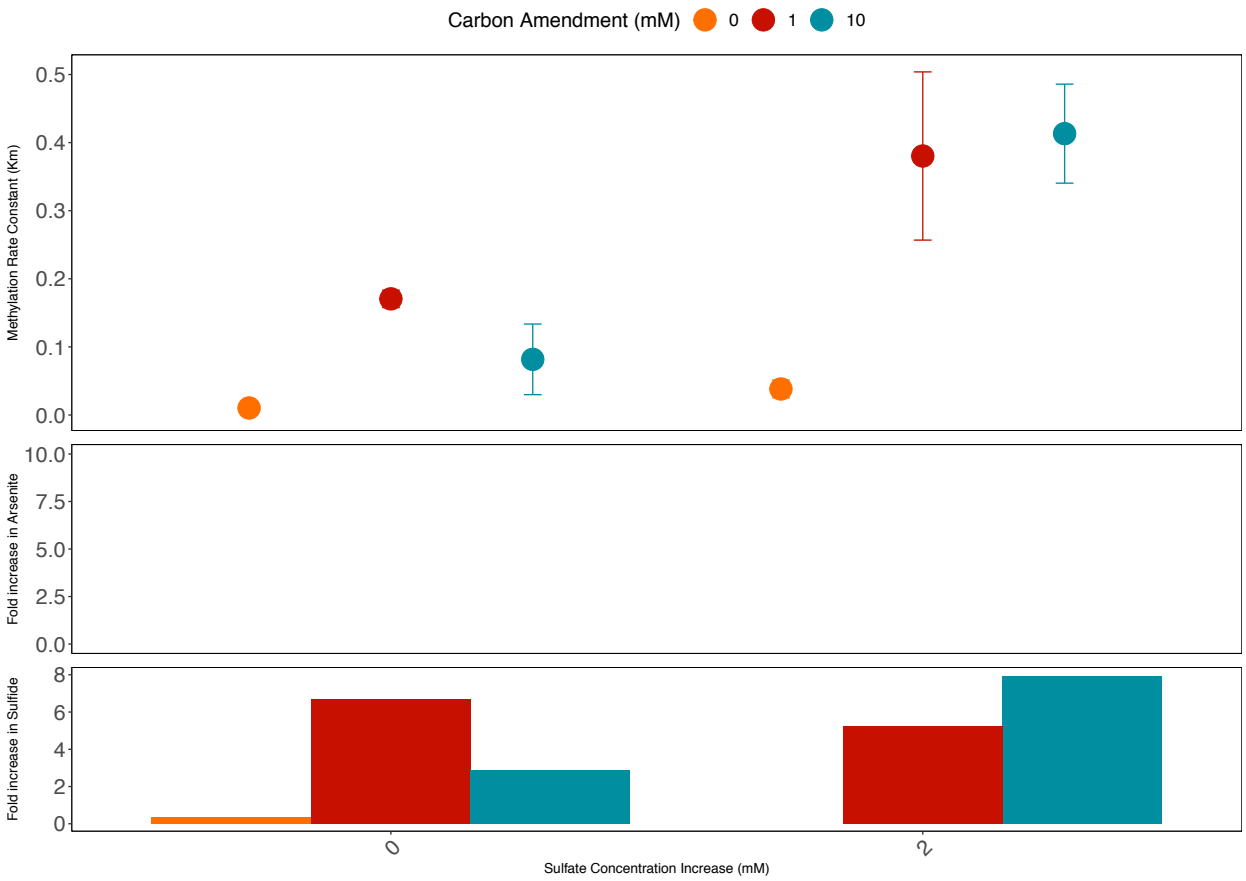


Figure S3.3: Methylation rates, arsenite increase, and sulfide increase of treatments without carbon, only with carbon, and with carbon and sulfate. These results are from preliminary experiments in which we tested the effect of 1 mM and 10 mM carbon amendments.

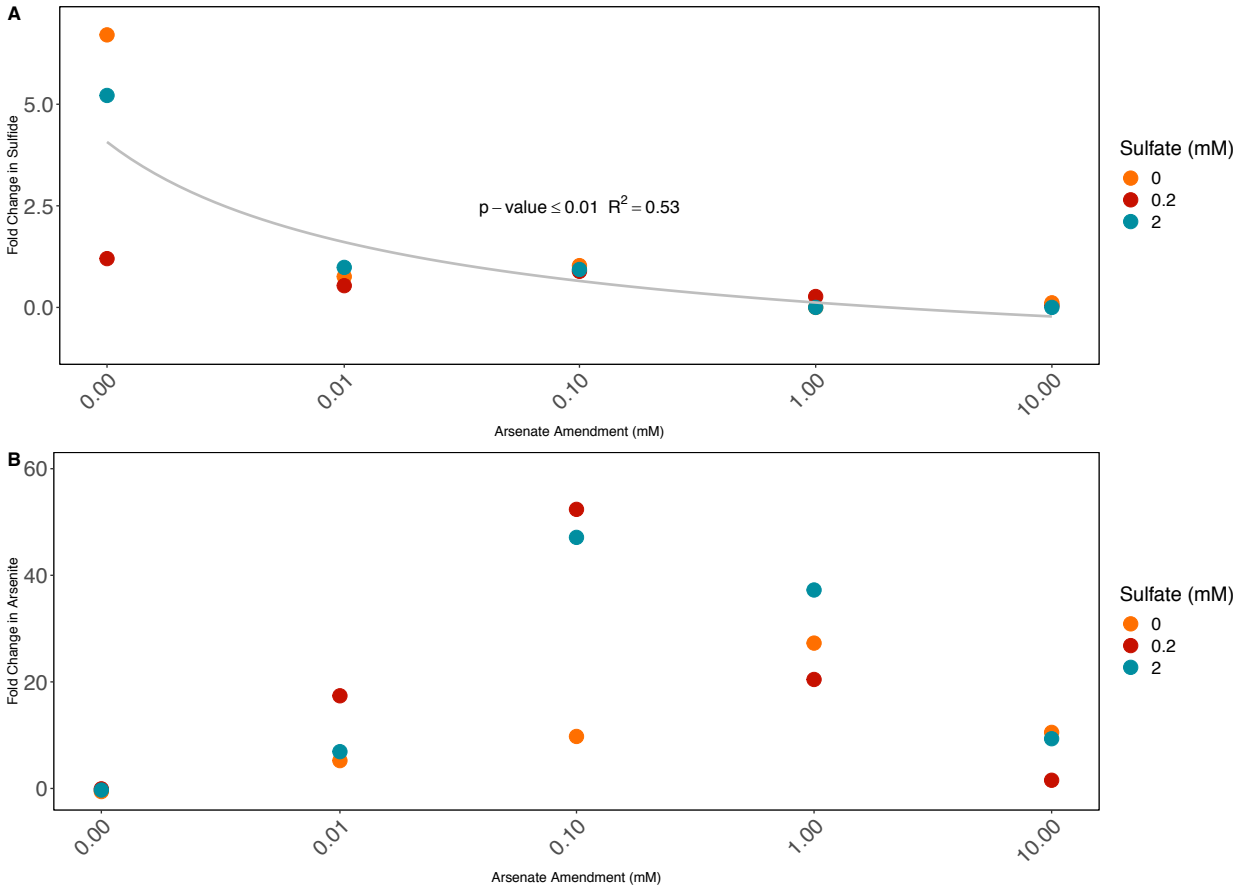


Figure S3.4: Fold change in sulfide (A) and arsenite (B) produced in overlying water during the incubation experiments.

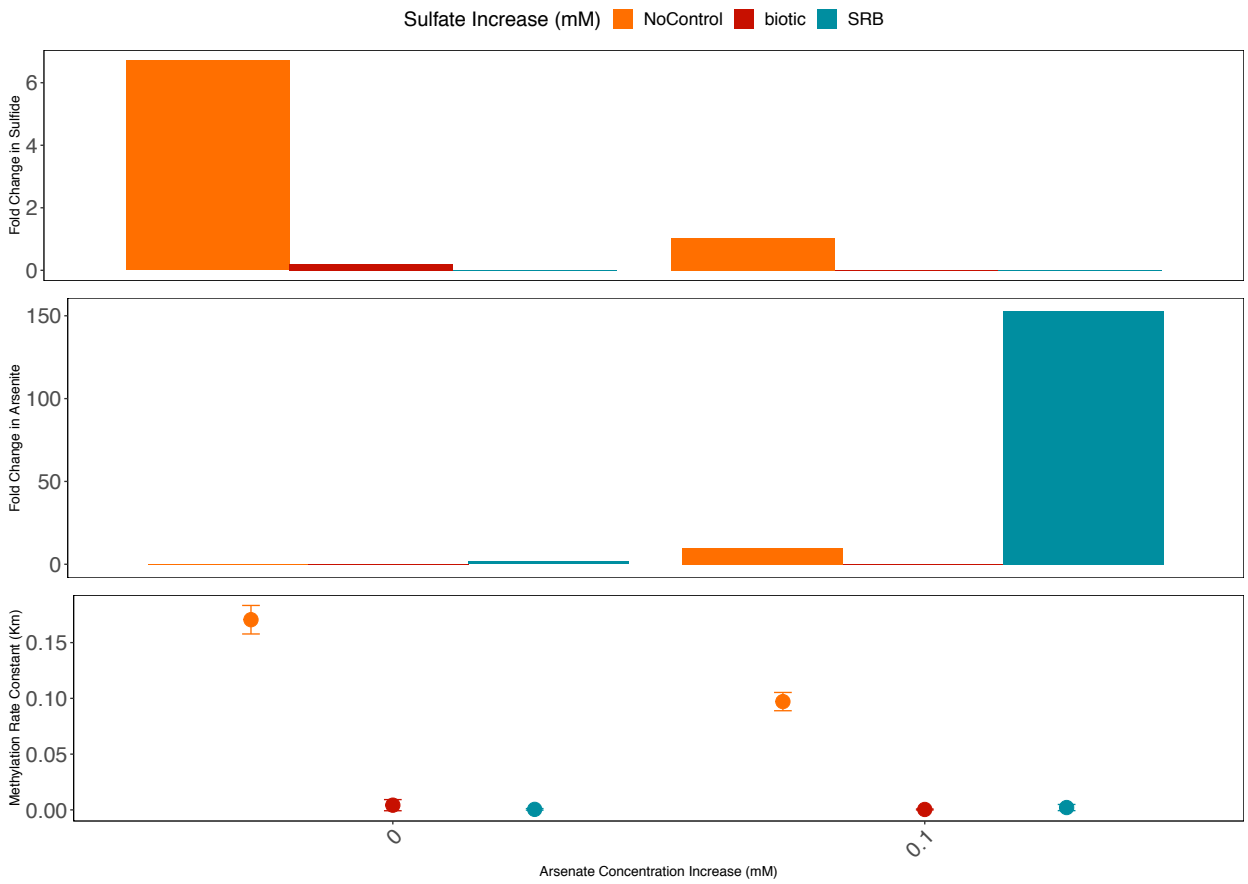


Figure S3.5: Results from control experiments. Top figure show the fold change in sulfide produced in overlying water, middle figure shows the fold change in arsenite produced in overlying water and the bottom figure show methylation rate constants (with standard deviations) for control experiments. Biotic controls (biotic) were tested when all microbial activity was suppressed while sulfate reduction (SRB) controls were tested when all microbial activity from sulfate reducing microbes were suppressed.

CHAPTER 4: EFFECTS OF A DECADE OF SELENIUM EMISSION REDUCTIONS ON MERCURY ACCUMULATION IN AQUATIC BIOTA IN THE SUDBURY REGION OF ONTARIO

Aždajić, Mija[1]; Belzile, Nelson[2]; Gunn, John M[3]; Blais, Jules M[1]; Poulain, Alexandre J[1]

[1] Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, Ontario K1N 6N5, Canada

[2] Cooperative Freshwater Ecology Unit, and Department of Chemistry and Biochemistry, Laurentian University, Sudbury, ON P3E 2C6, Canada

[3] Cooperative Freshwater Ecology Unit, and Department of Biology, Laurentian University, Sudbury, ON P3E 2C6, Canada

A modified version of this manuscript was published: July 16th, 2021
Canadian Journal of Fisheries and Aquatic Sciences | DOI: https://doi.org/10.1139/cjfas-2019-0196

Main Conclusions:

1) Total Se emissions have decreased following emission restrictions

2) Total Se concentrations in the water of the majority of lakes have increased despite decreases in Se depositions

3) Se in water continues to correlate with lower total Hg and MeHg accumulation in tissues of zooplankton, amphipods, mayflies, and perch

4) Se continues to exhibit a protective effect on Hg accumulation in biota, even years after emissions have greatly decreased

Contribution to Field:

In this study, we offer the first assessment of the effect of Se on Hg species accumulation over a decade after a significant reduction in Se emissions. Our results show that a reduction in Se emissions did not translate into less total Se in lake waters and emphasize the complexity of Hg accumulation in biota under these conditions. MeHg and total Hg burden in biota continues to

be correlated with the total Se in the water column, 15 years after the original findings by Belzile et al. (2006). These results are important as they can help manage long-term recovery of mining-impacted aquatic systems and can inform future remediation strategies involving Se additions.

4.1 ABSTRACT

Control of smelting emissions in the Sudbury (Ontario, Canada) area has dramatically decreased the amount of selenium (Se) deposited onto the surrounding landscape. Historically, Se emissions in Sudbury correlated with lower total mercury (Hg) and methylmercury levels (MeHg) in tissues of zooplankton, amphipods (*Hyalella azteca*), mayflies (*Stenonema femoratum*), and young of the year perch (*Perca flavescens*). In 2017, ten years following emission reductions, we evaluated whether changes in Se deposition affected total Hg and MeHg burden in lake biota. We show that total Se concentrations in the water of the majority of lakes have increased despite decreases in Se depositions, most likely due to the long residence time of Se in the watershed and the water column. As a result, Se in water continues to correlate with lower total Hg and MeHg accumulation in tissues of zooplankton, amphipods, mayflies, and perch. These results suggest that Se continues to exhibit a protective effect on Hg accumulation in biota, even years after emissions have greatly decreased. We expect this work to inform efforts aiming at long term recovery of aquatic environments affected by smelter emissions and aid in designing remediation strategies involving Se additions.

4.2 INTRODUCTION

The metal mining and smelting industry in Sudbury, Ontario, Canada, has been operating for more than 130 years[1], extracting primarily nickel and copper, but also large quantities of silver, gold, cobalt, and platinum group elements from the rich local ore body[2]. Although highly profitable over much of this period, the Sudbury smelters have severely impacted the local aquatic and terrestrial environments through extensive atmospheric deposition of sulfate and metals. Several studies have shown the impact of metal smelting on soil, vegetation[3–5], water quality[2], and biota[6]. Fortunately, Sudbury is also a great example of environmental reclamation that started in the 1970's[7]. One of the most famous efforts was the liming performed to counteract the acidification of the city's lakes and soils[1,8,9]. Furthermore, reduction of emissions occurred through the use of more efficient smelting techniques, periods of reduced production, and stricter emission regulations[8,10].

Particulate matter emitted from smelters contained significant quantities of nickel, copper, iron, but also considerable levels of selenium (Se) because Sudbury's nickel and copper sulfidic ores are rich in Se, which can replace sulfur in mineral structures[6,11,12]. As a result, lakes closer to the smelter exhibit [total Se] ranging from 2.9 to 5.1 nmol/L[11,13], levels well above most Ontario lakes that typically range from 0.63 to 1.3 nmol/L[11]. The decreasing [total Se] observed in lake water with increasing distance from the smelter sites provides a unique opportunity to investigate the response of aquatic biota to Se[14,15].

Se is an essential micronutrient in most animals[16,17], usually present as selenocysteine[18] and generally acquired through their diet[19]; however it can also become toxic in certain concentrations. Although inorganic species of Se such as selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) are present in the aquatic environment, organic species are thought to be the most bioavailable for uptake by periphyton, macrophytes[18,20,21], and fish[22]. Se can be an essential or toxic element, depending on concentrations and speciation, however, the concentration range at which Se is beneficial to fish is very narrow[23]. Due to this narrow range, and its persistent and bioaccumulative nature in freshwater biota, there has been discussion on the concentration thresholds in aquatic environment[24]. Due to this narrow range, it is important to ensure that [Se] in the lakes do not reach toxic levels.

The interaction between mercury (Hg) and Se has been the focus of several studies as Se can affect the mobility, bioavailability, and toxicity of Hg in aquatic ecosystems[25]. In particular, Se has been shown to have an antagonistic effect on Hg bioaccumulation in freshwater environments[26–28]. In the Sudbury area, Belzile et al. (2006) showed that [total Se] in Sudbury lake water were inversely related with total Hg and MeHg concentrations in four different freshwater components: zooplankton, amphipods (*Hyalella azteca*), mayflies (*Stenonema femoratum*), and young of the year perch (*Perca flavescens*)[16]. Other studies have shown similar inverse relationships between [Se] in lake water and [total Hg] in fish tissue (Belzile et al. 2009), and [MeHg] in young walleye[17] both in Sudbury and elsewhere[29].

Although the inhibitory effect of Se on Hg bioaccumulation is well known, there is no clear consensus on the mechanism involved. Earlier studies suggest that Se affects both Hg mobility and bioavailability. Inorganic Hg can form low solubility complexes with Se[4,26,30–32], which can (1) decrease the bioavailability of Hg to be methylated by microbes[33,34]; (2) decrease the bioaccumulation of Hg in organisms[35]; and (3) increase the elimination rates of Hg from the organism[27]. In addition, selenide's (Se^{2-} or HSe^{-}) strong binding affinity with MeHg[26] can (1) promote mercury demethylation; and (2) prevent MeHg from binding to proteins while increasing its excretion from the cell[36].

Due to Hg and MeHg toxicity to wildlife and humans[29], Se additions to lakes have been proposed as a remediation strategy to reduce Hg accumulation[37]. However, to the best of our knowledge, there have not been any studies assessing the long term effects of varying Se deposition on total Hg and MeHg accumulation in freshwater biota. Such data would aid in evaluating the feasibility of Se-based remediation strategies.

Over the past ten years, Se emissions in Sudbury have substantially decreased mostly because of a decrease in emissions from the Copper Cliff smelter which was the largest smelter site in Sudbury[1]. We hypothesized that changes in Se emissions in Sudbury will alter Hg accumulation in biota, and predicted that a decrease in emissions would lead to an increase in Hg and MeHg in biota. In this study, we examined (1) how [total Se] have changed in the water of lakes in the Sudbury area following an 85% decrease in emissions; and (2) whether total Se,

total Hg, and MeHg concentrations in aquatic biota have changed 15 years after a similar study was conducted[16].

4.3 MATERIALS AND METHODS

We compared the July 2002 sampling results reported in Belzile et al. (2006)[16] with new biota and unfiltered surface water samples collected in July 2017 and 2018. Complete information of the lake water chemistry and physical characteristics are available in Table S4.2.

4.3.1 Lake Selection

All eleven lakes from the original 2002 survey by Belzile et al. (2006)[16] were resampled for both water chemistry and biota in July 2017. The lakes ranged from 8-65 km from the smelters (Figure 4.1). In 2017, two samples (Lohi and Long) had very high [total Se] therefore a complete set of water samples were collected and analyzed again in 2018 for [total Se] and [total Hg]. All data are shown in Table S4.2.

4.3.2 Biological sample collection

Four different food web components were collected: zooplankton, amphipods (*Hyalella azteca*), mayflies (*Stenonema femoratum*), and young of the year perch (*Perca flavescens*). All samples were collected from the same area within the lake. Zooplankton were collected using an 80 µm net, then sorted with a mesh between 200 and 1000 µm. Amphipods were collected with a kick

net and picked by hand. Mayflies were picked by hand from the underside of stones. Yellow perch were collected with seine netting. All samples were frozen after sampling, freeze dried, homogenized with mortar and pestle, and kept in a dry and dark place until analysis.

4.3.3 Extraction and Metals Analysis

Total Hg for yellow perch, amphipods, and mayflies were analyzed using a Nippon MA-3000 Hg analyser with a method detection limit of 0.002 ng; zooplankton samples were analysed using Tekran-2600 with a method detection limit of 0.02 ng/kg. MeHg in biota was analysed using GC-CV-AFS with a limit of detection of $2.67 \cdot 10^{-5}$ ng/L (digestion solution). Total Se in biota was analysed using ICP-MS with a detection limit of 10 ng/L (digestion solution).

Extraction and analysis methods were maintained as similar as possible between the two study periods. Table 4.1 provides a summary and a comparison of techniques used in our study and that of Belzile et al. (2006)[16]. The quality of analysis between the two samplings was verified using the following certified reference materials: DORM2, DORM4, TORT2, IAEA407, and DOLT5. All five reference materials, in duplicate, were used in each extraction and analysed with the samples. The relative error on measurement was 0-14.8% for total Hg, 0-10.9% for MeHg, and 5.7-21.5% for Se (Table S4.1).

4.3.4 Statistical Analyses

All statistical analyses were done in the program R. Simple linear regression models and analyses of covariance (ANCOVA) were used to test the relationships between variables. Model assumptions (i.e. linearity, heteroscedasticity, and normality of residuals) were tested using formal tests and dependent variables were transformed accordingly. In certain instances, the relationship between variables was visually assessed and it was decided that a nonlinear relationship was more suitable. ANCOVAs were used to test the difference in concentrations between years with distance from smelter stack being the co-variate. This statistical analysis was chosen in order to address issues of spatial autocorrelation along the distance gradient when comparing sampling years.

4.4 RESULTS

4.4.1 [Total Se], [total Hg] and water quality variables differences between 2002 and 2017/2018.

Results from all sampling years showed that [total Se] was inversely related to distance from the closest smelter (Figure 4.2). The two ANCOVA models had distance as co-variate and tested for effect of year on [total Se], this was done for 2017 vs 2002 (p-value < 0.05, $r^2 = 0.86$, $F_{2, 17} = 61.41$), and 2018 vs 2002 (p-value < 0.05, $r^2 = 0.90$, $F_{2, 19} = 91.73$).

[Total Se] in 2017 decreases with distance from the closest smelter (GLM model, p-value = 0.046, r^2=0.38, $F_{1, 7}$ = 5.863) (Figure 4.2). When comparing 2002 and 2017, there was a significant difference in the intercepts between the years (p-value = 0.002) with the July 2017 intercept being significantly higher (Figure 4.2). For the 2017 samples, [total Se] in lake water ranged from 0.63 to 63.32 nmol/L with two lakes appearing as extreme outliers: Lohi (63.32 nmol/L) and Long (33.56 nmol/L). After excluding the Lohi and Long lake samples from the 2017 vs 2002 analysis (p-value < 0.05, r^2 = 0.82, $F_{3, 14}$ = 28.03), the intercepts were significantly different between the two years (p-value < 0.05). When the lakes were resampled again in 2018, [total Se] were no longer highly elevated (0.67 to 2.74 nmol/L) and [total Se] remained inversely correlated with distance from the closest smelter (p-value < 0.05, r^2=0.75, $F_{1, 9}$ = 31.63). When comparing the [total Se] results from 2018 with 2002 (p-value < 0.05, r^2=0.89, $F_{2, 19}$ = 31.63), there was once again a significant difference in the intercepts (p-value < 0.03), with the 2018 intercept being higher (Figure 4.2).

[Total Hg] in water in 2018 (Table S4.2) decreased significantly with distance from the smelters, although the relationship was weak (p-value = 0.0495, r^2 = 0.29, $F_{1, 9}$ = 5.147), and the effect size was small (m= 0.029 pmol/L·km^{-1}). When comparing the two years with an ANCOVA model (p-value < 0.05, r^2 = 0.91, $F_{2, 18}$ = 94.17), the 2018 samples had significantly lower concentrations than the 2002 samples (p-value < 0.05, m=-2.04 pmol/L of Hg). Unfortunately, no [total Hg] data are available for lake water samples in 2017.

Other chemical variables from 2017 were also inversely correlated with distance from the stacks such as [sulfate] (p-value = 0.004, r^2=0.58, $F_{1, 9}$ = 14.62, m=-0.019 mg/L·km^{-1}), [nickel] (p-value = 0.021, r^2 = 0.49, $F_{1, 7}$ = 8.753, m = -0.843 ug/L·km^{-1}), and [copper] (p-value = 0.007, r^2 = 0.62, $F_{1, 7}$ = 14.25, m=-0.16 ug/L·km^{-1}), however the effect sizes were small. [Calcium] decreased with distance (p-value = 0.04, r^2=0.32, $F_{1, 9}$ = 5.603) with a small effect size (m=-0.027 mmol/L·km^{-1}). Environmental variables such as dissolved organic carbon, pH, nitrogen, and phosphorous were not significantly different between 2017 and 2002 sampling seasons (ANCOVA models, p-value > 0.05), nor were they significantly correlated with distance from the smelters (p-value > 0.05).

4.4.2 [Total Se], [total Hg] and [MeHg] in tissues in 2017

[Total Hg] in organisms increased with distance from the smelters (Figure 4.3), for amphipods (p-value = 0.003, r^2= 0.65, $F_{1, 8}$ = 17.92), mayflies (p-value < 0.05, r^2= 0.85, $F_{1, 8}$ = 53.45), and perch (p-value < 0.05, r^2= 0.89, $F_{1, 7}$ = 66.07). However, [total Hg] in zooplankton were not significantly correlated with distance from the smelters (p-value > 0.05) (Figure 4.3).

In addition, [total Hg] in organisms decreased with increasing [total Se] in water (Figure 4.4) for zooplankton (p-value = 0.022, r^2=0.71, $F_{1, 4}$ = 13.17), amphipods (p-value = 0.017, r^2=0.58, $F_{1, 6}$ = 10.77), mayflies (p-value = 0.007, r^2=0.68, $F_{1, 6}$ = 16.06), and perch (p-value < 0.05, r^2=0.96, $F_{1, 5}$ = 183.9). When the two outliers (Lohi and Long) were removed, a significant inverse relationship for [total Hg] in zooplankton (p-value = 0.042, r^2= 0.73, $F_{1, 3}$ = 11.63), amphipods (p-

value = 0.039, r^2= 0.63, $F_{1, 4}$ = 9.102), mayflies (p-value = 0.016, r^2= 0.75, $F_{1, 4}$ = 16.21) and perch (p-value > 0.05, r^2= 0.96, $F_{1, 4}$ = 129.1) was observed with respect to [total Se] in water. [Total Hg] were not correlated with [total Se] in tissues (p-value > 0.05) of zooplankton, amphipods, mayflies, and perch (Figure S4.3).

[MeHg] in tissue samples increased with distance from smelter stacks (Figure 4.3) for amphipods (p-value = 0.001, r^2= 0.77, $F_{1, 7}$ = 28.08), mayflies (p-value = 0.001, r^2= 0.76, $F_{1, 8}$ = 29.67), and perch (p-value = 0.003, r^2= 0.66, $F_{1, 8}$ = 18.31). They decreased with respect to [total Se] in water for amphipods (p-value < 0.05, r^2= 0.89, $F_{1, 5}$ = 52.11), mayflies (p-value = 0.002, r^2= 0.78, $F_{1, 6}$ = 26.28), and perch (p-value < 0.05, r^2= 0.95, $F_{1, 6}$ = 144.9) (zooplankton were excluded due to missing data). Additionally, when the two outliers (Lohi and Long) were removed, [MeHg] was significantly inversely correlated with [total Se] in water for amphipods (p-value = 0.008, r^2= 0.91, $F_{1, 3}$ = 41.75), mayflies (p-value = 0.024, r^2= 0.70, $F_{1, 4}$ = 12.49), and perch (p-value < 0.05, r^2= 0.95, $F_{1, 4}$ = 98.53). Finally, only [MeHg] in perch tissues significantly decreased with [total Se] in perch tissues (p-value = 0.03, r^2= 0.40, $F_{1, 8}$ = 6.92). This is interesting because [total Se] in tissue samples did not significantly correlate with distance from the smelters stacks (p-value > 0.05) nor with [total Se] in water (p-value > 0.05) (Figure S4.2).

4.4.3 [Total Se], [total Hg], and [MeHg] in tissues between 2002 and 2017

We tested differences in concentrations of total Se in biota between the two years using analysis of covariance (ANCOVA). Two models identified significant results; the first model (p-value =

0.002, r^2 = 0.44, $F_{2, 18}$ = 8.84) identified that, for zooplankton, the intercept of [total Se] in 2002 was lower than the one in 2017 with respect to distance from the smelters (p-value = 0.021), meaning that [TSe] in zooplankton has overall increased. The second model (p-value < 0.05, r^2 = 0.41, $F_{2, 15}$ = 6.83) also identified the 2002 intercept as being higher than the value for 2017 for [total Se] in perch, with respect to distance (p-value = 0.046), therefore [total Se] also increased in the 2017 sampling. In addition, [total Hg] and [MeHg] in tissue samples were not significantly different, with respect to distance from the closest smelter, between sampling years for any of the organisms (p-value > 0.05). However, there was a significant difference in the slopes of [MeHg] (p-value = 0.001) in mayfly tissue samples with respect to distance from the smelters in 2002 vs. 2017 (ANCOVA analysis, p-value < 0.05, r^2 = 0.91, $F_{3, 15}$ = 61.73) (Figure 4.5).

4.5 DISCUSSION

[Total Se] in lake water samples were inversely related to distance from the closest smelter stacks in all years (2002, 2017, and 2018), consistent with other studies in the Sudbury area, which show the effect of metal smelting emissions on water chemistry[11,38–42]. Interestingly, despite almost an 85% decrease in Se emissions, [total Se] in lake water increased rather than decreased between 2002 and 2017/2018 (Figure 4.2). We also noted that [THg] in water decreased between 2002 to 2018, and although these concentrations are low, they do fall within the range of concentrations shown for Ontario lakes in the last few years[43,44]. However, these temporal results should be interpreted with caution as we have only compared data from 2002 and 2017/2018.

Several factors may explain why [total Se] did not decrease following a reduction by stack emissions. First, it has been previously shown that catchment soils near the smelters are heavily contaminated with metals and some studies have predicted that high metal concentrations in runoff water may continue for decades or even centuries after the smelting operations have come to an end[41]. Increased runoff of Se from soil may therefore explain the patterns we observed.

A second potential important source of Se is lake sediments. Se in sediments can be remobilized by oxidation processes, disturbance of the sediments by water turbulence or burrowing insects[20], decomposition of organic matter[45], or by the desorption of Se from mineral phases[15]. Remobilization of Se from sediments has been shown to occur in two lakes near Sudbury smelters (Clearwater and McFarlane), though the authors did not state that this process could account for large quantities of Se mobilized to the water column[46]. Desorption of Se from iron or manganese oxyhydroxides in sediments occurs mostly because of changes in pH[47]; we did not observe a significant change in lake water pH between the two sampling years, however this does not exclude a possible pH change in sediments which was not measured.

Finally, the overall increase in [total Se] in lake water between 2002 and 2017/2018 could be attributed to the retention time of Se in water, which studies have shown to vary widely from system to system[32,48]. Differences in Se retention time could be influenced by the retention time of lake water itself, by differences in primary productivity, or by chemical conditions that regulate the natural cycling dynamics and sedimentation rates[21]. Typically, lentic systems such as lakes

and wetlands allow Se to stay in the water column for longer periods of time[20,49] than for lotic systems such as rivers. In lakes, the slow moving water allows Se species to be reduced into selenite and organic selenium, which are more likely to be associated with particulates, making Se more bioavailable to biota[23,50]. Se tends to have a longer residence time once it is reduced as it can be recycled in the water column through trophic interactions preventing its deposition and burial in sediments[45,47,51]. Additionally, Se species have very slow oxidation rates[52], further increasing the residence time of more bioavailable reduced Se species in the water column[53].

Our study replicated trends observed by Belzile et al. (2006), showing that [total Se] is inversely correlated with the accumulation of total Hg and MeHg in zooplankton, amphipods, mayflies, and perch[16]. Only [MeHg] in perch tissue samples significantly decreased with increasing [total Se] in tissue samples. However, [total Se] in water remained the best predictor of [MeHg] in perch. Our results are in line with others that have shown an antagonistic effect of Se in water on total Hg and MeHg bioaccumulation in animal tissues[17,32]. In this study we offer the first assessment of the effect of Se on Hg species accumulation over a decade after a significant reduction in Se emissions.

A reduction in Se emissions and a reduction in [total Hg] in water between 2002 and 2017/2018 have not resulted in overall changes in [total Hg] or [MeHg] in biota. Mayflies appear to be the only biota for which the slope describing [MeHg] with respect to distance from the smelters changed between July 2002 and July 2017 (Figure 4.3). MeHg burden in mayflies was

greater in 2017 than in 2002 for lakes located further away from the stacks, but no change occurred in lakes located closer to the emission sources. Several other variables besides [total Se] may have affected [MeHg] in primary consumers such as mayflies. Such variables include [MeHg] in algae[54], pH[55], DOC[56], or feeding patterns[57]. It is also possible that the effects of a change in Se emissions in such a historically metal contaminated environment is first detectable in the less impacted lakes at the edge of the deposition zone. We predict that over time, with continued low emission, a response of the biota will increasingly apply to lakes closer to the emission sources.

In conclusion, our results showed that a reduction in Se emissions did not translate into less total Se in lake waters and emphasize the complexity of Hg accumulation in biota under these conditions. MeHg and total Hg burden in biota continues to the be correlated with the [total Se] in the water column, 15 years after the original findings by Belzile et al. (2006)[16]. These results are important as they can help manage long term recovery of mining impacted aquatic systems and can inform future remediation strategies involving Se additions.

4.6 ACKNOWLEDGEMENTS

The authors would like to thank researchers at the Vale Living with Lakes Centre who have provided the samples from July 2017 and 2018: Emily Smenderovac, Jasmine Louste-Fillion, and Jordon Courchesne. The authors would also like to thank the two anonymous reviewers for their comments, which has helped to improve the manuscript.

Table 4.1: Comparison of extraction methods for total Se, total mercury, and methylmercury

METHOD	July 2002 - Belzile et al (2006)	July 2017 – Present Study
TOTAL SELENIUM	Digested with HNO_3 and H_2O_2	Digested with concentrated HNO_3 and H_2O_2
	Analyzed by HG-AFS	Analysed by ICP-MS
TOTAL MERCURY	Digested in mixture of HNO_3 and H_2O_2	Digested with concentrated HNO_3 and H_2O_2 (Zooplankton)
	Analyzed by CVAFS (Tekran 2600)	Tekran 2600 (Zooplankton) or Nic MA-3000 mercury analyser (Perch, Amphipods, Mayflies)
METHYLMERCURY	Digested with 2.0mL of 25% KOH-methanol in Teflon vial at 85°C for 3h.	Digested with 2mL of 6N KOH, shaken for 4 hours
		2.0 mL of 6N HCl pH adjusted to 3.0 with 20% HCl and 20% KOH
	Back extracted with 6.0mL of CH_2Cl_2	Add 4mL acidic potassium bromide and copper sulfate (3:1) and 5mL of CH_2Cl_2, shake overnight
	CH_2Cl_2 is collected and ethylated	CH_2Cl_2 is collected into vial with 1mL of sodium thiosulfate, shaken
	MeHg collected on Tenax tube	Sodium thiosulfate added to 3:1 mixture of acidic potassium bromide and copper sulfate and CH_2Cl_2, shaken
		CH_2Cl_2 is collected
	Analysed by GC-CVAFS	Analysed by GC-AFS

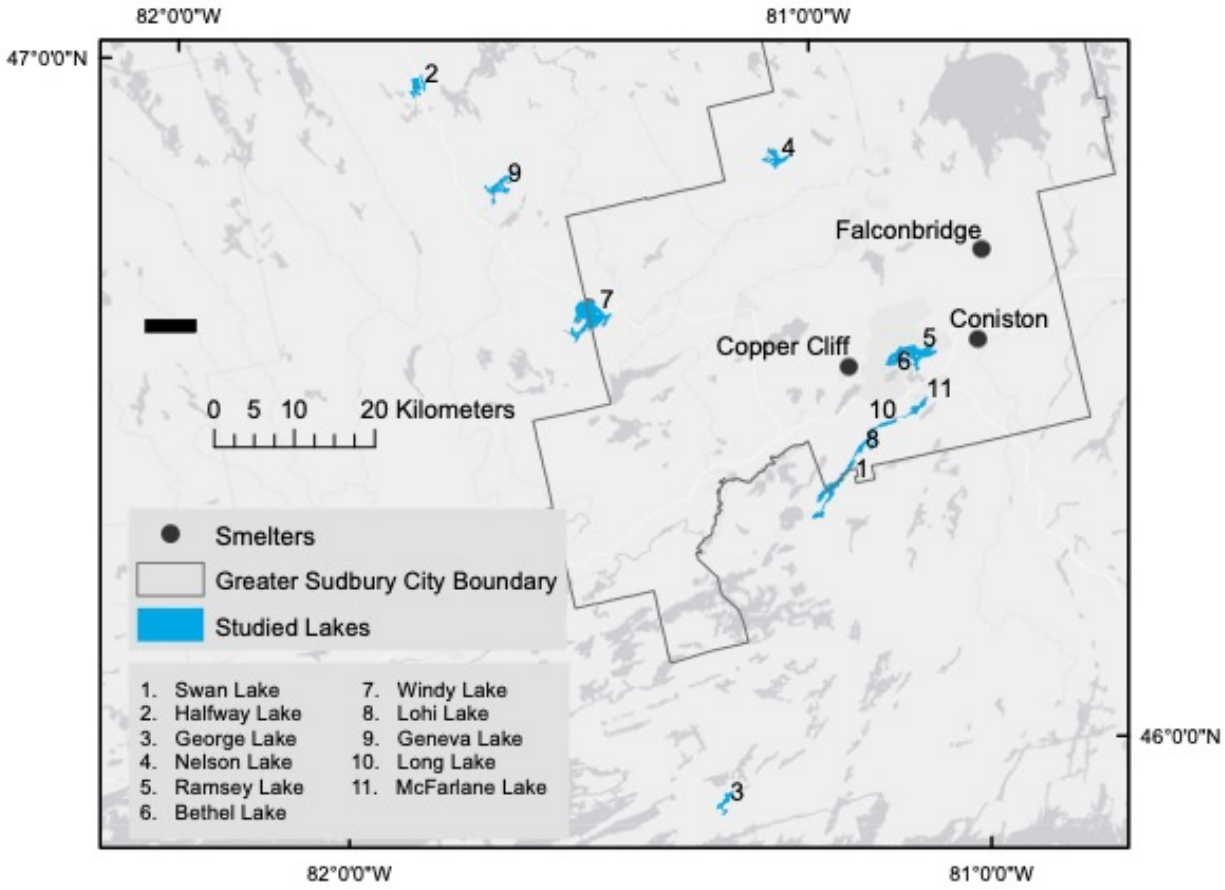


Figure 4.1: Map of the lakes selected for the study as well as the placement of the three smelter stacks in the greater Sudbury area. Spatial data was extrapolated from Belzile et al. (2006), the National Pollutant Release Inventory (Canada 2017), and the Ontario Integrated Hydrology Data (Forestry 2012).

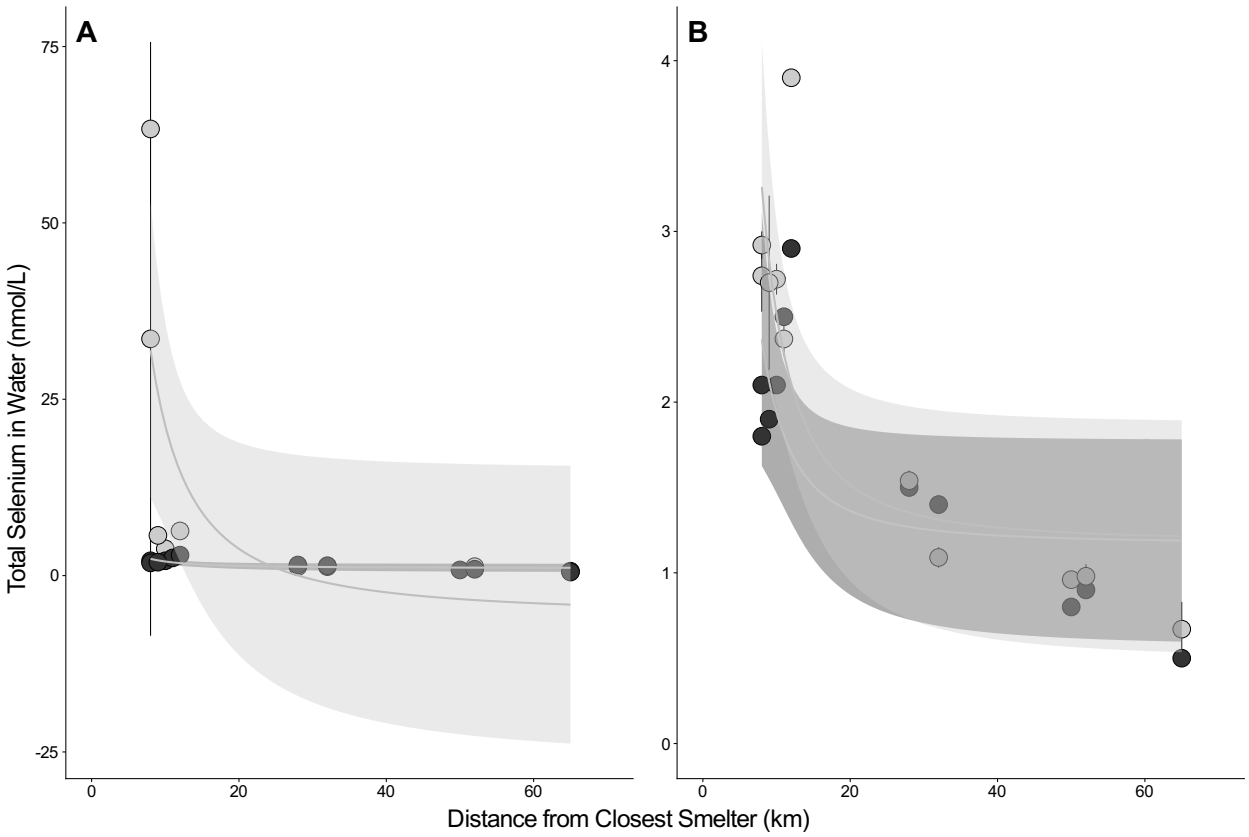


Figure 4.2: Concentration of total Se as a function of distance from the closest smelter stack, with and without outliers. Dark points represent the samples from July 2017 (A) and July 2018 (B) and the light points represent the samples from Belzile et al (2006). Standard deviations of analysis replicates are shown for samples from July 2017 and July 2018.

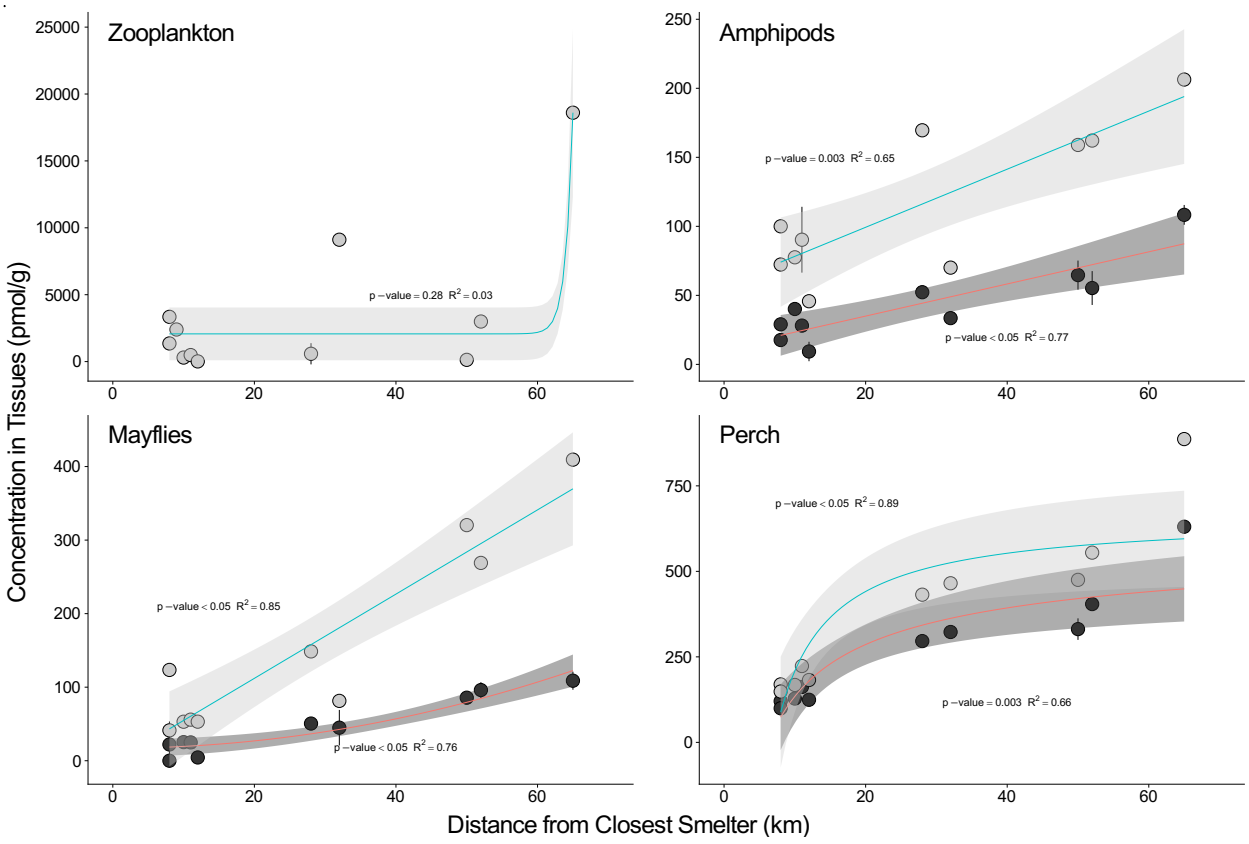


Figure 4.3: Concentrations of methylmercury and total mercury in tissues with respect to distance from the closest smelter in zooplankton, amphipods, mayflies, and perch. Black points represent methylmercury concentrations and grey points represent total mercury concentrations. Standard deviations of analysis replicates are shown for samples from July 2017.

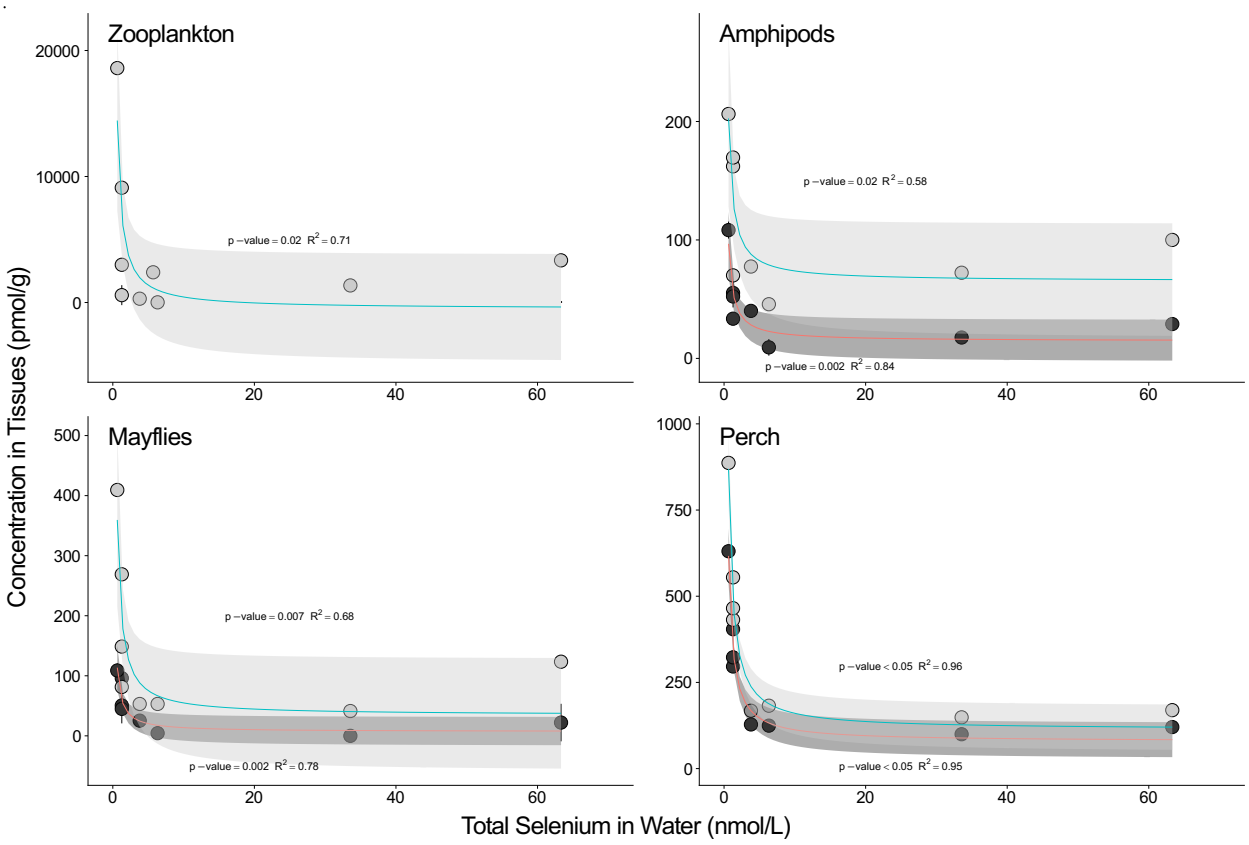


Figure 4.4: Concentrations of methylmercury and total mercury in tissues with respect to concentrations of total Se in water for zooplankton, amphipods, mayflies, and perch. Black points represent the methylmercury concentrations and grey points represent the total mercury concentrations. Standard deviations of analysis replicates are shown for samples from July 2017.

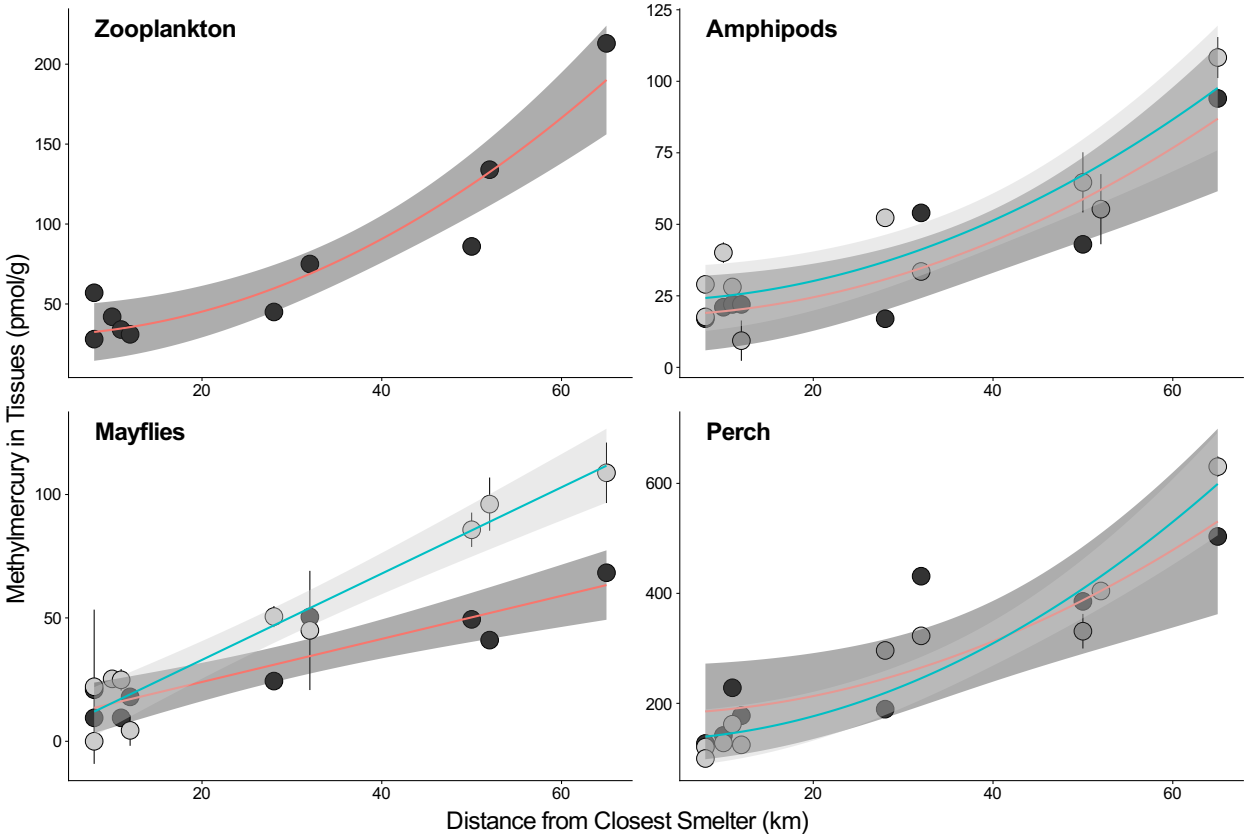


Figure 4.5: Concentrations of methylmercury in tissues with respect to distance from the closest smelter for zooplankton, amphipods, mayflies, and perch. Black points represent the results from the sampling in July 2017, grey points represent the results from Belzile et al. (2006). Standard deviations of analysis replicates are shown for samples from July 2017.

4.7 REFERENCES

1 O. W. Saarinen, *From meteorite impact to constellation city: A historical geography of greater Sudbury*, 2013.

2 B. Freedman and T. C. Hutchinson, *Canadian Journal of Botany*, 1980, 58, 108–132.

3 M. Mailman, L. Stepnuk, N. Cicek and R. A. Bodaly, *Science of The Total Environment*, 2006, 368, 224–235.

4 M. A. Turner and J. W. M. Rudd, *Canadian Journal of Fisheries and Aquatic Sciences*, 1983, 40, 2228–2240.

5 X. Wang, N. F.-Y. Tam, S. Fu, A. Ametkhan, Y. Ouyang and Z. Ye, *Annals of botany*, 2014, 114, 271–278.

6 E. Gorham and A. G. Gordon, *Canadian Journal of Botany*, 1960, 38, 477–487.

7 N. Conroy, K. Hawley, W. Keller and C. Lafrance, *Journal of Great Lakes Research*, 1976, 2, 146–165.

8 M. Boerchers, P. Fitzpatrick, C. Storie and G. Hostetler, *The Extractive Industries and Society*, 2016, 3, 793–801.

9 J. M. Gunn, *Restoration and recovery of an industrial region : progress in restoring the smelter-damaged landscape near Sudbury, Canada*, 1995.

10 K. Winterhaider, *Environmental Technology Letters*, 1983, 4, 209–216.

11 J. O. Nriagu and H. K. T. Wong, *Nature*, 1983, 301, 55.

12 J. O. Nriagu, H. K. Wong and R. D. Coker, *Environmental science & technology*, 1982, 16, 551–560.

13 J. E. Hawley and I. Nichol, *Economic Geology*, 1959, 54, 608–628.

14 J. M. Beatty and G. A. Russo, *Ambient water quality guidelines for Selenium Technical Report Update*, 2014.

15 V. K. Sharma, T. J. McDonald, M. Sohn, G. A. K. Anquandah, M. Pettine and R. Zboril, *Environmental Chemistry Letters*, 2015, 13, 49–58.

16 N. Belzile, Y.-W. Chen, J. M. Gunn, J. Tong, Y. Alarie, T. Delonchamp and C.-Y. Lang, *Canadian Journal of Fisheries and Aquatic Sciences*, 2006, 63, 1–10.

17 Y.-W. Chen, N. Belzile and J. Gunn, 2001, 46.

18 H. Ullah, G. Liu, B. Yousaf, M. U. Ali, S. Irshad, Q. Abbas and R. Ahmad, *Environmental Geochemistry and Health*, 2018.

19 R. F. Burk, *Selenium, an Antioxidant Nutrient*, 2002.

20 Lemly, *Selenium Assessment in Aquatic Ecosystems*, 2002.

21 A. D. Lemly and G. J. Smith, *Aquatic cycling of selenium: Implications for fish and wildlife*, 1987.

22 J. M. Besser, J. N. Huckins, E. E. Little and T. W. La Point, *Environmental Pollution*, 1989, 62, 1–12.

23 J. M. Besser, T. J. Canfield and T. W. La Point, *Environmental Toxicology and Chemistry*, 1993, 12, 57–72.

24 S. J. Hamilton, *Science of the Total Environment*, 2004, 326, 1–31.

25 D. J. Hoffman, *Handbook of Ecotoxicology*, 2003.

26 P. Bjerregaard, S. Fjordside, M. G. Hansen and M. B. Petrova, *Environmental Science & Technology*, 2011, 45, 9793–9798.
27 E. E. V. Chapman, J. Robinson, J. Berry and L. M. Campbell, *Water, Air, & Soil Pollution*, 2016, 227, 216.
28 S. Nuutinen and J. Kukkonen, *Biogeochemistry*, 1998, 40, 267–278.
29 D.-Y. Yang, Y.-W. Chen, J. M. Gunn and N. Belzile, *Environmental Reviews*, 2008, 16, 71–92.
30 K. Paulsson and K. Lundbergh, *Water Air & Soil Pollution*, 1991, 56, 833–841.
31 S. A. Peterson, N. V. C. Ralston, D. V. Peck, J. V. Sickle, J. D. Robertson, V. L. Spate and J. S. Morris, *Environmental Science & Technology*, 2009, 43, 3919–3925.
32 G. R. Southworth, M. J. Peterson and M. G. Ryon, *Chemosphere*, 2000, 41, 1101–1105.
33 J. G. Melnick, K. Yurkerwich and G. Parkin, *Journal of the American Chemical Society*, 2010, 132, 647–655.
34 H.-Y. T. Truong, Y.-W. Chen, M. Saleh, S. Nehzati, G. N. George, I. J. Pickering and N. Belzile, *Metallomics*, 2014, 6, 465–475.
35 Y. Wang, F. Dang, R. D. Evans, H. Zhong, J. Zhao and D. M. Zhou, *Sci Rep*, 2016, 6.
36 Y. Sugiura, Y. Tamai and H. Tanaka, *Bioinorganic Chemistry*, 1978, 9, 167–180.
37 K. Kidd and K. Batchelar, *Fish Physiology*, 2011, 31, 237–295.
38 O. M. of the Environment, *Sudbury environmental study synopsis, 1973-1980*, 1982.
39 L. D. Gignac and P. J. Beckett, *Canadian Journal of Botany*, 1986, 64, 1138–1147.
40 W. Keller, 1994.
41 J. O. Nriagu, H. K. T. Wong, G. Lawson and P. Daniel, *Science of The Total Environment*, 1998, 223, 99–117.
42 A. Valois, W. Bill Keller and C. Ramcharan, *Freshwater Biology*, 2010, 55, 2585–2597.
43 M. L. Brazeau, A. J. Poulain, A. M. Paterson, W. Keller, H. Sanei and J. M. Blais, *Environmental Pollution*, 2013, 173, 52–60.
44 Government of Canada, Freshwater Inventory and Surveillance of Mercury: Water Quality Data.
45 G. Bowie, J. Sanders, G. Riedel, C. Gilmour, D. Breitburg, G. Cutter and D. Porcella, *An International Journal of Environmental Pollution*, 1996, 90, 93–104.
46 N. Belzile, Y. W. Chen and R. Xu, *Applied Geochemistry*, 2000, 15, 1439–1454.
47 P. M. Chapman, W. J. Adams, M. Brooks, and Setac, 2010.
48 R. H. Hesslein, W. S. Broecker and D. W. Schindler, *Canadian Journal of Fisheries and Aquatic Sciences*, 1980, 37, 378–386.
49 T. S. Presser and S. N. Luoma, *Integrated Environmental Assessment and Management*, 2010, 6, 685–710.
50 A. Fernández-Martínez and L. Charlet, *Reviews in Environmental Science and Bio/Technology*, 2009, 8, 81–110.
51 P. L. Orr, K. R. Guiguer and C. K. Russel, *Ecotoxicology and Environmental Safety*, 2006, 63, 175–188.
52 G. A. Cutter and K. W. Bruland, *Limnology and Oceanography*, 1984, 29, 1179–1192.
53 Y. W. Chen, M. D. Zhou, J. Tong and N. Belzile, *Analytica Chimica Acta*, 2005, 545, 142–148.

54 P. C. Pickhardt, C. L. Folt, C. Y. Chen, B. Klaue and J. D. Blum, *Proceedings of the National Academy of Sciences*, 2002, 99, 4419–4423.
55 M. G. Clayden, K. A. Kidd, J. Chételat, B. D. Hall and E. Garcia, *Ecotoxicology*, 2014, 23, 273–284.
56 M. Rennie, N. Collins, C. Purchase and A. Tremblay, Predictive models of benthic invertebrate methylmercury in Ontario and Quebec lakes, 2005, 62.
57 A. Tremblay, M. Lucotte and M. Meili, Total Mercury and Methylmercury Contents of Insects from Boreal Lakes: Ecological, Spatial and Temporal Patterns, 1996, 31.

4.8 SUPPLEMENTARY INFORMATION

Order occurring in the text:

Figure S4.1: Emissions of Selenium from Copper Cliff Smelter in Sudbury ON, the primary source of emissions to the area. The emissions from 2003 to 2017 are quantified by engineered estimation. Data were collected from the National Pollutant Release Inventory provided by Environment and Climate Change Canada. The vertical blue lines are showing the two years of sampling compared in the study (2002 and 2017)

Table S4.1: Results of quality control for all biota analyses. DORM2, DORM4, TORT2, and DOLT5 is provided by Canadian National Research Council. IAEA407 is provided by the International Atomic Energy Agency

Table S4.2: Physical and chemical characteristics of the lakes

Table S4.3: Concentrations of total selenium, total mercury and methylmercury in organisms collected in the lakes around Sudbury. The samples from 2002 are taken from Belzile et al. (2006). All concentrations are given in dry weight.

Figure S4.2: Concentrations of total selenium in tissues with respect to distance from the closest smelter for zooplankton (A), amphipods (B), mayflies (C), and perch (D).

Figure S4.3: Concentrations of methylmercury and total mercury in tissues with respect to concentration of total Se in tissues for zooplankton (A), amphipods (B), mayflies (C), and perch (D). Black points represent the methylmercury concentrations and grey points represent the total mercury concentrations

Figure S4.4: Concentrations of methylmercury in tissues of organisms. The samples are pooled into two categories: near (<20km from the closest smelter) and far (>20km from the closest smelter). Grey boxes are 2002 samples and black boxes are 2017 samples.

Figure S4.5: Concentrations of total mercury in tissues of organisms. The samples are pooled into two categories: near (<20km from the closest smelter) and far (>20km from the closest smelter). Grey boxes are 2002 samples and black boxes are 2017 samples.

Figure S4.6: Concentrations of selenium in water. The samples are pooled into two categories: near (<20km from the closest smelter) and far (>20km from the closest smelter). Grey boxes are 2002 samples and black boxes are 2017 samples.

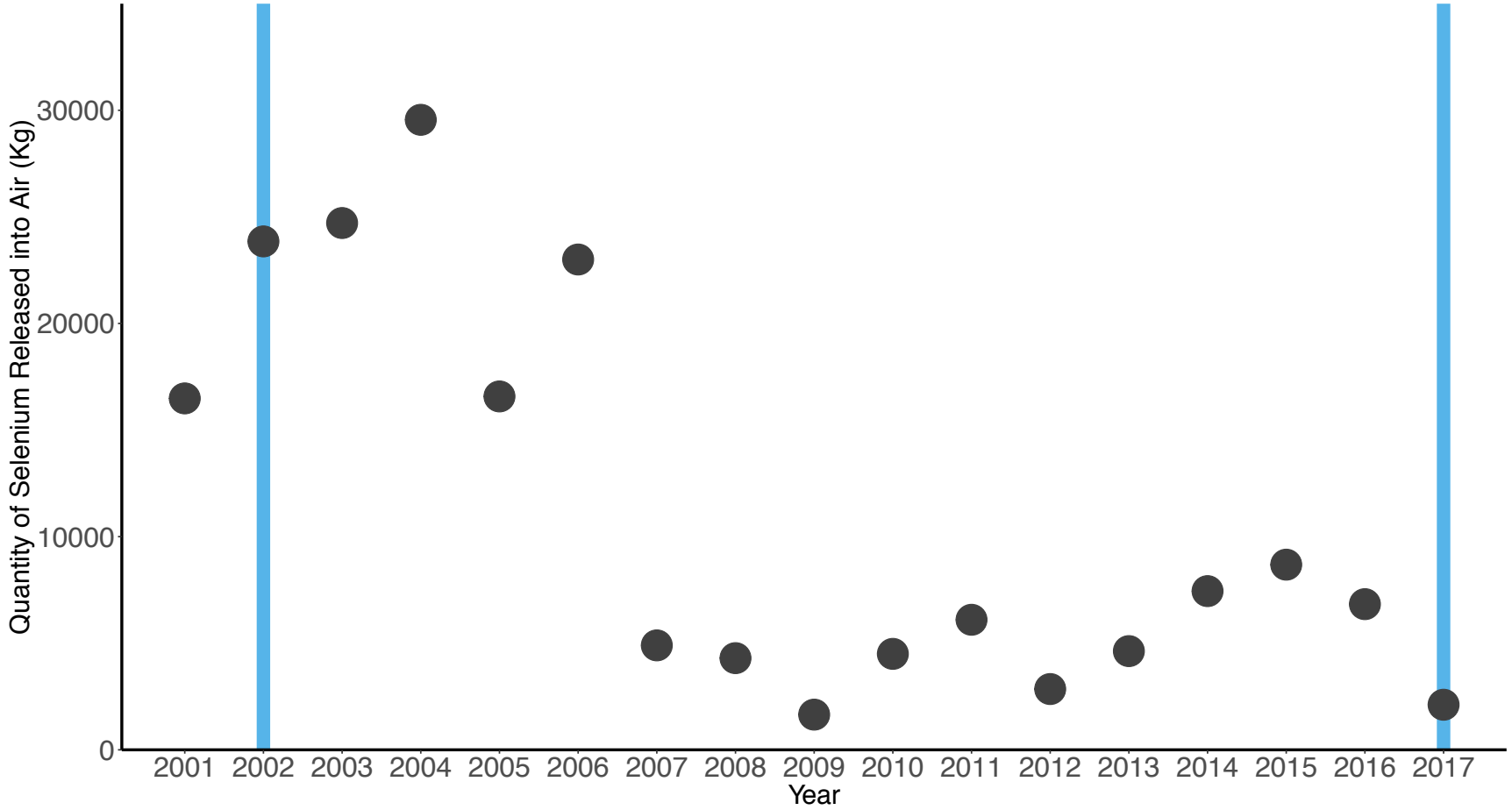


Figure S4.1: Emissions of Selenium from Copper Cliff Smelter in Sudbury ON, the primary source of emissions to the area. The emissions from 2003 to 2017 are quantified by engineered estimation. Data were collected from the National Pollutant Release Inventory provided by Environment and Climate Change Canada. The vertical blue lines are showing the two years of sampling compared in the study (2002 and 2017)

Table S4.1: Results of quality control for all biota analyses from 2017. DORM2, DORM4, TORT2, and DOLT5 is provided by Canadian National Research Council. IAEA407 is provided by the International Atomic Energy Agency

	Total Se			Total Hg			MeHg		
CRM[b]	Certified Value (mg/Kg)	Average Results (mg/Kg)	Relative Error[a] (%)	Certified Value (mg/Kg)	Average Results (mg/Kg)	Relative Error[a] (%)	Certified Value (mg/Kg)	Average Results (mg/Kg)	Relative Error[a] (%)
DORM2	1.4	1.32	**5.7**	4.64	4.06	**12.5**	4.47	3.976	**10.9**
DORM4	3.45	3.19	**7.5**	0.41	0.36	**12.6**	0.355	0.355	**0**
TORT2	5.63	4.42	**21.5**	0.27	0.31	**14.8**	0.152	0.159	**4.6**
IAEA407	2.83	2.29	**18.1**	0.22	0.22	**0**	0.2	0.20	**0**
DOLT5	8.3	7.03	**15.3**	0.44	0.39	**11.4**	0.119	0.116	**2.5**

Relative error = [(Actual Value - Measured Value) / Known Value]*100
CRM = certified reference material

Table S4.2: Physical and chemical characteristics of the lakes

		2002						2017							2018	
Lake	Distance (km)	pH	DOC (mg/L)	Ca (mmol/L)	Mg (mmol/L)	Se (nmol/L)	Hg (pmol/L)	pH	DOC (mg/L)	Ca (mmol/L)	Mg (mmol/L)	Na (mmol/L)	Sulfate (mg/L)	Se (nmol/L)	Se (nmol/L)	Hg (pmol/L)
Bethel	10	8.1	7.7	0.32	0.22	2.1	6.5	7.86	5.3	0.36	2.48	1.12	6.6	3.8	2.72	1.13
Geneva	50	6.8	4.2	0.1	0.02	0.8	13.5	6.85	4.1	0.06	0.24	0.04	3.9	-	0.96	3.66
George	52	6.3	2.2	0.04	0.02	0.9	10.4	6.36	2.6	0.03	0.21	0.03	4.35	1.27	0.98	1.28
Halfway	65	6.9	4.4	0.11	0.06	0.5	16	6.93	4.4	0.09	0.44	0.31	3.75	0.63	0.67	3.7
Lohi	8	6.2	3.4	0.1	0.05	2.1	10.9	6.71	3.5	0.09	0.46	0.19	7.55	63.32	2.74	2.42
Long	8	7	4.1	0.19	0.11	1.8	12.6	7.17	4.1	0.19	1.04	0.77	9.7	33.56	2.92	0.89
McFarlane	11	7.4	4.6	0.35	0.19	2.5	10.2	7.74	4	0.45	2.07	3.11	16.7	-	2.37	1.2
Nelson	28	6.6	2	0.06	0.03	1.5	9.3	6.56	2.2	0.05	0.23	0.03	5.65	1.27	1.54	1.64
Ramsey	12	7.4	3.5	0.33	0.18	2.9	12.3	7.6	3.6	0.4	1.94	2.34	14.7	6.33	3.9	1.01
Windy	32	6.5	2.8	0.08	0.05	1.9	16.5	5.9	2.8	0.08	0.26	0.07	7.05	5.7	1.09	1.77
Swan	9	5.7	2.8	0.08	0.02	1.4	10.9	6.64	2.6	0.07	0.36	0.29	5.4	1.27	2.7	2.5

Table S4.3: Concentrations of total selenium, total mercury and methylmercury in organisms collected in the lakes around Sudbury. The samples from 2002 are taken from Belzile et al. (2006). All concentrations are given in dry weight.

		Amphipods			Mayflies			Perch			Zooplankton		
Year	Lake	MeHg (pmol·g^{-1})	THg (pmol·g^{-1})	TSe (nmol·g^{-1})	MeHg (pmol·g^{-1})	THg (pmol·g^{-1})	TSe (nmol·g^{-1})	MeHg (pmol·g^{-1})	THg (pmol·g^{-1})	TSe (nmol·g^{-1})	MeHg (pmol·g^{-1})	THg (pmol·g^{-1})	TSe (nmol·g^{-1})
2002	**Bethel**	**21**	47	20	–	–	–	141.6	191	39.1	42	85	46
	Geneva	43	141	26	49.4	198	71	385.4	543	41.8	86	284	29
	George	–	–	–	41	239	102	–	–	–	134	364	70
	Halfway	94	174	12	68.3	419	56	503.5	1221	34.5	213	398	22
	Lohi	–	–	–	20.9	130	132	–	–	–	57	184	129
	Long	17	41	30	9.5	65	88	127.1	403	79.5	28	145	56
	McFarlane	22	55	21	9.5	50	69	228.3	294	68.4	34	95	48
	Nelson	17	135	76	24.4	145	155	189.4	340	135.2	45	150	86
	Ramsey	22	39	35	18	100	99	177.5	293	96.8	31	135	63
	Windy	54	116	17	50.4	105	90	431.2	543	76.7	75	281	80
2017	**Bethel**	40.11	77.56	19.37	25.3	53.04	41.12	128.05	167.85	31.44	73.88	301.02	28.66
	Geneva	64.67	158.96	27.8	85.71	320.37	49.21	331.16	475.17	34.77	–	130.04	33.16
	George	55.3	162.14	28.07	96.1	268.98	82.95	404.34	554.74	37.88	–	2994.58	37.37
	Halfway	108.31	206.33	12.76	108.8	409.41	20.8	630.49	886.73	23.36	–	18603.22	14.11
	Lohi	29	100.01	55.22	22.1	123.46	96.67	120.5	169.5	80.97	37.87	3345.35	44.67
	Long	17.63	72.32	37.55	0	41.29	85.62	99.69	148.66	52.45	55.49	1355.11	38.16
	McFarlane	28.09	90.3	22.22	24.91	55.86	61.71	162.03	223.22	37.11	67.93	487.94	27.68
	Nelson	52.28	169.58	53.63	50.54	148.65	127.28	296.15	431.83	67.21	–	584.49	58.4
	Ramsey	9.33	45.74	26.82	4.43	53.11	50.93	124.54	182.36	60.13	13.6	13.9	56.32
	Windy	33.51	70.08	22.46	44.92	81.37	64.15	322.72	465.18	39.91	–	9109.46	36

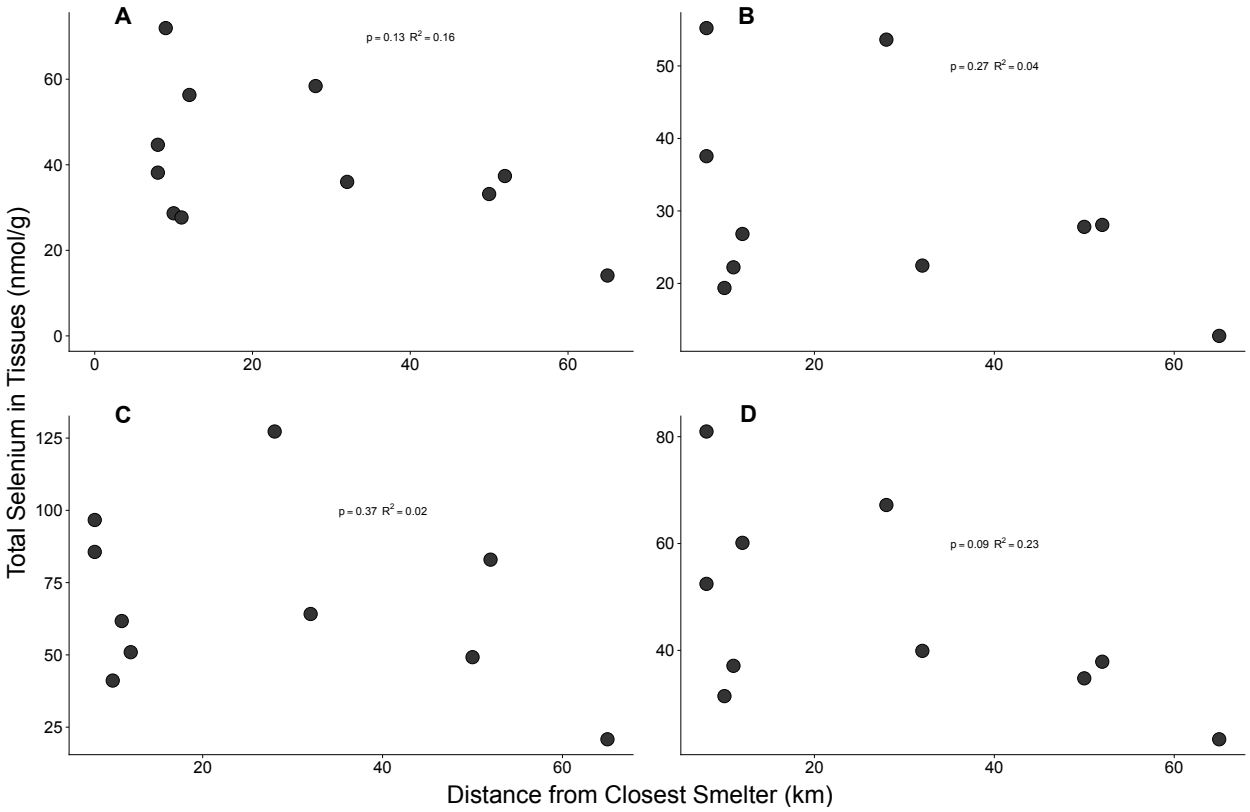


Figure S4.2: Concentrations of total selenium in tissues with respect to distance from the closest smelter for zooplankton (A), amphipods (B), mayflies (C), and perch (D).

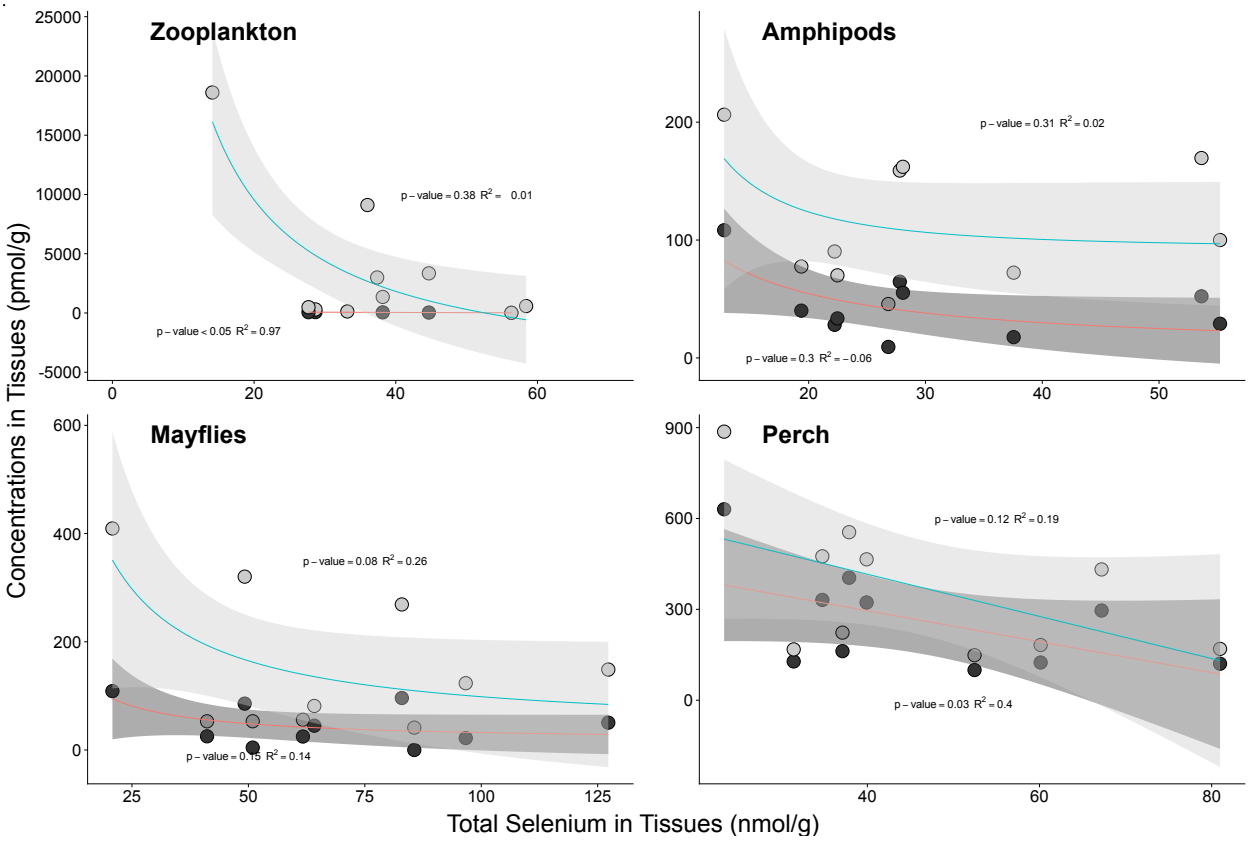


Figure S4.3: Concentrations of methylmercury and total mercury in tissues with respect to concentration of total Se in tissues for zooplankton (A), amphipods (B), mayflies (C), and perch (D). Black points represent the methylmercury concentrations and grey points represent the total mercury concentrations

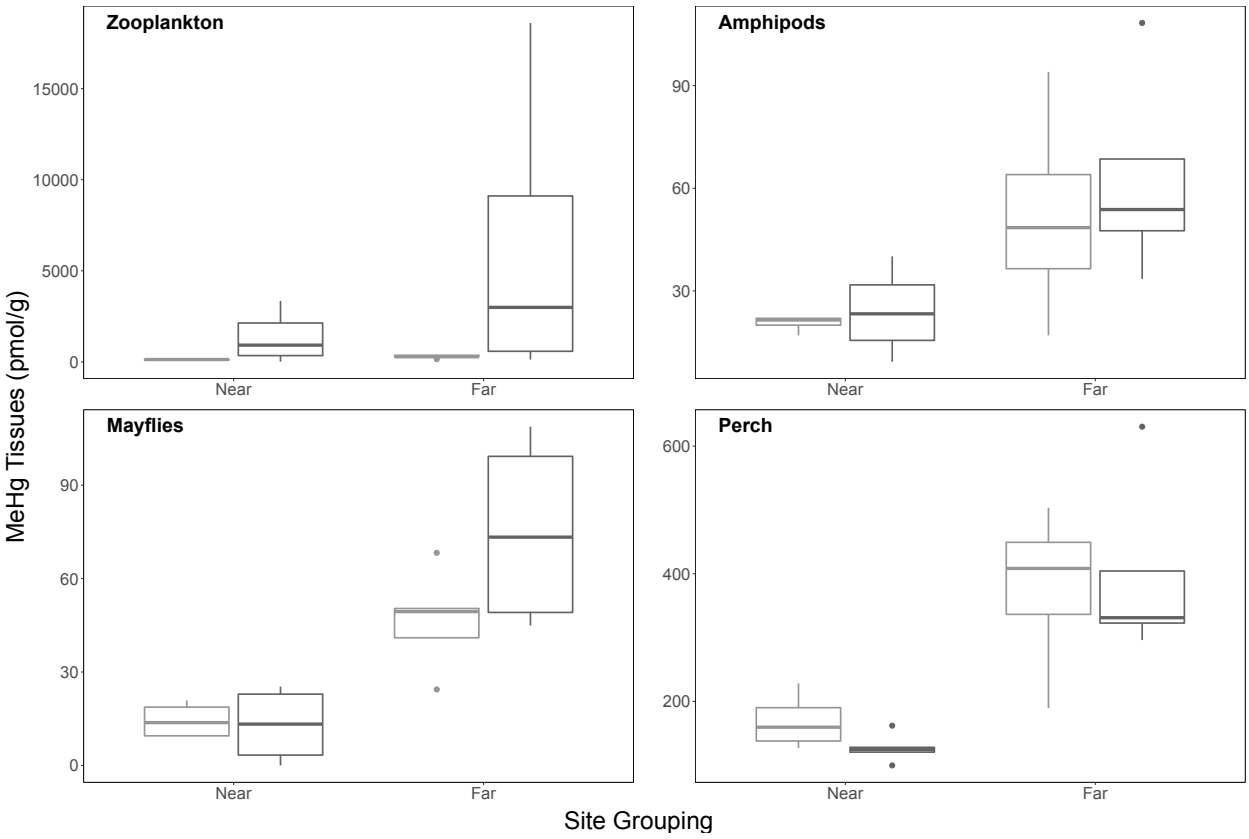


Figure S4.4: Concentrations of methylmercury in tissues of organisms. The samples are pooled into two categories: near (<20km from the closest smelter) and far (>20km from the closest smelter). Grey boxes are 2002 samples and black boxes are 2017 samples.

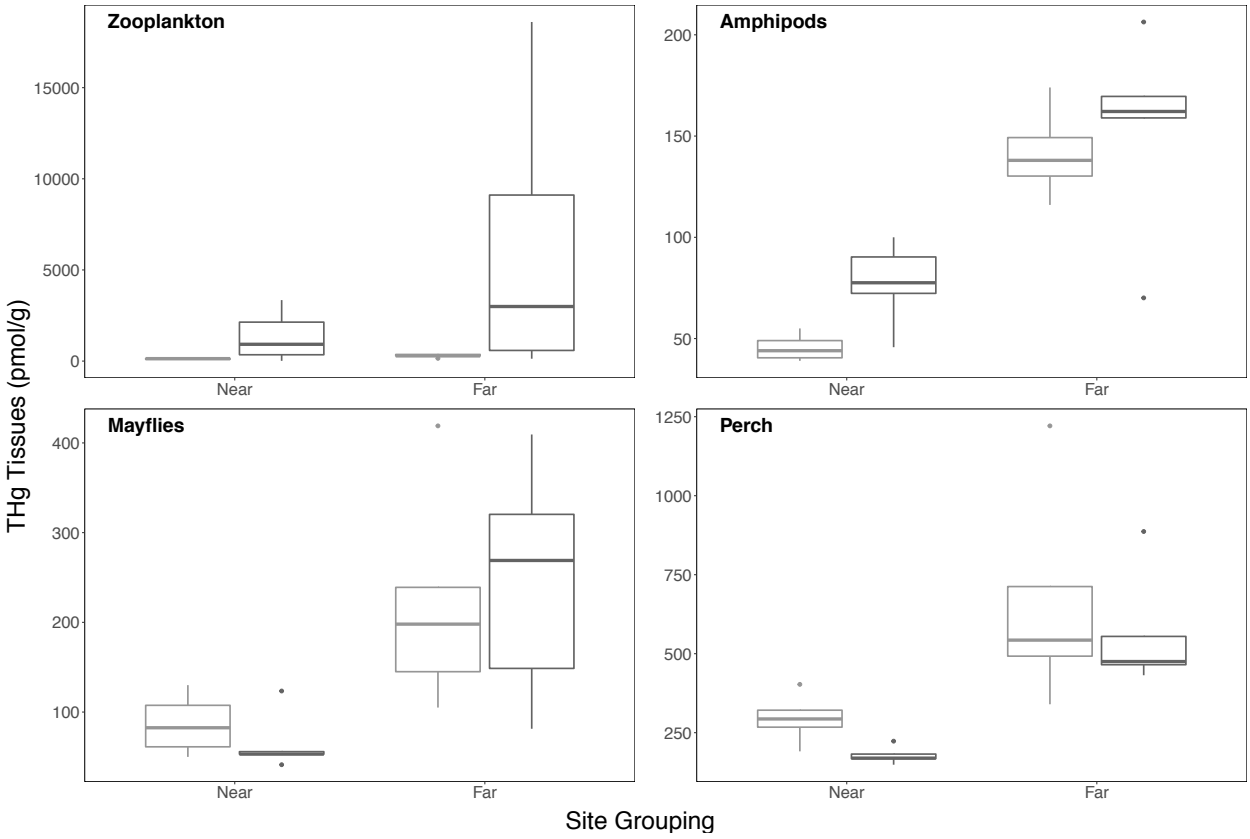


Figure S4.5: Concentrations of total mercury in tissues of organisms. The samples are pooled into two categories: near (<20km from the closest smelter) and far (>20km from the closest smelter). Grey boxes are 2002 samples and black boxes are 2017 samples.

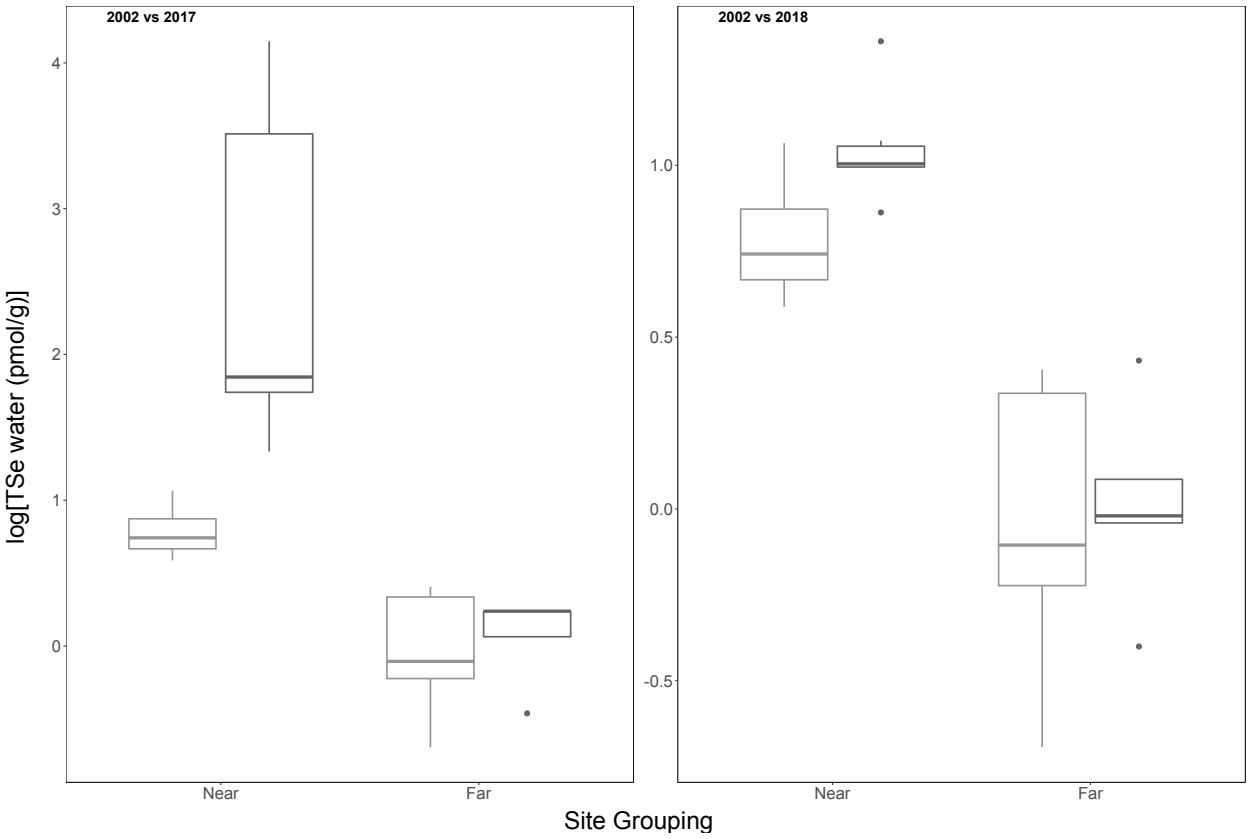


Figure S4.6: Concentrations of selenium in water. The samples are pooled into two categories: near (<20km from the closest smelter) and far (>20km from the closest smelter). Grey boxes are 2002 samples and black boxes are 2017 samples.

CHAPTER 5: CONCLUSION

5.1 STUDY OUTCOMES

The three research chapters of this thesis demonstrate how mining pollution is affecting the natural cycling of Hg in freshwater lakes. Chapter 2 and 3 examined the effect of sulfate and arsenic (As) pollution on microbial cycling of mercury (Hg), while Chapter 4 assessed the effect of varying selenium (Se) smelting pollution on Hg cycling in the food web (i.e. bioaccumulation). In each chapter the results demonstrated that pollution gradients do in fact affect the cycling of Hg in freshwater lakes. However, the size and direction of these effects is dependent on the type of pollutant (e.g. sulfate, As, Se).

The first two research chapters report on sampling sites located in Yellowknife, NWT, Canada. The first research chapter (Chapter 2) examined the effect of sulfate and As on the production/degradation of methylmercury (MeHg) as well as the changes in microbial community structure in lake sediments. We hypothesized that enhanced sulfate deposition from roasting affected MeHg dynamics in sediments of lakes surrounding Giant Mine. Assuming that sulfate reducing metabolism was the main variable limiting MeHg in the system and not the amount or delivery rate of bioavailable Hg, we predicted that sulfate would be positively correlated with both rates of MeHg production and %MeHg. The four main outcomes of this chapter were as follows: 1) %MeHg is significantly correlated with the sulfate concentration gradient, 2) K_m is controlled by total As, dissolved organic carbon, total phosphorous and %MeHg in the water

column, 3) K_d is controlled by dissolved organic carbon, and 4) microbial community changes are correlated in part by the pollution gradient (i.e. sulfate and As).

The goal of Chapter 3 was to explore the effects of As(V) on MeHg production in sediments of lake YK-E1. YK-E1 was chosen as it is a limnologically representative lake of the area and it is outside of the emissions gradient from Giant Mine[1]. Considering that SRMs are the main Hg methylators in freshwater systems and that As(V) can be used by SRMs as a terminal electron acceptor (TEA), we postulated that the presence of As(V) in the system could influence the production of MeHg. This study was exploratory by nature and the goal was to observe how As(V) addition to sediment slurries would affect the rate of production of MeHg in lake sediments. In this chapter, the conclusions were the following: 1) sediments were first limited in carbon, 2) once carbon was amended to sediments, addition of sulfate increase MeHg production, and 3) addition of As(V) had a significant negative correlation with MeHg production regardless of sulfate concentrations.

Finally, Chapter 4 was set in a different location: Sudbury in Ontario, Canada. This study was a follow up to Belzile et al. (2006)[2]. Our study revisited the same lake sites 15 years after the Se emissions had been reduced due to environmental regulations. We hypothesized that changes in Se emissions in Sudbury would alter Hg accumulation in biota, and predicted that a decrease in emissions would lead to an increase in Hg and MeHg in biota. We show that: 1) Se atmospheric emissions did decrease in Sudbury, 2) reduction in Se in the air did not decrease Se

concentrations in the water, and 3) Se still offers a protective effect on MeHg and total Hg bioaccumulation in the biota of the lakes in Sudbury. This study shows possible support for the use of Se as a remediation tactic in freshwater boreal lakes and highlights the need for long term studies on the cycling of Se in these freshwater lakes.

5.2 FUTURE DIRECTIONS

In Chapter 2, we characterized the microbial community using 16S rRNA gene amplicon sequencing, therefore a more detailed study of the Hg methylators that are present in sediments of each lake could be a potential future study. The next step in this environment would be to characterize the community with respect to *hgc*AB (i.e. Hg methylation genes). Most studies that have examined the distribution and phylogeny of *hgc*AB genes were done on non-impacted sites[3–5] or sites impacted by Hg pollution[6]. The strength of our study is the number of sampling sites (35) that are spread across a large landscape (2800 km^2) which offers an opportunity to study the change in *hgc*AB genes along a strong pollution gradient of As and sulfate, while also accounting for other environmental variables known to affect Hg cycling. This type of study would be a complementary to Chapter 2 as it could give us further insight on how the environmental variables correlated with K_m (i.e. total As, dissolved organic carbon, total phosphorous and %MeHg) are potentially affecting the community structure of Hg methylating microbes specifically in the lakes sediments.

In a similar vein, possible complementary experiments for Chapter 3 results would be two-fold: quantifying specific genes and testing other lake sediments. First, quantify As reduction (e.g *ars* and *arr*) genes would be a good indicator if microbes that contain these genes are suppressed or amplified during As(V) addition. One of the limitations in our study was that we could not identify which mechanism was responsible for the As(V) reduction (dissimilatory vs detoxifying). By quantifying As reduction genes (e.g. *ars* and *arr*), we can have a better understanding of the composition of the microbial community and how it changes along the sulfate and As(V) gradients. So far, a few other studies have investigated gene expression along As gradients[7–9], but not within the context of Hg methylation.

Next, future studies should test sediments from other environments. Considering that this is the first study that investigates the effect of As(V) on the kinetics of Hg methylation, it would be important to test if these results are replicable in other environments. Each lake has unique biogeochemical properties that could potentially affect the outcome of these experiments, therefore testing lakes with different limnological characteristics could yield a more holistic representation of the relationship between As(V) and Hg cycling. Studies show that As transformation genes are wide spread but that the distribution of these genes is not uniform across all environments[10], therefore it is possible that other lakes, with different microbial community compositions, could have a different response to As(V) addition than the ones observed in YK-E1.

Finally, Chapter 4 is a follow up to a study published in 2006[2], our results highlighted the importance of revising legacy sites. In the future, further monitoring would be an asset as we could identify the point in time at which Se will no longer have a protective effect on Hg bioaccumulation as our results showed that Se increased in the water column following decreased atmospheric loading. We had suggested that the increased Se in the water column could be attributed to: 1) Se from the catchment draining into the lake, 2) remobilization of Se from sediments, and 3) long retention time of Se present in the water column of the lake. The most recent study that has explored the Se geochemistry in the Sudbury area is Belzile et al (2000) in which the distribution of dissolved Se species were observed in two lakes (Clearwater and McFarlane) in the Sudbury area[11]. However, this study did not address the diffusion of selenium from the sediments to the water column in the system, nor the impact of watershed draining to these lakes. Therefore, a more in-depth and updated study would address some of the observations we have made in Chapter 4.

5.3 RESEARCH CONTRIBUTIONS

The three chapter in this thesis explore in various ways in which mining pollution can affect the natural cycling of Hg and MeHg in freshwater systems and offer insight on the effect of that pollution over a spatial extent (Chapter 2), along an unexplored metal gradient (Chapter 2 and 3) and over long period time with varying pollution emissions (Chapter 4). Considering the results from each chapter in the thesis, these findings could be used for planning future work in

the field of Hg biogeochemistry as well as help to inform future management of mining contaminated sites.

5.3.1 Field of Hg Research

The scientific community has made great strides in understanding which environmental factors can affect the cycling of Hg in freshwater systems[12]. However, most studies are done in the laboratory[13–15] or on very few selected lakes along a specific gradients (mostly Hg gradients)[16–23]. Moreover, the vast majority field studies are done only once[16,17,21,22] or for a short period of time (≤ year)[20,24]. Although these studies are instrumental in understanding Hg biogeochemistry, the results from this thesis provides a more holistic view of the cycling of Hg in dynamic landscapes such as the ones in Yellowknife (NWT) and Sudbury (ON).

5.3.2 Management of Contaminated Sites

Pollution gradients are an inevitable consequence of mining, particularly smelting and roasting practices create pollution gradients that can remain in the landscape for decades to come[25,26]. Lately, these large pollution gradients are being seen as a liability risk as they can have serious consequences on lake chemistry[25,27] and on public health[28,29]. The results from this thesis can help to better understand the controls of Hg cycling in freshwater lakes at sites impacted by mining pollution. By understanding what controls Hg cycling, we can be better equipped to assess and remediate future impacted sites.

5.4 CLOSING STATEMENT

Historically, before stricter environmental policies, there have been very few steps taken to mitigate the pollution produced and emitted by mining activities[30,31]. Lately, we have come to realize how devastating this legacy pollution is to the health of both the environment and its inhabitants[30,32]. The results from my thesis specifically demonstrate how mining emissions can alter the kinetics and bioaccumulation of MeHg in freshwater lakes, highlighting the complexity of natural Hg cycling in response to mining activities. As such, these results contribute to identifying the controls of MeHg production and bioaccumulation in environments impacted by roasting emissions from mining operations. It is imperative to understand the consequences of these emissions, as this is the first step to better mitigate its harmful effects on ecosystems and surrounding communities.

5.5 REFERENCES

1 M. Aždajić, E. Yumvihoze, J. M. Blais and A. J. Poulain, The effect of legacy gold mining on methylmercury cycling and microbial community structure in northern freshwater lakes, *Environ. Sci.: Processes Impacts*, 2021, **23**, 1220–1230.

2 N. Belzile, Y.-W. Chen, J. M. Gunn, J. Tong, Y. Alarie, T. Delonchamp and C.-Y. Lang, *Canadian Journal of Fisheries and Aquatic Sciences*, 2006, 63, 1–10.

3 A. G. Bravo, S. Peura, M. Buck, O. Ahmed, A. Mateos-Rivera, S. Herrero Ortega, J. K. Schaefer, S. Bouchet, J. Tolu, E. Björn and S. Bertilsson, *Applied and environmental microbiology*, 2018, 84.

4 J. K. Schaefer, R. M. Kronberg, F. M. M. Morel and U. Skyllberg, *Environmental Microbiology Reports*, 2014, 6, 441–447.

5 M. Podar, C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A. V. Palumbo, A. C. Somenahally and D. A. Elias, Global prevalence and distribution of genes and microorganisms involved in mercury methylation, *Science Advances*, , DOI:10.1126/sciadv.1500675.

6 Y. R. Liu, R. Q. Yu, Y. M. Zheng and J. Z. He, *Applied and Environmental Microbiology*, 2014, 80, 2874–2879.

7 V. H.-C. Liao, Y.-J. Chu, Y.-C. Su, S.-Y. Hsiao, C.-C. Wei, C.-W. Liu, C.-M. Liao, W.-C. Shen and F.-J. Chang, Arsenite-oxidizing and arsenate-reducing bacteria associated with arsenic-rich groundwater in Taiwan, *Journal of Contaminant Hydrology*, 2011, **123**, 20–29.

8 S.-Y. Zhang, F.-J. Zhao, G.-X. Sun, J.-Q. Su, X.-R. Yang, H. Li and Y.-G. Zhu, Diversity and Abundance of Arsenic Biotransformation Genes in Paddy Soils from Southern China, *Environ. Sci. Technol.*, 2015, **49**, 4138–4146.

9 P. S. Costa, L. L. S. Scholte, M. P. Reis, A. V. Chaves, P. L. Oliveira, L. B. Itabayana, M. L. S. Suhadolnik, F. A. R. Barbosa, E. Chartone-Souza and A. M. A. Nascimento, Bacteria and Genes Involved in Arsenic Speciation in Sediment Impacted by Long-Term Gold Mining, *PLOS ONE*, 2014, **9**, e95655.

10 T. K. Dunivin, S. Y. Yeh and A. Shade, *BMC Biology*, 2019, 17, 45.

11 N. Belzile, Y. W. Chen and R. Xu, *Applied Geochemistry*, 2000, 15, 1439–1454.

12 A. R. Paranjape and B. D. Hall, Recent advances in the study of mercury methylation in aquatic systems, *FACETS*, 2017, **2**, 85–119.

13 A. M. Graham, G. R. Aiken and C. C. Gilmour, Dissolved organic matter enhances microbial mercury methylation under sulfidic conditions., *Environ Sci Technol*, 2012, **46**, 2715–2723.

14 S. A. Chiasson-Gould, J. M. Blais and A. J. Poulain, Dissolved Organic Matter Kinetically Controls Mercury Bioavailability to Bacteria, *Environ. Sci. Technol.*, 2014, **48**, 3153–3161.

15 A. M. Graham, G. R. Aiken and C. C. Gilmour, Effect of Dissolved Organic Matter Source and Character on Microbial Hg Methylation in Hg–S–DOM Solutions, *Environ. Sci. Technol.*, 2013, **47**, 5746–5754.

16 A. Drott, L. Lambertsson, E. Björn and U. Skyllberg, Do Potential Methylation Rates Reflect Accumulated Methyl Mercury in Contaminated Sediments?, *Environ. Sci. Technol.*, 2008, **42**, 153–158.
17 C. G. J. Hoggarth, B. D. Hall and C. P. J. Mitchell, Mercury methylation in high and low-sulphate impacted wetland ponds within the prairie pothole region of North America., *Environ Pollut*, 2015, **205**, 269–277.
18 J. Stamenkovic, M. S. Gustin, M. C. Marvin-DiPasquale, B. A. Thomas and J. L. Agee, Distribution of total and methyl mercury in sediments along Steamboat Creek (Nevada, USA), *Science of the Total Environment*, 2004, **322**, 167–177.
19 I. Tjerngren, M. Meili, E. Björn and U. Skyllberg, Eight Boreal Wetlands as Sources and Sinks for Methyl Mercury in Relation to Soil Acidity, C/N Ratio, and Small-Scale Flooding, *Environ. Sci. Technol.*, 2012, **46**, 8052–8060.
20 L. Windham-Myers, J. A. Fleck, J. T. Ackerman, M. Marvin-DiPasquale, C. A. Stricker, W. A. Heim, P. A. M. Bachand, C. A. Eagles-Smith, G. Gill, M. Stephenson and C. N. Alpers, Mercury cycling in agricultural and managed wetlands: a synthesis of methylmercury production, hydrologic export, and bioaccumulation from an integrated field study., *Sci Total Environ*, 2014, **484**, 221–231.
21 B. Ouddane, M. Monperrus, M. Kadlecova, M. Daye and D. Amouroux, Mercury methylation and demethylation in highly contaminated sediments from the Deûle River in Northern France using species-specific enriched stable isotopes., *Environ Sci Process Impacts*, 2015, **17**, 145–155.
22 A. G. Bravo, S. Bouchet, S. Guédron, D. Amouroux, J. Dominik and J. Zopfi, High methylmercury production under ferruginous conditions in sediments impacted by sewage treatment plant discharges, *Water Research*, 2015, **80**, 245–255.
23 I. Lehnherr, V. L. St. Louis and J. L. Kirk, *Environmental Science & Technology*, 2012, 46, 10523–10531.
24 N. W. Johnson, C. P. J. Mitchell, D. R. Engstrom, L. T. Bailey, J. K. Coleman Wasik and M. E. Berndt, Methylmercury production in a chronically sulfate-impacted sub-boreal wetland, *Environ. Sci.: Processes Impacts*, 2016, **18**, 725–734.
25 A. J. Houben, R. D'Onofrio, S. V. Kokelj and J. M. Blais, Factors Affecting Elevated Arsenic and Methyl Mercury Concentrations in Small Shield Lakes Surrounding Gold Mines near the Yellowknife, NT, (Canada) Region, *PloS one*, 2016, **11**, e0150960.
26 J. M. Gunn, Restoring the Smelter-Damaged Landscape Near Sudbury, Canada, *Restoration & Management Notes*, 1996, **14**, 129–136.
27 E. Gorham and A. G. Gordon, *Canadian Journal of Botany*, 1960, 38, 477–487.
28 M. Sullivan, *Tainted Earth : smelters, public health, and the environment*, Rutgers University Press, New Brunswick, New Jersey ;, 2014.
29 J. S.-J. Cheung, X. F. Hu, R. P. Parajuli, R. Rosol, A. Torng, A. Mohapatra, E. Lye and H. M. Chan, Health risk assessment of arsenic exposure among the residents in Ndilǫ, Dettah, and Yellowknife, Northwest Territories, Canada, *International Journal of Hygiene and Environmental Health*, 2020, **230**, 113623.

30 J. Sandlos and A. Keeling, *Giant Mine : historical summary*, Memorial University, St. John's, N.L, 2015.

31 O. W. Saarinen, *From meteorite impact to constellation city: A historical geography of greater Sudbury*, Wilfrid Laurier University Press, 2013.

32 G. Bridge, CONTESTED TERRAIN: Mining and the Environment, *Annu. Rev. Environ. Resour.*, 2004, **29**, 205–259.