ABSTRACT

Title of Document: INTERACTIONS BETWEEN NATURAL ORGANIC MATTER COMPOSITION AND MERCURY TRANSPORT IN A BOREAL WATERSHED

Sarah Madelaine Ghorpade, Master of Science, 2010

Directed by: Dr. Andrew Heyes, University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory

Natural organic matter (NOM) composition affecting mercury (Hg) transport along a watershed transect were examined in the Lake 658 system at the Experimental Lakes Area, Canada. This watershed was dosed with an enriched stable isotope of Hg, allowing for distinction between recently deposited and historic Hg. Newly deposited Hg was not detected in significant quantities in upland flow or the lake, and occurred only in upper soil horizons, indicating that Hg has not reached steady state 8 years following deposition. Characterization of dissolved phase NOM was conducted by molecular weight fractionation, and analysis of spectral properties and lignin phenols. Low molecular weight compounds were more mobile in the upland, while high molecular weight fractions contained more Hg. Spectral properties were not consistent predictors of Hg, but supported findings on molecular weight distribution. Source material composition, as indicated by lignin phenols, did not vary widely and was not correlated with Hg.

INTERACTIONS BETWEEN NATURAL ORGANIC MATTER QUALITIES AND MERCURY TRANSPORT IN A BOREAL WATERSHED

By

Sarah Madelaine Ghorpade

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2010

Advisory Committee:
Dr. Andrew Heyes, Chair
Dr. Cynthia C. Gilmour
Dr. H. Rodger Harvey

Dedication

This work is dedicated to
Shri Mataji Nirmala Devi

Acknowledgements

I would first and foremost like to thank my advisor, Dr. Andrew Heyes, for guidance and support on every aspect of this project. You have been an engaged advisor and patient teacher and I especially appreciate the enjoyable work environment you create. I would also like to thank my committee members, Dr. Rodger Harvey, for assistance in the lab, guidance at various stages of lignin processing and advice on all things organic, and Dr. Cindy Gilmour for valuable insight and scientific perspective on this work throughout the project and especially for help with editing.

I would like to thank everyone in the Heyes lab: firstly, Lizzy Jenny, my labmate, roommate, officemate, fieldmate, teammate, travel mate, and dear friend. I cannot thank you enough for everything you have done for me personally, and in various ways to support the project throughout. Thanks to Laura Lockard for being such a good listener, phenomenal impressionist and great friend along this long and sometimes arduous journey of ours :) Thanks to Emily Stefansson for being a great hydrology partner. I would also like to thank the friends I have made in Solomons: special thanks to Kyle Matthew for being a great roommate, a generous friend and for putting up with a lot of lab/girl talk around the house, to my neighbors Mariana, Todd, Eli, Jason, Kathleen, Alison and Tammy and my cohorts/writing buddies Allison and Katherine.

There are many people to thank who contributed to acquiring, analyzing and processing data for this project. I would like to thank Kathy Wood and Nancy Kaumeyer in Analytical Services, Georgia Riedel, Tyler Bell and Taylan Morcol at SERC, and Dr. Craig Allan for sample analysis, Dr. Antonio Mannino, Dr. Rob Spencer and Dr. Roman Teisserenc for lignin advice and materials, Dr. Lisa Wainger for GIS help, Dr. Carrie Miller for insight on centrifuging methods, Dr. Jenny Graydon for GIS data, general mercury talk, and for giving me the fantastic opportunity to work at ELA all those years ago, and Dr. Vince St. Louis, Dr. Brian Branfireuen and Claire Oswald for providing LiDAR data. A special thank you to Rachel Pleuthner and Karen Taylor for a huge amount of help in the Harvey lab. I would also like to thank all the folks at ELA, especially Ken Sandilands, Madison Hornbeck, Justin Shead and Lynn Dupuis for help with the project, Lee Hrenchuk and Adam and Shantall McFee for their generosity and hospitality in Winnipeg, and Natalia Waldner for oh so many things. A special thank you to Claire Oswald for field help, lab help, thoughts on the project, insightful discussions and for being a great mentor and friend.

Thanks to many folks at CBL for helping out in various capacities: Dr. Lee Cooper for help in the lab, former committee members Dr. Johan Schijf, Dr. Sujay Kaushal and Dr. Chris Rowe as well as Kristlyn Araujo for assistance with previous work, Dr. Margaret Palmer and Dr. Tom Miller for much support and guidance in my initial year at CBL, to the GEC Committee and UMD Jacob K. Goldhaber Award for supporting fantastic travel opportunities to China and San Francisco, and to the Maintenance and Business staff at the lab for much assistance over the years, especially Elaine Proctor for always having my back.

I would like to the DC Sahaja group for being a surrogate family to me while in the States, and making me feel so at home here.

I would like to thank my parents for their love, support and encouragement in everything I do, for always taking an interest in my work, however “bo-ring”, and for being an inspiration to me in so many ways. To my siblings for always being loving, supportive and a lot of fun: Bill, Krasi, Mary, Marcel, Kath, Liz, Margie, Jimmy, Angie, Mike, Dana, Christine, Cory, Erin and a special thank you to Pat and Paula for their generosity. I would like to also thank my parents in-law, and Harshavardhan, Anuradha and Vikram for supporting me from afar. Special thanks to Jimmy, Sean, Rosa, Michaella, Jean-Luc and Tanisha for bringing so much light to our family.

Most of all I would like to thank Sangram, for everything you do for me, which I couldn’t possibly list here. I truly could not have done this without you and thank you especially for patiently waiting for me with unwavering support and for making our life together so full of joy.

Table of Contents

List of Tables

List of Figures

overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

Figure 2.7 Hg concentration in HMW and LMW fractions (mean $\pm$ std error) along a watershed transect in the Lake 658 watershed. LMW = low molecular weight (< 3 kDa); HMW = high molecular weight (3 kDa< HMW < 0.7 µm). Data sets not significantly different from one another are grouped by the same symbol. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

Figure 2.8. Hg/DOC concentrations across the watershed transect in HMW and LMW fractions demonstrating greater relative importance of HMW fractions in Hg binding. Data sets not significantly different from one another are grouped by the same symbol. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

Figure 2.9. Decreasing $CDOM_{440}$ in the <0.7 µm fraction along watershed transect in the UP1 sub-basin. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

Figure 2.10. Correlations between $CDOM_{440}$ (m^{-1}) and Hg concentration (ng L^{-1}) in <0.7 µm and LMW (<3 kDa) fractions in all subsurface flow samples in the Lake 658 upland.

Figure 3.1. Lignin compounds, composed of connecting units of monolignols cross-linked by carbon to carbon and the dominant B-O-4 aryl ether bonds. (From Bianchi, 2007)

Figure 3.2 a) Map of the Lake 658 watershed showing general forest types and contour lines.

Figure 3.2b) Map of the Lake 658 watershed showing forest types, subsurface flowpaths and location of sub-basins, and soil and water column collection sites.

Figure 3.2.c) Map of the Lake 658 watershed showing UW, UP1 and C2 sub-basins, location of subsurface flowpaths. The insert is an expanded map showing sites of collection of subsurface flow, flow at zones of hydrologic convergence, streamflow and lake water.

Figure B.9. Reaction mechanism of derivatization by TMAH thermochemolysis of G6 (vanillin).

Chapter 1: Introduction

1.1. Mercury as an Environmental Contaminant

Mercury (Hg) contamination of ecosystems is a global environmental concern and is the cause of the majority of fish consumption advisories in the United States (US EPA, 2010). Though Hg occurs naturally in the environment in soils, aquatic systems, rocks and the atmosphere, the amount "actively" cycled through the environment has increased as a result of anthropogenic activities (Fitzgerald et al., 1998; Lindberg et al., 2007). The majority of Hg in the environment exists in inorganic forms, predominantly as elemental gaseous Hg (Hg^0), and divalent ionic Hg (Hg(II)). Methylmercury (MeHg), an organic form, occurs as a relatively small fraction of the total Hg pool in the environment, but the occurrence of this toxic form is the driver of human and ecosystem health concerns. The prevalence of MeHg in the environment has implications for human health as MeHg is a neurotoxin and is classified as a potential carcinogen (US EPA, 2000). Additionally, MeHg is bioaccumulative and has been shown to have toxicological effects on various animals, particularly those feeding on fish or at higher trophic levels (Wolfe et al., 1998; Tan et al., 2009). Both abiotic and biotic mechanisms exist to form MeHg from Hg(II). Chemical methylation has been observed in aqueous, particulate and sediment environments in the presence of methyl donors, such as methyl iodide, dimethyl sulfide or certain components of organic matter (Celo et al., 2005). However, biotic methylation by anaerobic bacteria is the dominant pathway MeHg formation in the environment (Gilmour et al., 1992). Areas where anoxic conditions occur, such as aquatic sediments and bottom waters, are therefore sites of high MeHg production (Gilmour et al., 1992;

Gilmour and Riedel, 1995; Benoit et al., 2003; Heyes et al., 2004; Heyes et al., 2006; Hollweg et al., 2009).

Industrial activities such as coal combustion, waste incineration and mining activities have led to increases in levels of Hg in the atmosphere, which is in turn the primary pathway of Hg to most ecosystems (Lindberg et al., 2007; Mason et al., 1994; Fitzgerald et al., 1998). Hg^0, comprising approximately 98% of Hg species in the atmospheric pool, has a long residence time in the atmosphere, and can be transported over great distances, leading to contamination of even remote, unindustrialized areas (Wiener et al., 2003; Kolker et al., 2010). Atmospheric Hg(II) is operationally defined as reactive gaseous Hg (RGM) or particulate Hg (p-Hg). RGM is highly soluble and is delivered to surfaces as wet deposition in precipitation, while Hg^0 and p-Hg are more commonly removed from the atmosphere via dry deposition (Mason et al., 1994). Forested ecosystems retain a high proportion of atmospherically derived Hg, first primarily in canopy and ground vegetation, to be later transferred to soil pools in throughfall or litterfall (Graydon et al., 2008; Demers et al., 2007). This leads to the accumulation of Hg in soils with some release occurring as soil organic carbon (SOC) is mobilized into the dissolved phase (Grigal, 2002; Grigal, 2003). However, the timeline of Hg cycling within soils and vegetation following deposition is not well characterized and there is therefore uncertainty regarding the length of time it takes for Hg to be exported from watersheds (Hintelmann et al., 2002). Due to the large number of surfaces upon which Hg can be deposited and the capacity of soils to retain Hg, soils and vegetation together comprise a much larger pool of Hg than does the aquatic portion of most watersheds. This is particularly true in watersheds in which the surface area of the

terrestrial environment is much larger than that of the receiving water body (Kolka et al., 2001; Grigal, 2002). Concern over impacts of MeHg on human and ecosystem health has led governments and agencies to consider practices that would reduce atmospheric Hg emissions, for example by limiting emissions from coal fired power plants. However, lack of understanding about the timing of Hg release from terrestrial systems makes it difficult to predict the impact that such controls would have on MeHg levels in aquatic ecosystems. The Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS) in part seeks to address the uncertainty associated with this component of the Hg cycle.

1.2. METAALICUS

1.2.1. General Description

The METAALICUS experiment is being conducted at the Experimental Lakes Area, Canada, a field research facility located in the boreal ecoregion of northwestern Ontario, Canada (49°39'35.16"N 93°43'25.75"W). The ELA is located on the Superior Province of the Canadian Shield. Glacial erosion has created a complex landscape, with many ridges and valleys that have isolated and separated watersheds creating a seemingly disproportionate number of headwater watersheds. Exposed outcrops are a common site with forests dominated by jack pine (*Pinus banksiana*), balsam fir (*Abies cilicica*), black spruce (*Picea mariana*), red maple *(Acer rubrum)* occupying slopes valleys and treed islands and an understory of alder shrubs (*Alnus rugosa*) and juniper (*Juniperus spp*). Soils are classified as silt loams and probably of glacio-lacustrine origin (Brunskill and Schindler, 1971). Pilot studies for the METAALICUS experiment began in 1999, and the whole-ecosystem study began in 2001 at Lake 658 (49°44'2.31"N 93°44'14.35"W) a first

order lake located approximately 17 km north of the main field station (Figure 1). Lake 658 is an oligotrophic, dimictic lake, 18.4 ha in area and approximately 13 m deep. The METAALICUS experiment utilized stable isotopes in a loading experiment. The loading rate was approximately 22 μg Hg m^{-2} $year^{-1}$, which is roughly equivalent to deposition in contaminated regions of Europe and North America (Sandilands et al., 2008). Each year from 2001 to 2006, three enriched stable isotopes of Hg were added to the Lake 658 system; one each to the upland (^{200}Hg), the wetland (^{198}Hg) and the lake surface (^{202}Hg). The stable isotopes are discernable from ambient Hg present in the environment, allowing researchers to track Hg as it is transported through the terrestrial environment, and within the lake ecosystem.

1.2.2. Findings to Date

One of the immediate findings of the METAALICUS study was that the ^{202}Hg added to the surface waters of Lake 658 was rapidly methylated and incorporated into the food web. Within one month of addition, Me^{202}Hg was measured in sediments, zooplankton and benthic invertebrates, and within 2 months, Me^{202}Hg was detected in fish tissue (Harris et al., 2007). This observation demonstrates how quickly newly added Hg was quickly transported to sites of methylation (anoxic bottom waters and sediments), methylated and taken up by organisms. This implies that increased loading of Hg to lake systems results in increased MeHg in organisms in the very short term (Harris et al. 2007).

An important finding of the METAALICUS study has been that very little ^{200}Hg added to the upland has been detected in the lake ecosystem. Following deposition to the upland, concentration of ^{200}Hg in discharge from the catchment was only a fraction of

that added, being equivalent to approximately 1% of ambient Hg within the first 8 years (Harris et al., 2007, this study). While the plot scale studies of Hintelmann et al. (2002) suggested little Hg would be released in the short term, i.e. within a year, this extremely slow response was somewhat unexpected, as the terrestrial upland is the primary source of ambient Hg to the lake. This implies that in natural systems a lag phase of unknown duration occurs after Hg is deposited onto a catchment and before it is transported to receiving waters. The concentration of ^{200}Hg in the stream draining the UP1 sub-basin has gradually increased in discharge from UP1 each year from 2005 to 2009 (Krabbenhoft D., pers. comm; this study) and while it is expected to continue to increase in the short term, there is no definitive indication about when ^{200}Hg export will reach steady state.

This finding has raised several questions about the biogeochemical and hydrological controls on Hg transport from terrestrial to aquatic systems, and about the timing of the response of forested uplands to changes in Hg deposition. Because natural organic matter (NOM) appears to influence Hg binding, transport and bioavailability in the environment, an investigation of the characteristics of Hg-bound NOM in the Lake 658 watershed is relevant to addressing these questions.

1.3. The Role of NOM quality in Hg Binding and Transport

Natural organic matter in soils, sediments and aquatic matrices are comprised of a heterogenous mixture of plant, fungal and algal by products, microbial metabolites and exudates, and represent a variety of functional groups, molecular weights and structures (Grandy and Neff, 2008; Kaiser et al., 2002; Kögel-Knabner, 2002). NOM is generally

grouped into two pools: dissolved of organic matter (DOM) and particulate organic matter (POM) fractions. These fractions are operationally defined and designations may vary. In this study 0.7 micrometer (μm) glass fiber filters (Whatman, USA) were used to separate DOM. Both pools are in general reactive with respect to one another and to their surrounding environment, being susceptible to mineralization, physical degradation, leaching, sorption and fractionation (Kögel-Knabner, 2002). Biogeochemical cycling of NOM is complex and difficult to characterize in part because structure and form are dynamic. Additionally, techniques to characterize NOM are usually defined operationally, rather than functionally. NOM extraction techniques may influence the composition of the material, and analytical techniques provide information about only a portion of the material being examined (Grandy and Neff, 2008; Floge and Wells, 2007). Certain techniques, such as nuclear magnetic resonance (NMR), provide information about overall bonding structures but lack information on molecular level composition. Others, such as pyrolysis techniques, are useful in providing structural information at the molecular level but not on the macromolecular scale (Grandy and Neff, 2008). When attempting to characterize NOM, techniques should therefore be chosen with great consideration of the purpose of the characterization, the overall goals of the study and with an understanding of any drawbacks.

One method of DOM characterization involves the determination of the proportions of compounds in different molecular weight size classes. These are generally designated as low molecular weight (LMW) and high molecular weight (HMW) classes. HMW compounds are sometimes termed “colloids”; this generally implies the fraction between 10 kilodaltons (kDa) and 0.45 μm in size (Babiarz et al., 2003; Bianchi, 2007).

In this study, dissolved phase is operationally defined as those compounds passing through a 0.7 µm filter; within the dissolved phase, LMW compounds are present in ultrafiltrate of a 3 kDa ultrafilter and HMW compounds are between 3 kDa and 0.7 µm. A dalton (Da) is an atomic mass unit but can be generally correlated with diameter of macromolecules: a 1 nm spherical diameter is essentially equivalent to macromolecules with a nominal molecular weight cutoff of 1 kDa (Floge and Wells, 2007; Wells, 2002). Common methods to size fractionate DOM include centrifuge ultrafiltration (Miller, 2006) and tangential flow ultrafiltration (Babiarz et al., 2003; Babiarz et al., 2001; Mannino and Harvey, 2000). The molecular weight of DOM has been shown to influence DOM mobility in watersheds, with LMW fractions generally exhibiting greater mobility (McCarthy et al., 1996; Kaiser et al., 2002). HMW compounds have been demonstrated to have lower mobility in flow through terrestrial systems because they are more likely to be adsorbed to soil surfaces (Kaiser et al., 2002; McCarthy et al., 1996; Lajtha et al., 2005; Kalbitz et al., 2005). There is some evidence for a relationship between molecular weight distribution and the binding of metals (Sigg et al., 2000). With respect to Hg, HMW compounds generally have a higher affinity for Hg, though the reason for this is unclear (Cai et al., 1996; Babiarz et al., 2003; Chapter 2). Haitzer et al. (2003) indicate that HMW compounds in general contain a greater number of thiol groups, but since thiol content was not measured in this or other studies examining molecular weight distribution of Hg-bound DOM, the importance of thiols cannot be confirmed (Babiarz et al., 2003; Babiarz et al., 2001; Guetnzel et al., 1996; Cai et al., 1999; Choe et al., 2003). Another method of characterization of NOM is the analysis of lignin phenolic compounds. Lignin phenols are a class of compounds found in the hemicelluloses matrix

of the secondary cell walls of vascular plants (Wershaw, 2004). The lignin polymers found in NOM depend on the source and diagentic state of the material and are comprised of varying amounts of p-hydroxyphenolpropanoid, guaiacylpropanoid and syringylpropanoid monomeric units. The analysis requires cleavage of the dominant β-O-4 bond cross-linking monomeric units, commonly by CuO oxidation or thermochemolysis, to produce derived phenols (Bianchi, 2007). The derived lignin phenols are grouped into four families: vanillyls (V), parahydroxy phenols (P), syringyls (S) and cinnamyls (C), the relative proportions of which in a given sample matrix reflect the plant/tissue type and the diagenetic state of the original source material (Bianchi, 2007; Teisserenc, 2009). If the lignin signature of an NOM sample is known, the relative influence of different source materials, degradation state of material and the relative proportion of NOM derived from the terrestrial environment can potentially be established. In streams, NOM sources and the degree of DOM degradation may have implications for binding and transport of Hg, since the structure of DOM is altered with degradation and may impact availability of binding sites for metals.

In terrestrial and aquatic systems, the majority of Hg in oxygenated sample matrices exists as Hg(II) associated with NOM. In the dissolved phase, associations between Hg and dissolved organic carbon (DOC) concentrations have been observed in a variety of systems (Shanley et al., 2002; Brigham et al., 2009; Dittman et al., 2009; Dittman et al., 2010). There is increasing evidence that specific types or qualities of DOM correlate more strongly with Hg concentration than do total DOC concentrations (Shanley et al., 2002; Dittman et al., 2009; Ravichandran, 2004; Waples et al., 2005). Recent studies have focused on identifying specific characteristics of DOM that may

provide information on relative affinity of different types of DOM for Hg. Examples of methods applied to classify the types of compounds with which Hg is associated within NOM include XAD resins to fractionate DOM intro hydrophobic and hydrophilic acids and neutrals (Haitzer et al., 2003; Hall et al., 2008; Schuster et al., 2008), ^{13}C NMR to measure aromatic carbon content (Ravichandran et al., 1998; Waples et al., 2005; Haitzer et al., 2003), measurement of absorbance capacity of UV light by DOM as proxies of hydrophobicity (Ravichandran et al., 1998; Waples et al., 2005; Dittman et al., 2009), molecular weight size fractionation (Babiarz et al., 2003; Guetnzel et al., 1996; Cai et al., 1999; Choe et al., 2003), and application of lignin biomarkers to examine the role of degradation state in Hg binding and ascertain source areas of DOM (Ouellet et al., 2009; Teisserenc, 2009; Caron et al., 2008). These techniques separate DOM into different classes, and are followed by subsequent analysis of Hg and DOC within those fractions. The goal is to determine relative affinity of different types of DOM for Hg, which is expected to lead to more accurate understanding of the characteristics of DOM that drive Hg binding.

Studies focusing on characterization of NOM associated with Hg have provided some indication about the characteristics of NOM that may drive the association between NOM and Hg. Spectral properties generally correlate with molecular weight as compounds with a higher proportion of HMW compounds displaying a higher absorbance capacity (Helms et al., 2008; Chin et al., 1994; Weishaar et al., 2003). Several studies have also demonstrated a correlation between compounds with high absorbance capacity and Hg concentrations (Ravichandran et al., 1998; Waples et al., 2005; Dittman et al., 2009; Shanley et al., 2020). Similarly, XAD resin techniques separate DOM into

hydrophobic and hydrophilic acids and neutral fractions, and studies generally show a more pronounced association of Hg within the more hydrophobic acid fractions (Ravichandran et al., 1998; Hatizer et al., 2003; Shanley et al., 2010). HMW compounds as determined by ultrafiltration techniques also contain higher Hg concentrations (Chapter 2; Cai et al., 1996; Babiarz et al., 2003; Guentzel et al., 2003; Choe et al., 2003). Application of lignin phenol biomarkers to examine correlations between diagenetic state of NOM and Hg binding is a more recent technique, and relatively few studies exist (Teisserenc, 2008; Ouellet et al., 2009). Ouellet et al. (2009) found that Hg is more strongly correlated with fresher POM and DOM, as indicated by lignin parameters of degradation, while Teisserenc (2008) found that correlations between Hg and diagenetic state of sediment organic matter varied in different watersheds characterized by different land use types. In general, results from several studies have implicated lability of DOM as a predictor of binding in different environmental matrices across geographical areas. Though organic matter is difficult to characterize and techniques for size determination and specific linkages are not exact, information gained through studies can be applied to improve models of Hg cycling in specific environments. However, the evolution of Hg-DOM association within watersheds remains vague and to understand the controls on Hg flux and likely subsequent bioavailability in aquatic ecosystems, further advances in this field are required. Understanding the continuum and evolution of DOM and associated Hg during transport has major implications for predicting the long term impacts of changes in atmospheric Hg deposition.

The goal of this study was to characterize NOM associated with Hg along a watershed transect in the Lake 658 system to better understand biogeochemical controls

on Hg export from the upland. Samples were examined in the dissolved phase (in subsurface flow, streamflow and in the water column), and in the solid phase in soils in the Lake 658 watershed. Characteristics of DOM that influence the capacity of DOM to transport Hg from the point of entry into SSF to the receiving lake were examined. Molecular weight size fractionation was applied to determine the relative reactivity and mobility of LMW and HMW compounds in the Lake 658 upland and the implications for transport of associated Hg within those fractions. Lignin biomarkers were applied to determine degradation state of organic matter in soil and the DOM in subsurface waters, released from selected points within the watershed and surface waters along flowpaths to the lake. Biomarkers are also applied to examine the degradation state of the sediment as this is a long term repository for terrestrial NOM and Hg delivered to the lake. This study was conducted in the METAALICUS watershed and advances research towards one of the main goals of the project: to understand timing of release of deposited Hg from the terrestrial upland to the Lake 658 system.

1.4. Research Questions

The goal of this study was to investigate characteristics of NOM that are related to the binding, transport and fate of Hg in the terrestrial environment. Of more specific interest are broad spectrum characteristics of DOM that improve our ability to predict the affinity of Hg to DOM and qualities of DOM that influence its mobility through watersheds. In the Lake 658 watershed, I am interested in shallow subsurface and intermittent stream flow which is the most important transport pathway of water from the

Lake 658 upland to lake components of the ecosystem. Two main questions were investigated in this thesis:

1. What is the role of DOM quality in controlling the movement of Hg within and from the Lake 658 upland?

To understand the evolution of the Hg-DOM association, we measured dissolved phase Hg and DOM concentrations and UV absorbance properties, in different molecular weight size fractions of DOM along a watershed transect that encompassed subsurface water flow, stream water flow and the water column of Lake 658. This technique is advantageous because it allows the determination of DOM and Hg concentrations and UV absorbance properties in specific molecular weight size fractions from the same unique sample.

To further characterize NOM associated with Hg along this transect, we applied lignin biomarkers as proxies of source material of DOM. Specifically, we examined S/V ratios, an indicator of relative source of angiosperms and gymnosperms, in soils, soil leachate, SSF, streamflow and the lake to determine whether there were correlations with Hg concentration in those matrices. We investigated the potential for lignin biomarkers contained in NOM to be applied as proxies of the degradation state of NOM in the Lake 658 watershed. To do this we used C_{18} solid phase extraction, combined with TMAH thermochemolysis. The appropriateness of the method as an effective tool in the study of lignin phenol analysis in these environmental matrices will be discussed.

2. Does the relationship between natural organic matter and Hg binding and transport vary between recently deposited (isotopically labeled) and historic (ambient) Hg in the Lake 658 watershed?

Anthropogenic Hg deposited to watershed surfaces is a major source of Hg to lakes but evidence suggests there is a time lag between deposition and release from

terrestrial environments (Hintelmann et al., 2002; Harris et al., 2007; Munthe et al., 2007). The time lag appears to be controlled by processes associated with carbon cycling so we will use the isotopically labeled Hg in the METAALICUS watershed to examine how qualities of DOC influence the fate and transport of both newly deposited Hg and Hg that has resided in the watershed for a longer period of time.

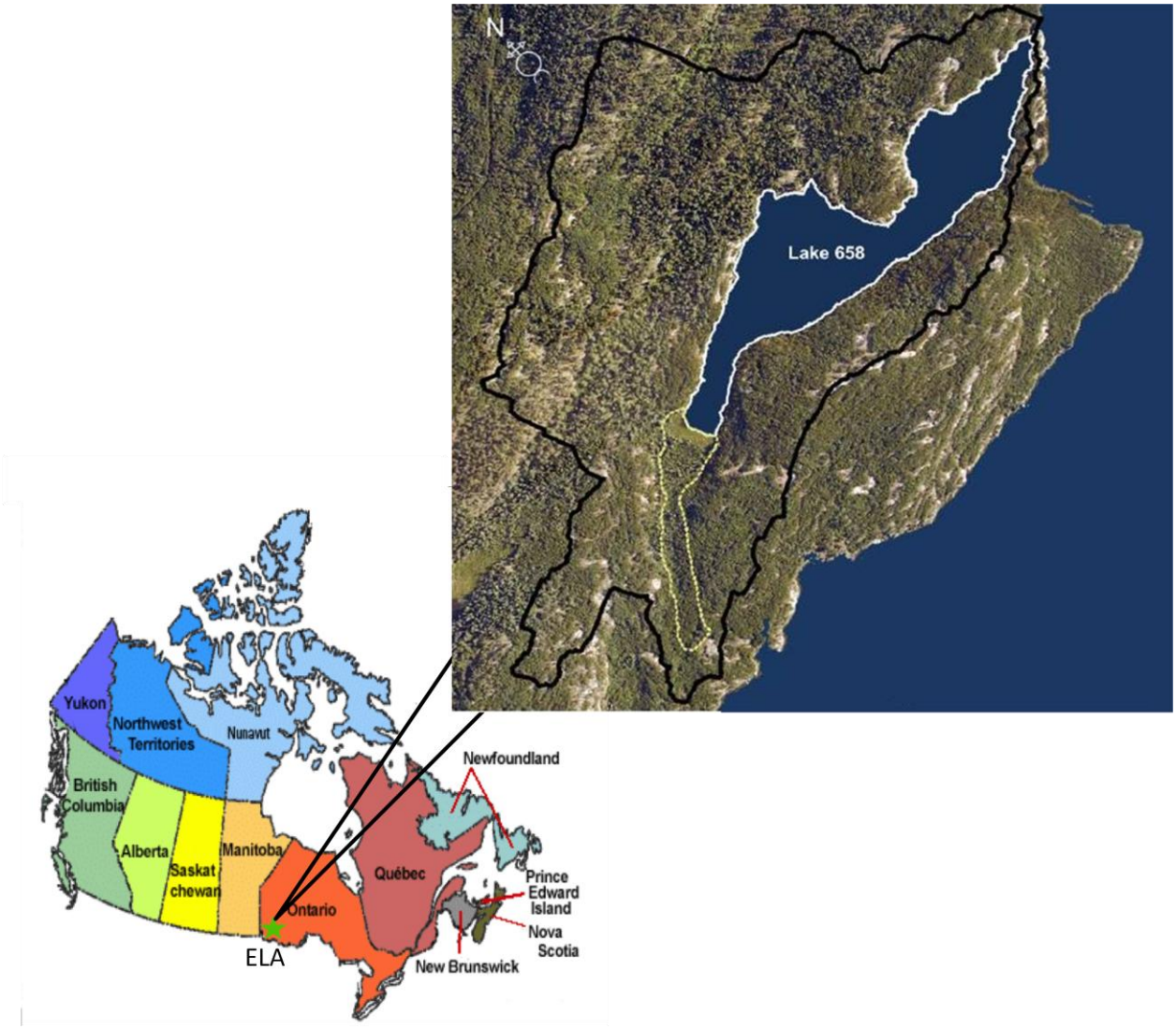


Figure 1.1. Location of ELA on a map of Canada and an aerial photograph of Lake 658 Watershed. The watershed boundary is indicated by the black line, the wetland portion by the dashed yellow line and the lake by the white line.

Chapter 2: Characteristics of Dissolved Organic Matter Controlling Hg Transport in the Lake 658 Upland.

2.1 Introduction

Mercury (Hg) transported from the terrestrial environment is a significant source of Hg to aquatic systems, though the hydrological and biogeochemical controls of Hg transport are not well characterized. The transformation of Hg to methylmercury (MeHg) occurs predominantly in anoxic aquatic environments, such as bottom waters and sediments (Gilmour et al., 1992; Gilmour and Riedel, 1995; Benoit et al., 2003; Heyes et al., 2004; Heyes et al., 2006; Hollweg et al., 2009). Health concerns over MeHg have led governments and agencies to consider practices to reduce anthropogenic Hg emissions in an effort to reduce MeHg production. However, the lack of understanding about the transport of Hg from terrestrial to aquatic ecosystems is a source of uncertainty in predicting the effect that such controls will have. It is important to understand the length of time Hg is retained in the terrestrial environment prior to export in order to accurately develop model predictions about long term effects of reduced atmospheric Hg emissions on resultant MeHg production and subsequent accumulation in food webs.

The Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS), being conducted in the Lake 658 watershed at the Experimental Lakes Area (ELA) Canada, is designed in part to address the question of the timing of the watershed response to reduced atmospheric emissions by examining Hg cycling within terrestrial systems following deposition. An enriched stable isotope of Hg (^{200}Hg) was added to the upland each year from 2001 to 2006, at rate of approximately 22 µg Hg m^{-2} $year^{-1}$, approximately equivalent to deposition in contaminated regions of Europe and North America (Sandilands et al., 2008). ^{200}Hg is discernable from ambient Hg occurring

in the environment, allowing researchers to track Hg as it is transported through the terrestrial environment and to the lake ecosystem. From this study, there is evidence that Hg resides in watersheds for a significant period of time before being delivered to aquatic systems. Three years following deposition of ^{200}Hg in the Lake 658 watershed, the majority of Hg in the uplands was bound within upper soil horizons, primarily the litter and upper organic layers that do not contribute significant amounts of water to flow (Harris et al., 2007; Oswald et al., 2010; submitted). As of 2009, ^{200}Hg was detected in trace quantities in streamflow from the Lake 658 upland, being equivalent to no more than 5% of ambient Hg concentration. Because it is known that ambient Hg from the upland is the dominant source of Hg to Lake 658, these studies indicate that a significant delay exists between the time Hg is deposited from the atmosphere to initial points of contact (canopy and ground vegetation), incorporated into soils and translocated to zones of flow generation.

Inputs of Hg to terrestrial systems occur as wet or dry deposition to ground and canopy vegetation, which then deliver Hg to soil surfaces as leaves and needles fall to the forest floor and senesce (Grigal, 2003; Graydon et al., 2009). Hg is also delivered to soils in direct precipitation and throughfall, as precipitation removes some particulate Hg from foliar surfaces with passage through the canopy (Graydon et al., 2008). Hg accumulates in soils, making them an important pool in terrestrial systems, and subsequent release from soils an important source of Hg to aquatic bodies in most watersheds (Obrist et al., 2009; Smith-Downey et al., 2010). As soil organic matter is degraded into dissolved organic matter (DOM) fractions, it may be transported in flow to downstream areas, carrying associated Hg with it (Akerblom et al., 2008; Skyllberg et al., 2003; Kalbitz and

Wennrich, 1998; Aastrup et al., 1991). Water passing through organic soils horizons generally contribute the majority of soil derived subsurface flow to downstream areas in many watersheds (Akerblom et al., 2008; Froberg et al., 2003; Tipping et al., 2005; Schiff et al., 1999; Oswald et al., 2010, submitted), therefore DOM-Hg compounds occurring in those horizons are more likely to be exported from the terrestrial system. The length of time it takes for Hg to reach soil layers where flow generation occurs is unknown, but results from the METAALICUS study do provide some indication. In 8 years following deposition, ^{200}Hg was detected only in trace quantities in upland flow, however, concentrations (in streamflow) have been gradually increasing since 2002 (Krabbenhoft, D., pers. comm.) implying that more ^{200}Hg has reached zones within the soil profile from which it can be mobilized and exported in greater quantities.

Release of Hg from soil layers occurs primarily with transport of natural organic matter (NOM) (Shanley, 2002; Brigham et al., 2009; Dittman et al., 2010; Dittman et al., 2009; Akerblom et al., 2008; Fleck, 1999; Allan and Heyes, 1998; Schuster et al., 2008; Shanley et al., 2008; Bushey et al., 2008). In the Lake 658 upland, DOM components, defined in this study as NOM passing through a 0.7 µm filter, are most important with respect to Hg transport, which is the case in some other forested systems (Bushey et al., 2008), while in others transport via particulate organic matter is also significant (Shanley et al., 2002; Schuster et al. 2008). There is increasing evidence that the chemical character of DOM influences the strength of the association between Hg and DOM, which in turn influences Hg transport and fate in watersheds (Ouellet et al., 2009; Dittman et al., 2009; Dittman et al., 2010; Ravchandran et al., 2004). The role of DOM in Hg binding and transport needs to be better understood in order to accurately model Hg

behavior in the natural environment and the response of watersheds to changes in atmospheric Hg deposition.

Natural organic matter in forested environments is a heterogeneous mixture of decomposition products of vascular plants, microbial metabolites, and fungal by-products. Common organic complexes represented include lignins, lipids, carbohydrates, proteins and amino acids, which span a wide range of molecular weights and chemical structures (Kalbitz et al., 2000). As it ages and moves downslope, terrestrial DOM continually undergoes dynamic processing through microbial and photochemical degradation, leaching, sorption and fractionation (Kaiser et al., 2002; Grandy and Neff, 2008; Kalbitz et al., 2000). Due to its complexity, the characterization of DOM has proven difficult. Most analytical methods in common use have inherent drawbacks, often relying on operationally defined measures, or providing information about only a portion of the material being examined (Grandy and Neff, 2008). It is therefore important that results are interpreted and applied with caution and with acknowledgement of any shortcomings.

The measurement of spectral properties of DOM, such as absorbance of ultraviolet light a specific wavelength (SUVA) and chromophoric DOM (CDOM) are commonly applied to describe DOM. The capacity of DOM to absorb UV light has been shown to correlate positively with the molecular weight and the aromaticity of DOM (Chin et al., 1994; Weishaar et al., 2003), qualities which affect both the mobility of DOM compounds and its capacity to bind metals (McKnight et al., 1994; McCarthy et al., 1993; Ravichandran et al., 1998). CDOM is defined as the absorption coefficient of DOM at a given wavelength; similarly, SUVA represents the absorption coefficient of DOM at

a specific wavelength, normalized to the concentration of dissolved organic carbon (DOC) of the sample. Both SUVA and CDOM are applied as proxies of the aromatic carbon content and lability of DOM (Del Vecchio and Blough, 2004; Ma et al., 2010; Weishaar et al., 2003; Chin et al., 1994). Chin et al. (1994) also demonstrated a strong positive correlation between $SUVA_{280}$ and molecular weight as determined by high pressure size exclusion liquid chromatography. In general, compounds with higher measured absorbance are expected to be more labile with respect to degradation, comprised of more aromatic structures and comprised of a greater proportion of HMW compounds. With respect to Hg, $SUVA_{254}$, along with aromatic carbon content of DOM, was correlated with the rate of dissolution of cinnabar, solid mercuric sulfide, induced by DOM (Ravichandran et al., 1998). In a similar study, $SUVA_{280}$, along with degree of aromaticity, as determined by ^{13}C-nuclear magnetic resonance (NMR), and molecular weight, as determined by high pressure size exclusion liquid chromatography (HPSELC), was correlated with rate of cinnabar dissolution by DOM (Waples et al., 2005). Recently, $SUVA_{254}$ was found to be strongly correlated to Hg concentrations in streams to such an extent that the authors postulate using $SUVA_{254}$ as a proxy of Hg concentration (Dittman et al., 2009).

The molecular weight distribution of DOM in natural waters has been shown to affect its mobility in terrestrial systems, and its ability to bind metals (Babiarz et al., 2003; Guentzel et al., 1996; Cai et al., 1999; Choe et al., 2003; Kaiser et al., 2002; Sigg et al., 2000). Though molecular weight fractions are operationally defined and size cutoffs may vary, in general, high molecular weight (HMW) compounds are generally comprised of a higher proportion of hydrophobic, aromatic compounds that have higher affinity for

both Hg and soil surfaces (Kaiser et al., 2002; Cai et al., 1999; Babiarz et al., 2003). Low molecular weight (LMW) compounds have been demonstrated to be more mobile in subsurface flow (Kaiser et al., 2002; McCarthy et al., 1993; Lajtha et al. 2005). Molecular weight distribution also provides information about the age, reactivity, and bioavailability of DOM, as predicted by the reactivity size continuum hypothesis (Mannino and Harvey, 2000; Tulonen et al., 1992; Amon and Benner, 1996, Amon and Benner, 1994, Tranvik, 1990). HMW compounds represent material that is younger in age, more labile and more reactive, while LMW compounds are generally older and more recalcitrant. In studies of Hg binding in different molecular weight size fractions, HMW compounds have been demonstrated to have greater importance in dissolved phase Hg binding (Babiarz et al., 2003; Guetnzel et al., 1996; Cai et al., 1999; Choe et al., 2003). The above studies generally demonstrate a correlation between Hg concentrations and measures of reactivity and molecular weight of NOM. However, the mechanism driving the apparent higher affinity of more reactive HMW compounds for Hg is not clear.

Though a correlation between HMW DOM and Hg has been demonstrated in a variety of environmental systems, no studies have specifically examined the role of molecular weight distribution of Hg and DOM in subsurface flow, which represents a conduit for Hg transport from terrestrial to aquatic systems. While HMW compounds are expected to more favorably bind Hg, they are also expected to have a greater affinity for soil surfaces (McKnight et al., 1992, Meier et al., 1999; Kaiser et al., 1996). The overall role of molecular weight distribution of DOM in Hg transport from terrestrial to aquatic systems will therefore depend on both the affinity of DOM for Hg and its susceptibility to

sorption on soil surfaces, which subsequently prevents its transport and that of associated Hg.

The purpose of this study was to investigate the relationships between DOM structural character and Hg transport in a first-order boreal watershed. The study was designed to assess the size and age of Hg-DOC complexes moving downslope in this environment. Molecular weight distribution and absorbance properties of DOC were measured in samples collected along a watershed transect: from subsurface flow in different areas of the upland, to areas of hydrological convergence (representing an intermediary between subsurface flow and the outlet), streamflow draining the catchment, and in the lake water column. Additionally, having the opportunity to distinguish between ambient Hg and ^{200}Hg in this watershed, the study investigates variations in the role of DOM character on newly deposited versus historic Hg in this watershed.

2.2 Materials and Methods

2.2.1. Study Site

The study was conducted as part of the METAALICUS project which is taking place in the Lake 658 watershed (49°43.95′ N, 93° 44.20′ W) (Figure 2.1a) situated approximately 18 km from the Experimental Lakes Area (ELA) base field station in northwestern Ontario, Canada. Lake 658 is a first order boreal lake, 8 ha in area, and the terrestrial upland and wetland comprise 43 ha. The terrestrial upland is comprised of 14 ha portion of old growth forest, dominated by mature black spruce (*Picea mariana*) and balsam fir (*Abies balsamea*), a 21 ha area which was burned in 1983 and now supports young jack pine (*Pinus banksiana*), and a 6 ha area deciduous stand, subject to logging in

1978 and comprised of red maple (*Acer rubrum*), white birch (*Betula papyrifera*) and trembling aspen (*Populous tremuloides*). A 2 ha wetland, which drains into the west basin, supports a mixed stand of black spruce, wetland alder (*Alnus sp*) and tamarack (*Larix laricnia*). Thin podzolic soils dominate the upland and are underlain by pink Precambrian granodiorite (Brunskill and Schindler, 1971).

2.2.2. Field Sampling

Zero tension lysimeters were installed in organic soil horizons throughout the upland of the Lake 658 watershed in 2006 and 2008. The zero tension lysimeters collect only soil pore water, indicated here as subsurface flow (represented in figures and tables as SSF) that is likely to drain saturated soil horizons and flow down slope. Location of lysimeter installation was determined with the use of Light Detection and Ranging (LiDAR) maps, which allowed us to identify subsurface flowpaths (Sandilands et al., 2008). Lysimeters were installed in three different sub-basins to include different forest-soil assemblage types present in the Lake 658 upland (Figure 2.1a, Richardson et al., 2009). Lysimeter samples were collected in double bagged clean 500 ml PETG (glycol-modified polyethylene terephthalate) bottles attached to lysimeters by acid washed C-flex® tubing. The bottles were placed within plastic boxes dug into the ground to protect the samples from heat and sun exposure. A total of 13 lysimeters were installed – five in the UW sub-basin, five in the Upland 1 (UP1) sub-basin and three in the C2 sub-basin (Figure 2.1b). Water was also collected from flow at two areas of hydrologic convergence where diversion walls were built to constrain subsurface flow through shallow moss layers but water passing through these sites may also contain an overland flow component (Figure 2.1b). These samples were collected using 500-ml PETG bottles

stored in doubled plastic bags. The hydrologic convergence sites are grouped as one sample type and denoted "HC" in figures and tables. Streamflow was also collected in double bagged PETG bottles at the gauged weir near the terminus of UP1 (Figure 2.1b). Samples were collected repeatedly during three sampling periods: May 7 to- 18; June 24 to July 6; and October 14 to 28; 2009. Lake water column samples were collected using acid cleaned Teflon tubing and a peristaltic pump at depths of 2 m at sites CB-W, UW, UP1, C2 and 13 m at CB-W once per field trip (Figure 2.1b).

Immediately upon collection, samples were transported to the chemistry laboratory at the ELA field site, stored refrigerated and in the dark, then filtered through pre-combusted 0.7 µm glass microfiber filters (GF/F, Whatman, USA) within 24 hours. Filtered water samples were analyzed for THg concentration, DOC concentration, and DOM spectral absorbance. Aliquots of the filtered samples were further size-fractionated using centrifuge ultrafiltration to 3kDa. The ultrafilters (regenerated cellulose, Amicon Millipore, USA) required cleaning prior to use (Miller, 2006). Filters were cleaned once with a 15 ml aliquot of dilute solution of ultra trace grade HCl, rinsed with three subsequent aliquots of Milli-Q de-ionized water to remove residual acid and finally conditioned with a 15 ml aliquot of the sample (Appendix A). Subsequent 15 ml aliquots of the sample were then centrifuged at 3000 rpm and a constant temperature of 25^0C on a fixed rotor centrifuge (IEC Model CL, GSR Technologies, Canada) for approximately 45 minutes. Three filtrates from each sample were combined into a single PETG bottle with subsamples taken for DOC, absorbance and Hg analysis. All samples for DOC and SUVA analysis were stored frozen in 20-ml amber vials until analysis. Samples for Hg analysis were acidified with ultra trace grade HCl (Baker Company, USA) to 0.5% by

volume. In the data presentation, samples analyzed after filtration through 0.7 µm filter are designated "<0.7 µm fraction", those ultrafiltered through 3 kDa ultrafilters are labeled "low molecular weight" (LMW). The fraction in between 3 kDa and 0.7 µm is labeled "high molecular weight" (HMW), and was calculated by difference.

2.2.3. Laboratory Methods

2.2.3.1 Reagent Preparation

De-ionized water used for reagent preparation was reagent grade Milli-Q. Bromine monochoride (BrCl) was prepared by dissolving 27 g of reagent grade potassium bromide (KBr) in 2.5 L concentrated hydrochloric acid (HCl). Prior to reagent preparation, KBr was muffled overnight at 250^0C, removed while still hot and immediately dissolved in HCl. Stannous chloride ($SnCl_2$) solution was prepared by dissolving 100 g of reagent grade $SnCl_2$ in 500 ml of de-ionized water and 50 ml of concentrated Baker Instra-analyzed HCl (Baker Company, USA). The solution was purged overnight with ultra high purity nitrogen (N_2) at 300 ml/minute. Hydroxylamine hydrochloride ($NH_2OH*HCl$) was prepared by adding 30 g of reagent grade hydroxylamine hydrochloride to 100 ml de-ionized water and 100 µl of $SnCl_2$. The solution was purged overnight with ultra high purity N_2 at 300 ml/minute. Mercury standards were prepared from a stock solution obtained from National Institute of Standards and Technology (NIST. Standards used for standard curve generation were prepared with concentrations of 0.5, 1, 5, 10, 20 and 50 ng L^{-1} into analytical vials containing 250 µl BrCl and 10 µl $NH_2OH*HCl$. The quality of the curve was based on attaining $r^2 > 0.999$.

2.2.3.2. Total and Isotopic Hg analysis

Total Hg was measured using cold vapor atomic fluorescence spectrometry (CVAFS) on a Tekran Model 2600 Preconcentration/Detector Unit with a Model 2620 Autosampler (Tekran Instruments, Canada). The instrument performs in-line reduction of Hg(II) to Hg^0 by $SnCl_2$ for capture of Hg^0 onto a gold trap and subsequent thermal desorption, and analysis by CVAFS. The Tekran is interfaced with a Hewlett Packard 4500 ICP-MS (Agilent Technologies, USA), to which the sample was carried after passing through the fluorescence cell in the Tekran, and analyzed for Hg isotopes. Concentrations of individual isotopes were measured by ICP-MS, then relevant isotope concentrations were calculated using the methods of Hintelmann et al. (2003). Briefly, ambient Hg is measured by using ^{201}Hg as an ambient Hg surrogate. Because a small amount of ^{201}Hg is present in the spike solution, contributions of ^{201}Hg from the spike were mathematically subtracted from the total ^{201}Hg measured in the sample and the remaining ^{201}Hg used to calculate the ambient concentration. Quantification was performed using the external standards, and quality assurance/quality control measures included matrix blanks, calibration blanks, sample spikes and replicate samples (every 10 – 12 samples). Replicate sample concentrations were required to be within 10% of each other and spike recoveries between 90 and 110%.

2.2.3.3. Measurement of Chemical Properties of Aquatic Samples

DOC concentration on all filtered and ultrafiltered samples was measured on a Shimadzu Total Organic Carbon Analyzer 5000 (Shimadzu Corporation, Japan) at Nutrient Analytical Services at the Chesapeake Biological Laboratory. Absorbance at wavelengths from 270 to 750 nm was measured on all filtered and ultrafiltered aquatic

samples using clean 1 cm quartz cuvettes on a Cary 4E UV-visible spectrophotometer (Varian Inc., USA) at the Smithsonian Environmental Research Center. CDOM was calculated as the absorption coefficient at 440 nm, which is the absorbance at 440 nm, adjusted for scattering and path length of the cuvette. SUVA was calculated for each sample as the absorption coefficient at 280 nm divided by the corresponding DOC concentration of the sample. Absorbance measurements were taken only on <0.7 µm and LMW fractions, as samples in the HMW fraction were not collected for analysis. For DOC and Hg, HMW concentration was obtained by subtracting the concentration in the LMW from that in the <0.7 µm fraction; since absorbance is not an additive property, as Hg and DOC are, the same method is not appropriate to obtain absorbance in HMW fraction.

2.2.4. Statistical Analyses

Student's t test was applied to test for the difference between two means in normally distributed data. For non-normally distributed data, the Mann-Whitney U test was applied to test for differences between two means and Kruskal-Wallis was applied to test for differences between more than two means. Simple linear regression was applied to examine correlations between DOC and Hg concentrations (Figure 2.2) and between spectral properties and Hg concentrations (Figure 2.10). In all cases, significance was evaluated at $p = 0.05$.

2.3. Results

To examine the relationship between Hg and DOM characteristics in the Lake 658 watershed, the data were examined in two general ways. First, the relationships between

Hg and DOC concentration and DOM spectral properties were investigated in water from subsurface flow using data from all three sub-basins. Second, changes in Hg-DOM properties in water collected along a flow path were examined by comparing subsurface water in the UP1 catchment with water from the downslope convergence areas and the main stream channel. Subsurface flow from the UW and C2 sub-basins could not be compared directly to water delivered to the lake since discharge from those catchments occurs as diffuse flow through shallow horizons and could not be sampled directly.

2.3.1. Relationships Between Hg and DOC Concentrations in the Lake 658 Upland.

Hg and DOC concentrations were significantly correlated in subsurface waters throughout the Lake 658 upland in water collected in the spring, summer and fall (Figure 2.2). Both Hg and DOC concentrations in subsurface waters spanned a wide range in all seasons (Figure 2.2) and were more variable than those at zones of convergence and in the stream (Figure 2.3, 2.4). Concentrations of Hg and DOC were generally highest during drier periods in the fall. In all three seasons, DOC concentrations were significantly higher in upland subsurface waters than in either streamwater or the downslope convergence sites (Figure 2.3). Hg concentrations followed the same general pattern, and were significantly higher in upland subsurface waters than in downslope water in summer and fall (Figure 2.4).

2.3.2. Molecular Weight Distribution of Hg and DOC Along a Watershed Transect

While the samples collected in the UP1 sub-basin were collected as being representative of a continuum of water transport from subsurface flow (collected in lysimeters upslope), through hydrologic convergence zones and then to the stream, the water collected may not actually represent a continuous transport pathway. The

subcatchments from which water may flow and contribute to the runoff that drains the catchment are not always continuously hydrologically connected (Oswald et al., 2010, submitted). The samples are however indicative of the differences in DOC character and DOC and Hg concentrations in these water flow classes.

DOC concentrations in both HMW and LMW fractions decrease from subsurface flow to flow at convergence zones and the stream (Figure 2.5). However, the proportion of DOC in the LMW fraction increases downslope, with the proportion in the stream greater than in the convergence zones, and greater than subsurface flow in all seasons (Figure 2.5 – 2.6). The proportion of DOC in LMW and HMW fractions is most consistent between sample types in the summer, when DOC concentrations are lowest, while in spring, streamflow contains a high proportion of LMW compounds (Figure 2.6). Through the summer and fall, the proportion of DOC in the LMW fraction continues to increase from the stream to the water column, while in the spring, the proportion of LMW DOC decreases through the season (Figure 2.6). Similar to DOC, Hg concentrations generally decrease from subsurface flow to flow at convergence zones and in the stream in both size fractions (Figure 2.7). Hg concentrations are consistently significantly higher in HMW fractions in all sample types along the watershed transect (Figure 2.7). In water from all sample locations and seasons, the Hg content (μg) relative to DOC (g) is significantly higher in the HMW (Hg/DOC_{HMW}) than in the LMW (Hg/DOC_{LMW}) fraction (Figure 2.8). Average Hg/DOC_{HMW} are slightly higher in the fall than in spring and summer, while Hg/DOC_{LMW} is lowest in the fall (Figure 2.8). Hg/DOC_{LMW} and Hg/DOC_{HMW} concentrations are relatively consistent between sample types and seasons (Figure 2.8). In comparison to sample matrices in the upland, Hg is

more evenly distributed between molecular weight size fractions of DOC in the Lake 658 water column: average annual Hg/DOC_{LMW} is 0.17 ug Hg g^{-1} C and average annual Hg/DOC_{HMW} concentration is 0.27 ug Hg g^{-1} C (data not shown).

2.3.3. Correlations Between Hg Concentration and Spectral properties in the Lake 658 Upland

There is a general trend of decreased chromophoric DOM along the watershed transect. <0.7 µm $CDOM_{440}$ values were highest in subsurface flow, and values were sequentially lower in convergence zones, streamflow and the water column sites (Figure 2.9). In general, there are correlations between spectral properties and Hg concentrations in subsurface flow throughout the Lake 658 upland (Figure 2.10). $CDOM_{440}$ is a better predictor of Hg binding than $SUVA_{280}$ (data not shown) and CDOM is significantly correlated with Hg concentration in all seasons in the <0.7 µm fraction but only in the LMW fraction in the spring (Figure 2.10).

2.3.4. ^{200}Hg in the Lake 658 Upland

^{200}Hg was detected in only 53 of a total of 121 water samples collected in the Lake 658 upland and not detected in any samples taken from the water column of the lake. Overall, ^{200}Hg concentrations were low in all sample classes; subsurface flow, convergence zone and streamflow water samples being 1.08 $\pm$0.09 ng L^{-1}, 0.75 $\pm$ 0.14 and 0.89 $\pm$ 0.13 (mean $\pm$ std error) respectively. Concentrations were highest in subsurface flow in UP1 being significantly higher than concentrations in flow at convergence zones and in the stream (Table 2.1). Of the MW fractionated samples in which ^{200}Hg was detected, it was detected in the LMW fraction in only 10 samples. ^{200}Hg was not observed in the water column in any season. There were no correlations between ^{200}Hg concentration and $CDOM_{440}$ or DOC concentration.

Samples containing ^{200}Hg were compared with those in which ^{200}Hg was not detected (Table 2.1). Significant differences in the DOC concentration and $CDOM_{440}$ value existed between the two groups of samples, with samples containing ^{200}Hg exhibiting higher DOC concentrations and higher CDOM. Samples containing ^{200}Hg were also characterized by higher $SUVA_{280}$ and a lower proportion of DOC in the LMW fraction, though differences were not significant.

2.4. Discussion

2.4.1. Variations in DOC and Hg Concentrations Along a Watershed Transect

In general, these findings support the widely recognized notion that dissolved phase complexation of Hg in oxic waters is controlled by DOC (Figure 2.2). DOC concentration accounted for about half of the variability observed in Hg concentrations in subsurface flow in the Lake 658 upland. The strength of the Hg-DOC correlation was not as strong as some studies that examined only streamflow (Dittman et al., 2009; Dittman et al., 2010) but was similar to those reported in studies examining a wide range of DOC and Hg concentrations as in subsurface flow (Babiarz et al., 2001, Akerblom et al., 2008; Fleck et al., 1999). Since it is expected that only a portion of DOC partakes in Hg binding, deviations in the correlation between Hg and DOC is not unexpected and implies that DOC concentration alone is not sufficient to predict Hg binding in these sample matrices.

Overall, DOC and Hg concentrations were higher and more variable in subsurface flow than in flow at convergence zones and in the stream (Figures 2.3-2.4). Overland flow, subject to direct precipitation, contains lower Hg and DOC concentrations since

precipitation contains relatively low Hg concentrations and negligible DOC. Samples containing an overland flow component, such as those in convergence zones and at the stream, are diluted by water lower in Hg and DOC, resulting in lower concentrations of both. Also, more continuous flow at those two site classes leads to more consistent solute concentrations. The concentration of Hg and DOM in subsurface flow is controlled by the amount of soluble Hg and soluble NOM available in the hydrologicaly conductive soil layer during a flow event. The concentrations will depend on the duration of an event, the duration of drying cycles between events allowing the resupply of Hg and NOM through oxidation, and the amount of water moving through the layer during an event. Thus changes in any of these factors could result in different concentrations of Hg and DOC observed (Boyer et al., 1996; McGlynn and McDonnell, 2003; Akerblom et al., 2008). Concentrations of DOC and Hg are therefore higher and more variable in subsurface flow than in flow at convergence zones and in the stream.

Composition of DOM in subsurface flow water also appears to differ from that of DOM in streamflow and in flow at zones of convergence. DOM in subsurface flow is consistently comprised of a lower proportion of LMW DOC (Figures 2.5, 2.6), and a high proportion of chromophoric DOM (Figure 2.9), implying that DOM exported in the stream is more degraded, more recalcitrant and older than that in subsurface flow in the UP1 sub-basin (Mannino, 2000; Amon and Benner, 1996; Del Vecchio and Blough; 2004, Weishaar et al., 2003; Chin et al., 1994). The organic matter leaving the Lake 658 watershed has undergone significant processing in the upland before being exported as DOM, which is consistent with observations made in other boreal forest systems (Schiff et al., 1997; Sanderman et al., 2008; Palmer et al., 2001). The continued processing of

DOM in transit may be in part explained by the findings of Oswald et al. (2010, submitted) that the movement of water in the watershed is controlled by "fill and spill". Water flows between subcatchments or contributing areas only when a storage threshold is reached; DOM is therefore subject to degradation prior to export from each component (Oswald et al., 2010, submitted). The primary source of water, and therefore DOC and Hg, in streamflow draining the UP1 sub-basin is a terminal depression near the stream that collects water from various contributing areas of the catchment. Thus, it can be expected that once DOM compounds reach the terminal depression, they are more degraded, having undergone significant processing in other depressions before reaching the stream.

The higher proportion of LMW compounds in the stream water, as compared to subsurface flow, suggests that in accordance with studies of mobility of different types of DOM (McCarthy et al., 1993; McKnight et al., 1994; Kaiser et al., 2002), HMW compounds have an overall reduced mobility along the watershed transect. This finding is supported by the higher CDOM recorded in water from subsurface flow than in water collected at the hydrologic convergence zones and in the stream. Thus, DOM with higher absorbance and more HMW compounds is less mobile in general. The trend is consistent across seasons except in the summer when $CDOM_{440}$ of water from convergence zones is equivalent to that of stream water in the <0.7 μm fraction, and lower in the LMW fraction of stream water (Figure 2.9). This deviation from the general trend of a lower proportion of HMW DOC further down the watershed transect is not unexpected, as during the summer when dry periods are likely to result in the hydrological isolation of many soil

pockets, and leave differences between the water character at convergence zones and the stream to be driven by local factors.

The observed depletion of HMW DOC with transport through the upland is expected to have implications for associated Hg. Despite the preferential loss of HMW DOC over LMW DOC, the HMW fraction transports a larger amount of Hg into the lake (Figure 2.7). Relative to DOC concentration, Hg content is much higher in HMW fractions across upland sample types and across seasons (Figure 2.8). Though the relative extent of this enrichment appears to vary somewhat with transport along the watershed transect, (Figure 2.8), Hg/DOC_{HMW} is not significantly different between subsurface flow, flow at convergence zones or the stream in any season. This implies that as LMW and HMW compounds sorb to soil surfaces during transport, associated Hg sorbs with it, resulting in consistent Hg/DOC_{HMW} and Hg/DOC_{LMW} concentrations. Greater relative importance of HMW fractions in Hg retention has been observed in a variety of other ecosystems (Guentzel et al., 1996; Cai et al., 1999; Stordal et al., 1996; Babiarz et al., 2003; Babiarz et al., 2001).

2.4.2. Export of Hg and DOC Compounds to Lake 658

Water in the Lake 658 water column is comprised of a higher proportion of DOC in the LMW fraction and lower CDOM values when compared with upland runoff (Figure 2.6). This implies that DOC in the lake is at a more advanced diagenetic state than that exported from the UP1 sub-basin. In comparison to sample matrices in the upland, the enrichment of Hg in HMW fractions of DOC was less pronounced. The fate of Hg entering the lake cannot be ascertained from this data, but variations in the relative proportion of Hg in different molecular weight fractions between the stream and the

water column imply that Hg is subject to further physical, chemical and biological processes upon entry into the lake. The distribution and size fractionation of Hg in the water column will affect lake Hg cycling, though exact implications are unclear from this study.

2.4.3. Spectral Properties and Hg Concentrations

$CDOM_{440}$ was a better predictor of Hg concentration (Figure 2.9) than $SUVA_{280}$, which was only significantly correlated with Hg/DOC concentrations in the 0.7 μm fraction in the spring ($p= 0.0087$) and in the LMW fraction in the summer ($p = 0.0008$). The results in this study tend to indicate that Hg binding may in part be controlled by the chromophoric portion of DOM, which generally represents HMW, aromatic structures (Weishaar et al., 2003; Chin et al., 1994; Spencer, R., pers comm). The weakness of the correlation between Hg/DOC and SUVA is somewhat in contrast to other studies. Studies that demonstrate a strong correlation between Hg and SUVA values in streams also contain a lower magnitude and range of spectral properties and DOC concentrations (Dittman et al., 2009; Dittman et al., 2010). This implies that DOM composition was more homogenous in those sample matrices, while in subsurface flow measured in our systems, a wider range of DOC concentrations and spectral properties was observed, implying a greater range of structures and compounds. Though in general, spectral properties appear to be related to Hg concentration in subsurface flow, spectral properties are not sufficient to predict the portion of DOM controlling Hg binding. $CDOM_{440}$ and $SUVA_{280}$ are not quantitative measures of molecular weight or aromaticity, though they are generally accepted as proxies of those characteristics (Weishaar et al., 2003; Chin et

al., 1994). Studies employing spectral proxies should therefore be coupled with other, more precise measures of DOM composition.

2.4.4. ^{200}Hg in the Lake 658 Watershed

Though the small sample number makes it difficult to examine patterns of molecular weight distribution and ^{200}Hg binding, the lack of detection in the LMW fraction in the majority of samples that contain ^{200}Hg implies that, like ambient Hg, ^{200}Hg is found largely in the HMW fraction. It also appears that like ambient Hg, ^{200}Hg concentrations are higher closer to sites of release and due to its reactivity may be readily lost via sorption to soil surfaces. The lack of correlation between ^{200}Hg and DOC concentrations or other parameters may be a result of the small sample number present in each season, and implies that ^{200}Hg has not yet reached steady state with the existing Hg pool since it does not appear to behave similarly to ambient Hg. However, though no significant correlations exist between ^{200}Hg concentrations and DOC qualities, data in Table 2.2 indicate that ^{200}Hg is generally found in samples comprised of a greater proportion of fresher and younger NOM. This data tends to support the hypothesis based on distribution of ^{200}Hg within the soil profile (Chapter 3, Oswald et al., 2010, in preparation) that ^{200}Hg is associated with fresher NOM in upper soil horizons. ^{200}Hg has not yet been translocated to lower organic horizons where the majority of flow within the soil profile is generated, and thus has not been exported from soils in significant quantities to a degree that it is not yet detected in the lake (Harris et al., 2007).

2.5. Conclusions

This study provides valuable insight about the role of characteristics of DOM that affect Hg binding and transport in the Lake 658 watershed. LMW compounds are more

mobile along a transport pathway through the UP1 sub-basin, while HMW compounds consistently contain more Hg per unit of DOC. Though the quality of DOM changes with transport in UP1, the Hg/DOC ratio does not vary widely within molecular weight fractions or between sample types, implying that despite the reactivity of DOC, association of Hg within molecular weight size fractions does not change with transport. While this gives the appearance that Hg does not move between DOC complexes or ligands, this finding does not imply that Hg is not actively cycled, but rather that the association of Hg with HMW compounds is maintained through long term diagenesis. The importance of HMW compounds is in general agreement with the relatively few studies that have examined Hg binding in molecular weight fractions of DOC, though none have examined this trend in subsurface flow. The cause of the observed higher affinity of HMW compounds for Hg is not clear from this study, but may be a function of relative thiol content. Future research should focus on further characterization of LMW and HMW fractions of DOC, including the measurement of thiol content to determine whether its concentration is limiting in LMW fractions.

This study demonstrates that DOM compounds present in flow at different points along a transport pathway increase in degradation state with transport, implying that DOM-Hg compounds being exported from the UP1 sub-basin are older and more recalcitrant than that flowing through upland soils. This finding is supported by the observation of low levels of ^{200}Hg detected in flow 8 years following depositions, and by results from other METAALICUS studies (Harries et al., 2007; Hintelman et al., 2002). This has implications for the recovery of terrestrial systems to reduced atmospheric Hg

deposition and suggests that the recovery time of watersheds in response to reduced atmospheric deposition is substantial.

DOC is exported from the UP1 sub-basin primarily as LMW compounds. DOC composition and concentration, as well as Hg concentrations vary between streamflow draining the UP1 sub-basin and the water column of Lake 658, implying that Hg-DOC compounds entering the lake are reactive and subject to various types of processing. The fate of terrestrially derived Hg-DOC compounds that enter the lake is unclear but has important implications for bioavailability of Hg to sulfate reducing bacteria in sediments of Lake 658. The proportion of HMW to LMW DOC may provide a means of identifying the proportion of Hg entering lakes from the terrestrial environment that is bioavailable. The application of ^{13}C and ^{14}C isotopes would provide more precise information on the age of the material being export from the upland. Future research to investigate the bioavailability of terrestrially derived Hg should focus on the relationship between the activity of sulfate reducing bacteria and molecular weight distribution of Hg-bound as well as age of Hg-bound DOC.

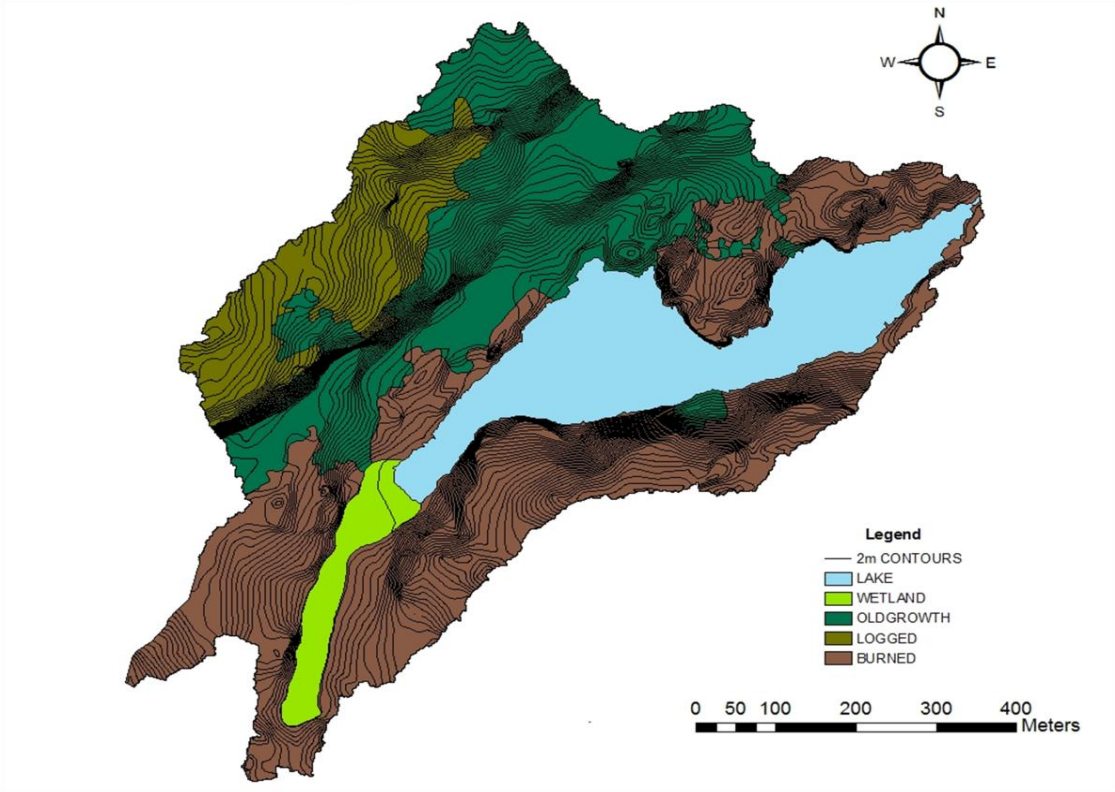


Figure 2.1a) Map of the Lake 658 watershed showing general forest types and contour lines.

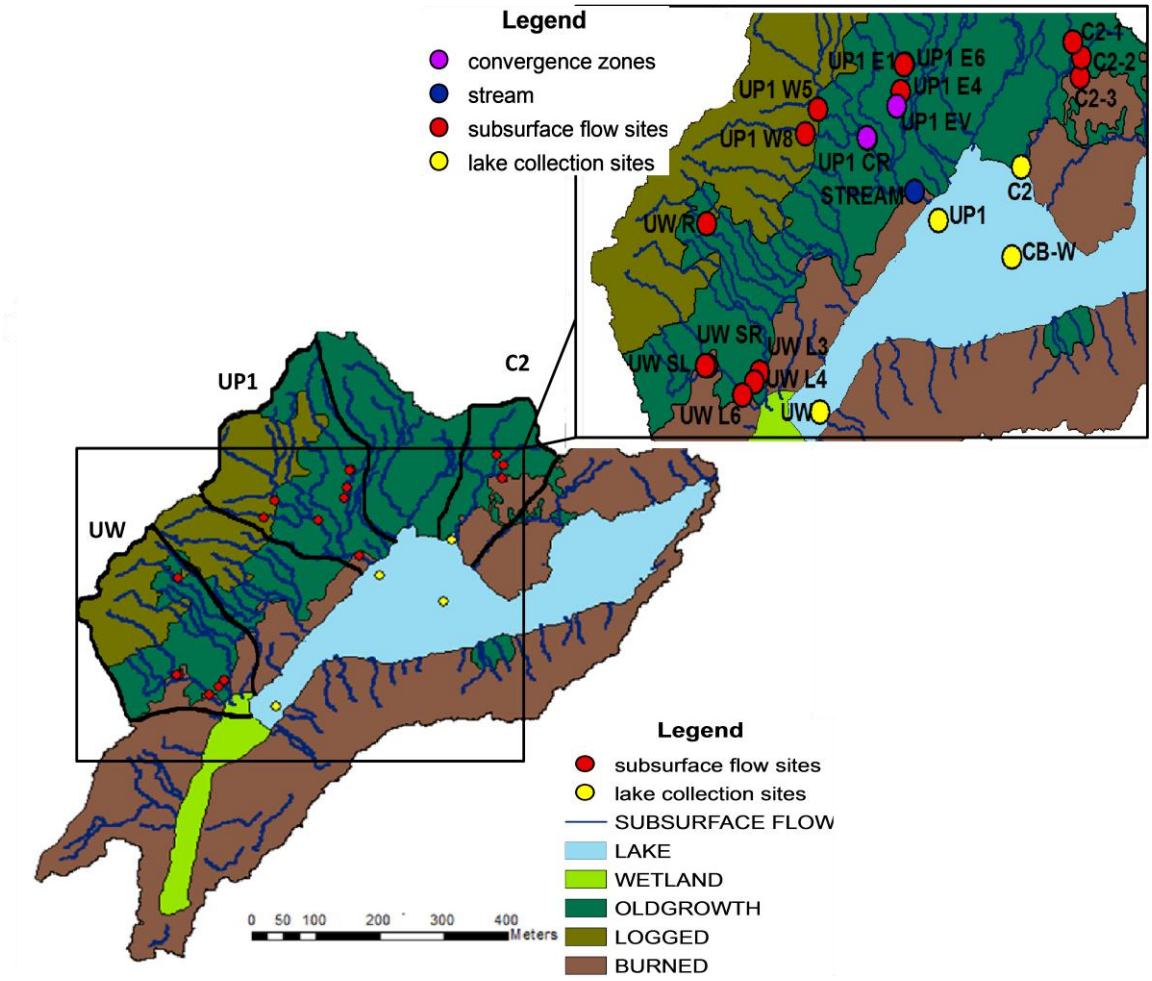


Figure 2.1b) Map of the Lake 658 watershed showing UW, UP1 and C2 sub-basins, location of subsurface flowpaths and zoomed in area of upland showing sites of collection of subsurface flow, flow at zones of hydrologic convergence, streamflow and lake water. Map courtesy of C. Oswald.

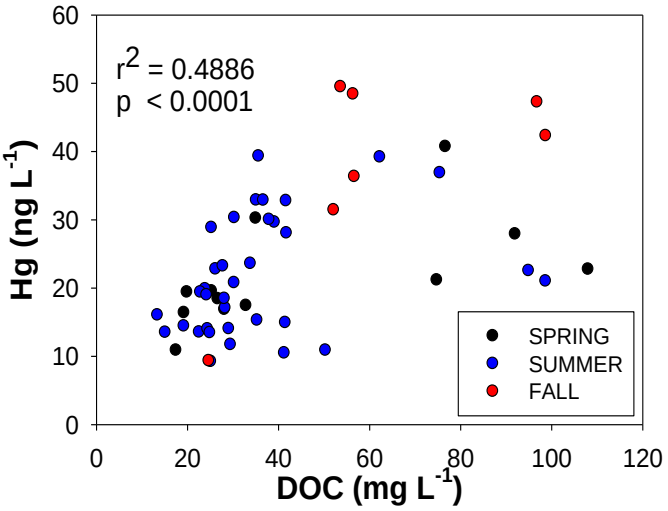


Figure 2.2. Hg and DOC concentrations in the <0.7 µm fraction in subsurface flow from all lysimeters in the Lake 658 upland sampled in 2009; the r^2 presented is for complete data set.

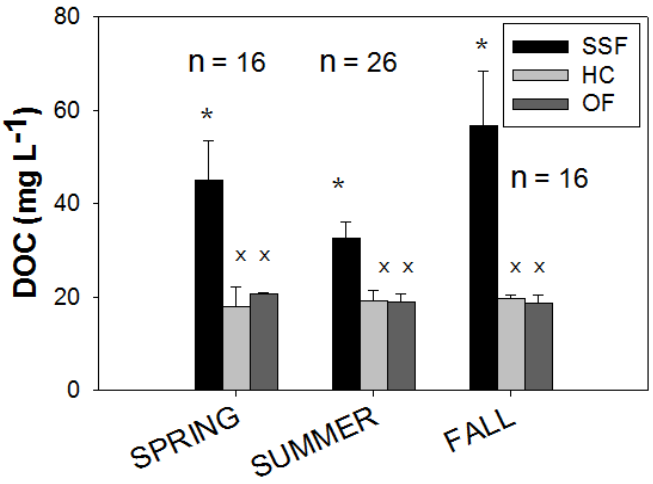


Figure 2.3. DOC concentration in the <0.7 µm fraction (mean ± std error) in different sample types in the UP1 sub-basin. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow, and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin. Mann-Whitney U test was applied to determine significance of difference in DOC concentrations between sample types and seasons. Data sets not significantly different from one another are grouped by the same symbol.

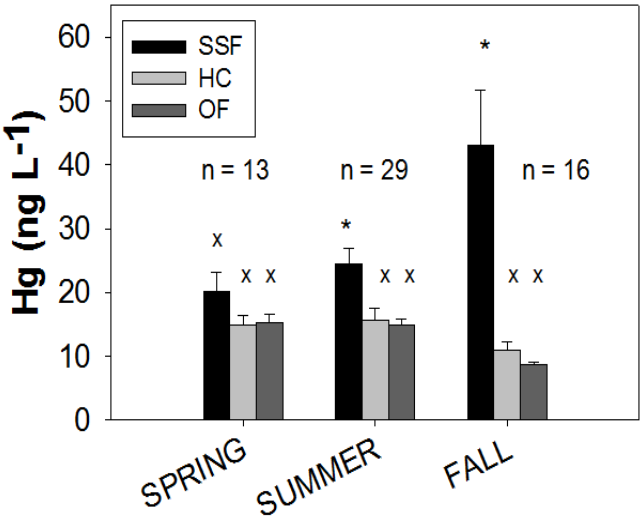


Figure 2.4. Hg concentration in the <0.7 µm fraction (mean± std error) in different sample types in the UP1 sub-basin. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow, and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin. Mann-Whitney U test was applied to determine significance of difference in DOC concentrations between sample types and seasons. Data sets not significantly different from one another are grouped by the same symbol.

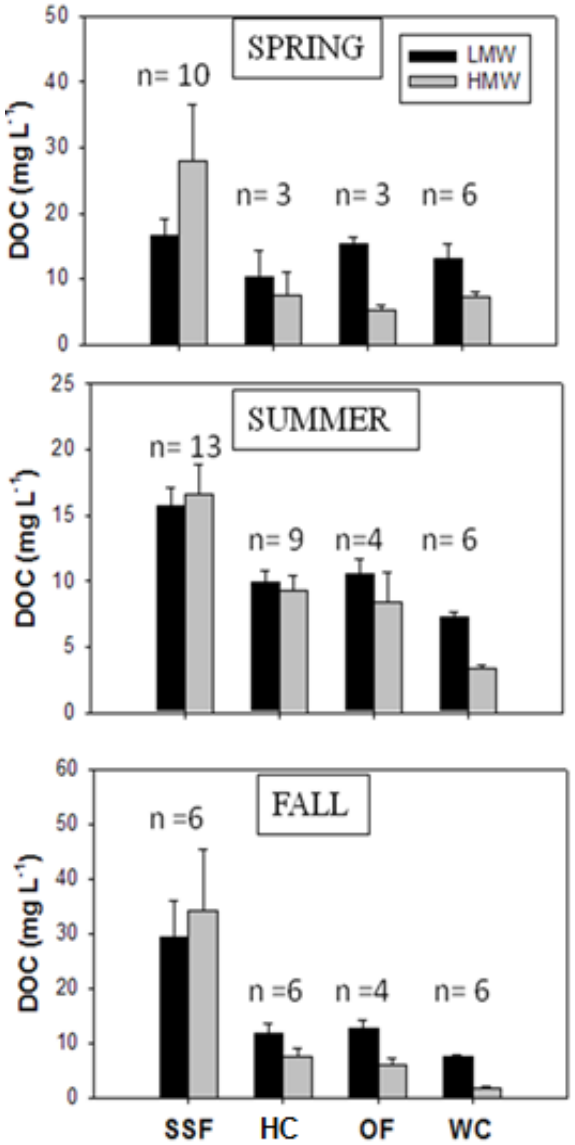


Figure 2.5. DOC concentration in HMW and LMW fractions (mean ± std error) along a watershed transect in the Lake 658 watershed. LMW = low molecular weight (< 3 kDa); HMW = high molecular weight (3 kDa< HMW < 0.7 µm). Sample types are subsurface flow, collected from lysimeters (SSF), mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

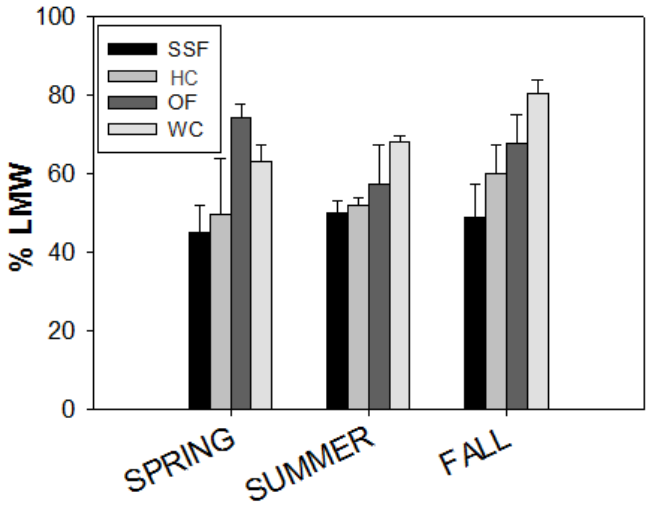


Figure 2.6. Proportion of DOC in the LMW fraction in different sample types in the UP1 sub-basin and in surface water of Lake 658. All bars represent at least 3 samples. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

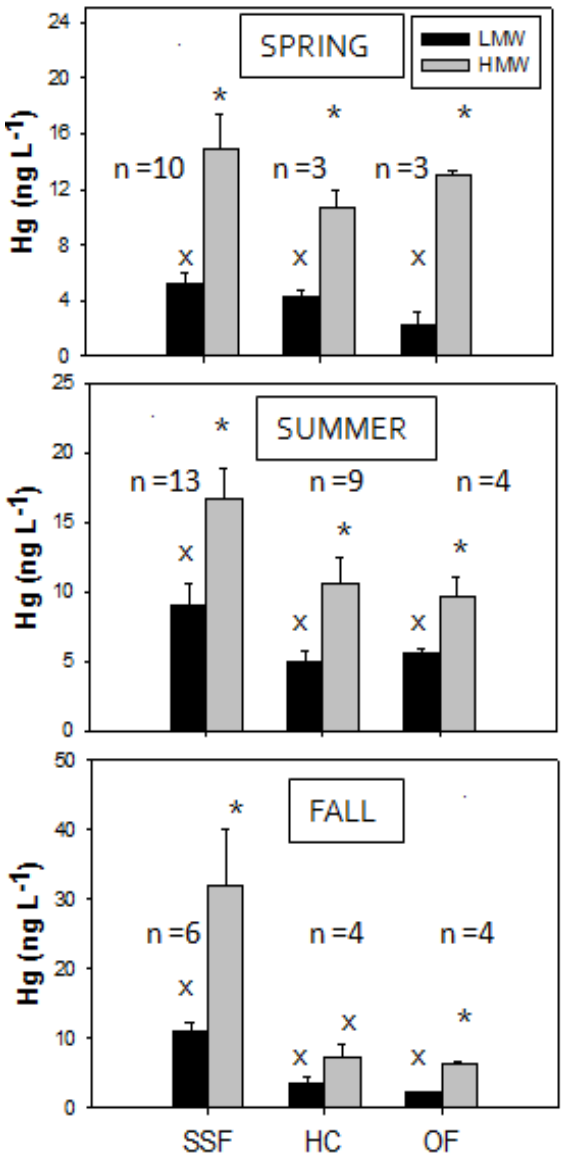


Figure 2.7 Hg concentration in HMW and LMW fractions (mean ± std error) along a watershed transect in the Lake 658 watershed. LMW = low molecular weight (< 3 kDa); HMW = high molecular weight (3 kDa< HMW < 0.7 µm). Data sets not significantly different from one another are grouped by the same symbol. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

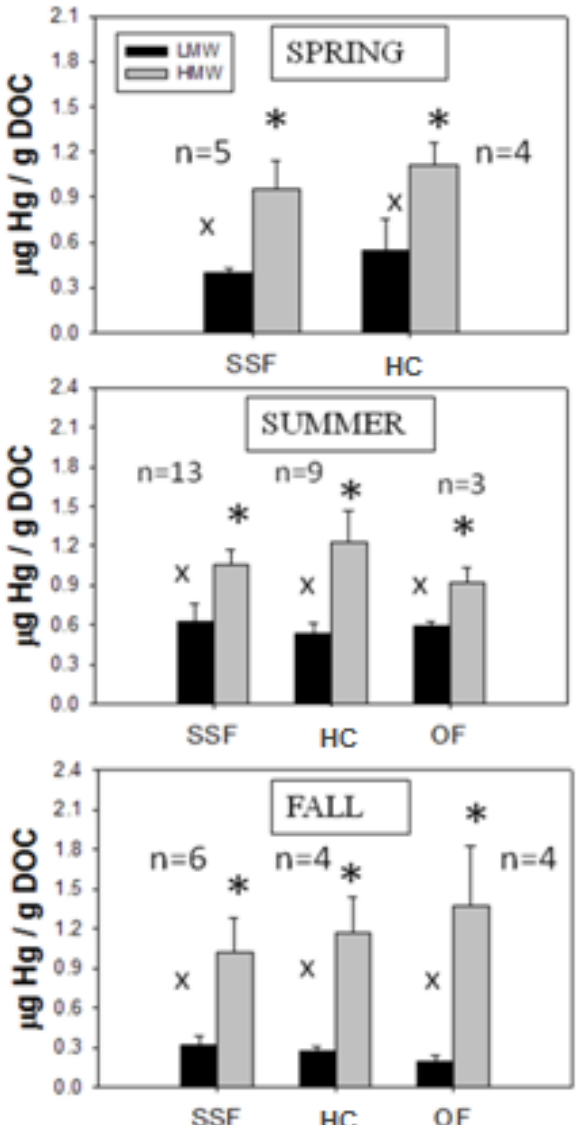


Figure 2.8. Hg/DOC concentrations across the watershed transect in HMW and LMW fractions demonstrating greater relative importance of HMW fractions in Hg binding. Data sets not significantly different from one another are grouped by the same symbol. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

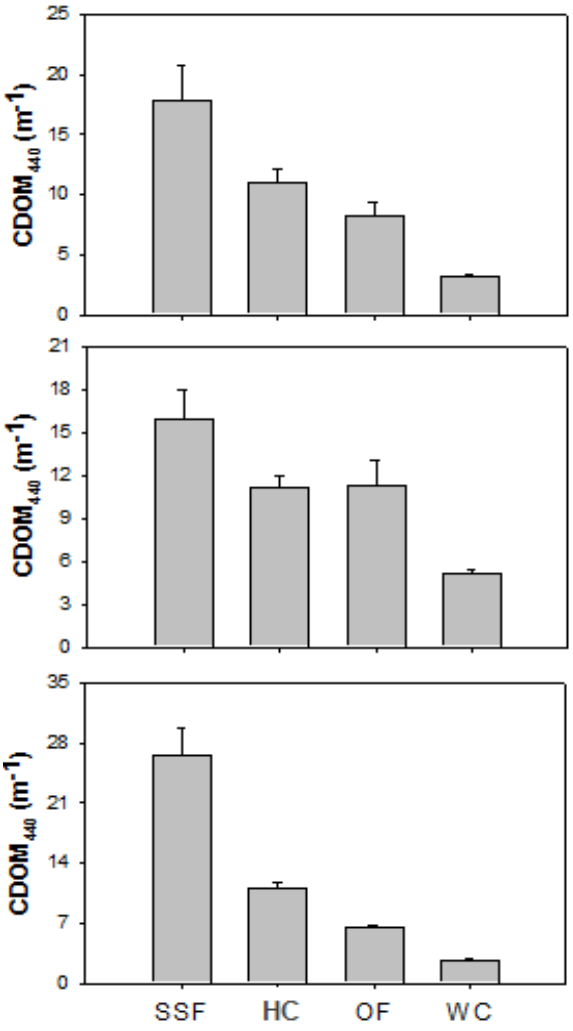


Figure 2.9. Decreasing $CDOM_{440}$ in the <0.7 µm fraction along watershed transect in the UP1 sub-basin. Sample types are subsurface flow, collected from lysimeters (SSF); mixed subsurface and overland flow through convergence zones (HC) collected directly from diversion walls constraining subsurface and overland flow; and pure overland flow (OF) collected from the gauged weir at the terminus of the UP1 sub-basin.

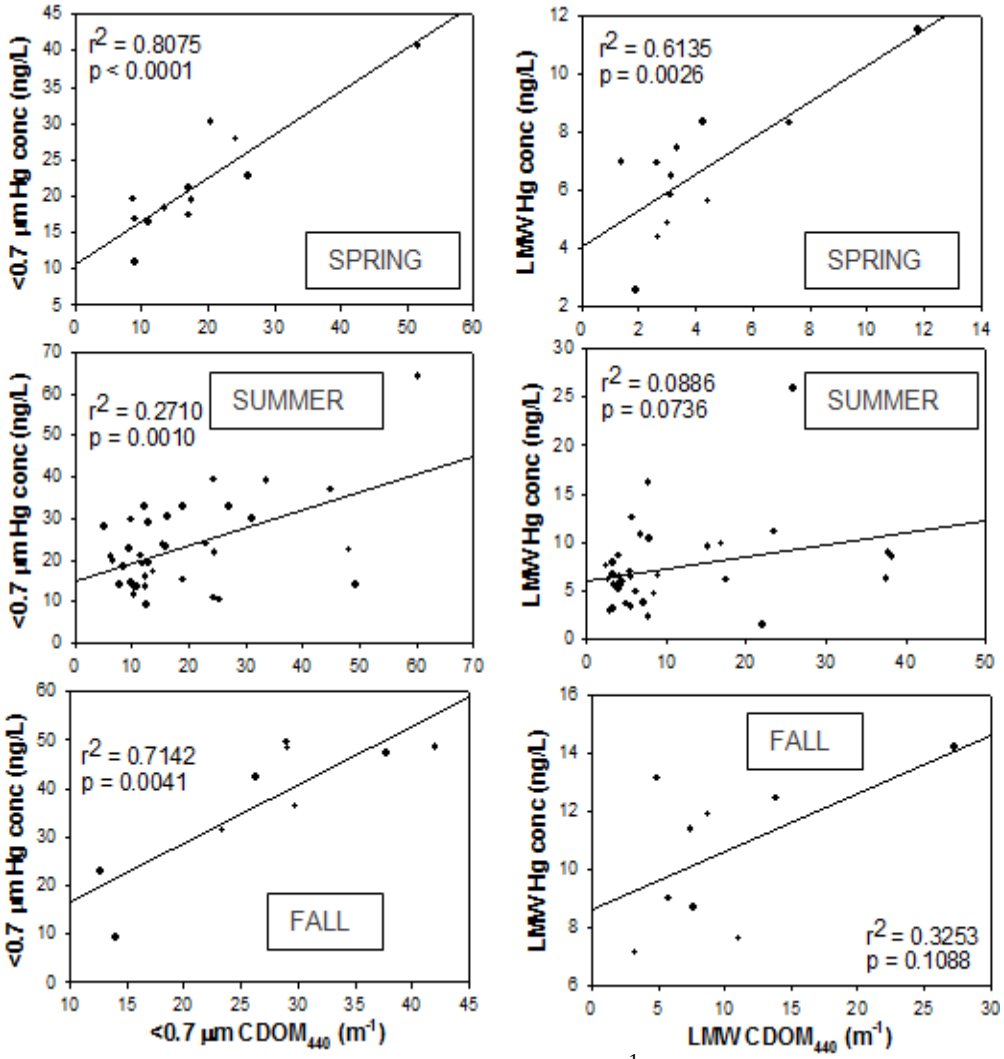


Figure 2.10. Correlations between $CDOM_{440}$ (m^{-1}) and Hg concentration (ng L^{-1}) in <0.7 µm and LMW (<3 kDa) fractions in all subsurface flow samples in the Lake 658 upland.

Table 2.1. Average ^{200}Hg concentration in samples from the Lake 658 upland.

Sample type	n	number of samples containing ^{200}Hg	Average ^{200}Hg conc (ng L^{-1}) (mean $\pm$ std error)
UP1 SSF	37	25	1.01 $\pm$ 0.12
UP1 HC	24	7	0.77 $\pm$ 0.14
UP1 OF	10	3	0.89 $\pm$ 0.13

Table 2.2. Contrast between upland flow samples containing ^{200}Hg and those that do not contain ^{200}Hg.

Parameter	**Samples with ^{200}Hg**	**Samples without ^{200}Hg**	**p value**	**Test**
% LMW	53.1 ± 2.5	56.7 ± 2.8	0.34	Student's t
$CDOM_{440}$ (m^{-1})	20.6 ± 1.9	12.9 ± 0.9	0.02	Mann-Whitney
$SUVA_{280}$(L mg^{-1} m^{-1})	3.0 ± 0.1	2.7 ± 0.2	0.15	Student's t
DOC (mg L^{-1})	42.3 ± 4.1	36.0 ± 4.7	0.03	Mann-Whitney

Chapter 3: Distribution of Ambient and Isotopically Labeled Hg in Soils in the Lake 658 Upland.

3.1. Introduction

Deposition from the atmosphere is the primary source of Hg to the terrestrial environment, which is in turn the dominant Hg source to the aquatic system in most watersheds (Grigal, 2002). Within terrestrial systems, soils comprise a significant pool of Hg, the magnitude of which is estimated to have increased substantially since preindustrial times (Smith-Downey et al., 2010). Hg complexation within soils is dominated by soil organic carbon (SOC), evidenced by the strong correlation between SOC and Hg concentrations in different soil horizons across land use types and geographical areas (Grigal, 2002; Smith-Downey et al., 2010; Biester et al., 2002). Release of SOC and associated Hg, particularly in the dissolved phase, is an important vector of Hg transport to aquatic systems, wherein Hg methylation occurs. However, the connections between Hg deposition to terrestrial systems and resultant concentrations of MeHg in aquatic systems are poorly understood. Thus, an investigation of SOC and Hg cycling within soils and biogeochemical factors affecting their release will contribute to understanding Hg dynamics in terrestrial systems.

In forested systems, SOC is produced from decomposition of canopy and ground vegetation and by leaching of carbon complexes from live standing biomass (Kaiser et al., 1996). Atmospherically derived Hg associated with those components is incorporated into soil pools as decomposition occurs (Smith-Downey et al., 2010; Hintelmann et al., 2002; Graydon et al., 2008). Hg deposition to soils is therefore delayed by its cycling within vegetation and litterfall. A pilot study in which a stable Hg isotope was applied to

a small catchment at the Experimental Lakes Area (ELA) demonstrated that one year after deposition, the majority of Hg was associated with vegetation, whereas soils contained only a small proportion (Hintelmann et al., 2002). 3 years following deposition of a stable isotope of Hg in the Lake 658 watershed, the majority of Hg in the uplands was bound within upper soil horizons, primarily the litter and upper organic layers (Harris et al., 2007). These litter horizons do not appear hydrologically important and do not contribute significant amounts of water to flow (Oswald et al., 2010, submitted). These studies indicate a significant delay exists between timing Hg is deposited from the atmosphere to initial points of contact (canopy and ground vegetation) and its incorporation into soils. During this time period, the Hg-organic matter complex is prone to significant environmental processing.

Within the soil horizon, SOC continues to undergo degradation during vertical translocation (Sanderman et al., 2008). This leads to increase in diagenetic state of organic matter with movement downwards in the soil profile and a concurrent decrease in SOC as carbon is mineralized. In general, Hg concentrations mirror SOC concentrations, and are higher in organic as opposed to the mineral horizons (Akerblom et al., 2008; Grigal, 2002; Oswald et al., 2010, in prep). Bulk density and SOC concentration are inversely correlated, which makes mineral layers, with low organic carbon concentrations but high density, a significant pool of Hg on a mass basis. Strong sorptive stabilization due to surface properties of mineral phases in these soil layers inhibits the release of dissolved organic matter (DOM) compounds (Kalbitz et al., 2005). Furthermore deeper soil layers generally do not contribute to lateral water flow, thus once the DOM and associated Hg migrate deep enough, the potential for DOM export is reduced (Biester et

al., 2002; Kalbitz et al., 2000; Kalbitz et al., 2005; Kiaser and Guggenberger, 2000). Conversely, Hg occurring in organic horizons could be mobile, as subsurface flow that supplies surface waters is primarily generated from those layers (Akerblom et al., 2008; Froberg et al., 2003; Tipping et al., 2005; Schiff et al., 1999). As SOC undergoes partitioning to the dissolved phase, associated Hg is also mobilized (Akerblom et al., 2008; Skyllberg et al., 2003; Kalbitz and Wennrich, 1998; Aastrup et al., 1991). Akerblom et al. (2008) measured consistent ratios of Hg to organic carbon in source area soils and subsurface flow, indicating that Hg-ligand binding does not change as partitioning into the dissolved phase occurs. However, this has not been widely observed since relatively few field studies contain data on Hg and organic carbon in both source area soils and flow generated through soils. In this study, field measurements of Hg and organic carbon were made in both soil and subsurface flow components to study changes in the nature of the Hg-NOM association in boreal soils.

Lignin phenols are a class of compounds found in the hemicelluloses matrix of the secondary cell walls of vascular plants (Wershaw, 2004) (Figure 1). Processing for analysis involves cleavage of the dominant β-O-4 bond that cross-links monomeric units by cupric oxide (CuO) oxidation or thermochemolysis. The derived phenolic compounds are grouped into four families: vanillyls (V), parahydroxy phenols (P), syringyls (S) and cinnamyls (C). Relative proportions of some compounds are specific to plant and tissue type: S compounds are present only in angiosperm tissue, while V compounds occur in both angiosperms and gymnosperms. The ratio of S to V can therefore be used to determine the relative influence of angiosperm and gymnosperm sources. S/V of pure source material (ie fresh tissue) is less than 0.1 for angiosperms and ranges between from

0.8 to 3.8 for gymnosperms (Bianchi, 2007). As tissues from various sources break down and are incorporated into soil organic matter, ratios are altered leaving soils with unique lignin signatures.

In addition to being applied as tracers of the source material for NOM, lignin signatures are useful as proxies of degradation since specific lignin phenol compounds are selectively degraded by bacteria and fungi during translocation in soil horizons (Teisserenc, 2009; Houel et al., 2006; Ouellet et al., 2009). Demethylation induced by brown rot fungal degradation leads to the selective loss of vanillyl and syringyl methoxylated groups, while p-hydroxy phenols (P) remain unaltered. Thus, P/(V+S) can be applied as a proxy of the diagenetic state of NOM. 3,5 dihydroxybenzoic acid (3,5-Bd), not defined as a lignin phenol, is a product of soil degradation derived from tannins and other flavonoids (Ouellet et al., 2009). Increase in 3,5-Bd in soils has been shown to occur with increased degradation of plant tissues, and specifically an increase in 3,5-Bd relative to V compounds has been applied as a proxy for the diagenetic state of NOM (Teisserenc, 2009). A final proxy of degradation that can be utilized from lignin phenols analysis is the ratio of vanillic acid to vanillic aldehyde, as aldehydes are converted to acids during degradation because of side chain oxidation by white rot and brown rot fungi or microbial processing. Lignin compounds are highly refractory compounds and undergo minimal alteration as they are transported from the terrestrial to the aquatic environment and delivered to sediments (Ertel and Hedges, 1984). Because lignins retain the unique chemical structure specific to the plant and tissue from which they are generated, they are useful as biomarkers of NOM, either with respect to source area material or as parameters of degradation (Teisserenc, 2009; Ouellet et al., 2009; Caron et

al., 2008; Dittmar and Lara, 2001). As stated, soils are likely the most useful indicator of source signature of lignins, since they contain information on the state of material present at the site of generation of DOC compounds, as opposed to raw source material (e.g. plant matter) which undergoes *in situ* processing before DOC is released.

With respect to Hg specifically, lignin biomarkers have been used to identify the source material for Hg-NOM compounds and to determine whether correlations between Hg concentration and diagenetic state of NOM occur (Teisserenc, 2009; Ouellet et al., 2009; Caron et al. 2008). Caron et al. (2008) applied lignin biomarker signatures to demonstrate that erosion of agricultural soils may be a source of elevated Hg in an aquatic system. Ouellet et al. (2009) found correlations between Hg concentrations and total lignin content in the water column of boreal lakes, implying that Hg in lakes is predominantly terrestrially derived. They also found an inverse correlation between Hg and diagenetic state of organic matter, which they postulate is a result of logging disturbance in the watershed which caused the release of fresh soil organic matter and associated Hg. Teisserenc (2009) found a direct correlation between Hg and diagenetic state of material as indicated by 3,5-Bd/V in some lake sediments of undisturbed watersheds, indicated that Hg is more strongly associated with highly degraded organic material in those matrices. These findings indicate that land use in the watershed has implications for transport of NOM and Hg and emphasize the importance of understanding the quality of the NOM moving through and from watersheds.

In Chapter 2 it was described that the molecular weight distribution of DOC was altered within soil profile, such that LMW compounds were released in greater proportions than HMW compounds. The majority of DOM migrating in subsurface flow was adsorbed, as

shown by the loss of the high molecular weight fraction, while older, LMW compounds were more mobile. There was also evidence that Hg transported by the DOM was also lost and the Hg reaching downstream areas was older. In this chapter, lignin biomarkers are applied to further test these hypotheses. S/V and $(Ad/Al)_{van}$ ratios were applied to determine whether Hg content in various matrices varies with respect to source area of NOM and whether these ratios could be applied as a tracer of source of subsurface flow within the soil profile. C_{18} solid phase extraction (SPE) was conducted for isolation of lignin compounds, which were subsequently processed for analysis by tetramethylammonium hydroxide (TMAH) thermochemolysis. Recovery of isolated lignin compounds with C_{18} SPE is generally high, ranging from 73 to 101% (Louchouarn et al., 2000; Spencer et al., 2010). Applicability of this method for the determination of degradation state of NOM is discussed in section, and details on the chemical reactions involved in TMAH thermochemolysis are given in Appendix B.)

3.2. Materials and Methods

3.2.1 Study Site

The study was conducted as part of the Mercury Experiment to Asses Atmospheric Loading in Canada and the US (METAALICUS) at the Lake 658 watershed (49°43.95′ N, 93° 44.20′ W) (Figure 2), situated approximately 18 km from the Experimental Lakes Area (ELA) base field station in northwestern Ontario, Canada. Lake 658 is a first order boreal lake, 8 ha in area, and the terrestrial upland and wetland comprise 43 ha. The terrestrial upland is comprised of 14 ha portion of old growth forest, dominated by mature black spruce (*Picea mariana*) and balsam fir (*Abies balsamea*), a 21

ha area which was burned in 1983 and now supports young jack pine (*Pinus banksiana*), and a 6 ha area deciduous stand, subject to logging in 1978 and comprised of red maple (*Acer rubrum*), white birch (*Betula papyrifera*) and trembling aspen (*Populous tremuloides*). A 2 ha wetland, which drains into the west basin, supports a mixed stand of black spruce, wetland alder (*Alnus sp*) and tamarack (*Larix laricnia*). Thin podzolic soils dominate the upland and are underlain by pink Precambrian granodiorite. As part of the METAALICUS project, the watershed was spiked with enriched stable isotopes of Hg at a rate of approximately 22 µg Hg m^{-2} $year^{-1}$, which is roughly equivalent to deposition in contaminated regions of Europe and North America (Sandilands et al., 2008). Each year from 2001 to 2006, one of each of the isotopes was added to the upland (^{200}Hg), the wetland (^{198}Hg) and the lake surface (^{202}Hg). The isotopes are discernable from ambient Hg present in the environment, allowing researchers to track Hg as it is transported through the terrestrial environment, and within the lake ecosystem. This study examines proportion of ^{200}Hg in upland samples.

3.2.2 Field Sampling

Zero tension lysimeters were installed in organic soil horizons throughout the upland of the Lake 658 watershed in 2006 and 2008 (Figure 3.2b, indicated as subsurface flow collection sites). The zero tension lysimeters collect only soil pore water, indicated here as subsurface flow that is likely to drain saturated soil horizons and flow down slope. Location of lysimeter installation was determined with the use of Light Detection and Ranging (LiDAR) maps, which allowed us to identify subsurface flowpaths (Sandilands et al., 2008). Lysimeters were installed in three different sub-basins to include different forest-soil assemblage types present in the Lake 658 upland (Figure

3.2a, Richardson et al., 2009). Lysimeter samples were collected in double bagged clean 500 ml PETG (glycol-modified polyethylene terephthalate) bottles attached to lysimeters by acid washed C-flex® tubing. The bottles were placed within plastic boxes dug into the ground to protect the samples from heat and sun exposure. A total of 13 lysimeters were installed – five in the UW sub-basin, five in the Upland 1 (UP1) sub-basin and three in the C2 sub-basin (Figure 3.2c). Water was also collected from flow at two areas of hydrologic convergence where diversion walls were built to constrain subsurface flow through shallow moss layers but water passing through these sites may also contain an overland flow component (Figure 3.2c). These samples were collected using 500-ml PETG bottles stored in doubled plastic bags. Streamflow was also collected in double bagged PETG bottles at the gauged weir near the terminus of UP1 (Figure 3.2c). Samples were collected repeatedly during three sampling periods: May 7 to- 18; June 24 to July 6; and October 14 to 28; 2009. Lake water column samples were collected using acid cleaned Teflon tubing and a peristaltic pump at depths of 2 m at sites CB-W, UW, UP1, C2 and 13 m at CB-W once per field trip (Figure 3.2b).

Soil samples for a leachate experiment were collected in May, 2009 from each of the three sub-basins (sites not shown). Soils were taken at a depth of approximately 10 cm and include an integrated sample of fresh litter/live layer and surface organic soil. Samples were placed in acid washed plastic containers (Ziploc) and submerged in Milli-Q water. Samples were stored in the dark and refrigerated at 4^0C for approximately 4 weeks, after which time leachate water was removed. Leachate and lysimeter waters were subsampled for Hg, DOC and lignin phenolic content. Samples for Hg and DOC analysis were filtered through pre-combusted 0.7 µm GF/F filter and acidified to 0.5% by volume

with ultra trace grade HCl in 250-ml PETG bottles. DOC samples were stored frozen in 20-ml amber vials. Sub samples for lignin phenol analysis were filtered through 0.2 polycarbonate filters (Millipore), acidified to pH 2 using ultra trace grade HCl and stored at 4 ^{0}C in preparation for C_{18} SPE extraction.

Soil samples were also collected from sites throughout the Lake 658 upland (Figure 3.2.b). Samples were taken from live/litter, organic and mineral horizons based on visual assessment of the soil profile. Designations were later made based on SOC concentration, measured after collection (Table 3.1)

3.2.3. Laboratory Methods

3.2.3.1. Processing of Samples for Hg Analysis

Soils and sediment samples were digested on a hot plate at 250 ^{o} F for 6-8 hours in 5 - 10 mL of 70:30 nitric acid: sulfuric acid. Samples were cooled and brought up to a total volume of 50 ml in Milli-Q water. Approximately 24 hours prior to analysis, digested soil extracts and water (subsurface flow and leachate) samples were brominated with bromine monochloride (BrCl) to oxidize residual organic matter. Hydroxylamine hydrochloride (NH_2OH*HCl) was added to neutralize excess BrCl prior to analysis. A Tekran Model 2600 Cold Vapor Atomic Fluorescence Spectroscopy (CVAFS) was used to perform inline reduction of Hg with tin chloride ($SnCl_2$) and Hg detection was done by both CVAFS and ICP-MS (for isotopic analysis) methods by plumbing the instrument in sequence. The standard reference material NIST 1944 (New York/New Jersey waterway sediment, NIST, USA) was analyzed along with digestion blanks, blank spikes, sample spikes and duplicates were measured as part of the quality assurance/quality control protocol. Duplicate SRM concentrations and samples concentrations were required to be

within 10% of each other and SRM concentrations were within 10% of the reported concentration of 3.4 mg kg^{-1}. Digestion blank concentrations were 2.22 $\pm$ 0.22 ng L^{-1} (mean $\pm$ std deviation) and calibration blanks were 0.12 $\pm$ 0.1 ng L^{-1}. Sample and blank spike recoveries were between 90% and 110%. Standards used for standard curve generation were prepared with concentrations of 0.5, 1, 5, 10, 20 and 50 ng L^{-1}.The quality of the curve was based on attaining $r^2 > 0.999$. Hg concentration in soil sample is denoted Hg_S and Hg concentration in aquatic samples in the dissolved phase is denoted Hg_D.

3.2.3.2. Lignin Phenol Processing by C_{18} SPE and TMAH Thermochemolysis

Concentration and isolation of lignin phenols in aquatic samples (leachate, subsurface flow, streamflow and water column samples) was conducted using the method outlined by Louchouarn et al. (2000). A C_{18} column with 10 g of sorption material composed of octadecyl carbon moieties (C_{18}) chemically bonded to a silica support (C_{18}-SPE Mega-Bond Elut, Varian, USA) and a bed volume of 60 ml was used for lignin phenol isolation. Methanol (MeOH, Ultra Resi-Analyzed, Baker, USA) was used to activate the columns and for subsequent sample elution. Columns were pretreated with 100 ml of MeOH, then 50 ml of Milli-Q water that was acidified to pH 2 with reagent grade HCl (Instra-Analyzed, Baker, USA). Filtered samples were also acidified to pH 2 using HCl, then 300-500 mL of sample were poured into the headspace of the pretreated C_{18} columns while the columns were still wet. Samples were drained through the column by gravity, allowing sufficient time for sorption of the hydrophobic DOM compounds. After extraction, a small amount of Milli-Q water was used to keep the column moist, the bottom and top of the column were wrapped with Parafilm, and the columns were stored

refrigerated. Prior to sample elution, columns were allowed to warm to room temperature, then 50 ml of MeOH was used to elute DOM into pre-combusted round bottom flasks. Samples were evaporated (Buchi R-210 Rotavapor, USA) to approximately 2-4 ml, then frozen at -80^{o}C for 3-4 hours prior to being freeze dried for 24 hours at a vacuum of 100 millitorres (Virtis Benchtop Freeze Dryer, USA). Soil (and sediment) samples, having been frozen after collection, were freeze dried directly. The freeze dried samples were transferred to pre-combusted 10-ml amber vials in 2.0 ml of MeOH. Samples were dried under nitrogen, brought up in a known volume and an aliquot of the sample containing approximately 2 mg was transferred to ampoules for TMAH thermochemolysis. Ampoules were made from 48 inch hollow Pyrex rods (9 mm o.d., VWR Scientific) by breaking the tube into 8 inch long sections. One end of each was fire polished, while the other end was sealed using a flame. A dimple was formed at approximately 6 inches from the sealed end to be used to flame seal the ampoule under vacuum following thermochemolysis.

After sample addition, approximately 1 mg of each of three internal standards was added to ampoules: ethyl vanillin, *p*-coumaric acid and nonadecanoic acid (Sigma-Aldrich, USA). 200 µl of TMAH (Sigma-Aldrich, USA, 25 wt % solution in MeOH) was added to each ampoule immediately before the samples were put under vacuum. Samples were evacuated for 3 hours and then flame sealed and heated in a GC oven at 250^{o}C for 30 minutes. Ampoules were cooled to room temperature, cracked open and samples were transferred with three rinses of dichloromethane to pre-combusted amber vials. Samples were dried under nitrogen, then brought up in 50uL of N,O-bis(trimethylsilyl)trifluoroacetamide **(**BSTFA, Sigma-Aldrich, USA) 10% by volume

solution in pyridine (Sigma-Aldrich, USA) and the sample was heated in a heating block at 50^0C for 30 minutes to induce trimethylsilation of any unmethylated hydroxyl groups. Samples were dried under nitrogen then brought up in a known volume of dichloromethane for subsequent GC-MS and GC-FID analysis.

3.2.3.3. Lignin Phenol Analysis by GC-FID and GC/MS

Lignin compounds were quantified and identified using the approach outlined by Mannino (2000). The procedure involves quantification by gas chromatography with flame ionization detection (GC-FID) (Agilent 6890N Network GC system, USA) and identification by Gas Chromatography Mass Spectrometry (GC-MS) (Agilent 6890 Series GC system coupled to an Agilent 5973 Network Mass Selective Detector, USA) operated in electron ionization mode. For GC-FID detection a 60 m DB-5MS fused silica column (0.32 mm internal diameter, 0.25 mm film thickness, J&W Scientific, USA) with carrier gas (hydrogen) flow rate at 2 ml min^{-1} and with four step temperature ramp of 10^0C min^{-1} from 50 ^{0}C to 120^0C followed by 3^0C min^{-1} to 200^0C and 4^0C min^{-1} to 300^0C was used. For GS-MS helium was used as the carrier gas.

The lignin compounds analyzed and quantified included vanillin (G4), acetovanillone (G5) and vanillic acid (G6) from the vanillyl (also known as guiacyl) family, and syringin (S4), acetosyringone (S5) and syringic acid (S6) from the syringyl family. Unlike the more traditional CuO oxidation method to measure lignin phenols, the TMAH thermochemolysis technique applied in this study induces methylation of all hydroxy groups, leaving compounds present in the sample as phenols indistinguishable from those present in the sample as 3,4-dihydrodxy or 3,4,5-trihydroxy compounds. As a result, the lignin signal measured from fresh and degraded lignin from the same source

material will be identical when measured by TMAH thermochemolysis. This precludes the application of ratios of lignin compounds that have been implicated as parameters of degradation in other studies. P/(V+S), for example, produces a different signal from fresh and degraded lignin, when measured by CuO oxidation, but is identical when measured by TMAH thermochemolysis (see Appendix B for details on TMAH thermochemolsyis reactions).

3,5-Bd/V is another parameter of degradation applied in studies (Ouellet et al., 2009, Teisserenc, 2009, Caron et al., 2008). 3,5-Bd, a product of soil decomposition, the likely precursors of which are tannins and other flavonoids with hydroxy groups present on alternate positions on the ring. 3,5-Bd has been shown to increase in proportion to diagenetic state of NOM in soil matrices (Teisserenc, 2009, Houel et al., 2006). Thus, the 3,5-Bd content is expected to increase with degradation, while the V content decreases, making it an effect tracer of degradation. However, tannin compounds, the parent material of 3,5-Bd are themselves subject to methylation by TMAH thermochemolysis, rendering them indistinguishable from 3,5-Bd. Additionally, TMAH thermochemolysis prevents the detection of changing V content with degradation, as stated above. The influence of tannins likely to affect the signal of 3,5-Bd and the inability to distinguish between degraded and fresh V compounds make TMAH thermochemolysis ineffective at detecting changes in 3,5-Bd/V with degradation. A more detailed explanation with specific test identifying the phenomena is presented in Appendix B.

3.2.4. Statistical Analysis

Student's t test was applied to test for differences in Hg and DOC concentrations between subsurface flow and leachate samples. These data were normally distributed.

The Mann-Whitney U test was applied to test for differences in Hg and organic carbon content among soils as these data were not normally distributed due to small sample size. Significance was evaluated at $p = 0.05$.

3.3. Results

3.3.1. Distribution of Ambient and ^{200}Hg in Soils

SOC concentrations were applied to designate soil horizons as live/litter, organic or mineral horizons (Table 3.1). The relatively low carbon concentration in the organic soil horizon implies that the material sampled represents lower organic horizons (A horizons). The samples were taken on bulk and reflect visual layer continuity rather than depth. This sampling approach may not have captured the gradient of Hg penetration resulting from increased anthropogenic deposition as upper organic horizons are not represented. Still, a wide range of SOC concentration was present and ambient Hg concentration was significantly correlated with SOC concentration across soil horizons (Figure 3.3), while $^{200}Hg_S$ concentration was not ($r^2 = 0.1598$, $p = 0.1250$). In general, ambient and $^{200}Hg_S$ exhibited different distributions in upland soils, indicating that ^{200}Hg does not reflect the behavior of the larger Hg pool. The concentration of ^{200}Hg in the live/litter horizon was more than 10 times that in the organic horizon, while ambient Hg concentration in the live/litter was approximately twice that of organic soils (Figure 3.4). Ambient Hg_S/SOC concentration was not significantly different between litter and organic horizons, while in the mineral horizon they were higher and significantly different than both litter and organic horizons (Figure 3.5). $^{200}Hg_S$/SOC concentrations were significantly different between live/litter and organic horizons, with live/litter

containing the highest Hg_S concentration; ^{200}Hg was not detected in mineral horizons (Figure 3.5).

3.3.2. Release of Hg from Soils in Subsurface Flow and Soil Leachate

Soil leachate from the upper soil horizon was obtained by submerging soil in water to enhance physical degradation. We assumed negligible biological degradation because samples were stored at 4^0C and negligible carbon and Hg loss via volatilization or photochemical reduction since samples were covered and stored in the dark. The soil leachate was compared to water obtained from lysimeters, which also contain DOM extracted from soils, however the lysimeters contain additive water, meaning it has passed between horizons and is less discrete than the leachate. Subsurface flow was also subject to dilution and antecedent moisture conditions will influence DOM constituents. Leachate on the other hand represents stagnant conditions, not likely to be encountered in these soil environments and thus absolute concentrations will likely exceed those of normal runoff. On average, leachate samples contained DOC, ambient Hg and ^{200}Hg concentrations well above those observed in subsurface flow waters (Table 2). However, ambient Hg/DOC and ^{200}Hg/DOC concentrations were not significantly different between subsurface flow and leachate samples.

3.3.3 Lignin Phenolic Compounds

S/V and $(Ad/Al)_{van}$ was measured in soils, soil leachate and subsurface flow in UP1 samples as biomarkers of source material. $(Ad/Al)_{van}$ values did not vary significantly between soil and subsurface flow samples, but values were significantly higher in leachate samples than both soil and subsurface flow waters (Table 3.3). S/V also did not vary widely between soil horizons, source areas or sample types, with values

falling between 0.03 and 0.55 (mean: 0.18 $\pm$0.06) (Figure 3.6). Average S/V was 0.12 $\pm$ 0.03 when soils were combined and 0.11 $\pm$ 0.02 in subsurface flow. In UP1 samples, S/V and $(Ad/Al)_{van}$ (leachate samples excluded) did not vary widely among sample types, implying little modification occurs along the watershed transect. Neither ambient nor ^{200}Hg concentration or Hg_S/SOC were related to S/V or $(Ad/Al)_{van}$ in any sample matrices.

3.4. Discussion

3.4.1. Distribution of Ambient and ^{200}Hg in Soils

As expected, ambient Hg_S complexation in soils was dominated by SOC, demonstrated by the significant correlation between ambient Hg_S and SOC concentrations across soil horizons and sample types (Figure 3.3). However, the strength of the correlation varied between live/litter (r^2 = 0.4481, p = 0.2165), organic (r^2 = 0.4200, p = 0.1154) and mineral (r^2 = 0.8672, p = 0.0069) horizons and was only significant in the mineral horizon. As expected, ambient Hg_S complexation in soils was dominated by SOC, as indicated by the significant correlation between ambient Hg_S and SOC concentrations across soil horizons and sample locations (Figure 3.3). However, the strength of the correlation varied between live/litter (r^2 = 0.4481, p = 0.2165), organic (r^2 = 0.4200, p = 0.1154) and mineral (r^2 = 0.8672, p = 0.0069) horizons and was only significant in the mineral horizon. This demonstrates that in upper soil layers, Hg_S concentration was less strongly correlated with total SOC concentration, consistent with other studies (Obrist et al., 2009). The increase in the strength of the correlation between Hg and SOC in mineral horizons as well as the enrichment of Hg with respect to SOC in

those layers may be driven by the mineralization of carbon during decomposition, which results in shrinking carbon pools with depth. During this process Hg accumulates and becomes associated with more recalcitrant organic carbon in the deeper soil horizons. The distribution of ambient Hg in the soil profile (Figure 3.3a) is somewhat in contrast to data from an extensive soil survey conducted in the Lake 658 upland (Oswald et al., 2010, in prep). The authors report that the concentration of ambient Hg is highest in the organic horizon, followed by the live/litter horizon, and the mineral horizon. The observation of enrichment of ambient Hg in the litter layer observed in this study may be a result of the timing of sample collection, the location from within the soil horizons samples were taken, and the relatively small sample set. Samples were collected only once in October, 2009, in the period following leaf fall. As leaf litter contains Hg, which is a source of Hg to the surface soil layers (Graydon et al., 2008; St. Louis et al., 2001), this sudden input of Hg in the fall could have temporarily influenced the Hg distribution. Schiff et al. (1997) indicate that a significant amount of organic carbon can be leached from fresh leaf litter within a week, thus transport of associated Hg is also likely.

The distribution of ^{200}Hg in this study (Figure 3b, Figure 4b) does correspond with data from Oswald et al. (2010, in prep), demonstrating that the concentration of ^{200}Hg in the live/litter horizon is consistently larger than in the lower organic horizons and present in undetectable or trace quantities in the mineral horizon at the majority of sites. This provides evidence that most of the Hg deposited between 2001 and 2006 has yet to migrate from litter and surface organic horizons into deeper soil layers. The relatively low ^{200}Hg concentration in subsurface flow and streamflow in this watershed indicates flow is largely generated from layers with low ^{200}Hg, and supports the observation that

organic horizons are the dominant source of flow within terrestrial systems (Froberg et al., 2003; Tipping et al., 2005; Akerblom et al., 2008). The distribution of $^{200}Hg_S$/SOC (Figure 3.4a) from this study also corresponds with Oswald et al. (2010, in prep), demonstrating highest concentrations in live/litter, then organic horizons. Enrichment of $^{200}Hg_S$/SOC in live/litter horizon is further evidence that ^{200}Hg has not yet fully been equilibrated into soils pools; this is also implied by the lack of correlation between ^{200}Hg and SOC.

Though there is some evidence that deciduous and coniferous tissues vary with respect to relative Hg concentration (Rasmussen et al., 1991; Moore et al., 1995), but in this study there was no relationship observed between Hg concentration and S/V in the sample matrices examined, in accordance with other studies (Teisserenc, 2009; Ouellet et al., 2009). This is not surprising as litterfall Hg concentration do not vary widely between forest types in the Lake 658 upland or elsewhere (Graydon et al., 2008; Maňkovská, 1996). This study provides further evidence that relative influence of angiosperm and gymnosperm sources is not an important factor controlling Hg cycling in boreal forest soils. Additionally, S/V content of different sample types was relatively consistent throughout the watershed and between different soil horizons, despite attempting to select sites with varying angiosperm and gymnosperm dominance. Thus S/V ratio is unsuitable as a tracer of source of flow within the soil horizon in this system.

3.4.2. Release of Hg from Soils in Leachate and Subsurface Flow

The soil leachate experiments indicate that ambient Hg_D and $^{200}Hg_D$ were easily solubilized from litter and upper organic horizons (Table 3.2). While DOC and Hg_D concentrations generated were much higher than those observed being released from soils

under ambient conditions, the ratio of Hg_D/DOC concentration was not significantly different between leachate and lysimeter samples (Table 3.2). Though the compounds represented in the two sample types are subject to different processing mechanisms, the Hg_D/DOC ratio produced is relatively consistent between them. This implies that in response to different types of environmental degradation, there is little to no change in the Hg to carbon ratio even as DOM is degraded. $^{200}Hg_D$/DOC concentrations are also similar between sample types, implying that though ^{200}Hg is present in much lower levels, its distribution with respect to DOC concentration is similar between sample types and that, like ambient Hg, the ratio of newly deposited Hg with respect to organic carbon is similar in different sample types.

These findings also demonstrate that it is feasible to use soils to generate substrates of DOC and Hg, which can be used to test bioavailability of Hg released from soils to methylation by sulfate reducing bacteria. In particular, studies of leachate produced from soils in the Lake 658 watershed would provide a particular advantage since there is the potential to generate both ambient and ^{200}Hg, allowing for the comparison of bioavailability of recently deposited and historic Hg.

3.4.3. Lignin Phenol Compounds

In some leachate samples examined, $(Ad/Al)_{van}$ ratios that are well beyond those observed in the natural environment, with $(Ad/Al)_{van}$ in leachate samples samples averaging 11.22 ± 2.27 (mean $\pm$ std error). In comparison, the highest values in mineral soils in a boreal system was measured as approximately 3.5 (Teisserenc, 2009) (Table 3.3), and maximum values observed in mangrove sedimentary organic matter was 1.3 (Dittmar and Lara, 2001). The elevated $(Ad/Al)_{van}$ content in this study appears to be the

result of very high vanillic acid signal in high DOC leachate samples (Table 3.3). Presumably, the DOC released in such samples is comprised of a greater diversity of compounds, and there is the potential for the presence of hydrolysable tannins to influence the vanillic acid signal.

A relatively narrow range of S/V values was observed in this study (Figure 3.6). S/V content of fresh plant material has been found to range from 0.01 to 3.5 (Teisserenc et al., Goni and Hedges, 1992), and Teisserenc (2009) observed S/V values between 0.2 and 0.9 in organic and mineral boreal soils. The even narrower range of values measured in this study implies that the relative influence of angiosperms and gymnosperm cannot be determined in this system. Alternately, this could be in part due to the sampling approach of this study as no fresh litter was examined and samples were taken from throughout the upland in areas in close proximity to lysimeter sites so that source material signatures could be compared with signatures of subsurface flow. A sampling scheme aimed at sampling isolated deciduous or coniferous stands within sub catchments would likely produce more varied S/V ratios. However, these signatures would soon be lost in subsequent downslope water mixing.

Hg was not related to S/V or $(Ad/Al)_{van}$ content of any sample matrices examined. This implies that Hg binding is not influenced by relative contribution of angiosperm versus gymnosperm sources. However, studies of vegetation in the Lake 658 upland have shown that the concentration of Hg in coniferous versus deciduous tissue does vary (Gradyon et al. 2009). The absence of any correlation observed between Hg and S/V in the samples examined was also likely due to the sampling approach. Samples were obtained from soil horizons at depths where NOM has undergone decomposition and

does not represent fresh material. All the sites sampled in this study contained both deciduous and coniferous vegetation in some amount, although proportions varied. The resulting S/V signature demonstrates that inputs of both are present to such a degree and did not vary widely enough between samples to capture any variation in Hg content resulting from vegetation types. Again a sampling approach targeting isolated deciduous and coniferous stands would be useful in identifying whether decomposed litter of the two types of vegetation vary with respect to Hg content, but again, this signature would soon be lost downslope.

3.5. Conclusions

This study demonstrates that concentrations of ambient Hg, when normalized to organic carbon content, do not vary widely between different sample matrices in the Lake 658 watershed. Newly deposited Hg, represented by ^{200}Hg in the METAALICUS watershed, resides primarily in surface layers for substantial periods, (greater than 5 years), and is unlikely to contribute significantly to subsurface flow. As Hg is translocated downward in the soil profile during decomposition of associated organic matter as part of the soil forming process. The subsequent DOM produced lies in the more hydrologically conductive soil. It is from these layers that OM can be solubilized and more readily mobilized, enhancing transport of associated Hg. Since this has not yet happened to OM carrying isotope, it is important that the distribution of ^{200}Hg within the soil horizons and its concentration in the different components of flow in the Lake 658 upland continue to be monitored. Thus the length of time it takes for Hg to be released from forested environments following atmospheric deposition can actually be quantified.

Leachate samples also demonstrate that a high concentration of ^{200}Hg, along with ambient Hg, occurs in surface and upper organic horizons, which has the potential to be released and transported in flow through the upland. However, lower Hg and DOC concentrations in lysimeter waters, representative of what is actually occurring in this system, implies that in the natural environment, biological degradation and volatilization are important loss mechanisms for DOC and Hg.

The relative influence of angiosperm and gymnosperm sources, as indicated by S/V, did not vary widely between different source areas in the Lake 658 upland and was not related to Hg binding in soils, subsurface flow, leachate or water column samples. This lack of relationship likely stems from coniferous and deciduous litter Hg concentrations being similar and relatively consistent influences of those litter types in the Lake 658 upland. TMAH thermochemolysis is effective for examining S/V but not for measurement of diagenetic state as it has a number of draw backs that restrict interpretation (Appendix B). The application of CuO oxidation is a better approach in this system and would allow for measurement of lignin phenolic compounds that provide information about diagenetic state of NOM, which is expected to be an important factor determining Hg binding (Chapter 2; Ouellet et al., 2009; Teisserenc, 2009; Dittmar and Lara, 2001).

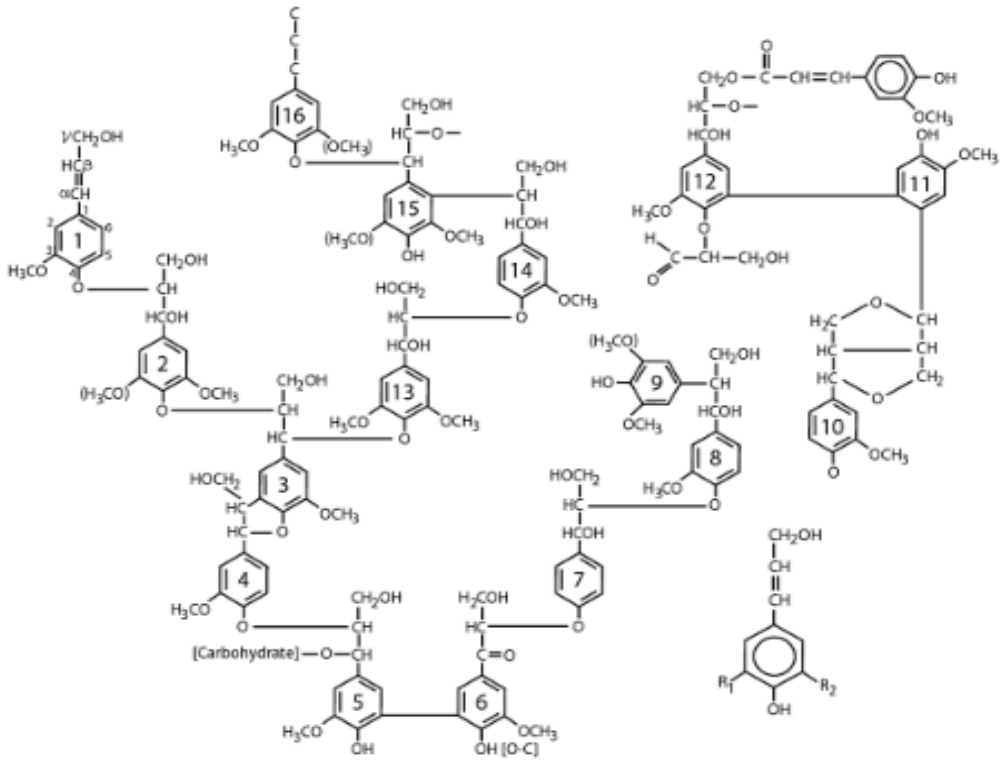


Figure 3.1. Lignin compounds, composed of connecting units of monolignols cross-linked by carbon to carbon and the dominant B-O-4 aryl ether bonds. (From Bianchi, 2007)

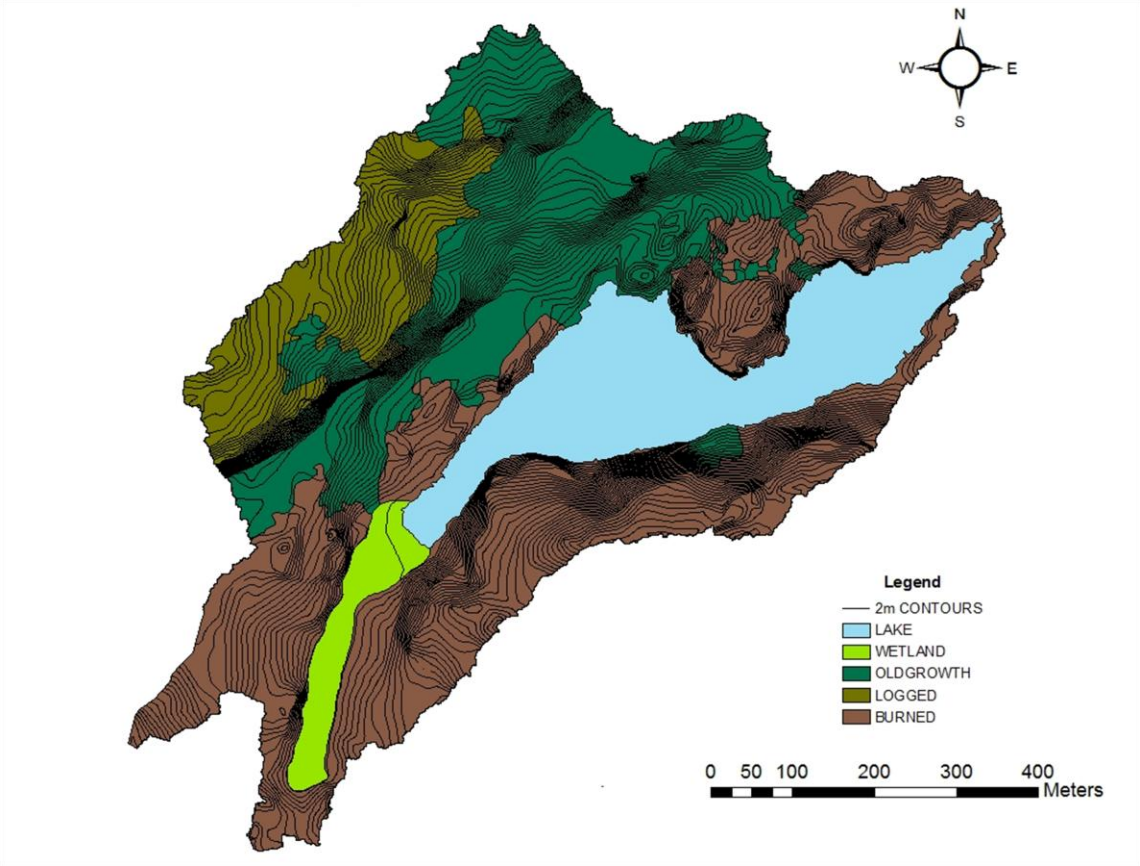


Figure 3.2 a) Map of the Lake 658 watershed showing general forest types and contour lines.

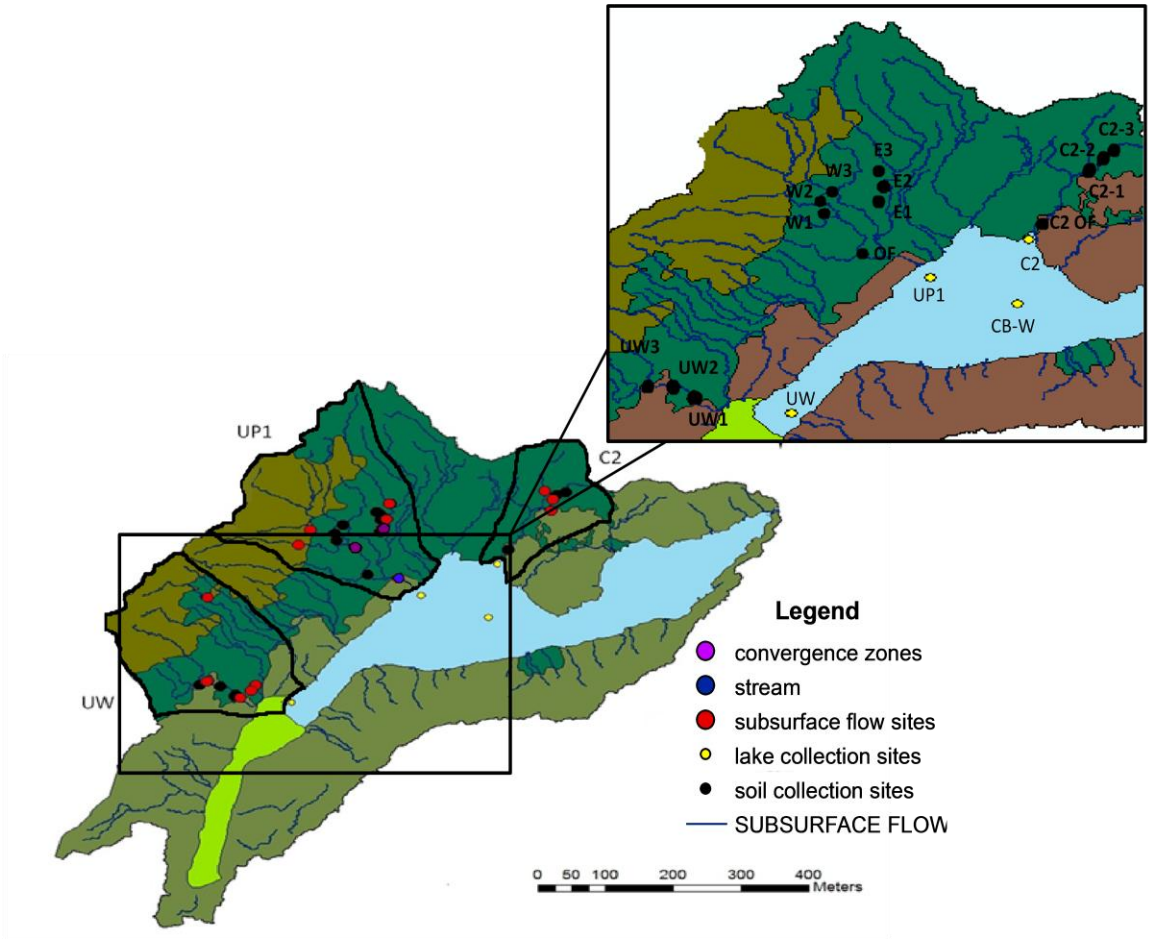


Figure 3.2b) Map of the Lake 658 watershed showing forest types, subsurface flowpaths and location of sub-basins, and soil and water column collection sites.

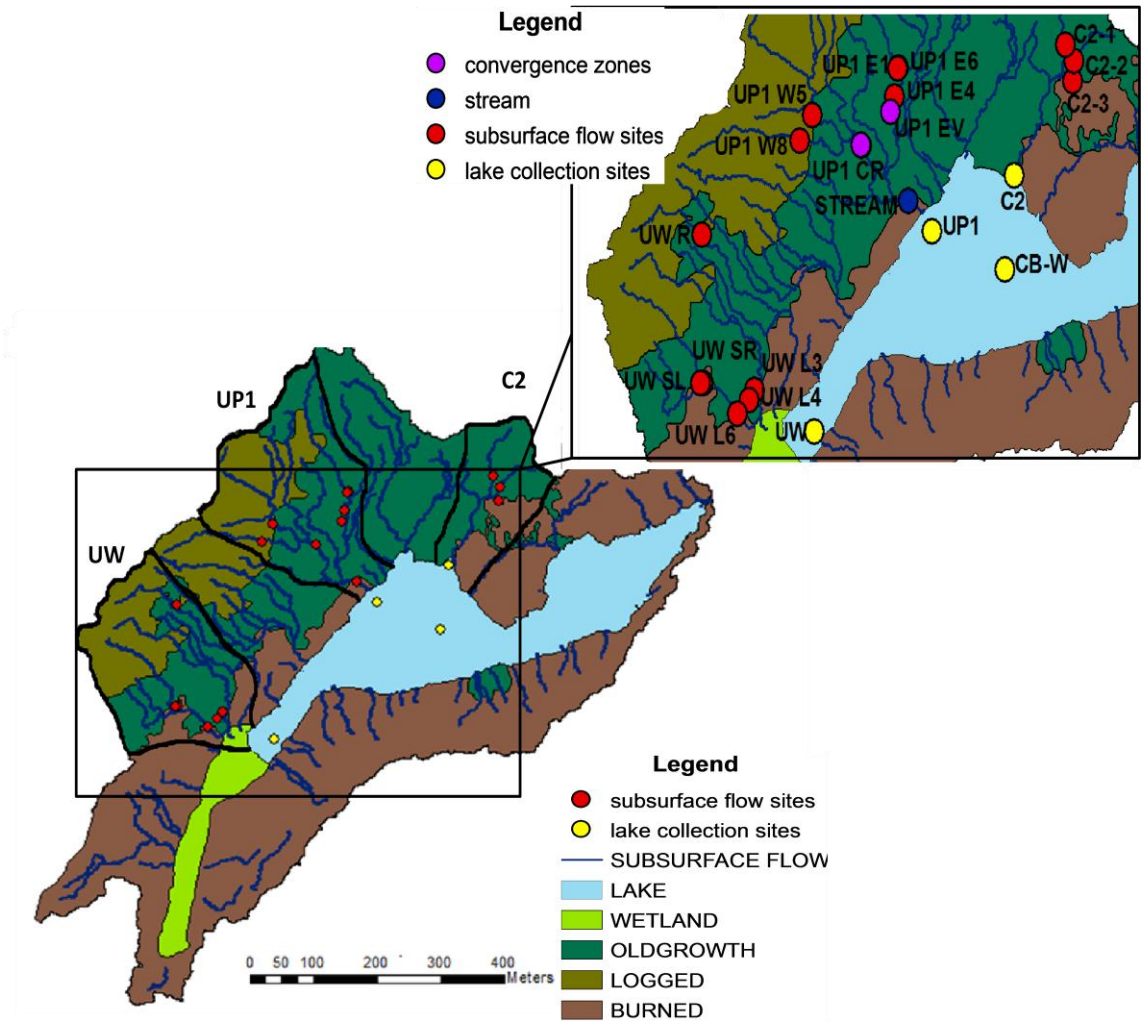


Figure 3.2.c) Map of the Lake 658 watershed showing UW, UP1 and C2 sub-basins, location of subsurface flowpaths. The insert is an expanded map showing sites of collection of subsurface flow, flow at zones of hydrologic convergence, streamflow and lake water.

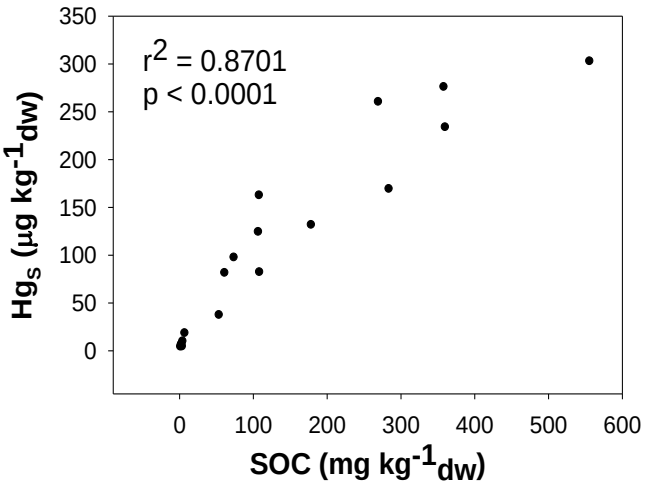


Figure 3.3. Correlation between the soil mercury (Hg_S) and soil organic carbon (SOC) concentrations for the Lake 658 upland.

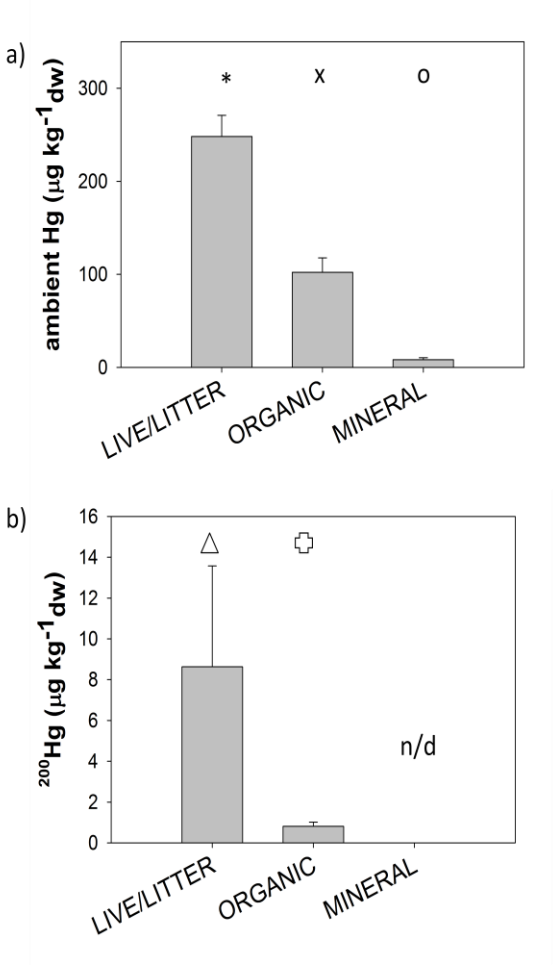


Figure 3.4. Concentration of a) ambient Hg and b) $^{200}Hg_S$ (mean $\pm$ std err) in soil horizons in the Lake 658 upland. For both ambient and upland spike, Hg concentrations were significantly different in each horizon tested by a Mann-Whitney U test ($p = 0.05$) $n = 6$ for all sample groups. n/d indicates non-detect.

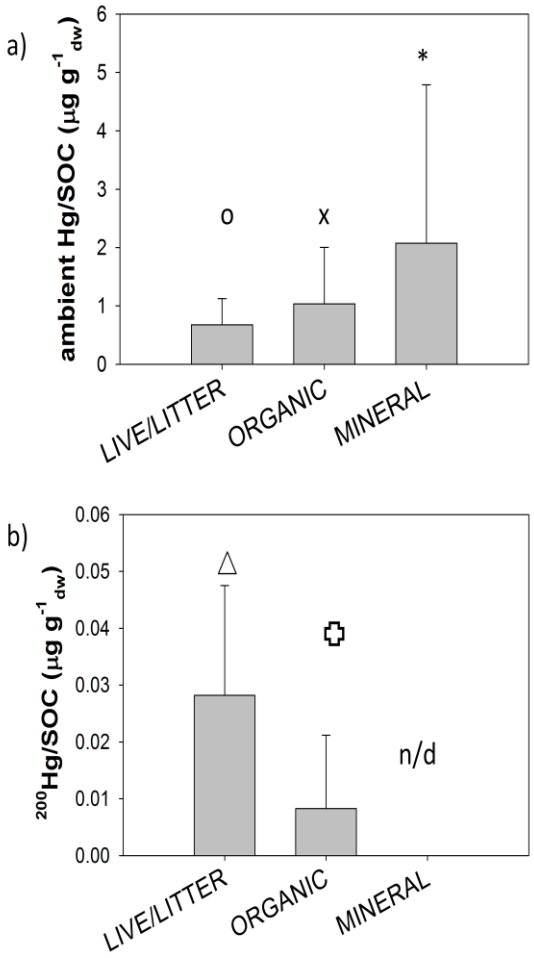


Figure 3.5. Concentration of a) ambient Hg/SOC and b)^{200}Hg /SOC (mean $\pm$ std err) in soil horizons in the Lake 658 upland. An identical symbol means the difference between the Hg concentrations is not significant as determined by Mann-Whitney U test. n = 6 for all sample groups. n/d indicates non-detect.

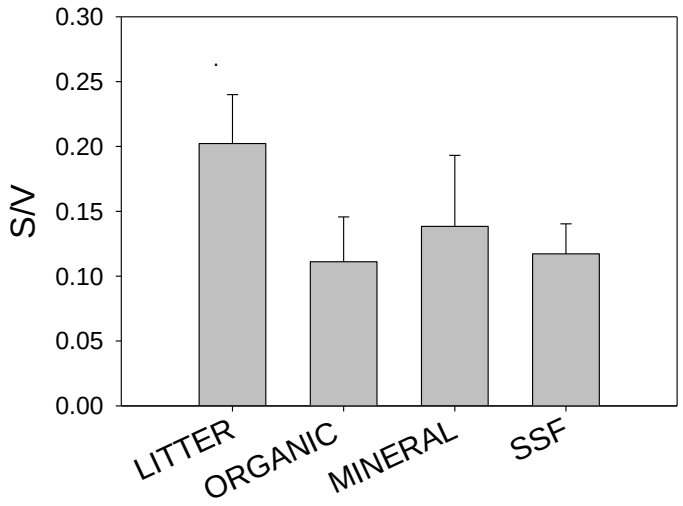


Figure 3.6. S/V ratios in different sample types demonstrating narrow range of S/V content across sample types and locations. Soils, soil leachate and subsurface flow (SSF) were collected from selected sites throughout the Lake 658 upland (see Figure 3.2a and 3.2b for site locations).

Table 3.1. Soil organic carbon concentrations in soil horizons from samples in the Lake 658 upland.

Soil horizon	Mean ± std error (mg g^{-1})	Minimum (mg g^{-1})	Maximum (mg g^{-1})
Live/litter	365.72 ± 51.10	269.71	556.10
Organic	98.85 ± 15.95	53.80	178.76
Mineral	3.95 ± 0.80	1.75	7.41

Table 3.2. Concentration (mean ± std error) of Hg, DOC and Hg/DOC concentrations in subsurface flow and leachate samples. p values indicated were calculated based on comparison of values in leachate versus subsurface flow using student's t test.

Type	**Hg (ng L^{-1})**	**^{200}Hg (ng L^{-1})**	**DOC (mg L^{-1})**	**Hg/DOC (µg g^{-1})**	**^{200}Hg/DOC (µg g^{-1})**
Leachate (n=10)	59.08 ±8.38	5.66 ± 1.49	89 ±13.59	0.75 ±0.09	0.07 ± 0.03
Subsurface flow (n=16)	19.19 ±3.39	0.79 ± 0.08	26.62 ± 2.52	0.61 ±0.06	0.03 ± 0.00
p value	<0.001	0.002	<0.001	0.333	0.142

Table 3.3. $(Ad/Al)_{van}$ content in different sample types demonstrating enhanced $(Ad/Al)_{van}$ in soil leachate samples.

Sample Type	**$(Ad/Al)_{van}$ (mean ± std err)**	**n**
Soil	1.18 ±0.33	12
Subsurface flow	2.65 ± 0.87	4
Leachate	11.22 ± 2.27	13

Chapter 4: Conclusions

4.1. Overview

NOM has been shown to control Hg complexation in a variety of environmental matrices in both pristine and contaminated areas (Skyllberg et al., 2003; Akerblom et al., 2008; Bushey et al., 2008; Shanley et al., 2008, Ouellet et al., 2009). This study provides further evidence that quality of NOM influences Hg binding and transport from terrestrial systems. Through an analysis of DOC and Hg concentrations in different molecular weight size fractions in subsurface flow, streamflow and lake water in the Lake 658 watershed it became clear that the molecular weight of DOC to which Hg was bound influenced Hg transport through the upland and to the lake. In a comparison of lignin phenolic composition of flow samples with source area material (soils), as determined by the ratio of syringyl to vanilliyl (S/V) compounds it was found that the source areas of Hg in the L658 watershed could not be separated because the source material in what were perceived to be different source areas was too similar for the method to work. Finally, recently deposited Hg, as represented by the Hg isotope (^{200}Hg) applied to the METAALICUS watershed between 2001 and 2006 remains bound to the upper soils horizons and little is being transported by DOM to downslope areas.

4.2. Research Questions

This research addresses two main questions:

1. What is the role of DOC quality in controlling the movement of Hg within and from the Lake 658 upland?

Three characteristics of L658 watershed DOC were examined. First, the relative concentrations of Hg and carbon of high molecular weight (HMW), defined as being

between 3 kDa and 0.7 µm in size, and low molecular weight (LMW) defined as being less than 3 kDa, fractions of NOM were studied. Centrifuge ultrafiltration was applied to separate DOC molecules based on size. Second, spectral properties of DOM were analyzed by absorbance of light at a wavelength of 280 nm and normalized to organic carbon concentration of the sample ($SUVA_{280}$), and absorbance of light at a wavelength of 440 nm ($CDOM_{440}$). Finally, lignin phenolic compounds were analyzed by tetramethylammonium hydroxide (TMAH) thermochemolysis. It was hypothesized that in subsurface flow, LMW compounds would have greater mobility and hence greater overall importance with respect to Hg transport. Results from Chapter 2 indicate that LMW compounds were more mobile in the UP1 sub-basin, as the relative proportion of DOC in the LMW fraction was higher in areas further downslope and the stream than in subsurface flow in close proximity to source material (i.e. soil organic carbon). However, HMW compounds consistently demonstrated greater relative importance with respect to Hg binding, as concentrations were significantly higher in HMW fractions across sample types and seasons. This finding supports other studies demonstrating greater sorption of HMW compounds to soil surfaces, causing their fractionation with transport (Kaiser et al., 1996; Lajtha et al., 2005; McCarthy et al., 1993; Kalbitz et al., 2005) and with studies demonstrating greater relative importance of HMW DOC in Hg binding (Choe et al., 2003; Babiarz et al., 2003; Cai et al., 1999). As HMW DOC is thought to represent fresher material, this is evidence that Hg binds preferentially to more reactive, labile compounds within DOM. This also implies that DOM-Hg compounds being exported to Lake 658 are more degraded and older than present in subsurface flow in the upland. However, Hg is still preferentially associated with the HMW fraction.

Spectral properties, which are generally accepted to be proxies of hydrophobicity and molecular weight of DOM, supported the trend observed of DOM being increasingly degraded with movement downslope in this system, as absorption values were on average higher in subsurface flow water in headwater areas than in subsurface water in hydrological convergence zones further downslope and in the stream. Though correlations between absorbance values and reactivity have been observed (Ravichandran et al., 1998; Waples et al., 2005; Dittman et al., 2009), correlations between Hg and $SUVA_{280}$ were not significant in all seasons and sample types in this study. Spectral properties are not quantitative measures of molecular weight or hydrophobicity of DOM and supply information about only the portion of DOM reactive to UV light. Size fractionation of DOM by ultrafiltration with known molecular weight size cutoffs provided an advantage in this study because it allowed for a more precise measure of the role of molecular weight distribution of DOM in Hg binding and transport. Centrifuge ultrafiltration was determined to be a viable method for the molecular weight size fractionation of DOM and associated Hg (Appendix A). Results demonstrate that DOC contamination was present on centrifugal ultrafilters and there was evidence of Hg binding of some ultrafilters in the initial sample aliquot. Inclusion of a rinse of the ultrafilters with an aliquot of sample following the standard cleaning procedure was sufficient to remove DOC contamination and to saturate Hg binding sites.

It was hypothesized that Hg concentrations would be related to diagenetic state of NOM as determined by lignin phenol composition, as discussed in Chapter 3. However, it was not possible to test this hypothesis using TMAH thermochemolysis, which was shown to be ineffective for the study of lignin phenols as parameters of degradation in

this case (Appendix B). TMAH thermochemolysis induces methylation of all hydroxyl groups present on the parent molecule, producing identical derivatized compounds from different parent molecules (Filley et al., 2006; Lara and Dittmar, 2001). This precludes the ability to distinguish between fresh and altered compounds, so degradation ratios were not applicable in this study. TMAH thermochemolysis was, however, an appropriate method for determining S/V content of different sample types (Chapter 3). We measured S/V content of soils, subsurface flow, streamflow and water column samples. This parameter did not correlate with Hg concentration in any sample matrices, supporting findings of other studies and demonstrating that Hg concentrations do not vary widely between vegetation assemblages (Graydon et al., 2008; Ouellet et al., 2009; Teisserenc, 2009; Caron et al., 2008; Maňkovská, 1996). S/V content was also consistent throughout source areas and sample types so did not provide information about source of subsurface flow generated within the soil profile, or about relative contributions of DOC from different sub-basins to the lake.

2. Does the relationship between NOM quality and Hg binding and transport vary between recently deposited (isotopically labeled) and historic (ambient) Hg in different sample matrices in the Lake 658 watershed?

Dissolved phase ^{200}Hg concentrations were low in subsurface flow and streamflow (mean $\pm$ std error: 1.21 $\pm$0.14 ng L^{-1}) and detectable in a relatively small number of samples (53 of 121 upland samples examined). Of those samples, ^{200}Hg was only detected in the LMW fraction on 10 occasions. Though the small sample number makes it difficult to examine patterns of molecular weight distribution and ^{200}Hg binding, the lack of detection in the LMW fraction in the majority of samples containing ^{200}Hg implies that like ambient Hg, ^{200}Hg is found in a higher proportion in the HMW fraction.

^{200}Hg was not observed in the water column in any season, confirming that terrestrial Hg deposited between 2001 and 2006, has not yet been exported to the lake in significant quantities (Harris et al., 2007).

^{200}Hg was detected in significant quantities in leachate and soil samples. Distribution of ambient and ^{200}Hg within the soil profile provided information about general distribution of Hg within a soil profile and how this varies between recently deposited and historic Hg pools. ^{200}Hg concentrations are highest in litter and surface organic horizons and appears to still be migrating to lower soil horizons. This finding supports the hypothesis that subsurface flow is primarily generated from organic horizons (Akerblom et al., 2008; Froberg et al., 2003; Tipping et al., 2005; Schiff et al., 1999), which would explain low ^{200}Hg concentrations in subsurface flow and streamflow. Concentrations of ^{200}Hg in leachate, though still only a fraction of ambient Hg concentration, were significantly higher than those in subsurface flow. Ambient Hg and DOC concentrations were also significantly higher in soil leachate than in subsurface flow. This implies that DOC and both pools of Hg are easily mobilized from litter and surface organic layers. This finding implies that soil leachate is a potentially useful tool in the study of bioavailability of Hg to aquatic organisms, as the substrate generated represents material that sulfate reducing bacteria may encounter in the natural environment thus can be used for laboratory based studies of Hg uptake by sulfate reducing bacteria.

4.3. Future Research

The findings of this study are further evidence that characterization of NOM provides insight about the biogeochemical controls of Hg transport from terrestrial to aquatic systems. Though Hg appears to bind favorably in HMW DOM fractions, the majority of compounds exported to the lake are in the LMW fraction. This implies that DOM released from the Lake 658 upland is older and more recalcitrant than that in soil porewater, while Hg binds preferentially to fresher, more labile material. However, while molecular weight distribution is expected to provide information about lability and age of material, it is not a definitive measure of either parameter. Further characterization of NOM is required to elucidate mechanisms controlling the observed lower mobility and higher affinity for Hg of HMW compounds, and to confirm whether LMW fractions of DOC do represent older, more recalcitrant material. Characterization of DOC in future studies should focus on identifying diagenetic state and age of material in different sample components as those parameters are likely to explain variations in mobility and Hg binding. It will also allow for the development of more accurate predictions of the length of time it takes for Hg to be transported to receiving waters after deposition to forested environments, since DOC export controls Hg export (Grigal, 2003; Dittman et al., 2009; Dittman et al., 2010). If the majority of DOC released from the terrestrial environment is older and more degraded, as indicated by this study, the lag between Hg deposition to a catchment and its export to receiving waters is likely to be significant. The application of a radioactive isotope of carbon (^{14}C) can be used to determine age of DOC. Thermonuclear testing activity, beginning in the 1940s, has resulted in varying levels of ^{14}C in the atmosphere over time, leaving organic carbon in the environment with

a distinct ^{14}C signature based on when it was fixed from the atmosphere (Schiff et al., 1997; Froberg et al., 2007). ^{14}C has been effectively applied as an indicator of age of NOM in terrestrial systems (Schiff et al., 1997; Froberg et al., 2007), as well as an indicator of origin of subsurface flow within the soil profile (Froberg et al., 2003). The analysis of ^{14}C and Hg in subsurface flow, streamflow and lake water in the Lake 658 watershed would lend support to the findings from Chapter 2 that DOC in subsurface flow through the UP1 sub-basin is younger and more labile than that delivered to the lake via the stream, and that Hg is more closely associated with younger, fresher DOM fractions. Analysis of ^{14}C in the lake itself would also provide information about processing of terrestrially derived DOC within the lake, i.e. whether DOM in the lake retains a signature similar to that exported from the upland, or whether it appears older, implying that it undergoes significant processing after delivery from the upland. ^{14}C analysis of LMW compounds of select samples would also demonstrate whether LMW compounds represent material that is older and more recalcitrant, as expected. This would specifically benefit this study as the results on molecular weight distribution will have greater relevance if supported by evidence that size fractions represent material of different ages.

Using lignin phenol compounds as proxies of degradation state of NOM, outlined in Chapter 3, could also provide a detailed description of the diagenetic state of DOM. Though TMAH thermochemolysis as applied in this study was ineffective for this purpose, applying thermochemolysis with ^{13}C labeled TMAH would allow for distinction between fresh and degraded lignin, as methoxy groups added by TMAH could be differentiated from methoxy groups present on the parent compound (Filley et al., 2006).

Alternatively, cupric oxide (CuO) oxidation has been demonstrated to be an effective technique for analysis of degradation state of NOM as indicated by lignin phenols (Ouellet et al., 2009; Dittmar and Lara, 2001; Teisserenc, 2009; Houel et al., 2006). Applying either of these methods, would confirm whether compounds present in subsurface flow are fresher than those that reach surface waters in this system, as indicated in Chapter 2. Corresponding measurement of Hg will indicate whether Hg content is a function of diagenetic state of compounds, as was implied by other studies (Ouellet et al., 2009; Teisserenc, 2009). This approach could be further enriched by using both ^{14}C and lignin phenol content of leachate of different soil horizons may be used to identify location of source of flow within the soil profile. Comparison of the ^{14}C and lignin signature from distinct soil horizons and that in subsurface flow will indicate whether, as expected, subsurface flow is primarily generated through organic horizons (Froberg et al., 2003).

Finally, the results of this study may have important implications for bioavailability of Hg to methylating bacteria in Lake 658 sediments, expected to be of primary importance in production of MeHg in this system. There is currently a lack of understanding of the link between Hg export from terrestrial systems and MeHg accumulation in food webs. The findings from this study may direct future research to assess bioavailability of terrestrially derived Hg and subsequent methylation. Greater characterization of DOC coupled with studies of Hg methylation will provide information about the bioavailability of Hg entering the water column from the upland sources. Though relatively few studies examine specifically the role of DOC quality in determining bioavailability of Hg to bacteria there is some evidence that molecular

weight distribution of DOC affects bacterial uptake in general (Mitchell and Gilmour, 2008, Golding et al., 2002; Tulonen et al., 1992). This study provides evidence that Hg is primarily associated with HMW compounds of DOC in the upland (Chapter 2). Therefore, experiments with a focus on determining relative bioavailability of Hg associated with HMW versus LMW DOC compounds will allow for a better understanding of the relative importance of terrestrially derived DOC-Hg compounds in MeHg production. Because molecular weight fractionation of Hg-DOC compounds in subsurface flow and streamflow has not been examined in other systems, it is unknown whether trends observed in this study are widely observed in other ecosystems. Additionally, measurement of ^{200}Hg in soil leachates in ratios consistent with subsurface flow imply that the Lake 658 soils are a potentially useful tool for generating substrate with which to examine bioavailability of DOC-Hg compounds representing those present in the natural environment. Because both ambient and ^{200}Hg are present in leachate, the use of soil leachates in these experiments will also allow for comparison of methylation of recently deposited versus historic Hg.

Appendix A: Validation of Centrifugal Ultrafiltration for Size Fractionation of Mercury and Dissolved Organic Carbon

Introduction

For the purposes of this research, a method was required for ultrafiltration of aquatic samples that uses minimal volumes, as sample volumes for subsurface flow are generally small (less than 500 ml). Centrifugal ultrafiltration has been effectively applied in studies of DOC and other metals (Bruggemann and Maes, 2010, Zhang and Wang, 2004) as well as mercury (Hg) (Miller et al., 2007), though has not been widely applied in studies of trace metals and dissolved organic carbon (DOC). Additionally, for the purposes of this study, ultrafilters were to be reused to process many aliquots of the same sample, because the small volume processed per use is insufficient for low level Hg analysis. It was therefore required that these methods were deemed appropriate for the intended analysis prior to application of centrifugal ultrafilters for Hg and DOC sample processing.

Two potential analytical issues with the application of centrifugal ultrafiltration were considered: 1) Hg or DOC contamination might exist on the ultrafilters that would result in artificially high concentrations in the ultrafiltrate; and 2) binding sites for Hg or DOC might be present on the ultrafilters that would lead to underrepresentation of the actual concentration of LMW DOC concentration in the ultrafiltrate. These effects were tested by ultrafiltering sequential aliquots of a sample from the natural environment through the same ultrafilter, with the presumption that effects of binding or contamination of Hg or DOC would result in variations in concentrations in ultrafiltrate from sequential sample aliquots.

Materials and Methods

Site description

St. Mary's lake is a 250-acre impoundment located in southern St. Mary's County, Maryland (38°15'12.82"N, 76°32'31.40"W). Inflow to the lake is primarily via the Western Branch of the St. Mary's River, and discharge from the lake is also to Western Branch. Land use distribution in the watershed is approximately 80% forested/herbaceous, 8% developed, 8% agricultural, and 4% open water (MDE, 2002).

Sample Collection

Surface water samples were collected in February, 2009 from St. Mary's Lake in three separate 1L clean PET bottles. The concentration of mercury in the samples was expected to be at or slightly above detection limits, as indicated by a previous experiment. Therefore, each 1 L sample was spiked with 3 ml of 10 µg/L ^{199}Hg isotope within one hour of collection and allowed to equilibrate overnight. The samples were filtered through a 0.7 µm Whatman GF/F pre-combusted filter and refrigerated. Following filtration, 200 ml of each sample was set aside for total Hg (THg) analysis, while the remainder was used for the ultrafiltration experiment.

Cleaning procedure for the centrifugal ultrafilters

Prior to sample collection, centrifugal ultrafilters (regenerated cellulose, Millipore Amicon Ultrafilters, Millipore Corporations, USA) were cleaned once with a 15 ml aliquot of a 10% solution (volume:volume) of ultra trace grade HCl (Baker, USA) : Milli-Q (Millipore, USA) de-ionized water, then with three subsequent aliquots of Milli-Q de-ionized water; for each aliquot, ultrafilters were spun at approximately 3000 rpm for 30 minutes on a swinging bucket centrifuge (Beckman GPR, GMI Inc., USA).

Experimental Design

Ultrafilters with three molecular weight size cutoffs were tested: 3, 10 and 30 kDa. Each sample bottle was split between four ultrafilters of the same size and a Milli-Q de-ionized water blank was passed through four 10kDa ultrafilters. Samples were spun at approximately 3 000 rpm at a constant temperature of 25^0C for 70 minutes. Because only 15 ml of sample could be processed through an ultrafilter at a time, a volume insufficient for Hg analysis, the filtrate of two ultrafilters in each bucket were composited to produce one sample and the other two composited to produce the duplicate. After each spin, the ultrafiltrate was removed and composites were combined. 5 ml of the total composite volume was removed for DOC analysis; the remainder was acidified to 0.5% (vol:vol) with Baker Instra-analyzed HCl (Baker, USA) for THg analysis.

Laboratory Analysis

Approximately 24 hrs prior to THg analysis, samples were brominated to 0.5% (vol:vol) with bromine monochloride (BrCl) for oxidation of organic matter. Immediately before analysis, hydroxylamine hydrochloride was added (10 µl per 250 µl BrCl) to neutralize any excess BrCl. Samples were added to clean glass bubblers containing Milli-Q de-ionized water, following the addition of 500 µl of stannous chloride ($SnCl_2$) for reduction of all chemical species of Hg to Hg^0. Samples were sparged with ultra high purity argon for 15 minutes to release Hg^0, which was captured onto attached gold traps. Gold traps containing the sample were then thermally desorbed and introduced to the inductively coupled plasma-mass spectrometer (Hewlett Packard 4500 ICP-MS, Agilent Technologies, USA) in the gaseous phase using argon as the carrier gas. DOC samples were kept cold and in the dark until analysis. Samples were analyzed using a Shimadzu

TOC-5000 Analyzer (Shimadzu Corporation, Japan) by Nutrient Analytical Services at the Chesapeake Biological Laboratory.

Quality Assurance/Quality Control

Quality control measures included centrifugal ultrafiltration of a Milli-Q de-ionized water blank through 10kDa ultrafilters. Blank concentrations were 0.42 $\pm$ 0.23 ng L^{-1} of THg and 0.31 $\pm$ 0.09 mg L^{-1} of DOC. All samples analyzed for THg and DOC were run in duplicate, in addition to instrument duplicates, and Hg sample spikes were analyzed. Spike recoveries were between 90 and 110% and duplicate samples concentrations were within 10% of each other.

Statistical Analyses

Statistical testing was employed to test for differences between DOC concentrations in ultrafiltrate of the first spin and DOC concentrations in the 0.7 µm filtered fraction, DOC and THg concentrations in ultrafiltrate of spin 1 and of spins 2-7, and DOC and THg concentrations between ultrafiltrate of spins 2-7. Mann-Whitney U tests were performed at a significance level of $p = 0.05$, except for tests of differences between individual spins, which were performed at significance of $p = 0.10$, the lowest p value capable of being detected with sample sets of this size.

Results

Concentration of DOC in Ultrafiltrate of a Series of Sequential Spins

The concentration (mean $\pm$ std error) of DOC in the 0.7 µm fraction of the three samples was 6.21 $\pm$ 0.07 mg L^{-1}. The concentration of DOC in ultrafiltrate of the initial spin was significantly higher than that in the <0.7 µm fraction in all three samples in all

ultrafilters (Table A.1, Figure A.1). Ultrafiltrate of the initial spin also contained DOC concentrations significantly higher than that of ultrafiltrate of subsequent spins in all molecular weight size classes, while concentrations in subsequent spins were not significantly different from one another. Mean proportion of DOC in the ultrafiltrate of spins 2-7 was 74.93%, 84.21% and 97.15% in the <3kDa, <10kDa and <30kDa fractions respectively.

Concentration of THg in Ultrafiltrate of a Series of Sequential Spins

The concentration (mean $\pm$ std error) of THg in the 0.7 µm fraction of the three samples was 36.34 $\pm$ 1.1 ng L^{-1}. THg concentration was significantly lower in the ultrafiltrate of the initial spin when compared to ultrafiltrate of subsequent spins of the 3 kDa ultrafilter (Table A.2, Figure A.2). However, the high standard error reported for that value illustrates the variation in that trend. For both duplicates, THg concentration was lower in the ultrafiltrate of the first spin than that of all subsequent spins, but the difference was much greater for one than for the other. In ultrafiltrate of the 10kDa and 30kDa molecular weight size filters, THg concentrations in a series of sequential spins were not significantly different from one another. As with DOC, all ultrafiltrate samples were characterized by THg concentrations below that in the <0.7 µm fraction. Proportion of Hg in the ultrafiltrate 24.87%, 37.47% and 43.76% in the <3kDa, <10kDa and <30kDa fractions respectively.

Discussion

DOC Concentrations in Ultrafiltrate of a Series of Sequential Spins

Results from the experiment show that ultrafiltrate from the first spin was characterized by an artificially high concentration of DOC, significantly higher than what is found in the 0.7 µm filtered sample (Figure A.1, Table A.1). This demonstrates that the combination of the weak HCl solution and Milli-Q water did not effectively remove DOC contamination from the ultrafilters and was ineffective for the purposes of this study. HCl may be ineffective at removing this contamination because decrease pH does not induce oxidation of organic matter (Heyes, A., pers. comm.) Additionally, the HCl solution may even cause coagulation of organic compounds on the filter, as a decrease in pH has shown to induce coagulation of DOC (Bratby, 2005, Howe and Clark, 2002, Bianchi, 2007). The sample, however, does appear to bind and remove any such contamination present, likely because the natural ligands present in the sample either complex and flush the DOM from the filter or bind it to the filter surface.

Excluding that from the initial spin, the data provide evidence that the ultrafilters did separate natural DOC compounds based on their actual molecular weight sizes. Ultrafiltrate from the 3kDa ultrafilters contained the lowest DOC concentration, followed by 10 kDa ultrafiltrate, then 30 kDa ultrafiltrate. This does not provide definitive evidence for accurate compound separation based on molecular weight size, but does support it, as variation in that trend (i.e. DOC concentrations in ultrafiltrate of lower molecular weight fractions exceeding those in higher molecular weight fractions) would prove that ultrafilters were not effectively separating natural DOC compounds based on size. Additionally, the consistency of the concentration of the ultrafiltrate of the final six

spins implies that there were no effects either of further contamination from the ultrafilters after the initial spin, or of binding of LMW DOC compounds to the ultrafilters. If there was residual contamination on the ultrafilters after the initial spin, there would likely be evidence of that in subsequent spins, i.e. a general decrease in DOC concentration in sequential spins. Similarly, if there was binding of natural low molecular weight DOC compounds onto the ultrafilters, that effect would likely be diminished after the first few spins, due to the presumably low number of binding sites potentially present on the ultrafilters that would become saturated. This is not surprising, since centrifuge ultrafiltration is commonly used to separate DOC and no such effects have been reported (Miller et al., 2007, Leblanc et al., 2004, Bruggemann and Maes, 2010). Finally, the results support the effective use of centrifugal ultrafilters to separate DOC based on molecular weight size distribution because concentrations reported are in the general range to be expected in such a system. LMW compounds generally comprise the highest proportion of DOC compounds in such systems, and a very small proportion exist in the dissolved fraction >30kDa (Leblanc et al., 2004, Bruggemann and Maes, 2010, Bianchi, 2007, Harvey, R., pers. comm.).

THg Concentrations in Ultrafiltrate of a Series of Sequential Spins

There is no evidence of Hg contamination of ultrafilters in any of the ultrafiltrate samples. This is not surprising because a weak acid solution at a pH <1 such as this will dissolve Hg rapidly, allowing it to pass into the ultrafiltrate in all molecular weight size fractions (Stumm and Morgan, 1996). It is therefore not unexpected that any Hg contamination that may be present on the ultrafilters would be removed by the current cleaning procedure. There is evidence, however, that there may be active Hg binding sites

on the surface of the ultrafilters. Miller et al. (2007) found binding of LMW Hg compounds by 5kDa centrifugal ultrafilters. Though they did not test binding of Hg to the filters in subsequent aliquots, we would expect that those sites would become rapidly saturated by the Hg or other cations (including metals) present in the initial sample aliquot, and subsequent aliquots would not be affected. There is some evidence in this study for Hg binding by some ultrafilters, as demonstrated by the lower concentration of THg present in the ultrafiltrate of the initial spin. The decrease of THg in subsequent spins and the lack of statistically significant difference between them indicate that any active binding sites are saturated in the first aliquot and subsequent aliquots are not affected.

Conclusions

The DOC contamination that occurs appears in the ultrafiltrate of all molecular weight size filters, implying that all ultrafilters are consistently affected by the contamination. Because DOC concentrations decrease and remain consistent after the initial spin, it appears that all the contamination is removed by the first sample aliquot. An organic solvent could potentially have been used as an effective cleaning agent for these ultrafilters but may have led to residual organic carbon contamination in the ultrafiltrate of the initial sample spin. There appears to be no Hg contamination from the filters. Hg binding by surfaces on the filter may occur, but evidence for this is inconsistent and appears to only the concentration in the ultrafiltrate of the initial spin. This study suggests that an effective way to remove DOC contamination and to prevent

any effects of Hg binding on the filters is to include a rinse of the filters with an aliquot of the sample in the ultrafilter cleaning procedure.

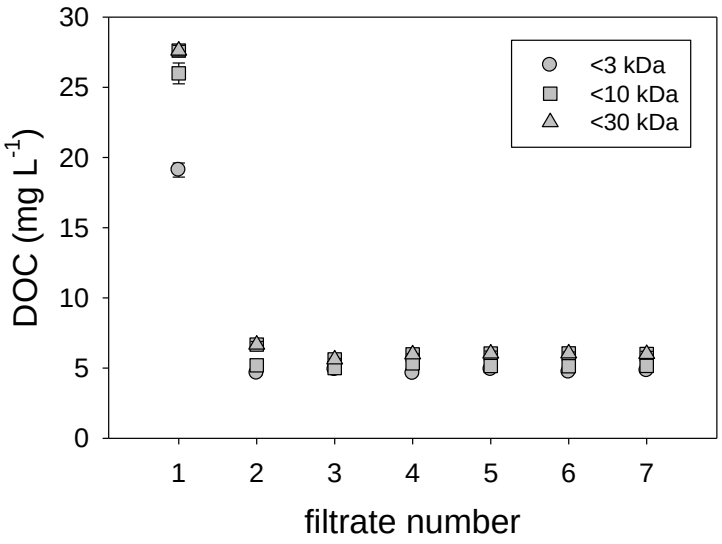


Figure A.1. DOC concentration (mean ± std error) (mg L^{-1}) in ultrafiltrate of sample in a series sequential spins.

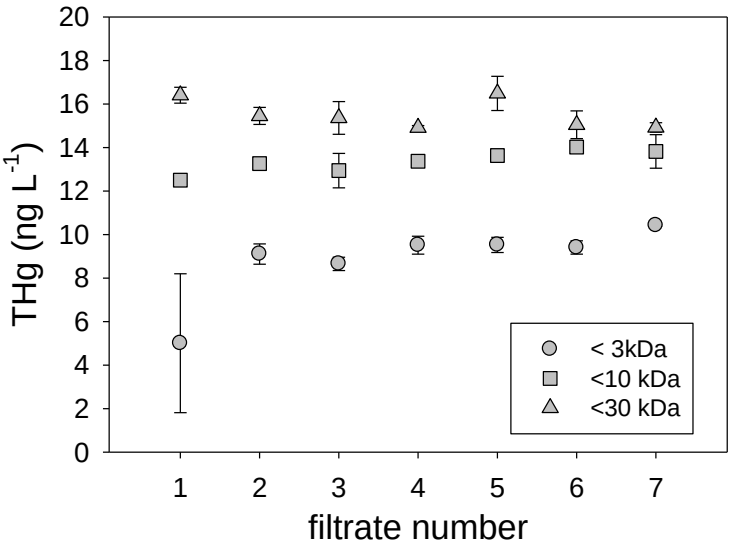


Figure A.2. Total Hg concentration (mean ± std error) (ng L^{-1}) in ultrafiltrate of sample in a series sequential spins.

Table A.1. DOC concentrations (mean ± std error) (mg L^{-1}) in ultrafiltrate of different molecular weight size ultrafilters.

	<0.7 um	**Spin 1**	**Spins 2-7**
<3 kDa	6.29 ± 0.04	19.11±0.51	4.77±0.05
<10 kDa	6.07 ±0.05	25.99 ±0.74	5.16±0.04
<30 kDa	6.26 ± 0.09	27.61±0.38	6.06±0.10

Table A.2. THg concentrations (mean ± std error) (ng L^{-1}) in ultrafiltrate of different molecular weight size ultrafilters.

	<0.7 um	**Spin 1**	**Spins 2-7**
<3 kDa	37.94 ± 0.91	5.01 ± 3.19	9.44 ± 0.19
<10 kDa	35.66 ± 0.54	12.50 ± 0.15	13.51 ± 0.18
<30 kDa	5.45 ± 0.77	16.40 ±0.37	15.62 ± 0.33

Appendix B: Applicability of TMAH Thermochemolysis for Analysis of Lignin Phenolic Compounds of Interest

Introduction

Tetramethylammonuim hydroxide (TMAH) thermochemolysis has been effectively applied as a method of sample processing for the determination of lignin phenolic compounds in a variety of sample matrices, including vascular plants (Clifford et al., 1995), fresh and degraded wood (del Rio et al., 1998, Kuroda and Nakagawa-izumi, 2005) and estuarine POM and DOM (Harvey and Mannino, 2001). The thermochemolysis reaction cleaves carbon to carbon bonds and the β-O-4 bond by which lignin compounds are cross-linked in the parent lignin molecule, and subsequently methylates carboxylic and acidic hydroxyl groups, leaving the derivatized compound amenable to analysis by GC/MS (Clifford et al., 1995, del Rio et al., 1996, Mannino, 2000). TMAH thermochemolysis produces a greater diversity of lignin products than traditional CuO oxidation and provides a greater degree of structural characterization (Mannino, 2000, Kuroda and Nakagawa-izumi, 2005). However, recoveries and total lignin yields are generally lower than those of CuO oxidation (Harvey, H.R., pers. comm., del Rio et al., 1998, Wysocki et al., 2008, Dittmar and Lara, 2001). Prior to addressing results of this study, it is important to address drawbacks of this method and analytical problems encountered.

Subsurface flow, soil and stream waters from the Lake 658 upland were collected for analysis of lignins. The sampling design was structured to examine differences in lignin composition of NOM to indicate degradation state and source of NOM in different sample matrices. However, analytical issues prevented the ability to achieve some of

those goals. In this appendix, the methods used for lignin analysis are described in detail, and the drawbacks of this method are discussed. The overall results of the lignin analysis, and their application to the broader study are given in Chapter 3.

Analytical Issues

Analysis of lignin phenols relied on spikes of known quantities of multiple standards as internal standards to determine concentrations. Unfortunately, recoveries of both p-coumaric acid and ethyl vanillin used as internal standards were variable and generally low; in some cases added standards were not observed. This did not appear to be a result of any specific sample matrix since it occurred in samples from different origins. In six of 52 samples, the added ethyl vanillin standard was not seen. Standard recovery in those samples in which the internal standards were seen was calculated based on the recovery of the blank standard; peak areas were normalized to the amount of sample were used to compare recoveries. Average recovery of the ethyl vanillin standard was 73.54 $\pm$14.39% (mean $\pm$ std error). As noted above there was a very high range of recoveries (min: 0.15%, max: 293%) and the recovery of internal standards exceeded 100% in 10 of 52 samples, the average recovery of which was 229%. Such variability precludes the use of recovery values to quantify concentrations of individual monomers in samples for ethyl vanillin. Similar issues were observed with p-coumaric acid standard which was not recovered in three samples, and recovery of which was very low in a number of others. Recoveries were more variable than those of ethyl vanillin standard, with a range in recoveries between 0.11 and 161% (mean $\pm$ std error of 50.49 $\pm$ 8.67 %). 100% recovery of the p-coumaric acid internal standard was exceeded in 7 samples,

which averaged 159% recovery, again implying that standard recovery in blanks could not be effectively used to determine quantitative concentrations.

Studies employing TMAH thermochemolysis are often characterized by low sample and standard recovery (del Rio et al., 1998, Wysocki et al., 2008, Dittmar and Lara, 2001), but complete loss of internal standards was not expected and prevented the determination of yields to allow different classes of compounds to be quantified. Given that ratios of compounds rather than total yields are the primary interest in this study, a comparison of peak area ratios remained sufficient to examine relative sources, and the inability to quantify concentrations of each product does not preclude application of the data. Nevertheless, the low or even absent recovery in some samples implies difficulty with the derivation process for some matrices.

In three sediment and soil samples, the ethyl vanillin standard was identified in the underivatized form at a retention time of 18 minutes, with identification from NIST reference library of 73% (Figure B.1). In these samples, ethyl vanillin was also identified in the derivatized form at the regular retention time of 21 minutes. The presence of this compound demonstrates that despite TMAH thermochemolysis and the addition of BSTFA, a portion of the ethyl vanillin was measured in the underivatized from, implying that the derivation process was incomplete or that an added TMS or methyl group was lost. The underivatized form was also identified in two blanks which contained only standards, confirming that the peaks observed were generated from the ethyl vanillin standard itself, rather than originating within the samples. The polarity of the compound is expected to prevent it from being measured using the column employed, but its low molecular weight and volatility appear to allow a portion to be resolved.

TMAH thermochemolysis is expected to completely derivatize lignin compounds, as it has been shown to be an effective tool for such reactions (Clifford et al., 1995, Harvey and Mannino, 2001, Wysocki et al., 2008, del Rio et al., 1998). However, trimethylsilation of functional groups was observed more frequently than expected, demonstrating that TMAH did not fully methylate hydroxyl groups of different compounds in several samples. No mass spectra of TMS'd derivatives of lignin phenolic acids are typically reported, thus we predicted mass spectra based on the structure of the derivatized compound were used in conjunction with identification of compounds by the NIST reference library. Using these techniques, we identified TMS'd derivatives of P4, P6, G4 and G6. The presence of these derivatives may be a result of the degradation state of the parent lignin material. As lignin compounds undergo degradation, methoxyl functional groups are converted to hydroxyl groups by white rot and brown rot fungi, which can lead to adjacent hydroxyl groups on the benzene ring (Teisserenc, 2009, Filley et al., 2006, Leonowicz et al., 1999). Due to steric effects, adjacent hydroxyl groups may be less likely to undergo methylation simultaneously, leading to methylation by TMAH of one group and trimethylsilation of the other. Thus, the presence of multiple hydroxyl groups and consequently TMS'd derivatives may be indicative of lignin material of a more advanced diagenetic state, though this cannot be confirmed.

The identification of 3,5-Bd and G6 derivatives presented difficulties because they are structurally similar. The fully methylated derivatives are distinguishable from one another because their mass spectra, though similar, include important fragment ions at m/z 181 among others (Figure B.2). In all samples, the fully methylated derivatives of both 3,5-Bd and G6 are present at retention times of 19.75 minutes and 22.9 minutes

respectively. Peaks representing what are expected to be derivatives of 3,5-Bd and G6 with one TMS substitution are also observed in almost all samples, implying that TMAH thermochemolysis did not result in complete methylation and BSTFA acted to produce a derivatized compound. Figures B.3 and B.4 illustrate the probable reaction mechanisms. Initial hydrolysis with TMAH induces subsequent methylation among all but one hydroxyl group. BSTFA completes the derivatization by substituting a TMS on the remaining hydroxyl group. This results in the formation of one of three possible structures (A, B or C) from 3,5-Bd (Figure B.3) and compound D or E from G6 (Figure B.4). Compounds A - C are structurally similar to D and E, but are expected to have different mass spectra and retention times because of differences in the position of the functional groups on the benzene ring, as in the case of the methylated derivatives (Figure B.2). A peak appears consistently at a retention time of 27 minutes that was positively identified as compound E by the NIST reference library (Figure B.5). Another peak appears consistently at a retention time of 23.75 minutes, which based on mass spectral interpretation and mass, appears to be one of compounds A –C. The identification of the G6 derivative in one sample and the chronology of the retention times provide evidence that the peak at 23.75 minutes is one of compounds A –C. To confirm the identity of this compound, a series of experiments were conducted using a 3,5-Bd std (Fisher Scientific, USA). BSTFA and boron trifluoride (BF3) were used as agents of trimethylsilation and methylation, respectively to attempt to produce one of compounds A –C. Details of the methods and results of the experiment are given below.

Methods

The method for TMAH thermochemolysis used here was based on Mannino, 2000. The detail procedure used for analysis is as follows. 50 µL BSTFA were added to a pre-combusted test tube containing 100 µg of the 3,5-Bd standard. The test tube was heated at 50^0C for 30 minutes, then blown down to dryness with N_2. 1 ml BF3 was added and it was heated at 70^0C for 30 minutes. The solution was allowed to cool to room temperature, after which time 1 ml de-ionized water and 2 ml of 9:1 hexane:diethyl ether were added. The test tube was capped, inverted three times and allowed to sit so the layers would separate. The top layer was transferred to an 8 ml amber vial; this was repeated twice. The solution was blown to dryness with nitrogen and transferred to an injection vial in 100 µl of DCM for analysis by GC/MS. See Chapter 3 for details on type and model of GC-MS and GC-FID instrumentation used . Three replicate standards were processed as above, and three replicates were processed using only BSTFA to verify the retention time and mass spectrum of the TMS derivative. The GC/MS program used was the same as outlined in Chapter 3.

Results

3,5-Bd derivatives were identified in the fully TMS'd form (Figure B.6) and with two TMS and one methyl substitution by NIST reference library in the standards processed with both BF3 and BSTFA. Neither the derivative with one TMS substitution nor the fully methylated derivative were identified, and the fully methylated derivative was not identified in the standards processed with only BF3. The experiment was repeated once exactly as above, then twice more with the temperature increased to 80^0C

and the heating time extended to one hour after addition of BF3 to catalyze the methylation reaction; there was no variation in the results.

Since the derivative with one TMS substitution was identified in the samples processed with TMAH but not in the standard processed with BF3, there may be an effect either of the sample matrix, or the methylation process preventing the formation of the methylated derivative. To test for the effect of sample matrix, a sample containing the derivatized compound of interest was spiked with the 3,5-Bd standard. The fully methylated derivatives and the derivatives with one TMS substitution appeared as they were present in the sample with no enrichment of either compound. To test for the effect of methylation process, the above experiment was repeated using TMAH as the methylating agent. To test for any effect of the strongly basic environment in the presence of TMAH, the pH was adjusted by dropwise addition of HCl to a pH of approximately 4 in three samples, while the pH was not adjusted in the other three. The procedure above was otherwise identical, with TMAH being added prior to the first heating step in place of BF3. Again there were no peaks identified as that of either compound with one TMS substitution. As with the original experiment, we identified the fully TMS'd derivative, as well as a derivative with one methyl substitution and 2 TMS substitutions.

Despite the inability to produce the desired derivatized compound, there is strong evidence that the peak at 23.75 minutes is compound A, B or C. This is indicated by the mass spectra, which matches that expected by the structure of the compound. Additionally, the retention times of the fully methylated derivatives vary by approximately 3.25 minutes; the retention times of the suspected 3,5-Bd peak and

compound E vary by the same amount and in both cases the 3,5-Bd derivative is eluted first.

An important constraint of TMAH thermochemolysis is the inability to distinguish whether methoxyl functional groups of derivatized compounds were present on the parent compound as hydroxyl or methoxyl functional groups, which makes hydrolysable tannins and other polyhydroxy phenols indistinguishable from lignin phenols (Filley et al., 2006, Figure B.7). The contribution from hydrolysable tannins is expected to be variable with respect to season, age and source of material, soil horizon and sample matrix (Filley et al., 2006). Filley et al. (2006) applied ^{13}C TMAH thermochemolysis to determine the relative contributions of non-lignin constituents, particularly hydrolysable tannins, to signals of specific lignin compounds. They found that as a result of methylation by TMAH, hydrolysable tannins produced derivatives identical to those of lignin compounds (Figure B.7). The relative contribution of hydrolysable tannins varied with sample type and the lignin compound measured, for example, the contribution of hydrolysable tannins in oak leaves, roots and bark represent 30%, 25% and 22% respectively of measured syringyl compounds. Figure B.7 demonstrates how compounds present in the natural environment, such as hydrolysable tannins, can form derivatives identical to those of lignin compounds.

Though the study was only conducted on different components of only one type of tree, the uncertainty of the contribution of non-lignin constituents warrants consideration. This problem is expected to affect sample types with high tannin content, such as organic soils, leachate and streamflow, (Filley et al., 2006, Ikeya et al., 2004), and has implications for this study. Though we did not measure tannin content in this

study, they are likely in several fractions of the sample suite examined and suggest a cautious interpretation is warranted.

Implications for this study

In this study, measuring ratios of lignin compounds have been proposed to provide an indication of diagenetic state of NOM (Houel et al., 2006; Dittmar and Lara, 2001; Teisserenc, 2008; Ouellet et al., 2009). However, in addition to the potential influence of tannins on various compound signals, the inability to distinguish whether functional groups of derivatized compounds were present on the parent compound as hydroxyl or methoxyl functional groups limit its application here for the study of degraded lignin contribution.

Lignins as parameters of degradation: Biological processing of lignin phenolic compounds occurs primarily by white and brown rot degradation by fungi. White-rot fungal degradation induces oxidation of the propyl side chain and aromatic ring cleavage, while brown-rot degradation induces demethylation of methoxy groups, leading to the formation of 3,4- and 4,5-dihydroxylated phenol groups (Dittmar and Lara, 2001). CuO oxidation is thought to induce cleavage of aromatic orthohydroxyl groups, thus the measured signal from fresh lignin material will have a signature different from degraded lignin. The degraded lignin will have a reduced signal of the compounds which are susceptible to demethylation, such as those with 3-hydroxy or 3-5 dihydroxy configuration (vanillyl and syringyl compounds respectively). Parahydroxyphenols, on the other hand, do not contain a methoxy group so do not undergo variation in structure with degradation. Relative P/(V+S) content is therefore a parameter of degradation:

higher values, produced from a reduction in S and V family compounds, represents material at a more advanced diagenetic state

Applicability for this study: We applied TMAH thermochemolsysis for lignin analysis. Unlike the more traditional CuO oxidation method, this technique induces methylation of all hydroxy groups, leaving compounds present in the sample as phenols indistinguishable from those present in the sample as 3,4-dihydrodxy or 3,4,5-trihydroxy compounds. For example, a vanillin compound undergoing degradation in the natural environment may form 3,4-dihydroxy benzaldehyde. The derivative of this compound following TMAH thermochemolysis is 3,4-dimethoxy benzaldehyde (Figure B.8). A vanillin compound not susceptible to degradation in the environment produces the same derivatized product following TMAH thermochemolysis (Figure B.9). This example demonstrates how the signal measured from fresh and degraded lignin from the same source material will not vary with respect to V or S content. As a result the P/(V+S) ratio from the same source material will not change as a result of degradation.

3,5-Bd/V is another parameter of degradation applied in studies (Ouellet et al., 2009, Teisserenc, 2009, Caron et al., 2008). 3,5-Bd, a product of soil decomposition, the likely precursors of which are tannins and other flavonoids with hydroxy groups present on alternate positions on the ring. It has been shown to increase with degradation in soil matrices (Teisserenc, 2009, Houel et al., 2006). Thus, 3,5-Bd content is expected to increase with degradation, while V content decreases, making it an effective tracer of degradation. However, tannin compounds, the parent material of 3,5-Bd are themselves subject to methylation by TMAH thermochemolysis, as described above, rendering them indistinguishable from 3,5-Bd. Additionally, TMAH thermochemolysis prevents the

detection of changing V content with degradation, as stated above. The influence of tannins likely to affect the signal of 3,5-Bd and the inability to distinguish between degraded and fresh V compounds make TMAH thermochemolysis ineffective at detecting changes in 3,5-Bd/V with degradation.

Another ratio applied that has been proposed as a parameter of degradation is content of vanillic acid to vanillic aldehyde $(Ad:Al)_{van}$ (Ertel and Hedges, 1984). White rot fungal degradation induces side chain oxidation, leading to an increase in this ratio. However, the application of this ratio has not been consistently shown to increase with diagenetic state. Teisserenc (2009) found that it was only applicable as a parameter of degradation in certain soils, potentially in areas where white rot fungi is expected to show higher activity, while Dittmar and Lara (2001) found that it was not applicable as a parameter of degradation as it was consistent between fresh leaf litter and sedimentary organic matter. Additionally, in this study, some samples are characterized by $(Ad/Al)_{van}$ ratios well beyond those observed in the natural environment, with $(Ad/Al)_{van}$ in samples averaging 11.8 ± 2.9 (mean $\pm$ std error), while the highest values in mineral soils in a boreal system was measured as approximately 3.5 (Teisserenc, 2009), and maximum values observed mangrove sedimentary organic matter was 1.3 (Dittmar and Lara, 2001). This appears to be the result of very high vanillic acid signal measured in some samples. This was most notably the case for high DOC leachate samples.. The DOC released is likely comprised of a greater diversity of compounds, and there is the potential for the presence of hydroysable tannins to influence the vanillic acid signal. Thus $(Ad:Al)_{van_}$is not applicable as a parameter of degradation in thus study.

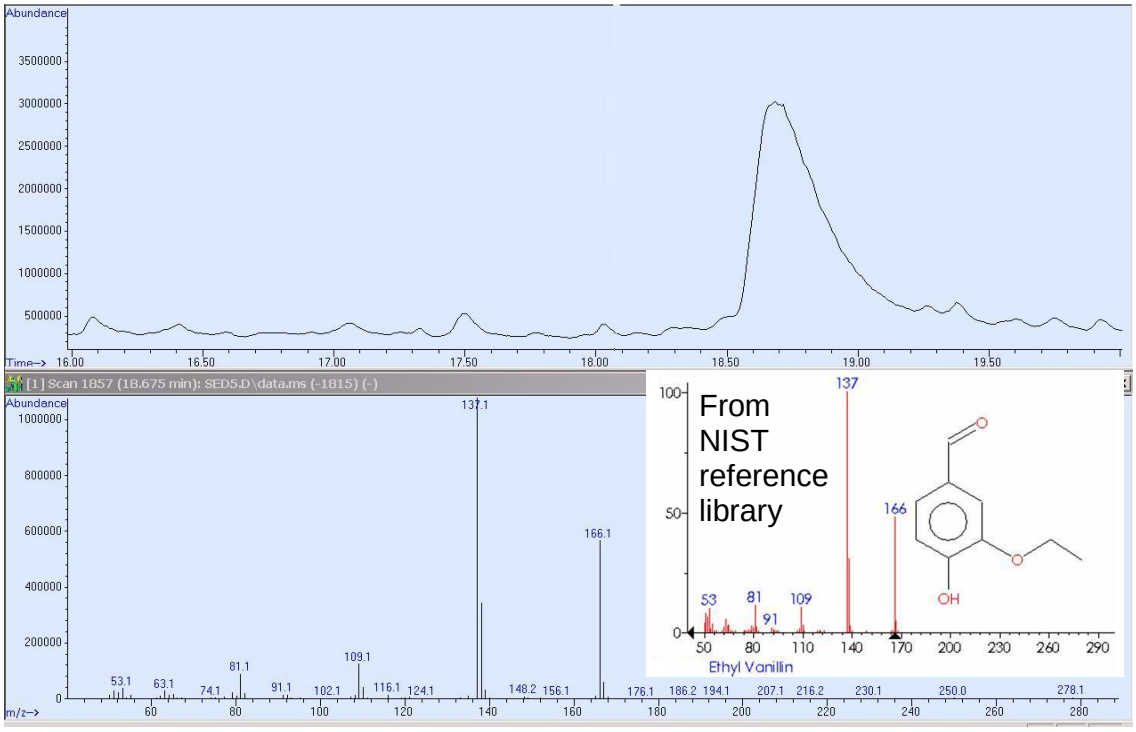
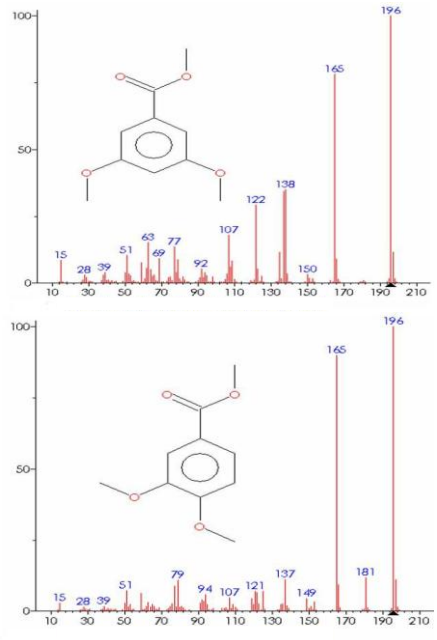


Figure B.1. Mass spectrum of suspected underivatized ethyl vanillin standard in sample SED5 and as indicated by NIST reference library.

a)



b)

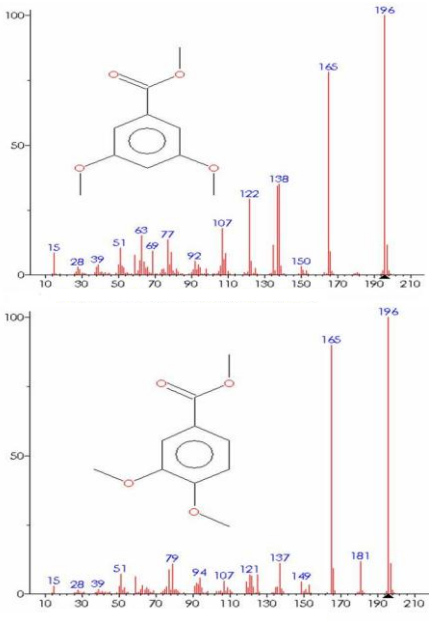


Figure B.2. Mass spectra of fully methylated derivatives of a) 3,5-Bd and b) G6 as indicated by the NIST reference library.

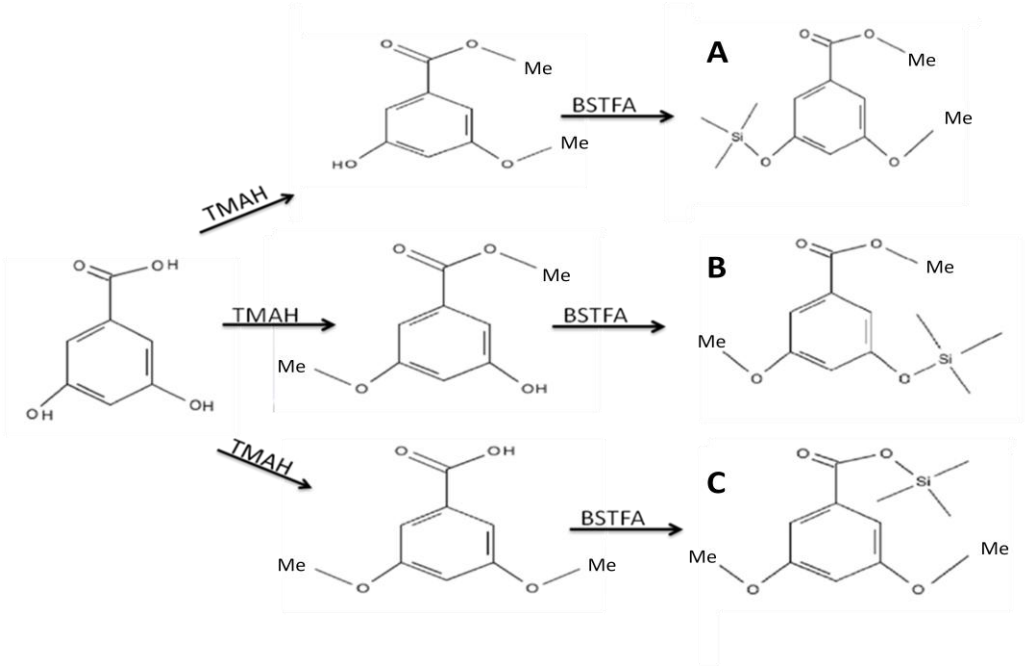


Figure B.3. Potential reaction mechanisms of the derivatization of 3,5-Bd**.**

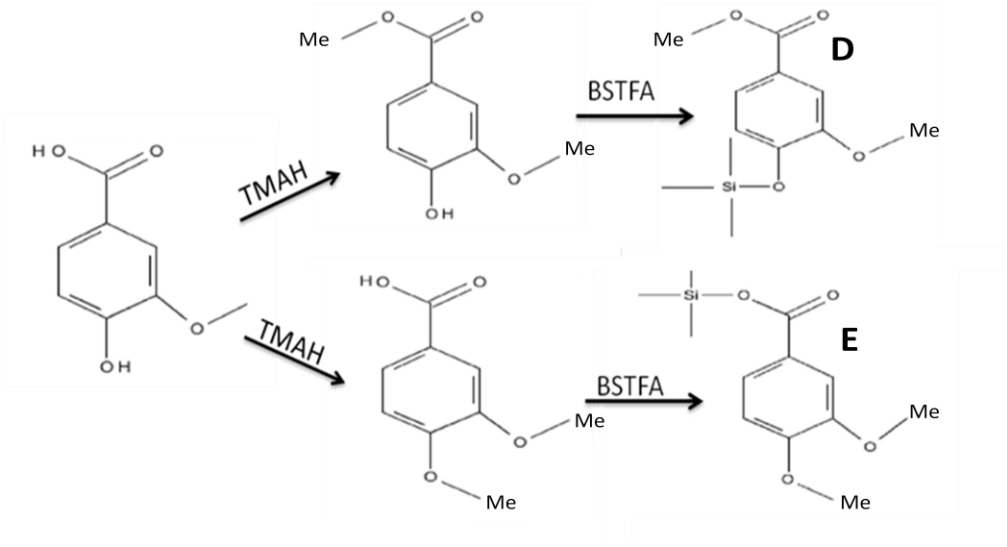


Figure B.4. Potential reaction mechanisms of the derivatization of G6.

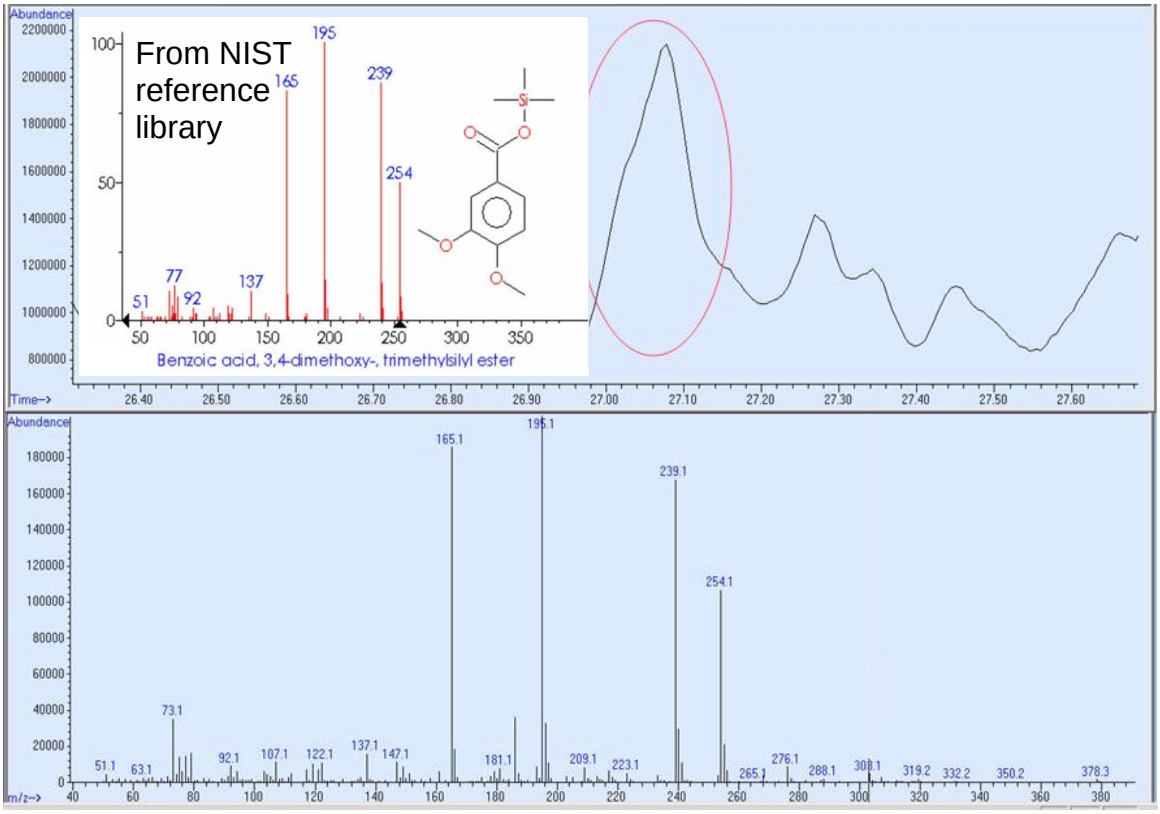


Figure B.5. Mass spectra of peak in sample LIG55 and mass spectra of TMS'd derivative of G6 as indicated by the NIST reference library.

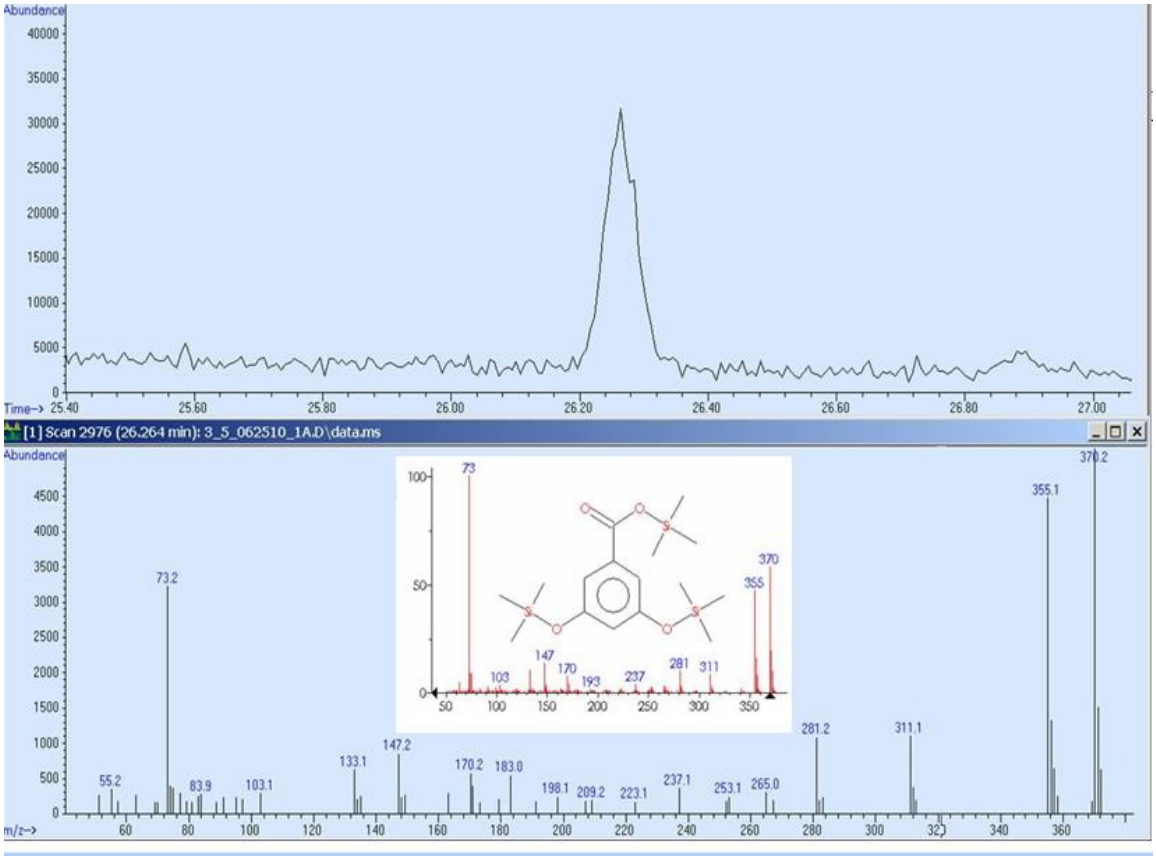


Figure B.6. Mass spectra of peak in a 3,5-Bd standard processed with BSTFA and BF3 and mass spectra of TMS'd derivative of 3,5-Bd as indicated by the NIST reference library.

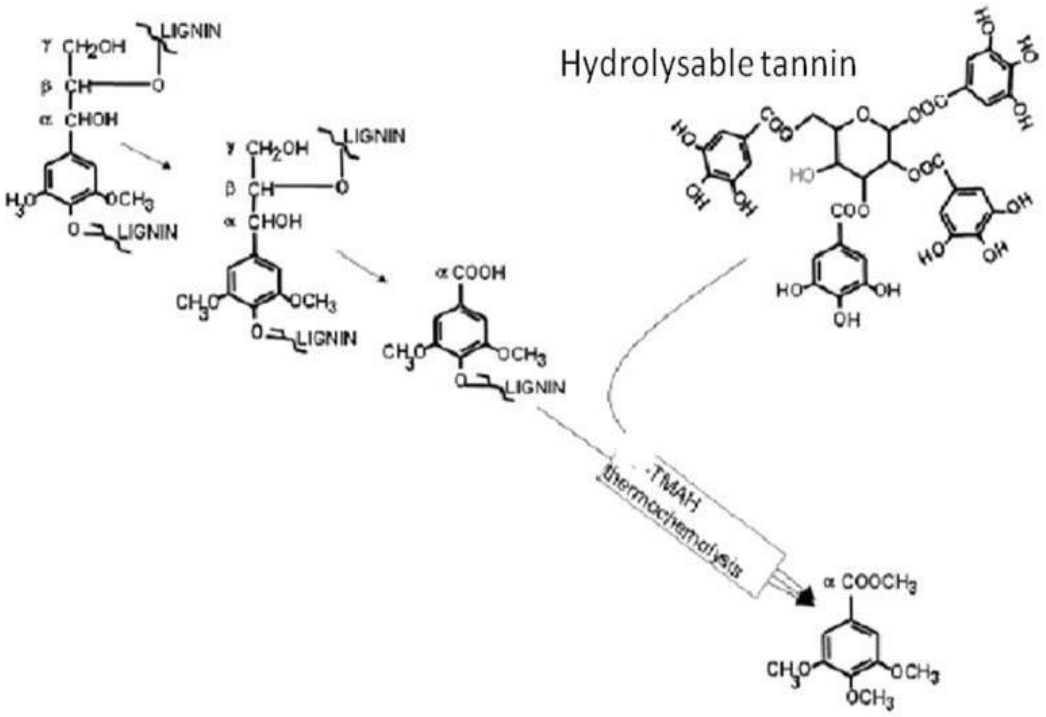


Figure B.7. The mechanism of formation of the same derivatized compound from different sources in the natural environment. Modified from Filley et al., 2006.

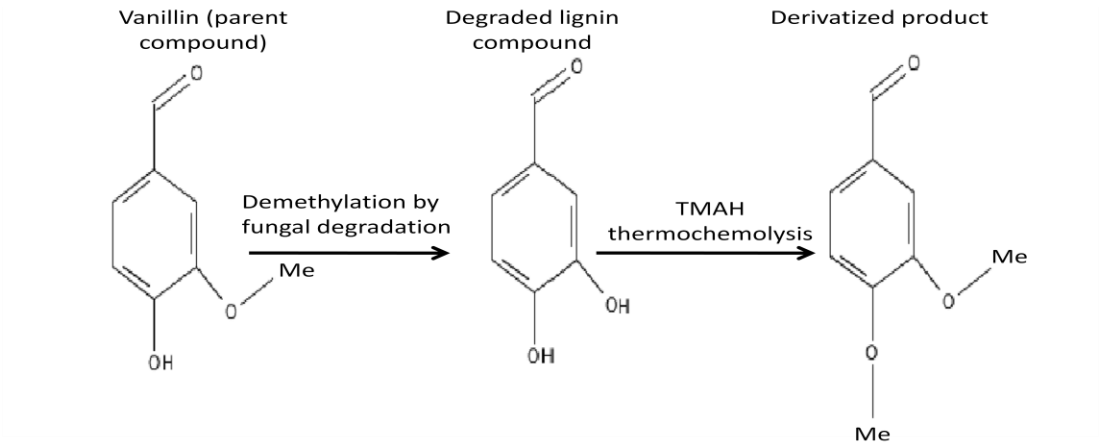


Figure B.8. Reaction mechanism of natural degradation of G6 (vanillin) and subsequent derivatization by TMAH thermochemolysis.

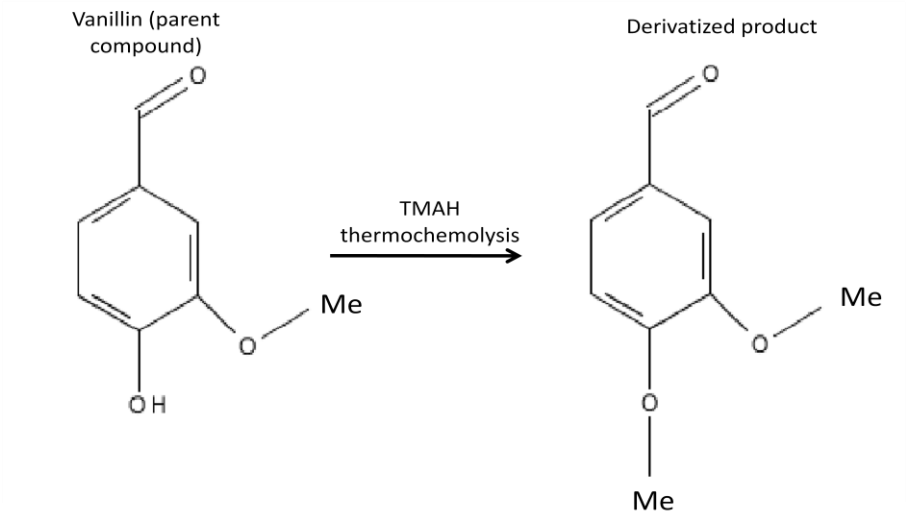


Figure B.9. Reaction mechanism of derivatization by TMAH thermochemolysis of G6 (vanillin).

References

Aastrup, M., Johnson, J., Brignmar, E., Bringmark, I. and A. Iverfeldt. 1991. Occurrence and transport of mercury with a small catchment area. Water, Air and Soil Pollution. 56: 155 – 167.

Aiken, G.R., Haitzer, M., Ryan, J.N. and Nagy, K., 2003. Interactions between dissolved organic matter and mercury in the Florida Everglades. Journal de Physique IV. 107: 29 - 32.

Akerblom, S., Meili, M., Bringmark, L., Johansson, K., Berggren, D. and Bergkvist, B. 2008. Partitioning of Hg between solid and dissolved organic matter in the humus layer of boreal forests. Water, Air and Soil Pollution. 189: 239 – 252.

Allan, C.J., Heyes, A., Roulet, N.T., St. Louis, V.L. and Rudd, J.W.M. 2001. Spatial and temporal dynamics of mercury in Precambrian Shield upland runoff. Biogeochemistry. 52: 13 – 40.

Allan, C.J. and Heyes, A. 1998. Episodic transport of total and methyl mercury from low order Blue Ridge watersheds, S.E. USA. Water, Air and Soil Polltuion. 105: 573 – 592.

Amirbahman, A., Reid, A.L., Haines, T.A., Kahl, J.S. and Arnold, C. 2002. Association of methylmercury with dissolved humic acids. Environmental Science and Technology. 36: 690-695.

Amon, R. M. W. And Benner, R. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography. 41: 41-51.

Babiarz, C.L., Hurley, J.P., Krabbenhoft, D.P., Gilmour, C.C. and Branfireun, B. 2003. Application of ultrafiltration and stable isotopic amendments to field studies of mercury partitioning to filterable carbon in lake water and overland runoff. The Science of the Total Environment. 304: 295 – 303.

Babiarz, C.L., Hurley, J.P., Hoffman, S.R., Andren, A.W., Shafer, M. and Armstrong, D.E. 2001. Environmental Science and Technology. 35: 4773 – 4782.

Babiarz, C.L., Hurley, J.P., Benoit, J.M., Shafer, M., Andren, A.W. and Webb, D.A. 1998. Seasonal influence on partitioning and transport of total and methylmercury in rivers from contrasting watersheds. Biogeochemistry. 41: 237 – 257.

Bianchi, T.S. 2007. Biogeochemistry of Estuaries. Oxford University Press.

Benoit, J., Gilmour, C.C., Heyes, A., Mason, R.P. and Miller, C.L. 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic systems. Biochemistry of Environmentally Important Trace Elements. Y. Chai and O.C. Braids. Washington, DC, American Chemical Society. ACS Symposium Series 835: 262 – 297.

Biester, H., Muller, G and H.F. Scholer. 2002. Binding and mobility of mercury in soils contaminated by emissions from chlor-alkali plants. The Science of the Total Environment. 284: 191 -203.

Boyer, E.W., Hornberger, G., Bencala, K. and McKnight, D. 1996. Overview of a simple model describing variation of dissolved organic carbon in an upland catchment. Ecological Modelling. 86: 183 – 188.

Bratby, J. 2006. Coagulation and Flocculation in Water and Wastewater Treatment, 2nd edition. Awwa Research Foundation, pp 100 - 105. IWA Publishing, London, UK.

Brigham, M.E., Wentz, D.A., Aiken, G.R, and Krabbenhoft, D.P. 2009. Mercury cycling in stream ecosystems. 1. Water column chemistry and transport. Environmental Science and Technology. 43: 2720 – 2725.

Bruggemann, C. and N. Maes. 2010. Uptake of uranium (VI) by pyrite under boom clay conditions: influence of dissolved organic carbon. Environmental Science and Technology. 44: 4210 – 4216.

Brunskill, G.J. and Schindler, D.W. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. Journal of the Fisheries Research Board of Canada. 28: 139 – 155.

Bushey, J.T., Driscoll, C.T., Mitchell, M.J., Selvendiran, P. and Montesdeoca, M.R. 2008. Mercury transport in response to storm events from a northern forest landscape. Hydrological Processes. 22 (25): 4813 – 4826.

Cai, Y., Jaffé, R. and R. Jones. 1999. Interactions between dissolved organic carbon and mercury species in surface waters of the Florida Everglades. Applied Geochemistry. 14: 395 – 407.

Caron, S., Lucotte, S. and Teisserenc, R. 2008. Mercury transfer from watersheds to aquatic environments following the erosion of agrarian soils: A molecular biomarker approach. Canadian Journal of Soil Science. 88: 801-811.

Celo, V., Lean, D.R.S. and Scott, S.L. 2005. Abiotic methylation of mercury in the aquatic environment. Science of the Total Environment. 368 (1): 126 – 137.

Chadwick, S.P., Babiarz, C.L., Hurley, J.P. and Armstrong, D.E. 2006. Influences of iron, manganese, and dissolved organic carbon on the hypolimnetic cycling of amended mercury. The Science of the Total Environment. 368 (1):177 -188.

Chin, Y-P., Aiken, G.R. and O'Loughlin, E. 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environmental Science and Technology. 28: 1853 – 1858.

Choe, K-Y., Gill, G.A. and Lehman, R. 2003. Distribution of particulate, colloidal and dissolved mercury in San Francisco Bay estuary. Limnology and Oceanography. 48 (4): 1535 – 1546.

Clifford, D.J., Carson, D.M., McKinney, D.E., Bortiatynski, J.M. and Hatcher, P.G. 1995. A new rapid technique for the characterization of lignin in vascular plants: thermochemolysis with tetramethylammonium hydroxide. Organic Geochemistry. 23 (2) : 169 – 175.

del Rio, J.C., McKinney, D.E., Knicker, H., Nanny, M.A., Minard, R.D. and Hatcher, P.G. 1998. Structural characterization of bio- and geo-macromolecules by off-line thermochemolysis with tetramethylammonium hydroxide. Journal of Chromatography A. 823: 433 – 448.

Del Vecchio, R. and Blough, N. 2004. On the origin of the optical properties of humic substances. Environmental Science and Technology. 38: 3885-3891.

Dittman, J., Shanley, J., Driscoll, C., Aiken, G., Chalmers, A., Towse, J. and Selvendirans, P. 2010. Mercury dynamics in relation to dissolved organic carbon concentration and quality during high flow events in three northeastern U.S. streams. Water Resources Research. 46: W07522-537.

Dittman, J., Shanley, J., Driscoll, C., Aiken, G., Chalmers, A. and Towse, J.E. 2009. Ultraviolet absorbance as a proxy for total dissolved mercury in streams. Environmental Pollution. 157: 1953 – 1956.

Dittmar, T. and Lara, R.J. 2001. Molecular evidence for lignin degradation in sulfate-reducing mangrove sediments (Amazonia, Brazil. Geochimica et Cosmochimica Acta. 65 (9): 1417 – 1428.

Downs, S.G., Macloed, C.L. and Lester, J.N. 1998. Mercury in precipitation and its relation to bioaccumulation in fish: a literature review. Journal of Water, Air and Soil Pollution. 108, 149-187.

Drexel, T., Haitzer, M., Ryan, J., Aiken, G. and Nagy, K. 2002. Mercury(II) Sorption to Two Florida Everglades Peats: Evidence for Strong and Weak Binding and Competition by Dissolved Organic. Environmental Science and Technology. 36: 4058-4064.

Driscoll, C.T., Blette, V., Yan, C., Schofield, C.L., Munson, R. and Holsapple, J. 1995. The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. Water, Air and Soil Pollution. 80: 499 -508.

EPA (2010). The National Listing of Fish Advisories. Washington, DC, US EPA.

EPA (2000). Integrated Risk Information System. Washington, DC, US EPA.

Ertel, J.R. and Hedges, J.I., 1984. The lignin component of humic substances: Distribution among soil and sedimentary humic, fulvic and base-insoluble fractions. Geochimica et Cosmochimica Acta. 48: 2065–2074.
Fan, Z., Neff, J.C. and Wickland, K.P. 2010. Modeling the production, decomposition, and transport of dissolved organic carbon in boreal soils. Soil Science. 175: 223 – 232.

Farella, N.M., Lucotte, M., Davidson, R. and Daigle, S. 2006. Mercury release from deforested soils triggered by base cation enrichment. Science of the Total Environment. 368: 19 -29.

Fellman, J.B., Hood, E., Edwards, R.T. and D'Amore, D.V. 2009. Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. Journal of Geographical Research. 114. G01021.

Filley, T.R., 2006. The contribution of polyhydroxyl aromatic compounds to tetramethylammonium hydroxide lignin-based proxies. Organic Geochemistry. 37: 711-727.

Fitzgerald, W.F., Engstrom, D.R., Mason, R.P. and Nater, E.A. 1998. The case for atmospheric mercury contamination in remote areas. Environmental Science and Technology. 32 (1): 1 -7.

Fleck, J.A. 1999. Mercury transport through northern forested watersheds: Dissolved and particulate pathways. M.S. thesis, University of Minnesota. St. Paul, MN.

Floge, S.A. and Wells, M.L. 2007. Variation in colloidal chromophoric dissolved organic matter in the Damariscotta Estuary, Maine. Limnology and Oceanography. 52 (1): 32 – 45.

Froberg, M., Jardine, P.M., Hanson, P.J., Swanston, C.W., Todd, D.E., Tarver, J.R. and Garten Jr., C.T. 2007. Low dissolved organic carbon input from fresh litter to deep mineral soils. Soil Science Society of America Journal. 71: 347 – 354.

Froberg, M., Berggren, D., Bergkvist, B., Bryant, C. and Knicker, H. 2003. Contributions of Oi, Oe and Oa horizons to dissolved organic matter in forest floor leachates. Geoderma. 113: 311 – 322.

Gilmour, C.C., Henry, E.A. and Mitchell, R. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environmental Science & Technology. 26: 2281 - 2287.

Gilmour, C.C. and Riedel, G.S. 1995. Measurement of Hg methylation in sediments using high
specific-activity Hg-203 and ambient incubation. Water Air and Soil Pollution. 80: 747.

Golding, G.R., Kelly, C.A., Sparling, R., Loewen, P.C., Rudd, J.W.M. and Barkay, T. 2002. Evidence for facilitated uptake of Hg(II) by Vibrio anguillarum and Escherichia coli under anaerobic and aerobic conditions. Limnology and Oceanography. 47(4): 967-975.

Grandy, A.S. and Neff, J.C. 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of the Total Environment. 404: 297-307.

Graydon, J.A., St. Louis, V.L., Hintelmann, H., Lindberg, S.E., Sandilands, K.A., Rudd, J.W.M., Kelly, C.A., Hall, B.D. and Mowat, L.D. 2008. Long-term wet and dry deposition of total and methyl mercury in the remote boreal ecoregion of Canada. Environmental Science Technology. 42: 8345 – 8351.

Graydon, J.A., St. Louis, V.L. Hintelmann, H., Lindberg, S.E., Sandilands, K.A., Rudd, J.W.M., Kelly, C.A., Tate, M.L., Krabbenhoft, D.P. and Lehnherr, I. 2009. Investigation of uptake and retention of atmospheric Hg(II) by boreal forest plants using stable Hg isotopes.

Grigal, D.F. 2002. Inputs and outputs of mercury from terrestrial watersheds: a review. Environmental Review. 10: 1 – 39.

Grigal, D.F., 2003. Mercury sequestration in forests and peatlands: a review. Journal of Environmental Quality. 32: 393 – 405.

Guentzel, J.L., Powell, R.T., Landing, W.M. and Mason, R.P. 1996. Mercury associated with colloidal material in an estuarine and an open-ocean environment. Marine Chemistry. 55: 177 – 188.

Haitzer, M., Aiken, G.R. and Ryan, N.J. 2003. Binding of Mercury (II) to aquatic humic substances: influence of pH and sources of humic substances. Environmental Science and Technology. 37: 2436 – 2441.

Hall, B.D., Aiken, G.R., Krabbenhoft, D.P., Marvin-DiPasquale, M. and Swarzenski, C.M. 2008. Wetlands as principal zones of methylmercury production in southern Louisiana and the Gulf of Mexico region. Environmental Pollution. 154: 124 – 134.

Harris, R.C., Rudd., J.W.M., Amyot, M., Babiarz, C.L., Beaty, K.G., Blanchfield, P.J., Bodaly, R.A., Branfireun, B.A., Gilmour, C.C., Graydon, J.A., Heyes, A., Hintelmann, H., Hurley, J.P., Kelly, C.A., Krabbenhoft, D.P., Lindberg, S.E., Mason, R.P., Paterson, M.P., Podemski, C.L., Robinson, A., Sandilands, K.A., Southworth, G.R., St. Louis, V.L. and Tate, M. 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. Proceedings of the National Academy of Science. 104 (42): 16586 – 16591.

Harvey, H.R. and Mannino, A. 2001. The chemical composition and cycling of particulate and macromolecular dissolved organic matter in temperate estuaries as revealed by molecular organic tracers. Organic Geochemistry. 32: 527 – 542.

Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber and Mopper, K. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnology and Oceanography. 53: 955–969.

Heyes, A., Miller, C.L. and Mason, R. 2004. Mercury and methylmercury in Hudson River sediment: impact of tidal resuspension on partitioning and methylation. Marine Chemistry. 90: 75 – 89.

Heyes, A., Mason, R.P., Kim, E-H, and Sunderland, E. 2006. Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes. Marine Chemistry. 102: 134 – 147.

Hintelmann, H., Harris, R., Heyes, A., Hurley, J.P., Kelly, C.A., Krabbenhoft, D.P., Lindberg, S, Rudd, J.W.M., Scott, K.J. and St. Louis, V.L. 2002. Reactivity and mobility of new and old mercury deposition in a Boreal forest ecosystem during the first year of the METAALICUS study. Environmental Science and Technology. 36: 5034 – 5040.

Hintelmann, H. and Ogrinc N. 2003. Determination of stable mercury isotopes by ICP/MS and their application in environmental studies. In Chai Y, Braids OC, eds, Biochemistry of Environmental
Important Trace Elements. American Chemical Society, Washington, DC, pp 321–338.

Holmes, R. M., McClelland, J.W., Raymond, P. A., Frazer, B.B., Peterson, B.J. and Stieglitz, M. 2008. Lability of DOC transported by Alaskan rivers to the Arctic Ocean. Geophysical Research Letters. 35. L03402.

Hollweg, T.A., Gilmour, C.C. and Mason, R.P. 2009. Methylmercury production in sediments of Chesapeake Bay and the mid-Atlantic continental margin. Marine Chemistry. 114: 86 – 101.

Houel, S., Louchouarn, P., Lucotte, M., Canuel, R. and Ghaleb, B. 2006. Translocation of soil organic matter following redervoir impoundment in boreal systems: implications for in situ productivity. American Society of Limnology and Oceanography. 51 (3): 1497 – 1513.

Howe, K.J. and Clark, M.M. 2002. Coagulation pretreatment for membrane filtration. Published by American Water Works Association, Denver, CO, pp 129 -131.

Hu, F.S., Hedges, J.I., Gordon, E.S. and Brubaker, L.B. 1999. Lignin biomarkers and pollen in postglacial sediments of an Alaskan lake. Geochimica et Cosmochimica Acta. 63 (9): 1421 – 1430.

Hu, C., Muller-Karger, F.E. and Zepp, R.G. 2002. Absorbance, absorption coefficient, and apparent quantum yield: A comment on common ambiguity in the use of these optical properties. Limnology and Oceanography. 47(4): 1261-1267.

Ikeya, K., Yamamotot, S., and Watanabe, A. 2004. Semiquantitative GC/MS analysis of thermochemolysis products of soil humic acids with various degrees of humification. Organic Geochemistry. 35 (5): 583 – 594.

Kainz M. and Lucotte, M. 2006. Mercury concentrations in lake sediments – revisiting the predictive power of catchment morphometry and organic matter composition. Water, Air and Soil Pollution. 170: 173 – 189.

Kaiser, K., Guggenberger, G., Haumaier, L. and Zech, W. 2002. The composition of dissolved organic matter in forest soil solutions: changes induced by seasons and passage through the mineral soil. Organic Geochemistry. 33: 307-318.

Kaiser, K., Guggenberger, G. and Zech, W. 1996. Sorption of DOM and DOM fractions to forest soils. Geoderma. 74: 281-303.

Kaiser, K., and Guggenberger, G. 2000. The role of DOM sorption to mineral surfaces in preservation of organic matter in soils. Geochemistry. 31: 711 – 725.

Kalbitz, K. and Wennrich, R. 1998. Mobilization of heavy metals and arsenic in polluted wetland soils and its dependence on dissolved organic matter. The Science of the Total Environment. 209: 27 – 39.

Kalbitz, K., Schwesig, D., Rethemeyer, J. and Matzner, E. 2005. Stabilization of dissolved organic matter by sorprtion to the mineral soil. Soil Biol. Biochem.

Kalbitz, K., Solinger, S., Michalzik, B. and Matzner, E. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Science. 165 (4): 277 – 304.

Karlsson, T. and Skyllberg, U. 2003. Bonding of ppb levels of methylmercury to reduced sulfur groups in soil organic matter. Environmental Science and Technology. 37: 4912 – 4918.

Khwaja, A., Bloom, P.R. and Brezonik, P.L. 2006. Binding constants of divalent mercury (Hg^{2+}) in soil humic acids and soil organic matter. Environmental Science and Technology. 40: 844-849.

Kogel-Knabner. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry. 34: 139-162.

Kolka, R., Grigal, D.F., Nater, E.A., and Verry, E.S. 2001. Hydrologic cycling of mercury and organic carbon in a forested upland-bog watershed. Soil Science Society of America Journal. 65: 897 – 905.

Kolker, A., Olson, M.L., Krabbenhoft, D.P., Tate, M.T. and Engle, M.A. 2010. Patterns of mercury dispersion from local and regional emission sources, rural Central Wisconsin, USA. Atmospheric Chemistry and Physics. 10 (10): 4467 – 4476.

Kuroda, K-I and Nakagawa-izumi, A. 2005. Tetramethylammonium hydroxide (TMAH) thermochemolysis of lignin: behavior of 4-O-etherifieed cinnamul alcohols and aldehydes. Journal of Agricultural and Food Chemistry. 53: 8859 – 8865.

Lajtha, K., Crow, S.E., Yano, Y., Kushal, S., Sulzman, E., Sollins, P. and Spears, J.D.H. 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry. 76: 261-281.

Leblanc, L., Schroeder, R.A., Orlando, J.L. and K.M. Kuivila. 2004. Occurrence, distribution and transport of pesticides, trace elements and selected inorganic constituents into the Salton Sea Basin, California, 2001-2002: U.S. Geological Survey Scientific Investigations Report 2004-5117, 40 p.

Leonowicz, A., Matuszewska, A., Luterek, J., Ziegenhagen, D., Woitas-Wasilewska, M., Cho, N-S. and Hofrichther, M. 1999. Biodegradation of lignin by white rot fungi. Fungal Genetics and Biology. 27: 175 – 185.

Lindberg, S., Bullock, R., Ebinghaus, R., Engstrom, D., Feng, X., Fitzgerald, W., Pirrone, N., Prestbo, E. and Seigneur, C. 2007. A synthesis of progress and uncertainties in attributing the sources of mercury in deposition. Ambio. 36 (1): 19 – 32.

Lui, G., Cai, Y., Mao, Y., Scheidt, D., Kalla, P., Richards, J., Scinto, L.J., Tachiev, G., Roelant, D. and Appleby, C. 2009. Spatial variability in mercury cycling and relevant biogeochemical controls in the Florida Everglades. Environmental Science and Technology. 43: 4361 – 4366.

Louchouarn, P., Opsahl, S. and Benner, R. 2000. Isolation and quantification of dissolved lignin from natural waters using solid-phase extraction and GC/MS. Analytical Chemistry. 72: 2780 – 2787.

Ma, J., Del Vecchio, R., Golanoski, K., Boyle, E. and Blough, N. 2010. Optical properties of humic substances and CDOM: Effects of borohydride reduction source. Environmental Science and Technology. 44 (14): 5395-5402.

Maňkovská, B. 1996. Mercury concentrations in forest trees from Slovakia. Water Air and Soil Pollution. 89: 267 – 275.

Mannino, A. 2000. Chemical Composition of Particulate and Macromolecular Dissolved Organic Matter in the Delaware Estuary and Experimental Diatom Blooms: Sources and Reactivity Patterns. PhD Dissertation, University of Maryland at College Park.

Mannino, A. and Harvey, H.R. 2000. Biochemical composition of particles and dissolved organic matter along an estuarine gradient: Sources and implications for DOM reactivity. Limnology and Oceanography: 45(4): 775-788.

Maryland Department of the Environment. 2002. Water Quality Analysis of Eutrophication of the St. Mary's Lake, St. Mary's County, Maryland.

Mason, R.P. and Gill, G.A. 2005. Mercury in the Marine Environment. Mineralogical Association of Canada Short Course 34, Halifax, Nova Scotia. Chapter 10. 179 -216.

Mason, R.P., Fitzgerald, W.F. and Morel, F.M.M. 1994. The biogeochemical cycling of elemental mercury: Anthropogenic influences. Geochimica et Cosmochimica Acta. 58 (15): 3191 – 3198.

McCarthy, J.F., Williams, T.M., Liang, L., Jardine, P.M., Louwanda, W.J., Taylor, D.L., Palumbo, A.V. and Cooper, L.W. 1996. Mobility of natural organic matter in a sandy aquifer. Environmental Science and Technology: 27: 667 – 676.

McGlynn, B.L. and McDonnell, J.J. 2003. Role of discrete landscape units in controlling catchment and dissolved organic carbon dynamics. Water Resources Resarch. 39(4): 1090.

McKnight, D., Andrews E.D., Spauldings, S.A. and Aiken, G.R. Aquatic fulvic-acids in algal-rich Antarctic ponds. Limnology and Oceanography. 39 (8): 1972 – 1979.

Meier, M., Namjesnik-Dejanovic, K., Maurice, P.A., Chin-Y-P. and Aiken, G.R. Fractionation of aquatic natural organic matter upon sorption to goethite and kaolinite. Chemical Geology. 157(3-4): 275 – 284.

Miller, C.L., Southworth, G., Brooks, S., Liang, L. and Baohua, G. 2009. Kinetic controls on the complexation between mercury and dissolved organic matter in a contaminated environment. Environmental Science and Technology. 43: 8548 – 8553.

Miller, C.L. 2006. The role of organic matter in the dissolved phase speciation and solid phase partitioning of mercury. PhD Thesis. University of Maryland College Park.

Mitchell, C.P.J. and Gilmour, C.C. 2008. Methylmercury production in a Chesapeake Bay salt marsh. Journal of Geophysical Research. 113: G00C04

Moore, T.R., Bubier, J.L., Heyes, A. and Flett, R.J. Methyl and total mercury in boreal wetland plants, Experimental Lakes Area, Northwestern Ontario. Journal of Environmental Quality. 24 (5):

Munthe, J., Bodale, R.A., Branfireun, B.A., Driscoll, C.T., Gilmour, C.C., Harris, R., Horvat, M., Lucotte, M. and Malm, O. 2007. Recovery of mercury-contaminated fisheries. Ambio. 36(1): 33 -44.

Obrist, D.D., Johnson, D.W. and Lindberg, S.E. 2009. Mercury concentrations and pools in four Sierra Nevada forest sites and relationships to organic carbon and nitrogen. Biogeosciences. 6: 765 – 777.

O'Donahue, M.J.H. and Dennison, W.C. 1997. Phytoplankton productivity response to nutrient concentrations, light, availability, and temperature along an Australian estuarine gradient. Estuaries. 20: 521-533.

Oswald, C.J., Richardson, M. and Branfireun, B. 2010. Water storage dynamics and runoff response of a Boreal Shield headwater catchment. Submitted to Hydrological Processes.

Oswald, C.J., Heyes, A. and Branfireun, B.A. 2010. Investigation of storage of ambient mercury and applied mercury isotope in a Boreal Shield soil landscape. In preparation.

Ouellet, J-F., Lucotte, M., Teisserenc, R., Paquet, S. and Canuel, R. 2009. Lignin biomarkers as tracers of mercury sources in lakes water column. Biogeochemistry. 94: 123 – 140.

Palmer, S., Hope, D., Billett, M.F., Dawson, J.J. and Bryant, C.L. 2001. Sources of organic and inorganic carbon in a headwater stream: Evidence from carbon isotope studies. Biogeochemistry. 52: 312 -338.

Qian, J., Skyllberg, U., Frech, W., Bleam, W.F., Bloom, P.R. and Petit, P.E. 2002. Bonding of methyl mercury to reduced sulfur groups in soil and stream organic matter as determined by X-ray absorption spectroscopy and binding affinity studies.

Pedosphere.com. 2002. Searchable Canadian System of Soil Classification, Third Edition. Available URL: http://www.pedosphere.com/resources/CSSC3rd/ Retrieved October 12, 2009.

Rasmussen, P.E., Mierle, G. and Nriagu, J.O. 1991. The analysis of vegetation for total mercury. Water, Air and Soil Pollution. 56: 379 -390.

Ravichandran, M. 2004. Interactions between mercury and dissolved organic matter – a review. Chemosphere. 55:319-331.

Ravichandran, M., Aiken, G.R., Reddy, M.M. and Ryan, J.N. 1998. Enhanced dissolution of cinnabar (mercuric sulfide) by dissolved organic matter isolated from the Florida Everglades. Environmental Science and Technology. 32: 3305 – 3311.

Richardson, M., Fortin, M-J. and Branfireun, B. 2009. Hydrogeomorphic edge detection and delineation of landscape functional units from lidar digital elevation models. Water Resources Research. 45. W10441.

St. Louis, V.L., Rudd, J.W.M., Kelly, C.A., Hall, B.D., Rolfhus, K.R., Scott, K.J., Lindberg, S.E. and Dong, W. 2001. Importance of the forest canopy to fluxes of methyl mercury and total mercury to boreal ecosystems. Environmental Science and Technology. 31 (15): 3089 – 3098.

Sanderman, J., Baldock, J.A. and Amundson, R. 2008. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. Biogeochemistry: 89: 181 – 198.

Sandilands, K., Kelly, C.A., Rudd, J.W.M., Tate, M.T., Hintelmann, H., Dimock, B. and Harris. R.C. 2008. Application of enriched stable mercury isotopes to the Lake 658 watershed for the METAALICUS project, at the Experimental Lakes Area, northwestern Ontario, Canada, 2001 – 2007. Canadian Technical Report of Fisheries and Aquatic Sciences 2813: vii + 40p.

Schiff, S., Aravena, R., Trumbone, S.E., Hinton, M.J., Elgood, R. and Dillon, P.J. 1997. Export of DOC from forested catchments on the Precambrian Shield of Central Ontario: Clues from ^{13}C and ^{14}C. Biogeochemistry. 36: 43 -65.

Schuster, P.F., Shanley, J.B., Marvin-Dipasquale, M., Reddy, M.M., Aiken, G.R., Roth, D.A., Taylor, H.E., Krabbenhoft, D.P. and DeWild, J.F. 2008, Mercury and organic carbon dynamics during runoff episodes from a northeastern USA watershed. Water, Air, and Soil Pollution. 187: 89-108.

Shanley, J.B., Mast, A., Campbell, D.H., Aiken, G.R., Krabbenhoft, D.P., Hunt, R.J., Walker, J.F., Schuster, P.F., Chalmers, A., Aulenbach, B.T., Peters, N.E., Marvin-DiPasquale, M., Clow, D.W. and Shafer, M.M. 2008. Comparison of total mercury and methylmercury cycling at five sites using the small watershed approach. Environmental Pollution. 154 (1): 143-154.

Shanley, J., Schuster, P.F., Reddy, M.M., Roth, D.A., Taylor, H.E. and Aiken, G.R. 2002. Mercury on the move during snowmelt in Vermont. EOS. 83(5): 45-48.

Shanley, J., Aiken, G., Dittman, J., Driscoll, C.T., Pellerin, B., Saraceno, J., Riscassi, A. and Burns, D. 2010. Proxy measures of mercury and carbon quality in forested upland streams. Northeastern Section (45th Annual) and Southeastern Section (59th Annual) Joint Meeting (13-16 March 2010)

Sigg, L., Xue, HB., Kistler, D. and Shonenberger, R. 2000. Size fractionation (dissolved, colloidal and particulate) of trace metals in the Thur River, Switzerland. Aquatic Geochemistry. 6(4): 413-434.

Skyllberg, U., Qian, J., Frech, W., Xia, K. and Bleam, W.F. 2003. Distribution of mercury, methyl mercury and organic sulphur species in soil, soil solution and stream of a boreal forest catchment. Biogeochemistry. 64: 53 – 76.

Skyllberg, U., Lin, C-M., Bloom, P.R., Qian, J. and Bleam, W.F. 2006. Complexation of mercury (II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulphur groups. Environmental Science and Technology. 40: 4147-4180.

Smith-Downey, N.V., Sunderland, E.M. and Jacob, D.J. 2010. Anthropogenic impacts on global storage and emissions of mercury from terrestrial soils: Insights from a new global model. Journal of Geophysical Reseach. 115. G03008

Spencer, R.G.M., Aiken, G.R., Dyda, R.Y., Butler, K.D., Bergamaschi, B.A. and Hernes, P.J. 2010. Comparison of XAD with other dissolved lignin isolation techniques and a compilation of analytical improvements for the analysis of lignin in aquatic settings. Organic Geochemistry. 41: 445 – 453.

Southworth G, Lindberg S, Hintelmann H, Amyot M, Poulain A, Bogle M, Peterson M, Rudd J, Harris R, Sandilands K, Krabbenhoft D. and Olsen, M. 2007. Evasion of added isotopic mercury from a northern temperate lake. Environmental Toxicology and Chemistry. 26 (1): 53 – 60.

Spencer, R.G., Aiken, G.R., Wickland, K.P., Striegl, R.G. and Hernes, P.J. 2008. Seasonal and spatial variability in dissolved organic matter quantity and composition from the Yukon River basin, Alaska. Global Biogeochemical Cycles. 22: GB4002

Tan, S.W., Meiller, J.C. and Mahaffey, K.R. 2009. The endocrine effects of mercury in humans and wildlife. Critical Reviews in Toxicology. 39 (7): 627 – 635.

Teisserenc, R. 2009. Dynamique de la matière organique terrigène et du mercure dans les lacs et réservoirs boréaux. PhD Dissertation. Université du Québec à Montréal.

Tipping, E., Froberg, M., Berggren, D., Mulder, J. and Bergkvist, B. 2005. DOC leaching from a coniferous forest floor: modeling a manipulation experiment. Journal of Plant Nutrition and Soil Science. 168(3): 316 – 324.

Town, R. and Filella, M. 2002. Size fractionation of trace metal species in freshwaters: implications for understanding their behaviour and fate. Reviews in Environmental Science and Biotechnology. 1 (4): 277 – 297.

Tulonen, T., Salonen, K. and Arvola, L. 1992. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. Hydrobiologia. 229: 239 – 252.

Waples, J., Nagy, K., Aiken, G. and Ryan, J. 2005. Dissolution of cinnabar (HgS) in the presence of natural organic matter. Geochimica et Cosmochimica Acta. 69 (6): 1575 – 1588.

Watras, C.J., Back, R.C., Halvorsen, S., Hudson, R.J.M., Morrison, K.A. and Wente, S.P. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. The Science of the Total Environment. 219: 183 – 208.

Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R. and Mopper, K. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science and Technology. 37: 4702 – 4708.

Wershaw, R.L. 2004. Evaluation of conceptual models of natural organic matter (Humus) from a consideration of the chemical and biochemical processes of humification: U.S. Geological Survey Scientific Investigation Report 2004-5121, 44 p.

Wiederhold, J.G., Cramer, C.J., Daniel, K., Infante, I., Bourdon, B. and Kretzschmar, R. 2010. Equilibrium Mercury Isotope Fractionation between Dissolved Hg(II) Species and Thiol-Bound Hg. Environmental Science and Technology. 44 (110): 4191 – 4197.

Wiener, J. G., Krabbenhoft, D. P., Heinz, G. H. and Scheuhammer, A. M. 2003. Ecotoxicology of mercury, Chap. 16, in: Handbook of Ecotoxicology, 2nd edn., edited by: Hoffman, D. J., Rattner, B. A., Burton Jr., G. A., and Cairns Jr., J., CRC press, Boca Raton, Florida, USA. 407–461.

Wolfe, M., Schwarzbach, S. and Sulaiman, A. 1998. Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry. 17 (2): 146 – 160.

Wysocki, L.A., Filley, T.R and Bianchi, T.S. 2008. Comparison of two methods for the analysis of lignin in marine sediments: CuO oxidation versus tetramethylammonium hydroxide (TMAH) thermochemolysis. Organic Geochemistry. 39: 1454 – 1461.

Xia, K., Skyllberg, U., Bleam, W.F., Bloom, P.R., Nater, E.A. and Helmke, P.A. 1999. X-ray absorption spectroscopic evidence for the complexation of Hg(II) by reduced sulfur in soil humic substances. Environmental Science and Technology. 33(2): 257 – 261.

Zhang, W. and W-X Wang. 2004. Colloidal organic carbon and trace metal (Cd, Fe, and Zn) releases by diatom exudation and copepod grazing. Journal of Experimental Marine Biology and Ecology. 307 (1) : 17-34.

Zheng, W. and Hintelmann, H. 2009. Mercury isotope fractionation during photoreduction in natural water is controlled by its Hg/DOC ratio. Geochimica et cosmochimica acta. 73 (22): 6704 -6715.

Zucker, W.V. 1983. Tannins: Does structure determine function? The American Naturalist. 121 (3) – 335 -365.