Riverbank Mercury Methylation Study

Technical Report

May 14, 2024

Submitted by:

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The numerical data presented in this report are validated and certified. The interpretations are based on best available information at this time, and may be subject to change pending further information and expert input.

The opinions expressed in this report are the opinions of the author and should not be represented as the opinions of ANA, the EWRR Panel, or any other party.

Brian Branfireun at London, Canada. May 14, 2024

1 Plain-Language Summary

As part of a larger study of where methylmercury is produced in the Wabigoon River system, a laboratory experiment was done to determine if the water that is discharged from the Dryden Mill to the Wabigoon River makes the methylmercury contamination problem in the Wabigoon River ecosystem worse than it would otherwise be. This water does not contain additional mercury, however it does have high levels **sulphate** and **organic matter**; both are ingredients that are well-known to feed the bacteria that produce methylmercury from inorganic mercury in the environment.

Mercury contaminated sediments were added to small bottles, and water with different chemistries were added to the bottles, including different levels of pure sulphate, and different levels of Mill effluent. Bottles were sampled at different times over a month, and methylmercury and other chemistry was measured in the experimental water.

The results of the experiment showed that when added to mercury contaminated sediments, the Mill effluent containing sulphate and organic matter results in the production of large amounts of methylmercury. At low levels of sulphate, ~50% more methylmercury was produced than with upstream Wabigoon River water that is low in sulphate. At higher levels of sulphate that are like those that are found in the Wabigoon River just downstream of the mill, almost 3 times more methylmercury was formed.

These results clearly indicate that the Dryden Mill industrial wastewater that is discharged to the Wabigoon River is making the mercury contamination in fish worse than it would be if it were not present. The increase in sulphate in the Wabigoon River from the mill source can been seen all the way to Ball Lake, so the impact of this chemistry on methylmercury levels is throughout the river system. Although both mercury and methylmercury levels are very high because of the historical release of mercury to the river in the 1960s which still requires remediation, the problem is amplified by the current mill wastewater discharges. If these discharges were eliminated, there would be a reduction in methylmercury produced in the river system, and a reduction of methylmercury in fish.

2 Acknowledgements

Eric Grimm conducted this experiment as part of his MSc thesis research at the University of Western Ontario under the supervision of Dr. Branfireun. He provided figures, data tables, and some methodological information that are included in this report. The written interpretation of the data in this report are solely that of Dr. Brian Branfireun.

List of Abbreviations

Cl^-	Chloride
DI	Deionized water
DMA	Direct Mercury Analysis
DOC	Dissolved Organic Carbon
EPA	Environmental Protection Agency
HCl	Hydrochloric acid
Hg	Mercury
IHg	Inorganic Mercury
MeHg	Methyl Mercury
QA/QC	Quality Assurance/ Quality Control
RPD	Relative Percent Difference
Sed	Sediment
SO_4^{2-}	Sulphate
S^-	Sulphide
SW	Surface Water
THg	Total Mercury

3 Introduction

3.1 Project Background and Overview

The overall purpose of this project was to characterize the impacts of elevated sulfate from Dryden Mill effluent on mercury methylation in the English-Wabigoon Rivers System, and evaluate porewater concentrations of Hg, methylmercury and relevant parameters affecting MeHg production (e.g. sulfate, DOC, sulfide, chloride) in the Wabigoon River sediments.

Recent studies have shown that there is significant methylation of mercury in the Wabigoon River downstream of the Dryden Mill (as shown by both the persistent increase in river water methylmercury concentrations, the percent of total mercury as methylmercury, and elevated methylmercury in biota). While there are several factors that could contribute to mercury methylation, one significant question is whether the current Mill wastewater effluent could enhance methylation, amplifying the existing issue of contamination by excess inorganic mercury from historical inputs. Addressing this knowledge gap has been identified as a high priority by EWRRP in the data priorities table: "Evaluation of water quality parameters affecting methylmercury production, e.g., sulphate, DOC, pH, chloride, temperature). Consider an experimental study to identify the contributions of these factors."

It has been established for nearly 40 years that sulphate-reducing bacteria (SRB) are methylators of inorganic mercury[1], and are the principal mercury methylators in freshwater ecosystems[2], responsible for forming the species of mercury that bioaccumulates and biomagnifies (methylmercury). As their name indicates, SRB require sulphate (to 'breathe'), and also organic matter (to 'eat') for their metabolism and growth. The 'respiration' product of sulphate-reduction is sulphide. When SRB are active in an environment where available inorganic mercury is present, the process of mercury methylation can occur within the bacterial cell, resulting in the conversion of inorganic mercury to the more toxic methylmercury. Therefore the mercury methylating activity of SRB is regulated by the supply of three ingredients: organic matter, sulphate and bioavailable inorganic mercury. It is well established that even in environments with background levels of mercury and sulphate, the addition of a sulphate can substantially increase the proportion of mercury that is transformed into methyl mercury in porewater and surface waters[3]; sulphate supply is typically the limiting factor on the mercury methylation process in most freshwater ecosystems under the required biogeochemical conditions. The availability of organic matter is equally important for the mercury methylation process[4]. Although often not limited in an absolute sense, all organic matter is not created equal for

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1 Compeau GC, Bartha R. **1985**. Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. Appl Environ Microbiol. 50(2):498-502.

2 Gilmour CC, Henry EA, and Mitchell R. **1992**. Sulfate stimulation of mercury methylation in freshwater sediments. Environmental Science & Technology, 26: 2281–2287.

3 Jeremiason, J. D.; Engstrom, D. R.; Swain, E. B.; Nater, E. A.; Johnson, B. M.; Almendinger, J. E.; Monson, B. A.; Kolka, R. K. **2006.** Sulfate addition increases methylmercury production in an experimental wetland. Environ. Sci. Technol. 2006, 40, 3800−3806.

4 Ravichandran, M. **2004**. Interactions between mercury and dissolved organic matter--a review. Chemosphere, 55(3), 319-331.

bacterial metabolism – for example the addition of easily digested dissolved organic matter can be just as important as the addition of sulphate, resulting in the amplification of the sulphate reduction and mercury methylation process[5].

Our prior research (Nearshore Riverbank Sediment and Pore Water Sampling near the Dryden Mill Final Report, 2021) clearly demonstrated that water concentrations of sulphate in the Wabigoon River increased from a mean of 1.61 mg/L upstream of the Dryden Mill to an overall mean of greater than 12 mg/L (with individual samples over 20 mg/L during summer flows) downstream of the continuous discharge of mill aeration stabilization basin (ASB) effluent from a diffuser in the middle of the Wabigoon River (an average increase of about ten times the upstream concentrations). We hypothesized in that report that these elevated sulphate concentrations likely contribute to increased methylation downstream.

3.2 Objectives

This project proposal identified the elevated sulphate concentrations in surface waters as an issue potentially contributing to MeHg production. The work done tested two central hypotheses relating to the role of Dryden Mill effluent discharge on methylmercury formation downstream:

Hypothesis 1: Given that the sulphate-reducing bacteria have been well-established as principle methylators of inorganic mercury in freshwater systems for nearly 40 years, the continuous addition of excess sulphate from the current mill operations in Dryden significantly increases methylmercury formation in this otherwise sulphate-limited environment at both local and more distant locations where conditions suitable for mercury methylation exist.

Hypothesis 2: Low-lying floodplain sediments and wetlands immediately adjacent to the river channel that are contaminated with inorganic mercury are "hot spots" of mercury methylation, with the highest concentrations of methylmercury associated with sediments receiving additional sulphate from upstream.

Information collected will help to understand:

- If the continuous addition of excess sulphate from current Dryden Mill operations significantly increases methyl mercury formation in an otherwise sulphate-limited environment
- If low-lying floodplain sediments and wetlands immediately adjacent to the river are potential hot spots of mercury methylation
- How methylmercury production potential (reflected by % of total mercury as methyl mercury, and partitioning between sediments and porewaters) varies downstream in the English-Wabigoon River System.

This technical report focusses on Hypothesis 1, which was tested using a laboratory approach. Field data associated with Hypothesis 2 will be reported on subsequently.

[5] Mitchell, CPJ, BA Branfireun, and RK Kolka, **2008**. Assessing sulfate and carbon controls on net methylmercury production in peatlands: An in situ mesocosm approach, Applied Geochemistry, 23, 503-518.

4 Methods – Sampling & Analysis

4.1 Laboratory Experiment Methods

Previous experiments in the Branfireun Lab have been successful in using glass chromatography columns to test the effects of sulphate addition upon peatland soils, and the current ANA methylation project proposed to use the same general approach. Sediments collected from the mercury contaminated riparian wetland upstream of the effluent discharge were uniformly much finer-grained than anticipated, making the use of a flow through column experiment unfeasible. During preliminary testing, very low hydraulic conductivity (water-passing ability) resulted in a build up of back-pressure in the columns even at flow rates that were minimally feasible to collect sufficient sample volumes in a reasonable time. This created very unrealistic flow conditions, leaks from sample lines, inconsistent flow between columns, and visual channelling of flow along the glass column bypassing sediments. These issues necessitate a change in experimental design and protocol.

A bottle incubation protocol was used to address the question of the effects of different levels sulphate and Mill Effluent containing sulphate on mercury methylation. The experimental treatments were effectively the same as proposed, however instead of columns, Hg contaminated sediments and the various solutions of pure sulphate Mill effluent containing sulphate and were placed in 500ml PETG bottles and destructively sampled over time. Bottle incubations such as this have also been used successfully in the Branfireun lab to assess mercury methylation processes in contaminated sediment.

70 g of sediment collected from a mercury-contaminated wetland downstream of the historic mercury source at the Dryden mill and upstream of the contemporary sulphate source at the operational mill discharge (**Hg concentration 49 mg/kg**), and 70 g of sediment and 140 mL of solution were added to each bottle and allowed to react for between 3 and 28 days. Each bottle was purged with nitrogen gas to expedite the establishment of reducing conditions. and 140 mL of the various solutions (Table 1) were placed in each bottle, mixed, and purged with nitrogen gas to promote reducing conditions. Triplicate samples (3 bottles) were destructively sampled at prescribed intervals (Table 2) from each treatment level on each sampling day, and bottles were gently agitated daily.

Table 2.1. Bottle experiment treatment levels and sample numbers.

Treatment	Description	Number of Samples
Deionized Water – Control	Control – Ultra clean filtered water.	33
Upstream Wabigoon River Water	Control – Unimpacted Wabigoon river water from upstream of the Dryden mill.	33
2 mg/L SO_4^{2-} in Deionized Water	Low Sulphate – Made with pure laboratory reagent (K_2SO_4).	33
10 mg/L SO_4^{2-} in Deionized Water	Medium Sulphate – Made with pure laboratory reagent (K_2SO_4).	33
30 mg/L SO_4^{2-} in Deionized Water	High Sulphate – Made with pure laboratory reagent (K_2SO_4).	33
2 mg/L SO_4^{2-} Effluent Solution	Low Sulphate – Made with diluted effluent collected from the Dryden mill effluent diffuser.	33
10 mg/L SO_4^{2-} Effluent Solution	Medium Sulphate – Made with diluted effluent collected from the Dryden mill effluent diffuser.	33
30 mg/L SO_4^{2-} Effluent Solution	High Sulphate – Made with diluted effluent collected from the Dryden mill effluent diffuser.	33
TOTAL		**264**

Table 2.2. Sampling schedule, including reaction time.

Sample #	*Reaction Time*	*Sampling Date*
Sample 1	3 Days	November 26th
Sample 2	4 Days	November 27th
Sample 3	5 Days	November 28th
Sample 4	7 Days	November 30th
Sample 5	9 Days	December 2nd
Sample 6	11 Days	December 4th
Sample 7	14 Days	December 7th
Sample 8	17 Days	December 10th
Sample 9	20 Days	December 13th
Sample 10	24 Days	December 17th
Sample 11	28 Days	December 21st

On each sampling day redox potential were measured directly using a platinum redox electrode in both the supernatant water and the sediment of each bottle. A subsample was taken for sulphide, sulphate and DOC analyses. Supernatant water was centrifuged at 4000 RPM for 20 minutes, filtered with a 0.45 um, then preserved with 0.5% v/v ultrapure HCl prior to analyses for THg and MeHg.

Water samples were analysed for Total Mercury (THg) and Methylmercury (MeHg), SO_4^{2-}, dissolved HS^- DOC, and dissolved organic matter characterization analysis using fluorescence and absorbance spectroscopy.

Benefits of the Bottle Experiment Approach: Although very different from the column experiment, this approach more closely mimicked the interaction of river water with interface sediments. We were also able to measure redox potential directly (not possible with the column experiment). We were also able to recover and measure THg and MeHg in sediment for each sampled bottle, providing a time series in sediment that would not be possible from the column experiment.

4.2 Laboratory Methods

THg and MeHg analyses were conducted in the Biotron Analytical Laboratory at Western University. This is an ISO17025-accredited laboratory and as such, all of the analytical processes that are within its accredited scope comply fully with accepted standard methods. Quality assurance and quality control measures are in place both during the analytical process that meet or exceed the standard methods, and all certified data are reviewed independent of analyses prior to release.

All water samples were filtered through a 0.45 um filter and one aliquot preserved with 0.5% v/v HCl for subsequent analyses for mercury and methylmercury, and another aliquot transferred into pre-cleaned 60 ml amber glass bottles and stored in the dark at 4 °C until analyses for DOC, DOM characteristics, and ions. Sulphide samples were transferred directly into a new, sterile vaccutainer® and analysed immediately.

2.1.1 Total Mercury - Water

Overlying water from the experimental bottles were all analyzed for THg, following EPA method 1631. The day before analysis, samples are brominated. 25 mL of sample is decanted into a glass vial. 125 µL of BrCl is pipetted into each sample vial (including blanks, duplicates, calibration blanks and calibration standards, IPR, and OPR), shaken, and left for analysis the next day. New calibration standards, IPRs, and OPRs are made the day of analysis. Quantification of THg is by CVAFS detection on a Tekran® 2600 total mercury analysis system. Before being loaded into the autosample rack, 30 µL of hydroxylamine hydrochloride and 60 µL of $SnCl_2$ is added to each vial, waiting 30 min between each addition and before analysis. For QA/QC, acceptable recoveries are 71-125% with an RPD < 24% for matrix spikes, < 0.2 ng/L for calibration blanks, < 0.5 ng /L for method blanks, and RPD < 20 % for duplicates.

2.1.2 Methylmercury - Water

Water samples were analyzed for methyl mercury, following EPA method 1630. To analyze MeHg there are two main steps, 1) distilling MeHg out of a sample to eliminate environmental interferences (e.g., organic matter), and 2) quantifying MeHg by aqueous phase ethylation, chromatographic separations, and CVAFS detection in the Tekran® 2700 methylmercury analysis system. 40 mL of sample is poured into a Teflon distillation vessel and capped with a distillation cap. For every 10 samples in the analysis run there is a duplicate sample, a matrix spike sample, a matrix spike duplicate sample, and a method blank. To each prepared Teflon vessel, 180 µL of 1% APDC is added before beginning distillation. Samples are distilled at 125 °C purging with nitrogen gas, in a Tekran distillation unit until 40-48 mL of sample is transferred in the chilled receiving vial.

Following distillation, 30 mL of distilled sample is decanted into a new instrument vial and loaded into the Tekran 2700 sample rack. Reagents (30 µL ascorbic acid, 225 µL buffer solution, 30 µL $NaBet_4$) are added to each sample vial and shaken after each addition to mix thoroughly. Each set of samples includes a set of sample duplicates, matrix spikes, and method blank. For QA/QC, acceptable recoveries are 100 +/- 35% with an < 35% for method spikes, < 0.01 ng/L for reagent blanks, < 0.045 ng CH_3Hg/L for method blanks, and RPD < 35% for duplicates.

2.1.3 Dissolved Organic Carbon

Overlying water in the experimental water samples were analyzed for dissolved organic carbon (DOC) using an OI Analytical Aurora 1030 total organic carbon (TOC) analyser (Xylem, College Station, TX) at the Branfireun Lab, in London, ON. Each run included Milli Q deionized (DI) water blanks, check standards (1 ppm, 5 ppm, 10 ppm, 25 ppm, and 100 ppm) and a sample duplicate at least every 8-10 samples. The minimum detection limit for TOC is 0.01 mg/L C.

2.1.4 Major Anions

Concentrations of target anions (chloride, sulfate) in surface water and porewater samples were analysed using a Dionex® DX-1600 Ion Chromatography System (ICS - Thermo Scientific, Waltham, MA) at the Branfireun Lab. A 0.5 mL aliquot of each pre-filtered sample was dispensed into a sampling vial and a filter cap was placed on top. Each run included two Milli Q deionized (DI) water blanks, matrix spikes, check standards (5 ppm, 10 ppm, and 25 ppm) and a sample duplicate at least every 8-10 samples. If the samples had analyte concentrations outside the range of calibration, they were diluted with Millipore DI water and rerun. The minimum detection limit of the ICS is 0.025 mg/L.

2.1.5 Sulphide

Sulphide analysis was completed immediately during destructive sampling of incubation bottles according to the methylene blue method described in Cline (1969)[6]. 5 mL of supernatant water was extracted under nitrogen and immediately injected into a vacutainer tube to prevent sample oxidation. Each sample reacted with 0.4 mL of a diamene reagent for 20 minutes before measuring absorbance at 670 nm with an integration time of 0.1 s using a Horiba Aqualog spectrofluorometer.

The diamene reagent contains N,N-dimethyl-p-phenylenediamine which reacts with sulphide to form a blue colour. Standard concentration curves were prepared using the same procedure described above, using deoxygenated water and differing concentrations of disodium sulfide nonahydrate.

[6] Cline, J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. Limnology and Oceanography, 14(3), 454-458.

5 Results & Interpretation: Laboratory Methylation Study

5.1 Reduction-Oxidation Conditions in Experimental Water and Sediment

In the first 5-6 days of the experiment measured redox values indicated that the incubation water in all treatments was still oxic or sub-oxic, which would not support sulphate reduction or Hg methylation (typically at ~ - 200mV) (Figure 1). By Day 7 all treatment waters (including those with no additional sulphate) were strongly anaerobic without significant differentiation and remained so throughout the experiment (Figure 3.1).

Figure 3.1: Measured redox potential (Eh) in the incubation bottle **water** over the course of the experiment. D = Deionized water. R = Wabigoon River water. S2, S10, S30 = sulphate only treatments at 2, 10 and 30 mg/L.

In contrast, redox potentials in sediments were all sub-oxic at the beginning of the experiment, becoming increasingly negative, settling into deeply anaerobic conditions -200 to -300mV) that would support sulphate reduction and methylation (Figure 3.2).

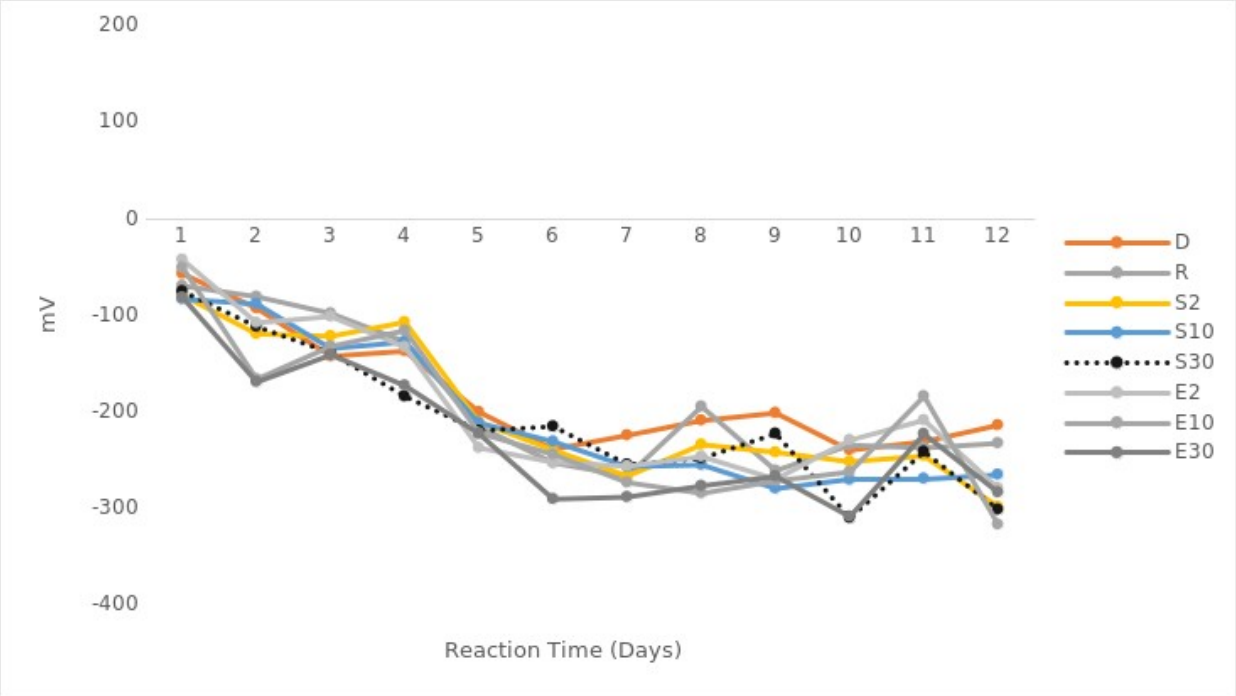

Figure 3.2: Measured redox potential (Eh) in the incubation bottle **sediment** over the course of the experiment. D = Deionized water. R = Wabigoon River water. S2, S10, S30 = sulphate only treatments at 2, 10 and 30 mg/L.

From these data, we can conclude all of the experimental treatments and replicates had suitable biogeochemical conditions to support sulphate reduction and mercury methylation by Day 7 of the incubation.

5.2 Methylmercury Concentrations in Incubation Waters

Methylmercury concentrations in all incubation bottles began clearly increasing at incubation day 7 when the redox potentials in both the sediment and overlying water were sufficiently negative (Figure 3A and 3B). Methylmercury concentrations even increased in the control treatment with only ultra-pure deionized water added, indicating that MeHg was released into overlying water through desorption from the sediments which contained a pre-existing pool of MeHg, or that there was a sufficient amount of oxidized sulphate present in the solid phase or in the field moist sediment to support reasonable rates of Hg methylation (Figure 3.3). The deionized water treatment produced a nearly equivalent amount of MeHg over the course of the incubation, indicating that the additional sulphate found in the Wabigoon River water (1.3 mg/L for the water used in the experiment) had no significant overall impact on MeHg production in this experiment. After Day 9, the average MeHg concentrations were 103.36 and 123.50 ng/L in Deionized water and Wabigoon River water treatments, respectively (Table 3.1).

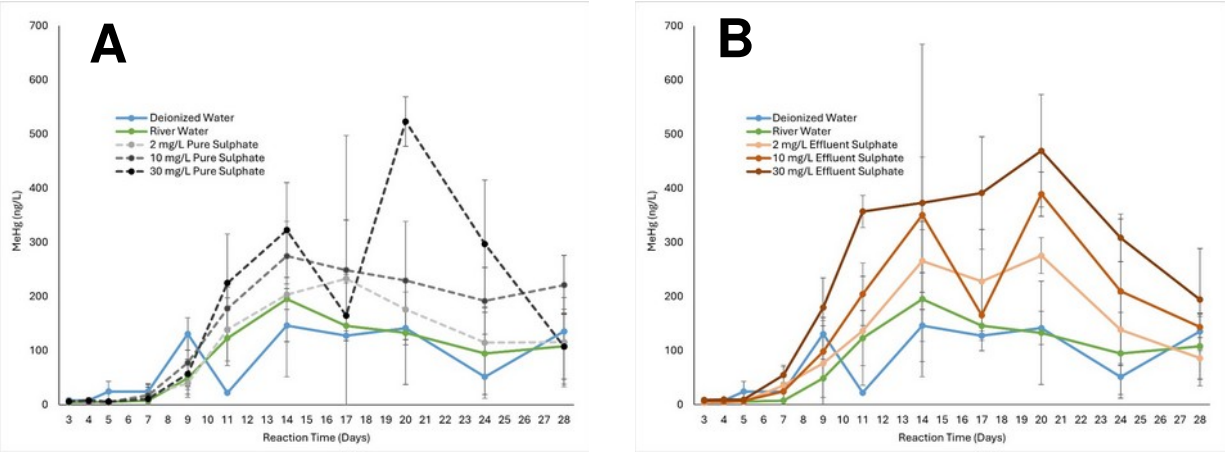

Figure 3.3: Methylmercury (MeHg) concentrations (ng/L) in supernatant water over the duration of the bottle incubation experiment. Sulphate in ultrapure water treatments are presented in Figure 3A, and the sulphate in mill-derived effluent treatments are presented in figure 3B. Deionized water and Wabigoon river water treatments are presented on both panels. Error bars are the standard deviation among replicates.

For the experimental treatments of both sulphate in ultrapure water and mill-derived effluent containing sulphate, average MeHg concentrations exhibited a pattern of clear increase between day 7-11, a stabilization until day 20-22, then some evidence of decline. This pattern is what would be expected in a microbial incubation, where bacteria have both nutrients and organic matter to increase their population and activity (exponential growth phase), and then maintain metabolism with available resources (stationary phase) until a required metabolite becomes limiting or the environment toxic. Since no additional nutrients (sulphate) or organic matter was added to the experiment, it was expected that at some point the experimental conditions would become unfavourable for bacterial metabolism. This would not occur in the natural environment where nutrient supply and organic matter inputs are replenished from advective water flows, plant roots, soil biota, etc. The achievement of stationary phase allows us to examine differences among experimental treatments under these (relatively) more stable conditions (i.e. between days 9 and 24 of the experiment).

Methylmercury concentrations increased in all of the sulphate addition treatments, with higher MeHg concentrations associated with the higher sulphate additions. MeHg concentration increases were not proportional with sulphate concentrations (i.e. they do not increase proportionally to the same degree) but are still in increasing order with treatment level (Table 5.1). Relative to Wabigoon River water with sulphate concentrations of 1.3 mg/L, it is consistent that the 2 mg/L pure sulphate treatment would result in slightly higher concentrations. Indeed, MeHg concentrations are 22% higher, in line with the 53% higher sulphate amount and the overall sub-linear response (Table 5.2). The relative percent difference between Wabigoon River water and the 2 mg/L mill effluent sulphate treatment was over twice as great as for the pure sulphate treatment alone (51% vs 22 %) indicating a dramatic and important additive effect of the Dryden Mill effluent chemistry.

Table 3.1. Average methylmercury (MeHg) concentrations (ng/L) measured in supernatant water from experimental samples during stationary phase of bottle incubation (Day 9-24).

Treatment Level	Average MeHg in Supernatant Water (ng/L)
Deionized Water	103.36
Upstream Wabigoon River Water	123.50
2 mg/L Pure Sulphate	151.01
10 mg/L Pure Sulphate	200.04
30mg/L Pure Sulphate	264.96
2 mg/L Mill Effluent Sulphate	186.80
10 mg/L Mill Effluent Sulphate	236.29
30mg/L Mill Effluent Sulphate	346.52

Table 3.1. % difference in methylmercury concentrations of experimental treatments relative to deionized water control and Wabigoon River water (1.3 mg/L sulphate) during the stationary phase of bottle incubation (Day 9-24).

Treatment Level	Increase in MeHg (%) above Deionized Water	Increase in MeHg (%) above Upstream Wabigoon River Water
2 mg/L Pure Sulphate	46%	22%
10 mg/L Pure Sulphate	94%	62%
30mg/L Pure Sulphate	156%	115%
2 mg/L Mill Effluent Sulphate	81%	51%
10 mg/L Mill Effluent Sulphate	129%	91%
30mg/L Mill Effluent Sulphate	235%	181%

This relative enhancement of Hg methylation by mill effluent over pure sulphate was clear for all treatments. At the highest levels of sulphate addition (30 mg/L), the mill effluent treated bottles had MeHg concentrations that were 2.8 times greater the Wabigoon River water treatment, and 3.4 times greater than the deionized water treatment. Even at the lowest level 2 mg/L sulphate treatment in mill effluent, these increases were 1.5 and 1.8 times greater, respectively. It is clear that the addition of mill effluent (which at the discharge point after mixing with river water can routinely exceed 50 mg/L) substantially enhanced MeHg production in the laboratory experiment. The addition of sulphate increased MeHg production consistent with pre-existing scientific knowledge; the additive effect of the other mill effluent chemistry on MeHg production paints an alarming picture for the conditions in the English-Wabigoon River system, where increases in sulphate and dissolved organic matter due to the mill discharges are evident as far down as data clearly exist (Ball Lake outflow, and potentially beyond).

5.3 Total Mercury and Percent of Total Mercury as Methylmercury

It would be expected that THg in overlying water in the bottle experiments would increase for two reasons: 1) Thg comprises all forms of mercury, and therefore contains BOTH inorganic Hg and MeHg. Since MeHg increased substantially in the experimental treatments, and we may reason that this MeHg was derived from sediment pore waters, then THg would increase at least proportionally to that increase in MeHg. 2) We may also expect the fraction of THg as IHg to increase given the level of Hg contamination in the experimental sediments. Given the concentration difference, there would be diffusion of IHg from sediment porewaters to the overlying waters due to the concentration gradient.

Consistent with this, changes in THg concentrations over time (Figure 3.4) mirrored the changes in concentrations in MeHg (Figure 3.3). The pattern over time and the relative magnitude of changes (increases) mirrored those of MeHg largely because a substantial fraction of THg in the overlying water was MeHg (Figure 3.5).

During the stationary phase of the experiment. the overall differences %MeHg generally fall in line with the relative amounts of sulphate added (30>10>2 mg/L) (Figure 3.5). The sulphate source (Mill Effluent Sulphate vs. pure Sulphate) trends were less differentiated, however all treatments subjected to Mill Effluent had more consistent, and higher %MeHg. During the stationary phase of the experiment, all Mill Effluent sulphate treatments had >60% MeHg; a very high fraction given that there is no fresh inputs of either sulphate or organic matter.

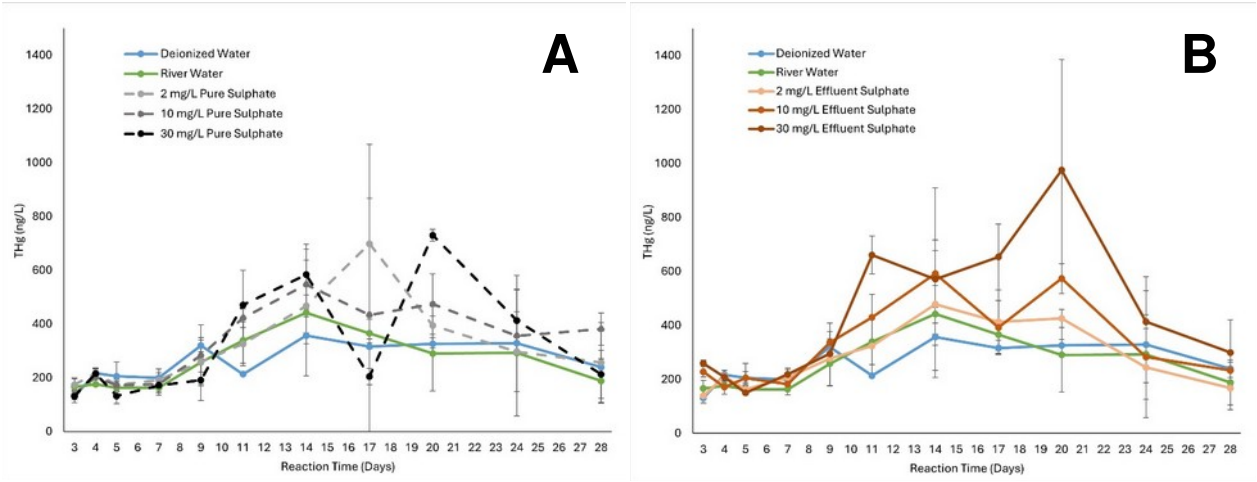

Figure 3.4: Total Mercury (THg) concentrations (ng/L)
in supernatant water over the duration of the bottle incubation experiment. Sulphate in ultrapure water treatments are presented in Figure 3A, and the sulphate in mill-derived effluent treatments are presented in figure 3B. Deionized water and Wabigoon river water treatments are presented on both panels. Error bars are the standard deviation among replicates.

The inorganic fraction of THg (IHg, calculated at THg – MeHg) showed an almost immediate partitioning of a substantial amount of IHg from sediment and sediment pore waters to the overlying water (Figure 3.6). Given that the deionized water treatment had no measurable Hg of any form, this partitioning was not only rapid, it remained relatively constant over time and across treatments suggesting that there was no other biogeochemical factor other than concentration difference that was driving the transfer of IHg into overlying water. This finding is relevant, because it revealed that the exchange of "clean" water with contaminated sediments resulted in the rapid partitioning of 100s of ng/L of IHg into overlying water in the bottle experiments; concentrations that are 2 orders of magnitude above background Wabigoon River water concentrations. Although not an explicit objective of this experiment, it demonstrates that the Hg in legacy contaminated sediments in the English-Wabigoon system is easily partitioned into pore waters and surface waters.

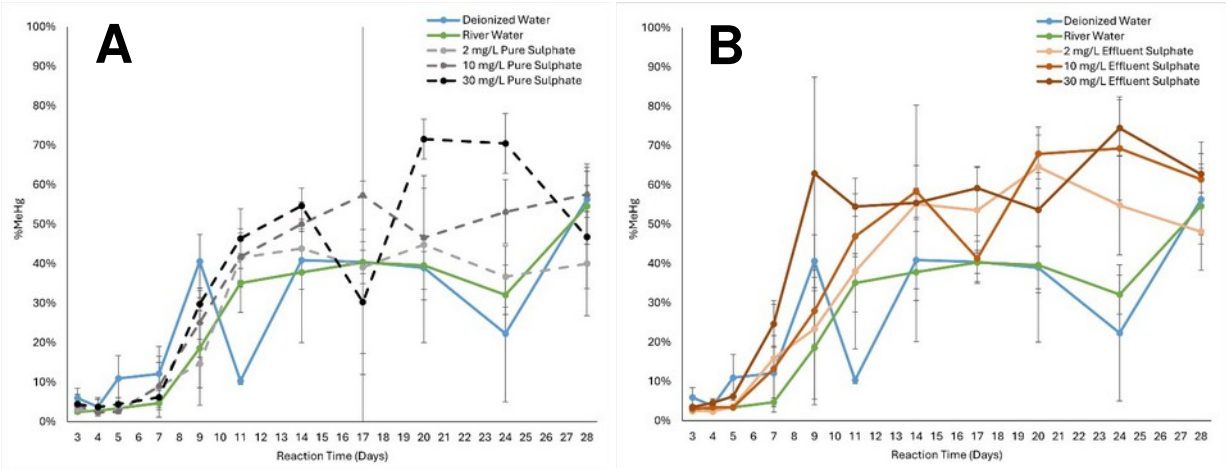

Figure 3.5: Percent of Total Mercury as Methylmercury (%MeHg) in supernatant water over the duration of the bottle incubation experiment. Sulphate in ultrapure water treatments are presented in Figure 3A, and the sulphate in mill-derived effluent treatments are presented in figure 3B. Deionized water and Wabigoon river water treatments are presented on both panels. Error bars are the standard deviation among replicates.

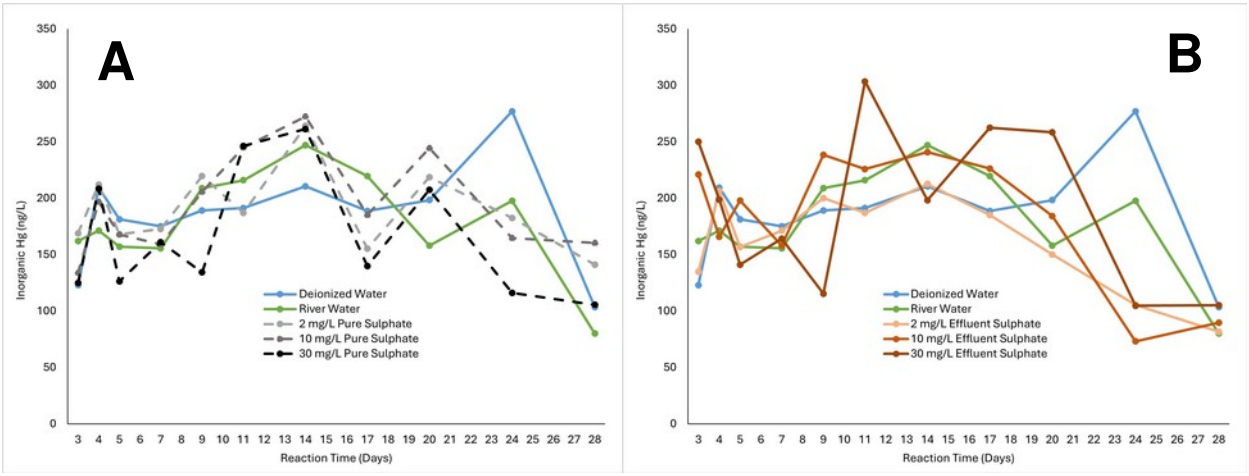

Figure 3.6: Inorganic mercury (IHg) in supernatant water over the duration of the bottle incubation experiment. Sulphate in ultrapure water treatments are presented in Figure 3A, and the sulphate in mill-derived effluent treatments are presented in figure 3B. Deionized water and Wabigoon river water treatments are presented on both panels. **Error bars to be added to this figure**

5.4 Sulphide Concentrations in Incubation Waters

Dissolved sulphide concentrations unsurprisingly increased in all of the incubation flasks, indicating that active sulphate reduction was occurring in all bottles, including the deionized water treatments (Figure 3.7). Since no additional sulphate was associated with these 'controls' it is clear that there was sufficient available sulphur in the incubation sediments to support Hg methylation and produce the MeHg concentrations measured (Figure 3.3).

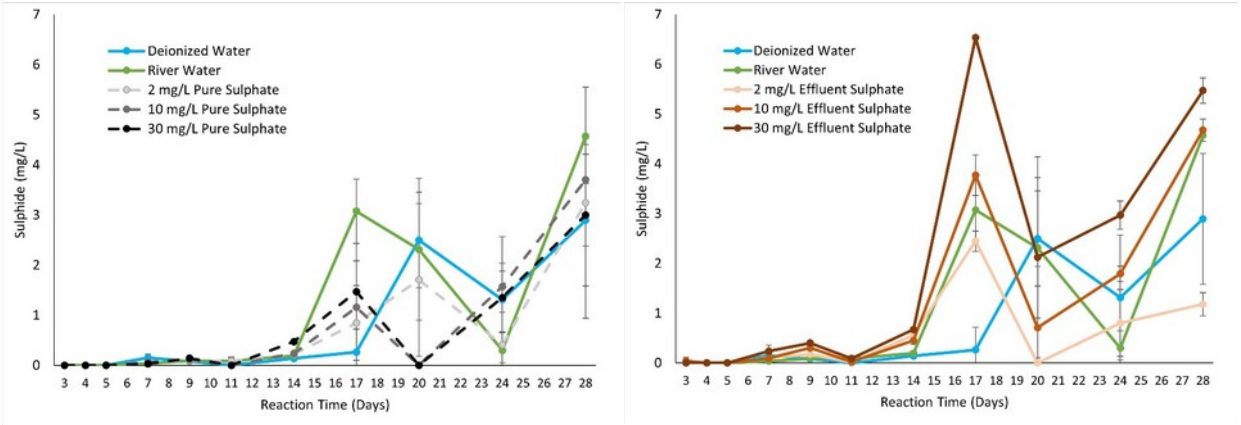

Figure 3.7: Dissolved sulphide (S^-) in supernatant water over the duration of the bottle incubation experiment. Sulphate in ultrapure water treatments are presented in Figure 3A, and the sulphate in mill-derived effluent treatments are presented in figure 3B. Deionized water and Wabigoon river water treatments are presented on both panels. Error bars are the standard deviation among replicates.

Dissolved sulphide concentrations in the experimental treatments did NOT correspond with sulphate addition levels, which indicates that at the highest sulphate concentrations there is likely the formation of precipitated insoluble S^- complexes, and/or an inhibition of sulphate reduction by sulphide toxicity. Indeed in the pure sulphate treatments, the sulphate addition treatments were no different, or even lower than the deionized water and Wabigoon River water treatments. In contrast, the provision of additional sulphate in Mill Effluent generally produced more sulphide than an equivalent treatment of pure sulphate, indicating that the sulphate reduction process was intensified by the Mill Effluent chemistry, or that sulphide was maintained in solution to a greater degree because of differences in water chemistry. **It is my opinion that the former is more likely, and that the provision of both sulphide and bioavailable organic matter in the Mill Effluent intensifies the activity of sulphate reducers (as indicated by the concentration of the sulphide product) and thus produces more MeHg (as indicated by the absolute concentration of MeHg, and the relative difference in %MeHg).** During the stationary phase, there is a clear, positive relationship between MeHg and S^- ($r^2 = 0.32$) confirming that sulphate reduction/active mercury methylation is driving the release of MeHg into overlying waters, not desorption from an existing pool of MeHg in sediments.

6 EXPERIMENTAL LIMITATIONS AND APPLICATION TO FIELD CONDITIONS

As a bottle incubation with destructive sampling of separate replicate samples at each time interval, there was considerable and expected variability among replicates, and between sampling times. Despite homogenization of sediments used for the experiment, the relatively small amounts placed in each bottle have variable amounts of organic matter, mercury contamination, and even biological activity. Rather than compromising the interpretation of the results, the fact that the experimental treatments resulted in clear patterns *despite* this variability lends confidence that the mechanisms (and magnitudes of effect) are real.

The sulphate (and diluted effluent) added to the experimental bottles was only a single 'dose' at the beginning of the experiment. This dose was metabolized by the bacterial community over the duration of the experiment until the replicate was sampled, and was not replenished. Therefore the amount of sulphate added (and the designation given to each treatment) is merely the initial amount, which would decline over time until such time that it was completely metabolized. Under field conditions, not only would the supply of sulphate (and organic matter) be effectively continuous to the sediment-water interface and hydrologically-connected riverbank locations, but sulphide would also be transported away, reducing the potential for sulphide toxicity which would limit sulphate reduction rates. Therefore under field conditions in locations where suitable biogeochemical conditions exist, methylmercury production would be more continuous and potentially less rate limited. In locations that are subjected to more episodic flooding, the dose-response pattern may be more similar to what was observed in this experiment.

The sediments used in this experiment (from Wetland 1 immediately downstream of the Mill but upstream of the mill effluent diffuser were lower in organic matter content relative to other wetland sites that were sampled as part of this project; riparian wetlands with higher natural organic matter content in sediments would likely support higher rates of Hg methylation.

7 CONCLUSIONS

The results of the laboratory incubation experiment component of this project showed that:

1) the addition of water with even small amounts of additional sulphate increases the net production of methylmercury in mercury-contaminated river bank sediments.

2) the amount of methylmercury produced is positively related to the amount of sulphate added, but the increase in methylmercury production is not proportional (i.e. double the sulphate does not produce double the methylmercury).

3) Sulphate added as a component of Mill Effluent resulted in approximately double methylmercury being produced than just sulphate alone. Data from this experiment suggests that this is a result of greater activity/growth of sulphate-reducing bacteria, which are the primary methylators of inorganic mercury in freshwater systems. The mechanism of this enhanced microbial activity is the provision of bioavailable organic matter in the Mill Effluent water.

We can extend these clear results to the English-Wabigoon River system, and draw several broader conclusions that have important implications for the recovery of mercury contamination in fish:

4) The experimental results clearly support Hypothesis 1: *The continuous addition of excess sulphate from the current mill operations in Dryden significantly increases methylmercury formation in this otherwise sulphate-limited environment at both local and more distant locations where conditions suitable for mercury methylation exist.* The large increase in sulphate and organic matter above Wabigoon River background levels by past and current Dryden mill operations amplifies the methylation of mercury in contaminated sediments throughout the English-Wabigoon River system. Even considering the effects of dilution, this effect extends as far as the sulphate input from the Mill can be detected (at least to Ball Lake outflow) since the data from this experiment show that even small increases in sulphate above background levels increased methylmercury production.

5) Given the record of sulphate concentration data from the Wabigoon River and the results of this experiment, there is likely *at least* twice as much methylmercury being produced than would be expected under background sulphate and organic matter conditions. Given that the amount of methylmercury in fish is ultimately proportional to the amount of methylmercury available to biota, fish methylmercury is likely at least twice as high as would be expected under background sulphate and organic matter conditions.

6) The elimination of excess industrial sulphate and organic matter inputs to the Wabigoon River from the Dryden Mill would reduce the amount of methylmercury produced throughout the English-Wabigoon system, and reduce methylmercury in

aquatic organisms and fish. Based on published scientific literature, the reduction in methylmercury concentrations in water and biota could be relatively rapid (several years).

7) Since sediments are still highly mercury contaminated and there will always be background levels of methylmercury production, the amount of methylmercury present will still be well above what would be expected under uncontaminated conditions even when industrial sulphate and organic matter inputs are eliminated. Ultimately a reduction in the pool of available inorganic mercury for methylation is required to return the ecosystem to levels of mercury in fish that would be present without past and present mill impacts.