QUANTIFYING MERCURY REDUCTION KINETICS IN SOILS

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ABSTRACT

Mercury emissions from soils significantly contribute to the global Hg cycle; however there is little research on the fundamental biotic and abiotic factors (soil temperature and moisture) controlling Hg reduction kinetics in soils. Specifically, it is not clear if biological processes contribute significantly to mercury reduction in soils. I hypothesized that biological processes play an important role in elemental mercury, Hg(0), formation in soil. The effects of soil temperature, percent water filled pore space and sterilization on the kinetics of Hg(0) formation in 10 different boreal soils of Nova Scotia, Canada were quantified using a novel quartz beaker system. This system provided a reproducible estimate of Hg(0) formation rate constants under a range of environmental conditions. I derived pseudo-first order rate constants by fitting the cumulative Hg(0) formed in soil over a 24 hour period (r^2 = 0.90 to 0.99, $p<0.001$, n = 10). The cumulative mass of Hg(0) formed and the k values increased linearly with increasing soil temperature (278 to 303 K) both in non-sterilized and sterilized soils. Sterilizing soils significantly ($p<0.05$) decreased the percent of total Hg reduced to Hg(0), with sterile soils on average reducing 3.4% (SE = 1.4) of total mercury as compared to 6.8% (SE = 1.4) for non-sterile soils with increasing soil temperature. The cumulative mass of Hg(0) formed in soils (Log cumulative Hg(0) formed = $5e^{(-0.5(x-40)/23.5)^2)}$; r^2 = 0.77, n =10) and the reduction rate constants (k values) (k = $0.6e^{(-0.5(x-39)/26)^2)}$; r^2 = 0.64, n =10) follows a three parameter Gaussian peak function equation, attains a maximum at 60 percent water filled pore space and decreases thereafter in non-sterilized and sterilized soils. Hg(0) formation did not occur at 80 percent water filled pore space. This research finds biotic contributions to be highly significant in the process of Hg reduction and I report the first estimates of Hg reduction rates in soils.

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LIST OF ABBREVIATIONS

µM	Micro mole
A	Surface area
ANOVA	Analysis of variance
As	Exposed surface area
DOM	Dissolved organic matter
DS	Deciduous soil
e^-	Electron
E_a	Activation energy
EC	Electrical conductivity
ɤ	Gamma
g	Gram
GHG	Greenhouse gases
Gy	Gray
Hg	Mercury
Hg(0)	Elemental mercury
$Hg(0)_{ambient}$	Hg(0) in ambient air
$Hg(0)_{soil}$	Hg(0) produced in soil
Hg(II)	Divalent mercury
$Hg(II)_{red}$	Reducible divalent mercury
Hg(p)	Particulate mercury
Hg_{flux}	Mercury flux
Hg_T	Total mercury

hr	Hour
K	Kelvin
k	Rate constant
kg	Kilograms
kGy	Kilo Gray
KNP	Kejimkujik National Park
MBR	Modified Bowen ratio
$\overline{X}$	Mean
MeHg	Methyl mercury
N	Nitrogen
ng	Nanogram
OC	Organic carbon
OM	Organic matter
pg	Picograms
Q	Air flow rate
RGM	Reactive gaseous mercury
RH	Relative humidity
RSD	Relative standard deviation
S	Sulfur
SD	Standard deviation
SDS	Sterilized deciduous soil
SE	Standard error
SOM	Soil organic matter

t	Time
T	Temperature
WFPS	Percent water filled pore space
WHC	Water holding capacity
λ	Wavelength

1. INTRODUCTION

1.1 Overview

Mercury (Hg) is a naturally occurring, highly toxic and very mobile element that is present throughout the environment. It is recognized as a global pollutant because it can undergo long-range transport with air masses, reaching even very remote regions such as the high Arctic. While mercury is distributed throughout the atmosphere, it's primary environmental and health impacts result through atmospheric deposition to terrestrial and aquatic ecosystems, bio-accumulation of organic mercury in aquatic organisms, and bio-magnification to high trophic levels such as humans (Banic et al., 2003; Pirrone et al., 2009; Smith-Downey et al., 2010). Elemental mercury, Hg(0) is relatively unreactive, has an atmospheric residence time on the order of one year, and can be distributed across regional and global distances (Lindberg et al., 2007; Munthe et al., 1995; Wangberg et al., 2007). Elemental mercury in the atmosphere can be oxidized to divalent mercury, Hg(II), which is then rapidly deposited to the land or ocean surface. Divalent mercury can then be biotically or abiotically reduced back to elemental mercury and re-emitted to the atmosphere. This imparts Hg with an atmospheric cycle largely controlled by its redox chemistry and it continuously cycles between terrestrial systems, the atmosphere, oceans, and living organisms.

Mercury emissions into the atmosphere can originate from natural as well as anthropogenic sources. According to recent modeling, the magnitude of Hg in the atmosphere has more than tripled over the past century (Eckley et al., 2011; Fitzgerald, 1995; Lin et al., 2012; Lindberg et al., 2007; Miller et al., 2011; Pirrone et al., 2009; Quinones and Carpi, 2011). Natural sources may contribute significantly to global Hg movements. Terrestrial ecosystems may be sources as

well as short and long-term sinks for atmospheric Hg. Estimates of the quantitative significance of Hg emission from natural soils relative to other pathways, including emission from the oceans, from geothermal or tectonically active areas and anthropogenic activities, are very uncertain.

Soils, in particular, have the potential to be a large source or sink in the Hg cycle and research has established the importance of natural soils in environmental Hg cycling (Coolbaugh et al., 2002; Engle et al., 2001; Engle and Gustin, 2002; Gustin and Lindberg, 2000; Gustin, 2003; Kim and Lindberg, 1995; Zhang and Lindberg, 1999). In a recent study from the Great Lake Basin of North America, Denkenberger et al., (2011) found that of a total Hg(0) emission of about 7.4 Mg yr^{-1}, agricultural soils, forest lands, Great Lakes, grasslands, urban lands and the inland waters contributed 55%, 25.1%, 15.4%, 0.4%, 1.5% and 2.4% respectively, to the total Hg(0) emissions. It is also worth noting that natural and Hg-enriched soils can contribute to the amount of Hg in the atmosphere for extended periods of time (10^3 to 10^9 years), whereas anthropogenic point sources have lifetimes up to 50 years (Gustin et al., 2008). Soil Hg accounts for 75% of the biogeochemically active element (Mason, 2009) and at sufficiently high levels can be toxic to microbes, invertebrates and plants (Rundgren et al., 1992; Tipping et al., 2010). Soils enriched in Hg by natural processes may contain concentrations on the order of 100 to 500 μg Hg g^{-1} while low Hg containing background soils are generally considered to contain levels < 0.1 μg Hg g^{-1} (Gustin et al., 2003; Gustin et al., 2006; Zehner and Gustin, 2002; Zhang et al., 2001).

Natural soils accumulate Hg(0) by wet and dry deposition, and release it by emission at soil-atmosphere exchange surfaces (Grigal, 2002). Mercury emission is an important process in controlling atmospheric Hg levels over time (Pirrone et al., 2008). Grigal (2003) and Schluter (2000) reported that as much as 80% of the mercury that is deposited on the terrestrial surface is

re-emitted to the atmosphere through surface emission. Thus, the development of global and regional Hg models is incomplete without quantification of the Hg emissions from natural soils. For tracing the fate of Hg emitted to the atmosphere, as well as for estimating the future of Hg burden of our environment and predicting the toxic consequences of that burden, it is important to investigate terrestrial Hg fluxes and the climate factors affecting Hg exchange. Mercury emission from soils is a significant part of the biogeochemical cycling of Hg, however there is little data available to develop mass balance or mechanistic models.

Modeled estimates of global mercury emissions from natural sources vary widely, largely due to a lack of sufficient quantitative data on Hg fluxes from natural soil surfaces (Lin et al., 2010; Smith-Downey et al., 2010; Valente et al., 2007). The variability associated with estimates of global Hg emissions from soils arise because (i) global Hg flux estimates are largely extrapolated from a few site-specific data points, (ii) the flux measurements that are available are subject to a wide array of meteorological, chemical, and physical processes specific to a site, and (iii) there are not enough direct Hg flux measurements under controlled conditions. An accurate assessment of Hg emissions from soils is crucial to quantifying and predicting the movements of natural and re-emitted Hg from natural sources. At a national level, we do not yet have accurate estimates of Hg inputs to atmosphere from natural sources due to the paucity of available data and, thus, we cannot create effective regional and global models.

1.2 Limitations of Previous Hg Kinetics Research

Previous studies have used flux chambers and micrometeorological techniques to measure the mercury flux rates (Almeida et al., 2009; Bahlmann and Ebinghaus, 2003; Bahlmann et al., 2006; Carpi and Lindberg, 1998; Eckley et al., 2010; Eckley et al., 2011; Kocman and Horvat, 2010;

Miller et al., 2011; Poissant et al., 2008). The results showed that the micrometeorological techniques measured flux rates two to three times those of the soil chambers at the same site (Magarelli and Fostier, 2005b; Poissant et al., 2004). A key limitation of current techniques for estimating Hg flux is that direct flux measurements over natural and vegetated soil surfaces are plagued with site specific variations (solar radiations, soil moisture, humidity, soil and air temperature gradient, wind speed etc.) and QA/QC issues (elevated blank problems, low background levels, precise and reproducible replicate measurement). There exist many potential errors (chamber shape, material makeup, air flow rate, air source etc.) associated with existing methods and *in-situ* estimations of Hg(0) emissions from soils are often complex, stationary, expensive, and subject to a wide range of above said ecosystem variables (Bahlmann and Ebinghaus, 2003; Gillis and Miller, 2000a; Gustin et al., 2008; Zhang et al., 2005). Currently, there is no standard methodology for measuring Hg release from soils, which makes it difficult to compare data from different sites. Improved laboratory methods are required to explore, understand and quantify the processes and mechanisms involved in Hg release from natural soils and refine global Hg transport models. While it is believed that inherent soil characteristics (soil texture, pH, EC, organic carbon and substrate Hg concentration etc.) and the climate parameters (solar radiations, soil moisture and soil temperature) are important variables controlling Hg flux from soils, there are no controlled studies available, which account for these processes.

1.3 Thesis Rationale

Studying the amount and rate of Hg(0) production in soils is different than studying the flux of Hg at the soil-atmosphere interface. For example, soil Hg(0) concentrations may change much more slowly and be affected by different biotic and abiotic factors than Hg fluxes at soil-air interface. The reduction of Hg(II) to Hg(0) occurs in soils under a variety of conditions and

many physico-chemical processes may influence the conversion of Hg(II) to Hg(0) (Moore et al., 2011; Zhang and Lindberg, 1999; Zhang et al., 2003). The physical and chemical processes controlling Hg reduction in soils mainly involve (i) distribution of different Hg species [Hg(II) and Hg(0)] among aqueous and gaseous soil phases, which is largely associated with sorption and desorption reactions, and (ii) Hg redox reactions, a variety of which could be induced by biotic and abiotic factors. Furthermore, the role played by soil microbes in Hg(0) reduction remains a subject of debate as no *in-situ* proof has demonstrated that Hg(II) reduction and emission of Hg(0) from soil is dominated by microbial activity (Schluter, 2000). Although a large body of literature is now available on the levels and behaviors of Hg(0) emissions at the soil-air interface, there are no investigations comprehensively addressing the physico-chemical processes controlling production of Hg(0) in contaminated as well as low Hg containing background soils.

1.4 Thesis Organization

This dissertation is structured into eight parts. The first part presents a general introduction to the research and overview of the structure. Part two presents a literature review of the concepts and methods that were used throughout this dissertation. The following three parts are experimental work. Part three aims to develop a simple, cost effective, accurate and reproducible laboratory method to study the kinetics of Hg(0) formation in soils. Parts four and five investigate how the changes in soil temperature, water content and sterilization affect reduction rates and the total amount of Hg(0) produced in soils. The dissertation ends with an overall discussion/conclusion (Part 6) followed by a list of cited references (Part 7) and an appendix (Part 8).

This dissertation represents the first systematic study of abiotic and biotic factors affecting Hg reduction in soils under controlled conditions. This work provides the fundamental rate constants required to increase the predictive capability of national and global Hg transport models and also to better predict the impacts of climate change on Hg emissions from soils across Canada.

2. LITERATURE REVIEW

2.1 Mercury Speciation in Soils

Soil mercury can be divided into three pools depending on its association in the soil matrix: (i) mineral mercury (contained in the soil mineral fraction), (ii) mercury bound to organic matter and (iii) mercury adsorbed to the surface of soil particles. Mineral mercury is derived directly from soil parent material, and although the total mercury content is generally low (<10 ng g^{-1}) (Friedli et al., 2003), the large area covered by mineral soils make this the largest pool of mercury in the global environment (Gustin et al., 2006; Schluter, 2000). Soil texture and clay mineralogy (kaolinite, montmorillonite, smectite and goethite) are important in determining the interaction of Hg with soil minerals. Mercury interacts with minerals by adsorption-desorption and precipitation-dissolution reactions (Schroeder et al., 2005). Mercury also associates with hydrated ferric oxides in soils by forming two bridges with hydroxyl groups and can also co-precipitate or adsorb to phosphate, carbonate and sulfate containing minerals (Schuster, 1991). In a study from the soils at a location in eastern Canada, the highest Hg concentrations were observed in Ah-horizon (466 ng g^{-1}) followed by the O horizon (415 ng g^{-1}) and then by C horizon (304 ng g^{-1}) (O'Driscoll et al., 2005). The release of mercury from the mineral pool to the atmosphere is controlled by weathering over long time scales and, intermittently, by large emissions from volcanic activity.

The second pool, organically bound mercury, is derived from atmospheric deposition to soils and vegetation. Substantial quantities of Hg derived from atmospheric deposition are associated with organic matter in terrestrial ecosystems, and large pools of atmosphere derived Hg can be retained in surface litter (Grigal, 2003; Munthe et al., 1995). The organically bound soil mercury

retains terrestrial mercury deposition on a time scale of months to years. The interaction of Hg and organic matter can partially be explained by its attraction to cation exchange sites and also to S, SO_4^{2-} and O containing active sites on organic matter. Divalent mercury binds to reduced sulfur groups in SOM with very high affinity (Haitzer et al., 2003; Khwaja et al., 2006; Skyllberg et al., 2000) and is protected against reduction until the SOM is decomposed (Fritsche et al., 2008b; Wickland et al., 2006) or emitted by fire (Friedli et al., 2003; Turetsky et al., 2006).

The third pool, loosely adsorbed/surface mercury, is derived from atmospheric deposition of Hg(II) and Hg(0) to soil and leaf surfaces. Hg(II) can weakly bind to negatively charged soil particles, but processes such as cation exchange and water addition can easily displace Hg(II) from soils and lead to emission (Farella et al., 2006). Reduction must occur for Hg(II) to be released from the soil in its elemental form, Hg(0). The reduction of Hg(II) to Hg(0) occurs via biotic processes (Barkay et al., 2003; Barkay et al., 1989; Siciliano et al., 2002b) as well as abiotic processes involving sunlight and redox reactions with organic acids such as fulvic or humic acids (Allard and Arsenie, 1991; Costa and Liss, 1999; Gu et al., 2011; Pirrone et al., 2001; Schluter, 2000; Smith et al., 2002; Terkhi et al., 2008; Yang et al., 2007). This Hg pool is labile and relatively short lived providing an important source of soil mercury emissions. Elemental mercury is not stored in soils on long time scales and is re-emitted to the atmosphere. Emissions from this pool are thought to consist predominantly of Hg from atmospheric wet and dry deposition processes originating from both anthropogenic and natural sources (Pirrone et al., 2009).

2.2 Mercury Emissions from Soils

Mercury is a highly toxic and mobile contaminant making it both a regional and global concern. It is present in ecosystems in several different forms, including gaseous elemental mercury Hg(0), reactive gaseous mercury (RGM), particulate mercury Hg(p), dissolved divalent mercury Hg(II), and methylmercury (MeHg) (Pehkonen and Lin, 1998). Methylmercury can bio-accumulate and bio-magnify under natural conditions and poses a great risk to humans, wildlife and aquatic habitats (Mergler et al., 2007). Atmospheric mercury predominantly occurs (95-99%) as Hg(0) (Wangberg et al., 2007), which is volatile at ambient environmental temperatures, relatively unreactive, has an atmospheric residence time on the order of one year and is subject to long-range transport (Lindberg et al., 2007; Munthe et al., 1995). Elemental mercury can be emitted from terrestrial surfaces, oxidized in the atmosphere to Hg(II), dissolved and deposited via rainfall, and eventually reduced to Hg(0) and re-emitted back into the atmosphere from soil, water and vegetation. Mercury vapors exists in the soil pore space, primarily as Hg(0), and in concentrations ranging from 1 to 53 ng m^{-3} (Johnson and Lindberg, 1995). Mercury is vertically well-mixed in the troposphere and concentrations at background sites are in the range of 1-4 ng m^{-3} (Iverfeldt and Lindqvist, 1986; Lin and Pehkonen, 1999).

Global Hg cycling models estimate that anywhere from 50% to 70% of Hg in the atmosphere is deposited via dry and wet deposition onto land surfaces (Lindberg et al., 2007; Mason et al., 1995; Mason and Sheu, 2002). These land surfaces have been estimated to re-emit anywhere from 14% to 24% of the total atmospheric burden (Mason et al., 1995; Mason and Sheu, 2002) thus making land surfaces an important atmospheric Hg source (Da Silva et al., 2009; Fitzgerald, 1995; Mason et al., 1995). Mercury emissions to the atmosphere can originate from numerous

natural as well as anthropogenic sources; however, the relative extent to which each contributes to the atmospheric mercury pool is still debated in the literature. Gustin et al. (2008) noted that estimates of global natural mercury emissions range from 800 to 3000 Mg yr^{-1} which is similar to estimates for global anthropogenic releases (2000 to 2400 Mg yr^{-1}). According to recent modeling, the concentration of mercury in the atmosphere has more than tripled over the course of the past century (Mason, 2009; Pirrone et al., 2009; Selin et al., 2007; Smith-Downey et al., 2010). Mercury emissions from natural soils have been identified as a major contributor to the global atmospheric mercury budget. Overall, it is estimated that terrestrial Hg inputs are 1850 Mg yr^{-1} while emission from the ocean is 2680 Mg yr^{-1}. On an area basis, emissions from land are higher than from the ocean. Forests constitute about 20% of these emissions, with total emissions from vegetated regions being about 60% of the total terrestrial input (Gustin et al., 2008; Lindberg et al., 2007; Pirrone et al., 2009). Generally, background Hg concentrations in soil vary widely from place to place, depending on the local tectonic and geothermal setting. Soils enriched in mercury by natural geologic processes may contain Hg concentrations of the order of 100 to 200 µg g^{-1}, whereas background soils are generally considered to contain Hg at concentrations between 0.01 and 0.05 µg g^{-1} (Gustin et al., 1995; Rundgren et al., 1992; Schluter, 2000). In a geothermal zone in China, soil mercury concentrations less than 60 µg Hg g^{-1} soil were defined as background concentration and higher concentrations as anomalous. In a mineralized area in Germany, mercury concentrations as high as 1800 µg Hg g^{-1} soil were found, clearly exceeding the background concentrations (100 µg g^{-1}) (During et al., 2009; Schluter, 2000).

Research during the past decade has established the importance of natural soils in environmental Hg cycling, demonstrating that emission from soils may contribute substantially (700-1000 Mg

yr^{-1}) to the global atmospheric load of Hg (Coolbaugh et al., 2002; Engle et al., 2001; Engle and Gustin, 2002; Gustin and Lindberg, 2000; Gustin, 2003; Zhang and Lindberg, 1999). Selin and Jacob (2008) estimate Hg emissions from land at 2200 Mg yr^{-1}. Other recent modeling efforts have used a value of 2000 Mg yr^{-1} (Lindberg et al., 2007; Seigneur et al., 2004; Selin et al., 2007). Based on these studies (i.e., on average), forests account for 22%, agricultural locations 8%, other vegetated regions 27%, deserts and metal rich locations 30% and volcanoes 5% of total emissions. Most of the emissions are from tropical regions (53%), compared to temperate regions (39%) with the Polar regions being a minor source (8%). The emission from oceans, which constitutes 70% of the surface of the earth is 2680 Mg yr^{-1}. In contrast, emission from the terrestrial environment (30% of the surface) is 1850 Mg yr^{-1}. Therefore, the average emission from the land exceeds the ocean on area basis (Bash et al., 2004; Pirrone et al., 2009). Modeled estimates of global mercury emissions from natural sources vary widely, largely due to a lack of sufficient quantitative data on Hg emission from natural surfaces (Pirrone et al., 2009). In Canada there are only localized estimates of Hg inputs to atmosphere from natural sources and thus we cannot create accurate estimates based on empirical data. Mercury biogeochemical cycling and budget estimates are currently based on mercury cycling models.

In-situ measurements of Hg(0) are time consuming, expensive, and subject to many environmental variables. Researchers have carried out soil Hg emission inter-comparisons using different methodologies and operating procedures (e.g., micrometeorological methods and dynamic flow through chambers) under field conditions with varying results (Carpi and Lindberg, 1998; Kim and Lindberg, 1995; Poissant and Casimir, 1998). Currently, there is no standard field methodology for measuring Hg(0) emission from soils, which makes cross-comparisons difficult. In natural terrestrial ecosystems, the behavior of Hg(0) at the soil-

atmosphere interface is believed to be controlled by the fundamental soil properties (total Hg content (Hg_T), pH, EC, OC and soil texture), biological processes and meteorological parameters (e.g., temperature, moisture, solar radiation, relative humidity, wind speed and wind direction) (Almeida et al., 2009; Bahlmann et al., 2006; Barkay et al., 1989; Baya and Van Heyst, 2010; Carpi and Lindberg, 1997; Choi and Holsen, 2009; During et al., 2009; Fritsche et al., 2006; Gu et al., 2011). The interactions between all these biotic and abiotic factors lead to highly variable Hg emissions, making it imperative to study these in a variety of landscapes over a sufficiently long time-scale. An improved natural emission inventory for Canada will lead to a better understanding of biogeochemical mechanisms regulating air-surface mercury exchange processes.

2.3 Discrepancies in Soil Mercury Emission Values

Mercury emission from the earth is considered to be a significant process on a global scale. However, emission estimates vary widely, with estimates of 500-3200 Mg yr^{-1} for emissions from soil, 770-2300 Mg yr^{-1} for ocean, 20-447 Mg yr^{-1} for volcanoes, 850-2000 Mg yr^{-1} for vegetation and up to 100 Mg yr^{-1} for emissions from fires (Ebinghaus, 1999; Fitzgerald, 1995; Lindberg et al., 1998; Nriagu, 1989; Pacyna et al., 2001). Conservative estimates of global Hg emissions into the atmosphere suggest a total of 700 Mg yr^{-1} emitted from soils, with 500 Mg yr^{-1} originating from the mercuriferous belt comprised of plate tectonic boundaries; areas of high crustal heat flow; precious and base metal mineralization; recent volcanism and organic-rich sedimentary rocks (Lindqvist et al., 1991).

Soil is considered to be enriched in mercury when concentrations are > 0.1 µg Hg g^{-1} (Connor and Schaklette, 1975). Carpi and Lindberg (1998) estimated that low Hg containing soils may

account for the emission of 1000 Mg yr^{-1} of Hg(0) to the atmosphere and total terrestrial Hg(0) emission may equal or exceed the total marine emission. Mason and Sheu (2002) estimated that global emissions of natural and previously deposited anthropogenic mercury from terrestrial ecosystems account for more than 1600 Mg yr^{-1} and are comparable in magnitude to annual anthropogenic emissions (2200 Mg yr^{-1}). Although the atmosphere is enriched by anthropogenic mercury emissions, the largest reservoirs of Hg are contained in terrestrial soils, sediments, and subsurface ocean waters (Mason, 2009; Selin et al., 2007; Sunderland et al., 2009). Anthropogenic emissions of Hg from major sources such as fossil fuel combustion, waste incineration and metal smelting have been compiled (Pacyna et al., 2010); however, only order of magnitude estimates of Hg emissions from natural soils, vegetation and water bodies are presently available (Schroeder et al., 1989). Therefore, there is considerable variation in estimates of the contribution of Hg to the atmosphere from natural soil surfaces and hence, a high degree of uncertainty exists with respect to Hg emissions from terrestrial soils.

2.4 Soil Mercury Flux Measurement Methods

Two main techniques are currently employed for *in-situ* soil-to-air flux measurements: (i) dynamic chamber methods (Campbell et al., 2003; Carpi and Lindberg, 1998; Poissant and Casimir, 1998) and (ii) micrometeorological or modified Bowen ratio (MBR) methods (Lindberg et al., 1995; Poissant et al., 2000). There is no standard shape, size or turnover time (volume of air in chamber/flow rate of air through chamber) used in field chambers. Chamber volumes have ranged from 1 to ~ 30 L, and turnover time from 0.1 to ~15 minutes. In addition, laboratory gas exchange chambers have been used to develop a minimum estimate of flux and quantitatively

characterize those factors controlling Hg emissions from various substrates (Gustin et al., 1997; Gustin et al., 1999; Lindberg et al., 1979).

Within each broad class there are many different analytical designs, mathematical assumptions, and sources of errors. No single method for the measurement of mercury flux from terrestrial landscapes has been endorsed by the Hg research community. As a result, field chambers have different shapes, sizes, and materials, and utilize different sampling parameters. Flow-through chamber methods all operate using the principle outlined by Carpi and Lindberg (1998). Flux from a surface can be measured from the concentration in inlet air and the concentration in outlet air from a sealed chamber (Equation 2-1).

$$F = \frac{(Ci - Co)}{A_s} Q \qquad (2\text{-}1)$$

where F is rate of flux (ng m^{-2} h^{-1}); Co is the mercury concentration outside the flux chamber (ng m^{-3}); Ci is the mercury concentration inside the flux chamber (ng m^{-3}); A is the area of substrate covered by the flux chamber (m^2); and Q is the flow rate of air through the chamber (m^3 h^{-1}). The distribution of the inside and outside Hg analysis is individual to each research design. However, alternate measurements on gold traps reduce the time interval between flux measurements from 10-20 minutes to 5-10 minutes; thus increasing the resolution of short time-scale processes (O'Driscoll et al., 2007). Since equation 2-1 does not require any mathematically estimated values or assumptions, this is considered to be a direct quantitative technique.

In contrast, the MBR method requires several assumptions in the model to derive a flux measurement. Micrometeorological methods are founded on the assumption that given a stationary (constant in time) and horizontally homogeneous turbulent field, there exists an

atmospheric layer near the surface (typically 10–20 m) wherein the vertical fluxes of conservative quantities are constant with height. As a result, the fundamental assumption is that the surface flux of the quantity in question is equivalent to the vertical flux measured at some height within this atmospheric surface layer (Duyzer and Fowler, 1994; Grünhage et al., 2000). This method requires the measurement of specific environmental conditions (such as wind velocity and turbulent mixing) for proper data acquisition, which introduces sampling bias and therefore the data may not be entirely representative of the sampling site (Gustin et al., 1999). Gustin et al. (1999) showed that the MBR technique measured daytime flux rates approximately three times higher than those of chambers at the same location.

Several flow-through flux chamber designs have been employed in mercury research. Lindberg et al. (1999) developed a rectangular Teflon box design that has since been employed for Hg flux studies. Other researchers have employed similar rectangular or spherical designs made from various plastic (García-Sánchez et al., 2006) and metal materials (Schroeder et al., 1989), some of which have been covered with Teflon (Poissant and Casimir, 1998). The material for chamber design will affect radiation transmission to the substrate and the dissipation of heat in the chamber, as well as the air flow rate through the chamber (Cobos et al., 2002). While flux chambers may be more accurate for small controlled areas, they may not represent heterogeneous field conditions as accurately as micrometeorological methods (e.g., aerodynamic methods, gradient methods, eddy accumulation methods and the MBR method). Micrometeorological techniques provide a means of calculating a continuous gas flux using the measurement of short-term changes in temperature and various gas concentrations with little disturbance of the study area surface. A comparison of Hg fluxes between studies should be interpreted with great caution. For example, in one of the very few studies that used two dynamic flux chambers

located at a small distance from each other, Magarelli and Fostier (2005a) found significant variability (maximum CV = 250%) between the duplicate Hg flux measurements.

2.5 Variables Affecting Hg Emissions from Soils

Another uncertainty in the area of soil Hg research concerns the abiotic and biotic mechanisms controlling Hg(0) formation and emission from natural soils. Soil physico-chemical characteristics (e.g., texture, organic matter, substrate Hg concentration, pH and EC) and environmental parameters (e.g., soil and ambient air temperature, solar radiations, UV-A and UV-B, soil moisture content, wind speed and direction) are believed to be important variables affecting Hg(0) emission from soils (Bahlmann and Ebinghaus, 2003; Bahlmann et al., 2006; Barkay et al., 1989; Baya and Van Heyst, 2010; Choi and Holsen, 2009; Engle et al., 2001; Ericksen et al., 2006; Feng et al., 2005).

2.5.1 Soil Moisture

In order to estimate Hg emissions from soils, those processes controlling emissions and their relative forcing potential must be understood. A number of studies have measured the rate at which Hg(0) is emitted or deposited to soil in comparison with different environmental parameters, such as air/soil temperature, relative humidity (RH), solar radiation, etc. Rising soil water content can promote the aqueous reduction of Hg(II) to Hg(0) with subsequent emission to the atmosphere (Gillis and Miller, 2000b; Johnson and Lindberg, 1995; Song and Van Heyst, 2005). Past studies have demonstrated that small additions of water can greatly enhance Hg emissions from soils (Gustin and Stamenkovic, 2005). Precipitation events also have been observed to result in Hg emission from natural soils indicating that the effect of precipitation

events on Hg flux may depend upon the pool of Hg available for release in the soil (Lindberg et al., 1999; Song and Van Heyst, 2005; Wallschlager et al., 2000). A similar moisture related flux effect has been observed with organic chemicals such as pesticides. Bardsley and Walker (1968) observed an immediate release of the pesticide Trifluralin with soil wetting and suggested that the addition of water facilitated it's desorption from binding sites of soils. Gillis and Miller (2000b) reported that mercury flux from a sandy loam soil increased from -0.4 ng m^{-2} h^{-1} to 0.15 ng m^{-2} h^{-1} with increasing soil water content, peaked at when two-thirds of the soil air pore spaces were filled with water and decreased slightly to saturation. Lindberg et al. (1999) proposed three mechanisms that could be associated with the enhanced release of mercury observed with a precipitation event on a dry desert soil: (i) physical displacement of Hg(0) enriched soil gas by water gradually filling the soil pores; (ii) replacement of Hg(0) adsorbed to the soil by water molecules; and (iii) desorption of Hg(II) bound to the soil and subsequent reduction to Hg(0) through abiotic or biotic factors.

The increase in Hg flux was suggested to be related to soil physical or chemical interactions and the shapes of the response curves of flux versus time suggest that the initial response to moisture may exhibit first-order behaviour although no exact process was identified with the increasing soil moisture effect. Song and Van Heyst (2005) ruled out the possibility of biological reactions as the main process responsible for the enhancement of Hg(0) emissions from the soil in response to precipitation event as the Hg emission process is rapid and biological processes require time for the microbial community to establish, reproduce and influence emission. Further research is required to reveal the mechanisms by which soil moisture affects Hg(0) formation and subsequent emission at soil-air interphase. In particular there has been no controlled analysis

of soil moisture manipulation studies and the effect of WFPS on Hg reduction needs to be investigated.

2.5.2 Soil Temperature and Radiation

Many studies report a strong relationship between temperature and Hg emission rates for background, contaminated, and geologically enriched soils (Carpi and Lindberg, 1998; Choi and Holsen, 2009; Engle and Gustin, 2002; Poissant et al., 1999; Poissant et al., 2004). Researchers found that the highest Hg emission rates were measured in summer and in the afternoon, while the lowest rates occurred in winter and during the night. Gustin et al. (2004) and Baya and Van Heyst (2010) speculated that these patterns are a result of temperature cycles; however, they could also be due to radiation cycles and or effects from biological factors. Furthermore, Gustin et al. (2006) and Carpi and Lindberg (1998) also suggested that solar radiation has a direct effect on soil-to-air fluxes; however, these measurements have been complicated due to correlations with soil heating in the field by ultra-violet (UV) radiation comprising UV-A (320 to 400 nm), UV-B (280 to 320 nm) and UV-C (100 to 280 nm) wavebands. Solar radiation below 300 nm is not significant at the earth's surface due to absorption by the upper and middle atmosphere (Woods, 2008).

Solar radiation is expected to cause an increase in Hg flux from soils as it is highly correlated with increased soil temperature (Scholtz et al., 2003), which has been known to enhance Hg flux from soil (Choi and Holsen, 2009). This radiation-induced Hg flux may be partially due to photo-chemical reduction mechanisms; however, the exact mechanism for photo-induced Hg emission is unknown. Many authors have hypothesized there is a radiation-induced mechanism separate from soil temperature that promotes the photochemical reduction of Hg(II) and

subsequent release of the newly formed Hg(0) into the atmosphere (Bahlmann et al., 2006; Carpi and Lindberg, 1997; Gustin et al., 2002). This radiation-induced reduction of Hg(II) has already been observed in aqueous solutions in the laboratory under simulated radiation (Liu et al., 2000; O'Driscoll et al., 2007; O'Driscoll et al., 2006; O'Driscoll et al., 2005; Siciliano et al., 2002a). The effect of solar radiation on mercury emission from soil has only recently been investigated; moreover, the precise mechanisms have yet to be determined and their importance quantified.

Exposure to UV-A radiation has been reported to have a very minimal effect on Hg flux from soil, similar to that observed during dark conditions (Choi and Holsen, 2009; Xin and Gustin, 2007). Choi and Holsen (2009) observed that UV-A radiation coming from a 365 nm, 4 W UV tube, did not have a significant effect ($p<0.001$) on Hg flux from any of the soils examined. UV-B radiation has been found to have a much greater effect on mercury flux from soils (Bahlmann et al., 2006; Xin and Gustin, 2007). To account for the differences in Hg flux from soil after being exposed to the two different wavebands of radiation, Xin and Gustin (2007) proposed that UV-A exposure promotes the release of Hg(0) that was adsorbed to soil particles while UV-B directly converts Hg(II) to Hg(0) in the soil. Solar radiation induced Hg flux, independent of soil temperature, is significant as it means that soils can release Hg even when temperatures are low (Gustin et al., 2002). Bahlmann and Ebinghaus (2003) found that under natural conditions, the short half-lives of solar radiation-induced Hg fluxes may not be an appreciably limiting factor as recharge of Hg in the soil is very probable, either by diffusion of Hg(0) to the soil surface or by wet and/or dry deposition of Hg-complexes.

Schluter (2000) proposed that most of the Hg emitted from soil is likely from those layers favoring the formation of Hg(0) and where little binding to the soil matrix will occur, especially

in the surface horizons. In these upper layers, Hg(0) may be desorbed by surface processes such as an increase in soil temperature, thermal exchange of adsorbed Hg(0) with water molecules or sunlight enhanced reactions through which Hg(II) is reduced by humic substances; thereby increasing the pool of gaseous Hg(0) available for emission (Pehkonen and Lin, 1998; Scholtz et al., 2003; Zhang and Lindberg, 1999). Gustin et al. (2006) investigated the effect of temperature on soil-air Hg flux exchange for low Hg containing soils (<0.1 μg Hg g^{-1} soil) in Nevada and Oklahoma, USA, under field conditions. They found small Hg emissions when soils were dry and temperatures were low. However, daytime mercury emissions were greater compared to night due to high day time soil temperatures (0.6 ± 0.9 ng m^{-2} h^{-1} vs. 0.2 ± 0.5 ng m^{-2} h^{-1}). Similarly, as temperatures increased in April compared to March, the soil Hg flux was also increased and a positive correlation was found with soil temperature. Even in mesocosm studies, the flux increased from -0.3 ng m^{-2} h^{-1} in winter to 4.2 ng m^{-2} h^{-1} in summer corresponding to an increase in temperature and declined from July to October due to lowering of temperatures. These results demonstrate the importance of seasonal temperature fluctuations on mercury emissions.

Since terrestrial soils with low Hg concentration cover large surface areas, even small fluxes of Hg will have an important impact on scaling calculations (Nater and Grigal, 1992). In a field study from the north-eastern United States, Sigler and Lee (2006) found higher Hg(0) emissions from soils (5-10 cm depth) during mid to late summer season, which decreased during the autumn and became negative during the winter months. They proposed that the Hg(0) bound to overlying soil layers may be desorbed by an increase in soil temperature, thereby increasing the pool of gaseous Hg(0) in soil air spaces available for emission. Gillis and Miller (2000b) showed that Hg emission rates in low mercury, fine sandy loam soil can be largely explained by

variations in surface soil temperature and the mercury concentration gradient between the soil air and the ambient air above it. They found Hg flux to be highly correlated with 24 hour soil temperature (r^2 = 0.88) and moderately correlated with air temperature (r^2 = 0.58). Poissant and Casimir, (1998) studied the temperature dependence of Hg fluxes over the soil surface by considering it as the thermally enhanced emission process and employed the Arrhenius equation.

$$k = Ae^{-Ea/RT} \qquad (2\text{-}2)$$

where, k is the mercury flux (ng m^{-2} hr^{-1}), R is the gas constant (kcal K^{-1} mol^{-1}), T is the temperature in degrees Kelvin, E_a is the activation energy (kJ mol^{-1}) which the system must absorb in order to initiate a Hg flux increase or emission, and A is the pre-exponential factor, which was independent of temperature for many reactions. They obtained a good correlation (r^2= 0.87) between the reciprocal of absolute temperature of the soil (1/T at 5 cm depth) and the natural log (ℓn) of Hg(0) flux, which gave a straight line of slope E_a/R and intercept equal to ℓn(A). The E_a term from the regression was 85 kJ mol^{-1}, which suggests that the Hg(0) emission over the soil surface is not controlled by direct surface emission but through transition mechanisms since the enthalpy of vaporisation of elemental Hg (58 kJ mol^{-1}) is lower than the measured E_a. This suggested that the presence of water molecules in the soils, which require large activation energy due to different diffusion characteristics, or intermediate steps such as biotic or abiotic reduction of Hg(II) to Hg(0) may also be involved. Activation energy (E_a) values of Hg(0) emission from different kinds of soils measured by researchers is given in table 2-1.

Table 2-1. Literature values of the Activation energy (E_a) of Hg(0) emission from different kinds of soils.

Reference	Location	Site	Total Hg	E_a	Factors responsible
			-- µg g^{-1}--	-- kJmol^{-1} --	
Kocman and Horvat, 2010	Idrija, Slovenia	Contaminated soil	4-251	82-109	Abiotic and biotic factors
Gustin et al., 2002	California, USA	Mining site	125	71	Temperature and substrate concentration
,,	Nevada, USA	Mining site	0.06	60	,,
,,	Nevada, USA	Mining site	0.18–0.43	70-80	,,
,,	Nevada, USA	Geothermal site	3 -5	<59	,,
,,	California, USA	Background soil	0.023	70	,,
Zhang et al., 2001	Michigan, USA	Background soil	< 0.1	55	Thermal process
Carpi and Lindberg, 1998	Tennessee, USA	Background soil	0.061	75	Temperature and solar radiation
Poissant and Casimir, 1998	Quebec, Canada	Background soil	<0.1	86	Abiotic and biotic factors
Kim and Lindberg, 1995	Tennessee, USA	Background soil	76.1	73	Abiotic and biotic factors

2.5.3 Soil Organic Matter

Mercury exhibits a great affinity for organic matter (both solid and dissolved forms) in terrestrial (Schuster, 1991) and aquatic (Ravichandran, 2004) environments due to complexes with OH^-, S^{2-} and S^- containing functional groups of organic molecules because of their high abundance and stable binding with Hg. Carboxylic and N groups are also present in high abundance, but are weak binding agents; conversely, the reduced S groups are low in abundance but have strong binding ability for Hg. Contrary to the above findings, O'Driscoll et al. (2006) found that DOM samples derived from lakes in Quebec contained relatively more ester bound sulphates. Less reduced forms of S (cysteine and methionine forms of reduced S) similar to thiol groups were observed; however, they proposed that other organic functional groups (e.g., carboxylic acid) were relatively more important to Hg dynamics in lake samples because of more available and reactive binding sites susceptible to pH changes.

Processes such as chelation, ionic exchange, inner and outer sphere complex formation, adsorption, and co-precipitation are likely to occur with SOM (Celi et al., 1997) and the type of interaction will mainly depend on the chemical structure of the organic matter. As such, the sorption capacity for Hg is dependent not only on the amount of organic matter but also on the types of constituent organic functional groups. Some strong organic ligands form highly stable bonds with Hg (e.g., reduced sulfide species) that lower the availability of Hg(II) for redox reactions or methylation (Dittman et al., 2010; Gu et al., 2011; O'Driscoll et al., 2005; Reddy and Aiken, 2001). Results from a SOM-Hg(II) complexation study by Skyllberg et al. (2006) indicated that organic components were even more relevant in Hg adsorption at higher Hg concentrations. This was attributed to a larger adsorption capacity of organic matter as compared

with mineral colloids. Lindberg et al. (1979) found that the organic-associated fraction (extracted with $NaHCO_3$) accounted for 200 times more Hg than the cation exchangeable fraction of Hg contaminated soil. Based on indirect experimental evidence, some speculate that S containing thiol functional groups on natural organic matter are the principle ligands binding Hg(II) (Schuster, 1991). This is consistent with Hg(II) being a soft Lewis acid and therefore should bind strongly with thiol which is a soft Lewis base (Xia et al., 1999). Skyllberg et al. (2006) conducted a study in soils with high organic matter but low in Hg_T contents and found that Hg was complexed by two reduced organic S groups (thiols) and on average 20% of the reduced organic S represented high-affinity sites for Hg complexation. These high-affinity S groups were thiols, sulphides, disulfides, polysulfides and thiophenes. Smith et al. (2002) compiled data from the literature and reported that the tendency of the formation of Hg-organic ligand complexes to decrease in the order thiol > amino acid > carboxyl acid at pH 7. Xia et al. (1999) found that that presence of S atoms in the first and second coordination shell of Hg in humic acid was strong evidence that not only thiol (R-SH) but also disulfide/disulfane functional groups in humic acid play an important role in the complexation of Hg(II). They concluded that Hg(II) prefers reduced S containing functional groups over other functional groups (carboxylic, phenolic etc.) in humic acid. The relationship, if any between organic functional groups and Hg cycling in terrestrial soils is unclear and gaps still exist in the literature in the characterization of relevant Hg binding organic functional groups and their relationship to Hg speciation and oxidation-reduction dynamics in soils.

2.5.4 Soil Microbes

As previously described, soil Hg fluxes from site-specific field measurements have been shown to correlate well with soil temperature, moisture, and solar radiation. The rate at which Hg(0) is emitted to the atmosphere depends on the pool size of Hg(0) in the soils, the supply rate of Hg(0) from the underlying bedrock, the soil characteristics such as Hg_T, porosity, soil moisture, soil temperature and pH (Gabriel and Williamson, 2004; Siciliano et al., 2002a; Zhang and Lindberg, 1999). The factors responsible for the reduction of Hg(II) to Hg(0) are believed to be mainly abiotic in nature, such as photo-reduction (Bahlmann and Ebinghaus, 2003; Carpi and Lindberg, 1997; Gustin et al., 2004), reduction in the presence of humic and fulvic substances (Ravichandran, 2004; Schluter, 2000) or reactive Fe^{2+} adsorbed to mineral surfaces which act as a reductant (Charlet et al., 2002). In addition to abiotic factors a wide range of bacteria are able to detoxify inorganic and organic Hg compounds through the reduction of Hg(II) to Hg(0), which is then lost in the vapour phase (Bahlmann and Ebinghaus, 2003; Summers and Silver, 1978). Schluter (2000) concluded that the induction of biotic Hg reduction seems to require high concentrations of bio-available Hg. While there is more research on abiotic factors, the relative importance of biotic factors is still unclear.

The reduction of Hg(II) to Hg(0) is a process that may limit the concentration of Hg used as substrate for the methylation reaction (Fitzgerald and Lamborg, 2003; Zhang et al., 2001) and is mediated by a bacterial enzyme, mercuric reductase (merA). Along with photochemical processes, merA affects mercury mobility and bioavailability by converting water-soluble inorganic mercury and methylmercury to Hg(0). This is a detoxification process as evidenced by the resumption of microbial growth after the removal of the gaseous form of Hg(0) (Barkay et

al., 2003). The merA enzyme is located in the cytoplasm (Summers and Silver, 1978), utilizes NADPH as source of electrons (Hamlett et al., 1992) and catalyzes the conversion of thiol-loving Hg(II) to volatile, uncharged Hg(0) that lacks significant affinity for any functional groups. In the cytoplasm, thiols of proteins and smaller molecules are also susceptible to tight binding by Hg(II). Consequently, the efficiency of the merA at competing with these cellular thiols to scavenge and reduce the Hg(II) is critical to the survival of the cell (Barkay et al., 2003). Poulain et al. (2007b) detected merA genes and transcripts in high Arctic microbial biomass that contained microbes inhabiting polar environments. This study suggests that mercury-resistant organisms were present and active in Arctic coastal environments where critical redox transformations of mercury occur and where methylmercury is accumulated in the marine food chain. Methylmercury, is an organic form of mercury which can bioaccumulate in aquatic systems and its high concentration in predatory fish, which, when consumed by humans, can result in an increased risk of adverse health effects. Furthermore, modeling efforts suggested an important role for the prokaryotic merA in the production of Hg(0) in the high Arctic. Rolfhus and Fitzgerald (2004) suggested that microbial reduction can account for a significant component of the mercury redox cycling in temperate coastal marine systems [up to 20% of the pool of Hg(0)]. The oxidation of Hg(0) to Hg(II) is an important process that decreases Hg(0) levels in the environmental systems and increases the concentration of Hg(II), the substrate for methylation. Abiotic oxidation of Hg(0) occurs in the atmosphere (Lindberg et al., 2007), natural waters (Siciliano et al., 2002b), and soils (Thöming et al., 2000). Biologically induced oxidation of Hg(0) is the least explored step in the Hg biogeochemical cycle (Lin et al., 2012). Smith et al. (1998) showed Hg(0) oxidation by bacterial hydroperoxidases (KatG and KatE) in *Escherichia coli*, a double mutant, lacking both enzymes, retained a low level of Hg(0) oxidation, suggesting

the existence of other bacterial Hg(0) oxidases. Aerobic soil bacteria, *Bacillus* and *Streptomyces*, had high levels of Hg(0) oxidizing activity suggesting a potential role for microbial oxidation in the cycling of Hg in the environment (Smith et al., 1998). The rate of Hg(0) oxidation in these Hg(II) sensitive, plasmid-free bacteria is at least 10-fold lower than the rate of MerA-mediated Hg(II) reduction observed in bacterial cells carrying a mer operon (Hamlett et al., 1992; Summers and Silver, 1978). Siciliano et al. (2002a), showed a relationship between Hg(0) oxidase activities, measured by the accumulation of Hg(II) after incubation of protein extracts of lake microbial biomass with Hg(0)-saturated water, and the rate of accumulation of dissolved gaseous mercury, mostly Hg(0), in lake water. Plant catalases are also capable of oxidizing metallic mercury vapours (Kim et al., 1997), as are those of animals (Magos et al., 1978). Together, these studies suggest that microorganisms play an important role in catalyzing the oxidation of Hg(0) to Hg(II) in a range of different environmental settings and may have significant implications for the production of methylmercury. Since ionic Hg(II) is rapidly absorbed by rain, snow and airborne particles, oxidation of Hg(0) enhances atmospheric deposition of Hg. Because research has focused on atmospheric transformations, little is known about the mechanisms of Hg(0) oxidation in natural waters and soils where this process may critically affect MeHg production by increasing Hg(II) concentrations.

Fritsche et al. (2008) found that Hg(0) fluxes switched from emissions (4 and 0.5 ng $m^{-2} hr^{-1}$) to negative fluxes of 1.2 and 0.5 ng $m^{-2} hr^{-1}$ ($p<0.001$) due to sterilization of soil samples by autoclaving. The collapse of microbial activity caused by autoclaving could have directly stopped mercury emissions from the soils. However, after sterilized soil samples were inoculated with a few grams of untreated soil, a significant Hg(0) emission flush from slightly negative values to 30 ng $m^{-2} hr^{-1}$was observed indicating intensified microbial activity after inoculation

process. Similarly, a significant reduction of Hg(0) fluxes from soil was observed in chloroform fumigated samples (5-10 ng m^{-2} hr^{-1} to 2-3 ng m^{-2} hr^{-1}) which also showed inhibition of microbial activity. Microorganisms might reduce Hg(II) either directly, to detoxify their immediate environment, or indirectly by either decomposing organic matter, a strong binding agent for Hg(II), or by converting their substrate into compounds capable of Hg(II) reduction; e.g., humic and fulvic acids (Fritsche et al., 2008; Obrist et al., 2010). In another study, Choi and Holsen (2009) irradiated deciduous soils (DS) with 3.4 MegaRads of gamma rays at a rate of 1.14 MegaRads per hour to eliminate biological activity and observed higher Hg emissions from deciduous soils than from sterilized deciduous soils (SDS) under both dark and outdoor conditions. The values of the emission differences between sterilized and unsterilized soils were found to be fairly constant suggesting the biotic processes have a relatively constant influence on the increase or decrease of Hg emissions. Biotic reduction contributes significantly to the Hg flux from natural water as well as soils (Barkay et al., 2003; Schluter, 2000). Various bacteria strains have been shown to mediate reduction of bioavailable Hg by the mercury reductase enzyme which is encoded by the merA gene and several studies have identified Hg reduction by heterotrophic bacteria (Mason et al., 1995; Rolfhus and Fitzgerald, 2004; Siciliano et al., 2002a). A gap in this area of research concerns the biotic mechanisms controlling volatile mercury formation and release in natural background soils. To date, the role of mercury-resistant microbes such as *Escherichia coli* and *Saccharomyces cerevisae* in redox cycling of mercury in natural, uncontaminated background soils has not been examined. The role of soil temperature, WFPS and biotic processes exclusively affecting mercury reduction in terrestrial background soils is not fully understood, and no controlled measurements of this kind have been made in the past, due to primarily the lack of a widely accepted experimental methodology.

3. EVALUATION OF TWO METHODS TO ASSESS KINETICS OF ELEMENTAL MERCURY FORMATION AND EMISSION IN BOREAL SOILS UNDER CONTROLLED CONDITIONS

Preface

This chapter represents the development of a simple, accurate and reproducible technique for quantifying the Hg reduction; Hg(II) → Hg(0) kinetics in soils under controlled conditions. Findings and data obtained from this chapter are used to determine the influence of soil temperature (Chapter 4) and WFPS (Chapter 5) on rates and amount of Hg(0) formation in non-sterilized and sterilized boreal forest soils of Atlantic Canada.

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Ravinder Pannu planned, developed and conducted research experiment, conducted major field and lab work, reviewed the literature and is primary writer. Nelson J O' Driscoll and Steve Siciliano are co-supervisors and provided experimental and technical guidance, helped in statistical analysis and editing. John Dalziel provided technical support and troubleshooting of instruments.

3.1 Abstract

Mercury emissions from soils significantly contribute to the global mercury cycle, yet understanding the processes leading to emissions is technically challenging. As a result, there is a paucity of knowledge regarding the fundamental kinetic controls on soil Hg flux. Thus, to shed light on these fundamental processes, a method to accurately quantify the kinetics of elemental mercury formation in soils under controlled conditions was developed. This method was then compared to a field-based gas flux system widely used for greenhouse gas (GHG) estimates; i.e., LiCor automated chambers. The purpose of this study was to evaluate if non-specialized systems could be used for studying soil Hg kinetics, and thus allow for Hg flux estimates to be combined with greenhouse gas monitoring, or if specialized systems are required. Mercury fluxes from 10 different boreal soils measured using a quartz beaker chamber were consistently and significantly ($p=0.02$) higher (ranging 3.56 to 1154.74 ng m^{-2} h^{-1}) than those obtained using a LiCor chamber (ranging 3.07 to 18.93 ng m^{-2} h^{-1}). The two measurement systems were tightly related ($r^2 = 0.81$), but the LiCor system consistently and substantially under-estimated Hg emissions compared to the quartz beaker system (Quartz beaker = 45 LiCor + 116). Further, the quartz flux chamber had higher accuracy [mean recovery = 93% for concentrations ranging from 43 to 170 pg Hg(0)] and higher precision (Relative Standard Deviation = 2.4%, n = 28) compared to LiCor chamber. Thus, because there was a strong correlation between the two systems, it appears that it may be possible to link Hg emissions to GHG estimates but because of recovery issues, substantial calibration would be required. In contrast, the laboratory based quartz beaker system allowed estimates of Hg reduction rates in soils that varied between 1 and 2.5 x 10^{-3} h^{-1}. These rates were

independent of soil properties and represent some of the first reported estimates of elemental mercury formation rates in soils.

3.2 Introduction

Mercury is present in ecosystems in several different forms, including gaseous elemental mercury Hg(0), gaseous divalent mercury (or reactive gaseous mercury RGM), particulate divalent mercury Hg(p), dissolved divalent mercury Hg(II), and methylmercury (MeHg). Atmospheric mercury is predominantly (95-99%) in gaseous elemental form (Wangberg et al., 2007) and is globally distributed (Poissant and Casimir, 1998). Mercury emissions from natural soils have been identified as a major component of the global atmospheric mercury budget (Fitzgerald, 1995; Kim et al., 1995; Mason, 2009).

The abiotic or biotic reduction of Hg(II) to Hg(0) and further emission of Hg(0) are two very important processes in terrestrial surfaces because they can regulate much of the mercury load to the atmosphere (Poulain et al., 2007; Schluter, 2000; Schuster, 1991). Ambient mercury emission is a significant process on the global scale, with estimates of 500-3200 tons annum^{-1} (ta^{-1}) for emissions from soils, 770-2300 ta^{-1} for ocean emissions, 20-447 ta^{-1} for volcano emissions, 850-2000 ta^{-1} for emissions from vegetation and up to 100 ta^{-1} for emissions from fires (Ebinghaus, 1999; Lindberg et al., 1995; Lindberg and Stratton, 1998; Lindberg et al., 2002; Nriagu, 1989; Poissant et al., 2004; Schluter, 2000; Schroeder and Munthe, 1998; Schroeder et al., 1989; Wallschlager et al., 2000; Wallschläger et al., 1998; Xiao et al., 1991a; Xiao et al., 1991b). Carpi and Lindberg (1997) and Feng et al. (2005) have suggested that the top 1 to 2 cm and 0 to 5 cm of soil, respectively, are of most importance in this process. Despite progress in Hg research;

however, little is known about the exact mechanism of reduction processes and subsequent emission from soil.

In-situ Hg flux estimations are often expensive, complex, and subject to a wide range of ecosystem variables (Bahlmann et al., 2006; Gillis and Miller, 2000b; Gustin et al., 2008; Zhang et al., 2001) and measurements on direct mercury reduction processes under controlled conditions are too few. Researchers in the past have conducted soil Hg emission inter-comparison studies using different types of dynamic, flow through chambers under field conditions (Carpi and Lindberg, 1998; Kim et al., 1995; Poissant and Casimir, 1998; Schroeder et al., 1989). These field experiments shed considerable light on soil Hg fluxes, but could not determine the dynamics of the processes involved in the formation and release of Hg(0). Teflon flux chambers are popularly used for measuring the Hg flux from soils under field conditions; however, they are large in size, used for large soil-air exchange surface, and often suppress the influence of environmental parameters (Wallschlager et al., 2000) and hence cannot be used for measuring the Hg flux from a small soil mass under laboratory conditions. The development of portable flow through chambers and an inter-comparison of laboratory methods for studying soil mercury kinetics is an important research need.

The objective of this research was to develop an accurate technique for the quantification of the kinetics of elemental mercury formation in soils under controlled conditions.

3.3 Methods and Materials

3.3.1 Soil Sampling

A sampling grid was established at a site near the smelter facility operated by HudBay Minerals Inc. in the Flin Flon, Manitoba, Canada. The site was set up using a Nikon total station (Nikon DTM-332). Ten soil samples were collected along the Northern transect point marked away from the stack identified by easting and northing based on a reference point (54° 46' 0" N / 101° 53' 0" W). The organic litter was removed and 2 kg of surface soil (0 to 10 cm depth) was collected and homogenized with a stainless steel spade. The samples were stored in Ziploc bags in the dark at -20^{o}C until analysis to retard microbiological activity and minimize changes in Hg speciation. Thawed soil samples were dried in the dark in a clean growth chamber (Conviron ® Model E15) at 20^{o}C and 0 % relative humidity in polypropylene plastic containers for 72 hours. The dried soil samples were sieved through a 2 mm stainless steel sieve and stored in polypropylene containers under dark conditions at room temperature.

Soil pH was measured in 0.01M $CaCl_2$ solution with 1: 2.5 soil:solution ratio (Mehlich, 1976) and electrical conductivity (EC) was measured in Milli-Q water with 1:2 soil:water ratio. Total Hg concentrations, (Hg_T) in the soils were quantified using aqua regia (37% HNO_3 + 63% HCl, 1:3) digestion and cold vapour – atomic absorption spectroscopy (CV-AAS) (FIMS, Perkin Elmer). Soils also were analyzed for organic matter (OM) (Walkley and Black, 1934) and water holding capacity (WHC) (Franzluebbers, 1999). Blanks, triplicate measurements of total mercury in extracts, and analysis of a mercury standard (Merck) were routinely included for quality control (mean RSD of triplicates <10% and percent recovery of check standards 100 ± 5%).

3.3.2 Mercury Flux Measurement Chambers

Two methodologies were used to measure soil Hg flux under controlled conditions: one employing a commercially available flux chamber (LiCor LI-8100) and the other a quartz beaker chamber adapted from a water measurement system (O'Driscoll et al., 2003; O'Driscoll et al., 2006). There are several physical differences between these systems (Table 3-1). Automated LiCor flux chambers have been used for air sampling of greenhouse gases over natural soils (Xu et al., 2006). The LiCor chamber was adopted as such (with pre-calibrated flow rates and turn over time) in this experiment to test if it can be combined with greenhouse gas sampling to simultaneously measure Hg(0) emissions. The LiCor chamber is an automated unit with stainless steel body and aluminum bellow with a 4.076 L chamber volume resting over a soil collar inserted into the soil. The quartz flux chamber has a small volume (0.3L) with capacity to hold smaller sample mass with Teflon cap and Hg-free air feed. Both systems use a mercury vapour analyzer (Tekran 2537B) for continuous analysis of elemental mercury.

Table 3-1. Differences between the LiCor and Quartz flux chambers.

Characteristics	LiCor	Quartz
Volume, L	4.08	0.27
Soil Area Exposed, cm^2	317.8	28.26
Dimensions, cm	48.3 L X 38.1 W X 33.0 H	6 dia X 9.6 H
Weight, kg	5.9	0.27
Flow rate, $Lmin^{-1}$	1.5	1
Air flow pattern	Circular with Hg removed	Continuous zero air feed
Usage	Field and laboratory	Laboratory
Air turnover time, minutes	2.7	0.3

All measurements were made in the dark at room temperature (20 ±0.1°C) under laboratory conditions. The laboratory and outside natural solar radiation spectra were quantified using an Ocean Optics USB 4000 Spectra radiometer with fiber optic cable (10 m, 200 µm diameter) and spectral diffusion probe (diameter 4.3 mm). The UV-B (radiation wavelengths, (λ) between 280-320 nm, 0 vs 0.9 Watts m^{-2}), UV-A (λ between 320-400 nm, 0.001 vs 11.5 Watts m^{-2}) and visible spectra (λ between 400-700 nm, 0.14 vs 200 Watts m^{-2}) were found to be negligible under laboratory conditions compared to outside conditions.

3.3.2.1 LiCor Flux Chamber System

The flux chamber consists of an aluminum hemispherical bowl shaped flux chamber (LiCor 8100-104 long-term chamber) with a lift-and-rotate drive mechanism that rotates the chamber to configurable open positions. The flux chamber fits on a fiberglass collar (20 cm internal diameter) that houses the soil sample. Ambient air is drawn through the chamber at a rate of 1.5 L min^{-1} (turnover time of 2.7 minutes) to a Tekran 2537B mercury vapour analyzer with a sample resolution of 5 minutes. All connections were made using short (<1 m) connections of ¼" diameter Teflon (FEP) tubing.

During analysis, 200 g of soil at 60 percent water filled pore space (WFPS) was placed in a 0.39 cm layer maintaining an approximate bulk density of 1.60 Mg m^{-3}. Soil water content and water holding capacity were determined using standard procedures (Topp and Ferre, 2002). The soil was watered to 60 WFPS before Hg flux data collection (Franzluebbers, 1999). Five ambient air readings were collected before running each sample to correct for background. The chamber was closed after measuring elemental Hg in ambient air and soil Hg flux readings were taken at five

minute intervals for a total of 25 minutes. Mercury flux over a 25 minute period was determined using Equation 3-1, modified from Carpi and Lindberg (1998).

$$Hg_{flux} = \frac{\sum(Hg(0)sample - Hg(0)ambient)}{A \times t} \quad (3\text{-}1)$$

Where: Hg_{flux} is the average mercury flux (ng m^2 h^{-1}) over 5 sampling periods of 5 minutes each; $Hg(0)_{sample}$ is the total cumulative Hg(0) released (ng) during the 5 sampling periods (25 minutes of sampling time or 7.5L of air); $Hg(0)_{ambient}$ is the cumulative mass of Hg(0) in ambient air over 5 sampling periods; A is the surface area of the soil in the chamber (0.0317 m^2); and t is the total sampling time in hours. The average concentration of Hg in laboratory air was 1.2 - 1.8 ng m^{-3} and was subtracted during blank correction.

3.3.2.2 Quartz Flux Chamber System

This system consists of a glass beaker (6 cm diameter, 9.6 cm height, 0.27 L) made of high quality fused silica quartz with Teflon inlet and outlet tubing through a platinum cured silicone stopper (O'Driscoll et al., 2003). A Tekran model 1100 mercury zero-air generator supplied mercury-free air (1 L $minute^{-1}$) in order to measure Hg emitted from the soil surface. The Hg(0) formed in soil over the analysis period includes the fraction of Hg(0) present in the soil air spaces, adsorbed to soil particles as well as the reducible Hg(II) likely to be converted into Hg(0) either biotically or abiotically. A high flow rate (1 $Lmin^{-1}$) was used in the quartz chamber so as to quickly remove Hg(0) formed in the soil. The effect of increased air humidity was tested by passing dry zero air through a bubbler containing Milli-Q water prior to entering the quartz chamber. The Milli-Q water was analyzed for traces of Hg(0) before usage. A typical soil analysis under dark conditions consisted of initial blanking of the chamber by passing mercury-free zero air through the chamber without soil in the chamber until mercury concentrations were not detected and then 20 g soil at 60% of WFPS was uniformly placed at the bottom of the quartz

glass beaker in a thin layer (0.44 cm) to maintain a bulk density of 1.60 Mg m^{-3} (Figure 3-1). Soil flux mercury readings were then taken every 5 minutes over a 24 hour period.

In order to compare fluxes to the LiCor chamber, the first 25 minutes of analysis (the linear portion on the curve) were used to derive the flux using Equation 3-1 with the following modifications: ambient air was mercury-free air (mercury content 0 ng m^{-3}); the analysis air volume was 5 L per sampling period; and the chamber surface area was 0.0028 m^2. The chamber air turn-over-time in the LiCor chamber is 2.7 minutes (with 1.5 L min^{-1} flow rate) and 0.3 minutes (with 1 L min^{-1} flow rate) in the quartz chamber (Table 3-1). Thus, the quartz system should be expected to have at least a 9 times faster removal rate for mercury.

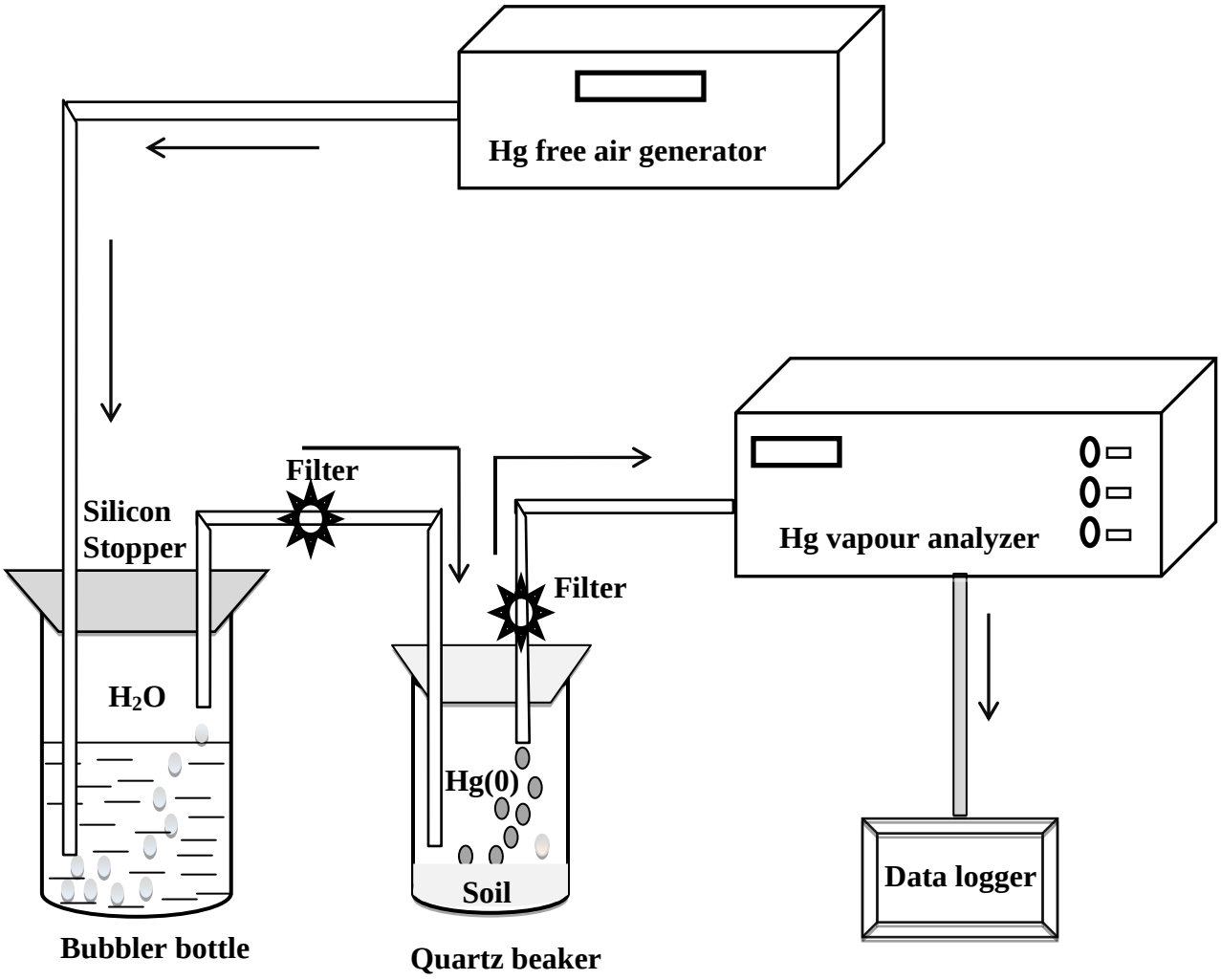

Fig. 3-1. Schematic diagram of quartz flux chamber system.

3.3.3 Calculation of the Apparent Pseudo First Order Reaction Rate for Mercury Reduction in Soil

The production of Hg(0) in soil can be modeled as a reversible first order reaction (Lindberg et al., 1999; O'Driscoll et al., 2006 and Schluter, 2000):

$$Hg(II)_{red} + SOM\ (\mathrm{n}e^{-}) \leftrightarrow Hg(0)_{soil} + SOM\ (\mathrm{n}-2)e^{-} \qquad (3\text{-}2)$$

where $Hg(II)_{red}$ is the reducible divalent mercury; SOM is soil organic matter; ne^- is the sink of electrons from the reducing agent, (SOM) and $Hg(0)_{soil}$ is the elemental mercury produced in soil. Since the reduction process seems to be facilitated when water is added to the soils, I analyzed highest (S1) and lowest (S10) Hg(0) forming dry soils (as determined from the flux study) in triplicate over a 24-h period and did not observe any Hg(0) formation. Thus, I have reason to believe that Hg(0) production in soils is due to the aqueous abiotic or biotic reduction, alone or in combination. To my knowledge, no values for Hg(0) fraction in soils have been reported and I assume that all of the Hg(0) produced in soil is effectively stripped from the quartz chamber at a fast rate. As such this reaction becomes dominated by the forward reduction reaction (Equation 3-3).

$$Hg(II)_{red} + 2e^{-} \rightarrow Hg(0)_{soil} \qquad (3\text{-}3)$$

The reduction rate constant can then be derived by fitting Equation 3-4 to the scatter plot of cumulative Hg(0) versus time.

$$Hg(0)_{cum\ in\ soil} = Hg(II)_{red}[1 - e^{-kt}] \qquad (3\text{-}4)$$

Where: $Hg(0)_{cum\ in\ soil}$ is the cumulative elemental mercury (pg) produced in soil at any analysis time t (hours); $Hg(II)_{red}$ is the total reducible mercury as calculated from plateau of the curve and

k is the reaction rate constant. Since my experimental design only quantifies Hg(0) flux from soil into air, I have to back-calculate the mercury present in soil $Hg(0)_{soil,}$ in order to derive $Hg(0)_{cum\ in\ soil}$. From Fick's first law we can calculate $Hg(0)_{soil}$ (Equation 3-5).

$$F = -D_e \frac{\Delta C}{\Delta z} = -D_e \frac{Hg(0)_{soil} - Hg(0)_{ambient\ air}}{\Delta z} \qquad (3\text{-}5)$$

Where F is the Hg(0) flux (the amount of Hg species crossing a certain area per unit time as expressed in ng m^{-2} sec^{-1}), D_e is the diffusivity of the soil layer, ΔC is the concentration difference between $Hg(0)_{soil}$ and $Hg(0)_{ambient\ air}$. In this case $Hg(0)_{ambient}$ is constant since mercury free air was passed over the soil sample and Δz is the length (0.44 cm) of the concentration gradient.

F, or flux (ng m^{-2} sec^{-1}) from soil, is directly measured by my experimental design and can be calculated using Equation 3-6 (Carpi and Lindberg, 1998).

$$F = \frac{Hg(0)}{A_s} Q \qquad (3\text{-}6)$$

Where: Hg(0) is the concentration of gaseous mercury (ng m^{-3}) released over the analysis period minus ambient air concentrations; Q is the air flow rate through the chambers (1.5 for LiCor and 1L min^{-1} for quartz chambers, respectively), A_s is the exposed surface area (0.0317 for LiCor and 0.0028 m^2 for quartz chamber, respectively) of the soil in the flux chamber.

Effective diffusivity (D_e) can be calculated using the Millington relationship (Millington, 1959) as modified by (McCarthy and Johnson, 1995) to include a term for aqueous diffusion (Equation 3-7):

$$D_e = \frac{\frac{\Theta_w^{10/3}}{H} D_{fw} + \Theta_g^{10/3} D_{fg}}{\Theta_T^2} \qquad (3\text{-}7)$$

where, D_{fw} is the diffusion coefficient of Hg(0) in free water (1.67×10^{-9} m^2 sec^{-1}), H is the dimensionless form of Henry's solubility constant (4.67×10^{-1}) in water, D_{fg} is the diffusion coefficient in free air (1.194×10^{-5} m^2 sec^{-1}), and Θ_w is water-filled porosity, Θ_g is air-filled porosity and Θ_T total porosity.

Concentration of $Hg(0)_{soil}$ at 5 minute resolution period in soil was calculated by combining Equations 3-5, 3-6 and 3-7, which gives equation 3-8.

$$Hg(0)_{soil} = \frac{\frac{\Theta_w^{10/3}}{H} D_{fw} + \Theta_g^{10/3} D_{fg}}{\Theta_T^2} \times \frac{\frac{Hg(0)}{A_S} Q}{\Delta z} \qquad (3\text{-}8)$$

From $Hg(0)_{soil}$ we can estimate $Hg(0)_{cum\ in\ soil}$ by multiplying the $Hg(0)_{soil}$ by the volume of air circulated over the sample. It should be noted that in this experiment we are dealing with very shallow soil layers and the diffusivity calculation results in only limited changes in the calculated Hg(0) mass formed after 24 hours analysis period.

3.3.4 Quality Assurance (QA)

Quality assurance (QA) included blanking of the analysis system, recoveries of gaseous mercury standard and replicate analyses. Initial chamber blanking involved passing Hg free air through the chamber until no detectable Hg(0) was liberated from the beaker or stopper surface (mercury levels usually fall below detection limits (< 0.1 ng m^{-3} within 0.5 hours). The method detection limit (MDL) was determined to be 0.184 ng m^{-3} with the procedure outlined by (Zhang, 2007). Recoveries of Hg(0) from the LiCor and quartz chambers were performed by direct external injections of 5, 10, 15 and 20 µL Hg(0) standards equivalent to 43.24, 86.52, 129.78 and 173.04

pg Hg(0) into both flux chambers at 19.03ºC over a period of 35 minutes each at time interval of 5 minutes.

3.3.5 Data Analysis

An orthogonal regression analysis technique, which has been widely used in environmental science (Kenneke and Weber, 2003; Leng et al., 2007), was used to compare the two measurement methods and define a line of best fit for a bivariate relationship between the Hg(0) flux (ng m^{-2} h^{-1}) collected from LiCor and quartz chambers as both variables were measured with error. It minimizes the sum of the squared perpendicular distances from each observation to the regression line. Since the data was not normally distributed, as determined using the Kolmogorov-Smirnov test, all data was log transformed prior to statistical analysis. Relationships between the log transformed mercury data and soil characteristics were assessed with multiple linear regression analyses.

3.4 Results and Discussion

3.4.1 Soil Physico-Chemical Properties

The soil chemical and physical results represent arithmetic means of triplicate samples. The soils investigated in this study had elevated levels (0.1-16 µg Hg g^{-1}) of Hg compared to terrestrial background Hg concentrations usually found in North American soils (Kuiken et al., 2008), (< 0.1µg Hg g^{-1}, Table 3-2). A wide range (12 and 160 g kg^{-1}) of total soil organic carbon contents was observed but which is representative of typical organic carbon contents found in Canadian Boreal soils (Perie and Ouimet, 2008). Fundamental soil properties (pH, EC, sand % and WHC etc.) are given in Table 3-2.

Table 3-2. Physico-chemical characteristics of the soils used in this study.

Soil	pH	EC	WHC	OM	OC	Total Hg
		-- dS/m --	-- ml kg^{-1} --	-- g kg^{-1} --	-- g kg^{-1} --	-- µg g^{-1} --
1	4.37	0.05	37.33	2.48	1.44	4.8
2	4.22	0.07	32.71	2.06	1.20	1.0
3	4.07	0.03	31.13	2.58	1.50	0.7
4	4.09	0.06	63.00	7.74	4.50	5.0
5	3.99	0.09	94.43	16.49	9.59	5.5
6	4.15	0.07	77.42	11.32	6.58	16.0
7	4.05	0.07	69.92	15.00	8.72	12.5
8	4.49	0.06	86.25	14.02	8.15	5.2
9	4.78	0.07	108.98	11.87	6.90	1.2
10	4.33	0.03	39.81	2.27	1.32	0.1

3.4.2 Comparison of Flux Systems

Mercury fluxes from the soils measured using both the LiCor and quartz flux chambers were generally large (mean flux = 145 ± 332 ng m^{-2} hr^{-1}), with relatively low coefficients of variation (i.e., 14%). Whereas Hg fluxes obtained using the quartz chamber ranged from 3.6 (± 2.03) to 1200 (± 87.8) ng m^{-2} hr^{-1}, Hg fluxes obtained using the LiCor chamber ranged from 3.1 (± 0.78) to 19.0 (± 1.75) ng m^{-2} hr^{-1} for the same set of soils (Figure 3-2). Although Hg fluxes obtained using the quartz chamber were generally one to two orders of magnitude greater ($p \leq 0.05$) than those obtained with the LiCor chamber, the two flux measurements were linearly correlated ($Hg\text{-}_Q = 45\ Hg\text{-}_{LC} + 116$; where $Hg\text{-}_Q$ = Hg concentration obtained using the quartz chamber and $Hg\text{-}_{LC}$ = Hg concentration obtained using the LiCor chamber).

The low Hg fluxes measured using the LiCor system reflects the fact that this system exhibited a very low recovery (2.2%) of Hg when gaseous elemental mercury standards were injected into the chamber. Indeed, Hg recoveries obtained using the quartz chamber system averaged 94% (or 43 times the recovery from the LiCor chamber) and presumably reflects the much greater turnover rate in the quartz chamber (i.e., turnover in the quartz chamber = 9 times that in the LiCor chamber). Eckley et al. (2010) found that at flow rates from 1.5 L min^{-1} up to 15 L min^{-1} (with equivalent turnover times of 0.1 – 13.9 min), the soil-to-air Hg flux increased with increasing flow rate (i.e., decreasing TOT). Moreover, Engle and Gustin (2002) found that high flow rates and short turnover times (TOTs) were more appropriate for measuring the Hg flux from soils with high Hg concentrations, whereas lower flow rates and long TOTs were more appropriate for soils with ambient Hg concentrations. Thus, the relatively long TOT (i.e., 2.7 min) characteristic of the much larger LiCor chamber (see Table 3-1) may have resulted in

elevated Hg levels in these soils that suppressed the Hg emission potential of these soils. Indeed, Zhang et al. (2010) reported that under low flow rates (or high TOTs), Hg accumulation within the chamber decreased the surface–air concentration gradient and increased the boundary layer resistance, resulting in a lower surface Hg emission potential.

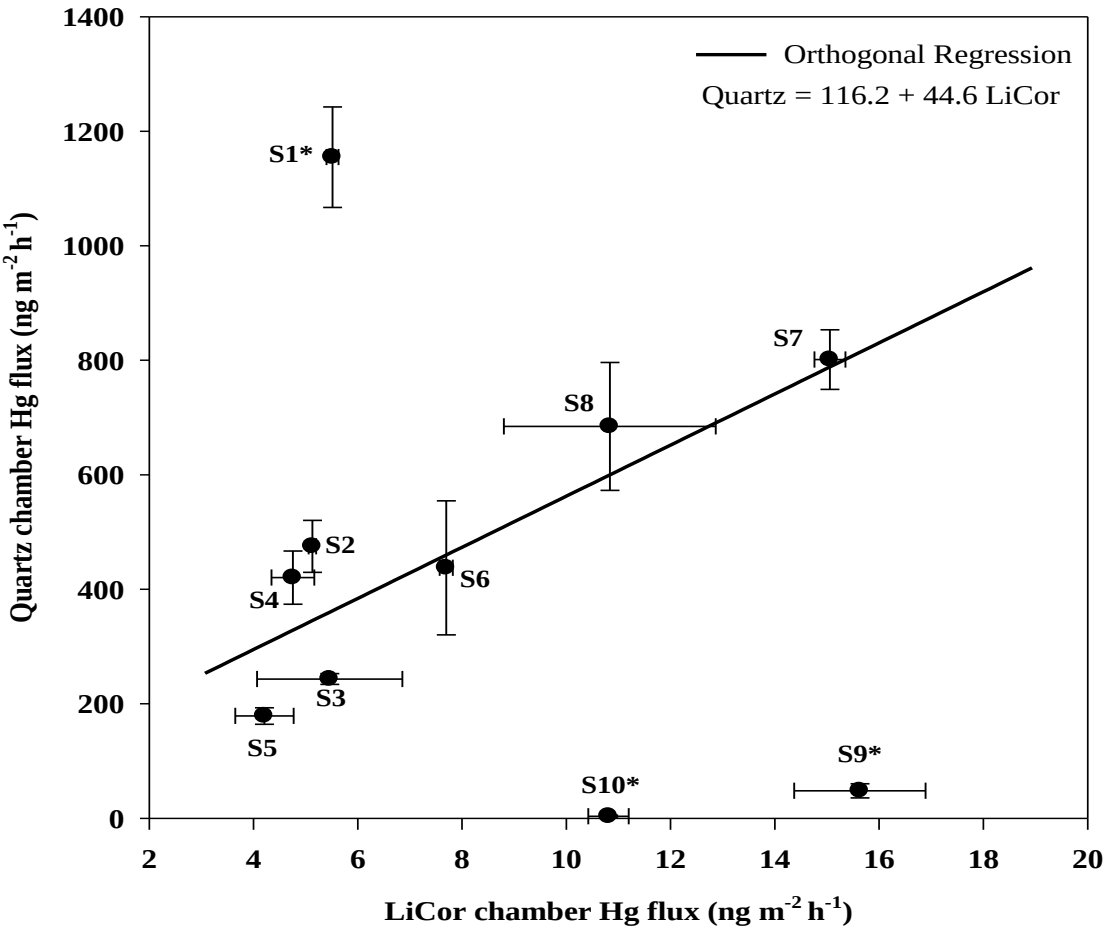

Fig. 3-2. Orthogonal least square regression plot of mercury flux (ng m^{-2} h^{-1}) obtained from 10 soils using LiCor and Quartz flux chambers. The individual points represent the ten soil samples marked from S1 to S10 analyzed in triplicates and the vertical and horizontal bars on each point represent standard error of the mean. The data points marked with an asterisk (S1*, S9* and S10*) indicate outliers.

On the other hand, the short TOT in the quartz chamber (0.3 min) may alter the chemical/physical conditions within the chamber beyond those expected under normal surface conditions resulting in an increase in surface emissions. During sample analysis under controlled conditions, a wide range of Hg emission fluxes were measured ranging from 3.3 ± 0.5 to 5800 ± 670 ng m^{-2} h^{-1}. These values exceed those reported for unaltered or background sites (1.4 ± 0.3 to 7.6 ± 1.7 ng m^{-2} h^{-1}) (Nacht and Gustin, 2004; Zhang et al., 2001) but are comparable to those measured in areas of Hg mine waste and areas disturbed by mining. Wang et al. (2005) measured higher emissions from mining areas in China, with maximum emissions of 11,544 ng m^{-2} h^{-1}.

The mercury flux measured using the quartz chamber, was significantly, though weakly, influenced by soil mercury concentration and organic matter content ($r^2 = 0.24$, $p<0.05$): however, soil pH and organic matter content were the best predictors ($r^2 = 0.48$, $p<0.05$) of the Hg flux measured using the LiCor chamber. Kim et al. (1995), Poissant et al. (2004), and Zhang et al. (2003) also observed that Hg flux from field soils was linked to soil Hg concentration as well as to soil temperature, soil moisture and wind speed, with soil mercury concentration, solar radiation and temperature as the rate-limiting factors (Carpi and Lindberg, 1998; Lindberg et al., 1995; Nater and Grigal, 1992).

3.4.3 Modeling Mercury Flux

The cumulative elemental mercury formation in the soils followed first order reaction kinetics reasonably well ($r^2 = 0.72$-0.96) with little difference observed when the mercury zero air sweeping the chamber was dry or humid (Figure 3-3). The coefficient of variation associated with my measurements (1.3-3.3%) was minimal at high Hg levels but increased at low Hg concentrations. The derived k's are for the reaction described in Equation 3-3:

$$Hg(II)_{red} + 2e^{-} \rightarrow Hg(0)_{soil} \qquad (3\text{-}3)$$

The reaction rates are not specifically linked to Hg concentrations in soil. Higher reaction rates, k, were associated with soils that have lower Hg contents. Thus, it is not clear if the k's derived from this laboratory system will be correlated with field fluxes where the reservoir of $Hg(II)_{red}$ in the soil profile is effectively infinite, in comparison to small, 20 g, soil that had a finite pool of $Hg(II)_{red}$ available to be converted to $Hg(0)_{soil}$ (O'Driscoll et al., 2003; O'Driscoll et al., 2006). Quinones and Carpi (2011) found that the first order model fits well to Hg emissions from sand samples of varying thickness and found rate constants in the range of 0.003 – 0.006 hr^{-1}. Hg flux was not dependent ($p=0.095$) on zero air humidity with similar levels of Hg evolved under humid (9.1 μg of Hg, standard error SE =4.8) and dry (3.2 μg, SE =1.8) zero air. Hg reduction rates were weakly ($p=0.025$) different under humid (1.1×10^{-3} h^{-1}, SE=0.13×10^{-3}) and dry (1.4×10^{-3} h^{-1}, SE=0.09×10^{-3}) conditions. Given the small difference in these values, I concluded that the results from humid and dry air should be combined for further analysis.

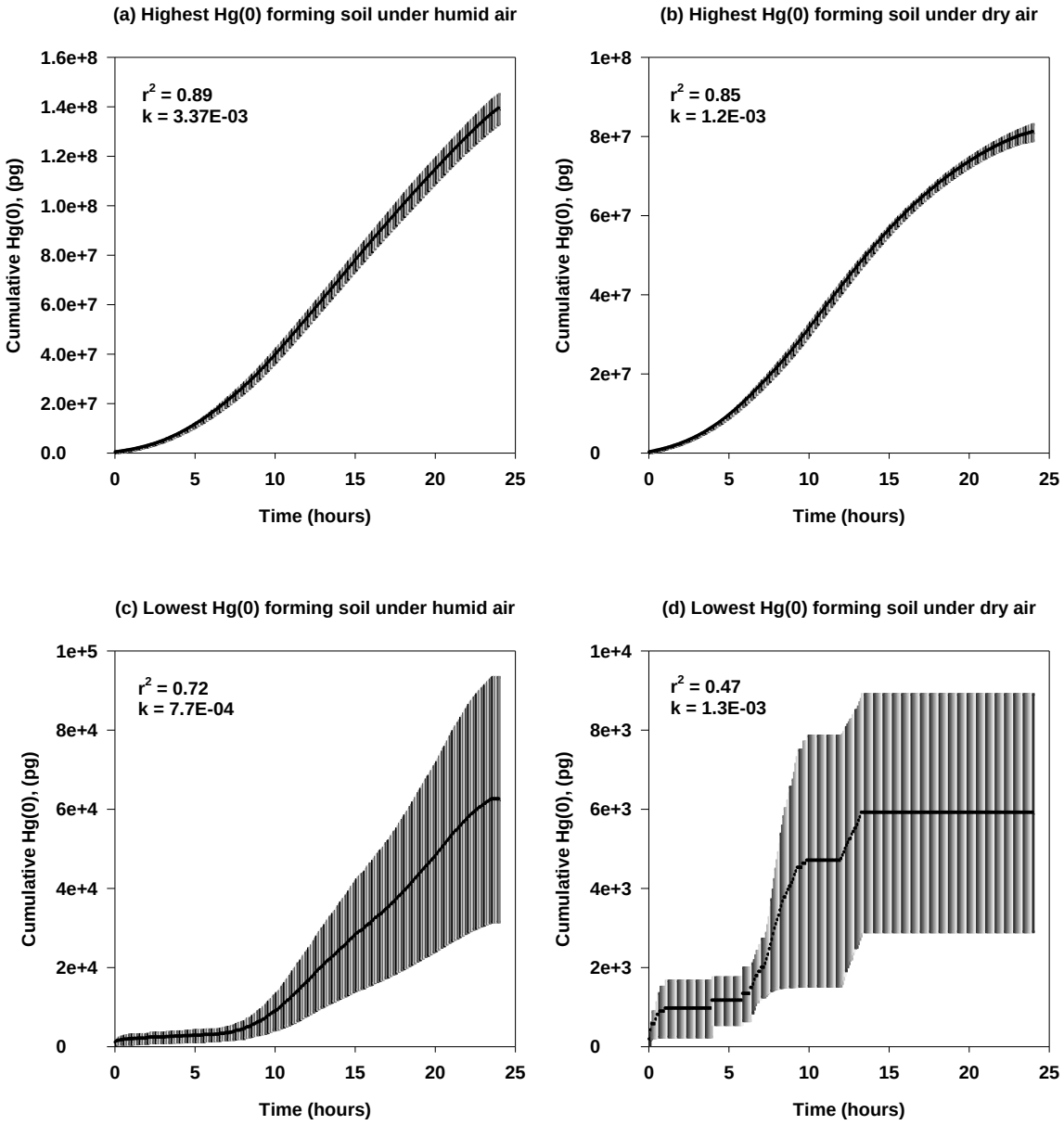

Fig. 3-3. Sample comparison of highest (soil 7, graphs a and b) and lowest (soil 10, graphs c and d) cumulative Hg(0) producing soils under humid (a, c) and dry (b, d) air conditions. k = reduction rate coefficient (h^{-1}), r^2 = regression coefficients. The solid black line (mean of three replicates) indicates the trend of Hg(0) production over 24 hours analysis period and the vertical bars represent standard error of the mean.

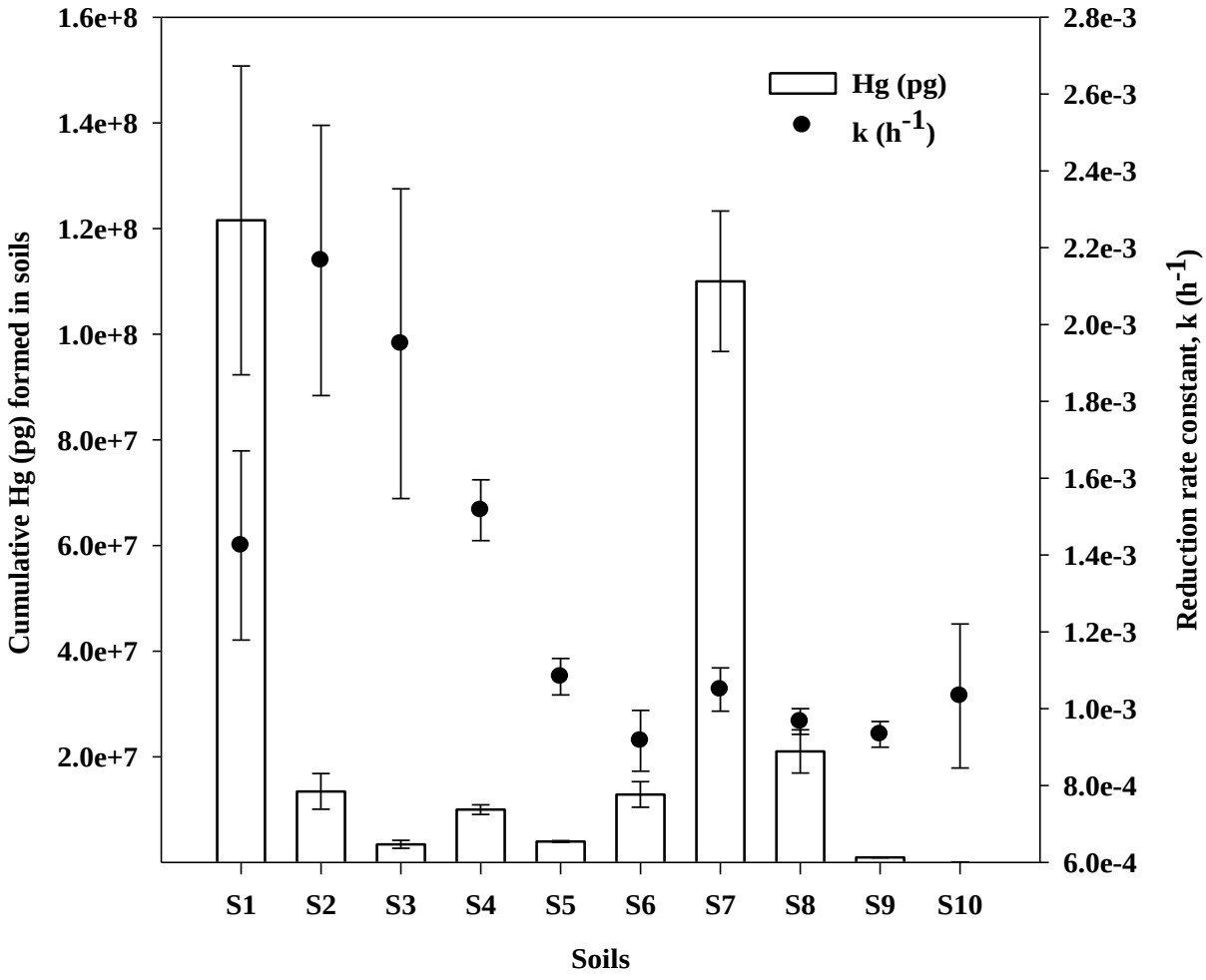

Fig. 3-4. Reduction rate constants (black circular dots) and cumulative mercury formed in soils (grey columns) over a 24 h analysis period. Each soil was analyzed in triplicate and the vertical black bars represent standard error of the mean.

Rate constants were largely independent of the amount of cumulative elemental mercury formed (Figure 3-4); suggesting that Hg(0) formation and emission is rapid, even in systems with relatively little Hg. The cumulative amount of Hg(0) formed in 20 grams of soil sample over a 24-h analysis period ranged from 0.049 to 120 ng, with k values (h^{-1}) ranging from 0.0009 to 0.0022. Lindberg et al. (1999) computed rate constants from Hg flux response curves which ranged between 0.04 ± 0.007 and 0.07 ± 0.023 hr^{-1}. Obrist et al. (2010) and Fritsche et al. (2008) observed that rates of Hg(0) emission increase or decrease with stimulated or inhibited microbial activity but no measurements of reaction rates are provided. Overall, 3.5 to 100% of total mercury contents were converted into elemental Hg in soils under controlled conditions. The reason for the high percent reduced may be due to the fact that the soils were collected from sites impacted by base metal mining and smelting area and with high levels of anthropogenic Hg present. This mercury may be present in easily reducible form and clearly more research is needed to determine the exact mechanism of mercury reduction and the speciation of easily reducible mercury.

The elemental Hg formation in the soil was only weakly correlated to total Hg content ($r = 0.52$, $p<0.05$). However, correlations between Hg(0) formation and other soil properties were non-significant. The lack of significant correlations between cumulative elemental mercury production and individual soil characteristics suggests that total mercury is the primary variable determining the mass of mercury reduced. The correlations observed between the reduction rate constants (k, hr^{-1}) and organic matter ($r = -0.47$, $p = 0.16$), pH ($r = -0.38$, $p = 0.26$), EC ($r = -0.22$, $p = 0.51$) and sand contents ($r = 0.04$, $p = 0.89$) were also non-significant. The negative correlation of reduction rates with organic matter, pH and EC suggests that a slow reduction of Hg(II) occurs within the soils having higher organic matter, EC and less acidity possibly due to

the enhanced binding of Hg(II) with negative organic functional groups, OH^- ions and anions. Mercury exhibits a great affinity for organic matter (both solid and dissolved forms) in soils due to complexes with OH^-, S^{2-} and S^-containing functional groups of organic molecules because of their high abundance and stable binding with mercury (Schuster, 1991; Schluter, 2000). Similarly, dissolved ionic species (Cl^- and S^-) play a major role in mercury speciation (Gabriel and Williamson, 2004).

My data indicates that humidity in the air above the soil surface did not have a controlling influence on the elemental mercury production during the experimental period for these samples. This supports the observation of Gillis and Miller, (2000b) who found no correlation between mercury emissions from soils and the air humidity under controlled conditions. Similarly, no significant correlation between Hg flux and relative humidity was found in desert and agricultural field soils under both dark as well as light conditions (Ericksen and Gustin, 2006; Lindberg et al., 1995). In another study; it was observed that the process responsible for the enhancement of Hg(0) emissions from the soil in response to simulated precipitation is connected to the aqueous soil phase and that the process is rapid (Song and Van Heyst, 2005). As such, relative humidity measures do not influence mercury flux in field experiments.

3.5 Conclusions

I achieved precise and repeatable results for elemental mercury formation in soil using the quartz flux chamber system. This system could be quickly and accurately blanked with excellent recovery (93 to 96%) of gaseous mercury standards. While this limited data set requires further validation, it suggests that this system can be effectively used to study the Hg reduction process

in soils under controlled conditions. Further work on the effects of soil temperature and moisture are needed to clarify these results.

4. DETERMINING THE CONTRIBUTION OF BIOTIC AND ABIOTIC FACTORS IN ELEMENTAL MERCURY FORMATION IN BOREAL SOILS WITH CHANGING TEMPERATURE

Preface

Chapter 3 developed a quartz flux chamber to accurately measure the rates and amounts of Hg(0) formation in soils. However, knowledge of the ability of the soil temperature, WFPS and microbes to affect Hg reduction process in soils is critical to predict and model future effects of climate change on Hg cycling in terrestrial ecosystems. This chapter represents a first investigation under controlled conditions to characterize the effects of soil temperature on rate of abiotic and biotic Hg(0) formation in soils and to estimate the proportion of Hg(0) production arising due to biological activity.

Ravinder Pannu, Steven D. Siciliano, Andy Rencz, John Dalziel, and Nelson J. O'Driscoll. 2012. Determining the contribution of biotic and abiotic factors in elemental mercury formation in boreal soils with changing temperature. Environmental Pollution (Submitted).

Ravinder Pannu planned, developed and conducted the experiment, conducted major field and lab work, reviewed the literature and is primary writer. Nelson J O'Driscoll and Steve Siciliano are co-supervisors and provided experimental and technical guidance, helped in statistical analysis and editing. Andy Rencz provided input relative to national assessment program. John Dalziel provided technical support and troubleshooting of instruments.

4.1 Abstract

There is a paucity of information regarding the fundamental biotic and abiotic mechanisms controlling soil Hg flux as well as on the effects of changing temperature on these fluxes. This research used a controlled analysis technique to quantify the effect soil temperature and sterilization on the kinetics of Hg(0) formation in forested soils of Nova Scotia, Canada. An apparent pseudo first-order model fit the data of cumulative Hg(0) formed in soil well (r^2= 0.90 to 0.99, $p<0.001$, n = 10). Both the logarithm of cumulative mass of Hg(0) formed in soils and the reduction rate constant (k values) increased linearly with soil temperature (r^2: 0.78-0.99 for Hg(0) formation and 0.47-0.98 for k values, respectively, $p<0.01$, n = 10). The percentage of Hg(0) formed in soil was linearly related to the logarithm of temperature (% Hg(0) = -3.0 + 4.5 ℓn (K-271.6), r^2=0.99, $p<0.004$, n=10). Sterilizing soil significantly ($p<0.05$) decreased the percent of total Hg reduced to Hg(0), with sterile soils on average reducing 3.4% (SE=1.4) of total mercury as compared to 6.8% (SE=1.4) on average for non-sterile soils. This research finds enhanced Hg(0) formation in soils with increased soil temperature, it also finds biotic contributions to be highly significant in this process.

4.2 Introduction

Mercury is ubiquitous in the environment and continuously cycles between terrestrial systems, the atmosphere, oceans, and living organisms. It is a global pollutant, and once released in its volatile elemental form, Hg(0), it can remain in the atmosphere for up to one year (Lindberg et al., 2002). Soils, in particular have the potential to be a large source or sink in the mercury cycle, depending on ambient conditions (Kim and Lindberg, 1995). Research during the past decade has established the importance of natural soils in Hg cycling, showing that emission from soils

may contribute substantially (700-1000 Mg yr^{-1}) to the global atmospheric load of Hg (Coolbaugh et al., 2002; Engle et al., 2001; Engle and Gustin, 2002; Gustin and Lindberg, 2000; Gustin et al., 2000; Zhang and Lindberg, 1999). Mercury vapour exists in the soil air space, primarily as Hg(0), and has been measured in concentrations ranging from 1 to 53 ng m^{-3} directly above soil surfaces (Johnson and Lindberg, 1995).

Experimental studies performed on natural and contaminated soils using dynamic flux chambers have demonstrated the strong dependence of Hg emission on climate factors (Gustin et al., 1997; Lindberg and Stratton, 1998; Poissant and Casimir, 1998; Scholtz et al., 2003). A principle driver of Hg(0) emissions appears to be temperature. For example, Gillis and Miller (2000b) found that mercury emission rates in low-mercury, fine sandy loam soil can be largely explained by variations in surface soil temperature ($r^2 = 0.88$) and the Hg concentration gradient between the soil air and the ambient air above it. This temperature dependence has been observed both in diurnal (Gustin et al., 2006) and seasonal studies (Sigler and Lee, 2006). In a factorial experiment, Lin et al. (2010) showed that the synergistic effect from the combination of air temperature and soil moisture was 30% greater than the additive Hg flux for the two individual effects. They proposed this synergistic effect was a result of enhanced water evaporation at higher temperatures promoting additional Hg emission from the soil surface; however, no particular mechanism was suggested. Sigler and Lee (2006) suggest that Hg(0) bound to upper soil layers may be desorbed by an increase in soil temperature, thereby increasing the pool of gaseous Hg(0) in soil air spaces available for emission. However, the mechanism by which Hg(0) is formed in soil is not well understood.

Elemental mercury in soil can be produced by abiotic or biotic processes. It is generally thought that most of the Hg emitted from soil originates from the A horizon and is produced by the high

microbial activity and abundance of reductants present in this soil horizon (Schluter, 2000). However, abiotic processes such as the desorption of Hg(0) sorbed onto soil particles or, alternatively, abiotic reactions in the soil can produce Hg(0) from available Hg(II) (Gu et al., 2011; Pehkonen and Lin, 1998; Scholtz et al., 2003; Zhang and Lindberg, 1999). Surprisingly, Hg(0) can readily sorb onto a soil surface and remain there; for example, Bouffard and Amyot (2009) found that 200 pg of Hg(0) adsorbed via Van der Walls type forces onto 1 g of sediment in less than one hour with maximum adsorption (approximately 85%) taking place in the first 5 min. Thus, there is a pool of Hg(0) available in the soil that can readily desorb into the soil atmosphere and be available for efflux to the atmosphere. In addition, there are a wide range of aqueous abiotic processes (e.g., reduction of Hg(II) mediated by humic acids, fulvic acids, free radical electrons and sunlight mediated photoreduction) that transform Hg(II) in the soil solution to Hg(0) (Gabriel and Williamson, 2004; Schluter, 2000). Further, these abiotic processes can be enhanced in the presence of mixed valence (Fe(II)/Fe(III)) iron oxide minerals and elevated pH (Wiatrowski et al., 2009).

Apart from physically and chemically mediated Hg(0) emission, microbial activity might contribute to Hg evaporation from soils (Schluter, 2000). This bacterial production of Hg(0) can occur at ambient Hg(II) concentrations via mercury-specific detoxification pathways (Barkay et al., 1991) or non-specific microbial reduction of Hg(II) linked to the microbial detoxification of reactive oxygen species (Siciliano et al., 2002b). The production of Hg(0) is linked not to total Hg(II) in soil but the bioavailable fraction of Hg(II) in soil. For example, the differences in response of the two mer-lux derivatives of *Escherichia coli* in agricultural and beech forest soil dosed with equal amounts of total Hg were likely due to differences in the bioavailability of Hg(II) (Rasmussen, 1994). Soil properties will not only influence the bioavailability of Hg(II)

but also the rate of microbial transformation and affinity of the soil for Hg(0). Organic matter content not only alters the affinity between Hg(0) and sediments under anoxic conditions but also accelerates biotic reduction of Hg(II) to Hg(0) (Bouffard and Amyot, 2009). Thus, it is plausible that biotic reduction may contribute to Hg(0) production in soil; however, the relative importance of abiotic to biotic process has not been quantified in detail.

Radiation has been used to sterilize, or partially sterilize, soil as a preliminary treatment for a wide range of soil microbiology research. Because it leaves soil structure intact and devoid of any residual toxicity, it is an attractive technique for ecological experiments with natural soils. Gamma (ɤ) irradiation allows the elimination of soil organisms by varying the dose applied, either directly by cell lyses or indirectly through the formation of mutagenic free radicals (McNamara et al., 2007; McNamara et al., 2003). A number of studies have suggested that ɤ irradiation is highly effective and is preferable to other methods as, in addition to being an effective biocide, it has less of an effect on soil chemical and physical properties (McNamara et al., 2007; Ramsay and Bawden, 1983; Stroetmann et al., 1994). Typically, a dose of ɤ irradiation of 10 kGy eliminates fungi, actinomycetes and invertebrates while, 20 kGy is considered to completely sterilize soil as verified by the absence of most culturable bacteria (McLaren, 1969; McNamara et al., 2007; Powlson and Jenkinson, 1976; Trevors, 1996). Jackson et al. (1967) irradiated 30 g soil samples in plastic culture dishes with ɤ irradiation ranging from 0 to 30 kGy. Ten kGy was required to kill all fungi, while 20-30 kGy were found to eliminate all bacteria. Soils can also be irradiated at a higher dose; for example, Van Elsas et al. (1989) exposed soils collected from Canada and The Netherlands to 40 kGy for use in studying survival of *Pseudomonas florescens* cells and serial dilutions of the ɤ irradiated soil plated onto tryptone yeast extract agar revealed no colony forming units. Similarly, four soils were placed in sealed

polythene bags (500 g per bag) and exposed to ɤ irradiation at 30 kGy at the Atomic Energy Authority Research Establishment, UK for sterilization. They were tested for sterility by inoculating 1 g samples into nutrient glucose broth and incubating at 24-26°C. No growth was observed even after 7 days and it was concluded that the treatment had completely sterilized the soils (Davis, 1975).

Because of the strong temperature dependence of Hg(0) flux from soil, one can quantify the influence of temperature on physical flux from soil and potentially examine the chemical reactions producing Hg(0) in the soil matrix itself. Early work suggested that the flux of Hg over the soil surface can be considered to be a thermally-enhanced emission process and the Arrhenius equation (Equation 4-1) can be used to quantify the activation energy associated with flux (Carpi and Lindberg, 1998; Poissant and Casimir, 1998).

$$k = Ae^{-(Ea/RT)} \qquad (4\text{-}1)$$

Where; k is the rate constant (hr^{-1}); R is the gas constant (kcal K^{-1} mol^{-1}); T is temperature in Kelvin; E_a is the activation energy (kcal mol^{-1}), which the system must absorb in order to initiate a reaction; and A is the pre-exponential factor, which is independent of temperature for many reactions. These authors obtained an apparent activation energy of 20.5 kcal mol^{-1} suggesting that Hg emission over the soil surface is not solely controlled by the vaporization of Hg(0), which has an enthalpy of vaporization of only 14 kcal mol^{-1}. Instead, Hg(0) emission likely includes intermediate steps such as biotic or abiotic reduction of Hg(II) to Hg(0).

The objectives of this study were to (a) characterize the effects of temperature on the rate of abiotic and combined abiotic/biotic Hg(0) formation in soil under controlled conditions and (b) to estimate the proportion of Hg(0) production arising due to biological activity.

4.3 Methods and Materials

4.3.1 Soil Sampling

Soil samples were collected from mature intact mixed forests in Kejimkujik National Park (KNP) and Antigonish County, Nova Scotia, Canada. Bedrock sampling within KNP indicates the mercury content (0.2 ng Hg g^{-1} to 38.9 ng Hg g^{-1} for surface outcrops) is generally within the range of other areas within Canada, with the exception of a few high concentration areas (>200 ng Hg g^{-1}) found within the park (O'Driscoll et al., 2005). Antigonish sites were previously used for conducting various carbon and soil respiration studies (Kellman et al., 2007; Risk et al., 2009). Surface soil samples (n=4, 0-15 cm depth) were collected from KNP on April 16-17, 2010, and from Antigonish County (n = 6, 0-15 cm depth) on June 28-29, 2010 (Table 4-1). The organic litter was removed and soils were homogenized with a stainless steel spade. Each sample was a pooled composite of four samples (approximately 0.5 kg each) collected within an area of 100 m^2. The 2 kg pooled bulk samples were further homogenized in the field and stored in Ziploc bags in the dark at -20˚C until analysis. Thawed soil samples were placed in polypropylene plastic containers and dried in dark for 72 h in a clean growth chamber (Conviron Model E15) at 20˚C and 0 % relative humidity. The dried soil samples were sieved through a 2 mm stainless steel sieve and stored in polypropylene containers under dark and dry conditions at room temperature.

Table 4-1. Locations and physical and chemical characteristics of the soils used in this study.

Soil ID†	Longitude	Latitude	pH	EC	WHC	OC	Total Hg
				-- dS/m --	-- ml kg^{-1} --	-- g kg^{-1} --	-- ng g^{-1} --
K1	44°26’45’’N	65°15’19’’W	4.6	0.03	46.2	36	105
K2	44°27’30’’N	64°59’01’’W	4.4	0.02	49.6	26	104
K5	44°19’49’’N	65°14’06’’W	4.8	0.02	31.1	15	66
K7	44°17’55’’N	65°14’54’’W	4.7	0.03	37.3	20	66
A11	45°45’06’’N	61°56’49’’W	4.2	0.04	43.7	16	28
A12	45°45’06’’N	61°56’46’’W	4.3	0.05	69.8	65	106
A13	45°42’14’’N	61°59’25’’W	4.4	0.08	59.3	35	69
A14	45°40’31’’N	61°43’29’’W	5.4	0.19	59.1	24	50
A15	45°39’22’’N	61°50’32’’W	5.1	0.18	56.3	37	96
A18	45°39’27’’N	61°51’25’’W	4.3	0.05	30.7	05	13

† K: Kejimkujik National Park; A: Antigonish County

Soil pH was measured in 0.01M $CaCl_2$ with 1:2.5 soil:solution (w/v) ratio (Mehlich, 1976) and electrical conductivity (EC) was measured in Milli-Q water with 1:2 soil:water (w/v) ratio. Total Hg concentrations of soil samples were quantified using aqua regia (37% HNO_3 + 63% HCl, 1:3) digestion and cold vapour – atomic absorption spectroscopy. Soils were analyzed for organic matter (OM) (Walkley and Black, 1934) and water holding capacity (WHC) (Franzluebbers, 1999). To eliminate microbial activity, 5 homogenized sub samples (K2, K5, K7, A11, and A12 respectively) were irradiated at the Canadian Irradiation Center (CIC), Laval, Québec, Canada to investigate the effect of soil sterilization (Thuerig et al., 2009). For this purpose, 500 grams each of air dried soil sample taken in a Ziploc bag further contained in an air sealed high quality Rubbermaid plastic container was placed inside a carrier irradiator (JS-8900, s/n: IR-147) and irradiated in continuous mode using Cobalt 60 as the radioactive source. Each soil sample was uniformly exposed to a maximum dose of 30.7 kGy measured using a Harwell Red 4034 dosimeter (Harwell Dosimeters Ltd., Oxfordshire, UK) at 640nm. The sterilized soils were stored and handled under sterile conditions in a laminar flow unit. The sterility of sterilized and non-sterilized soil samples was tested according to Berns et al. (2008) and the soil samples were considered to be sterile when there was no microbial growth after a 3 week incubation period at 20 ± 0.2°C. The irradiated sub samples were run parallel to the non-irradiated parent samples to study the effect of sterilization on Hg(0) formation.

4.3.2 Quartz Reaction Chamber System

A quartz beaker reaction system developed in Chapter 3 was used to quantify the effects of increasing soil temperature and soil sterilization on Hg(0) formation in soils under controlled conditions. A Tekran model 1100 mercury zero-air generator supplied mercury-free air (1 L min^{-}

[1]) to the chamber. Dry mercury-free zero air was used throughout this experiment to prevent the condensation of water vapours in the reaction chamber at low temperatures (278 and 283 K, respectively). To achieve the desired temperature, the lower portion of the quartz beaker was immersed in a water bath (ThermoHakke model K20) pre-set to the desired temperature. The soil surface temperature was monitored continuously with Teflon insulated, fine wire (0.013cm diameter) thermocouple inserted into the soil surface and connected outside to a HH806AU series digital thermometer obtained from Omega Engineering, Laval, Quebec, Canada. The soil Hg(0) readings were started immediately once the soil reached the desired temperature which typically took 5-10 minutes. Soil Hg flux readings were then taken every 5 minutes over a 24 hour period and repeated in triplicate.

A typical soil analysis under dark conditions consisted of initial blanking of the chamber by passing mercury free zero air through the chamber without soil until zero mercury concentration was detected. Soil (20 g) was brought to 45 percent water filled pore space (WFPS) and `uniformly placed at the bottom of the quartz glass beaker in a thin layer maintaining a bulk density of 1.60 g cm^{-3}. This was done by gently tapping the soil sample in the quartz beaker before analysis until a soil depth of approximately 0.44 cm was achieved. Temperature effects on Hg(0) emissions were evaluated at soil temperatures of 278, 283, 288, 293, and 303 K representative of ranges commonly found in upper 10 mm of soil (Carpi and Lindberg, 1998).

Reactions were allowed to proceed over a 24 hour period keeping all environmental parameters constant (soil temperature, radiation and air flow rate). No UV radiations [UV-B (λ = 280-320 nm), 0 Watts m^{-2}, UV-A (λ = 320-400 nm), 0 Watts m^{-2} and visible radiations (λ = 400-700 nm), 0.14 Watts m^{-2} respectively)] were detected in lab using an Ocean Optics USB 4000 Spectra

radiometer with a fiber optic cable (10 m, 200 µm diameter) and spectral diffusion probe (diameter 0.43 cm).

The production of Hg(0) in soil can be modeled as a reversible first order reaction (Lindberg et al., 1999; O'Driscoll et al., 2006; Schluter, 2000).

$$Hg(II)_{red} + SOM\ (\mathrm{n}e^{-}) \leftrightarrow Hg(0)_{soil} + SOM\ (n-2)e^{-} \qquad (4\text{-}2)$$

Where: $Hg(II)_{red}$ is the reducible divalent mercury present in soil; SOM is soil organic matter; ne^- is the sink of electrons from the reducing agent, (SOM) and $Hg(0)_{soil}$ is the elemental mercury produced in soil. I did not observe Hg(0) formation (all readings below stated method detection limit of 0.15 ng m^{-3}) in either non-sterilized or sterilized air-dried soil samples and therefore believe that Hg(0) production in soils is due to aqueous abiotic or biotic reduction alone or in combination. As such, this reaction becomes dominated by the forward reduction reaction (Equation 4-3) as Hg(0) produced in the soil is effectively stripped from the quartz chamber (turn-over time = 0.3 minutes).

$$Hg(II)_{red} + 2e^{-} \rightarrow Hg(0)_{soil} \qquad (4\text{-}3)$$

The cumulative mercury formed in soil placed in the quartz chamber and the pseudo first order reaction rate for mercury reduction in soil was calculated as described in Chapter 3.

4.3.3 Quality Assurance (QA)

Quality assurance (QA) included blanking of the analysis system, recovery of gaseous elemental mercury standards and triplicate analyses of all samples. Initial blanking of the flux chamber was performed by analyzing mercury-free air until no detectable Hg(0) was liberated from the acid-

cleaned beaker or stopper surface (mercury levels usually fell below analytical detection limits (<0.1 ng m^{-3}) within 0.5 hours.

A Tekran 2505 mercury vapor generation unit was allowed to equilibrate for a minimum of 2 hours prior to injections with acceptable error being ±5%. A Tekran Model 2505 mercury vapour calibration unit and Hamilton 700 series Microliter™ digital syringe were used to deliver seven external injections (5 µL) of a gaseous Hg(0) standard [equivalent to 43 pg or 9 ng m^{-3} Hg(0)] at 15˚C directly into the quartz chamber. Using this data, the method detection limit (MDL) was determined to be 0.15 ng m^{-3} (Zhang, 2007) and a recovery of 94% ± 2.2% (RSD, relative standard deviation) of elemental mercury was derived from direct external injections of 43, 87, 130, and 173 pg Hg(0) standards into the quartz chamber.

4.3.4 Data Analysis

Since the cumulative mass of Hg(0) formed and the rate constant data were not normally distributed, as tested by Kolmogorov-Smirnov test, all data were log transformed prior to statistical analysis. The relationships between percentage of mercury reduced with temperature and soil characteristics were assessed using multiple linear regression analyses. This was done because percentage of mercury reduced normalizes for differences in total soil mercury content. A step-wise backward exclusion approach was used to further minimize the number of soil variables to those most correlated with the percentage of mercury reduced and derived rate constants. One-way analysis of variance (ANOVA) was performed to test for significant differences ($p<0.05$) between cumulative Hg(0) produced in soils and reduction rate constants at different temperatures in natural and sterilized soils.

4.4 Results and Discussion

4.4.1 Activation Energies

The soils investigated in this study have background concentrations of mercury (13 to 106 ng g^{-1}) similar to those of typical soils in eastern North America (< 200 ng g^{-1}) (Kuiken et al., 2008) and have both organic carbon contents (5 to 65 g kg^{-1}) and pH (4.2-5.4) typical of boreal soils (Perie and Ouimet, 2008). The cumulative elemental mercury formed (Figure 4-1A) in soils, as well as the apparent pseudo-first order rate constants, increased linearly with temperature (Figure 4-1B). Activation energies (E_a's) varied between 14.2 to 55.4 (n=10, $\overline{x}$ = 35.2 kJ mol^{-1}) and there was a significant ($p<0.01$) sterilization interaction (Figure 4-2). However, the activation energies were not significantly correlated ($p>0.05$) with any of measured soil properties: pH (r = -0.18), EC (r = -0.19), WHC (r = -0.33), OC (r = -0.38) or Hg (r = -0.24).

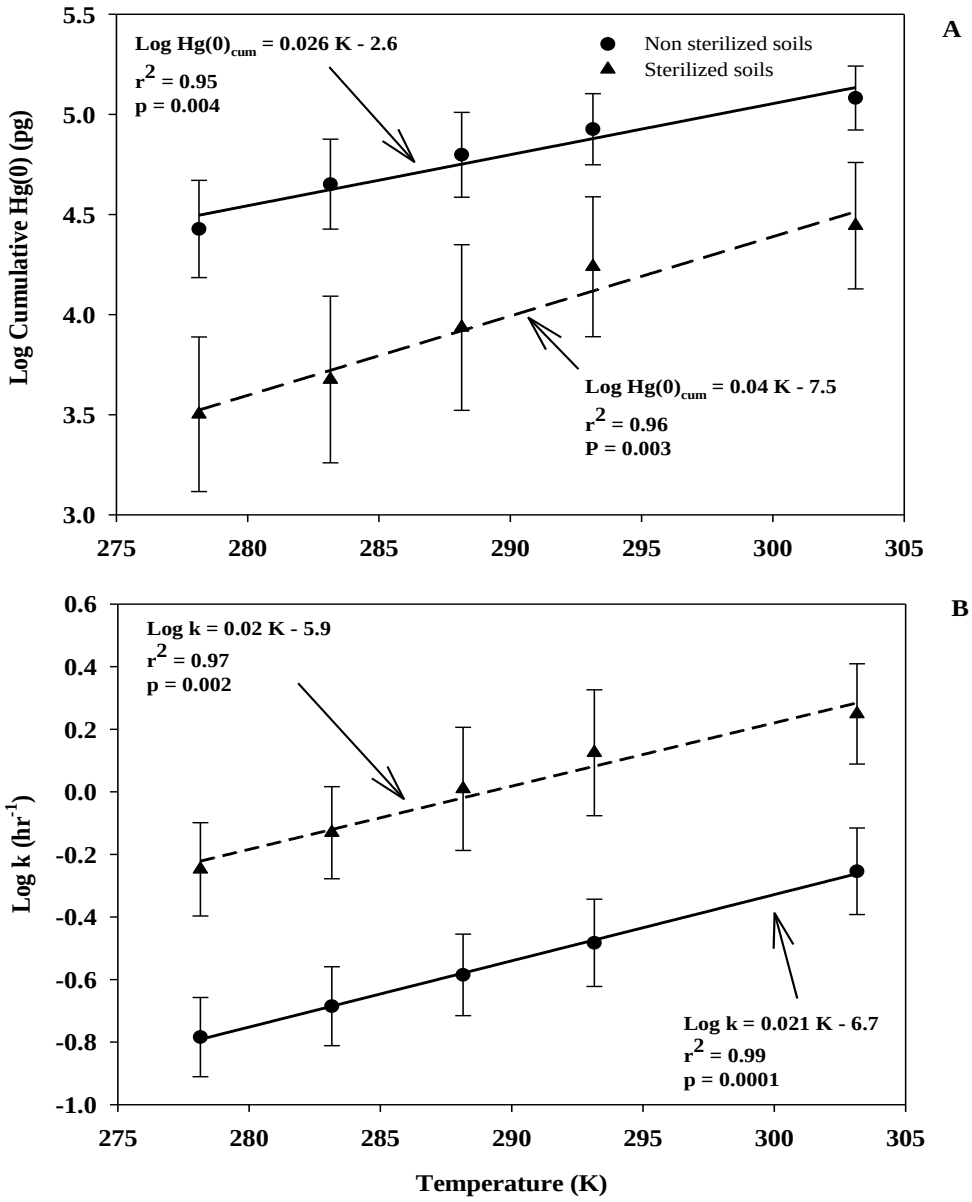

Fig. 4-1. Cumulative Hg(pg) formed (A) and reduction rates (B) in non-sterilized (black circles, n = 10) and sterilized soils (black triangles, n = 5) over 24 hour analysis period as affected by increasing soil temperature in studied soils. Each individual point represents average of five soil samples analyzed in three replications with errors bars indicating standard error of the mean of 15 samples.

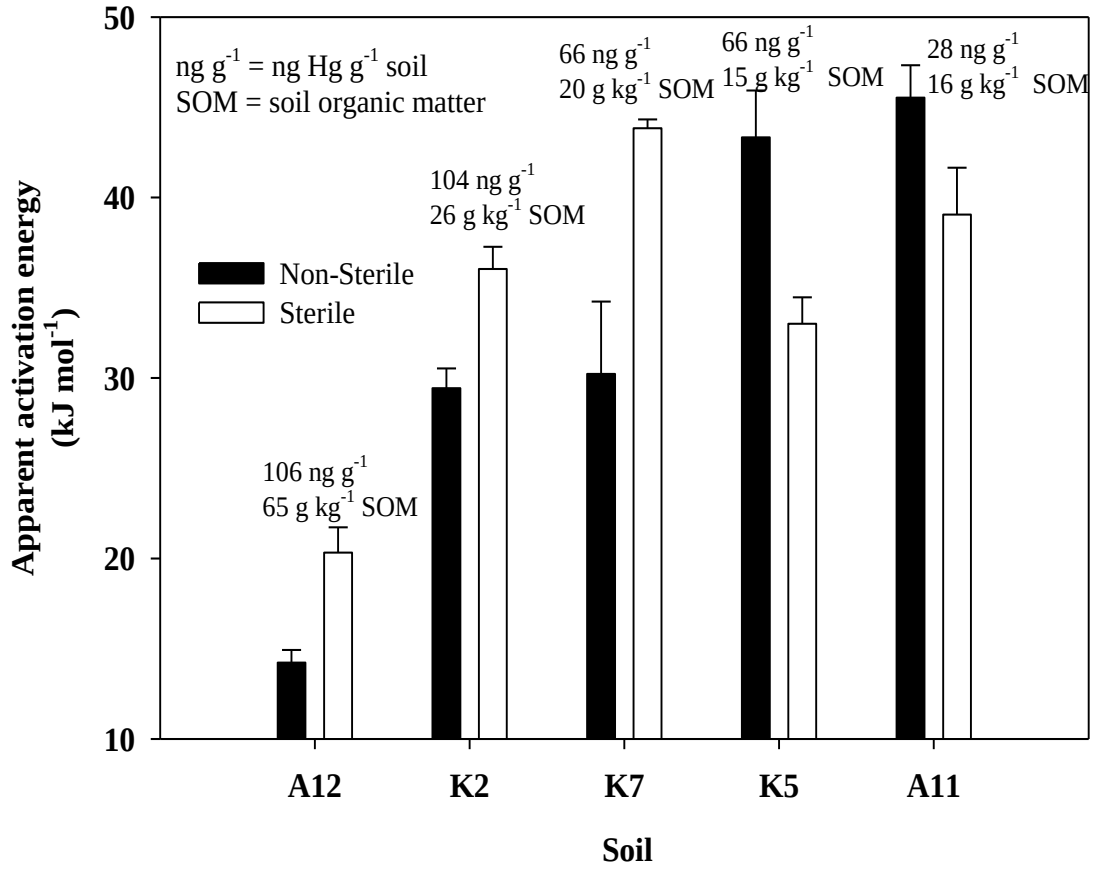

Fig. 4-2. A comparison of sterile (hollow transparent bars) and non-sterile (solid black bars) apparent activation energies across five (A12, K2, K7, K5 and A11) different soils. Soils and sterility interacted ($p<0.01$) but no soil factors explained the dependence of sterility effect on soil sample. Each bar represents the mean of triplicate estimates of apparent activation energy at increasing soil temperatures (278, 283, 288, 293, and 303 K respectively) and error bars are the standard error of the mean. Numbers above each bar are the total mercury content (ng g^{-1}) and soil organic matter (g kg^{-1}).

The activation energy (35 kJ mol^{-1}) of Hg(0) formation determined here is 50% lower than the activation energy obtained for Hg(0) emission under field conditions; e.g., 72 ± 32.2 kJ mol^{-1} for Tennessee forest soils (Kim et al., 1995); 75 ± 20.5 kJ mol^{-1} for Tennessee soils (Carpi and Lindberg, 1998); and 69 ± 23 kJ mol^{-1} for a southern Quebec soil (Poissant and Casimir, 1998). This suggests that under field conditions (intact soil profile), Hg(0) release is controlled by more than just Hg(0) formation; e.g., other chemical (precipitation-dissolution reactions) and physical processes (sorption-desorption). The E_a values are in the range of those reported for soils with a high fraction of Hg(0) (Sigler and Lee, 2006). These results indicate the importance of not only the total amount, but also the type of Hg species and their binding in soils. As reported by Schluter (2000), Hg evaporation occurs most easily in soils rich in Hg(0), followed by soils dominated by inorganic Hg(II), which is bound to soil components and probably relatively easily available for transformation to Hg(0) through abiotic and biotic processes. The highest activation energy of Hg evaporation is usually needed for soils whose Hg content is dominated by HgS, which is extremely insoluble, and therefore relatively unavailable for transformation (Kocman and Horvat, 2010). It should be noted that the previous researchers (Table 4-2) found temperature dependent mercury emission from soils as an exponential thermal desorption process and made an assumption of equating mercury flux values to rate constants and derived E_a of the combined reaction of Hg(0) formation and emission (Bahlmann et al., 2006; Carpi and Lindberg, 1998; Gustin et al., 2002; Zhang et al., 2001). My results on the other hand, have been calculated solely from Hg(0) formation (Chapter 3). Moreover, this study involved experimental conditions that may not accurately represent natural conditions (e.g., use of Hg free zero air instead of ambient air, disturbance of the soil profile and prevalence of different environmental conditions in the reaction chamber relative to background ambience).

My study was conducted under dark conditions to simulate Hg(0) formation in the bulk soil. Radiation is known to play an important role in Hg(0) emission. Zhang et al. (2001) observed that a reduction in ultraviolet radiation during field measurements reduced radiation-enhanced mercury emissions by ~24%. Activation energies for a mineral soil calculated using Hg fluxes and temperatures under solar radiation conditions were found to be greater (54 kJ mol^{-1}) than that needed under dark conditions (18 kJ mol^{-1}) (Gustin et al., 2002). Similarly, the E_a for Hg(0) emissions from a soil amended with municipal sewage sludge exposed to solar radiation was higher (110 kJ mol^{-1}) than that associated with a shaded chamber (95 kJ mol^{-1}) (Carpi and Lindberg, 1997). Thus, the application of my results for the activation energy of Hg(0) formation in soil should be limited to soil not exposed to solar radiation.

Table 4-2. Relationship between soil temperature and Hg emission as reported in the literature.

Reference	Relationship	Correlation	Lab/Field	Process	Fitting parameters
Moore and Castro, (2012)	Linear	$r^2 = 0.19$	Forest field	Abiotic / Biotic	Hg flux = 0.12(T) + 0.89
Moore and Castro (2012)	Linear	$r^2 = 0.29$	Grass field	Abiotic / Biotic	Hg flux = 0.07(T) + 0.50
Rinklebe et al., (2010)	Exponential	$r^2 = 0.99$	Lab	Thermal desorption	Hg flux = $178e^{0.03(T)}$
Rinklebe et al., (2010)	Exponential	$r^2 = 0.49$	Field	Thermal desorption	Hg flux = $69e^{0.07(T)}$
Sigler and Lee (2006)	Exponential	$r^2 = 0.77$	Forest field	Thermal desorption	Hg flux = $1.19e^{0.01(T)}$
Moore and Carpi (2005)	Exponential	$r^2 = 0.92$	Lab	Abiotic / Biotic	Hg flux = $7.4e^{0.07(T)}$
Poissant et al., (2004)	Linear	$r^2 = 0.70$	Field	Thermodynamic	Hg flux = 0.19 (T) + 0.73
Zhang et al., (2001)	Linear	$r^2 = 0.99$	Field	Physico-chemical	Hg flux = 0.64(T) – 9.4
Gillis and Miller (2000)	Linear	$r^2 = 0.80$	Lab	Abiotic / Biotic	Hg flux = 0.02(T) + 0.83

T, Soil temperature; Hg flux is in ng m^{-2} h^{-1}

4.4.2 Effect of Soil Sterilization on Hg(0) Formation in Soils

No significant ($p<0.05$) differences were observed between non-sterilized and sterilized soils with respect to the values of fundamental soil properties e.g., total Hg contents, pH, electrical conductivity and organic carbon. Previous studies on a number of different soils with ɤ irradiation dose reaching up to 65 kGy observed no significant changes in soil physical properties (structure, aggregate stability, surface area, particle size distribution) of sterilized soils (Chambers and Attiwill, 1994; Lensi et al., 1991; Rizzuti et al., 1996; Stroetmann et al., 1994; Wolf and Skipper, 1994). I did not find any significant change in total organic carbon between non-sterilized and sterilized soils; however, Marschner and Bredow (2002) reported structural changes in DOC of ɤ irradiated soils (UV absorbance of DOC extracted from irradiated soil samples was about 30% lower than from non-sterile samples) due to enhanced production of UV inactive, non-aromatic sugars, starches and pectins. They also reported that no culturable bacteria were detected in ɤ irradiated soils plated onto nutrient agar plates and incubated for 21 days. From this, it was concluded that ɤ irradiation was completely effective in destroying soil microorganisms. The pH of the sterile soils was compared with that of natural soil and found to be the same (+ 0.1 pH unit) in all cases (Davis, 1975).

Sterilizing soil reduced ($p<0.05$) the percent of total Hg in soil that was converted to Hg(0) by approximately 50%. That is, in sterile soil, the percent of total Hg converted to Hg(0) was only 3.4% (SE=1.4) compared to 6.8% (SE=1.4) in the non-sterile soils. The percent of total Hg converted to Hg(0) was tightly linked to temperature (Figure 4-3) and sterility, with the percentage Hg converted to Hg(0) in non-sterile soils showing a much greater temperature dependence compared to the sterile soils. One can extrapolate from this relationship between

percentage of Hg(0) reduced in soil and temperature that; at 283 K, only 1% of the total Hg would be converted to Hg(0) via abiotic processes, compared to 6.8% by biotic processes (i.e., 7.9% in non-sterile soil – 1% in sterile soil). This difference between sterile and non-sterile soil becomes more pronounced as temperatures increase, with abiotic processes reducing only 2% of total Hg in soil at 293 K compared to biotic processes reducing 11% of total Hg.

The large difference between Hg(0) formation in unsterilized and sterilized soils was likely due to differences in biological Hg reduction which was mediated by soil microbes. The reduction of Hg(II) to the highly volatile Hg(0), a process that may limit the concentration of the substrate for the methylation reaction, is mediated by mercuric reductase (MR) enzyme (Barkay et al., 1989; Barkay et al., 1991). Microbes have been shown to use a pathway to reduce Hg(II) to Hg(0) in contaminated systems through the expression of mercuric reductase genes (Barkay et al., 1989; Van Faassen, 1973). Biologically-induced Hg reduction also has been proposed to play a role in non-contaminated wetland and aquatic ecosystems (Mason et al., 1995; Rolfhus and Fitzgerald, 2004; Siciliano et al., 2002a).

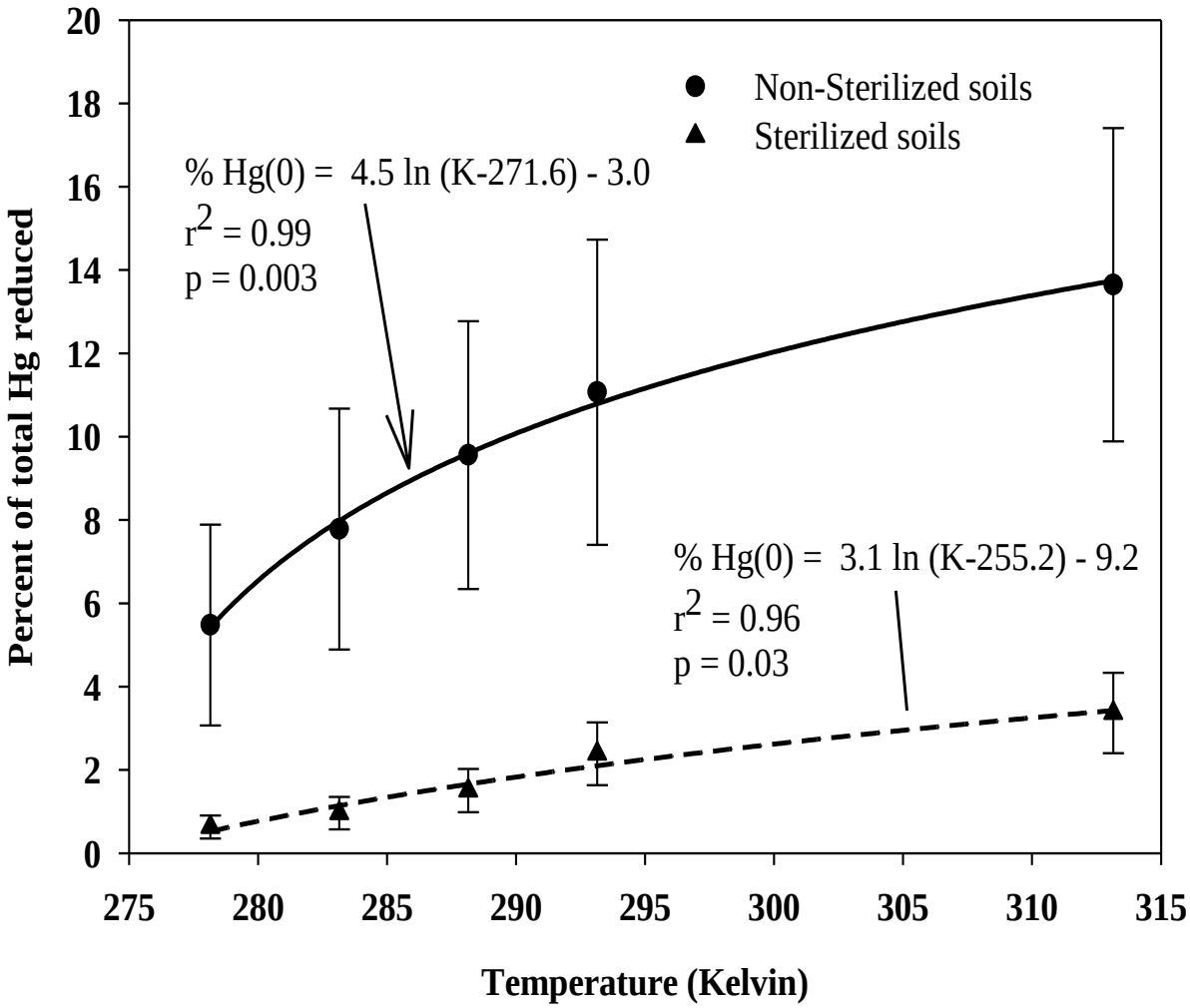

Fig. 4-3. A temperature dependence comparison of percent of total Hg reduced in non-sterile (solid black circles with error bars and solid line) and sterile soils (solid black triangles with error bars and dashed line). The percent of total Hg converted to Hg(0) is tightly linked to soil temperature and sterility with non-sterile soils showing a much greater temperature dependence compared to sterile conditions.

Others also have found that biological processes play a dominant role in Hg(0) emissions from soils (Fritsche et al., 2008). For example, in a laboratory study of deciduous forest soils from New York, USA, Hg emissions of 120 ng $m^{-2} h^{-1}$ in non-sterile soil were reduced by 70% (to 37 ng $m^{-2} h^{-1}$) in ɤ irradiated, sterilized soils at 308 K (Choi and Holsen, 2009). Under outdoor conditions and with a prolonged incubation time, these soils had lower emissions for the biological component but not the abiotic component. That is, Hg emissions were 52 ng $m^{-2} h^{-1}$ in non-sterile soil, but remained essentially unchanged (30 ng $m^{-2} h^{-1}$) in sterile soil at 306 K. Thus, it appears that in these soils the largest source of variance in net Hg(0) emissions was due to the biological component of soil. Another study also reported declines in Hg fluxes following autoclaving (Rogers and Mcfarlane, 1979). Rohlfus and Fitzgerald (2004) suggested that microbial reduction could account for a significant component of the mercury redox cycling [up to 20% of the pool of Hg(0)] in coastal marine systems from temperate zones, although no mechanisms were identified. A recent study from the high Arctic found that microbes expressed diverse merA genes (Poulain et al., 2007a) and that this mer-mediated Hg(II) reduction contributed up to 50% of Hg(0) which could reach a concentration 5-to 10-fold higher than that observed in coastal seawater during the ice-free season. Similarly, the interplay between microbial reduction and oxidation activities with photo-chemical processes controlled the levels of dissolved gaseous mercury in surface water of two lakes in Ontario (Siciliano et al., 2002a).

4.4.3 Relationship of Soil Properties With Temperature Dependence of Hg(0) Formation

The temperature dependence of the percent of mercury reduced in soil was closely linked to soil pH (Figure 4-4).

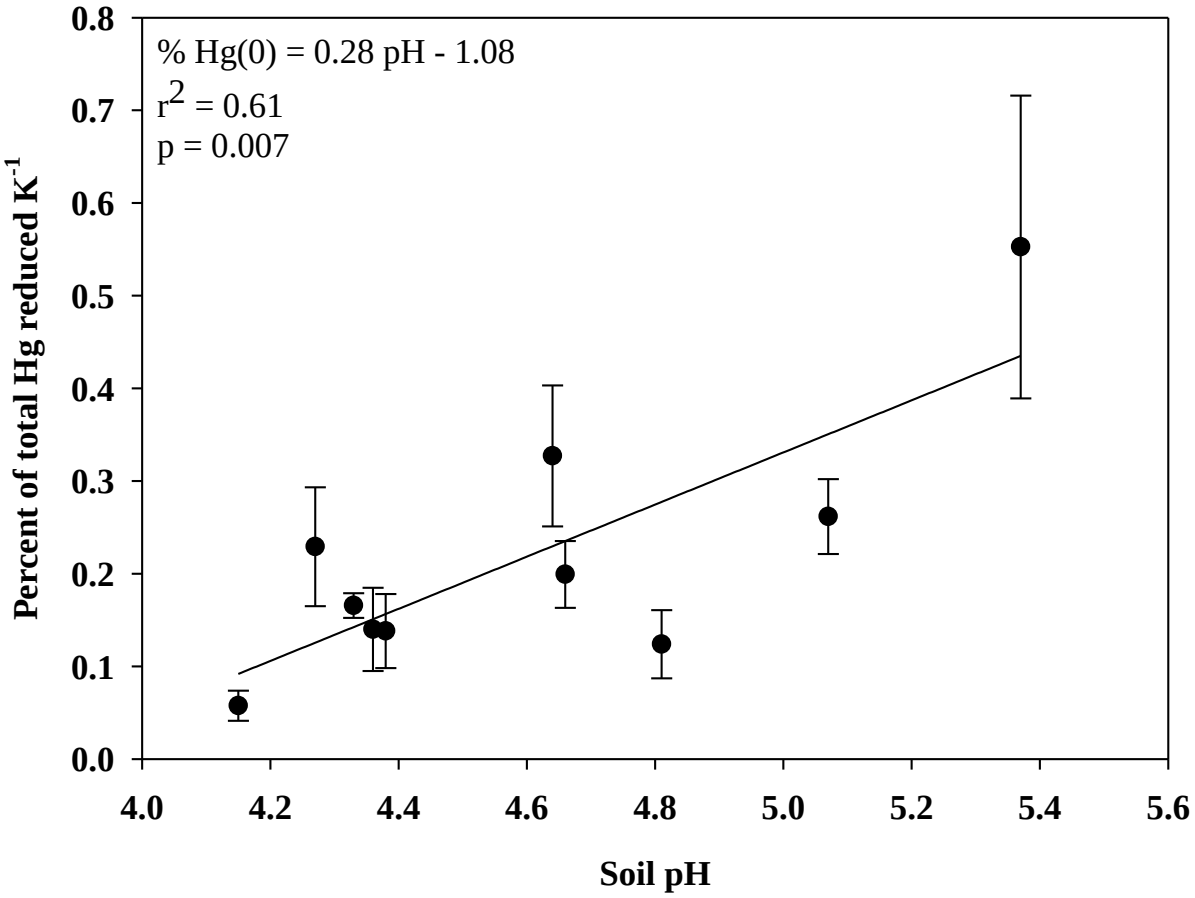

Fig. 4-4. Effect of soil pH on percent of total Hg reduced per Kelvin in studied soils. Each point represent mean of ten soil samples analyzed in three replications and error bars are the standard error of the mean.

Soil pH is found to have a large influence on Hg geochemistry under various conditions due to its strong effect on adsorption, speciation and exchange at the soil-air interface (Gabriel and Williamson, 2004; Semu et al., 1987; Wallschlager et al., 1999). Mercury adsorption generally decreases with decreasing pH, due to competitive binding by H^+ ions. Raising soil pH increases the quantity of negative charges on the exchange complex, which may attract and retain available Hg(II) ions for further biotic/abiotic reduction to Hg(0). Barrow and Cox (1992) found that the effect of pH on Hg(II) reduction rates in an Australian loamy sand under anoxic conditions depends upon the pH-dependent Hg(II) complexation with surface hydroxyl groups onto iron oxide surfaces with adsorption increasing at pH > 4 and decreasing at pH > 7. This adsorption behavior is controlled by the deprotonation of surface functional groups above pH 4, and the formation of neutral $Hg(OH)_2$ aqueous complexes above pH 7 . Wiatrowski et al. (2009) found that between pH 4 and 7, deprotonated surface hydroxyl groups generated negative surface charge and electrostatically attracted Hg(II) cations to adsorption sites at a magnetite-water interface. In another study, mercury evaporation from soils treated with $(CH_3)_2Hg$ was found to be highest for strongly alkaline soils and lowest for slightly acid soils (Schluter, 2000). Furthermore, soil acidity is also known to be an important factor determining the microbial population of a soil (Baath and Anderson, 2003; Lauber et al., 2009) and that microbialy produced organic substances can have different potentials and capacities for the reduction of Hg(II) to Hg(0). It should be noted, however, that my soil samples cover a small range in pH (4.2 to 5.4) and therefore, the pH dependence should be interpreted with caution. Mercury retention in soils is determined by the amount of organic matter present in the soil (Yin et al., 1996). In contrast, I calculated a positive, yet non-significant ($r = 0.50$, $p=0.38$), correlation between cumulative Hg(0) formed in the soil and soil organic carbon content. Obrist (2007) also

suggested that the decomposition of Hg-laden forest plant litter (organic carbon mineralization) in the soil represents a potential pathway for Hg return to the atmosphere. However, in my dataset, I found that soil parameters - alone or in combination - could only weakly predict Hg(0) formation in soils.

4.5 Conclusions

The experimental results presented in this study represent the first controlled systematic observations of rates of Hg(0) formation in soils as affected by soil temperature and sterilization in the samples obtained from remote environments of Nova Scotia, Canada. The results show that the Hg(0) formation in soils increases with increase in soil temperature and both biotic and abiotic mechanisms play an important role. The cumulative Hg(0) formed in natural soils is significantly higher compared to sterilized soils and indicates that microbial processes are a key factor regulating mercury emissions, either directly through microbial reduction or indirectly through microbial by-products (e.g., humic substances) that may stimulate Hg(II) to Hg(0) reduction.

5. QUANTIFYING THE EFFECTS OF SOIL WATER CONTENT ON ELEMENTAL MERCURY FORMATION IN NON-STERILIZED AND STERILIZED BOREAL SOILS

Preface

The previous two chapters showed that the quartz flux chamber precisely and accurately quantifies (Chapter 3) the effects of soil temperature and microbes (Chapter 4) on rates and amounts of Hg(0) formation in soils. This chapter integrates previous research sections and investigates how changes in WFPS and microbes affect Hg reduction process in non-sterilized and sterilized soils.

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Ravinder Pannu planned, developed and the experiment, conducted major field and lab work, reviewed the literature and is primary writer. Nelson J O'Driscoll and Steve Siciliano are co-supervisors and provided experimental and technical guidance, helped in statistical analysis and editing. Andy Rencz provided input relative to national assessment program. John Dalziel provided technical support and troubleshooting of instruments.

5.1 Abstract

Soils are a source of Hg(0) to the atmosphere; however, the effects of soil moisture on both biotic and abiotic Hg(0) formation are not well understood. In terrestrial soils, numerous factors control Hg(0) emissions, but it is still unclear if soil moisture induced biotic processes are important in Hg(0) formation in soils. This research quantifies the effect of varying soil moisture [15-80 percent water filled pore space (WFPS)] and sterilization on the kinetics of Hg(0) formation in soils. Both the cumulative mass of Hg(0) formed in soils (Log cumulative Hg(0) formed = $5e^{(-0.5(x-40)/23.5)^2)}$; $r^2 = 0.77$, $p<0.05$, $n =10$) and the reduction rate constants (k values) ($k = 0.6e^{(-0.5(x-39)/26)^2)}$; $r^2 = 0.64$, $p<0.05$, $n =10$) follow a three parameter Gaussian peak function equation, attain a maximum at 60% WFPS and decreases thereafter. On average, there was 90% ± 4% (n = 5) less cumulative Hg(0) formed in sterilized soils than in non-sterilized soils, highlighting the importance of microbes in the mercury reduction process. The mean percentage of total Hg reduced was larger (6 ± 1%) for non-sterilized soils as compared to sterilized soils (0.4 ± 0.1%). Our results highlight two processes contributing to Hg(0) formation in soil: (i) a fast abiotic process that peaks at 45% WFPS, and which depletes a relatively small pool of Hg(0) and; (ii) a much slower, rate limiting biotic process that generates a large pool of reducible Hg(II).

5.2 Introduction

Mercury is ubiquitous in the environment and can be globally dispersed due to its long (1.5 - 2 years) atmospheric residence time (Lindberg et al., 2007; Munthe et al., 1995). An accurate prediction of global and regional mercury flux is important for predicting the mercury burden of ecosystems. Although the atmosphere receives anthropogenic mercury emissions, the largest

reservoirs of mercury are contained in terrestrial soils, sediments, and waters (Mason, 2009; Selin et al., 2007; Sunderland et al., 2009). Research during the past decade has established the importance of natural soils in mercury cycling, showing that Hg(0) emission from soils contribute substantially (700-3200 Mg yr^{-1}) to the global atmospheric load of Hg (Carpi and Lindberg, 1998; Fitzgerald, 1995; Lindberg et al., 2002; Lindqvist et al., 1991). In order to estimate Hg(0) emissions from terrestrial soils, those processes controlling formation of Hg(0) in soils must be quantified.

Elemental mercury in soil can be produced by abiotic or biotic processes. It is generally thought that most of the mercury emitted from soil originates from the A horizon and is produced by the high microbial activity and abundance of reductants present in this soil horizon (Carpi and Lindberg, 1997; Schluter, 2000). Several field studies correlated various environmental conditions such as soil temperature, solar radiation, wind speed, and precipitation with the mercury emission from soil (Carpi and Lindberg, 1998; Frescholtz and Gustin, 2004; Gustin et al., 2004; Lindberg et al., 1995; Nacht and Gustin, 2004; Poissant and Casimir, 1998). Gustin and Stamenkovic (2005) demonstrated that small additions of water enhanced Hg emissions from dry desert soils and under field conditions, precipitation events increase Hg emission from natural soils (Lindberg et al., 1999; Song and Van Heyst, 2005; Wallschlager et al., 2000). Lindberg et al. (1999) proposed three mechanisms that could be associated with the enhanced release of Hg observed with a precipitation event on dry desert soil: (i) physical displacement of Hg(0) gas in the soil atmosphere by water filling the soil pores; (ii) desorption of Hg(II) bound to the soil and its subsequent reduction to Hg(0); and (iii) replacement of Hg(0) adsorbed to the soil by water molecules. Bouffard and Amyot (2009) observed that Hg(0) can readily adsorb onto a soil surface and remain there; for example, 200 pg of Hg(0) was adsorbed via Van der Walls type

forces onto 1 g of sediment in less than 1 h with maximum adsorption (approximately 85%) taking place in the first 5 min. Thus, there is a pool of Hg(0) available in the soil that can readily desorb into the soil atmosphere and be available for efflux to the atmosphere.

Rising soil water content also can promote the aqueous reduction of Hg(II) to Hg(0) with subsequent emission to atmosphere (Gillis and Miller, 2000b; Johnson and Lindberg, 1995; Song and Van Heyst, 2005). For example, Gustin and Stamenkovic (2005) found that with the addition of water and maintenance of 13% soil water content, mid-day and night-time mean Hg fluxes were double that of dry soils. Frescholtz and Gustin (2004) demonstrated that as the Hg concentration of the substrate was increased (0.01, 6.15, and 25.56 μg Hg g^{-1} soil) by spiking with Hg contaminated mill tailings (500 μg Hg g^{-1}), the amount of Hg(0) released with the addition of water increased. Thus, it appears that not only are physical effects occurring (i.e. Hg(0) displacement), but water also stimulates Hg(0) creation in the soil in some fashion. There are a wide range of aqueous abiotic processes such as reduction of Hg(II) mediated by humic acids, fulvic acids, free radical electrons and sunlight mediated photoreduction that transform Hg(II) in the soil solution to Hg(0) (Gabriel and Williamson, 2004; Schuster, 1991). The abiotic processes can be enhanced in the presence of mixed valence [Fe(II)/Fe(III)] iron oxide minerals and elevated pH (Wiatrowski et al., 2009).

Apart from physically and chemically mediated Hg(0) emission, microbes contribute to the transformation of inorganic and organic Hg(II) in to volatile mercury species, which then quickly evaporate into the atmosphere. Various bacteria strains have been shown to mediate reduction of bioavailable Hg by the mercury reductase enzyme which is encoded by the merA gene (Schluter, 2000). Furthermore, several studies have demonstrated Hg reduction by heterotrophic bacteria (Barkay et al., 2003; Mason et al., 1995; Rolfhus and Fitzgerald, 2004; Siciliano et al., 2002a). In

addition to the bacterial Hg(II) reductase, Devars et al. (2000) found an algae (*Euglena gracilis*) reducing Hg(II) to Hg(0) under dark conditions in a culture medium. Biotic reduction contributes significantly to the Hg flux from natural water as well as soils (Barkay et al., 2003; Gabriel and Williamson, 2004) for example a maximum Hg flux of 120 ng $m^{-2} h^{-1}$ was observed in a non-sterilized forest soil (Hg(II) → Hg(0) reduction due to biotic/abiotic processes combined) compared to 30 ng $m^{-2} h^{-1}$ in its sterilized counterpart (Hg(II) → Hg(0) reduction due to abiotic processes) at soil temperature of 308K under laboratory conditions. Similarly, a Hg flux of 52 ng $m^{-2} h^{-1}$ was observed in bare non-sterilized soil compared to 30 ng $m^{-2} h^{-1}$ in its sterilized counterpart under field conditions and at a soil temperature of 306K (Choi and Holsen, 2009). Fritsche et al. 2008 reported that manipulations of microbial activity by sterilization, followed by glucose addition and re-inoculation lead to consistent, parallel responses of Hg and CO_2 emissions. They reported that experimental sterilization of soils using chloroform fumigation and autoclaving leads to corresponding decreases in both CO_2 and Hg emissions and proposed that Hg emission from terrestrial soils is at least partially controlled by biotic processes. A review on evaporation of Hg from soils (Schlüter, 2000) concludes that microbial activity contributes to Hg evaporation from soils and that reduction of Hg(II) to Hg(0) with subsequent emission from soils may be a combined abiotic and biotic process, with possibly biologically meditated evaporation favored in soils of low Hg content (< 1 µg g^{-1}) and low SOM levels (soil organic carbon < 10 g kg^{-1}). In another laboratory experiment, Rogers and McFarlane (1979) amended soils with high levels (1µg g^{-1}) of mercuric nitrate and reported that autoclaving soil samples leads to a substantial decline in Hg emission, concluding that Hg emission was mediated by microorganisms. Poulain et al. (2007a) observed that the production of Hg(0) is linked not to total Hg(II) in soil but the bioavailable fraction of Hg(II) in soil. Soil properties will not only

influence the bioavailability of Hg(II) but also the rate of microbial transformation and affinity of the soil for Hg(0). Thus biotic processes were found to have a relatively constant influence on the Hg reduction process in soils than the more variable abiotic processes. However, the relative contribution of biotic processes in Hg(0) formation in terrestrial soils is still unclear.

The objectives of the current study were to (i) characterize the effects of water content on rate of abiotic and combined abiotic/biotic Hg(0) formation in soil and; (ii) estimate the proportion of Hg(0) production arising due to biological activity under controlled conditions.

5.3 Methods and Materials

5.3.1 Soil Sampling Locations

Soil samples collected from Kejimkujik National Park (KNP) and Antigonish County (AC), Nova Scotia, Canada were used in this experiment. Kejimkujik is a remote site with extensive mercury research, due to the high concentration of Hg found in loon blood [6-7 µg Hg g^{-1}which is higher than that found in most other areas in North America (1-3 µg Hg g^{-1})] (O'Driscoll et al., 2005). The sites were chosen to represent a range of different soil characteristics. The dominant surrounding land use at these sites is forest of varying ages. All sites represent the typical maritime climate, consisting of the mixed wood forests including coniferous and deciduous tree species (red spruce, sugar maple, paper birch and balsam fir). The region is described as humid to per-humid with potential evapotranspiration often exceeding precipitation from May through August. Summers are cool (~16ºC July mean) with moderate winters (~ -5ºC January mean) and approximately 1000–1600 mm of precipitation falls on the study sites; 15–30% of which occurs as snow. Soil samples were analyzed for organic carbon, pH, EC, total Hg content, and WHC as described in Chapter 4. Five soil samples (K1, A13, A14, A15, and A18) were ɤ irradiated

(Chapter 4) at the Canadian Irradiation Center (CIC), Laval, Québec, Canada to eliminate microbial activity (Thuerig et al., 2009).

5.3.2 Quartz Reaction Chamber System

A quartz beaker reaction system developed in Chapter 3 was used to quantify Hg(0) formation in soil. The quartz beaker system has previously been used for continuous measurement of dissolved gaseous mercury by O'Driscoll et al., (2006) and has been adapted previously for soil flux measurement (Chapter 3). Previous research has shown that there is no significant difference between cumulative Hg formed over a 24-hour analysis period under dry and humid zero air conditions after 24 hour analyses (Chapter 3). Humid air was circulated over the soil sample by passing dry zero air through a Milli-Q water containing bubbler bottle prior to the quartz chamber to maintain uniform moisture levels throughout the experiment.

Concentrations of Hg(0) emitted from each soil sample were measured using a Tekran 2537B mercury analyzer (Tekran Inc., Toronto, Canada). The analyzer operates two independent sampling paths and was set to adsorb Hg(0) onto solid gold bead traps during five-minute time periods, resulting in time resolutions of 5 min (average measurement of the one gold trap). Calibration of the Tekran 2537B was performed with a Tekran 2505 mercury vapor calibration unit and a Hamilton series 700 Microliter™, 25 µL digital syringe. The Tekran 2505 mercury vapor generation unit was allowed to equilibrate for a minimum of 2 hours prior to injections with acceptable error being ±5%. Using the data obtained from seven external injections each of 5, 10, 15 and 20 µL gaseous Hg(0) standards directly into the quartz chamber, the method detection limit (MDL) was determined to be 0.15 ng m^{-3}; recovery of total Hg(0) analyzed 94% ± 2.2% (Zhang, 2007).

A typical soil analyses under dark conditions consisted of initial blanking of the chamber by passing mercury-free humid air through the chamber without soil until zero Hg concentration was detected. Soil (20 g) maintained at 15, 30, 45, 60 and 80 WFPS were uniformly placed at the bottom of the quartz glass beaker in a thin layer. Before analysis, the soil sample was gently tapped in the quartz beaker to achieve a depth of 0.44 cm which represents a bulk density of 1.60 g cm^{-3}. Reactions were allowed to proceed over a 24 hour period while keeping environmental parameters constant (soil temperature; 20°C, radiation, air flow rate; 1Lmin^{-1}). Filter packs (47 mm with 0.2 μm pore size Teflon® filters) were placed after the soil chamber in sampling line to avoid contamination by particles. The system was regularly checked for contamination using a Tekran 1100 zero air generator, and fluxes were only measured when the system was completely free of contamination (i.e., no detectable Hg(0) levels). The samples were not exposed to any detectable levels of UV radiations [UV-B (λ = 280-320 nm, 0 Watts m^{-2}), UV-A (λ = 320-400 nm, 0 Watts m^{-2}) and visible (λ = 400-700 nm, 0.14 Watts m^{-2})] as measured by an Ocean Optics USB 4000 Spectra radiometer with a fiber optic cable (10 m, 200 μm diameter) and spectral diffusion probe (diameter 4.3 mm).

The formation of Hg(0) in soil can be described as a reversible first order reaction, as follows.

$$Hg(II)_{red} + SOM\ (\mathrm{n}e^{-}) \leftrightarrow Hg(0)_{soil} + SOM\ (n-2)e^{-} \qquad (5\text{-}1)$$

Where $Hg(II)_{red}$ is the reducible mercury in soil; SOM is soil organic matter; ne^- is the available electrons sink from SOM; and $Hg(0)_{soil}$ is the Hg(0) formed in soil. I assume that all of the Hg(0) formed in soil is effectively stripped from the soil due to the fast chamber turn-over-time (0.3 min). As such this reaction becomes dominated by the forward reduction reaction (Equation 5-2).

$$Hg(II)_{red} + 2e^{-} \rightarrow Hg(0)_{soil} \qquad (5\text{-}2)$$

The cumulative Hg(0) formed in soil placed in the quartz chamber and the pseudo first order reaction rate for mercury reduction in soil were calculated.

5.3.3 Data Analysis

All data were log transformed prior to statistical analysis as they were not normally distributed (assessed using the Kolmogorov-Smirnov test). Linear regression analyses of measured cumulative Hg(0) formed and k values to a series of variables (e.g., pH, EC, OC, WFPS, soil Hg concentrations) were performed using Minitab 6. This technique is direct gradient analyses that combine aspects of ordination and regression to detect the patterns of variation in data that can be explained best by soil properties. As part of these analyses, a step-wise backward exclusion approach was used to reduce the number of soil variables to those most highly correlated with Hg(0) formation. Analysis of variance (ANOVA) was performed to test the significant differences ($p < 0.05$) and interaction between cumulative mass of Hg(0) formed and reduction rate constants at different WFPS in non-sterilized and sterilized soils.

5.4 Results and Discussion

The soils investigated in this study had low or natural background levels of Hg (total mercury contents ranging 13 to 106 μg kg^{-1}). These concentrations are representative of most uncontaminated soils in North America (< 200 μg kg^{-1}, Kuiken et al., 2008) and globally (Gustin et al., 2008). Soil total organic carbon contents (50 to 65 g kg^{-1}) were similar to typical contents found in Canadian boreal soils (Perie and Ouimet, 2008).

5.4.1 Effect of Soil Sterilization on Hg(0) Formation in Soils

No significant ($p<0.05$) differences were observed between non-sterilized and sterilized soils with respect to fundamental soil properties e.g., pH, EC, OC and total Hg contents after γ irradiation (data not shown). Mass of cumulative Hg(0) formed and the Hg reduction rate constant were related to increasing WFPS in the soils studied (Figure 5-1).

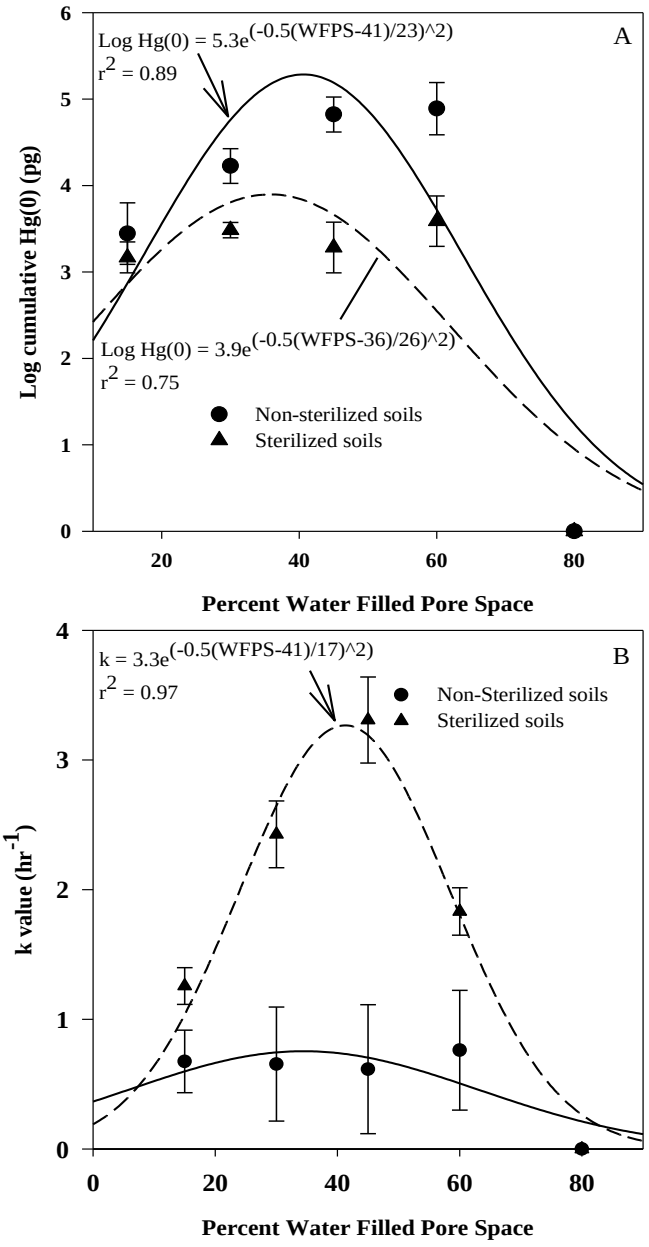

Fig. 5-1. Cumulative Hg (pg) formed (A) and k values (B) in non-sterilized (solid black circles, n = 5) and sterilized soils (solid black triangles, n = 5) over 24 hour analysis period as affected by increasing percent water filled pore space (WFPS) in the studied soils. Each point represents the mean of 5 soil samples analyzed in three replications and error bars represent standard error of the mean.

The WFPS in soils is a useful, simple, and reliable indicator of the relative potential for aerobic and anaerobic microbial activity in soil. Previous researchers have demonstrated that soil respiration correlates well with the soil matric potential with soil respiration rates generally increasing with increasing soil moisture (Carbone et al., 2011; Davidson et al., 2000; Moyano et al., 2011). Carbone et al. (2011) suggested that the optimum water content is usually somewhere near field capacity, since the macropore spaces are mostly air-filled, thus facilitating O_2 diffusion. In addition, the micropore spaces are mostly water-filled, thus facilitating diffusion of soluble substrates. The results of many studies, involving a wide range of soil types, indicate that a soil water content equivalent to 60% of a soil's water holding capacity delineates the point of maximum aerobic microbial activity (Breuer et al., 2002; Kiese and Butterbach-Bahl, 2002; Linn and Doran, 1984). A silty clay loam incubated in the laboratory at 60% WFPS supported maximum aerobic microbial activity as determined by CO_2 production and O_2 uptake (Linn and Doran, 1984). Similarly, N_2O and CO_2 production from a silt loam soil under no-tillage field conditions (0 -7.5 cm depth) in Kentucky, USA, was highly correlated with % WFP ($r= 0.90$, $p < 0.001$), increasing at water contents up to 60% WFPS. In an another field study from a tropical rain forest in Australia, Breuer et al. (2002) found a clear trend of rising N_2O emissions from dry to wet season (3.6 to 80.4 µg N_2O-N $m^{-2}h^{-1}$) with maximum N_2O emissions occurring between 56-62% WFPS and decreasing thereafter. They proposed the decline in nitrification with increasing WFPS due to appearance or extension of anaerobic microsites because of restricted O_2 diffusion into the soil at higher soil moisture contents. Kiese and Butterbach-Bahl (2002) observed a linear relationship between N_2O emissions and changes in soil moisture for values of WFPS <50 – 60%. For values of WFPS > 50-60%, a decrease in N_2O emissions rates was observed which is in good agreement with the observations by Breuer et al. (2002). Similarly, a

negative correlation between soil CO_2 emissions and soil moisture was observed for values exceeding 50-60% WFPS indicating that at values above of 60%, O_2 diffusion is limited in the soil matrix which in turn limits heterotrophic respiration and favours anaerobic denitrification. Thus, soil aeration is a major factor limiting microbial activity above 60% WFPS with obligate aerobic processes declining most rapidly with increasing soil water contents. Gillis and Miller (2000b) found increased Hg emissions when approximately two-thirds of the pore spaces were filled with water but no increase was noticed as the soil reached near saturation in a low mercury containing soil.

My research matches well with the previous work and in my case, the maximum mass of cumulative Hg(0) was formed at 60% WFPS (1083 to 283985 pg, $\bar{x}$: 83031 pg), the lowest at 15% WFPS (136 – 39321 pg, $\bar{x}$: 5206 pg) while no Hg (0) formation was observed at 80% WFPS (99% data points were absolute zeros and 1% observed Hg(0) values were below MDL, 0.15 ng m^{-3}) (Table 5-1). This increase is similar to other reported values. For example, Gustin and Stamenkovic (2005) observed an increase of 2-to 5-times in Hg flux for low Hg soil (20 ng g^{-1}) in controlled laboratory watering experiments. A field study of background Hg substrates (10 ± 5 ng g^{-1}) in Hungry Valley, Nevada, found a threefold increase in Hg flux from November (daytime mean = 0.6 ± 0.4 ng m^{-2} h^{-1}, 2.8% soil moisture) to March (1.5 ± 0.7 ng m^{-2} h^{-1}, 8.4% soil moisture) under slightly higher temperatures and higher soil moisture (Gustin et al., 2006). In contrast to my findings, Moore and Castro (2012) did not observe any correlation between soil total gaseous mercury (TGM) concentrations and soil moisture in forested and grass land sites in Maryland, USA. It should be noted that very short sampling times (1.5 to 3 hour) were used in the Moore and Castro (2012) study, and that soil moisture induced biotic/abiotic changes in the soil TGM concentrations were undetectable under such short sampling periods.

Table 5-1. Cumulative Hg(0) formed (pg) in non-sterilized soils at increasing WFPS.

Soil Label	Percent Water Filled Pore Space (WFPS)				
	-- 15 --	-- 30 --	-- 45 --	-- 60 --	-- 80 --
K1	39321 ± 2709	77264 ± 9295	198118 ± 35467	242268 ± 92791	0‡
K2	653 ± 263	2361± 347	675 ± 94	0‡	0‡
K5	395 ± 31	2081 ± 143	2170 ± 254	2645 ± 328	0‡
K7	571 ± 102	1895 ± 104	2704 ± 154	2564 ± 220	0‡
A11	255 ± 39	1361 ± 32	4718 ± 557	4705 ± 613	0‡
A12	136 ± 15	595 ± 49	1041 ± 123	1083 ± 173	0‡
A13	1342 ± 178	7844 ± 800	28410 ± 2460	8171 ± 951	0‡
A14	888 ± 44	11968 ± 279	173798 ± 1800	283985 ± 36521	0‡
A15	8065 ± 148	29228 ± 1639	67233 ± 589	173203 ± 9627	0‡
A18	436 ± 22	6337 ± 201	19518 ± 844	28651 ± 1958	0‡

† ± indicates SD
‡indicates Hg(0) value below MDL (0.15 ng m^{-3})

Eckley et al. (2011) assessed the effect of watering on mercury enriched mining substrates and found a 2 to 17 fold increase in Hg flux under moist conditions. However, rewetting these substrates to 20% soil water content shortly after they had dried resulted in a smaller increase in emissions compared to the initial wetting. The increase in flux with rewetting of the tailings suggests that the wetting process may have facilitated reduction of Hg(II) to Hg(0) in the soil water as suggested by others (Gustin et al., 2006). In contrast, a laboratory study with a Hg enriched loamy sand soil, found that if the soil water content is above 15%, the addition of more water does not enhance the Hg(0) emissions after a simulated rainfall event (Song and Van Heyst, 2005). In a laboratory study on the kinetics of the Hg emission from the contaminated soils of the Idrija Hg-mine region of Slovenia, Kocman and Horvat (2010) observed that the overall Hg flux response to simulated environmental conditions (radiation, soil temperature and moisture) depends not only on the amount but also the type or form of Hg species and their binding sites in the soils. The soil aqueous phase was found to be responsible for recharging the pool of Hg in the soil available for both the light and thermally induced flux.

The cumulative Hg(0) formed over 24-hours followed an exponential curve rising to a maximum peaking between 1 to 5 hours and achieving a stable plateau between 5 to 10 hours for all sterile and non-sterile soils at all moisture levels tested (Figure 5-2). Despite this, only a small fraction of the total Hg is converted to Hg(0) at each moisture level (0.3 ± 0.2% at 15%WFPS, 1 ± 0.4% at 30%WFPS, 4 ± 2% at 45%WFPS and 7 ± 3% at 60%WFPS). The apparent pseudo first-order exponential rise to maximum provided an excellent curve fit (r^2 values ranging from 0.8 to 0.9, $p < 0.0001$) and is in agreement with the exponential relationship reported by other researchers (Bahlmann et al., 2006; Gillis and Miller, 2000b; Gustin and Stamenkovic, 2005; Schluter, 2000).

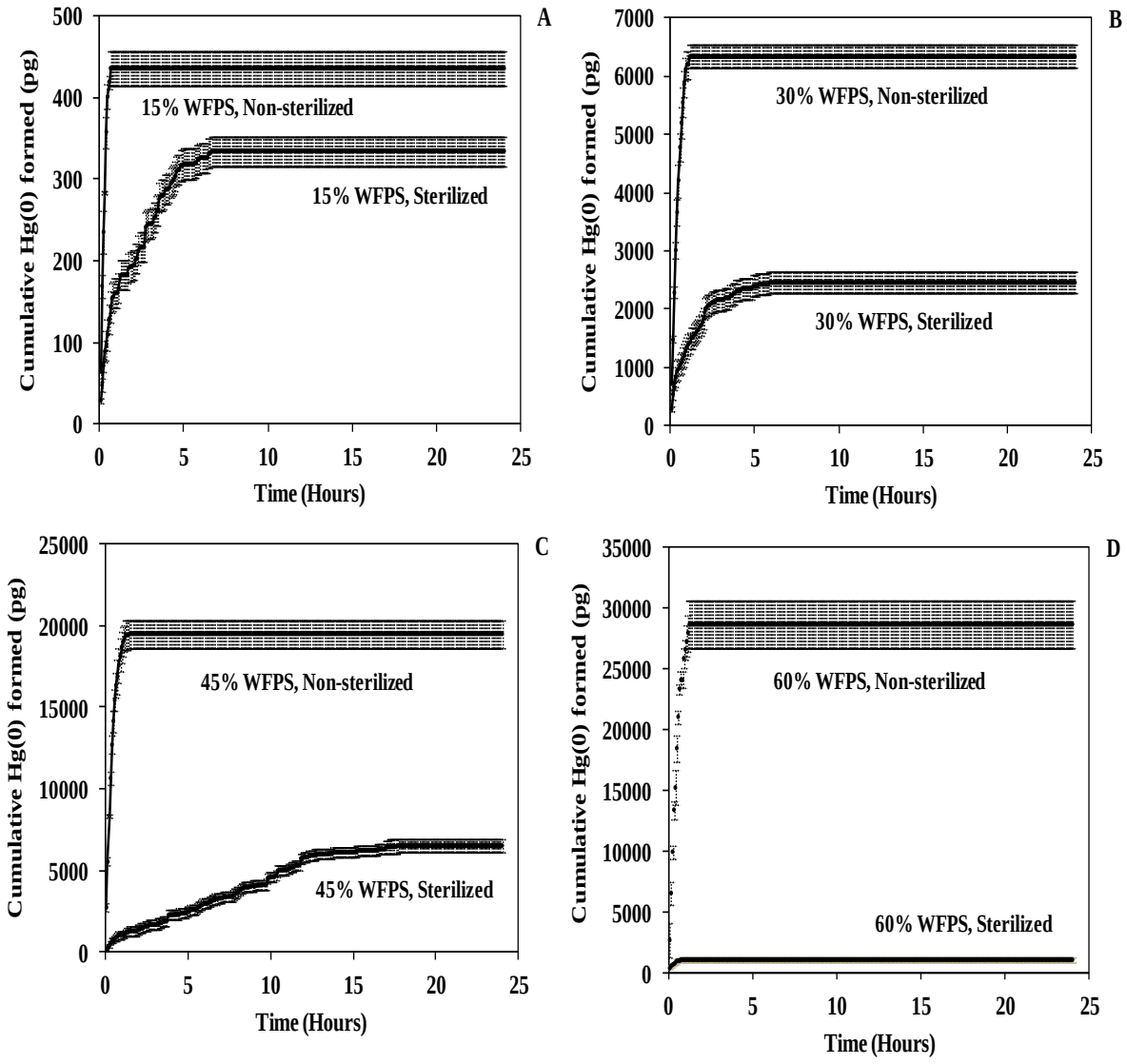

Fig. 5-2. Comparison of mass of cumulative Hg(0) formed in a soil (A18) at 15 (A), 30 (B), 45 (C) and 60 (D) WFPS respectively. The solid black line (mean of three replicates) both in non-sterilized (A18) and sterilized soils (A18) indicates the exponential rise to maximum trend of Hg(0) production over 24 hours analysis period and the vertical dark grey lines represent associated error on the mean. Results at 80% WFPS are not shown because they were below the detection limit.

The percent of total Hg in soil converted to Hg(0) was closely linked to WFPS under non-sterile conditions with a steady exponential rise to a maximum between 45 and 60% WFPS (Figure 5-3). In contrast, under sterile conditions, much less total Hg in soil was converted to Hg(0) and this conversion peaked at 45% WFPS. On average, 0.4 ± 0.1% of total Hg content was reduced in the sterilized soils compared to 6 ± 1% in non-sterilized soils. This difference between sterile and non-sterile soil became more pronounced as the WFPS increased, with abiotic processes reducing only 0.6% of total Hg in soil at 60 WFPS compared to biotic processes reducing 10% of total Hg (Figure 5-4).

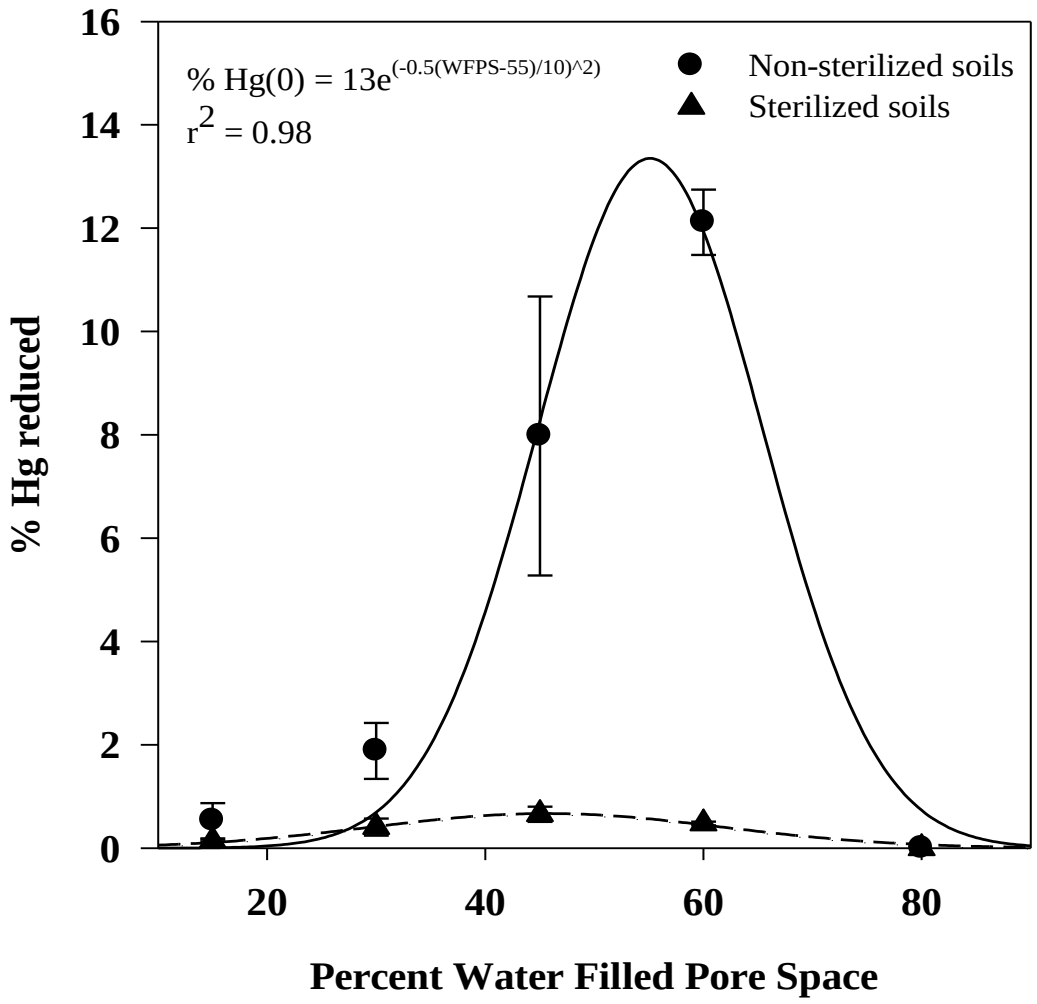

Fig. 5-3. The moisture dependence comparison of % of total Hg reduced in non-sterile (solid black circles with error bars) and sterile soils (solid black triangles with error bars). The % of total Hg converted to Hg(0) is linked to soil moisture and sterility with non-sterile soils showing a much greater moisture dependence compared to sterile conditions. Each point represents a mean of 5 different soils analyzed over 24 hour period in triplicates. The vertical bars indicate the standard error of the mean.

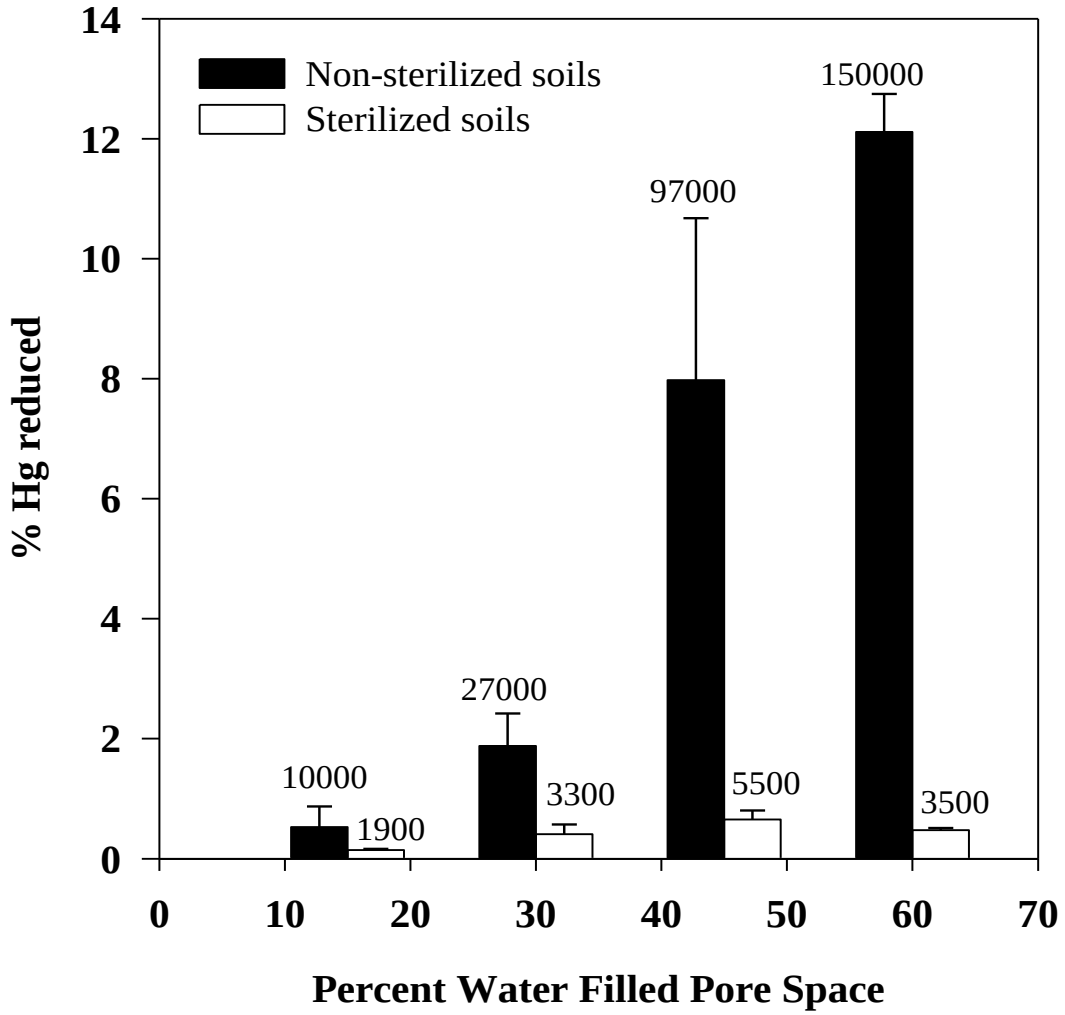

Fig. 5-4. The percent of total Hg reduced to Hg(0) in non-sterilized (Black bars) and sterilized soils (Hollow bars) at increasing WFPS in soils is compared. Each bar represents a mean of 5 different soils analyzed over 24 hour period in triplicates. The average amount of total Hg in these soils was 67 ng g^{-1} and their organic carbon (OC) content varied between 5 and 37 g kg^{-1} soil with an average OC of 30 g kg^{-1} soil. The figures above each bar indicate the cumulative mass of Hg(0)(pg) formed at respective WFPS and the vertical bars indicate the standard error of the mean.

5.4.2 Relationship of Soil Properties With Moisture Dependence of Hg(0) Formation

Only one soil parameter, EC, was a significant predictor of how the $Hg(0)_{cumulative}$ was related to soil moisture in 10 boreal forest soils studied here: $Log(Hg(0)_{cumulative}) = 0.176 + 0.88\ \ell n(WFPS) + 0.0067$ EC, n=10, $r^2=0.44$, $p<0.01$. Other soil parameters such as total Hg, OM or pH did not significantly influence the effects of soil moisture on $Hg(0)_{cumulative}$. Electrical conductivity is really an indicator of the amount of conductive ionic species (i.e. salt) in soil, and ionic species such as S^- and Cl^- ions, are well known to influence Hg speciation and adsorption to soil particles (He et al., 2007). It is surprising that background Hg levels were not found to be a key parameter, but my soils contained low levels of total mercury, and thus, the Hg concentration gradient may not have been sufficient to detect a relationship. Others have occasionally observed a link between soil Hg levels and Hg emission water dependence; for example, Frescholtz and Gustin (2004) demonstrated for one soil type, that as the Hg concentration of the substrate was increased, the amount of Hg released with the addition of water increased.

By manipulating soil moisture under sterile and non-sterile conditions, I was able to refine the conceptual models proposed by Gustin et al. (2006) and Schluter (2000). In essence, these researchers concluded that soil moisture drives adsorbed Hg(0) into the soil air and also moves adsorbed Hg(II) into the soil water, where it can be reduced to Hg(0), thus providing additional Hg(0) for flux to the atmosphere. These researchers also indicated that microbial activity likely plays a role in this process. Others have confirmed that there are not only detoxification pathways (Barkay et al., 1989; Barkay et al., 1991) that lead to Hg(0) formation but also that Hg emission is linked to soil respiration (Fritsche et al., 2008a; Rogers and Mcfarlane, 1979). Here I propose that the formation of Hg(0) in soil can be considered a two-step process:

STEP 1: $Hg(II)_{non-reducible} \leftrightarrow Hg(II)_{red}$

STEP 2: $Hg(II)_{red} + SOM\ (ne^-) \leftrightarrow Hg(0)_{soil} + SOM\ (n-2)e^-$

Under the conditions in my reaction vessels, where $Hg(0)_{soil}$ is immediately stripped, Steps 1 & 2 can be considered irreversible. Step 1 is mediated by soil microorganisms in some fashion and has a rate-limiting, apparent pseudo-first order rate constant of 0.66 h^{-1} (SE=0.10, n=40). Step 2 is largely an abiotic process and has an apparent pseudo-first order rate constant of 2.25 h^{-1} (SE=0.56, n=18). The assignment of two steps is necessary to explain why, under non-sterile conditions, the total $Hg(II)_{reducible}$ in soils increases with increasing soil moisture whereas under sterile-conditions, there is no increase in $Hg(II)_{reducible}$ with increasing soil moisture. I postulate that the increasing soil moisture stimulates microbial respiration which increases the pool of reducible Hg(II) that can be rapidly converted to Hg(0) by a fast abiotic process. However, this increase in soil respiration does not increase the rate of Hg(0) formation because it is likely that this Hg(0) formation is due to a by-product of microbial metabolism. Thus, using Occam's razor, I suggest that microbial activity only occurs in Step 1, and not Step 2.

However, my results do not rule out a direct microbial role in Step 2. It is possible that microbes play a direct role in Hg(II) reduction in terrestrial soils and consequent Hg(0) soil evaporation. Microbes have been shown to use a pathway to reduce Hg(II) to Hg(0) in contaminated systems through the expression of mercuric reductase genes (Barkay et al., 1989; Barkay et al., 1991; Van Faassen, 1973). Biologically-induced Hg reduction also has been proposed to play a significant role in non-contaminated wetland and aquatic ecosystems e.g., (Mason et al., 1995; Rolfhus and Fitzgerald, 2004; Siciliano et al., 2002a). Barkay et al. (1989) found that mercury-resistant microorganisms, isolated from lake and estuarine water by culturing at µM Hg

concentrations, were able to reduce Hg at rates of 1 to 10% per hour. This study showed that most of the biotic reduction in the isolate from the estuary was due to bacteria (the isolation procedure killed the eukaryotes in the sample) and that other pathways beside the mer genes were involved. Moreover, chemical modeling of Hg cycling in the high Arctic suggested that MerA mediated reduction could account for 90% of Hg(0) production at depth where photoreduction could not take place owing to reduced light penetration (Poulain et al., 2007).

5.4.3 Conclusions

This controlled laboratory research using low Hg containing ambient terrestrial soils confirms the key role played by soil moisture in influencing gaseous mercury emissions at the soil-air interphase. It appears that soil moisture stimulates microbes to increase the size of the reducible pool of Hg(II) in soil. Coupled with the effect of increased soil moisture on Hg(0) flux out of the soil, rainfall can be seen to be a key driver of Hg emissions from soil. The microbial population that would recover from such rainfall events might generate heavy Hg(0) pulses and could affect the long-term Hg emission budgets considerably.

6. OVERALL CONCLUSIONS

The development of controlled chambers and an inter-comparison of methods for studying soil Hg reduction kinetics addresses an important research need to better quantify the effects of environmental variables on Hg reduction process. I succeeded in developing a simple, portable and accurate laboratory quartz flux chamber system that can be used to precisely and accurately measure the effect of different environmental parameters on Hg reduction kinetics (Chapter 3). Using this system, the effect of soil temperature, soil moisture and biotic factors on Hg reduction kinetics was isolated by varying one parameter of interest while keeping others constant (Chapter 4 and 5). Based on my laboratory tests, I am confident that new system can be used to study Hg reduction dynamics in a wide range of natural soils. This system has several advantages and strengths. It allows one to measure Hg(0) emissions from a small amount of soil, it is portable and can be used under both lab and field conditions, eliminates sample/container interaction and blanking problems, and has a low detection limit.

Using the quartz flux chamber system, the effect of soil temperature (Chapter 4), water content (Chapter 5), and sterilization on (i) the rate of abiotic and combined abiotic/biotic Hg(0) formation in soils and (ii) the proportion of Hg(0) production arising due to biological activity in the low Hg containing background soils was investigated. Both in the non-sterilized as well as sterilized soils, the cumulative Hg(0) formed (and the apparent pseudo-first order rate constant) increased linearly with increasing soil temperature. The cumulative Hg(0) formed in natural soils was significantly greater than that in sterilized soils, indicating that microbial processes are a key factor regulating mercury emissions.

The mass of cumulative Hg(0) formed, as well as the k values, were found to increase with increasing WFPS both in non-sterilized and sterilized soils (Chapter 5). The difference in Hg(0) formation between 15 and 60% WFPS (unsaturated, high percentage of air filled soil pore spaces, aerobic conditions) and 80%WFPS (partially saturated, high percentage of water filled soil pore spaces, anaerobic conditions) suggests that the soils harbored different types of bacteria which are responsible for Hg(II) → Hg(0) reduction. Bacteria in the 15-60% WFPS range primarily reduced soil Hg(II) resulting in high Hg(0) formation whereas at 80% WFPS, anaerobic bacteria dominate the system resulting in low Hg(0) formation. Comparing Hg(0) formation in sterilized and unsterilized forested soils indicates that the biotic process had a relatively constant and larger influence on reduction of Hg, and the biotic process dominated the reduction of Hg in these experiments.

A limitation of my work is that the experiments were conducted under controlled conditions in the laboratory only; hence, these experiments need to be confirmed under field conditions. A question that follows from this work is that what kinds of microbes dominate the stimulation of Hg reduction in soils. I propose that aerobic microorganisms may dominate; however, the specific bacteria involved have yet to be determined. The proper identification, population biology and quantification of Hg reducing bacteria in soils are largely unknown. A final question relates to the mechanisms by which temperature, moisture and bacteria, induce a Hg flux from the soil. I believe that the soil moisture and temperature affect the abiotic/biotic reduction of divalent mercury, Hg(II) to elemental mercury, Hg(0) in soil; additional research on the role of other environmental variables important to this process, and the mechanisms involved in the emission of mercury are necessary to further understand this important phenomenon.

In conclusion, my results confirm the key role played by soil temperature, moisture and microbes in the production of Hg(0) in natural forested soils of Atlantic Canada. Recent predictions (Change, 2007) estimate that the global surface temperature will increase approximately 1.5 to 4 °C over the next century. Thus, these results suggest that the effects of climate change on Hg(0) emissions from the low mercury containing soils will affect the global atmospheric Hg(0) levels. These results also are in agreement with previous studies conducted in the North America.

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8. APPENDIX

Table A-1. Cumulative Hg(0) formed (pg) at increasing soil temperature (Kelvin).

Soil Label	Soil Temperature (K)				
	-- 278 --	-- 283 --	-- 288 --	-- 293 --	-- 303 --
K1	501447 ± 13117	577501 ± 46135	617370 ± 9633	701053 ± 10717	754733 ± 50657
K2	90470 ± 3188	157255 ± 3331	168015 ± 8667	176579 ± 3446	214038 ± 7865
K5	14035 ± 520	29560 ± 1630	47552 ± 383	66017 ± 4056	73768 ± 1009
K7	2798 ± 159	3464 ± 233	4916 ± 500	17120 ± 855	89266 ± 957
A11	1546 ± 200	3791 ± 331	6511 ± 395	11444 ± 420	12809 ± 918
A12	69083 ± 4854	83451 ± 4211	117527 ± 8761	120373 ± 9510	192744 ± 3294
A13	29185 ± 3907	37312 ± 3515	64020 ± 921	85167 ± 5460	95537 ± 7771
A14	140026 ± 15454	204647 ± 27688	265374 ± 10721	315117 ± 7412	348061 ± 27546
A15	20379 ± 1084	59604 ± 1657	111979 ± 4208	132171 ± 7119	203652 ± 4884
A18	11923 ± 2606	24338 ± 1539	27051 ± 2327	26558 ± 3424	36933 ± 4211

† ± indicates Standard Deviation

Table A-2. Pseudo First-order rate constants (k, h^{-1}) for Hg(0) formation with soil temperature.

Soil Label	Soil Temperature (K)				
	-- 278 --	-- 283 --	-- 288 --	-- 293 --	-- 303 --
K1	0.233 ± 0.008	0.313 ± 0.012	0.468 ± 0.034	0.579 ± 0.016	1.097 ± 0.043
K2	0.251 ± 0.004	0.236 ± 0.008	0.206 ± 0.008	0.270 ± 0.026	0.633 ± 0.042
K5	0.145 ± 0.020	0.231 ± 0.007	0.285 ± 0.005	0.400 ± 0.022	0.699 ± 0.007
K7	0.313 ± 0.016	0.294 ± 0.030	0.430 ± 0.041	0.580 ± 0.027	0.824 ± 0.094
A11	0.233 ± 0.008	0.508 ± 0.092	0.816 ± 0.028	1.355 ± 0.042	1.148 ± 0.008
A12	0.256 ± 0.010	0.207 ± 0.008	0.179 ± 0.006	0.224 ± 0.013	0.412 ± 0.011
A13	0.104 ± 0.007	0.178 ± 0.067	0.243 ± 0.017	0.429 ± 0.029	0.627 ± 0.030
A14	0.016 ± 0.001	0.019 ± 0.002	0.028 ± 0.003	0.030 ± 0.001	0.039 ± 0.002
A15	0.133 ± 0.002	0.168 ± 0.015	0.183 ± 0.018	0.194 ± 0.020	0.464 ± 0.013
A18	0.421 ± 0.036	0.472 ± 0.066	0.660 ± 0.053	0.548 ± 0.056	1.333 ± 0.258

† ± indicates Standard Deviation

Table A-3. Activation energies (E_a, kJ mol^{-1}) of Hg(0) formation in non-sterilized and sterilized soils.

Soil Label	-- Non-sterilized soils --			-- Sterilized soil --		
	Slope	r^2	E_a	Slope	r^2	E_a
K1	-5221 ± 453	0.98	43.4 ± 2.2	-	-	-
K2	-3540 ± 229	0.81	29.4 ± 1.1	-4335 ± 256	0.88	36.0 ± 1.2
K5	-5212 ± 546	0.97	43.3 ± 2.6	-3969 ± 305	0.93	33.0 ± 1.4
K7	-3636 ± 832	0.87	30.2 ± 4.0	-5272 ± 101	0.74	43.8 ± 0.5
A11	-5476 ± 372	0.74	45.5 ± 1.8	-4697 ± 542	0.83	39.1 ± 2.6
A12	-1721 ± 136	0.38	14.2 ± 0.7	-2446 ± 292	0.97	20.3 ± 1.4
A13	-6668 ± 149	0.89	55.4 ± 0.7	-	-	-
A14	-3135 ± 95	0.95	26.1 ± 0.5	-	-	-
A15	-3905 ± 269	0.87	32.5 ± 1.3	-	-	-
A18	-3859 ± 343	0.83	32.1 ± 1.6	-	-	-

† ± indicates Standard Deviation

Table A-4. Pseudo First-order rate constants (k, h^{-1}) of Hg(0) formation in non-sterilized soils at increasing WFPS.

Soil Label	Percent Water Filled Pore Space (WFPS)				
	-- 15 --	-- 30 --	-- 45 --	-- 60 --	-- 80 --
K1	0.13 ± 0.01	0.11 ± 0.02	0.19 ± 0.02	0.12 ± 0.03	0
K2	0.48 ± 0.15	0.32 ± 0.02	1.59 ± 0.41	0	0
K5	0.44 ± 0.13	0.44 ± 0.11	0.58 ± 0.12	1.1 ± 0.1	0
K7	0.62 ± 0.14	0.7 ± 0.04	0.57 ± 0.01	1.23 ± 0.15	0
A11	0.7 ± 0.05	0.54 ± 0.02	0.41 ± 0.01	0.44 ± 0.04	0
A12	0.57 ± 0.07	0.46 ± 0.02	0.68 ± 0.08	0.30 ± 0.08	0
A13	1.23 ± 0.1	0.22 ± 0.01	0.2 ± 0.03	1.28 ± 0.26	0
A14	0.44 ± 0.37	0.46 ± 0.08	0.02 ± 0.001	0.02 ± 0.002	0
A15	0.3 ± 0.02	0.09 ± 0.01	0.06 ± 0.01	0.03 ± 0.003	0
A18	1.28 ± 0.3	2.39 ± 0.13	2.6 ± 0.2	2.35 ± 0.4	0

† ± indicates Standard Deviation

Table A-5. Cumulative Hg(0) formed (pg) in sterilized soils at increasing WFPS.

Soil Label	Percent Water Filled Pore Space (WFPS)				
	-- 15 --	-- 30 --	-- 45 --	-- 60 --	-- 80 --
K1	2427 ± 172	2951 ± 819	5340 ± 1170	10129 ± 1384	0‡
A13	1227 ± 298	3170 ± 355	205 ± 24	0‡	0‡
A14	1884 ± 582	6282 ± 1845	4587 ± 1215	5372 ± 550	0‡
A15	3685 ± 360	1818 ± 143	788 ± 63	0‡	0‡
A18	335 ± 18	2458 ± 184	6518 ± 437	1062 ± 162	0‡

† ± indicates Standard Deviation
‡ indicates Hg(0) value below MDL (0.15 ng m^{-3})

Table A-6. Pseudo First-order rate constants (k, h^{-1}) of Hg(0) formation in sterilized soils at increasing WFPS.

Soil Label	Percent Water Filled Pore Space (WFPS)				
	-- 15 --	-- 30 --	-- 45 --	-- 60 --	-- 80 --
K1	3.19 ± 0.51	5.71 ± 0.1	3.40 ± 0.8	1.29 ± 0.2	0
A13	0.75 ± 0.4	0.41 ± 0.05	2.91 ± 0.6	0	0
A14	1.21 ± 0.09	0.38 ± 0.07	0.51 ± 0.05	0.82 ± 0.01	0
A15	0.62 ± 0.1	4.87 ± 0.5	9.59 ± 1.8	0	0
A18	0.51 ± 0.03	0.76 ± 0.06	0.13 ± 0.02	3.39 ± 0.2	0

† ± indicates Standard Deviation