Dissolved Organic Matter Kinetically Controls Mercury Bioavailability to Bacteria in Lake Water from the Canadian Arctic

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Abstract

The repercussions of rapid climate-change are felt worldwide, but particularly in Arctic and Subarctic regions. Evidence of recent changes in water chemistry is being recorded in Arctic aquatic ecosystems, bringing further attention to contaminant dynamics in these environments.

I assessed the role of dissolved organic matter (DOM) in controlling the bioavailability of mercury (Hg), a top priority among Arctic contaminants, to aquatic food webs using a bacterial bioreporter under oxic conditions. Experiments were performed under pseudo- and non-equilibrium conditions, in both defined media and water samples from tundra lakes with a large gradient in DOM. Inorganic Hg^{II} was considerably more bioavailable under non-equilibrium conditions than when DOM was absent or when Hg^{II} and DOM had reached pseudoequilibrium (24h). Under these enhanced uptake conditions, Hg^{II} bioavailability followed a bell shaped curve as DOM concentrations increased, both for defined media and field samples, suggesting that complexation kinetics and binding thresholds on DOM determine Hg^{II} bioavailability to methylating bacteria, and likely MeHg concentrations, the bioaccumulative neurotoxic form of Hg. Experiments also suggest that DOM may alter cell wall properties to facilitate the first steps toward Hg^{II} internalization via facilitated or active transport, and yet without altering overall cell wall permeability.

While further research on ternary (Hg^{II}-cell-DOM) interaction is warranted, I propose a molecular shuttle model for DOM in facilitating bacterial Hg^{II} uptake, and the existence of a short-lived yet critical time window (<24h) during which DOM facilitates the entry of newly deposited Hg^{II} from the atmosphere into aquatic food webs.

Résumé

L'impact des changements climatiques est perçu partout dans le monde, mais particulièrement dans les régions arctiques et subarctiques où le réchauffement climatique est plus prononcé. Les écosystèmes aquatiques polaires ont récemment subi des changements sur le plan de la chimie des eaux, ce qui suscite un intérêt accru pour le comportement, la biodisponibilité et le sort des contaminants anthropiques dans cet environnement.

Cette étude examine le rôle de la matière organique dissoute (MOD) quant au contrôle de la biodisponibilité du mercure (Hg) dans les réseaux trophiques aquatiques en utilisant un biorapporteur bactérien sous conditions oxyques de pseudo- et non-équilibre avec la MOD. Que ce soit dans un milieu d'exposition synthétique ou dans les eaux naturelles des lacs de la toundra, le Hg était plus biodisponible en conditions de non-équilibre avec la MOD que lorsque la MOD était absente ou quand le Hg et la MOD avaient atteint un état de pseudo-équilibre (24h). Dans ces conditions de prise en charge accrue, la biodisponibilité du Hg suivait une courbe en forme de cloche suivant une augmentation de la concentration de MOD. Ces données suggèrent qu'un contrôle cinétique sur la spéciation du Hg par la MOD ainsi qu'un seuil de concentration de la MOD déterminent la biodisponibilité du Hg aux bactéries productrices de méthylmercure (MeHg). Les résultats de tests additionnels suggèrent que la MOD peut modifier les propriétés de la membrane biologique afin de faciliter l'internalisation du Hg par transport facilité ou actif, sans toutefois changer sa perméabilité.

Selon ces données préliminaires, je propose un modèle de navette moléculaire qui contribue à la prise en charge du Hg par les bactéries. De plus, je propose qu'il existe une fenêtre temporelle de courte durée (<24h) durant laquelle la MOD facilite l'entrée du Hg nouvellement déposé de l'atmosphère dans les réseaux trophiques aquatiques.

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Sincerely,

Sophie Chiasson-Gould

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Acronyms and Abbreviations

[x]	Concentration of x
AMDE	Atmospheric mercury depletion event
C	Carbon
CVAFS	Cold vapour atomic fluorescence spectroscopy
DOM	Dissolved organic matter
DOC	Dissolved organic carbon
FA	Fulvic acids
GMM	Glucose minimal medium
H^+	Hydrogen ion
HA	Humic acids
Hg	Mercury
Hg^{II}	Divalent mercury
Hg^0	Elemental, volatile mercury
Hg^R	Bacterial mercury resistance
$HgCl_2$	Mercury(II) chloride
$Hg(Cys)_x$	Mercury(II) cysteine complex
HgS	Mercury(II) sulfide, cinnabar
HS	Humic substances
IHSS	International Humic Substances Society
IPR	Initial precision and recovery
IRB	Iron-reducing bacteria
K_2HPO_4	Dipotassium phosphate
LB	Lysogeny broth
LPS	Lipopolysaccharide
lux	Genes conferring bacterial bioluminescence
MeHg	Methylmercury, monomethylmercury, CH_3Hg
mer	Genes conferring bacterial mercury resistance
merR	Gene which encodes the transcriptional regulator
NaH_2PO_4	Monosodium phosphate
$(NH_4)_2SO_4$	Ammonium Sulfate
NOM	Natural organic matter
NSERC	Natural Sciences and Engineering Research Council of Canada

NWT	Northwest Territories
OPR	Ongoing precision and recovery
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
S^{2-}	Sulfide ion
SRB	Sulfate-reducing bacteria
T_C	Threshold concentration
THg	Total mercury
U.S. EPA	U.S. Environmental Protection Agency

Glossary

Active transport	The movement of ions or molecules across a cell membrane assisted by enzymes and requiring energy
Anthropogenic	An effect or an object resulting from human activity
Bile salts	Products of bile acids compounded with a cation, usually sodium, occurring in the bile of mammals
Bioaccumulation	The build-up of a substance in an organism
Bioassay	A measurement of the concentration or effect of a substance by the response of living cells or tissues
Bioavailability	The proportion of a substance that is readily available to a living organism, therefore having a biological effect.
Biogeochemical cycle	The cycling of a chemical between living organisms and the physical environment.
Bioluminescence	The biochemical emission of light by living organisms.
Biomagnification	The non-linear increase in the concentration of a bioaccumulating substance as the food chain is ascended
Bioreporter	A living cell genetically engineered to produce a measurable signal in response to a chemical or physical agent
Biouptake	The process by which a substance crosses the cell membrane(s) to be taken up by a living organism.
Complexation	The bonding of an atom or ion to a surrounding array of molecules
Competitive ligand exchange	The exchange of ions and/or competitive interaction between ligands due to their varying binding strengths
Fractionation	The process of separating a mixture into component parts or fractions during a phase transition
Facilitated transport	The passive movement of ions or molecules across a cell membrane via transmembrane protein, down their concentration gradient
Functional groups/sites	A group of atoms or bonds within molecules responsible for the characteristic chemical reactions of a compound

Gram-negative bacteria	A class of bacteria characterized by a thin peptidoglycan layer between an inner and outer membrane containing lipopolysaccaride.
Heterogenous	An object diverse in content or character
Kinetic stability	The rate of chemical processes
Labile	Readily degradable and/or easily removable
Ligand	An ion or molecule that binds to a central metal atom or ion
Luciferase	An enzyme present in the cells of bioluminescent organisms that catalyzes the oxidation of luciferin and ATP, producing light.
Luciferin	A pigment present in the cells of bioluminescent organisms that emits light when undergoing oxidation
Mer operon	A section of genomic DNA containing a cluster of genes that encode the proteins essential to bacterial mercury resistance
Methylation	The process of adding a methyl group (CH_3) to a substrate
Minamata disease	Methylmercury poisoning
Minamata convention	An international treaty designed to protect human health and the environment from anthropogenic sources of mercury
Molecular shuttle	A molecule capable of transporting ions or other molecules from one location to another
Nanoparticule	A particle between 1 and 100 nanometers in size with unique size-related properties
Non-equilibrium	The state of a dynamic system, i.e., having not yet reached thermodynamic equilibrium
Oxidation	The loss of electrons in a redox reaction and consequent increase in oxidation state
Passive diffusion	The passive movement of ions or molecules directly across a cell membrane, requiring no input of energy or transmembrane protein
Polyelectrolytes	A polymer that has several ionizable groups along the molecules
Prokaryotic cells	A cellular organism that lacks a membrane-bound nucleus

Proxy	The measurement of a physical quantity as an indicator of the value of another
Pseudo-equilibrium	The state of a system in which thermodynamic equilibrium is assumed and apparent
Redox	Reduction-oxidation reactions in which atoms have their oxidation numbers altered; involves the transfer of electrons between species
Reduction	The gain of electrons in a redox reaction and consequent decrease in oxidation state
Refractory	Difficult to degrade
Thermodynamic equilibrium	A state of balance, in which there are no net flows of matter or energy, and no phase change
Threshold concentration	A concentration above which an effect or response will be produced, and below which it will not
Transcriptional regulator	A protein that binds to specific DNA sequences, thereby controlling gene expression
Trophic level	The position of a species in the food chain
Volatilization	The process by which a substance is transformed into vapour/gas
Transport vector	An agent that contains or carries a compound, contributing to its movement in the environment
Speciation	The distribution of an element between the different chemical forms or compounds in which it may occur, in both non-living and living systems
Xenobiotic	A chemical compound that is foreign to a living organism or to an ecological system.

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Chapter 1.0: Introduction

1.1 Changes in Arctic and Subarctic Environments

Rapid climate changes are currently affecting Arctic and Subarctic ecosystems. Although climate warming is a global phenomenon, over the past few decades, the average temperature in polar regions has risen at twice the rate of the rest of the world (ACIA, 2004). Accordingly, dramatic changes have been observed in the Arctic cryosphere (i.e. sea ice and permafrost), aquatic and land systems since the 1970s. In the Northern Hemisphere, the average annual sea ice area decreased by 7.4% between 1978 and 2003 (Johannessen *et al.*, 2004), a 3% loss per decade. Likewise, the duration of the ice-free growing season has increased in Arctic freshwater systems, resulting in changes to the light and thermal structures, as well as ensuing effects on primary producers (Smol and Douglas, 2007a). On land, permafrost degradation has strongly altered the northern landscape. At present, total permafrost occupies a vast area of 26 million km^2 in the Northern Hemisphere, and is expected to decrease by an area of between 5-9 million km^2 by 2100 (ACIA, 2005). The effects of permafrost thawing include the loss of land surface integrity, and subsequent changes to the water quality of adjacent water bodies (Kokelj et al., 2005).

The changing ice/light climate, and ensuing dramatic changes to lentic conditions in the northern landscape, have also led to alterations in the dynamics of anthropogenic contaminants, including their fate and availability to Arctic freshwater ecosystems. For example, Carrie *et al.* (2010) reported on polychlorinated biphenyls (PCBs) and mercury (Hg) in burbot (*Lota lota*) from the Mackenzie River near Fort Good Hope, NWT. Despite stable or declining atmospheric Hg and PCBs trends, contaminant levels in fish have doubled between 1985 and 2008. Climate changes are believed to accelerate the mobilization of contaminants historically sequestered in soil to Arctic lakes and rivers. For instance, peat subsidence from thawing permafrost may contribute to higher contaminant concentrations in tundra lakes. According to Klaminder *et al.* (2008), a significant amount (40-95%) of Hg stored in hummock peat is released to surface waters during subsidence from permafrost thawing. Likewise, Rydberg *et al.* (2010) showed that changes in northern peatland structure from underlaying permafrost thawing resulted in increase Hg loading to Subarctic lakes. In Lake Inre Harrsjon, adjacent to the Stordalen Mire in northern Sweden, sediment Hg accumulation rates were higher during a period of permafrost warming in the pre-industrial past than at the peak of atmospheric Hg input in the 1970s.

In recent decades, most scientific research in the Arctic and Subarctic has focused on the Western Canadian Arctic and the Canadian Archipelago, as these geographic areas have experienced more significant and rapid climate change than others. In Western Canada,

underlain by continuous and discontinuous permafrost, winter temperatures have increased as much as 3-4 ºC in the past 50 years (ACIA, 2004). As a result, permafrost thawing is affecting up to 15% of water bodies in the region via the development of shoreline retrogressive thaw slumps that increase the delivery of surficial materials to adjacent lakes (Deison *et al.,* 2012; French *et al*., 2014). Changes to lake chemistry from thermokarst erosion, including elevated ionic concentrations and water clarity (Kokelj *et al*., 2009), are spatially variable in distribution and magnitude. Therefore, tundra lakes in the western Canadian Arctic are characterized by extremely large pH (6.6-8.1) and dissolved organic carbon (DOC; 6.8-30.0 mg C L^{-1}) gradients (Kokelj *et al*., 2005). Likewise, evidence of recent changes in water chemistry, such as increased specific conductance, were recorded in Arctic lake and high Arctic pond ecosystems as a result of recent warming (Deison *et al.,* 2012; Smol and Douglas, 2007b).

Anthropogenic contaminants and climate change are high priority issues for Arctic residents, as well as academic and governmental institutions. Mitigating synergistic problems and reducing both human and ecosystem health risks in northern regions requires a better understanding of interactions between climatic-related factors and pollutant cycling in Arctic aquatic ecosystems. Mercury is recognized as a top priority among arctic contaminants, and will be the main focus of this thesis. Hg-climate interactions are yet largely uncharacterized, including ones pertaining to DOC, an important vector for contaminant delivery to aquatic systems.

1.2 Objectives and Hypotheses

Specifically, this research will assess how DOC affects the potential for Hg^{II} bioavailability in defined media and in a selection of 18 lakes from Canada's Western Arctic using a whole-cell bacterial bioreporter.

1.2.1 Dissolved Organic Matter and Bacterial Hg^{II} uptake

The first objective is to investigate the role of DOM in controlling bacterial Hg^{II} uptake and hence intracellular Hg^{II} levels in defined medium. From the results of Miller *et al.* (2009) on the chemical reactivity of Hg-DOM complexes over time, I hypothesize that, following the addition of DOM in solution, Hg^{II} will remain available for bacterial uptake (as indicated by the bioreporter signal) within the time-window required for thermodynamic equilibrium to establish between Hg^{II} and DOM (< 24h). I also hypothesis that DOM will decrease Hg^{II} bioavailability once pseudo-equilibrium is reached (> 24h), even at low concentrations.

1.2.2 In situ Bacterial Hg^{II} uptake

The second objective is to determine the bioreporter's response to DOM in natural water samples spanning a wide DOC concentration gradient, and whether this response is consistent with that of results in defined media. Field samples were collected from lakes of the Northwest Territories in the Western Canadian Arctic.

1.2.3 Dissolved Organic Matter and Cell Wall Permeability

The last objective is to assess the effect of DOM on the permeability of Gram-negative bacterial cell walls to passive diffusion of small, neutrally charged Hg^{II} species.

1.3 Dissolved Organic Matter in Natural Waters

Ubiquitous in aquatic environments, dissolved organic matter (DOM), measured as dissolved organic carbon (DOC), is a heterogenous mixture of organic compounds of poorly-defined chemical structure. The term ' dissolved ' (vs particulate) refers to the physical size classification of matter, as defined operationally by use of 0.45 μm pore filter (Leenheer and Croué, 2003), or smaller (0.1 μm; Chow *et al.,* 2005). In aquatic chemistry and water quality studies, 'dissolved' is broadly employed, but not restricted to the characterization of natural organic matter (NOM). About 20% of aquatic DOM consists of carboxylic acids, carbohydrates, amino acids, hydrocarbons and other identifiable compounds (Ravichandran, 2004). The remaining fraction of DOM consists of a complex mixture of residues from the decomposition of plants and animals referred to as humic substances (HS; Aiken *et al.*, 1985; Leenheer, 1994; Thurman, 1985). These are heterogeneous mixtures of polyelectrolytes with varying molecular size/weight (Remucal *et al.*, 2012), substructures and functionalities (Stevenson, 1994) that can only be operationally defined by the techniques used for their extraction and fractionation; near 80-90% of HS consist of hydrophobic and hydrophilic acids, the most reactive fractions in trace metal binding. Hydrophobic acids in DOM are usually further fractionated into humic acid (HA) and fulvic acid (FA) based on their acid-base solubility behaviour: HA precipitates upon acidification to pH 2, while FA remains soluble even below pH 2 (Ravichandran, 2004). Fulvic acid has less complex structure and composition then HA, featuring lower molecular mass and aromaticity. Dissolved organic matter concentration, composition, and chemistry are highly variable and dependent on sources of organic matter, as well as numerous environmental variables, processes and precursors (Leenheer and Croué, 2003; Malcolm, 1990).

Dissolved organic matter plays a regulatory role in freshwater ecosystems. Firstly, it is a major reactant and product in biogeochemical processes, providing carbon and energy sources for biota, as well as controlling levels of dissolved oxygen, nitrogen, phosphorus, sulfur, numerous trace metals, and acidity in surface waters (Leenheer and Croué, 2003). For example, several studies have described the utilization of DOM by bacteria (Tranvic, 1990; Tulonen *et al.*, 1992), and subsequent transfer to higher trophic levels (e.g., amphipods; Hargeby, 1990; Hargeby and Petersen, 1988). Dissolved organic matter is often divided into two components based on nutritional quality (Webster and Benfield, 1986), and importance to the food web (Koetsier *et al.*, 1997): a labile fraction that is available for uptake by organisms, and a recalcitrant or refractory component that is generally more resistant to biological attack. Secondly, DOM plays a central role in the biogeochemical cycling of organic and inorganic contaminants, including Hg. Chemical interactions with DOM occur via various modes of binding and absorption: ion exchange, hydrogen bonding, charge transfer, covalent binding, and hydrophobic adsorption and partitioning. As a result, speciation, solubility, transport, bioavailability and toxicity of contaminants in the environment are affected. Strong complexation facilitates the mobility of contaminants from watersheds into lakes (Driscoll *et al.*, 1995), streams (Mierle and Ingram, 1991) and groundwater (Krabbenhoft and Babiarz, 1992). In conjunction with this mechanism, DOM has been shown to enhance water column concentrations by altering the partitioning of contaminants to suspended solids, thereby reducing their sequestration to sediments (Ravichandran, 2004) and lessening their volatilization (Gschwend and Wu, 1985; Hassett and Milicic, 1985; Mackay, 1979). Likely, changes to the residence time of contaminants in lentic systems will ensue from their increased solubility.

There are also competing effects of DOM on contaminant toxicity to aquatic biota. In freshwater, the bioavailability of contaminants, including their toxicity, is thought to be related to their ability to cross biological barriers, and is mostly predicted by the concentration of internalized compounds. The uptake process depends greatly on the physiocochemistry of the environment, as well as on the nature of the organism and applicable internalization pathways. Chemical speciation exerts a major control on the bioavailability of contaminants, and their ensuing toxic effects within aquatic food webs. Competition (e.g. H^+, S^{2-}, etc) or complexation (inorganic and organic ligands) by various environmental variables result in a decreased interaction of xenobiotics with uptake sites on the surface of the organism. Dissolved organic matter is believed to directly reduce contaminant bioavailability by the latter process, i.e., forming complexes of large molecular size, unavailable for biological uptake. This assumption is

extensively supported and accepted by the scientific community. For example, data compiled by Haitzer *et al.* (1998) from 27 studies showed that, in general, DOC leads to a decrease of the bioaccumulation of organic compounds, including various polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons. Several studies have also demonstrated that DOC decreases trace metal bioaccumulation by aquatic biota (Hung, 1982; Sedlacek *et al.*, 1983; Stackhouse and Benson, 1989), although data are more ambiguous in this case. Additionally, DOM may affect the bioaccumulation of contaminants by altering the physiology of exposed organisms (Haitzer *et al.,* 1998), or by influencing the light-induced transformation pathways of these compounds (Wenk *et al.*, 2011).

Humic substances from the International Humic Substances Society (IHSS) such as HA and FA are commonly used as surrogates for whole-DOM or unconcentrated natural waters, mostly to save time and expense from field-sampling or DOM isolation. In these preparations, DOC consists of nearly 100% HS, i.e., the fraction of DOM mainly responsible for the binding of organic and inorganic chemicals. Conversely, HS only accounts for 50-75% of total DOC in natural waters or whole–DOM preparations (Thurman, 1985). On a DOC basis, IHSS HS-preparations are likely to have more pronounced effects than the latter, comparable to 50-100% higher DOC concentrations (when excluding effects of DOM quality). Therefore, measuring the effect of DOC from IHSS HA and FA on the binding of contaminants may overestimate the corresponding effect of DOM in natural waters.

1.4 Mercury Contamination

Hg is characterized by its great toxicity and serious health risks for humans and wildlife. Natural transformations and environmental pathways of Hg are very complex, and include a wide range of biogeochemical interactions, warranting the need for separate consideration relative to other metals. Moreover, Hg has several unique properties. For example, it is the only metal to be liquid at room temperature, and to volatilize relatively easily, becoming a colourless, odourless gas. Mercury, which occurs naturally in the Earth's lithosphere in a variety of mercury-sulphur (HgS) binary minerals (Barnes and Seward, 1997), is emitted to the atmosphere as gaseous elemental Hg^0 through natural processes of erosion, volcanism and forest fires (Selin *et al.*, 2007). The atmospheric lifetime of Hg^0 against oxidation extends to ~1 year, resulting in global-scale deposition to terrestrial and aquatic ecosystems. Over the past two centuries, anthropogenic processes have significantly disturbed the natural biogeochemical cycling of Hg (Mason and Sheu, 2002; Selin *et al.*, 2007); coal combustion, waste incineration, and mining

(Streets et al., 2009), chiefly, have augmented at least 3-fold deposition Hg (Mason *et al.*, 1994). The time scale for the return of anthropogenic Hg to mineral reservoirs (sinks) has been estimated to be ~ 2000 years (Selin *et al.*, 2008; Sunderland *et al.*, 2007), henceforth representing an enduring global pollution legacy with continuous addition from present-day human activities. During the past two decades, control measures have substantially reduced the Hg emissions of developed countries (Pirrone *et al.*, 2009). However, on a global scale, these reductions are mostly offset by Hg releases due to rapid industrialization in many developing countries (Pacyna *et al.*, 2010; Streets *et al.*, 2009). Notably, Hg^0 emissions from Asia are expected to increase in coming decades (Pacyna and Pacyna, 2002; Streets *et al.*, 2009).

Among Hg species, methylmercury (MeHg) is the most toxicologically relevant. Research has shown that Hg contamination of high trophic levels of food chains is almost exclusively attributable to its methylated form (Campbell *et al.,* 2005; Loseto *et al.*, 2008), which has the well-known ability to bioaccumulate in organisms, and biomagnify throughout foodwebs. As such, human and wildlife exposure to Hg occurs primarily via aquatic and marine food webs as the potent neurotoxic compound MeHg. The first documented case of widespread MeHg poisoning was in Minamata Bay, Japan, in the early 1950s; nearly 3000 cases of what became known as Minamata disease were recorded by the Japanese government after the release of Hg from a nearby industry (Takizawa and Kitamura, 2001). Symptoms of MeHg toxicity in humans adults range from mild numbness of the extremities, blindness, impaired development of memory, attention and language skills (Krummel *et al.*, 2005), and in severe cases, death (Mergler *et al.*, 2007). A considerable reduction in brain size, overactive reflexes and severe motor and mental impairments also commonly occur from pre- and post-natal MeHg exposure (Sanfeliu *et al.*, 2001); developing fetuses and infants are exposed to MeHg from its penetration through the placental barrier, or its excretion in breast milk.

The importance of Hg as a chemical of global concern for human and ecosystem health was further emphasized by the recent adoption of the Minamata Convention on Hg. Under this global legally binding treaty, governments agencies are required to address anthropogenic emissions to air and releases to water and land, as well as monitor processes affecting environmental cycling, transport, transformation and fate of Hg and Hg-compounds.

1.4.1 The Mercury Cycle

Global biogeochemical cycling of Hg involves several abiotic and biotic pathways (Figure 1.1), leading to its distribution among several chemical forms (elemental, inorganic and organic)

and oxidation states (+2, +1 and 0). Gaseous elemental Hg^0 is deposited within terrestrial or aquatic ecosystems in its oxidized Hg^{II} form, chiefly throughout wet and dry depositions (i.e. rain, snow and air-borne particles; Fitzgerald *et al.*, 1998; Selin *et al.*, 2007). Some of this Hg^{II} is reduced back to Hg^0 and re-volatilizes to the atmosphere. Alternatively, Hg^{II} can be subject to sequestration in sediments, or potentially methylation to the more toxic MeHg form, thereby entering aquatic food webs (Selin *et al.*, 2009). Production of MeHg is a biologically mediated process facilitated by several groups of bacteria, typically anaerobes, including sulfate-reducing bacteria (SRB; Benoit *et al.*, 2003; Gilmour *et al.*, 1998), iron-reducing bacteria (IRB; Kerin *et al.*, 2006) and methanogens (Han *et al.*, 2010). Microbes (e.g. algae, bacteria and archaea) are involved in almost all reactions affecting Hg cycling (Barkay *et al.*, 2003). In most cases, transformations are thought to be intracellular processes limited by the uptake of Hg species, that occurs through passive diffusion (Benoit *et al.*, 2001; Benoit *et al.*, 1999; Bienvenue *et al.*, 1984) and/or facilitated transport (Golding *et al.*, 2002; Golding *et al.*, 2008; Schaefer *et al.*, 2011). Over recent decades, there have been many key advances in the field of Hg microbiology. However, the mechanisms of Hg^{II} availability to microbes remain unclear, particularly in ternary interactions with various environmental variables in aquatic ecosystems (Benoit *et al.*, 1999; Driscoll *et al.*, 1995; Graham *et al.*, 2012, Hall *et al.,* 1997; Miskimmin *et al.*, 1992; Schaefer and Morel, 2009; Zitko and Carson, 1976). Microorganisms respond strongly to environmental drivers such as temperature (Barkay *et al.*, 2011; Lefebvre *et al.*, 2007; Zogg *et al.*, 1997), salinity (Compeau and Bartha, 1984; Witt *et al.*, 2011), DOM (Judd *et al.*, 2006) and pH (Meron *et al.*, 2011); fluctuations in these variables, due to ongoing global environmental changes (Kokelj *et al.*, 2005; Macdonald *et al.*, 2005), will likely affect microbial activity and their role in the biogeochemical cycle of Hg.

1.4.2 Mercury in the Arctic and Subarctic Regions

Volatile elemental Hg^0 undergoes long-range atmospheric transport, following global air currents from lower latitudes to high latitudes and altitudes (Steffen *et al.*, 2008). As a result, Arctic and Subarctic regions are particularly prone to the accumulation of global Hg pollution, and ensuing ecological and human health concerns. At present, Asia is the dominant source of Hg^0 emissions to polar regions (Durnford *et al.*, 2010). Moreover, Hg deposition in the Arctic is augmented by springtime atmospheric Hg depletion events (AMDE), i.e., rapid and massive deposition of Hg^{II} from the atmosphere presumably due to oxidization of gaseous elemental Hg^0 by halogen radicals and oxidized forms of halogens formed in sea salt aerosols by

photochemical transformations (Ariya *et al.*, 2008; Brooks *et al.*, 2006; Lindberg *et al.,* 2002). Atmospheric Hg depletion events are estimated to contribute as much as 30-55% of annual Hg deposition to the Arctic (Ariya *et al.*, 2004; Christensen *et al.*, 2004; Dastoor *et al.*, 2008; Skov *et al.*, 2004). Despite there being no major Hg sources nearby, anthropogenic Hg contributes from 74% to 94% of the total body burdens of wildlife and humans alike across the Arctic (Dietz *et al.*, 2009). In fact, indigenous people in Canada's North are currently amongst the most Hg exposed population on Earth (Donaldson *et al.*, 2010). Evidence of chronic sub-lethal MeHg toxicity has been documented in arctic wildlife, and ultimately in Inuit people who subsist primarily on traditional country foods. The levels of MeHg measured in umbilical cord serum of Canadian Inuit fetuses are also consistent with cognitive deficits reported in other studies (Muckle *et al.*, 2001). Under the circumstances, further investigation of Hg contamination patterns is warranted.

1.4.3 Mercury Speciation and Bioavailability

A wide variety of Hg compounds exist in aquatic ecosystems. Indeed, dissolved aqueous Hg^{II} cations make only a very small portion of total Hg (THg). Rather, Hg^{II} is predominantly adsorbed to particle surfaces or coordinated to other molecules (e.g. chloride, sulphide or NOM) to form aqueous Hg^{II}-ligand complexes. The relative partitioning of Hg^{II} in various dissolved and particulates forms governs the overall mobility and bioavailability of Hg^{II} in these systems. Total Hg^{II} is in fact a poor estimate of Hg bioavailability in the environment, as only a small proportion of Hg^{II} is believed to be readily taken up by methylating microbes. When modelling Hg^{II} bioavailability, the most established approach assumes that uptake by the cell is limited to passive diffusion pathways. As such, only small, neurally charged dissolved Hg^{II} complexes (i.e. lipophilic Hg species) are presumed to be internalized by methylating microbes, and their concentration to be estimable from thermodynamic equilibrium models of Hg^{II} complexes (Figure 1.2). When employing an equilibrium model, it is implied that the partitioning of Hg^{II} between various chemical species can be represented by equilibrium chemistry. Yet, this assumption is unrealistic in many environments, including systems receiving continued Hg^{II} inputs. Miller *et al.* (2009) suggested the need to further consider the kinetics of Hg-ligand complexation in aquatic systems (hitherto overlooked) relative to the stability of complexes at a presumed equilibrium state; in kinetic experiments, the binding of Hg^{II} to "strong" ligands in DOM appeared to be a slow process (e.g. ~1 day or longer). Therefore, the ability to directly relate geochemical speciation and bioavailability of Hg^{II} remains limited.

1.4.4 Mercury and Dissolved Organic Matter

The interaction of Hg with inorganic and organic ligands strongly influences its speciation, fate, transport and bioavailability in aquatic ecosystems, like other environmental pollutants. Dissolved organic matter has a complex role in Hg cycling, For example, DOM is involved in the delivery of Hg from watersheds to freshwater ecosystems. In general, field measurements show that total Hg^{II} and MeHg concentrations increase in tandem with DOC concentrations (Babiarz *et al.*, 2001; Driscoll *et al.*, 1995). Nonetheless, these concentrations are not indicative of biological Hg concentrations. Typically, DOC is inversely correlated to Hg levels in fish (Wren and Maccrimmon, 1983), either by influencing bacterial methylation of Hg^{II}, the first step toward bioaccumulation of MeHg in aquatic food webs, or by influencing the relative accumulation of Hg^{II} and MeHg at higher trophic levels. In surface waters, DOM-Hg^{II} complexes dominate due to the high affinity of Hg for reduced sulfur functional groups on the NOM (Ravichandran, 2004); Hg is believed to preferentially bind with trace quantities of reduced sulfur, despite the greater abundance of carboxylic acids and other oxygen-containing functional groups in NOM. Additionally, DOM has been shown to also influence the formation of dominant sulphide-Hg^{II} complexes under anaerobic environments (Ravichandran, 2004). DOM-Hg^{II} and DOM-MeHg complexes are believed to be less bioavailable (Sjoblom *et al.*, 2000) due to their large molecular size, hence lowering the concentration of labile Hg forms. Likely, DOM directly inhibits bioaccumulation of MeHg in fish and high tropic levels. Indirectly, the effect of DOM on Hg levels in aquatic food webs is ambiguous (Figure 1.3). Dissolved organic matter has the known potential to promote toxic and bioaccumulative MeHg formation by stimulating microbial growth, or rather to inhibit methylation by reducing Hg^{II} availability to methylating bacteria through complexation (Barkay *et al.*, 1997). In addition, DOM enhances the photochemical reduction of Hg^{II} to volatile Hg^{0} in surface waters (Ravichandran *et al.*, 2000), likely influencing MeHg accumulation into aquatic food webs. The interaction between Hg^{II} and DOM has yet to be completely understood.

1.4.5 Whole-cell Bioreporters

Living whole-cell bioreporter assays are amongst very few toxicologically-relevant approaches that have the ability to report on chemical bioavailability rather than mere total concentrations; traditional analytical methods measure both bioavailable or non-bioavailable forms. They consist mostly of prokaryotic cells (bacteria) that serve as living sensors for harmful

pollutants and chemical toxicants in the environment, and in the process act as higher animal proxies of contaminant bioavailability and toxicity. Thus far, the response of whole-cell bioreporters has proven to be consistent with observed effects of environmental drivers on fish Hg levels, including water hardness, salinity and pH (Barkay *et al.*, 1997; Daguene *et al.*, 2012; Kelly *et al.*, 2003). Bacteria are advantageous biosentinels as they thrive under various environmental conditions and are easily genetically manipulated to include bioreporter constructs. Bacterial strains native to contaminated systems have evolved unique genetic traits that permit detoxification or resistance against chemical agents; the genes that respond to a chemical or toxin are regulated by a sensing component, i.e., a genetic element or promoter. Bioreporter constructs referred to as "lights-on" are essentially promoter/reporter gene linkages that emit light upon exposure to the target of interest. For example, whole-cell bioreporters detect cytoplasmic Hg^{II} (Selifonova *et al.*, 1993) by exploiting the metal-responsive regulator controlling the bacterial Hg resistance (H^{R}) *mer* operon (Figure 1.4). Briefly, the transcriptional regulator *merR*, and the *mer* promoter/operator region are fused to promoterless reporter genes that now, when activated by Hg binding to the regulator, are transcribed and translated to reporter proteins emitting light. The induction and intensity of light production are indicative of intracellular Hg^{II} concentration, and hence bioavailability (Golding *et al.*, 2002). Commonly incorporated as signalling element in whole-cell bioreporters is the *lux* operon encoding for bacterial bioluminescent, a chemical reaction catalyzed by the luciferase enzyme that reacts with a luciferine substrate to produce an excited state molecule that emits photons as it relaxes back to its ground state (Xu *et al.*, 2013). Alternatively, "lights-off" bioreporter constructs are designed to constitutively (continuously) emit light (Xu *et al.*, 2013). They reflect changes (stimulation or disruption) in general metabolism following cell exposure to chemical agents or altered physical conditions. As such, "lights-off" bioreporters are frequently employed as controls in bioassay to verify that the light production by "lights-on" bioreporters is attributable to Hg^{II} accumulation and not other experimental variables.

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1.6 List of Figures

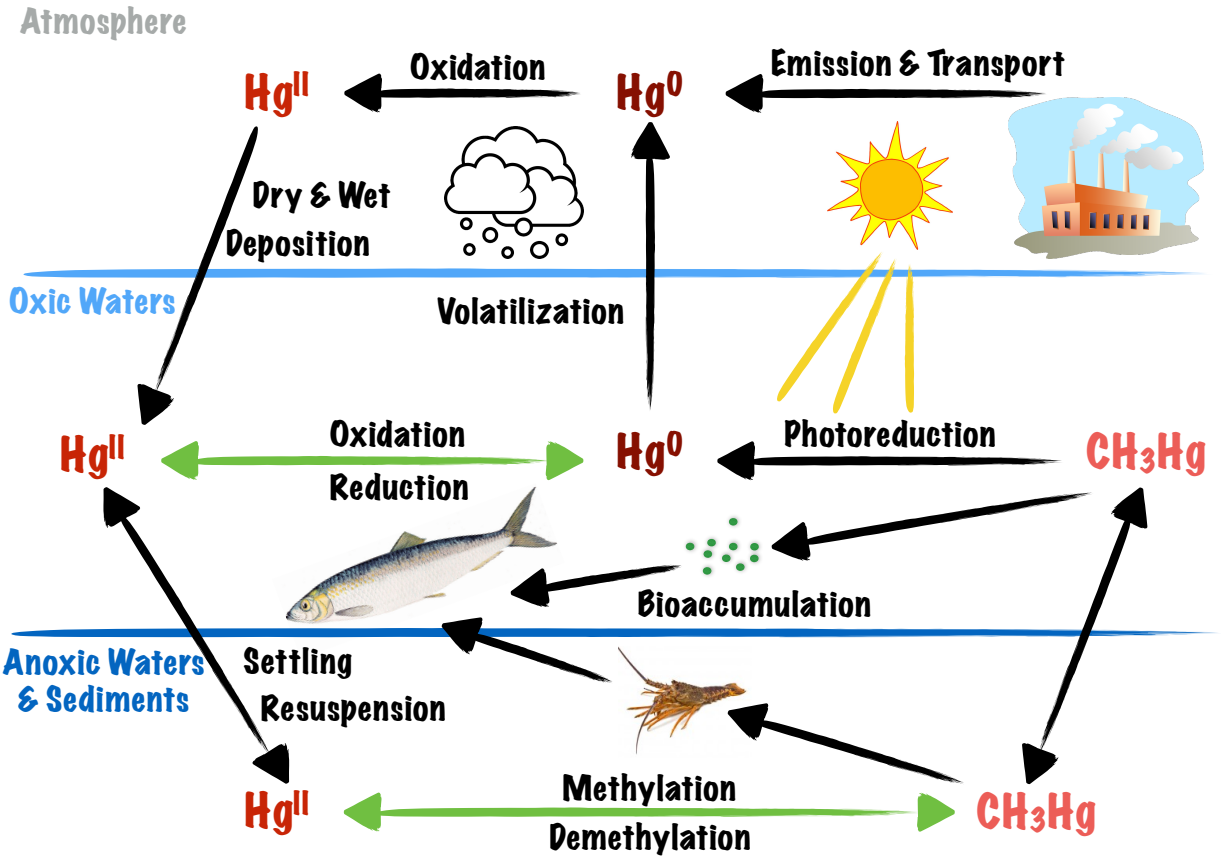

Figure 1.1: Summary of the mercury (Hg) cycle in aquatic environments. Hg^0: elemental mercury, Hg^{II}: ionic mercury, CH_3Hg: methylmercury. Green arrows represent biologically mediated reactions.

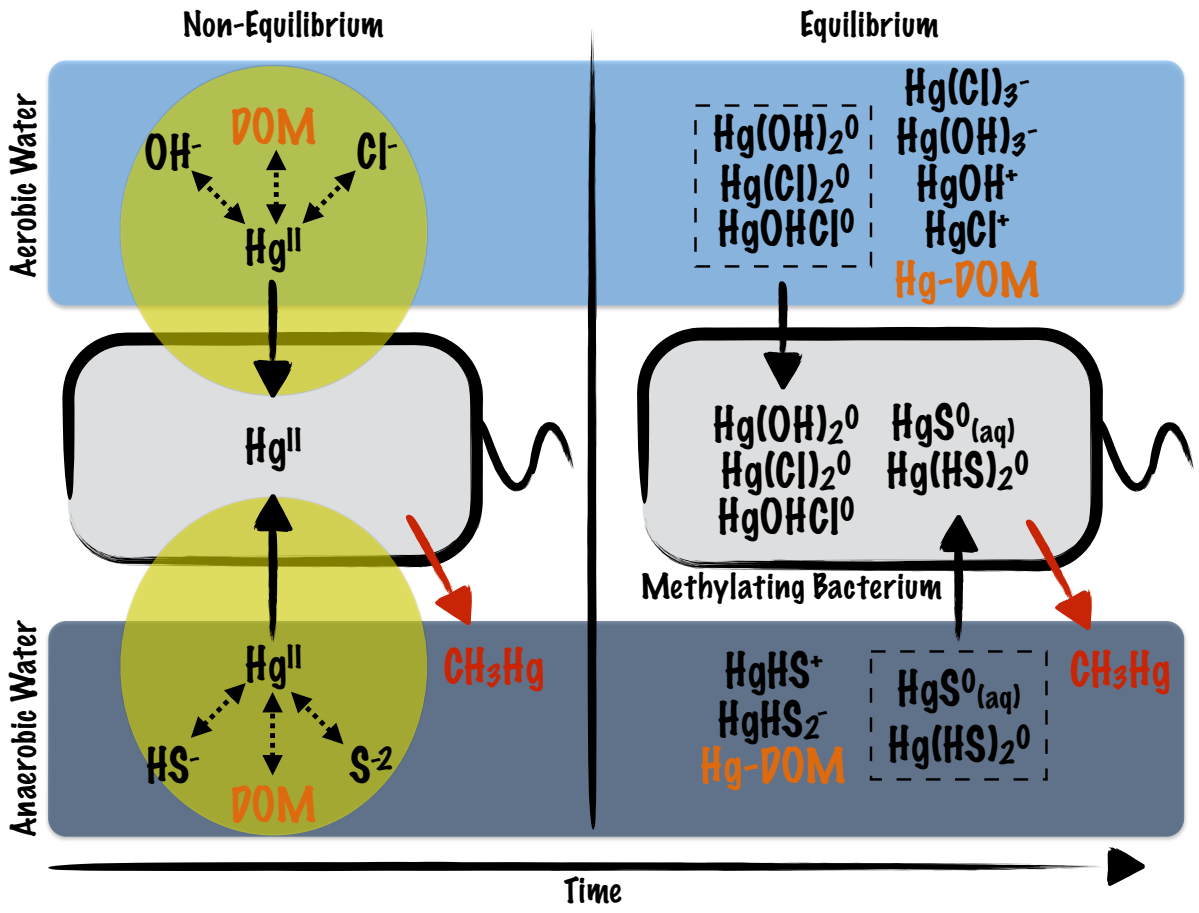

Figure 1.2: Summary of inorganic mercury (Hg^{II}) complexes available for uptake by methylating bacteria, according to the current consensus based on thermodynamic equilibrium speciation. The net production of CH_3Hg is mainly related to the concentration of neutrally charged Hg^{II} complexes able to passively diffuse into the cell. Equilibrium chemistry predicts that Hg-DOM complexes are less bioavailable, and that aqueous Hg-S complexes are the predominant form of dissolved Hg^{II} in anaerobic environments. Processes occurring when thermodynamic equilibrium is not reached are often overlooked. Yellow tones represent uncertainty as to the bioavailability of Hg^{II} complexes. S^{2-}: sulfide, HS^-: bisulfide, Cl^-: chloride, OH^-: hydroxide, DOM: dissolved organic matter, CH_3Hg: methylmercury.

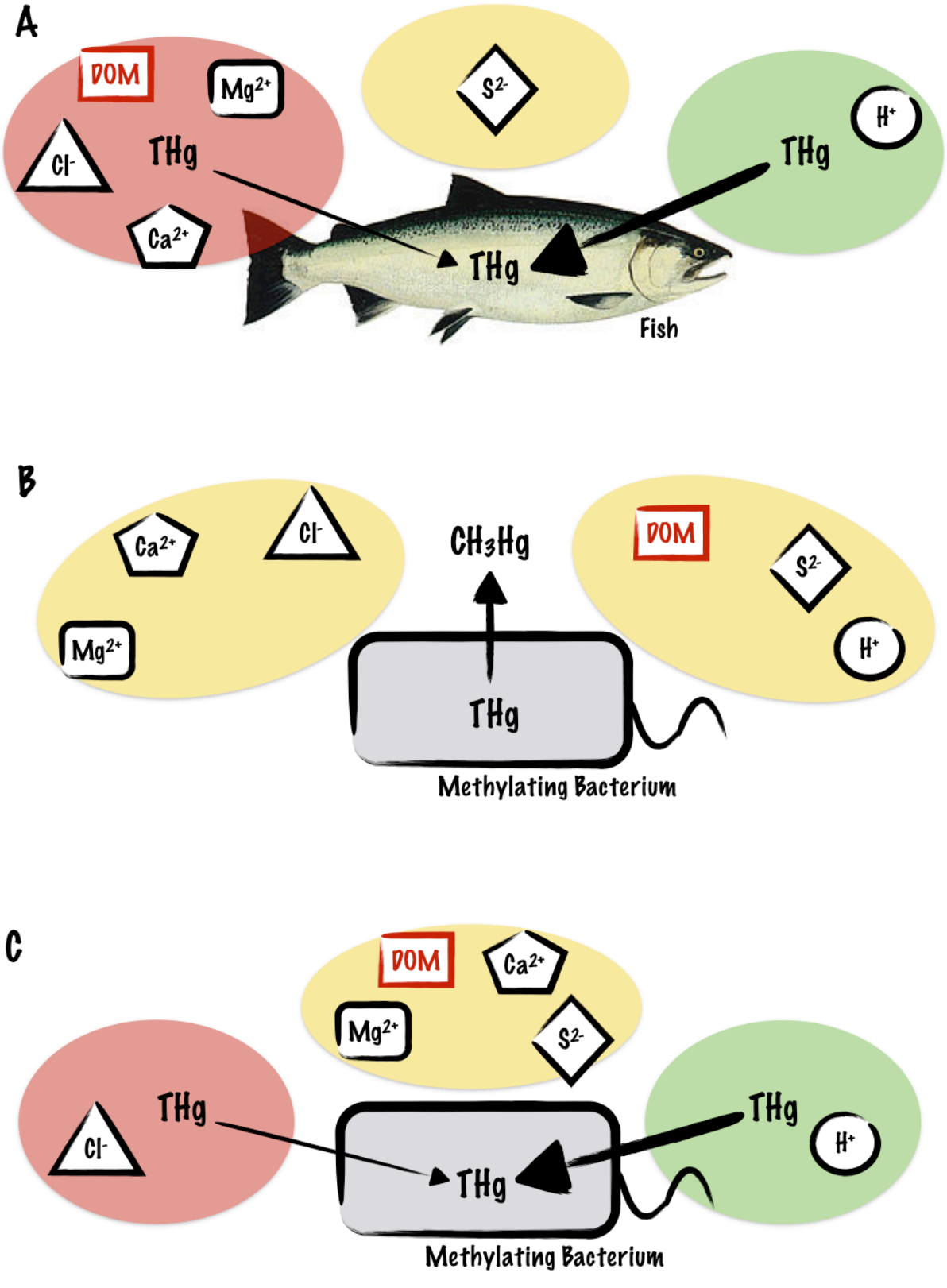
A
DOM
Mg2+
Cl-
THg
Ca2+
S2-
H+
THg
THg
Fish
B
Ca2+
Cl-
Mg2+
CH3Hg
DOM
S2-
H+
THg
Methylating Bacterium
C
DOM
Ca2+
Mg2+
S2-
Cl-
THg
THg
H+
THg
Methylating Bacterium

Figure 1.3: Schematic representation of environmental variables affecting total mercury (THg) interactions with biota. Total mercury bioaccumulation in fish (A), THg methylation by bacteria (B), and THg uptake by bacteria (C) were compiled from previous studies (Barkay et al., 1997; Benoit et al., 1999; Driscoll et al., 1995; Graham et al., 2012; Hall et al., 1997; Kelly et al., 2003; Mason et al., 1996; Miskimmin et al., 1992; Ramlal et al., 1985; Schaefer and Morel, 2009; Wang and Wang, 2010; Wren and Maccrimon, 1983; Zitko and Carson, 1976). Arrows depict THg uptake from water, as well as food in the case of fish. Green and red tones respectively represent enhancing and decreasing effects by environmental variables. Yellow tones represent uncertainty, or conflicting information regarding the effect of environment variables. S^{2-}: sulfide, Cl^{-}: chloride, Ca^{2+} and Mg^{2+}: water hardness, H^{+}: pH, NOM: natural organic matter, CH_3Hg: methylmercury.

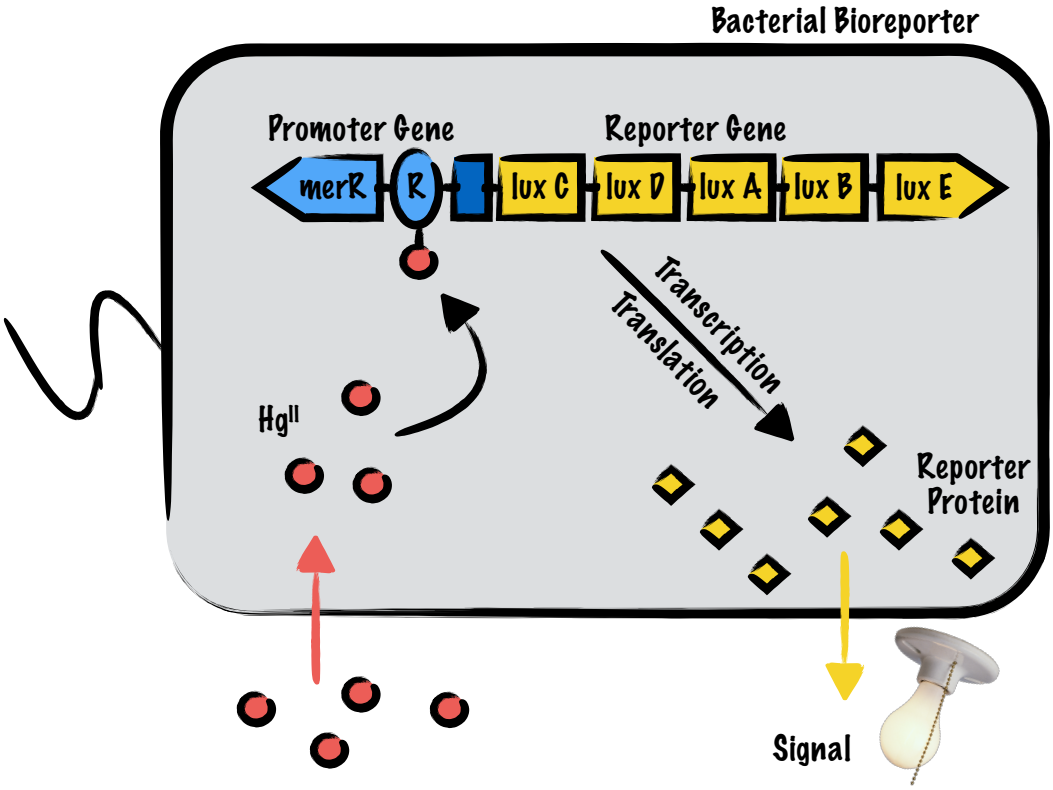

Figure 1.4: Simplified representation of a whole-cell bacterial bioreporter detecting cytoplasmic inorganic mercury (Hg^{II}). Intercellular Hg^{II} (red circules) binds to MerR upstream of the *mer* promoter/operator region (dark blue square), thereby switching on the *lux* system and synthesizing bioluminescence-emitting proteins (yellow diamonds). merR: *merR* gene, R: transcriptional regulator MerR, lux A-E: luciferase (*lux*) system.

Chapter 2.0: Dissolved organic matter kinetically controls mercury bioavailability to bacteria.

Modified with permission from:

Chiasson-Gould, S.A., J.M. Blais and A.J. Poulain. 2014. Dissolved organic matter kinetically controls mercury bioavailability to bacteria. Environmental Science & Technology 48: 3153-3161.

2.1 Abstract

Predicting the bioavailability of inorganic mercury (Hg) to bacteria that produce the potent bioaccumulative neurotoxin monomethylmercury remains one of the greatest challenges in predicting the environmental fate and transport of Hg. Dissolved organic matter (DOM) affects mercury methylation due to its influence on cell physiology (as a potential nutrient) and its influence on Hg^{II} speciation in solution (as a complexing agent), therefore controlling Hg bioavailability. We assessed the role of DOM on Hg^{II} bioavailability to a gram-negative bacterium bioreporter under oxic pseudo- and non-equilibrium conditions, using defined media and field samples spanning a wide range of DOM concentrations. Our results showed that Hg^{II} was considerably more bioavailable under non-equilibrium conditions than when DOM was absent or when Hg^{II} and DOM had reached pseudo-equilibrium (24h) prior to cell exposure. Under these enhanced uptake conditions, Hg^{II} bioavailability followed a bell shaped curve as DOM concentrations increased, both for defined media and natural water samples, consistent with recent field-reports of bioaccumulation in amphipods. Experiments also suggest that DOM may not only provide shuttle molecules facilitating Hg uptake but also alter cell wall properties to facilitate the first steps towards Hg^{II} internalization. We propose the existence of a short-lived yet critical time window (<24h) during which DOM facilitates the entry of newly deposited Hg^{II} into aquatic food webs, suggesting that the bulk of mercury incorporation in aquatic food webs would occur within hours following its deposition from the atmosphere.

2.2 Introduction

Contamination of fish and other aquatic predators by mercury is attributable almost exclusively to monomethylmercury (MMHg; Bloom 1992). Although mercury methylation is mostly catalyzed by anaerobic microbes, among which sulfate reducers (Gilmour *et al.,* 1992), iron reducers (Fleming *et al.*, 2006, Kerin *et al.*, 2006) and methanogens (Hamelin *et al.*, 2011) are key players (Hsu-Kim *et al.*, 2013; Parks *et al.*, 2013), recent evidence suggests that mercury methylation may not solely be confined to anoxic environments (Larose *et al.*, 2010; Lehnherr *et al.*, 2011). Parks *et al.* (2013) recently identified some of the genetic determinants of anaerobic mercury methylation at the cellular level, but it remains difficult to predict the timing and magnitude of MMHg production, at an ecosystem scale. Total mercury is a poor predictor of MMHg concentrations in aquatic systems (Kelly *et al.*, 1995) as it does not always correlate to the total amount of MMHg in sediments or water (Benoit *et al.*, 2003). A better estimation relies on estimating Hg^{II} speciation in solution, i.e., how it interacts with inorganic or organic ligands

and how these species interact with cellular targets. Cellular uptake most often limits rates of MMHg production (Benoit *et al.*, 2003; Graham *et al.*, 2012; Schaefer *et al.*, 2011), underscoring the need to define both Hg^{II} speciation in solution and the uptake strategy. Mechanisms underlying Hg^{II} uptake may include passive diffusion of neutrally charged species (Barkay *et al.*, 1997; Benoit *et al.*, 1999), facilitated transport (Golding *et al.*, 2002; Schaefer and Morel, 2009, Schaefer *et al.*, 2011), and/or active transport (Schaefer *et al.*, 2011).

Dissolved organic matter (DOM) is one of the most important Hg complexing agents in natural waters (Hsu-Kim *et al.*, 2013) but Hg^{II} binding constants with DOM and the kinetics of these interactions remain difficult to accurately describe. Over the last few decades, studies aiming at predicting the bioavailability and toxicity of metals in aquatic ecosystems assumed that a state of pseudo-equilibrium was reached between the metal and its various ligands in solution or at the cell surface (Paquin *et al.*, 2002). Under equilibrium conditions, DOM is usually thought to decrease Hg bioavailability (Barkay *et al.*, 1997; Gorski *et al.*, 2008; Miskimmin *et al.*, 1992), as, under equilibrium conditions, it reduces free metal ion activity as well as the proportion of metal bound to biological uptake sites (Bell *et al.*, 2002; Hudson, 2005; Paquin *et al.*, 2002). However, Miller *et al.* (2009) proposed that kinetic reactions, and not only thermodynamics, needed to be considered to accurately predict Hg-DOM complexation, where the formation of Hg-DOM complex is a slow process associated with competitive ligand exchange among various functional groups within DOM molecules (Miller *et al.*, 2012; Miller *et al.*, 2009). Recently, Graham *et al.* (2012; 2013) provided a possible mechanism for the positive correlations observed between DOM and MeHg production in aquatic ecosystems (Driscoll *et al.*, 1995) by showing that DOM enhances Hg^{II} methylation in an anoxic environment under low sulfide conditions by favouring the formation of HgS nanoparticles/clusters that may facilitate the release of chemically labile Hg species immediately adjacent to the cell surface and therefore facilitate Hg uptake (Deonarine and Hsu-Kim 2009; Hsu-Kim *et al.*, 2013; Zhang *et al.*, 2012). These studies underscore how complex Hg-DOM interactions are and challenge existing paradigms used to predict metal uptake and toxicity, in particular whether equilibrium chemistry is relevant to accurately describe Hg bioavailability and therefore toxicity (Hsu-Kim *et al.*, 2013).

Although cellular uptake is an important limiting step for subsequent intracellular Hg transformations, very few approaches allow quantification of intracellular Hg^{II} levels. One such approach is to use whole-cell bioreporters to detect cytoplasmic Hg^{II} (Selifonova *et al.*, 1993). So far, bioreporters have responded to environmental variables such as pH, salinity and water

hardness consistently with how these variables affect Hg levels in fish (Barkay *et al.*, 1997; Daguene *et al.*, 2012; Kelly *et al.*, 2003; Miskimmin *et al.*, 1992; Wang and Wang, 2010).

In light of the recent and important advances in our understanding of Hg cycling in the environment, we used a whole-cell bioreporter to investigate the role of DOM in controlling bacterial Hg^{II} uptake and hence intracellular Hg^{II} levels, in defined medium and natural lake water samples collected in the Western Canadian Arctic. Here we show that DOM enhanced Hg^{II} bioavailability under non-equilibrium conditions and decreased it once pseudo-equilibrium is reached.

2.3 Materials and Methods

2.3.1 DOM Isolates and Lake Water Samples.

The effect of DOM on Hg bioavailability was tested using fulvic (FA) and humic (HA) acid fractions isolated from the Suwannee River, and water samples from the Northwest Territories, in the Western Canadian Arctic. Suwannee River humic and fulvic acids (HA and FA, Standard II) were obtained from the International Humic Substances Society (IHSS), solutions were prepared from freeze-dried stocks diluted using ultra pure water (MilliQ) to a final concentration of 0.5 $g.L^{-1}$. Dissolved organic matter stock solution was filtered through a 0.22 μm polycarbonate filter prior to being used to prepare working solutions. Dissolved organic carbon (DOC) levels in our stock solutions was determined using an OI Analytical "TIC-TOC" Analyser Model 1030; DOC expressed as ppmC content was ca. 45%. Triplicate water samples were collected in September 2012 from 18 lakes (n = 18, Figure 2.1A and B) in the Tathlina Lake, Cameron Hills and Yellowknife regions (NWT; Figure 2.1B, Table S2). Marked permafrost thawing in the area has resulted in a wide dissolved organic carbon gradient among lakes; accordingly, field-collected waters of different DOC concentrations (0.8 to 38.1 $mg.L^{-1}$) were obtained. Samples were collected in acid-washed fluorinated bottles, and filtered in the field through 0.22 μm polyethersulfone sterile disposable filters (Stericup® Millipore Filter Unit), kept in amber 60 ml borosilicate containers in the dark and cold and analyzed within 7 days.

2.3.2 Modeling of Hg speciation.

Equilibrium speciation calculations were conducted using Visual MINTEQ Version 3.0 (Gustafsson 2012) to model Hg interactions with inorganic and simple organic ligands in the dissolved phase. A NICA-Donnan model predicted the extent of Hg binding to DOM. This model enabled simulation of cation complexation to constituents that are highly heterogeneous with

respect to binding affinity, such as humic (HA) and fulvic (FA) acids. As modeled, each FA and HA fraction had two types of binding sites: one with a weak affinity (FA1 and HA1) for Hg and one with a strong affinity (FA2 and HA2) for Hg (Drexel *et al.*, 2002). The weak binding sites are representative of interaction with carboxyl groups while the strong binding sites are representative of binding with organic thiol functional groups (Drexel *et al.*, 2002, Haitzer *et al.*, 2003; Ravichandran, 2004). The generic NICA-Donnan model parameters for fulvic and humic acids were based on those recommended by Milne *et al.* (2003) and the estimated binding affinities for Hg are described by the equations below:

$Hg^{2+} + 2H_2O \Rightarrow Hg(OH)_2 + 2H^+$ log K = 6.2

$H\text{-}FA1 + Hg(OH)_2 + H^+ \Rightarrow [FA1\text{-}Hg]^+ + 2H_2O$ log K = 14

$H\text{-}FA2 + Hg(OH)_2 + H^+ \Rightarrow [FA2\text{-}Hg]^+ + 2H_2O$ log K = 30

$H\text{-}HA1 + Hg(OH)_2 + H^+ \Rightarrow [HA1\text{-}Hg]^+ + 2H_2O$ log K = 14

$H\text{-}HA2 + Hg(OH)_2 + H^+ \Rightarrow [HA2\text{-}Hg]^+ + 2H_2O$ log K = 30

We also ran simulations for which the binding constants for Hg to the strong binding sites onto fulvic and humic acids (FA2 and HA2) varied from log K = 25 to log K = 32 to reflect variability observed in conditional constant estimates (Dong *et al.*, 2011; Haitzer *et al.*, 2003). In all cases, results are consistent with the observation that under oxic conditions when DOM is used as a ligand, virtually all of the Hg is bound to DOM and most (>95%) is bound to either strong binding sites onto fulvic or humic acids (Table S2.1).

2.3.3 THg analyses in water.

Total mercury was analyzed using dual gold trap pre-concentration and cold vapour atomic fluorescence spectroscopy (CVAFS). Analyses were conducted on a Tekran 2600 system following the modified U.S. EPA Method 1631. The method detection limit was 0.1 $ng.L^{-1}$ (3σ of all blanks). Initial precision and recovery (IPR) and ongoing precision and recovery (OPR) were 105 ± 1.2% and 104 ± 4.6%, respectively. Field and travel blanks had concentrations ≤ 0.1 $ng.L^{-1}$.

2.3.4 Bacterial strains and media.

We used *Escherichia coli* HMS174 (pRB28), a gram-negative bacterium hosting a *mer-lux* construct as our bioreporter (Selifonova *et al.*, 1993). An *E. coli* strain, HMS174, containing plasmid pRB27 and constitutively expressing bioluminescence, was used as control in our experiments to test for changes in bioluminescence unrelated to the presence of Hg^{II}, such as

alteration in overall cell physiology (Golding *et al.*, 2002). Growth and assay media composition were adapted from Golding *et al.* (Golding *et al.*, 2002) with the final concentration of $(NH_4)_2SO_4$ in the assay medium set at 0.9 mM (Golding *et al.*, 2002). Kanamycin was added to a final concentration of 100 $\mu g.mL^{-1}$ in all growth media but not in assay media. Briefly, 5 mL of lysogeny broth (LB) was inoculated with a single colony and incubated at 37°C with shaking until late log phase (typically, 6-7h). Fifty microliters of the LB culture was transferred to a serum bottle containing 5 mL of glucose minimal medium (GMM), and incubated overnight. In the morning, 20 mL of fresh GMM was added to the serum bottles and incubated for another 3 hours. Cells were harvested by centrifugation, washed and re-suspended in phosphate buffer (67 mM Pi composed of NaH_2PO_4 and K_2HPO_4 set at pH = 6.8). Cell density was set at a final OD_{600} of 0.4 (~3.10^8 cells.mL^{-1}). A dilution of 1/100 of this cell suspension was used for the assay.

2.3.5 Hg^{II}-NOM complexation Bioassays.

Assays were prepared in borosilicate scintillation vials filled with 10 mL of phosphate assay medium (see Table S2.3). Dissolved organic matter amendments were 2, 10, 25 and 50 $mg.L^{-1}$ of each of the HA and FA fractions. Mercury was added as $HgCl_2$ to a final concentration of 250 pM after cells and DOM were mixed in assay medium (t = 0), or after 24 hours incubation time in the presence of HA or FA without cells, prior to being exposed to the cells in the assay medium (t = 24h); pseudo-equilibrium conditions were presumed to be established following 24-hour contact between mercury and DOM prior bioassay initiation based on previous studies (Miller *et al.*, 2009); incubations were performed at 4°C in the dark in Teflon bottles and all interactions between Hg and DOM occurred at the same ionic strength for all experiments. pH of the solution was measured throughout the assay period and no significant changes in pH were observed over time or DOM levels (Table S2.4). Bioluminescence was measured using a multimode plate reader (Tecan F200 Pro) from a 96-well plate (Teflon PFA); 200 μL of assay medium were transferred from the scintillation vials to the wells. Light production was monitored continuously for up to 3 h at 5 min intervals; values were recorded individually for each well for 5 sec. No-DOM, no-DOM-no-Hg^{II} and no-Hg^{II} controls, as well as controls with *E. coli* HMS174 (pRB27) matching all treatments were included for each experimental set.

The bioavailability of Hg^{II} in in the presence of DOM was also investigated across a wide gradient of lake DOC (Figure 2.1A). These bioassays were performed as described above except that lake water was substituted for MilliQ-water in the preparation of the assay medium.

Therefore, all lake water samples were buffered at pH = 6.8 using phosphate buffer and amended with 5 mM glucose and 0.9 mM $(NH_4)_2SO_4$. Spiking was also required in these bioassays because THg concentrations in field samples were very low (Figure 1E), and could not be detected by the bioreporter *E. coli* HMS174 (pRB28). The results of three independent experiments are presented (i.e., each started from a single colony). As previously described (Daguene *et al.*, 2012; Golding *et al.*, 2002), net Hg^{II} accumulation is proportional to the maximum light produced for a given Hg^{II} concentration (Figure S1A) as well as the maximum slope of induction in light production. In this study, we used the maximum amount of light produced by the bioreporter as a proxy for the net accumulation of Hg^{II} in the cytoplasm. Examples of typical time series are presented in Figure S2.1 (B-F).

2.3.6 Cell Membrane Permeability Bioassays.

The effect of DOM on the cell wall permeability of the bioreporter was evaluated in bioassays similar to those described above. The difference was that cells were preconditioned with DOM for 20 minutes prior to being harvested, washed and re-suspended in fresh phosphate buffer to insure removal of DOM in solution or poorly adsorbed to the cell wall prior to exposure to Hg^{II}. We tested cells at 10 and 50 $mg.L^{-1}$ of HA, or 10 and 50 $mg.L^{-1}$ of FA. Bioassays were performed in triplicate. Cell wall permeability was tested specifically for Hg using bioreporter cells in the presence of Hg^{II} but also using bile salts (final concentration 0.5%) to test whether cells were more sensitive to the effect of bile salts after treatment by DOM by comparing their growth rates under the various conditions tested (Figure S2.3).

2.4 Results and Discussion

2.4.1 DOM enhances Hg^{II} bioavailability to bacteria under non-equilibrium conditions.

Because the formation of Hg-DOM complex is a slow process thought to be associated to competitive ligand exchange among various functional groups within DOM molecules (Miller *et al.*, 2012; Miller *et al.*, 2009), we assessed how Hg^{II} bioavailability varied in the presence of increasing DOM concentrations over time.

When Hg^{II} and DOM were left to reach pseudo-equilibrium for 24h, Hg^{II} bioavailability decreased with increasing [HA] and [FA] concentrations (Figure 2.2A and B); this was expected as chemical modeling of Hg^{II} speciation at equilibrium predicted that under the assay conditions, all Hg^{II} was mostly bound to strong binding sites onto HA and FA (Table S2.1), forming species

thought to be poorly reactive and non bioavailable (Barkay *et al.*, 1997; Kelly *et al.*, 2003; Miller *et al.*, 2012; Miller *et al.*, 2009). However, when Hg^{II} was added to a solution already containing cells in the presence of FA or HA, we observed an unexpected pattern where Hg^{II} bioavailability first significantly increased 10 fold when [FA] or [HA] varied from 0 to 10 $mg.L^{-1}$ (Figure 2.2C and D), before declining between 10 $mg.L^{-1}$ and 50 $mg.L^{-1}$ (Figure 2.2C and D) .

To test the relevance of this surprising observation with lake water, we assayed the response of the bioreporter using samples collected from a series of 18 lakes located in the Northwest Territories (NWT, Canada, Figure 2.1B) with DOC concentrations ranging from 0.8 to 38.1 $mg.L^{-1}$ (Figure 2.1A). Under non-equilibrium conditions, Hg bioavailability in lake water first increased 10 fold when DOC concentrations increased from 0.8 to 8.6 $mg.L^{-1}$ and followed the same bell shaped curve that was observed with defined medium (closed black symbols Figure 2.3A). No bioavailable Hg^{II} was detected when the bioreporter was exposed to lake water prior to any Hg^{II} addition. This was probably due to the very low THg concentration present in these samples (Figure 2.1A) as previously observed for lakes from the Canadian Boreal Shield (Kelly *et al.*, 2003); furthermore, Hg^{II} bioavailability greatly decreased when Hg^{II} was incubated for 24h with lake water prior to exposure to the cells (open symbols, Figure 2.3C). French *et al.* (2014; Published as a companion paper) observed a similar threshold type relationship for Hg bioaccumulation in aquatic invertebrates collected from a wide range of DOC concentrations from tundra lakes, suggesting that DOC affects Hg bioaccumulation similarly in bacteria and aquatic invertebrates. Expanding on these results, we later assayed the bioreporter response using newly collected lake water samples from these same study sites; the bioreporter data obtained also agreed with the results from French *et al.* (2014) for aquatic invertebrates. Under non-equilibrium conditions, Hg^{II} bioavailability increased between 6.8 and ~13.0 $mg.L^{-1}$ DOC, and then declined at higher DOC (~13.0 to 35.0 $mg.L^{-1}$; Figure 2.4), further supporting analogous effects between these trophic levels.

Components of DOM are both potential ligands for Hg^{II} and nutrients for bacteria and, as such, can alter Hg^{II} bioavailability by either affecting its speciation in solution or by affecting bacteria cell physiology or cells' physical and chemical properties. All experiments previously described were also performed using a control strain that constitutively emitted light. This strain was originally designed as a way to control for variation in light production unrelated to the presence of Hg but possibly due to alteration in cell physiology from experimental conditions (Barkay *et al.*, 1998). Light production by the control strain did not follow a bell-shaped curve but increased when exposed to lake water or exposed to the assay medium prepared in milliQ with

increasing [DOM] (Figure 2.3B and D, Figure S2.2). Constitutive light production, through the luciferase activity, is a good indicator of cell physiology (Golding *et al.*, 2007), but bioluminescence is also a very costly cellular process. We suggest that cells metabolized some of the most labile forms of carbon present in the pool of either fulvic or humic acids or in the natural water sample as a source of energy, increasing constitutively expressed light production.

The bell-shaped curve, observed for both defined media and lake water samples and describing the response of Hg^{II} availability to increasing DOM concentrations, is reminiscent of the role that cysteine exerted on mercury methylation rate by an anaerobic bacterium, *Geobacter sulfurreducens* (Schaefer and Morel, 2009). By carefully manipulating the composition of their assay medium, Schaefer and Morel (2009) demonstrated that variations in methylation rates, in response to increasing cysteine concentrations, were correlated to the formation of a bioavailable $Hg(Cys)_2$ complex. It was further suggested that methylation rates subsequently declined, at the tipping point, upon formation of an unavailable $Hg(Cys)_3$ complex. A similar model, implicating molecular shuttles, may apply to our data. While we did not perform detailed analyses of the organic composition of HA and FA or lake water, it is reasonable to speculate that increasing DOM and DOC concentrations corresponded to increased concentrations of labile molecules that bacteria can use as carbon or energy source (as suggested by the increased constitutive light production in the presence of DOM or DOC) and that were therefore co-transported with Hg^{II}.

In further support of the shuttle model is the fact that the bell-shaped curve was only observed under non-equilibrium conditions. We hypothesize that the nature of Hg interactions with DOM affects Hg^{II} bioavailability (Figure 2.5A) because of the heterogeneous nature of DOM that includes functional groups of various binding strength, but also of varying accessibility and abundance. First, Hg bioavailability increases upon interacting with DOM, because it binds to a fraction of the DOM pool that can be internalized by the cells, co-transporting Hg^{II} during the process (the fraction of the DOM pool that is available to cells is termed DOM1, Figure 2.5A); in this case Hg may interact with DOM1 via weak (e.g., carboxyl) or strong (e.g., biogenic thiols; Ndu *et al.*, 2012; Schaefer and Morel, 2009) binding sites. Indeed, Suwannee River fulvic acids, for instance, contain organic molecules spanning a wide range of molecular weight (100-1500 Da; Aiken and Malcolm, 1987; Remucal *et al.*, 2012) with metal chelating properties that are present individually or in loosely bound assemblies and that can be available to microbial cells (Remucal *et al.*, 2012). Alternatively, Hg could bind onto DOM1 sites that can be easily

exchanged for metal transport sites onto the bacterial cell wall via ligand exchange, without necessarily requiring internalization of the organic molecule (Figure 2.5A).

Over time and over increasing [DOM], bioavailability decreases (Figure 2.2C and D and 2.3A) as Hg is preferentially distributed towards less bioaccessible binding sites in molecules that are more refractory and unavailable to cells or bound to sites that do not allow for ligand exchange to occur (DOM2, Figure 2.5). This model is in agreement with experimental data showing that Hg^{II} interaction with DOM is kinetically controlled, involving first Hg^{II} binding to more accessible labile reactive molecules before being subsequently redistributed towards binding sites on larger hydrophobic molecules with binding microenvironments that limit competitive ligand interactions (Miller *et al.*, 2012; Miller *et al.*, 2009). Finally, Hg bioavailability to the bacterial cells may be controlled by diffusion through the boundary layer; indeed, if the presence of organic matter on the cell wall enhances Hg uptake (see below) so that Hg internalization is faster than its diffusion from the bulk solution to the uptake site, then diffusion through the boundary layer becomes limiting and the uptake would decrease.

Further investigation into the importance of ternary (Hg^{II}-cell-DOM) interactions is warranted to further elucidate the mechanisms involved in the NOM-dependent increase of Hg^{II} bioavailability. Indeed, degradation of DOM is key in meeting energy and carbon demands to sustain a heterotrophic metabolism, whether under oxic or anoxic conditions (Axmanova *et al.*, 2006). In freshwater systems, low-molecular-weight compounds are generally more readily assimilated by bacteria relative to the high-molecular-weight fraction of dissolved natural organic matter (Saunders, 1976). These simpler compounds (mostly carboxylic acids, amino acids, and carbohydrates) are less costly to process, making them more labile than macromolecules and ideal Hg shuttles (Golding *et al.*, 2002; Schaefer and Morel, 2009).

2.4.2 Interactions between DOM and bacterial cell walls.

Dissolved organic matter plays a central role in aquatic ecosystems (Hassett, 2006) and while it can be metabolized as a carbon or energy source, it can also interact with living surfaces and influence chemical and physical processes at the cell-solution interface (Campbell *et al.*, 1997). For instance, humic substances can act as surfactants when adsorbed to natural surfaces, increasing the permeability of biological membranes to passive uptake of neutral chemical species (Vigneault *et al.*, 2000) but can also decrease the uptake of charged metals and therefore their toxicity (Koukal *et al.*, 2003). We first determined that at all FA and HA concentrations tested, there was a great excess of DOM to cover the cell wall of all bacteria

present in solution, suggesting that the increase in Hg bioavailability as [DOM] increased was not due to a variable proportion of the cell wall covered by DOM. Our cell suspension assay contains 10^6 cells in a final volume of 10 mL, the surface area of an *E. coli* cell (assuming a cylindrical body with spherical ends) is ca. 6 μm^2 corresponding to a total of 6.10^{-4} $m^2.L^{-1}$. Assuming that some of the DOM added formed a colloidal fraction, we estimated DOM colloids surface area to be ranging from 3 to 80 $m^2.L^{-1}$ (Gueguen *et al.*, 2004) or six orders of magnitude greater than the cell surface area.

We tested the role of HA and FA in affecting the permeability of the bioreporter's cell wall because humic substances can act as surfactants and because the uptake of neutrally charged species is one possible route for Hg^{II} internalization (Benoit *et al.*, 2001; Benoit *et al.*, 1999). Note that, in the assay medium at pH = 6.8, neutrally (32%, $Hg(OH)_2$) and positively (68%, $Hg(NH_3)_2^{2+}$) charged Hg species were predicted to co-exist. We first performed a series of experiments, during which cells were pre-conditioned for 20 minutes with [HA] or [FA] at 10 or 50 $mg.L^{-1}$, then rinsed and washed prior to being exposed to Hg^{II}. Whether FA or HA were used to condition the cells, Hg^{II} uptake increased up to two fold compared to the no-DOM treatment (Figure 2.6A and B). Light emitted by the control strain was not affected by the conditioning, rinsing or washing steps (Figure 2.6C and D). Second, bile salts are commonly used to test how a given set of experimental conditions alter cell wall permeability (Hofmann and Roda, 1984; Welander *et al.*, 2012); a more permeable cell wall makes cells more sensitive to bile salts (e.g., cholic acid) leading to a decrease in cell growth rates. Although it affected Hg uptake, we did not observe that bioreporter cells pre-conditioned with DOM were susceptible to bile salts (no significant difference was detected between the various growth rates, see Figure S2.3). This differential response of the bioreporter may indicate that overall cell wall permeability was unaltered but its ability to internalize Hg via facilitated or active transport may have been enhanced. For instance, interaction of DOM with the bacterial cell wall may lead to destabilization of the LPS layer thus promoting access to transport sites.

2.4.3 Environmental Implications.

We propose a new model for the role of DOM in controlling Hg bioavailability to bacteria (Figure 2.5). Our data first obtained using defined media and reproduced with lake water, confirm the kinetically important and complex nature of Hg^{II} interaction with DOM. More strikingly, it also suggests that Hg bioavailability to bacteria under non-equilibrium conditions could be predicted by [DOM]. French *et al.* (2014; Published as a companion paper) used the

wide gradients in lake water DOC amongst tundra lakes to determine DOC effect on Hg bioaccumulation by aquatic invertebrates in Arctic lakes. Similarly to the response of the bioreporter, they showed that DOC promotes Hg bioaccumulation by aquatic invertebrates in lakes having low DOC (<8.6 – 8.8 mg $C.L^{-1}$; DOC threshold concentration, T_C) whereas DOC inhibits Hg bioaccumulation in lakes having high DOC (>DOC T_C). Subsequently, we obtained a consistent threshold-type relationship between bacterial Hg bioavailability and lake water [DOC] for the study sites of French *et al.* (2014); T_C corresponded to ~13.0 mg $C.L^{-1}$ (Figure 2.4). Together these two studies demonstrate how the biological uptake of Hg in lakes is determined both by complexation kinetics and binding thresholds on DOC, a water quality variable predicted to change markedly with future environmental change.

In an environmentally relevant situation, we propose a model that describes how DOM affects Hg bioavailability to bacteria and that can be summarized as follows: originating from the atmosphere, newly deposited Hg^{II} binds rapidly to biologically labile molecules present in the pool of DOM, forming Hg-DOM1 complexes that are bioavailable (Figure 2.5A). Over time (but no more than 24h), Hg^{II} becomes much less available, due to a series of ligand exchanges within complex molecules of the DOM pool (Figure 2.5A). Furthermore, DOM directly interacts with bacterial cell walls to affect Hg uptake (Figure 2.5B). This model implies that several routes for bacterial Hg^{II} internalization exist, involving both facilitated or active transport as well as passive diffusion.

It is hard to predict whether newly deposited Hg^{II} reaches the anoxic hypolimnetic waters or profundal sediments, where Hg methylation mostly occurs, within the first 24h after deposition. It is hard, because most lakes become rapidly stratified during the open water season or covered with ice during the winter. However, MMHg production from newly added stable isotopes to lake water was detected in anoxic bottom water within 72h after Hg^{II} addition (Harris *et al.*, 2007). Regardless of how fast newly deposited Hg^{II} can reach methylation sites, several processes may occur that can "reset" Hg speciation within lake water column or sediments, far from the air/water interface (Figure 2.5C). First, DOM can be rapidly degraded by photochemical oxidation or slowly broken down by the aerobic or anaerobic activity of microbial heterotrophic communities and upon degradation, release strongly bound Hg^{II}, as shown for other metals (Masson *et al.*, 2011; Wengel *et al.*, 2006). This process allows for Hg to dissociate from DOM2 and bind to DOM1, forming a more bioavailable fraction. Second, we hypothesize that Hg itself can be recycled from the pool of natural organic matter following a series of reduction and oxidation reactions, together constituting the "Hg redox wheel". Redox reactions

lead to the release of elemental Hg or newly oxidized mercury that can bind to DOM1, or under anoxic conditions, sulfides, forming dissolved or nanoparticle species. All these species are currently thought to be involved in Hg uptake (Deonarine and Hsu-Kim, 2009; Graham *et al.*, 2012; Zhang *et al.*, 2012) or directly bioavailable (Benoit *et al.*, 1999; Colombo *et al.*, 2013; Golding *et al.*, 2002, Schaefer *et al.*, 2011; Figure 2.5C). Altogether our data suggest that i) kinetic controls of Hg speciation by natural organic matter are key in predicting Hg bioavailability and ii) offer a possible mechanism for the greater availability of newly deposited Hg to aquatic food webs (Harris *et al.*, 2007), suggesting that the bulk of mercury incorporation in aquatic food webs would occur within hours following its deposition from the atmosphere.

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2.6 List of Figures

A

Lake	[DOC] ($mg.L^{-1}$)	[THg] (pM)
September 15, 2012 1. Cameron Hills, NWT		
CH2	17.4	7.88
CH4	18.8	9.92
CH5	20.3	7.28
CH7	21.4	9.77
CH6	25	12.46
CH3	35	17.85
CH1	37.4	16.05
September 16, 2012 2. Kakisa lake, NWT		
KAK4	33.8	4.29
KAk3	38.1	5.38
September 17, 2012 3. Tathlina lake		
TA1A	21.4	7.38
September 19, 2012 4. Yellowknife, NWT		
DQ2	0.8	1.00
DQ3	1.1	1.00
DQ1	2.3	1.25
ING27	8.6	2.54
ING28	12.6	3.54
ING24	27.8	1.99
ING19	30.6	16.05
DET15	33.4	3.69

B

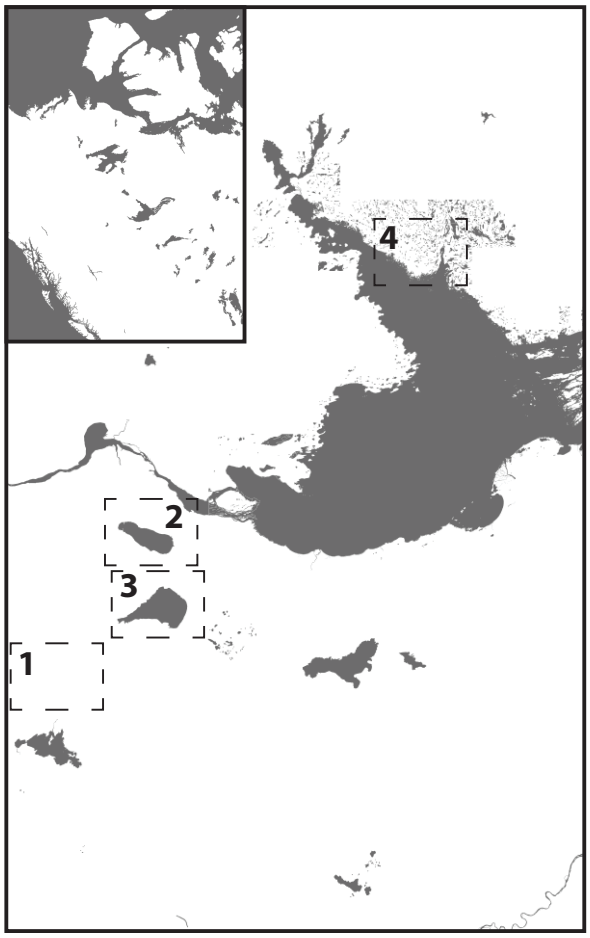


Figure 2.1: Study lakes (n = 18) on the northern and south-western shores of Great Slave Lake, Northwest Territories, Canada. Table (A) provides the DOC concentration ($mg.L^{-1}$) and THg concentration (pM) of the study lakes. Map (B) illustrates the location of the study lakes, according to four areas: (1) Cameron Hills, denoted "CH"; (2) Kakisa Lake, denoted "KAK"; (3) Tathlina Lake, denoted "TA"; and (4) Yellowknife, denoted "DQ", "ING", or "DET". Lake water samples were collected from September 15, 2012 to September 19, 2012,

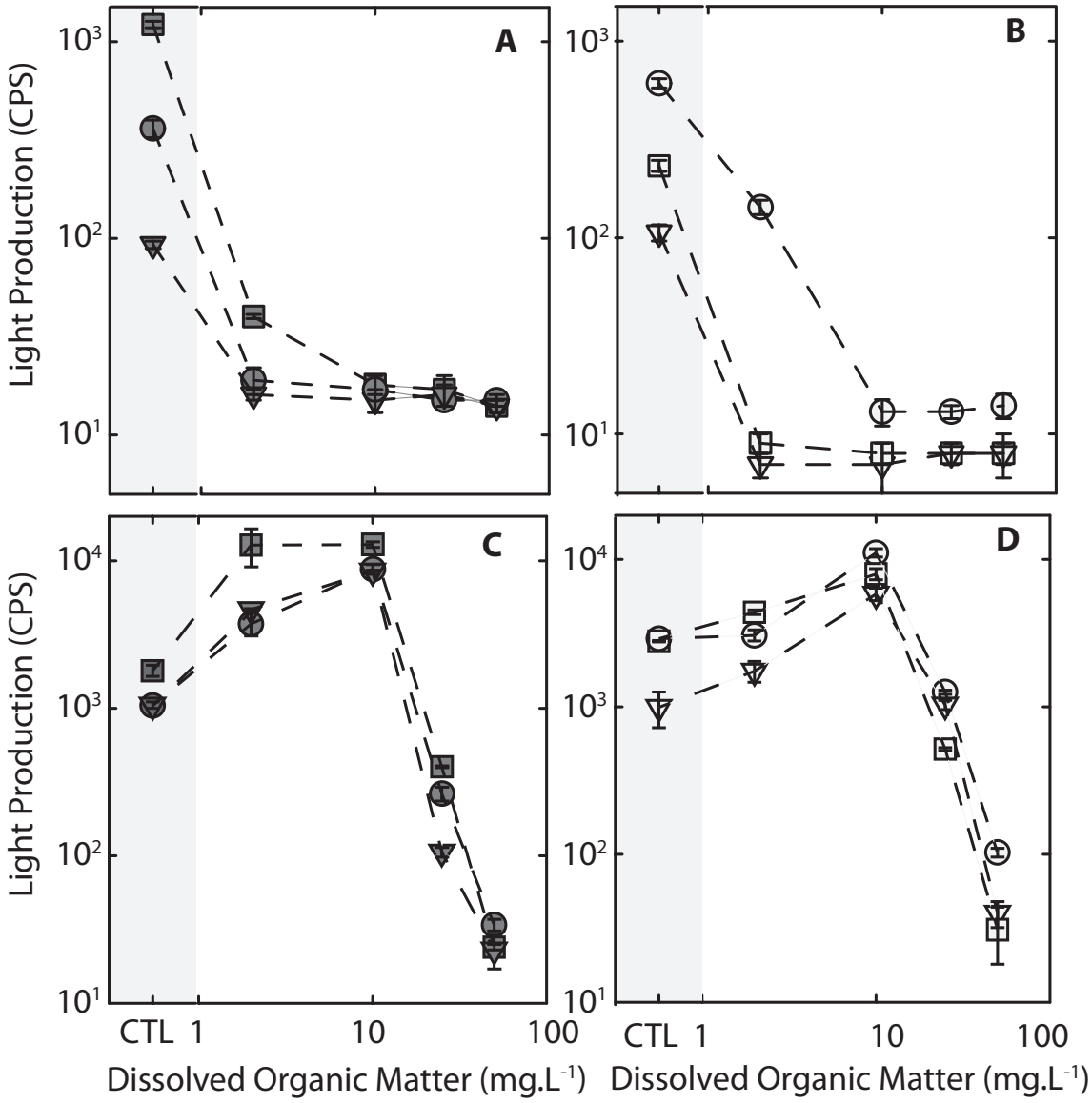


Figure 2.2: Effect of DOM on Hg^{II} bioavailability to the bioreporter. Light production is a proxy for the amount of intracellular Hg^{II}. Experiments were performed under pseudo-equilibrium conditions with humic (A) and fulvic (B) acids; or under non-equilibrium conditions with humic (C) and fulvic (D) acids. CTL represent control experiments for which no DOM was added to the assay. Solutions were prepared in milliQ water buffered at pH=6.8 with phosphate buffer and amended with 5 mM glucose and 0.9 mM $(NH_4)_2SO_4$ and 250 pM $HgCl_2$. Three independent experiments are presented for each panel; error bars represent the standard deviation of at least six analytical replicates.

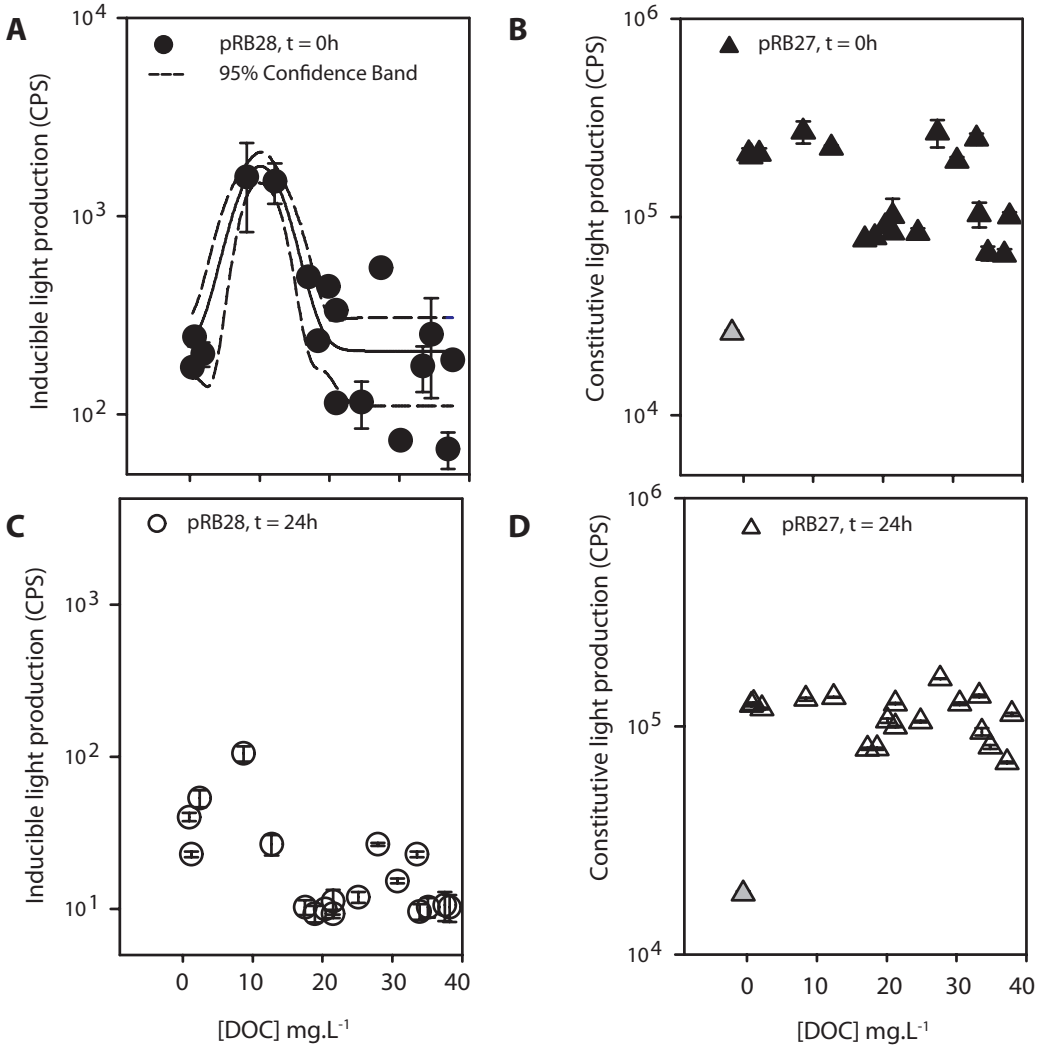
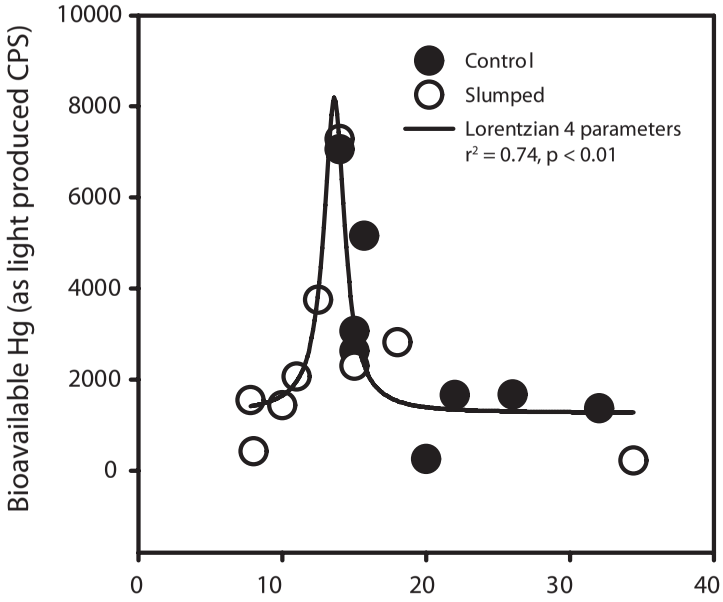
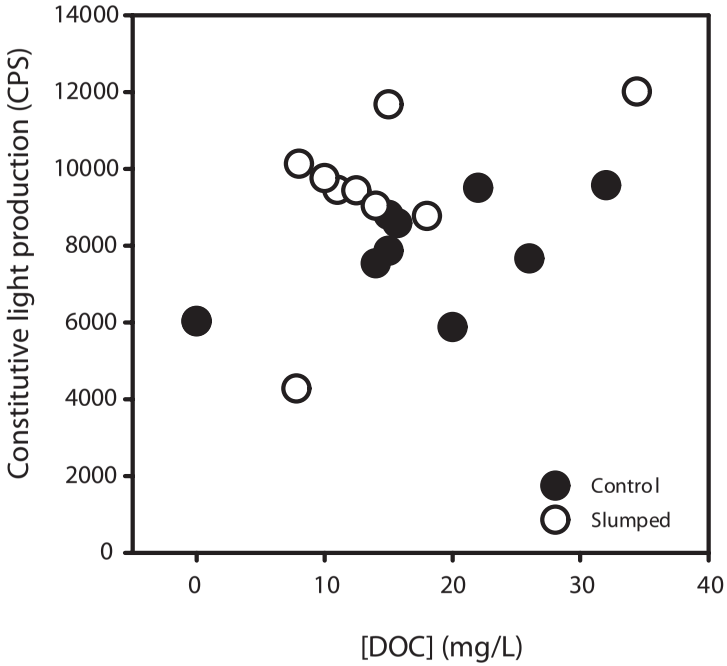


Figure 2.3: Bioavailability of Hg^{II} in lake water spanning a range in DOC concentrations. Light production is a proxy for the amount of intracellular Hg^{II}. Under non-equilibrium conditions, evolution of bioavailable Hg^{II} (A) or constitutive light expression (B) over a natural DOC gradient. Under pseudo-equilibrium conditions, evolution of bioavailable Hg^{II} (C) or constitutive light expression (D) over a natural DOC gradient. Note that grey triangle symbols in panels B and D represent the cells constitutively expressing light in defined assay medium. Plasmids pRB28 and pRB27 were used as bioreporter and control respectively. Lake water samples were buffered at pH=6.8 with phosphate buffer and amended with 5 mM glucose and 0.9 mM $(NH_4)_2SO_4$ and 250 pM $HgCl_2$. Each point represents data obtained in triplicate; error bars represent the standard deviation of at least six analytical replicates. Samples were collected in September 2012 from 18 lakes (n = 18) near Yellowknife, Tathlina Lake and Cameron Hills, NWT. The best fit model corresponded to a peak Gaussian at four parameters of the general formula $f(x) = y_0 + a*\exp^{(-0.5.((x-x0)\div b)\hat{}2)}$, $r^2 = 0.92$, $p < 0.001$.


Bioavailable Hg (as light produced CPS)
10000
8000
6000
4000
2000
0
0
10
20
30
40
Control
Slumped
Lorentzian 4 parameters
r² = 0.74, p < 0.01


Constitutive light production (CPS)
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
[DOC] (mg/L)
Control
Slumped

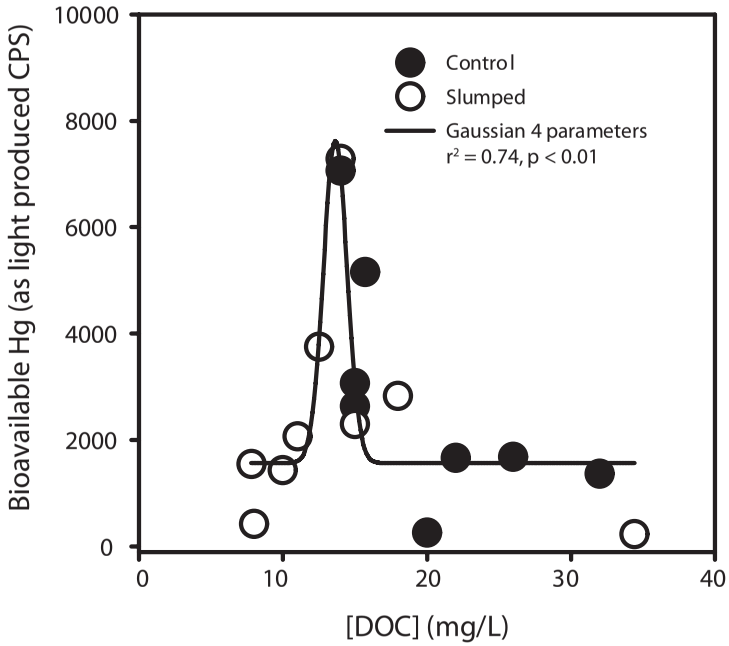
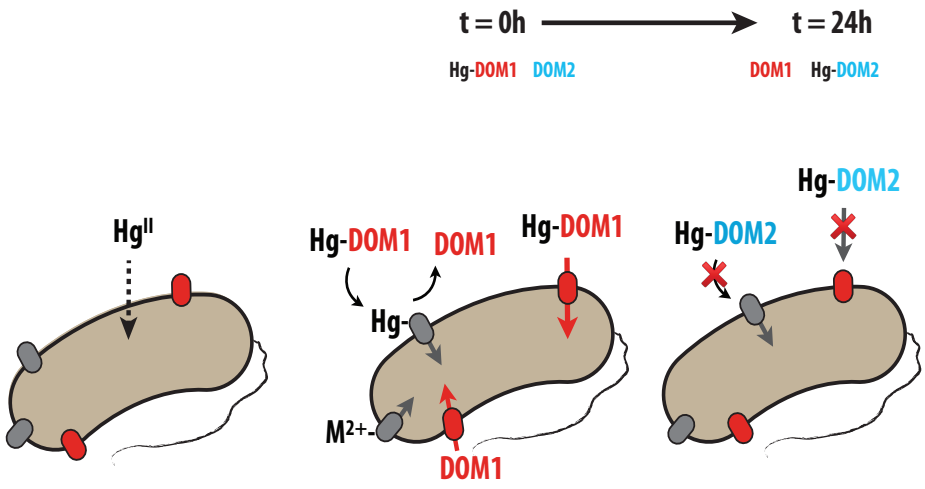
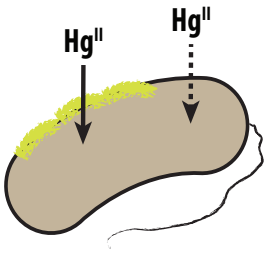

Bioavailable Hg (as light produced CPS)
10000
8000
6000
4000
2000
0
0
10
20
30
40
[DOC] (mg/L)
Control
Slumped
Gaussian 4 parameters
r² = 0.74, p < 0.01

Figure 2.4: Bioavailability of Hg^{II} in lake water (6.8 to 35.0 $mg.L^{-1}$ DOC) corresponding to study sites by French *et al*. (2014) under non-equilibrium conditions. Light production is a proxy for the amount of intracellular Hg^{II}. Evolution of bioavailable Hg^{II} (A, B) or constitutive light expression (C) over a natural DOC gradient. Note that open circle symbols represent lakes disturbed by permafrost thaw slumps, while closed (black) circle symbols represent undisturbed lakes. Lake water samples were buffered at pH=6.8 with phosphate buffer and amended with 5 mM glucose and 0.9 mM $(NH_4)_2SO_4$ and 250 pM $HgCl_2$. Each point represents data obtained in triplicate; error bars represent the standard deviation of at least six analytical replicate. Samples were collected in July 2013 from 18 lakes (n = 18) east of the Mackenzie River Delta, NWT. The best fit models corresponded to a peak Lorentzian at four parameters of the general formula $f(x) = y_0+a/(1+((x-x_0)/b)^2)$, $r^2 = 0.74$, $p < 0.01$(A), and to a peak Gaussian at four parameters of the general formula $f(x) = y_0+a*exp^{(-0.5*((x-x0)/b)^2)}$, $r^2 = 0.74$, $p < 0.01$ (B).

A - Shuttle, ligand exchange and aging of the Hg-DOM complexes



B - Alteration of cell wall properties



C - Reseting Hg speciation

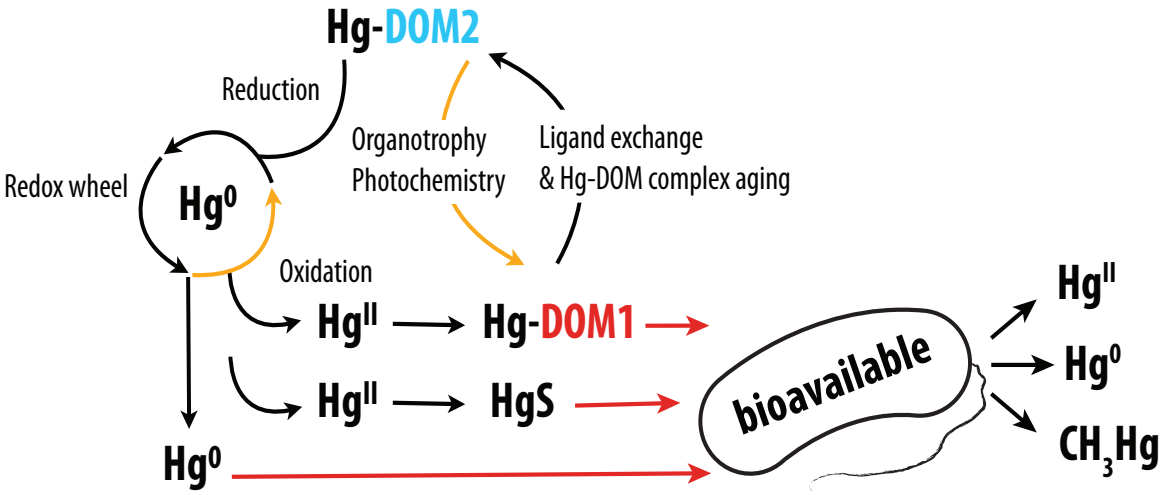


Figure 2.5: Proposed model for how DOM affects Hg bioavailability. Details are provided in the text. It is assumed here that DOM is not represented by a single type of molecule of a given size but rather by a continuum of molecules of varying molecular masses with varying properties with respect to their bioavailability to microbial cells as carbon and/or energy sources. Within a larger pool of DOM, DOM1 represents small molecules with metal chelating properties that can be taken up by microbial cells; DOM2 represents recalcitrant molecules that are unavailable to microbial cells.

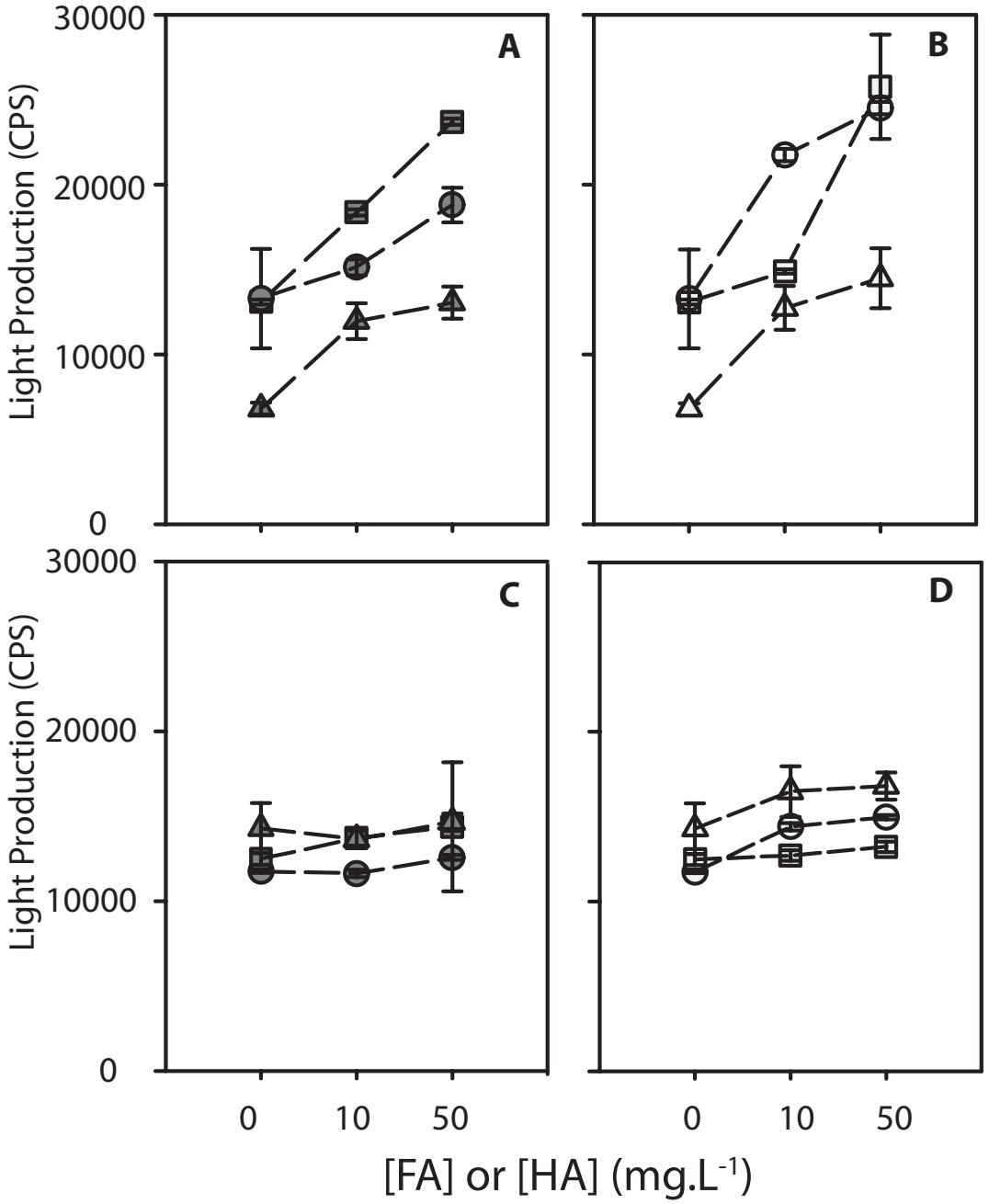


Figure 2.6. Effect of DOM on the permeability of bacterial cell wall and its influence on Hg^{II} bioavailability. Light production by the bioreporter following pre-conditioning of the cell for 20 min with humic (A) or fulvic (B) acids and rinsed with phosphate buffer. Constitutive light production by the control bioreporter strain following NOM-conditioning of the cell wall with humic (C) or fulvic (D) acids and rinsed with phosphate buffer. Experiments were performed at $[Hg^{II}]$ = 250 pM. Three independent experiments are presented in each panel; Error bars represent the standard deviation of at least six analytical replicates.

2.7 Supplemental Information

2.7.1 Material and Methods

2.7.1.1 Lake Water Samples from the Study Sites of French *et al.* (2014)

Study by French *et al.* (2014, Published as a companion paper) was undertaken in the tundra uplands east of the Mackenzie River Delta, Northwest Territories, Canada**.** In the western Arctic**,** tundra lakes display extremely wide pH (6.6-8.1) and DOC (6.8-30.0 mg C. L^{-1}) gradients due to spatial variability in the distribution and magnitude of shoreline retrogressive thaw slumps. French *et al.* (2014) sampled 26 lakes in Summer 2009, both with and without shoreline thaw slumps, to determine the effect of DOC in regulating Hg bioaccumulation (i.e. BAFs) in amphipods. In July 2013, we conducted a new sampling campaign in collaboration with French *et al.* (2014) to compare data of Hg bioavailability between aquatic invertebrates and bacteria. Triplicate water samples were collected from some of the same study lakes, including "Reference Lakes" without shoreline thaw slumps (n = 9) and "Thaw Lakes" with shoreline thaw slumps (n = 9). Samples were collected in acid-washed fluorinated bottles, and filtered in the field through 0.22 μm polyethersulfone sterile disposable filters (Stericup® Millipore Filter Unit), kept in amber 60 ml borosilicate containers in the dark and cold and analyzed within 7 days.

2.7.1.2 Bacteria Strains, Media, and Culture

We used *E. coli* HMS174 (pRB28) and *E. coli* HMS174 (pRB27) respectively as inducible and constitutive strains in our experiments. Selifonova et al. (1993) provided us with cryostocks of both strains, i.e., culture stocks frozen down at -80°C in 50% glycerol. Growth and assay media composition, as well as growth conditions were adapted from Golding *et al.* (2002). Briefly, a lysogeny broth (LB) culture plate with the appropriate antibiotic (kanamycin 100 μg.mL^{-1}) was inoculated with cryostocks of either strain, and incubated for a 36-hour period at 37°C. Kanamycin was added to a final concentration of 100 μg.mL^{-1} in all growth media to ensure selection of the transformants carrying the bioreporter construct. Under sterile conditions, a test tube with 5 mL of LB and antibiotics (kan-LB) was inoculated with a single isolated colony from the culture plate. After an 8-hour incubation period at 37°C with shaking (200 rpm), a 50 μL of the LB culture was transferred into 5 mL of glucose minimal media and antibiotics (kan-GMM) in a pre-autoclave serum bottle and grown overnight at 37°C with shaking (200 rpm). In the morning, 20 mL of kan-GMM was added to the overnight culture, and grown again for 4h until

cells reach mid-exponential phase at an optical density measured at 600 nm (OD_{600}) of 0.6-0.7. Cells were then ready to be harvested. At the end of the 4-hour growth period, 3-6 mL of GMM culture was centrifuged at 13400 rpm for 60s, and the supernatant was removed to leave behind only the pellets of cells. Cells were washed in 500 μL of fresh phosphate buffer (67 mM Pi composed of NaH_2PO_4 and K_2HPO_4 set at pH = 6.8). Following the washing step, cells were suspended in 5 mL of phosphate buffer to a final OD_{600} of 0.4, which is equivalent to ~3.10^8 cells.mL^{-1}. A dilution of 1/100 of this cell suspension was used for the assay. Similar cell population densities are found in aquatic ecosystems (~10^6 cells.mL^{-1}; Jordan, 1985).

2.7.1.3 Cell Membrane Permeability Bioassays

One treatment involved the cells to be preconditioned with DOM for 20 minutes prior to being harvested, washed and used for the assay. Once cells had reached mid-exponential phase, 3-6 mL of GMM culture was amended with 10 and 50 mg.L^{-1} of each of the HA and FA fractions. After 20 minutes, cells were harvested by centrifugation, and washed twice with 500 μL of phosphate buffer to insure removal of DOM in solution or poorly adsorbed to the cell wall prior to exposure to Hg^{II}. Both the inducible and constitutive strains were subject to the same conditioning and washing treatments. After being washed, cells were suspended in 5 mL of phosphate buffer to a final OD_{600} of 0.4. A dilution of 1/100 of this cell suspension was used for the assay that tested cell wall permeability specifically for Hg.

Additionally, non-specific cell wall permeability was tested using a bile salt mixture. A more permeable cell wall makes cells more sensitive to bile salts (e.g., cholic acid) leading to a decrease in cell growth rates. Two millilitres of glucose minimal media with 0.5% bile salts was amended with 2, 5, 10, 25 and 50 mg.L^{-1} of each of the HA and FA fractions. After vigorous mixing, 200 μL aliquot of the DOM-GMM medium was transferred into a 96-well plate (Teflon PFA). Wells were inoculated with 1% inoculum of LB culture with cells at mid-exponential phase, i.e., 2 μL of LB culture into 200 μL of DOM-GMM medium. Growth rates were determined by measuring OD_{600} using a multimode plate reader (Tecan F200 Pro).

2.7.1.4 Supplementary References

Jordan, M.J. 1985. Mirror lake – biologic considerations. In: G.E. Likens (ed.) An ecosystem approach to aquatic ecology. pp. 156-160, Springer-Verlag.

2.7.2 List of Tables

Table S2.1 : Inorganic and organic speciation of Hg at equilibrium modeled using Visual MINTEQ v 3.0. Relative abundance of each species (%). As modeled, each FA and HA fraction had two types of binding sites: one with a weak affinity (FA1 and HA1) for Hg and one with a strong affinity (FA2 and HA2) for Hg.

			OR			
Treatment	**$Hg(OH)_2$**	**$Hg(NH_3)_2^{2+}$**	**Hg-HA1**	**Hg-HA2**	**Hg-FA1**	**Hg-FA2**
No DOM	37.9	62.1	0	0	0	0
[DOM]=2 mg.L^{-1}	0	0	12.9	87.1	0.2	99.8
[DOM]=10 mg.L^{-1}	0	0	8.7	91.2	0.2	99.8
[DOM]=25 mg.L^{-1}	0	0	6.9	93.1	0.1	99.9
[DOM]=50 mg.L^{-1}	0	0	5.8	94.2	0.1	99.9

Table S2.2: Water chemistry data from sampled lakes.

Lake/ ID	pH	SpCond.[a] (μS.cm^{-1})	TA[b] (mg.L^{-1})	Ion Concentration (mg.L^{-1})						
				$NO_2^- + NO_3^-$ [c]	Ca^{2+}	Cl^-	Mg^{2+}	K^+	Na^+	SO_4^{2-}
Cameron Hill, NWT										
CH1	6.05	28.4	3.8	0.07	7.4	<0.7	1.6	0.3	0.7	1
CH2	7.75	96.1	48	<0.01	18.1	<0.7	3.8	0.2	1	1
CH3	6.86	33	9.1	<0.01	9.8	<0.7	1.7	0.1	0.4	<1
CH4	7.58	76.6	39.8	<0.01	16.3	<0.7	3.2	<0.1	0.4	<1
CH5	7.14	49.8	15.1	<0.01	9.9	<0.7	2.8	0.3	0.7	5
CH6	7.11	40.2	14.3	<0.01	10.2	<0.7	1.8	0.2	0.9	<1
CH7	6.98	32.9	9.4	<0.01	8.2	<0.7	1.5	0.3	0.6	2
Kakisa, NWT										
KAK3	8.35	268	143	0.02	47.7	<0.7	10.5	0.6	4.6	5
KAK4	8.45	234	129	0.01	39.8	<0.7	10.2	0.7	3.7	<1
Tathlina, NWT										
TA1A	8.33	331	144	<0.01	51.2	3.8	11.1	0.7	10	29
Yellowknife, NWT										
DET15	7.36	105	55.7	0.01	11.2	<0.7	6.7	0.9	4.5	2
DET24	8.00	195	89.3	<0.01	17.2	11.6	11.2	2.1	7.4	4
ING19	8.01	73	36.3	0.02	6.5	<0.7	4.9	1.8	3.3	4
ING27	7.94	67	32.8	0.01	9	<0.7	2.5	1.1	1.4	3
ING28	8.23	176	38.5	<0.01	10.1	<0.7	3.2	1.2	1.7	1

From left to right (excluding pH), detection limits correspond to 0.4 μS.cm^{-1}, 0.4 mg.L^{-1}, 0.01 mg.L^{-1}, 0.1 mg.L^{-1}, 0.7 mg.L^{-1}, 0.1 mg.L^{-1}, 0.1 mg.L^{-1}, 0.1 mg.L^{-1}, 1 mg.L^{-1} respectively.

[a] Conductivity, specific @ 25°C; [b] Total alkalinity measured as $CaCO_3$; [c] Nitrate and nitrite measured jointly as nitrogen.

Table S2.3: Composition of assay medium.

Medium components	**Final Concentration (M)**
Phosphate Buffer	
Glucose	5.0×10^{-3}
Inorganic PO_4^{3-}	6.7×10^{-2}
Na^+	2.3×10^{-2}
K^+	8.15×10^{-2}
NH_4^+	1.8×10^{-3}
SO_4^{2-}	9.0×10^{-4}
[DOM]	Varying

Table S2.4: Variation in pH of phosphate assay medium amended with Suwannee River humic and fulvic acids during 3h-bioreporter assays.

	Humic Acids			Fulvic Acids		
Treatment	**T = 0 h**	**T = 1.5 h**	**T = 3 h**	**T = 0 h**	**T = 1.5 h**	**T = 3 h**
No DOM	6.86	6.90	6.89	6.86	6.90	6.89
[DOM]=2 mg.L^{-1}	6.90	6.85	6.91	6.92	6.84	6.90
[DOM]=10 mg.L^{-1}	6.94	6.87	6.78	6.86	6.90	6.86
[DOM]=50 mg.L^{-1}	6.92	6.88	6.80	6.91	6.89	6.86

2.7.3 List of Figures

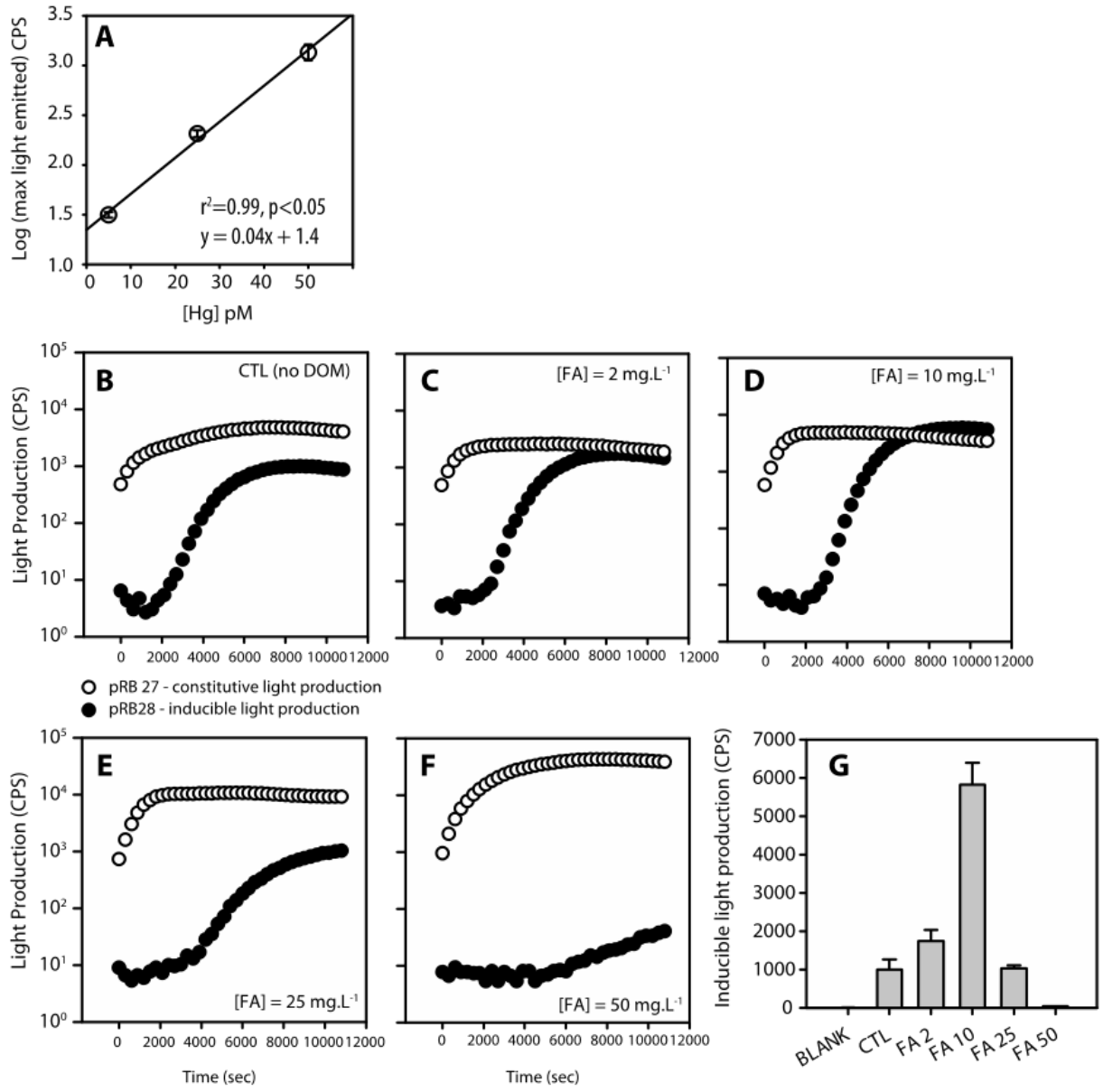


Figure S2.1: A) Maximum light production in response to increasing Hg^{II} concentration. Panels B) to F), time series of inducible light production and constitutive light production over the course of a typical experiment. G) Inducible light production for various fulvic acid concentrations under non-equilibrium conditions. Experiments were performed at $[Hg^{II}]$ = 250 pM.

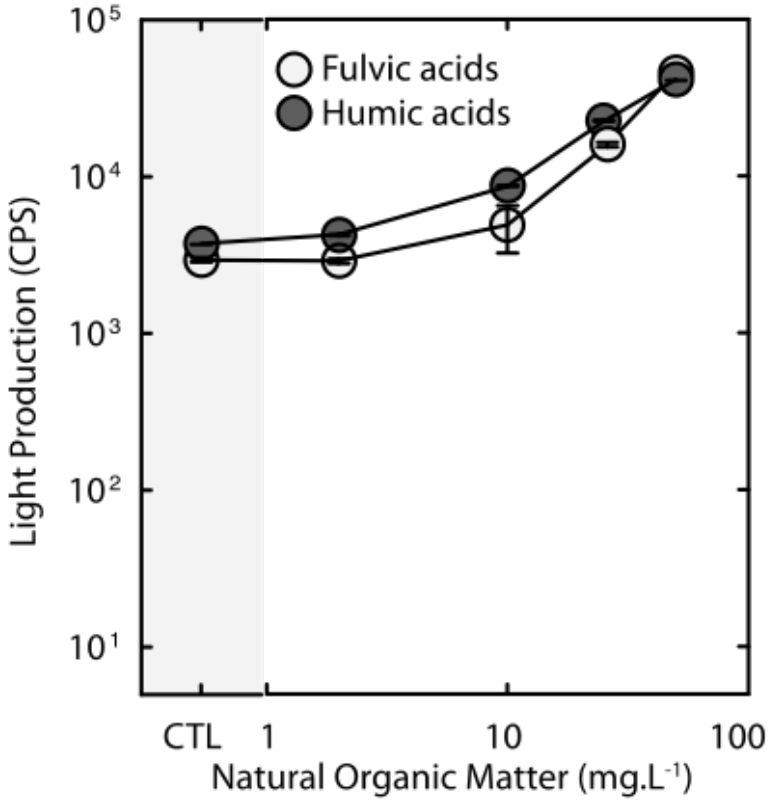


Figure S2.2: Effect of NOM on constitutive light production by the control strain *E. coli* HMS174 (pRB27). Control assays were conducted in humic acids (closed symbols) and fulvic acids (open symbols) solutions (>0-50 mg L^{-1} NOM) spiked with Hg^{II} as $HgCl_2$ (250 pM); Both Suwannee River humic and fulvic acids were obtained from the International Humic Substances Society (IHSS). No-NOM controls (0 mg L^{-1} NOM) identified as CTL. Error bars represent the standard deviation of analytical triplicates.

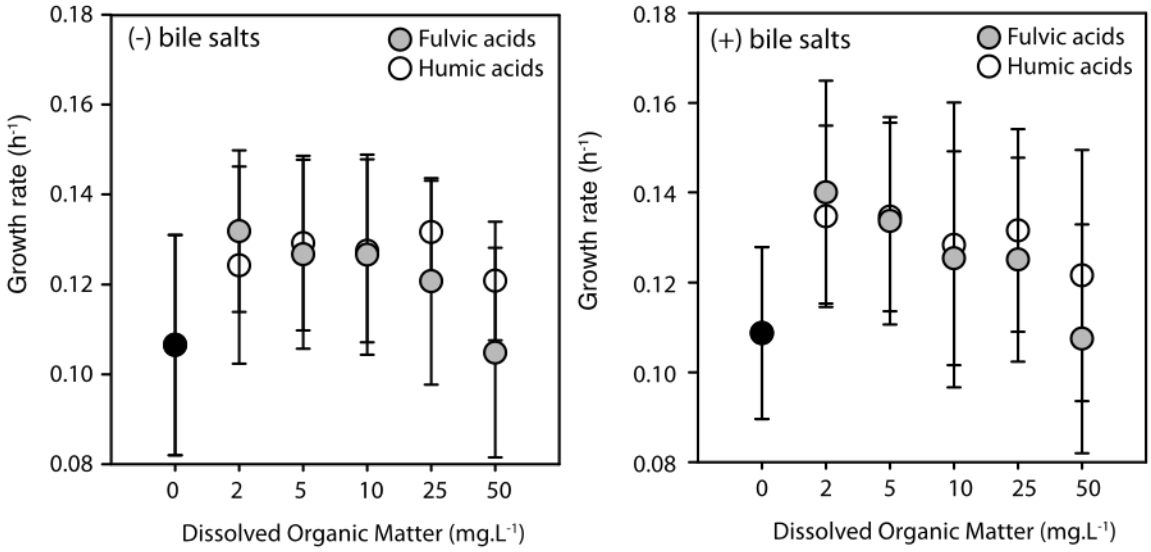


Figure S2.3: Effect of NOM on the bioreporter's sensitivity to bile salts. Bioreporter's growth rates in glucose minimal medium in the presence and in the absence of bile salts (0.5%) are presented. Experiments were performed with both Suwannee River humic and fulvic acids from the International Humic Substances Society (IHSS). One representative error bars represent the standard deviation of triplicates. No difference is noted whether or not bile salts are present. Experiments were also performed with cells conditioned with DOM, rinsed and exposed to bile salts; results showed that there were no significant differences in the growth rates of cells exposed or not to bile salts with or without conditioning with DOM.

Chapter 3.0: Conclusions and Perspectives on Future Research

3.1 Conclusions and Perspectives on Future Research

The present research set out to investigate how [DOM] affects Hg dynamics in lakes from Canada's western Arctic. Despite known effects of DOM on microbial and complexation processes, its role as a mediating factor in the bioavailability and bioaccumulation of Hg in aquatic ecosystems remains enigmatic.

This study is one of the first to consider kinetics, rather than thermodynamics, as the driving force of Hg-DOM interactions. In defined medium, I quantified Hg^{II} bioavailability over increasing [DOM], using a bacterial bioreporter response as proxy. At pseudoequilibrium, I recorded an inverse relationship between [DOM] and Hg^{II} bioavailability, as well as the inhibition of cellular Hg^{II} uptake at low concentration, consistent with the current consensus on the effect of DOM. It is believed that DOM complexation renders Hg^{II} poorly available to aquatic biota once equilibrium is reached. Moreover, the complexation stability at equilibrium is considered more important than the kinetics of Hg-DOM interactions. However, I also showed that, at non-equilibrium, Hg^{II} bioavailability followed a threshold-type relationship with [DOM], such that Hg^{II} bioavailability increased between 0 and 10 $mg.L^{-1}$ DOM, and then decreased between 10 and 50 $mg.L^{-1}$ DOM. These bioreporter results support the hypothesis that effects of DOM on Hg^{II} bioavailability to bacteria differ between equilibrium states, while remaining concentration dependent. Moreover, this is the first report of a fully opposing relationship, where low to medium DOM concentrations promote Hg^{II} uptake at non-equilibrium. Although a single time point was examined between first exposure of Hg^{II} to DOM and establishment of complexation equilibrium (<24h), I believe these data will be instrumental for Hg modelling studies (predicting MeHg availability to food webs), which have, until now, overlooked the kinetic controls on Hg^{II}-DOM complexation (Figure 3.1).

Further bioreporter assays revealed mechanistic insight into ternary (Hg^{II}-cell-DOM) interactions at non-equilibrium. In defined media, I tested bacterial cell wall permeability to both Hg^{II} and bile salts, when pre-conditioned with increasing [DOM]. While Hg^{II} uptake increased in tandem with [DOM], susceptibility of bacteria to bile salts remained unchanged following DOM-cell surface interactions, suggesting that alterations to overall cell wall permeability did not ensue. Rather, these results indicate that, under non-equilibrium conditions, the ability of bacteria to internalize Hg^{II} via facilitated or active transport may be enhanced by the action of DOM. Likely, at non-equilibrium, competitive ligand exchange occurs between the different functional groups within DOM that possess varying binding strength and accessibility for Hg^{II}, allowing for exchanges to occur with metal transport sites onto the bacterial cell wall. This

process would account for enhanced Hg bioavailability prior to reaching complexation equilibrium. Yet, to explain the role of DOM in increasing Hg bioavailability, I propose a molecular shuttle model that occurs through the use of a labile fraction of the DOM pool as a food or energy source for bacteria. In the process of competitive ligand exchange, Hg may bind to labile DOM molecules, and be co-transported into cells, under low to medium [DOM]. Above a certain [DOM] threshold, Hg would bind preferentially to more refractory DOM molecules present in large amount, thereby decreasing Hg bioavailability. Further studies should focus on further elucidating the mechanisms involved in the DOM-dependent increase of Hg^{II} bioavailability, as my present contribution is very modest, and mostly speculative.

In another part of this study, I tested the relevance of these observations to the Arctic environment, specifically to a series of lakes from the Northwest Territories, Canada. In lake water samples, I measured Hg^{II} bioavailability across a wide, natural gradient of [DOC], again using a bacterial bioreporter response as proxy. Under non-equilibrium conditions, Hg^{II} bioavailability followed the same threshold-type relationship with [DOC] as was observed in defined medium, including a [DOC] threshold near 10 $mg.L^{-1}$ DOC. Meanwhile, bioavailability greatly decreased at equilibrium. These results are relevant of Arctic lakes receiving new Hg inputs, such as Hg^{II} originating from the atmosphere subject to non-equilibrium interactions with DOM within the first few hours following deposition to surface waters. Therefore, I believe that [DOC] is key in predicting Hg^{II} bioavailability to methylating bacteria, a critical step in MeHg production, and bioaccumulation in aquatic food webs. Further studies should focus on bacterial Hg^{II} methylation, as it is the following step (after Hg^{II} uptake) in bacterial MeHg production. Gaining insight into both processes is required to accurately describe the effect of DOM on MeHg production in aquatic systems.

Unparalleled change in the Arctic and Subarctic environments are predicted for the coming decades, as temperature increases are likely to be double that of the global average by the end of the 21st century (AMPA, 2011). Warming temperatures are likely to produce a cascade of effects to the physical environment, many of which will impact on Hg cycling. Some predicted changes include a shift in the atmospheric pressure patterns across the Arctic, altering the transport of Hg into and out of polar regions by prevailing winds (AMPA, 2011). However, from a human and ecological health perspective, of predominant concern are climate-driven changes to environmental variables that influence the production of MeHg in freshwater ecosystems. In recent years, effects on Hg-relevant water chemistry have been recorded in Arctic lakes due to permafrost degradation in northern landscapes, including changes in pH,

SO_4 and dissolved organic carbon (DOC) concentrations (Kokelj et al., 2005). At present, many indigenous people exceed (U.S. and Canadian) blood mercury guidelines; this proportion may further increase as levels of Hg in biota, including species used for country food, show increasing trends (AMPA, 2011).

The amount of MeHg in an aquatic system is determined primarily by the rate of Hg^{II} methylation relative to MeHg demethylation (Hsu-Kim et al., 2013). As a result, any reaction modulating the availability of Hg^{II} to methylating microbes has the potential to directly affect MeHg concentrations and its toxicity. In this thesis, I demonstrated (i) kinetic controls of Hg speciation by DOM in predicting bacterial Hg^{II} bioavailability, and (ii) potential for greater availability of newly deposited Hg^{II} to aquatic food webs in Canada's Western Arctic. Below an intermediary threshold concentration (T_C = ~ 10 $mg.L^{-1}$), DOC has the potential to promote Hg^{II} bioavailability to methylating bacteria within hours following its deposition to surface waters, i.e. when complexation equilibrium has yet to establish between Hg^{II} and DOC. I believe that [DOC] is key in predicting Hg fate and bioavailability in Arctic lakes under a rapidly changing climate. This variable is predicted to change markedly with future environmental change, in accordance to climate factors such as temperature and precipitation (Drexel *et al.*, 2002). Many lakes in Canada's North, currently characterized by low [DOC] (<10 $mg.L^{-1}$; Lim *et al.*, 2001; Pienitz *et al.*, 1997a,b), have increasing [DOC] expected due to humidification (Macdonald *et al.*, 2005), hence approaching the threshold [DOC]. Alternatively, decreasing [DOC] has been reported in tundra lakes affected by retrogressive thaw slumps, thereby shifting from high [DOC] (>10 $mg.L^{-1}$; Kokelj et al., 2005) to intermediary [DOC] (~ 10 $mg.L^{-1}$; Kokelj et al., 2005).

3.2 References

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3.3 List of Figures

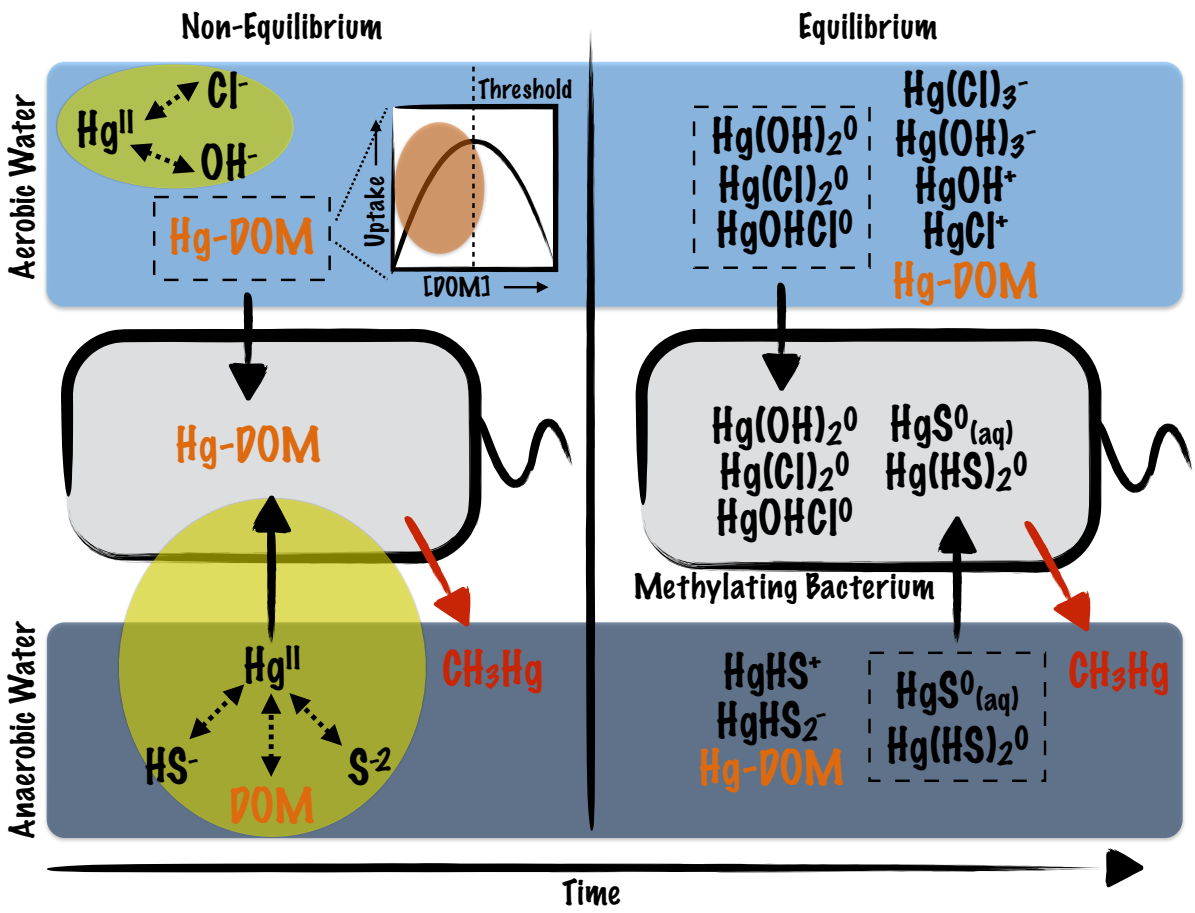

Figure 3.1: Summary of inorganic mercury (Hg^{II}) complexes available for uptake by methylating bacteria, updated with results from this study. Before thermodynamic equilibrium is reached, Hg-DOM complexes are available to cells when [DOM] is low (i.e. below a intermediary concentration threshold). Current research does not extend to anaerobic conditions, or other Hg^{II} complexes at non-equilibrium. Yellow tones represent uncertainty as to the bioavailability of Hg^{II} complexes. S^{2-}: sulfide, HS^-: bisulfide, Cl^-: chloride, OH^-: hydroxide, DOM: dissolved organic matter, CH_3Hg: methylmercury.