

**Using epiphytic lichens as biomonitors of atmospheric mercury and dust at a
historical gold mine tailings site in Nova Scotia, Canada**

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Abstract

Historic gold mining in Nova Scotia, Canada, produced mercury (Hg)-contaminated tailings from the 1860s to 1940s that were deposited into the environment and subsequently abandoned upon mine closures. Today, these degraded landscapes are potential sources of contaminated dust, posing risks to human and ecosystem health. The primary objective of this thesis was to use epiphytic lichens (*Usnea* and *Platismatia* spp.) as biomonitors of airborne Hg in the Montague Gold District. Spatial distribution patterns of Hg in lichens showed hotspots near tailings deposits, reflecting greater inputs of Hg from windblown tailings, volatilization processes, throughfall, and/or stemflow. The Hg in the lichens was assessed in two ways, including surface-deposited and absorbed Hg fractions. These results suggested that gaseous Hg from the tailings was a more important source of the element compared to particulate-bound Hg. These lichens proved to be effective biomonitoring tools at Montague for assessing Hg pollution and identifying risk areas.

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Firstly, I would like to begin by recognizing and acknowledging that this work was conducted in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq people.

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Note: This thesis is organized into four chapters and is a manuscript-style thesis. Chapter 1 includes background information and outlines the thesis objectives. Chapter 2 examines the spatial distribution patterns of mercury in lichens and from the manufactured passive air samplers. Chapter 3 discusses the mercury data pertaining to the washed and unwashed lichen samples. Some repeating methodologies are outlined in Chapters 2 and 3, both of which also include similar background information from Chapter 1. The final chapter, Chapter 4, provides a summary of this work with conclusions.

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Chapter 1: Introduction and Objectives

1.0 Introduction

1.1 History of Gold Mining in Nova Scotia

Mining and smelting of metalliferous ores have been taking place all over the world, potentially for up to 7000 years, providing wealth to many nations (Batty, 2005). In particular, gold (Au), a naturally occurring yet rare metal, has been valued by many human societies as it does not corrode and is highly malleable, durable, and attractive. It is also non-toxic, conducts electricity, and reflects heat and light. These features have made gold very useful for manufacturing electronics, jewellery, as well as for use in dentistry, medicine, engineering, and aerospace (Butt & Hough, 2009). These unique qualities and various uses of gold, in addition to its extreme rarity, have consistently led to its high value over time. The discovery of gold in California in 1849 caused “gold fever” to spread across countries and continents, leading to the discovery of additional gold deposits and the rise of many additional gold rushes (Mountford & Tuffnell, 2018).

In Nova Scotia, Canada, historical gold mining was first established in the Mooseland Gold District in 1858, with multiple operations along the Tangier River (Malcolm, 1929). Early exploration was prompted by the gold rushes in California and Australia in 1849 and 1851, respectively (Malcolm, 1929; Mountford & Tuffnell, 2018). With the initial discovery of gold in 1858 came the search for gold across the province, soon thereafter initiating the province’s first gold rush in 1861. To avoid the lawlessness and chaos seen in other gold-rush regions around the world (Mountford & Tuffnell, 2018), the provincial government established 64 gold mine districts with administration offices in each district to lease mining land and to encourage mining operations

(Malcolm, 1929). Official documents indicate that gold mining operations started in 1861 and ended by the mid-1940s. Over 360 mines were in operation and later abandoned during this time (Malcolm, 1929). Collectively, these mines produced 1.2 million troy ounces of gold based on official documents (Parsons et al., 2012).

This first gold rush peaked in 1867, yielding 27,358 ounces of gold, primarily extracted with the help of mercury (Hg) amalgamation, from Sherbrooke, Wine Harbour, Lawrencetown, Oldham, Waverley, Gold River, Isaacs Harbour, and Country Harbour (Malcolm, 1929; Bates, 1987). As mining continued into the early 1870s and with most of the accessible and high-grade ore worked, poor mining methods and management led to declines in gold production (Bates, 1987).

The second gold rush in the province lasted eight years, between 1896 and 1903, with highest gold yields reaching 31,113 ounces in 1898 (Bates, 1987). During this gold rush, smaller deposits of gold could be extracted from lower-grade ore due to the adoption of advanced mining technologies and practices that included the use of dynamite for blasting, cyanide for concentrating gold, and more effective grinding machinery.

In Nova Scotia, most gold is found in shallow quartz veins within slates and greywackes associated with the Cambro-Ordovician Meguma Supergroup, which comprises the majority of Nova Scotia's southern mainland (Bates, 1987; Parsons et al., 2012). The majority of mining activities involved small-scale open-cut mining and underground shafts, where mineralized rock was brought to the surface (Malcolm, 1929; Trip & Skilton, 1985). The use of crushers and stamp mills eventually became the most common method for extracting gold, leading to the development of more efficient large-

scale mining operations. Auriferous ore was broken up and crushed in stamp mills into sand- or silt-sized particles by mechanically lifting and dropping 5-20 vertically-aligned weighed-metal rods onto the ore (Bates, 1987; Parsons et al., 2012).

Once the ore was crushed, gold could be extracted through gravity separation, amalgamation, or cyanidation. The most common method for gold extraction in Nova Scotia was Hg amalgamation (Mudroch & Clair, 1985). The crushed quartz was mixed with water, initiating flow atop copper plates coated with a thin film of Hg (Mudroch & Clair, 1985; Bates, 1987; Art Gallery of Nova Scotia, 2013). Free gold dissolved in the liquid mercury, forming an amalgam. The gold amalgam would later be heated, causing the Hg to evaporate, and leaving the gold behind (Parsons & Percival, 2005).

In addition to gold, Nova Scotian bedrock deposits (i.e. slates and greywackes) naturally contain elevated levels of the sulfide mineral arsenopyrite (FeAsS), of which arsenic (As) is a component (Drage, 2015). Throughout the 1920s, an increase in the demand for arsenical insecticides in the United States, along with decreasing energy costs and an increase in the price of gold, led to the beginning of the third gold rush in 1932 (Malcolm, 1929; Bates, 1987; Parsons et al., 2012). This gold rush ended in 1942, producing a total of 158,000 ounces of gold (Bates, 1987) and approximately 1000 tonnes of arsenical concentrates for the market (Hurst, 1924; Parsons et al., 2012).

The sand- to silt-sized waste particles that remained after the milling and extraction operations are known as “tailings”. Early mining operations were particularly inefficient and wasteful; it is estimated that as much as 30% of gold was left behind in the tailings from loss during the crushing and concentrating processes (Bates, 1987; Mills, 1997). The gold extraction process also resulted in significant amounts of local Hg

pollution due to the careless handling of elemental Hg (Hg⁰) by mill operators and inexperienced miners (Trip & Skilton, 1985; Parsons et al., 2012). It is estimated that approximately 10-25% of all Hg used was lost to the mill tailings and to the atmosphere, with only a portion being captured and reused (Bates, 1987; Art Gallery of Nova Scotia, 2013). On average, one ounce of Hg was used to extract one ounce of gold from ore, however, up to three times this amount of Hg may have been used at some mines throughout the province (Parsons et al., 2012). Thus, an estimated 9,100 kg of Hg may have been lost from gold mining operations in Nova Scotia, ending up in the tailings and/or atmosphere (Parsons et al., 2012). In addition, the disruption of arsenopyrite when extracting the gold from auriferous veins resulted in significant concentrations of As released via tailings to the surrounding environments (Bates, 1987; Art Gallery of Nova Scotia, 2013). Cyanidation, the process of using cyanide solutions to leach gold from sulfide minerals, was more effective at extracting gold than Hg amalgamation, subsequently being employed at some mines towards the end of the 19th century (Bates, 1987; Parsons et al., 2012).

Over 80 years of historical gold mining operations in Nova Scotia, an estimated three million tonnes of tailings were generated across the province (Parsons et al., 2012). During those early mining operations, there were no regulations governing the disposal of mining waste after milling and processing via amalgamation or cyanidation. As a result, low-lying areas, including freshwater ecosystems and adjoining coastal areas downstream of mining operations, became convenient or unintended disposal sites for the tailings. By the mid-1940s, most gold production in the province had ceased. This resulted in the abandonment of mine sites, leaving a legacy of Hg and As contamination in surrounding

environments, as well as other contaminants such as lead (Pb) and organic residues.

Today, many of these former mining sites are located on private and public lands and are hazardous because they include surface-exposed tailings deposits, water-submerged tailings, waste rock piles, and open mine shafts.

1.2 Mercury

Mercury is a naturally occurring element found in the Earth's crust which can be released naturally from sources such as volcanoes and forests fires, for instance. However, elevated background levels and the ubiquitous distribution of Hg in the environment today are largely a result of anthropogenic activities. This includes the burning of coal, oil, and wood, but also results from the widespread use in amalgamation at historical gold and silver mining operations (Schroeder & Munthe, 1998; Keeler et al., 2006). Mercury was first used on an industrial scale for silver mining in Spain in 1554, then extensively used at gold mines in North America during the gold rushes of the 18th and 19th centuries, including in Nova Scotia (Parsons & Percival, 2005). The close association of Hg with silver and gold mining throughout history has led to the persistent contamination of soils, water, and air surrounding mining districts, as well as the bioaccumulation of methylmercury (MeHg) in local food chains world-wide (Nriagu, 1994; Lodenius & Malm, 1998; Lecce et al., 2008). Mercury is persistent in the environment and thus can be released from source areas for tens to thousands of years after mining operations have ceased (Parsons & Percival, 2005).

Mercury exists in various forms which vary in toxicity, including Hg⁰, organic Hg compounds, and inorganic Hg compounds, of which the latter can exist in two oxidative states (Hg⁺ and Hg²⁺) (Figure 1) (Park & Zheng, 2012). The highly volatile Hg⁰, which is

liquid between -38.9°C and 356.7°C (Government of Canada, 2013), was used for Hg amalgamation at historical gold mines in Nova Scotia starting in the early 1860s and declining somewhat when cyanidation processes became more common starting in the 1890s (Malcolm, 1929; Parsons et al., 2012). Elemental Hg is released either in its liquid form directly from disposed mining wastes or volatilized into the surrounding atmosphere (Schroeder & Munthe, 1998). Over 90% of globally atmospheric Hg consists of gaseous Hg^0 , which has an atmospheric lifetime of approximately 1-2 years, and is deposited mainly by dry deposition (Lin & Pehkonen, 1999; Kocman et al., 2011). The other main forms of Hg in the atmosphere include gaseous oxidized Hg (GOM) and particulate-bound Hg (Figure 1) (Pacyna & Pacyna, 2005; McLagan et al., 2016; Huang et al., 2020). Elemental Hg can be oxidized in the atmosphere to form GOM, which commonly comprises divalent Hg (Hg^{2+}) species that typically become dissolved in atmospheric water droplets or adsorbed onto dust particles as particulate-bound Hg (Kocman et al., 2011; Huang et al., 2020). Divalent Hg is deposited to the Earth's surface only within days to weeks through both wet and dry deposition (Lin & Pehkonen, 1999; Huang et al., 2020). Gaseous oxidized Hg and particulate-bound Hg become deposited and bound in terrestrial and aquatic ecosystems and not released unless reduced. Mercury is a Class B element and Hg ions bind very strongly to protein and enzyme components of cells, often interfering with their function (Nieboer & Richardson, 1980; Porett & Bollinger, 2018). In contrast, Hg^0 is bidirectional, capable of continuous exchange between the air and soil, water, or biota via deposition and volatilization (Bargagli, 2016).

In aquatic systems, Hg^0 can be oxidized to form Hg^{2+} which can then be transformed primarily by microorganisms into organic forms, such as MeHg, through the

process of methylation (Figure 1) (Kim, 2005; Parsons & Percival, 2005). Methylmercury is accumulated by fish and marine mammals, biomagnifying up the food chain to large fish, birds, and even humans. For humans, the most common exposure pathways to Hg are from MeHg through the consumption of fish and shellfish (Park & Zheng, 2012) and through inhalation of Hg vapour (US EPA, 2020). Inorganic Hg compounds are water soluble with a bioavailability of 7 to 15% once ingested, typically leading to kidney damage (Park & Zheng, 2012). In contrast, organic Hg compounds (e.g. MeHg) are rapidly absorbed by the brain and kidneys upon entering the body (Park & Zheng, 2012). Elemental Hg and MeHg are of particular concern as they can cross the blood-brain and placental barriers, acting as a neurotoxin and can persist in the brain for as long as 20 years (Gupta et al., 2005; Park & Zheng, 2012).

1.3 Mercury Contamination at Historical Gold Mine Tailings Sites in Nova Scotia

In Nova Scotia today, tailings continue to contaminate the environment with Hg (and As) more than 80 years after the last historical gold rush. In a large survey of 14 historical gold mine sites in the province, more than 480 tailings samples were collected, many of which were found to be elevated in Hg and exceeding the Canadian Council of Ministers of the Environment (CCME) soil (residential/parkland) and sediment (freshwater) quality guidelines of 6600 µg/kg and 486 µg/kg (dry weight), respectively (CCME, 1999a, b; Parsons et al., 2012). The tailings have also been shown to contaminate soils (Eaton, 1978; Parsons & Little, 2015), lake sediments (Mudroch & Sandilands, 1978; Mudroch & Clair, 1985; Clark et al., 2021), and waters (Eaton, 1978; LeBlanc, 2019, unpublished) with Hg at and further downstream of mine sites (Figure 1). In addition, as reviewed by LeBlanc et al. (2019), Hg from the tailings at various gold

districts across the province has bioaccumulated in plants, fish, terrestrial and aquatic invertebrates, marine molluscs, amphibians, and small mammals. In spite of these results, the bioaccumulation of Hg from gold mine tailings in small and large mammals, amphibians, reptiles, and lichens remains understudied or unstudied (LeBlanc et al., 2019).

1.4 Air Quality

Tailings deposits in former gold mining areas can act as a continuous source of Hg pollution to the air from both dust emissions and gaseous Hg evasion. This occurs because these contaminated sites typically lack nutrients, soil structure, and organic matter (Mendez and Maier, 2008; de-Bashan et al., 2010). Such conditions, in conjunction with disturbances, create landscapes devoid of vegetation, as observed at many historical gold mine sites in Nova Scotia (Figure 2). As a result, the exposed fine-grained tailings material can be subject to wind erosion (de-Bashan et al., 2010).

Furthermore, climate change in Canada is projected to increase annual and seasonal temperatures from 2 to 6°C by the end of the 21st century, as well as lead to changes in precipitation (Bush & Lemmen, 2019; Government of Canada, 2020). These changes may increase the number of extreme events, such as wildfires and droughts (Bush & Lemmen, 2019). When combined with sparse vegetation cover, such warmer, drier conditions may lower moisture levels, decrease soil particle cohesion, and increase the likelihood of dust emissions (Csavina et al., 2012). These will aid in the remobilization and dispersion of mine contaminants surrounding historic mine sites (Mendez & Maier, 2008). Exposure to these dusts can adversely impact human and environmental health (Noble et al., 2017).

1.4.1 Transport Pathways for Contaminants by Air

Many abandoned mine sites across Canada and around the world contain unconfined tailing deposits or ponds, open pits, or waste rock piles, which are a major source of mineral dust into the surrounding environment (Noble et al., 2017). Wind erosion is the primary process generating dust, along with anthropogenic activities and related disturbances (Csavina et al., 2012). Wind erosion is dependent on climatic conditions, rock mineralogy, land-use, vegetation cover, and soil characteristics (Biolders et al., 2001; Csavina et al., 2012; Noble et al., 2017). Mine tailings deposits can give rise to a wide size range of particles, including coarse airborne particles (~3-100 μm) and ultra-fine particles (0.001-0.1 μm) (Csavina et al., 2012; Noble et al., 2017).

Atmospheric suspended particles can adsorb, transport, and deposit contaminants in the environment, especially those with a low volatility, a low aqueous solubility, and those that remain attached to soil particles (Csavina et al., 2012; Noble et al., 2017). The long-distance transport of contaminants by atmospheric particles is of global concern because air masses can carry small particles over long periods of time and across continental and international boundaries. It is estimated that approximately 60% of globally atmospheric As originates from mining operations, and is transported and dispersed by atmospheric particulates (Csavina et al., 2011, 2012; Garrison et al., 2014). However, in general, atmospheric particulates originating from mine tailing sites are typically larger in diameter and deposit locally on spatial scales from meters to kilometers.

Many studies have shown historic mine tailing sites to be major sources of contaminated dust. For instance, at the historical Rio Tinto mining district in Spain, the

resuspended mine waste dust was estimated to contribute 32% to the total concentration of trace metals in the atmosphere. The elements included As, copper (Cu), and zinc (Zn) (Sánchez de la Campa et al., 2011). Also in Spain, mechanically resuspended particulate matter from the tailings at an abandoned gold mine site was enriched in antimony (Sb) and As (Moreno et al., 2007). Both studies concluded that there was potential for adverse environmental and human health effects from exposure to these metal-bearing particles.

In addition to the generation of metal-bearing dust particles, elements from contaminated sites can be released in the gas phase by direct transfer of volatilized species (Beamer et al., 2014). Mercury can be released as gaseous Hg^0 following the transformation of Hg^{2+} at redox boundaries via photoreduction or microbial communities (Krabbenhoft et al., 2005; Moreno et al., 2005). Many studies have shown that Hg-contaminated sites are significant sources of gaseous Hg. In urban and suburban areas in Guiyang, China, a city located in the Circum-Pacific Global Mercuriferous Belt and containing elevated soil Hg concentrations, the natural Hg emissions from soils to the ambient air (408 kg/yr) was comparable to emissions from coal combustion sources (639 kg/yr), the latter estimated to be the largest anthropogenic Hg source in the region (Feng et al., 2005). Similarly, at a landfill site in this same city in China, the gaseous Hg emission fluxes from both the exposed wastes to air and from the landfill soil surface to air were 2 to 3 orders of magnitudes higher than those from soils at a background site (Feng et al., 2004).

Metalliferous dust and gases pose significant risks to the local environment because the elements they contain can increase the concentrations in soils and surface waters (Meza-Figueroa et al., 2009; Kargar et al., 2012; Cleaver et al., 2021) as well as

biomagnify, having adverse physiological effects in biota (e.g. reduction in plant photosynthesis) near mine sites (Søndergaard, 2013; Pichhode & Nikhil, 2015). Fine particles from mining sites are of concern because of their high surface area, which results in higher dissolution rates and greater bioavailability (Csavina et al., 2012). The impact of metalliferous dust on human health depends on particle size and shape, composition, and solubility (Noble et al., 2017). Children are at increased risk of contaminated dust and vapours as they not only breathe more air and ingest more dust on a body weight basis than adults, but also because their developing bodies are more susceptible to the effects of metal exposure (Beamer et al., 2014). Coarse airborne particles (~3-100 μm) from the wind erosion of mine tailings can be inhaled and deposited in the upper respiratory system, or swallowed and absorbed in the digestive tract (Csavina et al., 2012). Fine airborne particles (0.001-0.1 μm) are respired deep into the lungs and contaminants such as Hg are generally absorbed directly into the bloodstream (Csavina et al., 2012; Stovern et al., 2014; Deng et al., 2019). Once inhaled or ingested, the dust can adversely impact human health.

1.4.2 Air Quality at Historical Gold Mine Tailings Sites in Nova Scotia

In Nova Scotia, studies investigating dust and air quality at the many legacy gold mine tailings sites have been limited. At the Goldenville, Caribou, and Lower Seal Harbour gold districts, the gaseous Hg flux rates from the tailings to the ambient air have been measured (Beauchamp et al., 2002; Wong et al., 2002; Dalziel & Tordon, 2014). These tailings sites released Hg at a faster rate and in greater magnitudes in comparison to soils and waters from control sites that were not close to gold mining areas. For instance, the daily average Hg flux rates from the tailings to the atmosphere at the Lower

Seal Harbour district were measured at 17.4 ng/m²/hr and 652 ng/m²/hr on tailings from a former cyanide plant and stamp mill, respectively, compared with 1-2 ng/m²/hr measured over undisturbed control soils (Dalziel & Tordon, 2014). It was concluded that these tailings sites are still important sources of Hg transfer to the atmosphere even decades after mining operations have ceased.

Recreational activities (e.g. off-road vehicle racing) were identified as major sources of airborne dust at the Montague and Goldenville districts, with most tailing grain diameters measuring between 125 and 500 µm at Montague (Corriveau et al., 2011a, b). This dust at Montague was found to contain many mobile, As-bearing mineral species and high total As concentrations in the respirable fraction of <8 µm (1040 ng/m³), suggesting that As may become bioavailable, potentially affecting human health of those living near the tailings or using the site for recreational activities (Corriveau et al., 2011a, b). In a soil sampling survey at the Montague Gold District in 2007, the spatial distribution of Hg and As in soils was investigated (Parsons & Little, 2015). For Hg, highest concentrations were found in the humus layer, thought to result from gaseous Hg flux from the soils in combination with atmospheric Hg deposition from both distant and local sources (i.e. windblown tailings, past airborne emissions from former stamp mills) (Parsons & Little, 2015).

Overall, these studies have demonstrated that abandoned gold mine tailing sites in Nova Scotia can still be important sources of hazardous dust and vapours. This is of concern as there may be increased human exposure to these mine wastes in the future as a result of increasing land development (e.g. residential subdivisions) and continued frequent recreational use (e.g. off-road vehicle racing) at and surrounding some mine

sites. It is important to document the atmospheric dispersion of the mine tailing contaminants to assess the pollution source, manage any potential risks, and to inform land use decisions as well as future mine planning.

1.5 Biomonitoring

For spatial air pollution monitoring, manufactured active and passive air monitoring systems (e.g. high-volume samplers, continuous particle monitors, dust deposition gauges) usually require power sources or may be expensive or difficult to deploy in remote areas and across large spatial scales. Biomonitoring, or biological monitoring, is an alternative that can provide an effective method to determine the spatial and temporal distribution of atmospheric contaminants at any desired scale, the occurrence of contaminant hotspots in the environment, and pollutant emission sources (Bargagli et al., 2002; Pignata et al., 2007; Boamponsem et al., 2010; Søndergaard et al., 2010). Biomonitoring involves the use of a living organism to provide information on the sphere of influence and level of contamination in the environment. Monitoring organisms accumulate pollutants in their tissues and may reveal changes in appearance or community composition (e.g. species diversity, abundance, distribution) when exposed to various environmental stressors (Lodenius, 2013). For example, the mollusk species *Viviparus bengalensis* was an effective biomonitor of metal contamination in river Gomti, India, accumulating high concentrations of Cu, Zn, and manganese (Mn) while persisting in the polluted environment without suffering mortality (Gupta et al., 2014). Other studies have successfully used spiders, seaweed, mussels, and fish to assess metal contamination from abandoned mines (Søndergaard, 2013; Hansson et al., 2019),

freshwater macrophytes to assess lake elemental pollution (Nirmal Kumar et al., 2006), and snails for biomonitoring soil contamination (Gomot de Vaufleury & Pihan, 2000).

1.5.1 Background Information about Lichens

Lichens are widely used as biomonitors of air quality, providing information on the spatial distribution and concentration levels of airborne contaminants at both local and regional scales. A lichen is a symbiotic relationship between a fungal partner (mycobiont) and an algae or cyanobacteria (photobiont) (Lodenius, 2013; Bargagli, 2016). The fungus provides the algae with minerals and protection from external stress factors such as high light levels. The algae conduct photosynthesis, providing organic carbon to the fungus. In cyanolichens species, cyanobacteria are also present and can fix nitrogen from the air and synthesize ammonia (NH₃) that can move to the fungus and be converted into amino acids and proteins (Bargagli, 2016).

Lichens are ubiquitous around the world, covering approximately 6% of the Earth's surface (Brodo, 2015). Lichens can grow on natural substrates such as soils (epigeic), rocks (epilithic), and trees (epiphytic), as well as on man-made structures (Nimis et al., 2002; Leavitt & St. Clair, 2015). These organisms can live in a wide range of habitats, climates, and environments, including the tropics, temperate and boreal forests, urban areas, and deserts (Nimis et al., 2002). They are also the dominant vegetation in polar regions such as the subarctic woodlands and the arctic tundra, being a major food source for caribou (Brodo, 2015; Bargagli, 2016). Lichens are perennial organisms and typically grow at very slow rates. Some arctic species only grow at a rate of about 0.1 mm per year while the margins or length of most of the larger temperate species extend by 5-8 mm per year (Brodo, 2015). Lichens photosynthesize and grow

when there is light, moisture, and humidity, and become dormant when dry (Shukla et al., 2014). Consequently, many lichen species grow along fog belts, coastlines, and near lakes, typically becoming more sparsely populated when approaching the drier conditions farther inland (Lutzoni & Miadlikowska, 2009).

The vegetative body of a lichen is called the thallus (plural: thalli). Lichen thalli may be fruticose forms (shrub-like thallus), foliose forms (leaf-like thallus), or crustose forms (crust-forming) (Shukla et al., 2014; Bargagli, 2016). Generally, lichens have an upper cortex (protective outer layer of the thallus), beneath which is an algal layer and then the medulla made up of fungal tissue (Figure 3) (Shukla et al., 2014). Below the medulla is a lower cortex and sometimes a basal attachment (rhizines or holdfast) that attaches the lichen to its substratum (Shukla et al., 2014).

Many lichens reproduce asexually, through fragmentation or the production of specialized growth structures, such as soredia and isidia, that are comprised of fungus and photosynthetic components and dispersed by wind, water, or animals (Lutzoni & Miadlikowska, 2009). However, sexual reproduction also occurs in many species. Lichen fungi develop fruiting bodies (apothecia) which produce spores that are shot into the air and will germinate and grow into a new lichen if they land on a suitable substratum on which is a compatible photosynthetic component (Lutzoni & Miadlikowska, 2009).

Unlike plants, lichens lack roots, stems, leaves, vascular systems, waxy cuticles, stomata, and other specialized structures for water retention, photosynthesis, and gas exchange (Lodenius, 2013; Bargagli, 2016). As a result, lichens obtain almost all nutrients required for growth by absorbing water and minerals by both wet and dry atmospheric deposition (Bargagli, 2016). Some species of lichens are also thought to

absorb and accumulate nutrients and metals from the substratum (Fahselt et al., 1995; Sueoka et al., 2015), however, this is not the primary source for most lichens.

Precipitation events drive wet deposition, depositing dissolved airborne aerosols as well as particles on lichen thalli (Tyler, 1989; Leavitt & St. Clair, 2015). Furthermore, dry deposition of airborne gases or particles can also be significant (Leavitt & St. Clair, 2015). However, with no waxy cuticle, lichens lack a physical barrier and can thus take up atmospheric contaminants in greater amounts than needed for growth (Shukla et al., 2014).

The accumulation of minerals in lichens, including contaminants, can occur via entrapment of particulates originating from soil, rock, dust, or anthropogenic activities (Figure 3). These can become trapped amongst the mycobiont hyphae (branching fungal body) and in intercellular spaces (Tyler, 1989). Particulates are the primary source of airborne trace elements to the lichen surface (Vannini et al., 2017). The ability of a lichen to intercept airborne particles depends on the particle size as well as the species-specific features of the thallus (e.g. presence/absence of a cortex, size of pores, etc.) (Bargagli, 2016). Air particles from soils, volcanic eruptions, forest fires, pollen, mining, and the combustion of fossil fuels are just some of the particles that can be intercepted by lichens (Figure 3).

Once particulates are trapped, they can be solubilized with the help of some of the secondary products produced by lichen fungi, such as oxalic acid (Tyler, 1989; Chiarenzelli et al., 1997; Bargagli, 2016). Metal ions released from dissolved particulates and those in rainfall are adsorbed to sites on the cell wall or the outer layer of the plasma membrane, predominantly of the fungal component, via reversible passive ion exchange

(Nash, 1989). In this process, low binding affinity hydrogen or metal ions bound to negatively charged anionic exchange sites can be displaced by ions with a higher binding affinity or with higher concentrations of ions (Figure 3) (Tyler, 1989; Bargagli, 2016; Vannini et al., 2017). Thus, these metal ions become bound to the cell walls. Extracellular metal ion exchange is a rapid process and unaffected by metabolic inhibitors (Garty, 2001).

Cations bound to these extracellular exchange sites can then be incorporated into the living cells of the lichen thallus with time (Hauck et al., 2002). Intracellular uptake occurs when soluble ions are transferred inside mycobiont and photobiont cells through energy-dependant and plasma membrane-controlled systems (Figure 3) (Garty, 2001; Vannini et al., 2017). It is the intracellular component of trace element accumulation in lichen thalli that affects the lichens at the physiological level, with the potential to result in visible symptoms of damage or death of the lichen (Vannini et al., 2017). Unlike extracellular uptake, intracellular metal uptake is a slow process that accelerates over time and depends on the species, cell metabolism, and availability of intracellular functional groups (Garty, 2001). Also, the nature of the metal ion (e.g. bioavailability), the permeability properties of the cell membrane, as well as the number of extracellular ligands all influence intracellular uptake (Tyler, 1989). In contrast to the extracellular fraction, the metals in the intracellular fraction tend to be indicative of long-term metal deposition (Johnson and Gunnar, 2018, unpublished). From the accumulation of contaminants in thalli, lichens may therefore act as vectors of contaminants to terrestrial wildlife as they are consumed by numerous invertebrates (e.g. springtails, beetles) and

vertebrates (e.g. caribou), or used by species for habitat and camouflage (Asplund & Wardle, 2017) (Figure 3).

1.5.2 Lichens as Biomonitors

Lichens have a ubiquitous distribution, long life spans, and the ability to grow continuously for decades, being a valuable organism for monitoring atmospheric deposition as well as changes in air quality, land management practices, and climate (Leavitt & St. Clair, 2015). For example, the disappearance or shifting of lichen populations and changing community compositions in an area can assist in monitoring climate change. With regards to air pollution, elevated atmospheric pollutant levels do not impose adverse effects on the survival and growth of some lichens that are pollution tolerant like *Lecanora conizaeoides* and *Parmelia sulcata*. Such species may be effective bioaccumulators and biomonitors of air quality around highly contaminated sites (Boamponsem et al., 2010). In comparison with high-volume samplers, continuous particle monitors, or dust deposition gauges, lichens are a relatively inexpensive method for determining the spatial and temporal distribution of atmospheric contaminants in relation to pollutant emission sources.

Lichens were first identified as indicators of air quality in 1866 by William Nylander who studied their distribution in Paris, France. Subsequent studies have shown sensitive lichen species to decrease in population size or disappear in the presence of consistently elevated atmospheric pollutants (Leavitt & St. Clair, 2015). Studies of lichen biodiversity in relation to air quality to assess ecosystem health has been used extensively, with permanent lichen monitoring programs having been established in

many countries, including Canada, the United States, United Kingdom, Switzerland, and the Netherlands (Brodo et al., 2001; Conestoga-Rovers & Associates, 2007).

Lichens can also be used as passive biomonitors of environmental contaminant concentrations (e.g. metals, sulfur dioxide, ozone, etc.) by measuring concentrations in lichen thalli. Pollutant concentrations in thalli achieve an equilibrium with the average pollution levels in the ambient air within three months of exposure (Kularatne & de Freitas, 2013; Loppi et al., 2015; Loppi & Paoli, 2015). Lichens can also be used in active biomonitoring, where specimens are transplanted from an unpolluted site to a polluted site (lichen bag or moss bag approach), and the changes in appearance, biochemistry, physiology, growth rates, or elemental content used to assess the air quality (Leavitt & St. Clair, 2015). Thus, transplanted lichens are useful for monitoring temporal trends in air pollution concentrations whereas *in situ* lichens can be effective for spatial air pollution monitoring (Kularatne & de Freitas, 2013).

Lichens have been used for monitoring dust and air quality from both natural and anthropogenic sources of pollution, including volcanoes, agricultural operations, chlor-alkali plants, and coal-fired power electricity generating stations (Garty & Hagemeyer, 1988; Bargagli & Barghigiani, 1991; Davies & Notcutt, 1994; Sensen & Richardson, 2002; Saat et al., 2016; Bernardo et al., 2019). The utility of lichens to effectively biomonitor airborne contamination from both historical and active mining operations has been demonstrated in numerous studies globally (Bargagli et al., 1987; Fahselt et al., 1995; Dolgoplova et al., 2006; Wilson & Pyatt, 2007; Saunier et al., 2013; Odumo et al., 2014; Landis et al., 2019). With respect to Hg, lichens were collected along a gradient at a historical Hg mine, Pinchi Lake, in British Columbia, Canada. It was generally found

that there were higher Hg concentrations in the lichens collected at the mine site, with decreasing concentrations measured in some samples collected at more distal sites (Plouffe et al., 2004). Similar results were noticed for lead (Pb) at the abandoned Pb-Zn “Black Angel Mine” in West Greenland, with background Pb levels in lichens not found until 12 km from the mine (Søndergaard et al., 2010). In both studies, the enrichment of elements in lichens were attributed to wind-borne dust from the tailings deposits and other waste materials at the sites. Overall, these studies not only support lichens as effective air quality biomonitors, but also further demonstrate the utility of lichen to assess continual contamination to the atmosphere which may occur at some abandoned mine sites.

In Nova Scotia, lichen-based air quality studies have included surveys from as early as 1968. Most of those investigations examined species distributions, abundance, and diversity in various urban areas, national parks, and near other potential point sources of pollution or areas with poor air quality (Ward, 1968; Brawn & Ogden, 1976; Selva, 1999; Cameron, 2003; Cameron et al., 2007; Conestoga-Rovers & Associates, 2007; Gibson et al., 2013; McMullin et al., 2017). Several studies conducted in the province have measured contaminants in lichen thalli to identify the spatial extent of airborne contamination and emission sources. Those studies include Pb from the long-range transport from the United States (Carignan et al., 2002), vanadium (V) from local oil-fired power plants (Juichang et al., 1995), deposition of atmospheric radionuclides (^{131}I , ^{103}Ru , ^{137}Cs) from the 1986 Chernobyl nuclear plant accident (Smith & Ellis, 1990), and Hg as part of an ecosystem assessment of Hg cycling in Kejimikujik National Park (Rencz et al., 2003). A small number of studies using lichens have been conducted at abandoned

mine sites in the province. Sedgewick (1998, unpublished) found elevated Cu, Zn, Pb, and cadmium (Cd) in lichens associated with a small historical Pb-Cu-Zn mine dump in Musquodoboit Harbour which ceased operations in 1920. More recently, Klapstein et al. (2020) used roadside sampling of the lichen *Usnea* to determine the spatial distribution and sources of airborne Hg and other elements across the entire province. Lichens collected near historical gold mine sites in Halifax and Guysborough counties were shown to contain elevated As and selenium (Se), but not Hg (Klapstein et al., 2020). However, lichen sampling from these gold mine sites was not the focus of this study as sampling was not conducted on a fine, local scale around the selected tailing sites.

2.0 Thesis Objectives

With a changing climate and increasing land use and development around contaminated sites, it is becoming more important to monitor dust and air quality. Further research is needed to assess the airborne contamination around the many abandoned gold mine tailings sites in Nova Scotia. The primary objective of this thesis is to examine the spatial distribution patterns of airborne Hg on a detailed, local scale using epiphytic lichens (*Usnea* and *Platismatia* spp.) around a historical gold mine tailings deposit in the Montague Gold District that is relatively close to the city of Dartmouth, Nova Scotia. In addition, total Hg (THg) flux rates were determined using passive air samplers and rain collectors to enable comparisons to the lichen biomonitors. Finally, the aim was to distinguish between the extracellular versus intracellular concentrations of Hg in the collected lichens to assess the influence of the tailings on atmospheric dust emissions.

3.0 Chapter 1 Figures

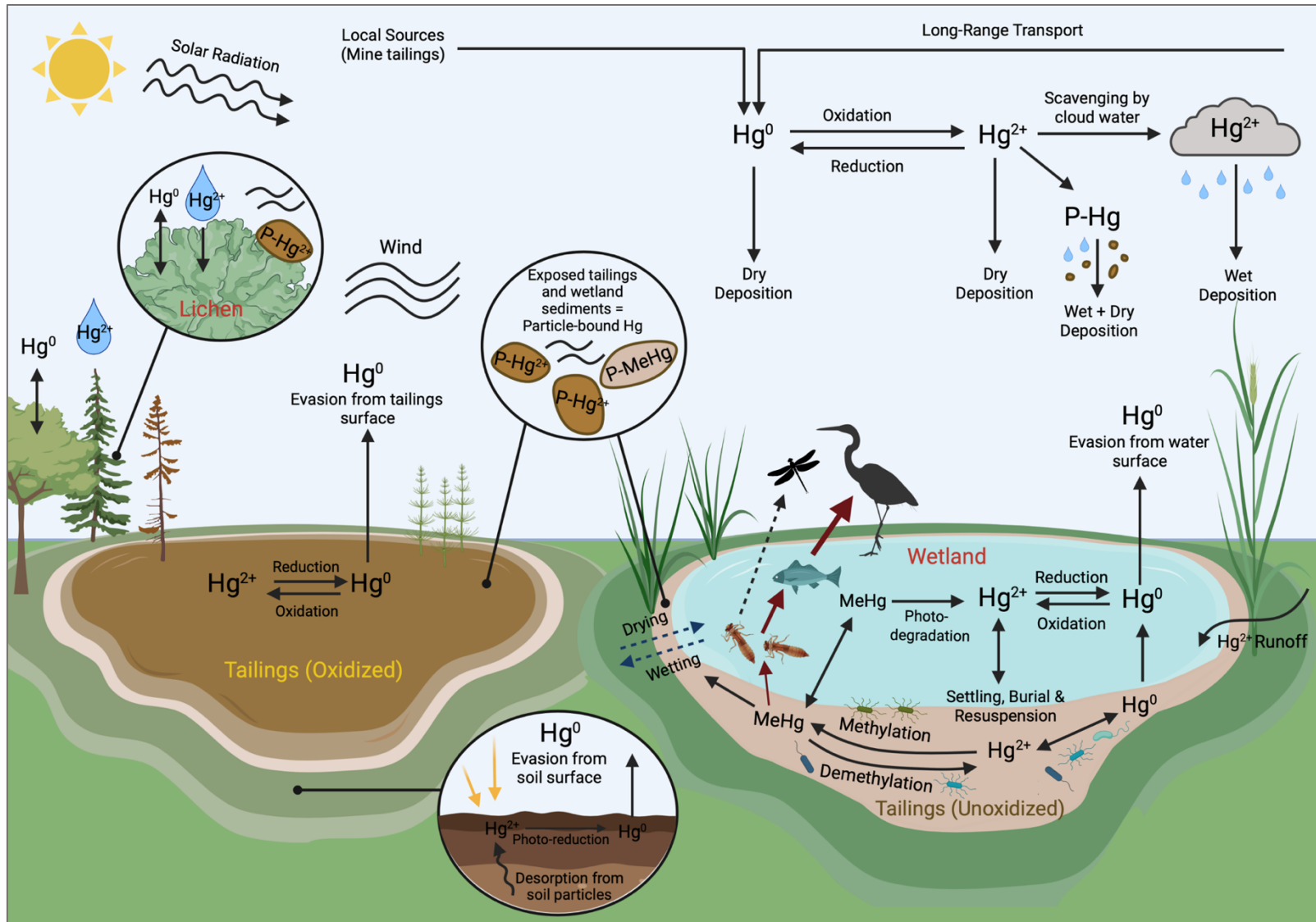


Figure 1. Conceptual diagram of mercury cycling and species transformations at historic gold mine tailings sites in Nova Scotia. Created in BioRender.com.



Figure 2. Tailings field at the Montague Gold District in Nova Scotia.

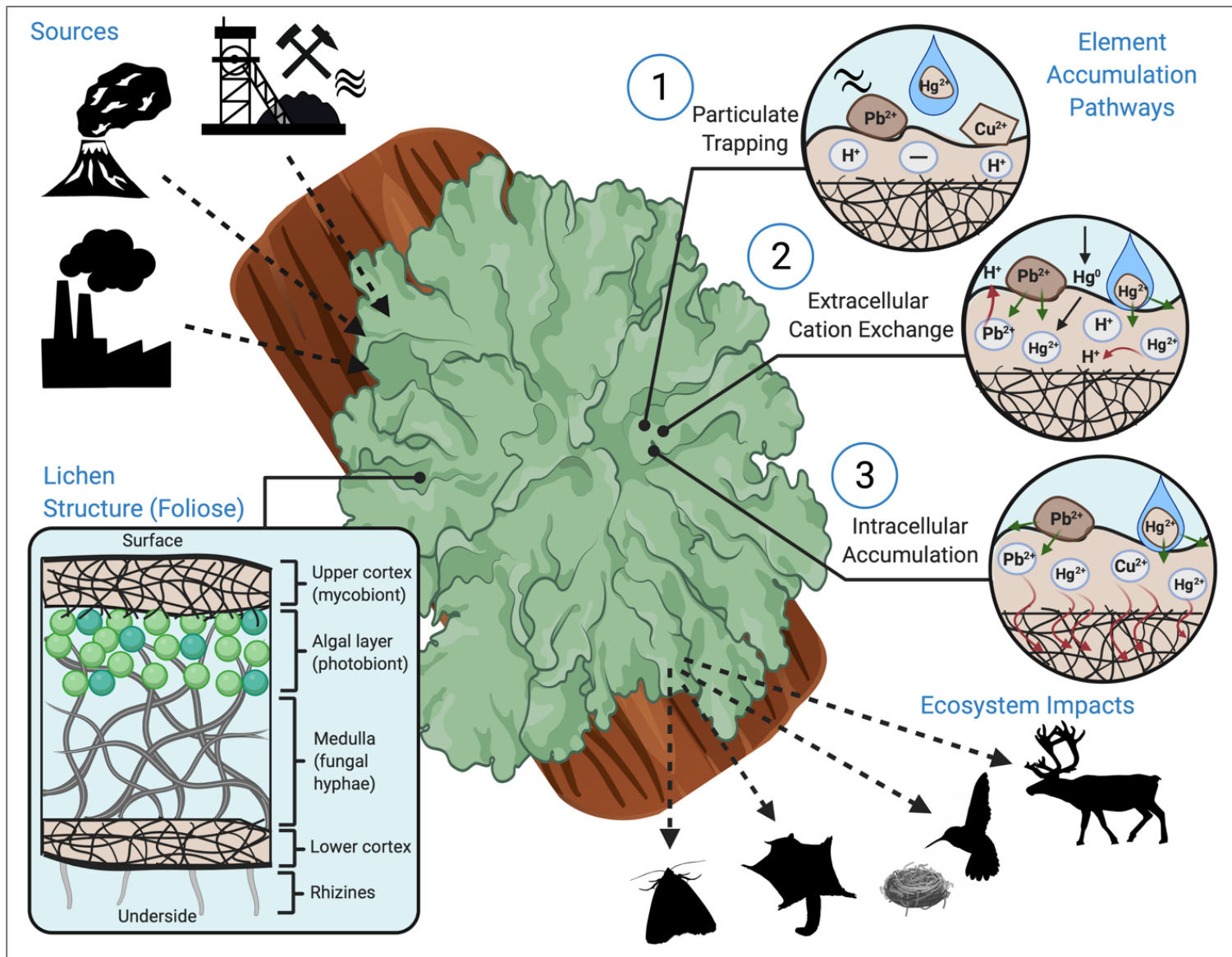


Figure 3. Conceptual diagram showing the general structure of lichens, sources of contaminants to lichens, element accumulation pathways, and the potential transfer of contaminants to biota in terrestrial ecosystems. Created in BioRender.com.

4.0 Chapter 1 References

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Chapter 2: Spatial distribution and patterns of airborne mercury using lichen biomonitors and passive dry deposition collectors at the Montague Gold District in Nova Scotia

Abstract

Mercury (Hg)-contaminated gold mine tailings deposits in Nova Scotia, Canada, are potential sources of contaminated dust, posing risks to surrounding environments. This study sought to determine the spatial distribution of airborne Hg surrounding the Montague Gold District near Dartmouth, Nova Scotia, using epiphytic lichens as biomonitors. *Usnea* and *Platismatia* spp. were sampled over a series of gridded transects and analyzed for Hg concentrations. Seasonal Hg flux rates were also determined using passive dust samplers situated along a stream-based transect. Mercury concentrations in lichens ranged from 69 to 320 $\mu\text{g}/\text{kg}$ for *Usnea* and 49 to 195 $\mu\text{g}/\text{kg}$ for *Platismatia*. Interpolation modelling of the Hg data showed the same two major hotspots between the two lichen genera. The level of Hg contamination in lichens was dependent on proximity to the tailings, likely receiving Hg from volatilization processes, windblown tailings, throughfall, and/or stemflow. Lichens collected from trees near wetlands and bogs showed elevated concentrations of Hg, demonstrating the role these environments have in methylating and demethylating Hg. From the passive dust samplers, the warmer seasons had the highest Hg flux rates, reflecting the increased temperatures and solar radiation that enhance the evasion of gaseous Hg into the air. It was concluded that *Usnea* and *Platismatia* were effective spatial biomonitors of Hg that can provide complementary information to these physical dust samplers. The results from this study can be used to inform future development decisions, provide guidance to mine and risk evaluators, and help to predict the impacts of this Hg contamination on nearby food webs.

1.0 Introduction

Nova Scotia, Canada, has a history of gold mining between 1861 to the mid-1940s. Gold was first discovered in 1858, soon thereafter in 1861 initiating the province's first of three major historical gold rushes (Malcolm, 1929). By the 1940s, over 360 mines had been in operation over these 80 years, located within 64 formal gold mining districts (Malcolm, 1929). Over this time, gold was predominantly extracted by crushing the ore to sand- or silt-sized particles using stamp mills, followed by mercury (Hg) amalgamation processing using Hg-coated copper plates (Mudroch & Clair, 1985; Bates, 1987; Parsons et al., 2012). Beginning in the 1890s, mine operators began to use cyanidation to extract gold from ore, yet Hg amalgamation was still used at many sites up until the 1940s (Bates, 1987; Parsons et al., 2012). These historical mining operations produced approximately 1.2 million troy ounces of gold across the province, resulting in the production of an estimated three million tonnes of fine-grained mine waste, called tailings (Bates, 1987; Parsons et al., 2012; Drage, 2015).

Early ore amalgamation operations were particularly inefficient and wasteful, with 10-25% of all Hg used (9100 kg) estimated to have been lost to the tailings and to the atmosphere, with only a portion of the elemental Hg (Hg^0) being captured and reused (Bates, 1987; Parsons et al., 2012). In addition, the tailings are elevated with arsenic (As) which was released from the disruption of the sulfide mineral arsenopyrite (FeAsS) when extracting gold from these ore deposits (Drage, 2015). Most tailings were disposed of, untreated, into surrounding environments such as lakes, wetlands, and streams. Mining operations eventually became uneconomical once most high-grade ore was processed, resulting in the abandonment of mine sites and leaving a legacy of environmental

contamination. With regards to Hg, tailing samples collected in recent years from numerous sites across Nova Scotia have been shown to be elevated in Hg, up to 350,000 µg/kg, significantly exceeding the Canadian Council of Ministers of the Environment (CCME) soil and sediment quality guidelines of 6600 µg/kg and 486 µg/kg (dry weight), respectively (CCME, 1999a, b; Parsons et al., 2012). The tailings have been repeatedly shown to contaminate soils (Eaton, 1978; Parsons & Little, 2015), lake sediments (Mudroch & Sandilands, 1978; Mudroch & Clair, 1985; Clark et al., 2021), and waters (Eaton, 1978; LeBlanc, 2019, unpublished) at and near mine sites. Mercury in all ecosystems also bioaccumulated in biota including plants, fish, terrestrial and aquatic invertebrates, marine molluscs, amphibians, and small mammals (LeBlanc et al., 2019 and references therein).

Mercury is a naturally occurring element found in the Earth's crust, existing in various forms; the main forms being Hg^0 , inorganic Hg compounds, and organic Hg compounds (Park & Zheng, 2012). The highly volatile and liquid form of Hg, Hg^0 , was the form used for Hg amalgamation at these historic gold mining operations in Nova Scotia. In aquatic systems, Hg^0 can be oxidized to divalent Hg (Hg^{2+}) which can then be transformed into organic forms, such as methylmercury (MeHg), primarily by methylating bacteria (Kim, 2005; Parsons & Percival, 2005). Methylmercury is accumulated by fish and marine mammals, biomagnifying up the food chain, even to wildlife in terrestrial environments and to humans.

Today, the tailings deposits are potential sources of airborne Hg via direct transfer of the volatile Hg^0 (gaseous) or Hg-containing dust emissions, potentially redistributing this contaminant into surrounding areas (Csavina et al., 2012). Any of the Hg^0 lost to the

tailings or nearby aquatic environments can undergo reversible transformations, being oxidized to Hg^{2+} and then reduced back to gaseous Hg^0 via photoreduction or microbial communities. The gaseous form of Hg can be volatilized from the tailings to the atmosphere (Krabbenhoft et al., 2005; Moreno et al., 2005). In addition to being reduced, Hg^{2+} may adsorb to dust particles, forming particulate-bound Hg (Kocman et al., 2011; Huang et al., 2020). With the contaminated tailings deposits in the province typically lacking soil structure, nutrients, and organic matter, this has created landscapes devoid of vegetation, exposing the fine-grained tailings material to wind erosion and increasing the potential to generate hazardous dust containing Hg. Once in the atmosphere, Hg^0 has an atmospheric lifetime of approximately 1-2 years and is deposited mainly by dry deposition, whereas Hg^{2+} is deposited through both wet and dry deposition within days to weeks of formation (Lin & Pehkonen, 1999; Kocman et al., 2011; Huang et al., 2020).

Previous studies at abandoned gold mine tailings sites in the province have shown these sites to be potential sources of contaminated dust and vapours. Several research teams have measured gaseous Hg flux rates from tailings fields in the Caribou Gold Mine District (Wong et al., 2002; Beauchamp et al., 2002), Lower Seal Harbour Gold District (Dalziel & Tordon, 2014), and Goldenville Gold District (Beauchamp et al., 2002). Results from the Caribou Gold District indicated that the gaseous Hg flux rate from the tailings to the atmosphere was 20 to over 100 times greater than that measured over natural, undisturbed soils (Wong et al., 2002). At the Montague, Goldenville, and Lower Seal Harbour districts, dust particles, including those generated from recreational activities, were found to contain many mobile and highly-soluble As bearing mineral species, posing risks to humans exposed to this tailings dust due to these more

bioavailable As components (Corriveau et al., 2011a, b). A soil survey at the Montague Gold District found elevated Hg concentrations in the near-surface soil layers, suggested to be, in part, from deposition of windblown tailings and the Hg flux from the soils (Parsons & Little, 2015). More recently, Cleaver et al. (2021) showed that tailings dust from the abandoned Stirling mine in Cape Breton, Nova Scotia, may be a potential source of metals (e.g. zinc (Zn), lead (Pb), copper (Cu)) to nearby surface waters. Even so, no studies, to the best of our knowledge, have documented the spatial distribution and extent of this tailings-generated dust and air contamination on a fine, local scale at any historical gold mine site in Nova Scotia. Such work is necessary for managing any potential risks to human and ecosystem health, as well as to inform future land use decisions.

Manufactured passive air samplers are one option to assessing atmospheric pollution. Passive air samplers do not require power and allow for dust deposition rates to be calculated as well as to understand and characterize the dust particles (i.e. size, mobility, mineralogy, etc.) if enough sample is collected. However, this equipment can be expensive or difficult to deploy on a fine resolution scale in remote areas and/or across very large regions.

As an alternative to passive air samplers, or to obtain complementary data to this equipment, lichens can be used as biomonitors. Lichens are ubiquitous in the environment, have long life spans, and have widely been used for biomonitoring atmospheric pollution from both natural and anthropogenic sources, providing information on the sphere of influence, level of contamination, and location of hotspots in the environment (Bargagli & Barghigiani, 1991; Davies & Notcutt, 1994; Pignata et al., 2007; Saat et al., 2016; Bernardo et al., 2019; Loppi et al., 2021). The lack of roots, waxy

cuticles, and stomata in lichens results in the accumulation of both essential nutrients and contaminants, such as metals, from wet and dry atmospheric deposition rather than uptake from the underlying substratum (Lodenius, 2013; Bargagli, 2016). In general, lichens may accumulate elements via particulate entrapment, extracellular cation exchange, and intracellular accumulation, reflecting atmospheric pollutant concentrations in thalli within approximately three months of exposure (Nash, 1989; Tyler, 1989; Kularatne & de Freitas, 2013; Loppi et al., 2015; Loppi & Paoli, 2015). With respect to Hg, gaseous Hg⁰, Hg ions (e.g. Hg²⁺), or particulate-bound Hg may be intercepted by lichens from dry and wet deposition (Bargagli, 2016). Lichens have been shown to be effective biomonitors of Hg (Weiss-Penzias et al., 2019; Klapstein et al., 2020). For example, Sensen and Richardson (2002) showed that lichens in the vicinity of a chlor-alkali plant in New Brunswick, Canada, indicated the Hg emissions to extend 2.4-3.4 km from the plant. Many other studies have also shown lichens to be valuable monitoring tools at abandoned mine sites in other regions, further demonstrating the continual contamination to the atmosphere that can occur from these contaminated sites years after operations have ended (Bargagli et al., 1987; Fahselt et al., 1995; Plouffe et al., 2004; Dolgoplova et al., 2006; Søndergaard et al., 2010; Demková et al., 2019).

We investigated dust contamination surrounding the historical Montague gold mine tailings site in Nova Scotia, Canada, using *in situ* epiphytic lichens (*Usnea* and *Platismatia* spp.) as biomonitors. Spatial distribution patterns of airborne Hg around this contaminated site were determined from fine-scale field sampling of these lichens followed by spatial analysis and modelling of the analytical data. Seasonal dust levels and Hg flux rates were also measured across this site using manufactured passive air

sampling equipment, providing complementary data and enabling comparisons to the lichen trends. Furthermore, the Hg accumulation capacity and distribution patterns observed in the two lichen genera were compared to assess the utility of these lichens for future biomonitoring work in Atlantic Canada and Nova Scotia.

2.0 Methods

2.1 Study Site History and Description

This study was conducted at the Montague Gold District near Dartmouth, Nova Scotia (44.71541, -63.52332) (Figure 1). The Montague Gold District was one of Nova Scotia's most productive former gold mines (Malcolm, 1929; Bates, 1987). Gold deposits were first reported in 1862, with a total of 134,278 tonnes of ore being crushed and producing 65,196.9 troy ounces of gold by the time of closure in the 1940s (Trip & Skilton, 1985; Mills, 1997; Parsons et al., 2012). Most ore was processed using Hg amalgamation, and the Hg- and As-contaminated tailings were deposited in the low-lying areas surrounding, and directly into, Mitchell Brook. Mitchell Brook consists of a series of interconnected streams and wetlands originating from Loon Lake, extending through the Montague tailings site, and emptying downstream into Lake Charles (Figure 1A).

The tailings deposits in the Montague Gold District cover over approximately 270,000 m² of land, with the main tailings deposit containing over 120,000 tonnes of tailings covering approximately 150,000 m² of land alone (Mills, 1997; Drage, 2015; Intrinsic Corp et al., 2019). A significant portion of this main tailings deposit is exposed with little or no vegetation cover (only grasses and shrubs). The tailings in this area are poorly consolidated and the erosion and transport of dry, surface tailings has been

observed on windy days (Parsons et al., 2012). The areas surrounding the main tailings deposit are largely forested with some smaller and dead trees located on the boundaries of the tailings. A highway (Forest Hills Extension (Highway 107)) is located to the south of the main tailings deposit, with residential roads and properties located to the north. The surrounding areas also contain numerous filled and open mine shafts, old mine building foundations, and a waste rock site. Recreational activities (e.g. off-road vehicle racing) occur on parts of the tailings and residential developments have taken place nearby.

In addition to the main tailings, there are smaller tailings deposits located in the study area (Figure 1A). A deposit to the southeast is referred to as “Old Stamp Mill” (OSM), and those tailings resulted from the activities of a small historical stamp mill processing site located just north of the deposit and where Hg amalgamation activities occurred. This is one of the oldest tailings deposits within this gold district and this site is elevated in Hg (Parsons et al., 2012). This OSM tailings deposit also overlaps a wetland that is under water for most of the year. Another small tailings deposit is located further northeast and is covered with low vegetation and only a few deciduous and coniferous trees.

2.2 Weather Information

Wind and weather data (March 2007 to May 2019) were obtained from the Halifax-Shearwater Environment and Climate Change Canada (ECCC) weather station (44.63922, -63.50147), located approximately 8 km away from the study sites (Government of Canada, 2021a). The annual average wind direction was predominantly from the south and southwest, with strong winds also from the northwest (Figure 2). The annual average wind speed was 18.5 km/h. The colder months (October to April) were

characterized by higher average wind speeds of 20.6 km/h, predominantly from the northwest. The warmer months (May to September) had average winds of 15.9 km/h with the prevailing wind direction being from the south and southwest. Average wind speeds and directions were similar for the period between 1971 to 2000 (Pieridae Energy (Canada) Ltd., 2013).

2.3 Lichen Species

Two epiphytic lichen genera were selected for this study, *Platismatia* and *Usnea*. These lichens were selected due to their abundance in the study area and their ability to be easily identified to the genus level in the field. *Platismatia* is an epiphytic lichen with a flat, leaf-like thallus that has a well-defined upper and lower surface (St. Clair et al., 2002b). *Platismatia* thalli have extensive contact with the substrate, typically growing on both branches and trunks of trees. They are attached to the substrate by rhizines which act only as an anchoring mechanism and are thought not to absorb nutrients from the bark surface (Bosserman and Hagner, 1981). The relatively flat surface of foliose lichens results in less surface area being exposed and possibly resulting in a lower capability to intercept precipitation (Bosserman and Hagner, 1981). In contrast, *Usnea* is an epiphytic lichen which consists of a densely branched and open three-dimensional shrubby thallus and may have a slower growth rate than *Platismatia* (Esseen et al., 1996). *Usnea* species have little contact with their substratum as they are attached at only a single point (i.e. holdfast). In combination with their shrub-like thallus, growth takes place out and away from bark of the host trees. This allows for a greater capability to intercept precipitation, throughfall, and dust.

2.4 Sample Collection

2.4.1 Lichen

An 800,000 m² (1000 x 800 m) study area was delineated to encompass the main gold mine tailings field at Montague using geographic information systems (GIS), ESRI's ArcGIS (ArcMap 10.7). The study area was divided into 100 by 100 m grid cells with a total of 138 proposed lichen sampling sites located at each of the intersection points (Grid 1; Figure 1A). A 100 m distance between each sampling location was chosen because it was feasible and provided samples to achieve data with a relatively high spatial resolution over the study area. Three additional areas measuring 60,000 m² (300 x 200 m), divided into 100 by 100 m grid cells, and located at significant distances from the main Montague tailings field were also chosen as study sites (Grids 2, 3, & 4; Figure 1A). The locations of these grids were selected to correspond with the direction of the prevailing winds (southerly; Grid 2), near a smaller gold mine tailings deposit (Grid 3), as well as near a previously used reference site located upstream of the tailings (Grid 4). All grids were finalized based on accessibility via roads and trails as well as land access (via Crown Land access permits or written approvals by landowners, Halifax Water, or Nova Scotia Transport & Infrastructure Renewal).

The latitude and longitude of each grid intersection point was extracted remotely in ArcMap 10.7, and a handheld global positioning system (GPS) was used to locate each collection site in the field during July and August of 2019. At each site, living samples of both *Platismatia* and *Usnea* were collected from both living or dead, standing or fallen spruce (*Picea* sp.) tree trunks and branches, at heights of approximately 1-2 m above the ground. Sample collection started at one tree near the geographically referenced point. To

allow for greater sample numbers and a high spatial resolution, lichen collections were not always made on the same sides of trees between sites. Also, if lichens or trees were sparse, or an exact site was inaccessible (e.g. deep waters, private property), lichen collection was extended to additional trees within a 25-m radius surrounding each collection site. In such cases, sample collection was only extended until sufficient biomass (~3 g) of lichen were collected. If the entire 25-m sampling radius surrounding the grid point was inaccessible or located entirely within private property (and access was not granted), or such area hosted no trees or lichens, that point was omitted from sampling.

Using powder-free nitrile gloves, lichens were collected from trees and transferred to a labelled, re-sealable food-grade plastic bag. At each site, all collected lichen thalli of the same genera were grouped into one composite sample. The GPS coordinates were recorded. The samples were then temporarily stored in a cooler for transport from Montague to the laboratory at Saint Mary's University in Halifax, Nova Scotia.

Additional sites were also included to provide reference control samples. Non-tailing sites included Albro Lake in Dartmouth (October 2020; 44.69082, -63.57547; Reference Site 1), and Saint Mary's University in Halifax, Nova Scotia (October 2020; 44.62854, -63.58156; Reference Site 2), both of which are urban sites. Additional tailing-impacted sites included downstream of Montague at Barry's Run (June 2019; 44.71633, -63.54395), Lake Catcha Gold District (July 2020; 44.73564, -63.19807) and Oldham Gold District (July 2020; 44.92070, -63.49310). Note that Reference Sites 1 & 2 and the

Barry's Run site were also locations for passive air samplers and rain catchers (see below).

2.4.2 Passive Air Samplers and Rain Catchers

To assess seasonal dust levels and Hg flux rates, passive air samplers were installed within and surrounding Montague in June and July of 2019. The passive air samplers included a glass fibre filter (GFF) atop a polyurethane foam filter (PUF), both of which were positioned between two plates with openings to allow for air flow. Only particles and dry gases are deposited on the GFFs and PUFs from air passing through. A large plate covers the top of the PUF and GFF, protecting these from direct sunlight and wet deposition. For this study, only the GFF was used for subsequent analyses, and used to assess dust deposition and Hg flux rates.

A total of six passive air samplers were installed along the Mitchell Brook stream system (Figure 1A). Two passive air samplers were installed at the main Montague tailings deposit (MO-1, MO-2), two at the OSM tailings deposit (OSM-1, OSM-2), one located upstream of Montague near Loon Lake (LL) and one further downstream at Barry's Run near Lake Charles (BR). The air samplers were installed at a height of approximately 2 m from the ground on standing living or dead, coniferous or deciduous tree trunks located on forest edges, either facing the adjacent water bodies (i.e. wetlands) and/or the tailings deposits. In addition, two passive air samplers were installed at the reference sites; one on a spruce tree at Albro Lake (ABL) (Figure 1B), and one on a wooden post that was 2 m in height placed on the roof of the O'Donnell Hennessey Student Centre at Saint Mary's University (SMU) (Figure 1C). All site names, sampler codes, and locations for each passive air sampler are shown in Table 1. Once installed,

new air filters (GFF + PUF) were placed in the air samplers wearing powder-free nitrile gloves. The air filters were weighed with a microbalance in the lab and recorded before being placed in the samplers.

In addition, rain catchers were used to determine total and dissolved concentrations of Hg in precipitation, collecting elements from wet and dry deposition, such as rainwater and dust particles settling from the air. The rain catchers consisted of a 4L plastic bottle with a funnel attached to the opening, secured in a wooden box. A total of three rain catchers were installed along Mitchell Brook at Montague in June of 2019, including one upstream near Loon Lake (LL), one at the main Montague tailings deposit (MO-1), and one downstream near Barry's Run (BR) (Figure 1A). The rain catchers were placed at heights of approximately 2 m from the ground on spruce tree trunks that were located on forest edges, and on trees that were relatively open to the atmosphere, being unobstructed by a dense canopy. An additional rain catcher was placed at the reference site at Saint Mary's University in July of 2019 (SMU) (Figure 1C). At the sites with both passive air samplers and rain catchers, separate trees were chosen for each sampling device. All site names, sampler codes, and locations of each rain catcher are shown in Table 1.

Air filters and rain bottles were collected and replaced approximately every three months from June/July 2019 to October 2020 (Table 2) and included the following sampling periods: June to September 2019 (Summer), September to December 2019 (Fall), December 2019 to March 2020 (Winter), March to July 2020 (Spring/Summer), and July to October 2020 (Summer/Fall). At the scheduled times, rain bottles were collected, capped, labelled, and refrigerated until sample processing. For the air filters,

100% Teflon forceps were used to remove the filters from the samplers, which were then placed and sealed in a labelled petri dish until further processing. The air samplers were cleaned with wet wipes between air filter changes, and the forceps rinsed with reverse osmosis (RO) water in the field between use at each passive air sampler. Air filter travel and field blanks routinely accompanied sampling trips to evaluate potential contamination of filters during field work at the sites and from travel to the appropriate laboratories. Overall, the seasonal sampling trips resulted in the collection of five filters and five rain bottles from each site. Slight variations in sampling resulted from the COVID-19 field restrictions in 2020, loss of the September to December 2019 BR rainwater sample due to freezing, and an additional rainwater bottle added to the MO-1 site for June to September 2019 following heavy rains.

2.5 Sample Preparation

Lichens were stored in a freezer in the laboratory until identification and further sample preparation. Lichens were first identified to the species level with a dissecting microscope and using dichotomous keys (Hinds & Hinds, 2007; Brodo, 2016), chemical spot tests, and ultra-violet light. Spot tests consisted of placing a drop of a chemical (e.g. 10% potassium hydroxide, household bleach, or para-phenylenediamine) on various parts of the lichen thallus and using the change in colour, along with presence or absence of morphological features, to identify the species.

After identification, further lichen sample preparation was conducted in a clean room laboratory at Saint Mary's University. For each site, the lichen sample was separated by hand to remove as much extraneous material (e.g. bark, twigs, leaves, invertebrates, etc.) as possible. Once cleaned of debris, each lichen sample was divided

into two portions of approximate equal mass, with one half reserved for washing (Chapter 3). The unwashed portion of the lichen was air-dried at room temperature for 48 hours and then homogenized to a powder in jars using a Retsch mixer mill MM 400. The jars were thoroughly cleaned in a Citranox and RO water solution between milling of each individual sample. Each ground sample was then transferred to a labelled 50 mL Falcon tube and stored in a dark and dry environment (i.e. storage bins) until analysis. Powder-free nitrile gloves were worn while working with each lichen sample and changed between samples.

For the collected passive air sampler filters, each GFF was air dried at room temperature for a minimum of 72 hours at CanmetMINING Natural Resources Canada (NRCan) in Ottawa, Ontario. The GFFs were first weighed whole and then cut into equal portions (4) using Teflon scissors, which were wiped with 70% ethanol between filters. Each portion of the GFFs were also weighed individually and one portion sent back to Saint Mary's University for Hg analysis. These portions were homogenized to a powder in jars using the ball mill and stored in glass vials until analysis.

For each rainwater sample, unfiltered and filtered samples were collected at CanmetMINING NRCan to determine total Hg (THg) and dissolved Hg (DHg) concentrations, to provide information on the soluble and insoluble Hg fractions in dust from the tailings area. For THg, 50 mL of each rainwater bottle was sampled by syringe and added to a labelled 50 mL Falcon tube, along with 1% (500 μ L) nitric acid (trace level grade) for preservation. For DHg, 50 mL of each rainwater bottle was sampled by syringe, where a 0.45 μ m syringe filter was then attached and the water dispersed through the filter and into a labelled 50 mL Falcon tube, along with 1% (500 μ L) nitric acid (trace

level grade) for preservation. The rainwater samples were stored upright and refrigerated until analyses.

2.6 Mercury Analyses

The lichen and GFF samples were all analyzed for THg concentrations, and the unfiltered and filtered rainwater samples were analyzed for THg and DHg concentrations, respectively, using a Milestone Direct Mercury Analyzer (DMA) 80.3 at Saint Mary's University. The weight or volume of each sample was measured using a microbalance, placed in an acid-washed quartz boat, and then entered into the DMA software. During each analytical run, Hg standards of varying concentrations along with certified reference materials (CRMs) were processed to validate proper calibration of the instrument. The CRMs used with the lichen and GFF samples were TORT-3, DORM-4, and BCR-482 Lichen (Willie et al., 2012, 2013; European Commission, 2021). An in-house standard reference material (SRM), a homogenized *Usnea* sample from a previous field season, was also prepared for comparison with the lichen samples and to ensure consistency within and between runs. For the rainwater samples, along with the Hg standards, quality control included a spiked and unspiked matrix and a spiked and unspiked RO water sample. The spiked matrix included lichen wash-water that was collected for another experiment (Chapter 3). Furthermore, blank boats were run at the start and end of each analytical run, as well as before and after each set of quality control samples. Within runs, blank boats and duplicate samples were run every 10 and 20 samples, respectively.

2.7 Spatial Analyses

To identify spatial patterns of Hg across the study area, an interpolation model was applied in ArcMap 10.7 using lichen THg concentrations. Interpolation is a technique used to estimate unknown values within sampled regions using nearby, known datapoints (Li & Heap, 2014). Due to the dense and relatively even spacing of the sampling points (Li & Heap, 2014), as well as the small spatial extent of the study area, the inverse distance weighted (IDW) model was selected to interpolate the data. The IDW model assumes that points closer together are more similar than those further apart (Llyod, 2010). Thus, the THg concentrations at unsampled locations were estimated from a weighted average of the surrounding concentrations from sampled locations. Sample points at a *greater* distance to the point being predicted have *less* influence (weight) on the predicted value (Llyod, 2010). There is no minimum number of data points required for IDW interpolation. Rather, it is the size of the study area that is important; generally, the larger the size of the study area, the greater number of points required to obtain optimal results. Using this interpolation model has created smooth, predicted surface outputs within each of the sampling grids at Montague displaying estimated THg concentrations in lichens ranging only between the observed minimum and maximum values (Li & Heap, 2014).

Additional spatial analyses included creating buffer polygons of various distances (e.g. 100 m, 200 m, 300 m) around the Montague tailings layer using the Buffer tool in ArcMap 10.7 (Figure 3). Sampling sites located within each buffer were then classified into that distance category, denoting their approximate distance to the nearest tailings edge (e.g. on tailings, 0-100 m, 100-200 m, 200-300 m, and >300 m). The tailings edge

refers to the bounds of the tailings polygons, as shown in Figure 3. More specifically, the exact distance of each sampling site to the nearest tailings edge was also calculated using the Near tool in ArcMap 10.7 and compared to the THg concentrations measured in lichens.

Furthermore, the lichen THg concentrations were compared to elevation data for each sampling site, extracted from a 1 m digital elevation model (DEM) from 2019 that was overlain on the study area in ArcMap. These data were from the Nova Scotia Elevation Dataset and extracted from GeoNOVA using the Elevation Explorer DataLocator tool (Government of Nova Scotia, 2021). Forest polygon layers from the Nova Scotia Forest Inventory were also used to classify the lichen sampling sites into six land types, which was a dataset interpreted from aerial photographs (Province of Nova Scotia, 2017). These six land types included lichens growing on trees near (1) roads, (2) urban areas, (3) wind throws, (4) natural forest stands, (5) treed bogs, and (6) wetlands. The wind throw zones included areas where more than 25% of the trees had been pushed over by wind action (Province of Nova Scotia, 2017).

2.8 Data Analyses

Descriptive statistics for the Hg datasets for each lichen genus, *Usnea* and *Platismatia*, were calculated, based on the various data classifications specified above (i.e. sampling grid, distance to nearest tailings edge, and land type). For each lichen genus and within each classification scheme, Hg concentrations were first tested statistically using parametric tests, including two-sample t-tests, one-way analysis of variance (ANOVA), and Pearson correlation analysis. The model residuals were tested for normality with the Shapiro-Wilk test as well as for homogeneity of variance with the

Levene’s test. Based on varying results between the two lichen genera both before and after \log_{10} transformations (e.g. normally distributed model residuals for *Usnea* but not *Platismatia*), non-parametric statistical tests were applied to all untransformed datasets so to ensure consistency and to allow for comparisons between lichens. These included the Mann-Whitney U test and the Kruskal-Wallis test to detect significant differences between Hg concentration means of the classified data. Spearman-rank correlation analysis was performed to examine the strength of relationships between Hg concentrations in paired samples, between different sampling methods, and against geographical variables. All graphs were generated, and statistical analyses performed, in RStudio.

2.9 Deposition and Flux Calculations for GFFs

Using a similar calculation to Cleaver (2020, unpublished), the total seasonal dust deposition rates ($\text{mg}/\text{m}^2/\text{day}$) were calculated from the GFFs from each passive dust sampler using equation (1):

$$\text{Total Dust Deposition Rate} = \frac{\text{Post filter weight (mg)} - \text{Before filter weight (mg)}}{\frac{\text{Area of filter (m}^2\text{)}}{\text{Exposure time (days)}}} \quad (1)$$

Field and travel GFF blanks were also weighed before and after sampling, and these differences in weight were subtracted from the corresponding GFFs for that sampling trip. A negative dust deposition rate was calculated for seven of the GFFs due to a greater before versus after filter weight. This was likely a result of the small differences in weights of filters before and after exposure and the sensitivity of the analytical mass balance in detecting such small changes. In such cases, these deposition

rates were corrected back to zero, indicating negligible total dust deposition rates for those seasons.

The THg flux rate ($\mu\text{g}/\text{m}^2/\text{year}$) for each season was calculated from the GFFs from each passive dust sampler site using equation (2):

$$THg \text{ Flux Rate} = \frac{\frac{THg \text{ in sample } (\mu\text{g}/\text{kg}) * \text{Post } \frac{1}{4} \text{ filter weight (kg)} * 4}{\text{Area of filter (m}^2)}}{\text{Exposure time (days)}} * 365 \quad (2)$$

Prior to this calculation, GFF lab blanks were also analyzed for THg concentrations, which were then subtracted from the THg measured on each GFF. After this blank correction, this resulted in negative THg concentrations for five filters, mainly from those where THg concentrations were already relatively low, or even below Method Detection Limits (MDLs; 0.001 ng). In such cases, these concentrations were corrected back to zero, indicating a negligible THg flux rate for that season.

The majority of the THg and DHg concentrations measured in the rainwater samples measured below MDLs (n=29/38). Thus, these sampling periods were not included in the results and the Hg flux rates not calculated for this dataset.

3.0 Results

3.1 Lichen

3.1.1 Lichen Species

A total of 112 *Usnea* and 113 *Platismatia* samples were collected from 138 lichen sampling points in the sampling grids across the Montague Gold District. The actual location of each *Usnea* and *Platismatia* sample collected, along with the identified

species, are shown in Figures 4 and 5, respectively. A total of ten *Usnea* species and two *Platismatia* species were identified, with *Usnea filipendula* and *Platismatia glauca* being the most common sampled (Table 3). At the reference sites, the lichen species included *Platismatia glauca* at both sites, with *Usnea subfloridana* and *Usnea filipendula* at Saint Mary's University and Albro Lake, respectively. From Barry's Run (downstream of Montague) and at the Oldham Gold District, the lichen samples were *Usnea filipendula* and *Platismatia tuckermanii*, while the lichen collected from the Lake Catcha Gold District were *Usnea subscabrosa* and *Platismatia glauca*.

3.1.2 Mercury Concentrations in Lichens

Total Hg concentrations in lichens varied greatly across the study area and within grids (Table 4). *Usnea* THg concentrations ranged from 69.7 µg/kg measured at a site in the northwest section of Grid 1 adjacent to Mitchell Brook to 320 µg/kg measured near the OSM tailings deposit. Total Hg concentrations measured in *Platismatia* spp. had a smaller range of values, from 49.9 µg/kg measured in Grid 3 to 195 µg/kg also near the OSM tailings deposit in Grid 1. Lichen THg concentrations also varied widely within the smaller three grids (Table 4). For both lichen genera, the THg concentrations were, on average, higher in Grid 3 nearer the smaller tailings deposit than those in Grids 2 and 4, though for both lichen species, this was not statistically significant (*Usnea*: $H = 1.36$, $df = 2$, $p\text{-value} = 0.72$; *Platismatia*: $H = 1.39$, $df = 2$, $p\text{-value} = 0.71$).

At the reference sites, Saint Mary's University and Albro Lake, THg concentrations for most of *Usnea* and *Platismatia* samples were lower than the median lichen THg values for each of the sampling grids at Montague, except for the Albro Lake *Usnea* sample, which was higher than the median THg from Grid 2 (Table 4). In contrast,

most of the THg concentrations in the single lichen samples from Barry's Run, Lake Catcha, and Oldham were higher than the median lichen THg values for each of the sampling grids at Montague (Table 4).

3.1.3 Lichen Genera Comparisons

The average *Usnea* spp. THg concentrations were higher than those for *Platismatia* spp. in each of the four sampling grids (Table 4), though statistically significant results were only observed in THg between *Usnea* and *Platismatia* within Grid 1 ($Z = 1488$, $p\text{-value} < 0.0001$) and Grid 2 ($Z = 22$, $p\text{-value} < 0.005$). When plotting *Usnea* THg concentrations against those of *Platismatia* samples from the same sites (Figure 6), most data points fell below the reference line with a 1:1 slope (black line), indicating that *Usnea* consistently had higher THg concentrations. Focusing on all the Montague sampling grids combined, *Usnea* had significantly higher average THg concentrations ($149 \pm 43.0 \mu\text{g/kg}$) than *Platismatia* ($112 \pm 26.7 \mu\text{g/kg}$) ($Z = 2910$, $p\text{-value} < 0.0001$). While both genera were not sampled from all sampling points in each grid, THg in co-sampled *Usnea* and *Platismatia* samples were positively correlated ($r = 0.55$, $p\text{-value} < 0.0001$).

3.1.4 Mercury Spatial Distribution Patterns in Lichens

Using IDW interpolation, it was observed that areas with highest lichen THg concentrations in Grid 1 (*Usnea*: 184-320 $\mu\text{g/kg}$; *Platismatia*: 124-195 $\mu\text{g/kg}$) were directly on or in the vicinity of the known tailings deposits (red zones in Figures 7 & 8). The same two main elevated THg regions were identified using both *Usnea* and *Platismatia*: one along the OSM tailings deposit in the southeast area of the sampling grid

that is associated with the location of a former stamp mill and amalgamation operations, and the second in the northern section of the main tailings deposit (Figures 7 & 8). Total Hg concentrations generally decreased beginning at the edge of these tailings, which, from field observations and satellite base maps, were confirmed to be forested areas. Other areas of elevated THg in both lichen genera were at exposed areas with lower vegetation cover (Grid 3) or where there had been significant tree cutting (Grid 4). Areas with the lowest lichen THg concentrations in Grid 1 (*Usnea*: 69-120 µg/kg; *Platismatia*: 49-91 µg/kg) were located on the northeastern and southern boundaries of the sampling grid (green zones in Figures 7 & 8). These were all located near residential roads or highways and were shaded and forested.

The two lichen genera did not always exhibit the same patterns throughout the sampling region. In Grid 1 for *Platismatia*, smaller hotspots of THg (124-195 µg/kg) were detected in areas adjacent to the other two former stamp mills (eastern- and western-most areas of the main tailings deposit), as well as in relatively open areas surrounding water features of the Mitchell Brook stream (Figure 8). Also, a slightly elevated region for *Usnea* (142-183 µg/kg) was through the middle section of Grid 1 north of Mitchell Brook (orange zone in Figure 7). In Grid 3, elevated concentrations of THg were observed in *Platismatia* at the southeastern corner of the sampling grid (Figure 8) unlike that observed from *Usnea* (Figure 7). This zone contained low vegetation (e.g. shrubs and grasses) and few trees and was located next to a tailings deposit.

3.1.5 Mercury Spatial Analysis Patterns in Lichens

Sampled lichen were classified based on their distance to the nearest tailings edge (Figure 3), terrestrial elevation, and land type. On average, a decreasing trend in THg

concentrations was observed in both lichen genera with increasing distances from the tailings and tailings edge (Figure 9). Average THg concentrations in *Usnea* ranged from $134 \pm 32.9 \mu\text{g/kg}$ from samples located within 100-200 m from the nearest tailings edge to $187 \pm 50.4 \mu\text{g/kg}$ for samples located directly on tailings (Table 5). *Usnea* samples collected from trees on tailings were found to have significantly higher average THg concentrations than samples collected from trees at each of the other four distance categories (p -values < 0.05). For *Platismatia*, the highest average THg concentrations were measured from trees growing on the tailings ($129 \pm 38.4 \mu\text{g/kg}$) with the lowest at sampling sites located >300 m from the nearest tailings edge ($108 \pm 22.5 \mu\text{g/kg}$) (Table 5). Average THg concentrations in *Platismatia* did not differ significantly between any of the five distance categories ($H = 5.12$, $df = 4$, p -value = 0.28). For both lichen genera, a wide range of THg concentrations were measured in the lichens collected from trees growing on or near the tailings (Figure 9). A more marked decline from elevated concentrations with increasing distance from the tailings was evident for *Usnea* relative to *Platismatia*. In relation to distance from the tailings edge, *Usnea* had significantly higher THg concentrations than *Platismatia* within each distance category (p -values < 0.005) except for the 200-300 m distance group ($Z = 53$, $p = 0.12$).

Terrestrial elevation in relation to the collection sites was not significantly correlated with THg concentrations in *Usnea* ($r = -0.10$, p -value = 0.31) or *Platismatia* ($r = -0.12$, p -value = 0.20). However, wind appeared to be important. *Usnea* samples located downwind in Grid 1 (north) had significantly higher average THg ($154 \pm 41.9 \mu\text{g/kg}$) compared to samples collected from upwind locations in Grid 1 (south; $133 \pm 37.1 \mu\text{g/kg}$) ($Z = 192$, p -value = 0.047). For *Platismatia*, average concentrations did not vary

as widely between downwind ($111 \pm 29.3 \mu\text{g}/\text{kg}$) and upwind ($109 \pm 22.2 \mu\text{g}/\text{kg}$) sites within Grid 1.

Lichens sampled in each land type as categorized by the Nova Scotia Forest Inventory dataset indicated that THg in *Usnea* sampled from trees near bogs ($185 \pm 67.0 \mu\text{g}/\text{kg}$) and wetlands ($177 \pm 38.5 \mu\text{g}/\text{kg}$) was the highest while *Usnea* THg concentrations were lowest near roads ($112 \pm 21.7 \mu\text{g}/\text{kg}$; $p\text{-values} < 0.05$) (Figure 10). Conversely, there were no significant differences in THg concentrations between any of the land type categories for *Platismatia* ($H = 6.82$, $df = 5$, $p\text{-value} = 0.23$) in spite of the fact that the highest mean was also from treed bogs ($130.0 \pm 41.1 \mu\text{g}/\text{kg}$) and lowest near roads ($101 \pm 23.6 \mu\text{g}/\text{kg}$; Figure 10). As shown in Figure 11, most treed bogs were located within 0-100 m of any tailings edge, with all wetland sampling sites located directly on tailings. Also, both *Usnea* and *Platismatia* samples collected from natural forest stands and wind throw zones showed a wide range of THg concentrations (Figure 10).

3.2 Passive Dust Samplers and Rain Catchers

Between June 2019 and October 2020, a total of 40 filters (GFF + PUF) and 20 rainwater bottles were collected from the passive air samplers and rain catchers, respectively. Weather data for each sampling season was calculated from the ECCC Halifax-Shearwater weather station (44.63922, -63.50147) and is shown in Figure 12 (Government of Canada, 2021b, c).

3.2.1 Passive Dust Samplers

3.2.1.1 Total Dust Deposition Rates

The total dust deposition rates were calculated for each site using equation (1), with seasonal trends shown in Figure 13. The highest seasonal total dust deposition rate was calculated at BR, 21.7 mg/m²/day, during the December 2019 to March 2020 sampling period, followed by 16.7 mg/m²/day measured at the OSM-2 passive sampler during the September to December 2019 season. Mean total dust deposition rates at each site over the study period were in the order of BR > ABL > SMU > OSM-2 > OSM-1 > MO-2 > MO-1 > LL, ranging from 2.6 ± 3.2 mg/m²/day at LL to 8.5 ± 8.0 mg/m²/day at BR. Thus, sites further from the Montague tailings deposits generally showed greater dust deposition rates than those located in closer proximity.

Overall, when averaged, the colder seasons measured higher total dust deposition rates, with the warmer seasons reporting lower rates. The ECCC Halifax-Shearwater weather data indicates that in general, the warmer seasons reported the lowest wind speeds and precipitation levels, with the colder seasons showing an increase in average wind speeds and total precipitation (Figure 12). The June to September 2019 season was the driest season, with lowest precipitation in addition to the lowest average wind speeds, with the highest average temperature. The strongest average wind speeds occurred from September to December 2019, with the lowest mean temperature during the winter season from December 2019 to March 2020. The September to December 2019 season measured the highest dust deposition rates for the SMU Reference, ABL Reference, OSM-1, and OSM-2 passive samplers, with the maximum at BR measured during the Winter season (December 2019 to March 2020).

The samplers at LL, MO-1, and MO-2 showed highest dust deposition rates during the first summer season (June to September 2019). Some samplers located adjacent to the tailings, including OSM-1, MO-1, and MO-2, showed an additional peak in dust deposition during the second warm season (March to July 2020). In contrast, lowest dust deposition rates for the OSM samplers were observed during the coldest season, December 2019 to March 2020. At sites with two samplers (OSM and Montague), similar seasonal trends and deposition rates were observed between these samplers within sites.

3.2.1.2 Total Mercury Flux Rates

The THg concentrations measured from the GFFs were not correlated with the mass of the dust deposited on the GFFs ($r = -0.006$, $p\text{-value} = 0.97$). For THg flux rates, the highest seasonal GFF THg flux rate (Figure 14) was at ABL during June to September 2019 ($2.6 \mu\text{g}/\text{m}^2/\text{year}$), followed by the sampler at MO-2 from December 2019 to March 2020 ($1.8 \mu\text{g}/\text{m}^2/\text{year}$). Mean GFF THg flux rates at each site over the study period were in the order of $\text{MO-2} > \text{ABL} > \text{OSM-2} > \text{SMU} > \text{OSM-1} > \text{MO-1} > \text{LL} > \text{BR}$, ranging from $0.22 \pm 0.20 \mu\text{g}/\text{m}^2/\text{year}$ at BR to $0.83 \pm 0.71 \mu\text{g}/\text{m}^2/\text{year}$ at MO-2. Despite high mean GFF THg flux rates at the two reference sites, the other sites located closer to the study area did tend to show an inverse relationship in GFF THg flux rates with distance: those located directly adjacent to tailings deposits (Montague and OSM) were higher compared to those at greater distances away (LL and BR).

Seasonal trends of average GFF THg flux rates for most sites were lower during the colder seasons, with higher rates reported at the beginning and end of the study period, aligning with the warmer seasons. The GFF THg flux rates were highest for most

sites during the first sampling season, June to September 2019, all significantly decreasing into the following Fall season. The Montague passive dust samplers showed the greatest fluctuations between all seasons, being the only sites showing relatively large peaks in GFF THg flux rates in the Winter season (December 2019 to March 2020). The OSM-1 and OSM-2 passive dust samplers showed a slight peak in GFF THg flux rates into the Spring/Summer 2020 sampling season unlike most of the other samplers. Similar seasonal trends and flux rates were observed between the two samplers at OSM, with peaks observed in the warmer sampling seasons, on average. However, between the two Montague samplers, seasonal trends were similar yet the GFF THg flux rates were not (Figure 14).

3.2.2 Rain Catchers

It is important to note that evaporative losses from these rain catchers were not expected to be significant due to their relatively closed designs. The funnel cap on the rain bottle reduced the size of the bottle opening and limited the open water surface that was directly exposed to the atmosphere. Also, from being hosted in a wooden box, most of the outside of the rain bottles were protected from the influence of weather conditions, such as exposure to UV radiation. Gröning et al. (2012) designed a relatively similar rain catcher and showed that its design resulted in negligible water evaporative losses. Due to the similar design of the rain catchers used in the current study, evaporative losses from the rainwater samples can also be expected to be low.

Most rainwater samples had DHg (n=19/20) and THg concentrations (n=12/20) below MDLs, so thus, comparisons across all seasons or sites were not possible. The rainwater sample from Fall of 2019 at MO-1 was the only sample with DHg above MDLs

(0.078 µg/L; Table 6). Rainwater collected at MO-1 had higher THg concentrations in the summer of 2019 (0.27 µg/L) than those from BR (0.16 µg/L) and the SMU reference site (0.03 µg/L; Table 6). The rain catcher at LL measured slightly higher THg in Fall of 2019 (0.23 µg/L) in comparison to downstream at the MO-1 rain catcher during this season (0.21 µg/L).

3.3 Mercury in Lichens Compared to Passive Dust Samplers

Comparing concurrently sampled GFF THg data (June to September 2019 for Montague; July to October 2020 for Reference Sites) with lichen THg results (Figure 15), a significant positive correlation was observed for *Platismatia* spp. ($r = 0.86$, $p\text{-value} < 0.05$), with only a weak positive correlation for *Usnea* spp. ($r = 0.36$, $p\text{-value} = 0.39$). In both correlations with each lichen genera, both the lichen biomonitors and the passive dust samplers had the highest THg concentrations at the OSM-2 sampling site, while the lowest were found at BR located further downstream from the main tailings deposit at Montague.

4.0 Discussion

4.1 Concentrations of Total Mercury in Lichens

The mean THg concentrations and ranges for *Usnea* samples were generally in agreement with those reported in the literature for various fruticose lichen species globally (Table 7). The maximum THg measured in *Usnea* spp. at Montague (320 µg/kg) was lower than the maximum values reported from an area with known elevated levels of Hg in Nova Scotia, Kejimikujik National Park (660 µg/kg) (Rencz et al., 2003). Yet, the range of Hg values reported from this study falls within very similar ranges to *Usnea*

samples collected in 2017 from other historical gold mining areas in the province (Klapstein et al., 2020). At a former Hg mine (mainly cinnabar) in British Columbia, Canada, Hg concentrations in fruticose lichens measured up to 600 µg/kg at distances greater than 2km from the mines (Plouffe et al., 2004); the higher concentrations likely reflecting the cinnabar ore that was being mined as opposed to the current study where Hg was only introduced for the Hg amalgamation processing of gold-bearing ore.

Total Hg concentrations in *Platismatia* from the Montague sampling grids were generally lower than those values reported from other foliose lichens worldwide (Table 7). A background range of Hg of 88-148 µg/kg was determined for the foliose lichen *Hypogymnia physodes* collected in a remote forested area in New Brunswick, Canada (Sensen and Richardson, 2002). This background range aligns with the range of THg measured in most of the *Platismatia* samples from Montague (49-195 µg/kg). Higher Hg emissions are expected near active sources of pollution, as reflected in the foliose lichens collected in the vicinity of chlor-alkali plants in Europe (>870 µg/kg) (Lodenius & Laaksovirta, 1979; Grangeon et al., 2012). The THg ranges and means were also lower in *Platismatia* from Montague compared to the foliose *Parmelia caperata* that was sampled near an abandoned mining region in Italy; the latter reflecting Hg contributions from both Hg degassing from the mining soils and active emissions from the modern-day geothermal power stations in the area (Bargagli et al., 2002).

When comparing the mean THg concentrations in lichens between Grids 2, 3, and 4 around the Montague Gold District (Table 4), Grid 3 lichens showed higher concentrations than lichens in the other two grids, although were not significantly higher. The slightly higher values are likely due to the smaller tailings deposit located adjacent to

Grid 3, while Grids 2 and 4 are at greater distances from any of the tailings deposits at Montague.

Usnea sampled from the other historical gold mine tailings sites in Nova Scotia, Lake Catcha and Oldham, were within the ranges as those sampled from Montague. However, the *Platismatia* samples from these other gold districts were both higher than the maximum THg value measured in *Platismatia* samples from Montague. Thus, these other abandoned gold districts in the province, like Montague, may be contributing to elevated Hg concentrations in lichens. However, additional biomonitoring at these sites is needed.

The reference lichen samples for both *Usnea* and *Platismatia* from SMU and ABL tended to be much lower than the mean and median THg concentrations reported in those lichens collected from within most of the grids at Montague (Table 4) and from most of the groupings indicating the various distances from the tailings edge (Table 5). Although only one sample of each lichen was collected at each reference site, these lower THg concentrations indicates that, at least within close proximity to the Montague tailings, the tailings are likely enriching the lichens with Hg.

4.2 Lichen Genera Comparisons

A positive correlation ($r = 0.55$, $p\text{-value} < 0.0001$) was measured between THg in *Usnea* and *Platismatia* samples collected from the same sites, which suggests that the Hg originated from a common source. Where sampled at the same location, concentrations were generally higher in the fruticose lichen (*Usnea*) as compared with the foliose lichen (*Platismatia*). This indicates that these lichen genera both respond similarly to available

atmospheric Hg vapour, particulates, and dissolved ions, yet the capacity to sequester the Hg differs (Glenn et al., 1991).

However, many studies have found foliose lichens to accumulate higher concentrations of elements than fruticose lichens, primarily being attributed to morphological differences (Chiarenzelli et al., 1997; St. Clair et al., 2002a, b; Pereira et al., 2018). Foliose lichens have a larger continuous, as well as flattened, surface, with some genera, like *Platismatia*, containing numerous surface depressions. These features can promote the efficient interception of both wet and dry deposition, such as windborne dust and canopy-leached elements (Richardson, 1995; Garty, 2001; St. Clair et al., 2002b; Monaci et al., 2012). Additionally, foliose lichens are in closer contact with substrates, with a higher capability of accumulating available elements from the substratum that originate from stemflow, thus increasing total elemental loads (Davis et al., 2002; St. Clair et al., 2002a; Loppi et al., 2021). The open, branching structure of fruticose lichens allows for more air flow and fall-through potential which may result in a decreased capability to intercept airborne elements in comparison to foliose species (St. Clair et al., 2002a, b). However, Bosserman and Hagner (1981) measured higher concentrations of precipitation elements in *Usnea* compared to a foliose lichen, suggesting that the elemental contents in *Usnea* species are more so influenced by wet, rather than dry, deposition.

In the current study, a possible reason for higher Hg concentrations in *Usnea* versus *Platismatia* samples at Montague could be the result of the primary physical form of this element in the atmosphere in combination with lichen growth form. Globally, the majority (>95%) of atmospheric Hg is in the gaseous phase (Hg^0), depositing onto

lichens primarily in this form (Bargagli, 2016; Huang et al., 2020). This, in combination with the fact that *Usnea* has more surface area per unit biomass than *Platismatia* due to its more finely divided thalli, allows for a greater capability to intercept this Hg⁰ and provides a wider surface for gaseous exchange (Monaci et al., 2012).

This observation is consistent with literature on elements that are primarily in the gaseous form for which concentrations are higher in fruticose lichens compared with foliose lichens. For example, in a remote region in Chile, Monaci et al. (2012) found that Hg, being the only gaseous element of a total of 19 elements analyzed, was the only element that was higher in *Usnea* sp. as compared with the foliose *Nephroma antarcticum*. In New Brunswick, Canada, THg concentrations were higher in *Usnea* than in the foliose *Parmelia* sp. where collected along an island to inland transect (Nasr and Arp, 2015). Similarly, fruticose lichens near the Athabasca Oil Sands Region in Alberta, Canada, had higher concentrations of sulphur (S) and nitrogen (N) than in foliose lichens, of which the compounds containing these elements were emitted as gases (NO_x and SO₂) from this source area (Addison and Puckett, 1980; Graney et al., 2017).

Other potential reasons for higher THg concentrations in *Usnea* compared to *Platismatia* could be differing functional groups on the cell walls, oxalates, and acid compounds produced by these lichens, all of which may contribute to the uptake and immobilization of elements extracellularly (Tyler, 1989; Pawlik-Skowrońska and Bačkor, 2011; Rola et al., 2016). In particular, usnic acid, produced by *Usnea*, may aid in binding metals to the surface of the fungal partner (Rola et al., 2016). Additionally, *Usnea* has a different wetting behaviour than foliose lichens, taking up moisture rapidly and at a faster rate owing to its higher surface area to weight ratio (Bosserman and Hagner, 1981;

Larson, 1981; Esseen et al., 2015; Asplund and Wardle, 2017). Lichens grow and take up minerals and soluble ions when wet, as this is when they are more metabolically active (Bačkor and Loppi, 2009). Precipitation can bring water-soluble and insoluble particulates as well as dissolved gases to lichens (Addison and Puckett, 1980). Since solutes in precipitation are in higher concentrations at the beginning of a rain event (Minger and Krähenbühl, 1996), there is a potential for *Usnea* to absorb any soluble Hg that is intercepted. Short precipitation events may fully saturate the thinly branched *Usnea* lichens but not *Platismatia*, reducing metal uptake in the latter.

4.3 Mercury Spatial Distribution Patterns in Lichens

4.3.1 Sites of Elevated Lichen Total Mercury Concentrations

Generally, THg spatial patterns were similar for both *Usnea* and *Platismatia* within each grid, including two areas of elevated THg in Grid 1 associated with amalgamation activities at a documented stamp mill site (OSM) and at the northern section of the main tailings deposit. These areas of elevated lichen THg coincide with elevated THg soil samples from previous soil surveys at Montague (Parsons & Little, 2015; Parsons, personal communication, 2021). Parsons et al. (2012) measured Hg in the tailings at OSM as high as 70,000 µg/kg, with the northern section of the larger tailings deposit at Montague having the highest Hg concentration (8390 µg/kg) in comparison with all other tailings samples that were collected south of this area. These samples exceed the Hg guideline for residential and parkland soils for the protection of environmental and human health (6600 µg/kg) (CCME, 1999a).

4.3.1.1 Mercury Volatilization

Various mechanisms and pathways have probably contributed to these elevated THg concentrations in lichens at those two hotspots, including the transfer of volatile Hg⁰ from tailing, soil, and water surfaces to the lichens (Figure 16) (Bargagli et al., 2002). Elemental Hg is volatile so can be re-emitted into the atmosphere after it has been deposited in soils, water, and vegetation (Beauchamp et al., 2002). In fact, Hg is primarily removed from, or transferred through, an ecosystem via Hg volatilization (O'Driscoll et al., 2005; Selvendiran et al., 2008). Mercury volatilization can be facilitated abiotically or by biotically-mediated mechanisms, such as solar radiation or bacteria, respectively (O'Driscoll et al., 2005).

Gaseous Hg flux rates have been measured at abandoned gold mine tailing sites in Nova Scotia (Beauchamp et al., 2002; Wong et al., 2002; Dalziel & Tordon, 2014). At the Caribou and Goldenville districts, the Hg flux rates ranged from 63-640 ng/m²/h between the two tailings sites as compared to the much lower flux rates observed over natural forest soils (-0.4-2.2 ng/m²/h) (Beauchamp et al., 2002). These studies concluded that abandoned gold mine tailings sites in Nova Scotia are significant sources of Hg to the air surrounding these mine sites. It was also shown that between tailings sites that differ in Hg concentrations, those with higher Hg concentrations showed higher Hg flux rates than those with known lower concentrations of Hg (Dalziel and Tordon, 2014). In the current study, due to the higher Hg contents present in the tailings near these observed hotspots as mentioned previously (Parsons et al., 2012), the flux of gaseous Hg from the tailings to the air in these areas is likely greater, making more Hg locally available for interception by lichens.

The lichens at the OSM tailings deposit are from trees edging a tailing-contaminated wetland. This tailings deposit is typically inundated with water. So, gaseous Hg evasion in this area would most predominantly be occurring from the water surface to the air (Zhang et al., 1995; O'Driscoll et al., 2018), with greater amounts of Hg likely being released in this area due to the elevated Hg concentrations in the underlying tailings (Parsons et al., 2012). The export of Hg from wetlands is discussed further in subsequent sections (4.4.2). Moreover, releases of gaseous Hg from adjacent forest soils are also likely occurring as Hg contamination at Montague has been shown to extend past the boundaries of tailings material and into nearby soils (Parsons and Little, 2015). This may be a factor contributing to the elevated Hg in the lichens at OSM and supported by Bowes (2021, unpublished) that suggested that, after comparing Hg concentrations measured in tree cores from the OSM versus the Montague tailings, higher rates of Hg revolatilization from soils was likely occurring at OSM.

4.3.1.2 Windblown Tailings

The link between elevated THg in lichens with these tailings in these areas may also reflect the impact of windblown tailings. Although the majority of Hg in the atmosphere is in the gaseous phase, the presence of mine wastes (containing Hg) have been suggested to be significant sources of particulate-bound Hg, particularly when local winds are present (Rimondi et al., 2020). Windblown tailings were suggested by Parsons and Little (2015) to have played a role in elevating the Hg concentrations in near-surface soils around Montague. In addition, particle size has a large influence on the elemental contents in lichens at a site. Larger particles (>20 µm) can originate from tailings deposits

and are typically deposited locally, with any smaller particles capable of being transported over longer distances (Zschau et al., 2003).

At the OSM site, the water levels in the surrounding tailing-contaminated wetlands fluctuate greatly depending on weather patterns. As a result, large sections have been observed to dry out during hot, dry summers such as in the summer of 2019, which exposes the underlying sediments and tailings along the boundaries. When exposed, these tailings are more susceptible to dust generation (Figure 16), potentially transporting Hg to the adjacent trees and epiphytes, which highlights the impacts of a changing climate on water resources and dust generation.

The lichens at the hotspot in the northern section of the main tailings deposit may also be collecting large windblown particulates containing Hg. This is because the tailings in this area are loosely consolidated and completely bare or only sparsely vegetated, thus likely being impacted by wind erosion (Figure 16). In support of this, a slightly elevated zone of THg for *Usnea* was observed extending north of Mitchell Brook and past this hotspot (Figure 7), with significantly higher mean THg in *Usnea* sampled from the northern section as compared to the southern section of Grid 1. These trends agree well with the average annual prevailing winds in the area that primarily move from the south to the north (Figure 2). We suggest that these winds may be transporting loose tailings material and contributing to the elevated THg concentrations in these lichens. These results demonstrate that lichens are effective biomonitors for these contaminated sites and may be indicating elevated THg in surrounding ecosystems as well.

4.3.1.3 Influence of Trees and Forest Cover

The elevated THg sites for *Usnea* and *Platismatia* were both located on or near tailings, with concentrations tending to decrease quite sharply once the boundaries of the tailings were met and into the surrounding denser forests. Forest cover increases the roughness of the surface and promotes deposition and accumulation (Hugenholtz and Wolfe, 2010). For example, at Jasper Lake in Alberta, Canada, the sediment carrying capacity of wind greatly decreased as it moved from the edge of the open lake and into the forest, resulting in decreased dust accumulation away from the lake (Hugenholtz and Wolfe, 2010). A similar process might be occurring near the tailing boundaries at Montague, demonstrating the role of forests in reducing the dispersion of airborne contaminants in dust surrounding this tailings site.

The sampling sites are near the Atlantic coast, and as such, are subject to frequent fog, with the Halifax-Dartmouth area experiencing 121 days of fog each year, on average (Toth et al., 2010). The majority of Hg in the atmosphere is in the vapour form, which, when oxidized to the water-soluble Hg^{2+} , can be intercepted by fog (Evans & Hutchinson, 1996; Ritchie et al., 2006). When a cloud encounters a forest, the deposition of the particles and gases scavenged by droplets within the cloud significantly increases (Lovett, 1984; Nasr & Arp, 2015). At Montague, the Hg emitted from these nearby tailings may come in contact with fog, especially at these relatively open and unvegetated landscapes. This fog may subsequently transport and deposit more Hg onto the surrounding vegetation along edges of the forests, which may be further increasing Hg deposition on lichens near the contaminated tailings deposits.

In addition, forest canopies intercept gaseous and particulate elements, such as Hg, on leaves, needles, and branches which then can be deposited on the surface or be absorbed by foliage through stomata (Rea et al., 2001; Nasr & Arp, 2015; Gauslaa et al., 2021). During rain events, any loose particles and water soluble or leached Hg may be washed off from leaves or branches and intercepted by lichens from throughfall or stemflow, the latter including run-off water flowing down the tree trunks (Loppi & Bonini, 2000; Loppi & Pirinrsos, 2003; Nasr & Arp, 2015). So, with the potentially greater deposition of Hg to the forest canopy at these tailing-forest boundaries, more Hg may be available for wash-off and foliar leaching, subsequently enhancing the THg contents of the epiphytic lichens below.

At the OSM location in particular, Bowes (2021, unpublished) found that spruce tree cores from OSM had significantly elevated concentrations of Hg in comparison to reference sites and even to cored trees in other tailing areas within the Montague Gold District. It was suggested that the Hg was deposited on the trees in gas or particulate-bound forms and either absorbed passively through the bark or foliage before being transported by the phloem and incorporated into the sapwood (Bowes, 2021, unpublished). Numerous other studies have also shown that Hg primarily accumulates in trees from atmospheric deposition on the foliage and tree barks, with root uptake being a negligible contribution (Zhang et al., 1995; Rea et al., 2002; Poissant et al., 2008). Furthermore, with regards to lichens, several studies have found strong correlations between Hg concentrations in lichens and their host trees and bark, suggesting that trees and epiphytic lichens have similar uptake mechanisms (i.e. atmospheric deposition on surfaces) (Becnel et al., 2004; Rimondi et al., 2020). Thus, with known elevated Hg

concentrations in the tree cores at OSM likely resulting from the uptake of atmospherically deposited Hg, the trees at this site are likely transferring washed off or leached Hg from foliage to the lichens via throughfall and stemflow. This pathway of Hg transfer from trees to the surrounding lichens is likely also occurring at other areas throughout Montague yet varying in degree based on site-specific factors such as exposure to winds and proximity to tailings.

4.3.2 Other Areas of Elevated Lichen Total Mercury Concentrations

Smaller zones of elevated THg in *Usnea* and *Platismatia* were observed near the other former stamp mills in Grid 1 (Figures 7 & 8), reflecting the past amalgamation activities that took place in those areas and the resulting losses of Hg to the environment (Parsons et al., 2012; Parsons and Little, 2015). This trend was expected and aligns with the findings of Winch et al. (2008a) that found decreasing THg concentrations in tailings with increasing distance from stamp mills at the Seal Harbour Gold District in Nova Scotia. Winch et al. (2008a) attributed this trend to the higher-grade gold ores that were first processed using Hg amalgamation and deposited near the stamp mills with the lower-grade ores being processed later in time using cyanide and being channeled further away.

Additional areas of high THg were predominantly observed in *Platismatia* concentrated in some open areas along water features of the Mitchell Brook stream on Grid 1 (Figure 8). This stream that runs through Montague contains a mix of wetlands and fast- and slow-flowing interconnected channels that vary considerably in width and depth over its course. Over time, tailings have dispersed into downstream waters, alluvial deposits and/or bottom sediments of the Mitchell Brook stream, Barry's Run, and Lake

Charles (Eaton, 1978; Dale and Freedman, 1982; Jacques Whitford and Associates Limited, 1984). Wetlands also produce large supplies of dissolved organic carbon (DOC) which binds Hg species and transports them in stream waters, possibly also contributing to the dispersion of Hg into downstream environments (Driscoll et al., 1998; Selvendiran et al., 2008). Thus, Hg in the waters or sediments along the stream's course may be volatilized and emitted to the atmosphere via biotic or abiotically mediated reactions, providing a localized source of airborne Hg to the nearby lichens. This is supported by Maprani et al. (2005) who estimated that high atmospheric Hg flux rates were originating from the stream water surface within the first kilometre of Gossan Creek, a first-order stream in northern New Brunswick, Canada, contaminated from a leached gold mine tailings pile located upstream. These observed spatial patterns demonstrate that any dispersed Hg may be influencing the air quality even at distances away from the main deposits.

4.3.3 Zones of Low Lichen Total Mercury Concentrations

For both *Usnea* and *Platismatia* in Grid 1, similar areas showed low THg concentrations in lichens, such as towards the northeastern and southern boundaries of the grid. From field observations and inspection of satellite images, these areas were confirmed to be shaded with dense forest cover. Dense tree stands will provide more protection to lichens from the deposition of windblown metal-bound particles in comparison to those growing on isolated trees (Bačkor and Loppi, 2009). In addition to this, the lower THg concentrations in these lichens may also reflect the greater distances of these areas to the exposed tailings and the reduced Hg volatilization that occurs under cooler, shaded forest canopies (Nasr and Arp, 2015).

It is important to note that Nova Scotia is located downwind of many urban and industrial air pollution sources originating from the northeastern United States, southern Ontario, and Quebec. The long-range transport of these source emissions has led to enhanced Hg deposition in Nova Scotia (Temme et al., 2007; Roberts et al., 2019). From a province-wide *Usnea* lichen sampling survey in Nova Scotia from 2015 to 2017, it was suggested that most of the Hg measured in these lichens was from historical and ongoing long-range transport of Hg⁰ rather than from localized pollution sources (Klapstein et al., 2020). Also, at the Montague Gold District, Parsons and Little (2015) suggested that the enriched Hg in the humus layers of soils resulted partially from the atmospheric deposition of Hg from distal sources. In the current study, it is expected that some of the THg detected in our lichen samples reflects deposition from the long-range transport of atmospheric Hg, as suggested in these previous works. However, this long-range transport contribution is likely masked by the fact that we conducted this study over a small area and collected samples close together, thereby being able to detect hotspots on a finer spatial scale. Thus, the THg spatial patterns determined from our lichen samples are more likely reflecting the local pollution sources in this study area, such as the mine tailings.

4.4 Mercury Spatial Analysis Patterns in Lichens

4.4.1 Distance from Mine Tailings

In both *Usnea* and *Platismatia*, THg concentrations generally showed a decreasing trend with increasing distances from the nearest tailings edge (Figure 9; Table 5). The higher mean THg concentrations in lichens collected directly on the tailings can be attributed their close proximity to the main Hg source and consequently, the lichens

receiving greater contributions of Hg from windblown tailings and evasion from the surfaces of the bare tailings. This inverse relationship with elemental concentrations in lichens and distance to a pollution source is a common trend and has been observed in numerous pollution studies using lichens as biomonitors (Addison and Puckett, 1980; Takala and Olkkonen, 1981; Walther et al., 1990; Bylinska et al., 1991; Søndergaard et al., 2011; Saunier et al., 2013).

Total Hg concentrations in both lichens generally leveled off by the time sites located >300 m (up to 1 km) from the tailings were reached. This trend indicates that the tailings are a local source of atmospheric Hg pollution, impacting the environment up to at least a few hundred metres, and possibly up to 1 km, from the tailings deposits. Other biomonitoring studies using lichens have shown areas more distant from pollution sources to be impacted by the related emissions before levelling off (Lodenius and Laaksovirta, 1979; Sensen and Richardson, 2002), including up to 2 km from an abandoned Hg mine in Italy (Bargagli et al., 2002). Yet, these other studies were impacted by different types of sources (i.e. active point sources), different levels of pollution, and would have varying site characteristics than in the current study (e.g. topography, exposure to winds). Additional lichen samples collected >1 km from the mine tailings at Montague, and in various directions, would be required to better understand the sphere of influence of the mine tailings on atmospheric Hg contamination.

Both *Usnea* and *Platismatia* collected on or close to the tailings showed a wide range of THg concentrations (Figure 9). This can be attributed to varying site-specific characteristics such as the Hg concentrations in nearby tailings, surrounding vegetation cover, and exposure to winds. For *Usnea*, sharp declines in THg were observed

approximately 25-50 m from the nearest tailings edge (Figure 11). This may result from particulate fallout of coarser windblown grains at greater distances from the tailings (Hocking et al., 1978; Hugenholz and Wolfe, 2010), leading to increased deposition of particles onto surrounding forests, which typically occurred within this distance of the tailings edge. This trend of high contaminant levels in lichens closest to a source with steep declines within short distances and a levelling off of concentrations is consistent with previous studies of Hg near a chlor-alkali plant (Steinnes and Krog, 1977) and an abandoned cinnabar mine (Bargagli et al., 1987), lead (Pb) near a major highway (Laaksovirta et al., 1976), As near gold smelters (Hocking et al., 1978), and microplastics near a landfill (Loppi et al., 2021). The detection of this steep decrease of Hg in *Usnea* lichens with distance from the tailings demonstrates that the mine tailings have a greater influence on the Hg content in these lichens than long-range atmospheric transport (Loppi et al., 2021).

4.4.2 Land Type

Lichens collected near roads had the lowest THg concentrations of all sampling environments (Figure 10). This trend may reflect the greater distances of roads relative to the tailings. The wide range of THg concentrations in lichens growing on trees from natural forest stands and wind throw zones may be explained by the varying distances that these habitats are located from the tailings (Figure 11) in combination with other site-specific factors, such as microclimates that are produced by different tree stands (Sloof and Wolterbeek, 1993). Trees can impact soil and air temperatures as well as exposure to solar radiation, precipitation, and winds (Eckley et al., 2008). In turn, ambient conditions within a forest can change quite significantly even over short distances, which can affect

the amount of Hg available for interception by lichens via processes such as the deposition of windblown particles or gaseous Hg evasion.

Lichens sampled from trees near wetlands and bogs showed the highest concentrations of THg. This trend is likely due to being in closest proximity to the tailings, as all these classified wetland and bog habitats at Montague were located directly on tailings or within approximately 100 m of the tailings edge (Figure 11). As previously mentioned, the Hg may originate from windblown tailings along the wetland boundaries exposed during the dry seasons or be volatilized from the water surface (Figure 16) (Zhang et al., 1995).

In general, Hg volatilization would be enhanced in these environments because wetlands and bogs typically have anaerobic conditions due to the high water contents in the underlying sediments and soils (Rinklebe et al., 2010). These saturated conditions decrease the redox potentials in sediments which promotes the reduction of Hg^{2+} to Hg^0 by biotic and abiotic processes (Du Laing et al., 2009; Rinklebe et al., 2010; Zhou et al., 2020). Once produced, Hg^0 can diffuse from the underlying tailing sediments into the water column and then into the atmosphere for potential interception by nearby lichens (Zhu et al., 2011). This demonstrates that inundated tailings may still be an important source of atmospheric Hg.

More importantly, wetlands and bogs are known sites of high *in situ* MeHg production (Driscoll et al., 1998; Yu et al., 2010; Strickman and Mitchell, 2017). Methylmercury is formed when Hg^{2+} reacts with a methyl group, induced by abiotic or biotic processes, such as by light or microorganisms, respectively (Figure 16) (Segade et al., 2011; Figueiredo et al., 2018). Microbial methylation is widely accepted as the main

mechanism for most of the MeHg production in a natural environment (Hall et al., 2008; Segade et al., 2011). Methylmercury production is thought to be predominantly mediated by sulfate-reducing bacteria (SRB) (Loseto et al., 2004; O'Driscoll et al., 2005), which are typically abundant in organic-rich, anaerobic aquatic sediments, like those in wetlands (Hall et al., 2008; Selvendiran et al., 2008).

Methylmercury can be associated with particulates in wetlands, rivers, and lakes (Hill et al., 2009). With the water levels decreasing at some wetlands at Montague during the dry seasons and exposing the underlying sediments, including at the OSM site for example, there is potential for any particle bound MeHg to be eroded by wind and transferred to the nearby epiphytes or forest canopies (Figure 16). Also, the rewetting of dried wetland sediments can further promote MeHg production at these sites. The drying-out of wetland sediments increases the oxidation of sulfides so that when rewetted, high levels of sulfate are available to SRB, thereby increasing the abundance of these methylating communities (Bigham et al., 2017). So, the fluctuating water levels observed at wetlands at Montague may also be enhancing the Hg methylation rates in the sediments, possibly making more particle bound MeHg available for wind transport following a wetting cycle. With a changing climate and the subsequent impacts to the hydrologic cycle, MeHg production may increase at these wetland sites in the future.

However, demethylation processes also occur simultaneously with methylation processes in highly Hg-contaminated environments (Winch et al., 2008b). This process transforms the MeHg to less toxic, yet more mobile forms for transfer to the atmosphere (Figure 16). Similar to methylation, this can occur via abiotic processes, like photodegradation (Sellers et al., 1996; O'Driscoll et al., 2005; Hill et al., 2009), or

biotically by Hg-resistant microorganisms via reductive demethylation (RD) or oxidative demethylation (OD) (Segade et al., 2011). Reductive demethylation is the process thought to dominate in aerobic environments or under anaerobic conditions with highly contaminated sediments, producing Hg^0 and methane (CH_4) (Segade et al., 2011; Lu et al., 2016; Figueiredo et al., 2018). Thus, RD may result in the immediate volatilization and loss of Hg^0 from the system. In OD, MeHg is converted to Hg^{2+} and carbon dioxide (CO_2), a process primarily occurring in anaerobic environments (Lu et al., 2016; Figueiredo et al., 2018). Oxidative demethylation may result in the re-methylation of this Hg^{2+} under favourable conditions, or if subsequently reduced to Hg^0 , the Hg may be removed from the system via volatilization like RD (Segade et al., 2011). At Montague, it can be expected that the elevated Hg concentrations in the tailings (Parsons et al., 2012) would be sufficient to allow for the presence of Hg-resistant bacteria in these wetland environments and thus for demethylation processes to also occur. As a result, these processes would produce Hg products for potential volatilization from the water surface into the atmosphere (Winch et al., 2008b).

At the Lake Catcha and Upper/Lower Seal Harbour gold mine tailings sites in Nova Scotia, high concentrations of MeHg were measured in the organic-rich layers of some tailings from wet, bog-like sites, suggested to have been produced *in situ* by SRB (Winch et al., 2008a). Mercury demethylation was also suggested to be occurring at the Lake Catcha tailings site. Winch et al. (2008a) indicated that demethylation via microbial activity was likely contributing to significant releases of Hg^0 to the atmosphere from these Nova Scotian mine tailings. Due to the similar type of sites investigated by Winch

et al. (2008a), similar processes can be expected at the Montague tailings deposits that are located beneath wetlands and bogs.

Additional conditions of these water features may be promoting enhanced Hg emissions from these areas. Mercury flux has been found to be strongly correlated with solar radiation (Poissant and Casimir, 1998; Boudala et al., 2000; Zhou et al., 2020). Solar radiation induces the photochemical reactions that reduce Hg to more volatile species, resulting in further exchanges to the atmosphere at the air-water interface (Poissant et al., 2004; Zhou et al., 2020). These wetland environments at Montague are typically open and may be receiving more intense solar radiation than the surrounding shaded forested areas.

Furthermore, the wetland environments may be exposed to higher wind speeds due to the unobstructed landscape above the water surface, which, in combination with the presence of solar radiation, can increase the flux of Hg (Boudala et al., 2000). This is because windier conditions can increase the vertical mixing of the water column, transporting more Hg^0 closer to the surface and subsequently increasing the potential for transfer to the atmosphere and to nearby lichens (Boudala et al., 2000; Beauchamp et al., 2002). This trend of higher Hg flux rates from the water surface under stronger wind conditions was observed by Beauchamp et al. (2002) after collecting repeated Hg flux measurements at Big Dam West Lake in Nova Scotia. This is a testable hypothesis which can be examined in more detail at key tailing-impacted wetland sites in Nova Scotia.

4.5 Passive Dust Samplers

4.5.1 Total Dust Deposition Rates

Sites that generated the most dust, on average, were located furthest from the tailings deposits at Montague, which included BR and the two reference sites, ABL and SMU. This suggests that these sites are impacted by other, larger sources of dust. These areas are located closer to roads, urban areas, and city centres, so may be receiving inputs of dust from vehicular traffic (i.e. resuspended soil, wear material), construction or demolition activities, domestic fuel burning, or industrial activities, for example (Karagulian et al., 2015; Jose and Srimuruganandam, 2020). The samplers at OSM and Montague, located directly adjacent to the tailings deposits and surrounded by forests, may therefore be more influenced by the tailings dust and less by dust originating from other sources outside of the study area. The sampler at LL received less dust in comparison to these other samplers that are located directly adjacent to the tailings, reflecting its slightly greater proximity to a tailings deposit (Figure 1).

Seasonal dust deposition calculations revealed that, when sites were averaged collectively, the colder seasons measured higher total dust deposition rates than the warmer seasons, although at some sites, deviations from these general trends did exist (Figure 13). Many studies have found contrasting results with higher dust deposition rates during summer seasons when temperatures were highest and precipitation levels were lowest (Reheis and Urban, 2011; Aghasi et al., 2019; Ahmadi Foroushani et al., 2021). The adhesion of dust particles decreases with higher temperatures, lower rainfall, and the subsequent lack of moisture, which were the main reasons suggested for increased dust generation during the warmer seasons in these studies.

In the current study, the colder seasons reported highest wind speeds and precipitation levels in comparison to the warmer seasons (Figure 12). These high precipitation levels would lead to an increase in soil moisture, thus enhancing the ability to withstand erosion (Jiang et al., 2016). However, when the wind shear velocity at the soil surface exceeds the shear strength of the soil aggregates, particles may begin to separate from the surface and become entrained by wind (Csavina et al., 2012). So, during the colder months of our study, although precipitation levels were highest, the stronger wind velocities may have exceeded the shear strength of the soil particles at the surface and mobilized more dust. In Alberta, Canada, Hugenholtz and Wolfe (2010) measured highest dust generation from exposed river sediments during the colder months of the year, in part due to the greater speeds and transport capacity of winds. Additionally, vegetation cover stabilizes the soil surface and decreases the mobility of dust particles (Kutiel & Furman, 2003; Norouzi et al., 2017). This may also explain the observed seasonal trends of lower dust deposition rates in the warmer seasons for some sites, as in Canada, vegetation biomass increases during these seasons. The reduction of plant biomass in the winter may be exposing more soils and tailings material to greater wind action and subsequent dust generation.

In combination with the climatic conditions, site-specific factors may have also played a role in the observed seasonal dust deposition trends at some sites. The OSM samplers measured highest dust deposition rates in the cooler fall season (Figure 13). The water levels at the OSM wetland typically decrease slightly along the boundaries and aeri ally expose some of the underlying tailings for several weeks after the dry, summer season. Coupled with an increase in wind speeds into this fall season (Figure 12), these

exposed tailings at OSM may have been mobilized and contributed to the higher dust levels during this season. However, the OSM-1 and OSM-2 samplers did report lowest dust deposition rates during the winter season, unlike the average seasonal trends previously discussed. The increased precipitation levels into the winter (Figure 12) would have increased water levels at this wetland and completely submerged any tailings exposed from the previous warmer seasons, thereby reducing sediment availability for uplift.

The samplers at Montague (MO-1 & MO-2) showed highest dust deposition rates during the first summer season and another peak during the second summer season, contrasting the average trends determined from all sites (Figure 13). These trends might be attributed to the fact that these samplers are in closest proximity to the large tailings deposit at Montague. The main tailings deposit is largely unvegetated and has vast areas of loose and unconsolidated tailings material. With the higher temperatures and lower precipitation levels that would further dry out and decrease the adhesion of the particles during the summer seasons, these tailings may easily become lifted from the surface even under the lower measured wind speeds. Also, dust generation near these tailings would be enhanced by occasional off-road vehicle activity on the tailings during these summer seasons.

4.5.2 Total Mercury Flux Rates

At sites within the Montague Gold District, the passive air samplers located closer to tailings deposits (MO-1, MO-2, OSM-1, & OSM-2) measured higher mean GFF THg flux rates than those at LL and BR, which were located further from the tailings. These findings suggest that the tailings are a local source of atmospheric Hg which diminishes

with distance. The reference site samplers, ABL and SMU, also showed some higher and intermediate average GFF THg flux rates. These samplers, located much further from the tailings deposits, may therefore be influenced by other sources of Hg more common near urban centres, such as from waste plants, industrial and metal manufacturing facilities, and local fossil fuel combustion (McLagan et al., 2018). However, at these reference samplers, the GFF THg flux rates were highest mainly during the first season, with the following seasons being comparable to the fluxes from the other samplers within Montague.

The GFF THg flux rates were generally higher during the warmer seasons than the colder seasons (Figure 14). These trends were opposite to those observed for total dust deposition, which can be attributed to the different physical and chemical properties of Hg versus dust; Hg being highly volatile and mainly released from soils as a gas rather than attached to particles (Gustin et al., 1997; Wang et al., 2006; Cizdziel et al., 2019; Shi et al., 2020). Increased solar radiation, which can also increase the temperature of water and soils, will facilitate photochemical reactions that transform Hg^{2+} to gaseous Hg^0 , which increases the release of Hg to the atmosphere (Ma et al., 2013; Shi et al., 2020). Additionally, higher temperatures can allow Hg to move up the soil column due to desorption from soil particles, thereby further enhancing this photoreduction process and the subsequent Hg emissions (Figure 16) (Rinklebe et al., 2010; Cizdziel et al., 2019). At Montague, the spring and summer seasons measured the highest average temperatures (Figure 12). These conditions would favour the reduction of Hg^{2+} to Hg^0 and lead to greater emissions from the tailings and surrounding soils during these months. The lower GFF THg fluxes during the winter season reflect the colder air temperatures observed

(Figure 12), which would inhibit Hg^0 formation. It is important to note that some of the THg may also have originated from particle-bound Hg during these warmer seasons of which the meteorological conditions may have promoted some uplift of soil and tailing particles.

The GFF THg fluxes observed at the samplers at the OSM wetland may also be influenced by methylating and demethylating species that are likely more prevalent in these wetland environments, as mentioned previously. For instance, at these samplers, a small peak in THg flux rates was observed into the Spring/Summer 2020 season. At a wet, bog-like site at the Lake Catcha Gold District in Nova Scotia, demethylation processes in the tailings, and the subsequent production of more volatile Hg, dominated in the spring due to changing redox conditions induced by an influx of oxygenated waters after snowmelt (Winch et al., 2008a). Thus, this smaller peak in this season at the OSM samplers may reflect the potentially greater rates of demethylation of MeHg in the nearby wetlands, initiated by the changing seasonal conditions.

The two samplers adjacent to the main tailings deposit at Montague (MO-1 & MO-2) were the only samplers measuring distinct peaks in GFF THg flux rates in the winter season (Figure 14). These samplers were in closest proximity to the large tailings deposit. This, combined with reduced plant biomass covering these tailings and the higher winds during the colder season may have contributed more particulate-bound Hg from the tailings, as volatile Hg emissions would be lower during this colder season, as previously discussed. At rural sites in Michigan and Vermont, US, the winter seasons measured higher concentrations of particulate Hg in the atmosphere in comparison to the summer seasons (Keeler et al., 1995), which supports this proposed reasoning. More

particulate-bound Hg may also originate from these tailings during this windier winter season as Hg^{2+} is able to diffuse onto particle surfaces at low temperatures (Cole et al., 2014).

Despite both samplers at Montague showing the same seasonal trends, the MO-2 sampler showed higher levels and greater fluctuations between seasons than the MO-1 sampler. The MO-2 sampler is located closer to the sections of bare tailings while also having less vegetation cover across this distance. This would allow for more particle uplift from wind erosion and increased Hg volatilization during warmer months promoted by the increased light penetration to the surrounding soils (Rinkelbe et al., 2010; Ma et al., 2013). Therefore, this sampler may have received greater inputs of Hg year-round in comparison to the MO-1 sampler. The two samplers at OSM showed similar seasonal trends and GFF THg flux rates, reflecting their similar proximities to tailings and surrounding environments (i.e. forest edges along wetland).

4.6 Mercury in Lichens Compared to Passive Dust Samplers

When comparing the THg concentrations measured from the lichen samples to that measured from the nearby passive air samplers from the time of sampling (Figure 15), the THg measured in *Platismatia* was more strongly correlated to that measured on the corresponding GFFs relative to *Usnea*. This may reflect differences in accumulation mechanisms favoured by these foliose and fruticose lichens in combination with the design of the passive air samplers. Although *Usnea* has a wider surface area for gas exchange, these lichens also favour moisture and are greatly influenced by wet deposition, receiving significant quantities of elements from rainfall and dewfall (Bosserman and Hagner, 1981). Foliose lichens on the other hand, like *Platismatia*, have

a larger continuous surface and have been shown to capture elements more efficiently from dry deposition, such as windblown particles (Bosserman and Hagner, 1981; Monaci et al., 2012; Graney et al., 2017). These distinctions are important because the passive air samplers are only collecting elements from dry deposition (gases and particles) as a large plate covers the air filters which protects from wet deposition. So, since elements from wet deposition were not captured on the GFFs, unlike in *Usnea*, this may explain the lack of correlation between THg in the filters and these lichens.

Another possible related reason for the weaker correlation of THg between *Usnea* and the filters may reflect the physical characteristics of the filter matrices. In this study, each passive air sampler hosted both a GFF and PUF during each sampling period, yet only the GFF was analyzed. Despite both matrices being capable of collecting both dry gases and particles, the GFFs are hypothesized to more so reflect the particulate components of dry deposition due to its more densely woven matrix, of which only particles mainly deposit on the surface (Eng et al., 2014). This may explain why the THg in *Platismatia* samples showed similar trends to that from the corresponding GFFs, as this foliose lichen is also more efficient at capturing particles (Bosserman and Hagner, 1981; Monaci et al., 2012; Graney et al., 2017). In contrast, the PUFs more so reflect the gaseous components of dry deposition (Loppi et al., 2015), in part due to the greater pore spaces within which allow for scavenging of gaseous species released from entrained particles (Eng et al., 2014). Thus, if the PUF was also analyzed, more gaseous Hg may have been measured. In such case, the THg concentrations from the filters may then have correlated better with that of *Usnea* which is already known to have a higher ability to sequester gaseous Hg due to its greater surface to volume ratio (Monaci et al., 2012).

Previous studies have shown strong linear correlations between concentrations of polycyclic aromatic hydrocarbons (PAHs) measured in transplanted fruticose lichens and PUF passive air samplers in Europe (Domínguez-Morueco et al., 2015; Loppi et al., 2015). Loppi et al. (2015) suggested this was largely due to the ability of the PUFs to accumulate mainly gaseous species over particulates, of which is the common form of PAHs.

Overall, these findings indicate that, in future work, it would be important to also measure the Hg concentrations on the PUF in addition to the GFF, as this may capture more gas-phase species which can contribute to the THg load. Analyzing both the GFFs and PUFs together may provide stronger correlations with lichens that also accumulate species in both the particle and gas phases.

It is important to note that the trends in THg concentrations in the lichens as compared to the passive air samplers may also differ because of the different exposure times between the two sampler types. The passive air samplers were deployed for a known amount of time, approximately three months, so only reflect THg fluxes during those time periods. In contrast, the *in-situ* lichens have been growing at these locations prior to the deployment of the passive air samplers and may reflect more long-term trends in THg (Rimondi et al., 2020).

4.7 Ecosystem Implications

Overall, the lichens have been shown to sequester Hg throughout the study area. This may have direct biological implications as lichens contribute to the vitality of many terrestrial ecosystems. For instance, beetles, grasshoppers, ants, flies, spiders, moths, slugs, and springtails, many of which are common in Nova Scotia, eat, live on, or use

lichens for camouflage (Asplund and Wardle, 2017). These species form the base of terrestrial food chains and are consumed by higher trophic level species, like birds. The transfer of contaminants from lichens to lichen herbivores and up to higher trophic level species has been shown in many studies, including to caribou and wolves in the Canadian Arctic (Kelly and Gobas, 2001) and to deer and pumas in California (Weiss-Penzias et al., 2019). Thus, these lichens at Montague may act as vectors of Hg to terrestrial food webs, contributing to Hg bioaccumulation and biomagnification.

In addition, the passive dust samplers showed varying dust deposition and THg flux rates across the study area. Along with the Hg contamination in lichens, these results further demonstrate that Hg may be spreading to surrounding ecosystems and depositing on vegetation, for example. This may also pose a risk to other ecosystem compartments, such as soils and waters, with Hg capable of being washed off or leached from vegetation and lichen thalli during rain events. In particular, Nova Scotia is impacted by acid rain, predominantly in the southwestern region of the province (Government of Nova Scotia, 2017). By altering the pH of the environment, acid rain can differentially affect the absorption and desorption of metals on vegetation and lichen thalli held by ion exchange, as well leach elements held in particulates (Garty and Hagemeyer, 1988; Čučulović et al., 2014). Under these acidic conditions, the surrounding vegetation and lichen may become a secondary source of metal pollution with the potential to spread any accumulated Hg to surrounding environments.

5.0 Summary and Conclusions

In summary, this study has shown the remobilization of tailings material and Hg transfer throughout the environment at the historical Montague gold mine tailings site in Nova Scotia. *Usnea* and *Platismatia* were effective spatial biomonitors of Hg that aided in identifying risk areas. The distribution of Hg hotspots in lichens primarily around tailing deposits suggested that the tailings are likely a local source of Hg to the atmosphere and into surrounding environments. Similar to previous studies of former gold mines in the province, this study shows that the generation of windblown tailings dust and Hg volatilization are important processes contributing to the Hg concentrations in the air surrounding these sites.

Mercury concentrations in lichens are influenced by many factors, including distance to the tailings, Hg concentrations of underlying or nearby tailings, surrounding forest cover, and land type. Surface vegetation and forest cover are important in reducing dispersion of Hg, indicating revegetation and phytostabilization to be a potential remediation option requiring further research (Chapman et al., 2019). In particular, the wetlands at Montague proved to be important reservoirs of Hg, acting as a long-term source for both the atmosphere and downstream aquatic environments, especially due the large role these environments have in methylating and demethylating Hg. Our findings have highlighted the impacts of a changing climate on dust generation and air contamination stemming from these contaminated sites, reinforcing the need to monitor and/or remediate both the tailing and wetland areas within these mine districts.

This study found that the fruticose *Usnea* had a higher capacity to sequester Hg in comparison to the foliose *Platismatia*, owing to the fact that, globally, most of the Hg in

the atmosphere is in gaseous form. Thus, when selecting lichen species for future biomonitoring work, it is important to consider both the lichen growth form as well as the primary physical forms of the target elements. Despite these differences between lichens, both *Usnea* and *Platismatia* were found to exhibit similar Hg distribution patterns, reaffirming the location of hotspots. For future biomonitoring work at contaminated sites, we recommend a sampling approach pairing two lichen genera, particularly of two different growth forms. This can be especially important when sampling on a high-density, local scale when the effects from within and between site elemental variation in lichens become more easily detected. Additionally, this can help to better understand the total pollution load in the area as lichens of different growth forms can have different abilities to capture and absorb airborne contaminants, such as those in gaseous or particulate forms.

The passive dust samplers proved to be a simple tool for determining spatial and seasonal dust deposition and THg flux rates at this contaminated site. Higher rates of dust deposition were measured in the colder seasons, whereas THg flux rates were greater in the warmer seasons. These different seasonal trends between the dust and THg flux rates can be attributed to the different meteorological conditions that enhance Hg generation and emissions.

The THg concentrations measured by the passive dust samplers correlated well with those measured from the nearby foliose lichens (*Platismatia*), but not the fruticose lichens (*Usnea*). This likely reflected the lower capacity of the GFFs to effectively capture gaseous species unlike in *Usnea*. To improve correlations with *Usnea* and the passive dust samplers, incorporating Hg data from both the GFF and PUF is

recommended for future studies. Regardless, both lichen biomonitors provided information on atmospheric Hg levels and are recommended as a complementary measurement method to passive air samplers, as both methods have provided a better understanding of the transport of contaminants in dust. Additional work improving the correlation between the Hg in lichens to these manufactured samplers is required before being able to quantitatively translate values from lichen biomonitors to equivalent air concentrations. This is challenging and would be species dependent, varying across space and time.

In conclusion, *Usnea* and *Platismatia* proved to be suitable genera for future spatial dust monitoring programs in Nova Scotia, and are relatively inexpensive tools with a widespread, natural distribution. This study has demonstrated that using *in situ* lichens may be a worthwhile dust monitoring approach at other isolated legacy mine sites in the province where employing conventional air sampling equipment would not be feasible, and when only relative estimates of atmospheric Hg concentrations are required. Additionally, interpolation of the analytical data in ArcMap was a powerful tool that helped to identify spatial patterns and the levels of contamination across the entire study area. Thus, lichen sampling and subsequent spatial analysis may be a simple approach that can be used to prioritize the remediation of contaminated sites or identify priority areas requiring management across larger regions. Overall, the results from this study can provide guidance to mine and risk evaluators and help to predict the impacts of this Hg contamination on nearby food webs.

More research is needed to assess the spatial extent of contamination from the Montague tailings deposits. In future, lichen sampling at further distances from the source

as well as collecting additional samples from reference areas would help in order to understand the tailings' atmospheric sphere of influence. Further research could also focus on Hg contamination in lichen herbivores, especially invertebrates, to understand the transfer of the mine tailing contaminants in terrestrial food webs.

6.0 Chapter 2 Figures

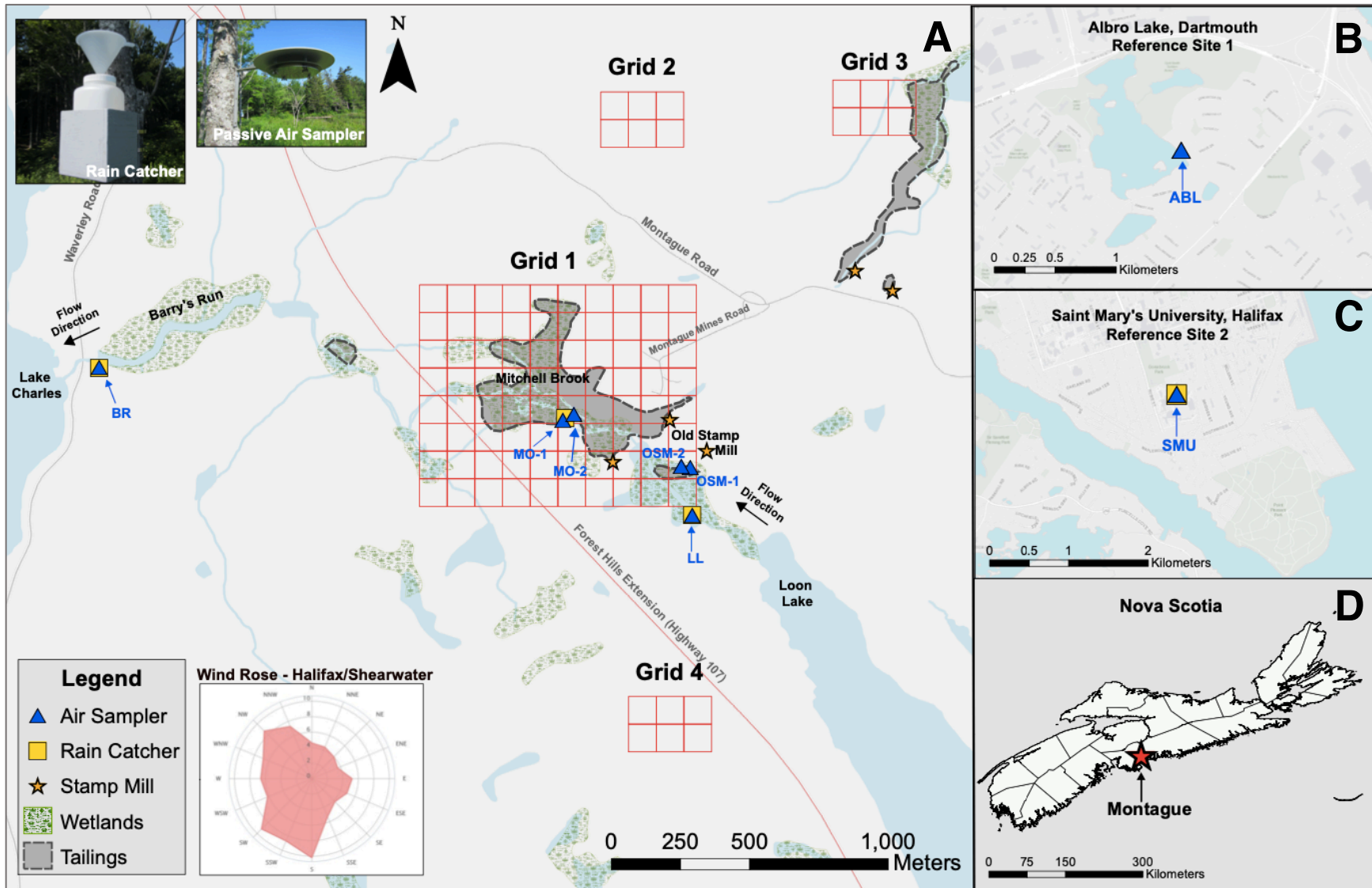


Figure 1. (A) Locations of grids, proposed lichen sampling sites (grid intersections), passive air samplers, and rain catchers at the historical Montague gold mine tailings site near Dartmouth, Nova Scotia. Site codes for passive air samplers are labelled in blue. (B) Location of the passive air sampler at Reference Site 1 at Albro Lake, Dartmouth, Nova Scotia. (C) Location of the passive air sampler and rain catcher at Reference Site 2 at Saint Mary's University in Halifax, Nova Scotia. (D) The relative location of the Montague gold mine tailings site in Nova Scotia, indicated by a red star.

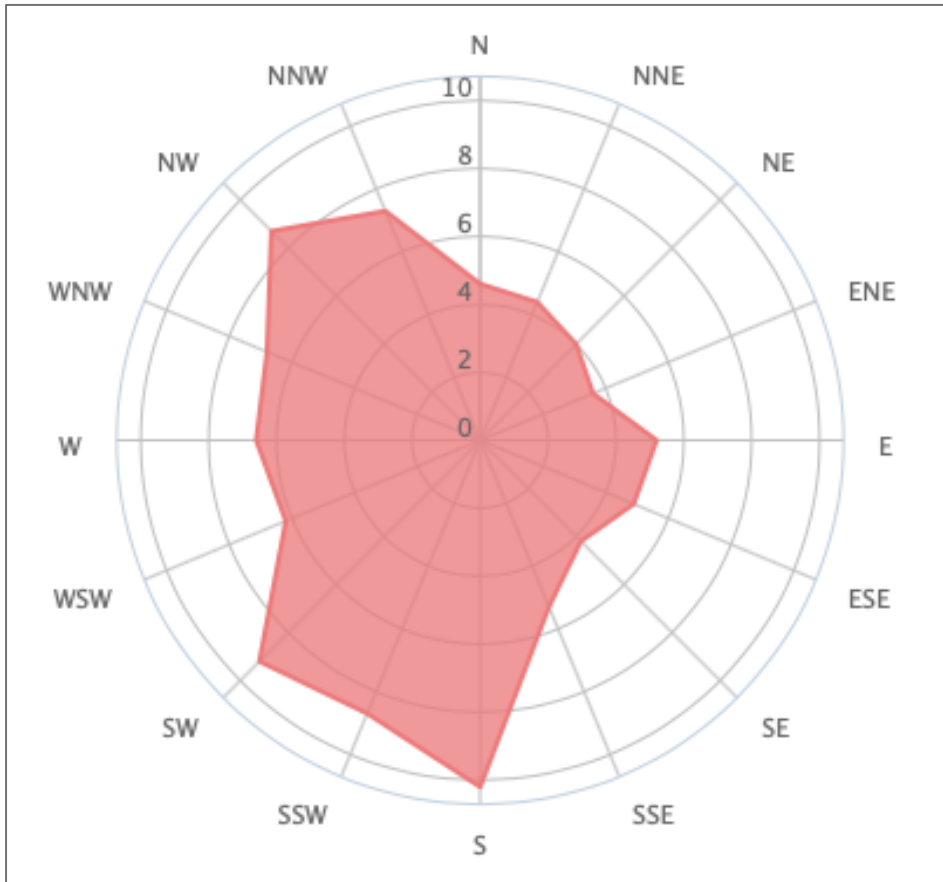


Figure 2. Wind rose from the Halifax-Shearwater weather station, which shows the annual average wind direction distributions and the associated time in percentages that winds blew from each direction. Data averaged from 2007 to 2019. Image source: https://www.windfinder.com/windstatistics/shearwater_halifax.

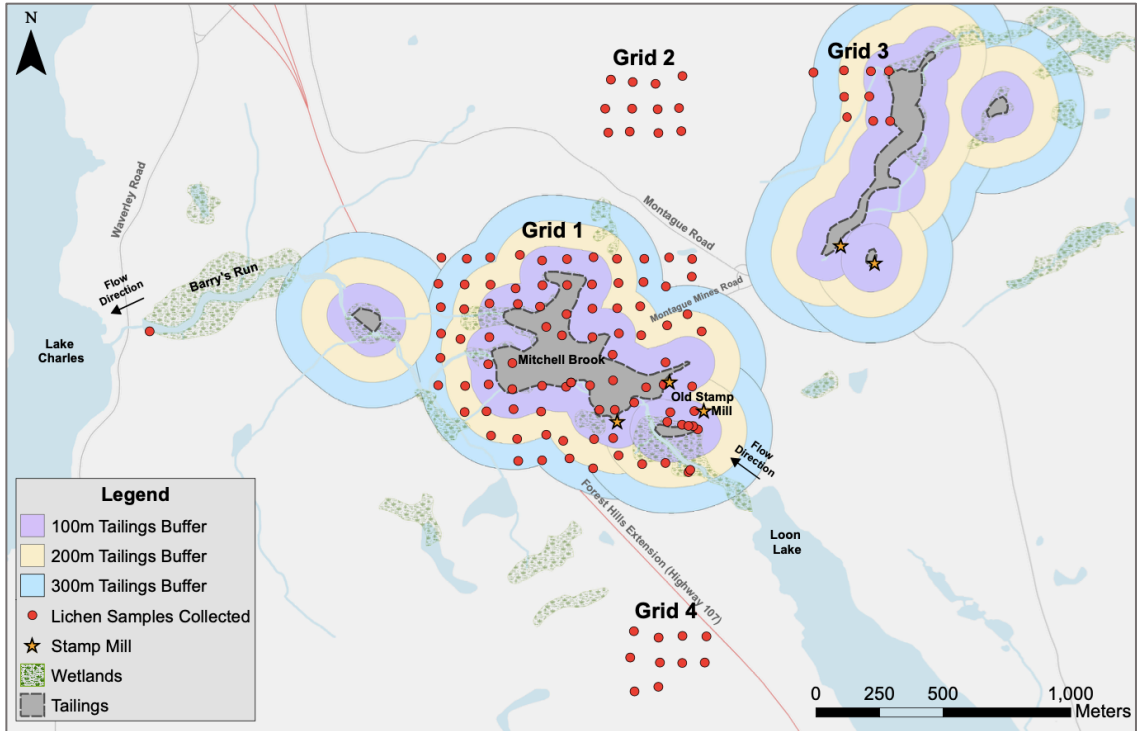


Figure 3. Map of the Montague gold mine tailings site with spatial buffers surrounding the known tailings deposits, used for classifying the lichen sampling sites based on distance to the nearest tailings edge.

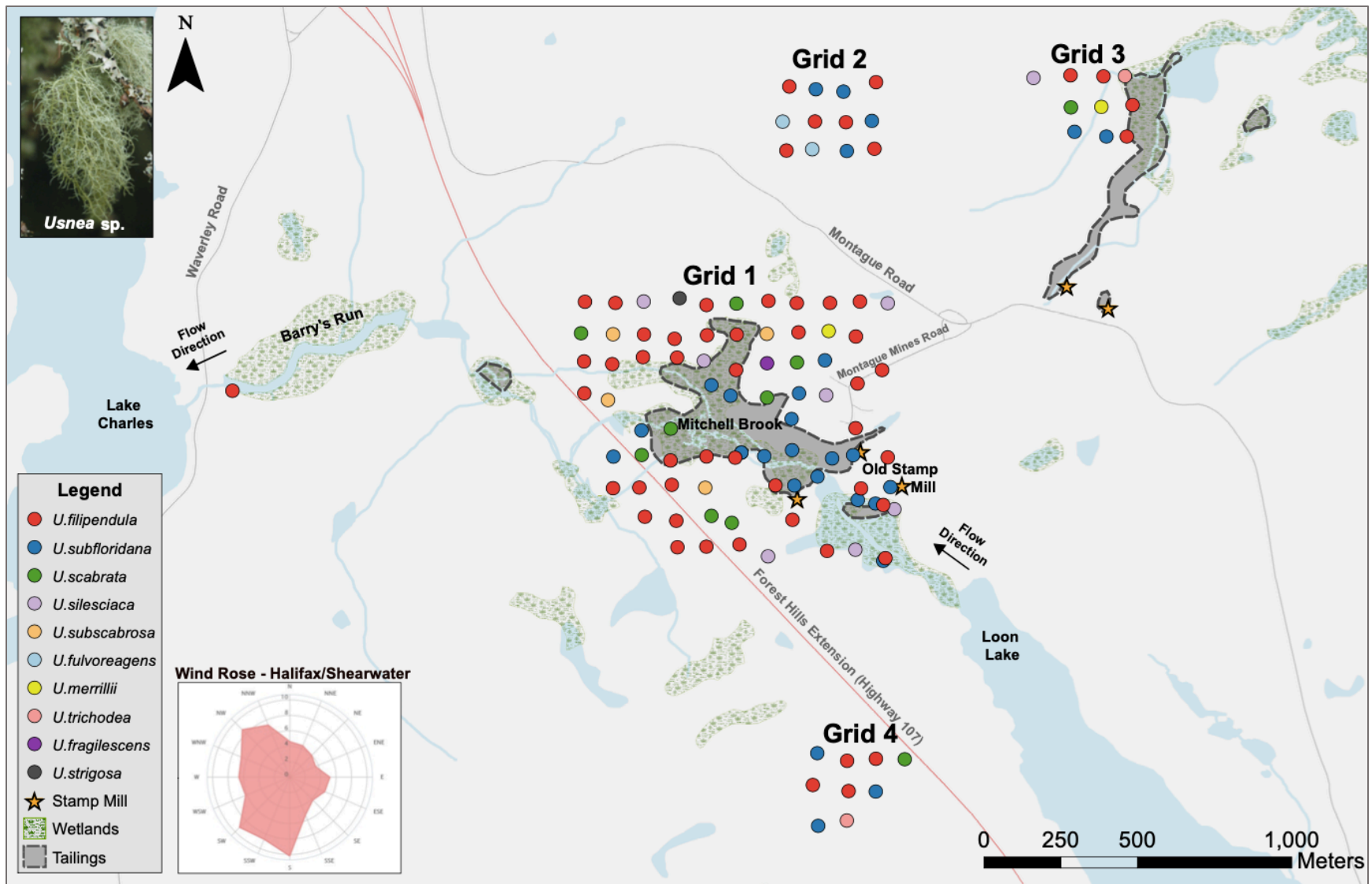


Figure 4. Actual sampling locations of *Usnea* and the associated identified species in Grids 1, 2, 3, and 4 at the historical Montague gold mine tailings site near Dartmouth, Nova Scotia.

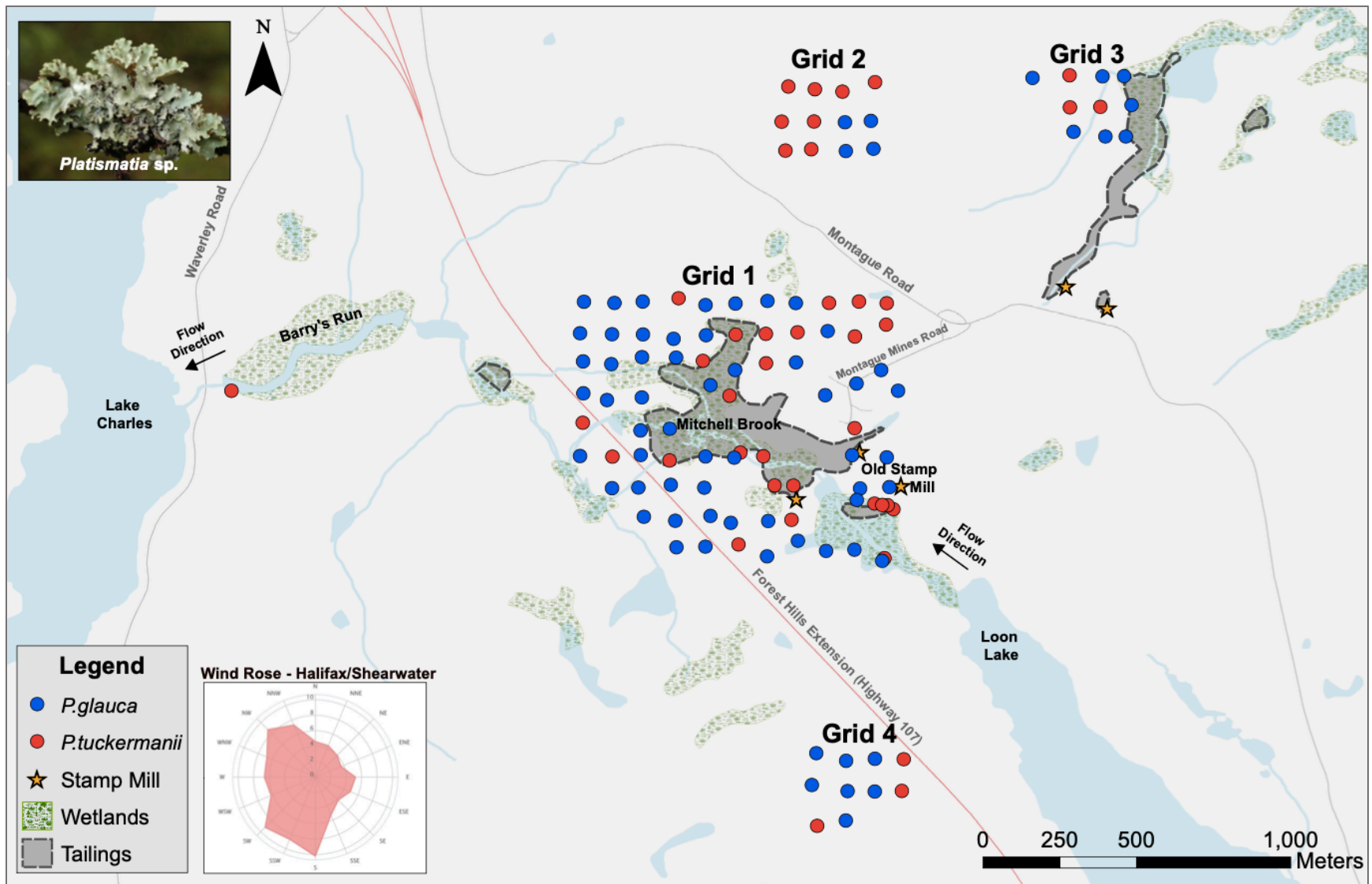


Figure 5. Actual sampling locations of *Platismatia* and the associated identified species in Grids 1, 2, 3, and 4 at the historical Montague gold mine tailings site near Dartmouth, Nova Scotia.

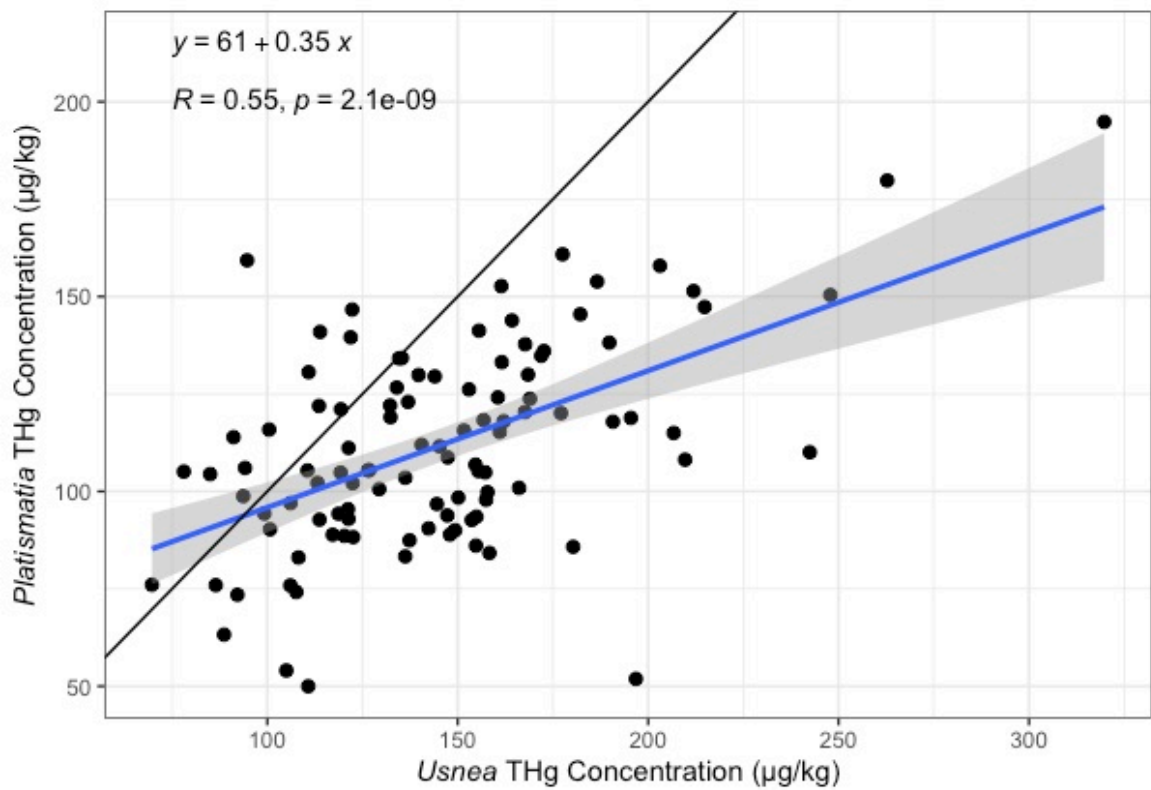


Figure 6. Total mercury concentrations ($\mu\text{g}/\text{kg}$) in *Usnea* versus *Platismatia* collected from the same sampling sites surrounding the Montague gold mine tailings site. Regression line is plotted in blue and a reference line with a 1:1 slope is plotted in black.

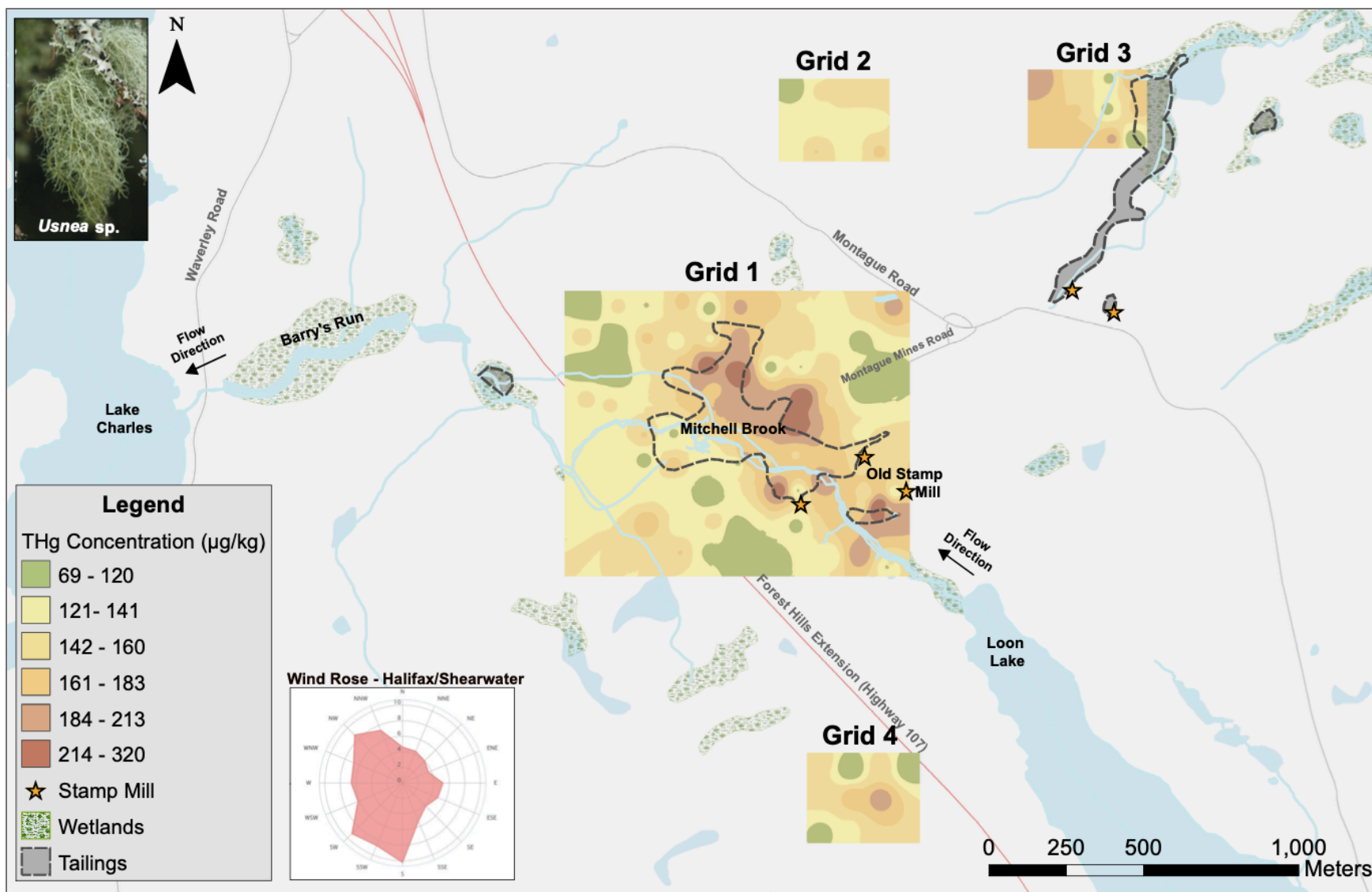


Figure 7. Interpolated surface displaying the predicted total mercury concentrations ($\mu\text{g}/\text{kg}$) in *Usnea* at the historical Montague gold mine tailings site near Dartmouth, Nova Scotia. Note: colour scale is different from that for the *Platismatia* interpolated map (Figure 8).

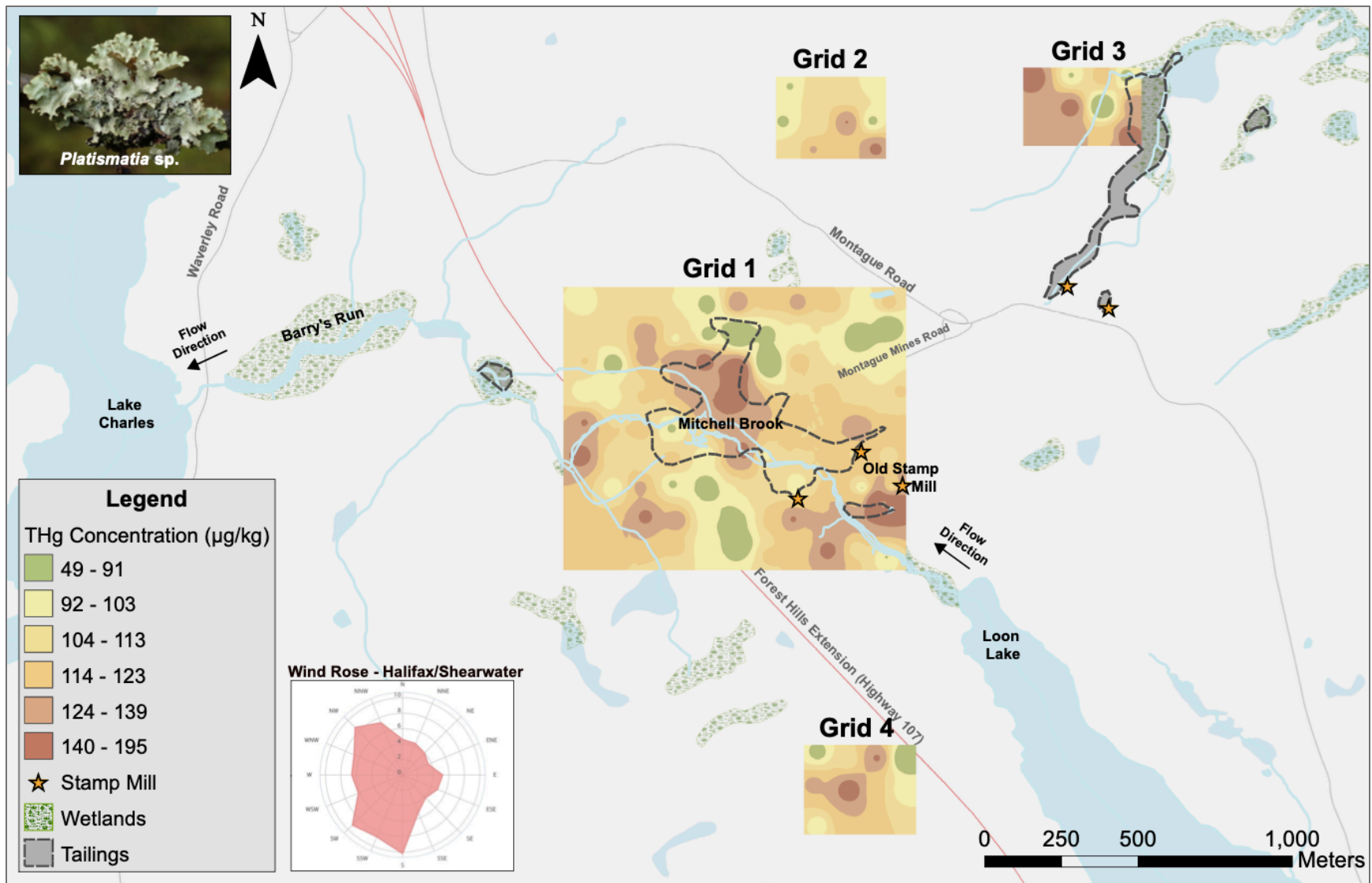


Figure 8. Interpolated surface displaying the predicted total mercury concentrations ($\mu\text{g}/\text{kg}$) in *Platismatia* at the historical Montague gold mine tailings site near Dartmouth, Nova Scotia. Note: colour scale is different from that for the *Usnea* interpolated map (Figure 7).

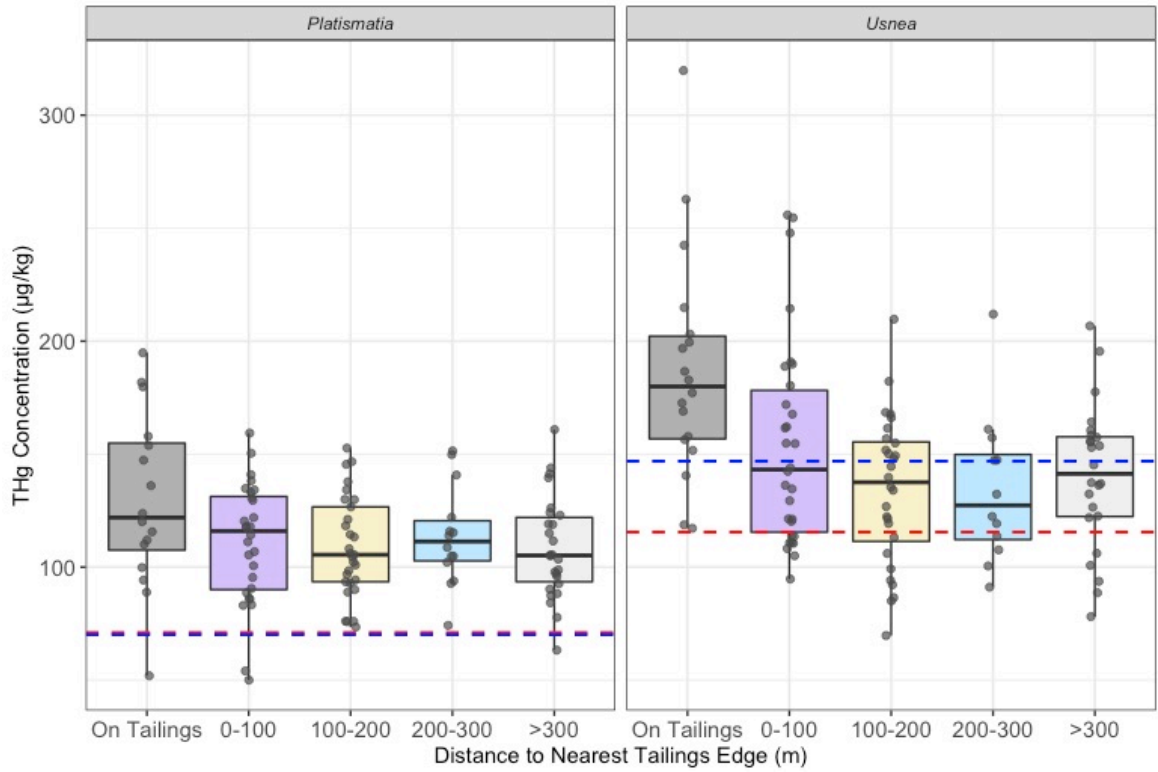


Figure 9. Total mercury concentrations ($\mu\text{g}/\text{kg}$) in *Usnea* and *Platismatia* from the Montague gold mine tailings site. Data are summarized into categories based on distance to the nearest tailings edge: On tailings, 0-100 m, 100-200 m, 200-300 m, and >300 m. The colour of each box corresponds to the colours shown in the buffers map (Figure 3). Reference lichen samples are plotted as dashed lines for each lichen genera (Saint Mary's University reference site: red, Albro Lake reference site: blue).

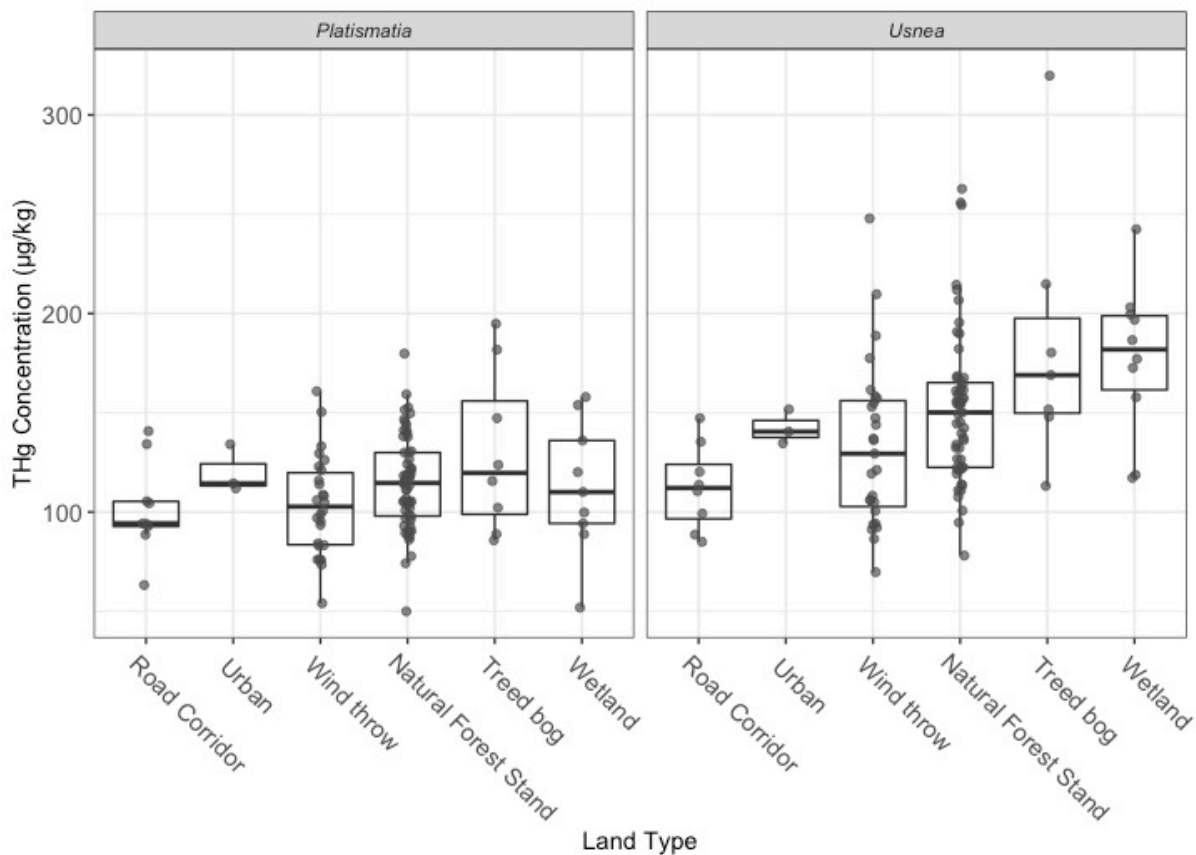


Figure 10. Total mercury concentrations ($\mu\text{g}/\text{kg}$) in *Usnea* and *Platismatia* from the Montague gold mine tailings site, summarized into categories based on the land type of the sampling site: Roads, urban areas, wind throws, natural forest stands, treed bogs, and wetlands.

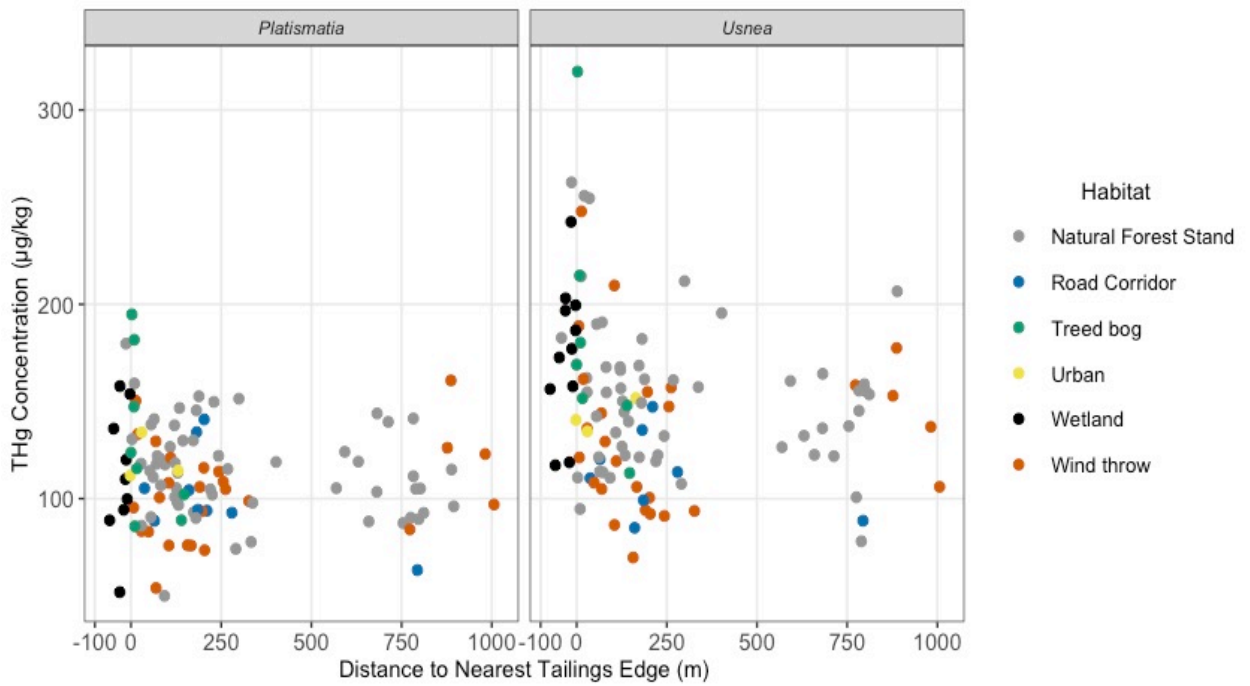


Figure 11. Total mercury concentrations ($\mu\text{g}/\text{kg}$) in *Usnea* and *Platismatia* versus distance to the nearest tailings edge (m) at the Montague gold mine tailings site. Data points are colour-coded into categories based on the land type of the sampling site: Roads, urban areas, wind throws, natural forest stands, treed bogs, and wetlands. Negative distance values refer to distances from the known tailings edge, but from samples located directly on tailings.

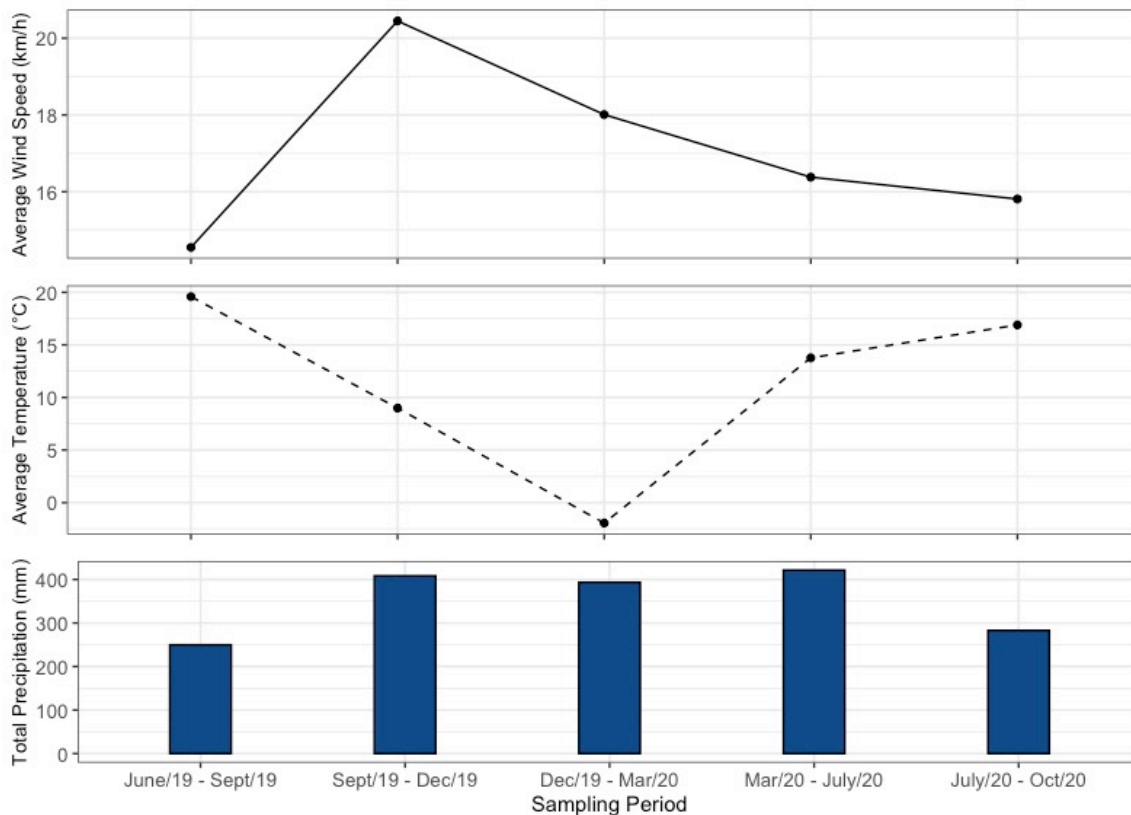


Figure 12. Average wind speeds (km/h), temperatures (°C), and total precipitation (mm) during each sampling period for the passive dust samplers at Montague. Data are from the Halifax-Shearwater weather station.

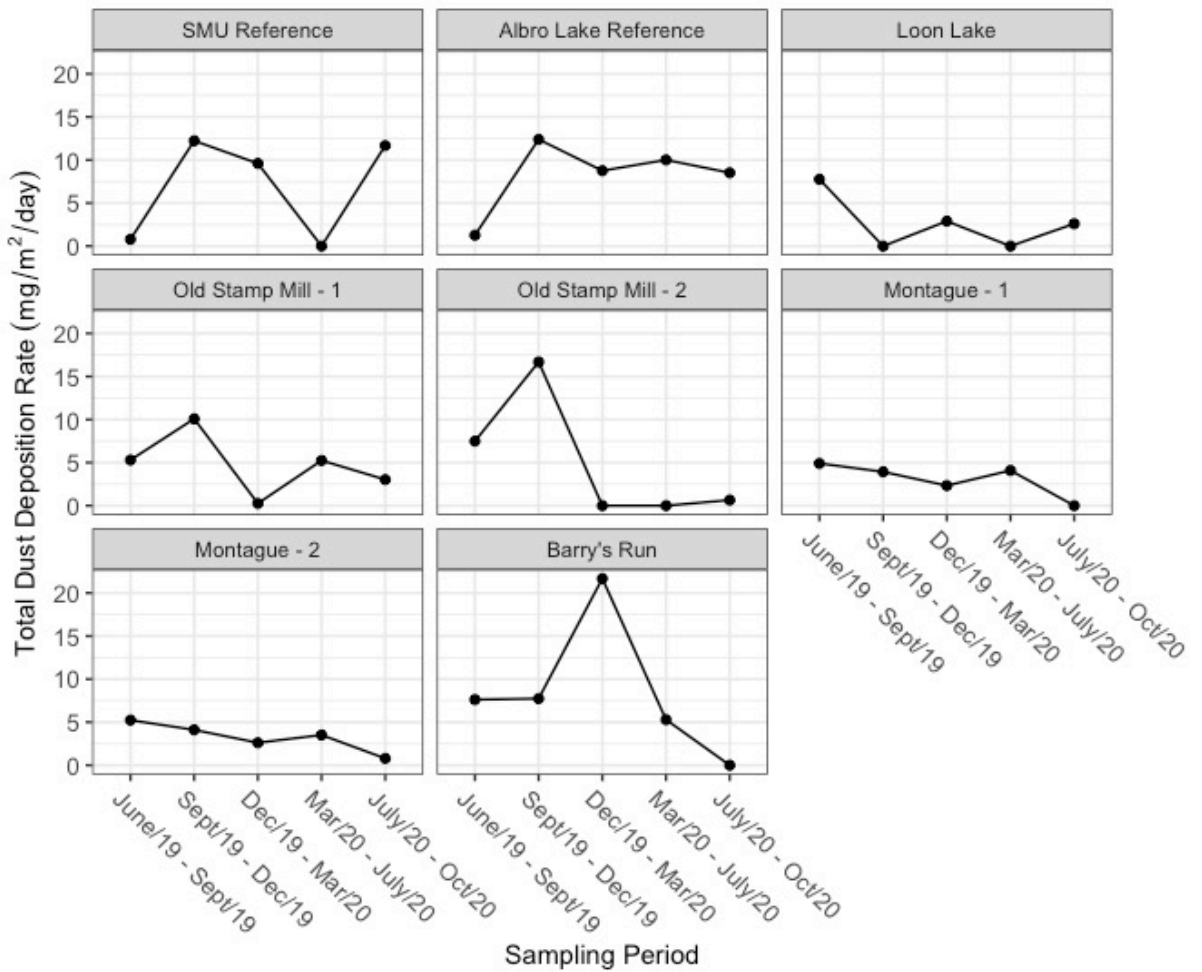


Figure 13. Total seasonal dust deposition rates ($\text{mg}/\text{m}^2/\text{day}$) measured on the passive dust sampler GFFs collected from sites surrounding the Montague gold mine tailings and reference areas from June 2019 to October 2020.

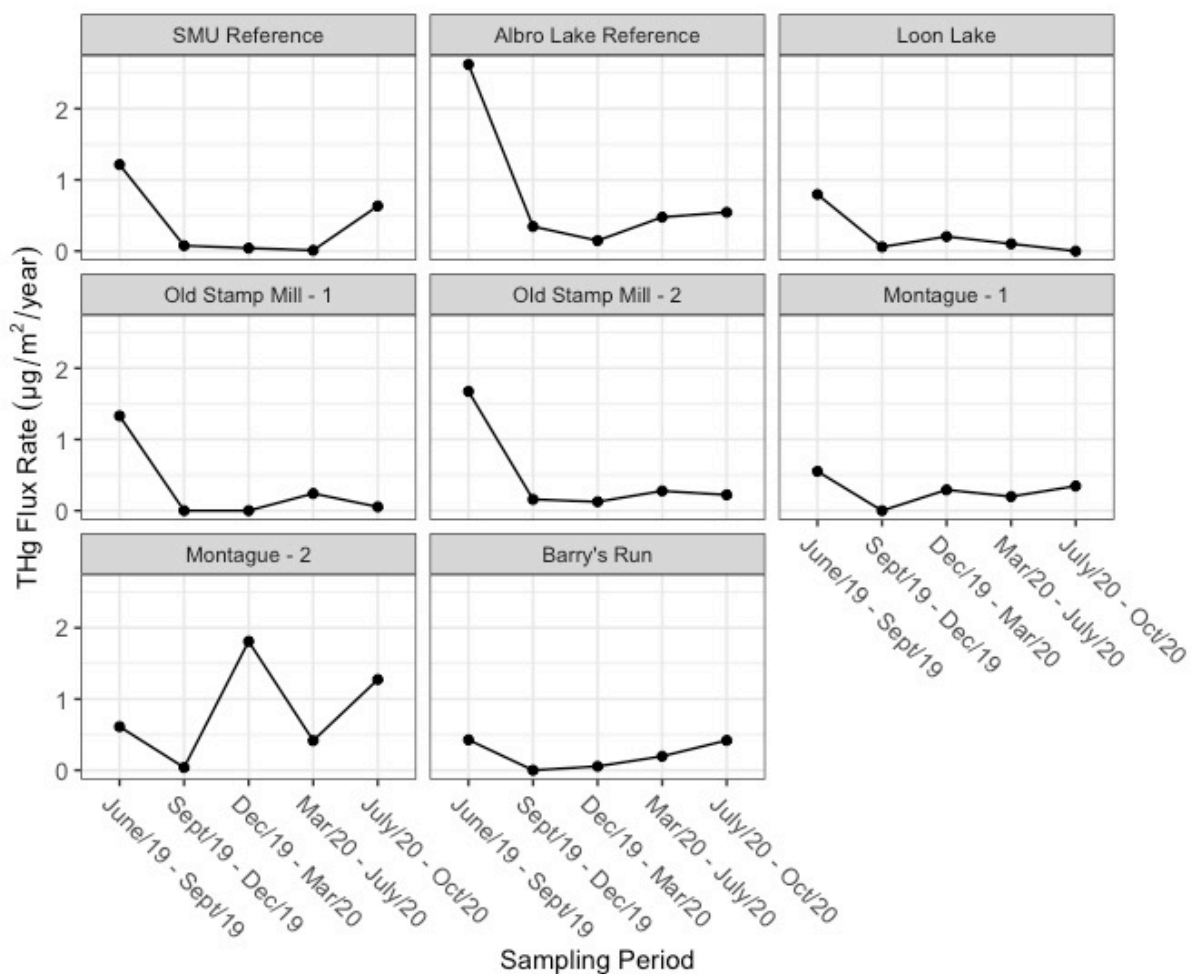


Figure 14. Seasonal total mercury flux rates ($\mu\text{g}/\text{m}^2/\text{year}$) measured on the passive dust sampler GFFs collected from sites surrounding the Montague gold mine tailings and reference areas from June 2019 to October 2020.

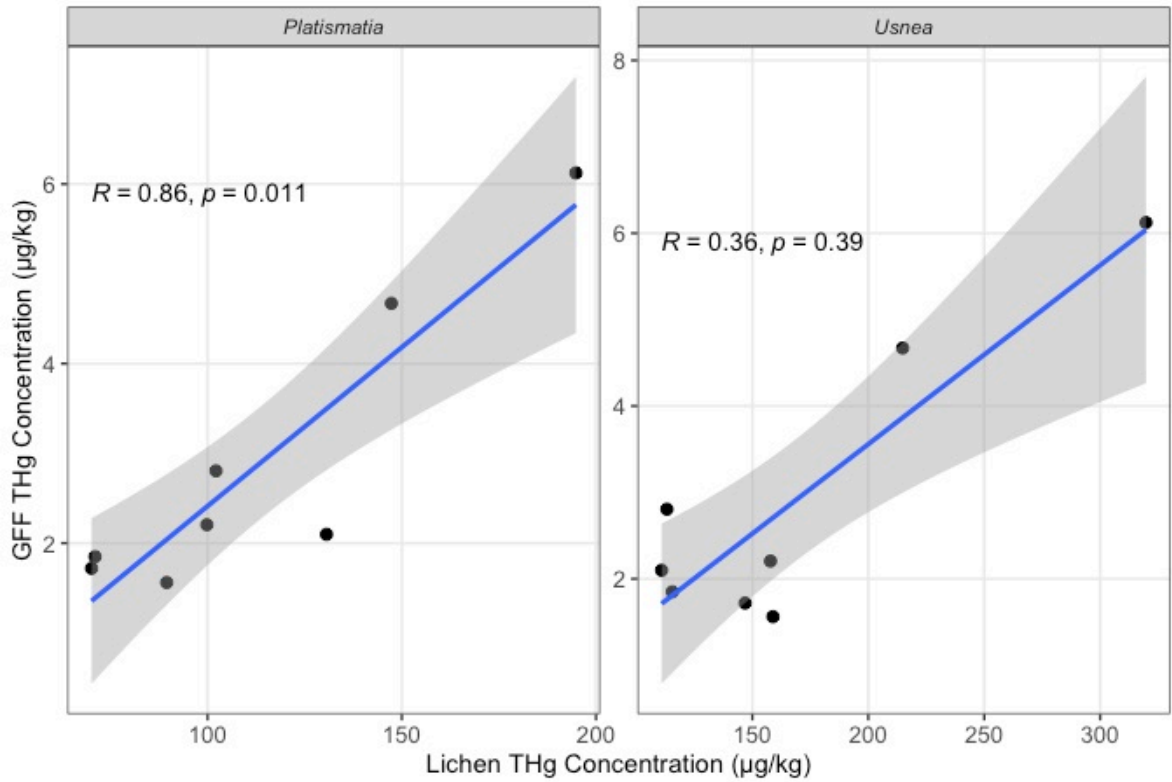


Figure 15. Total mercury concentrations ($\mu\text{g}/\text{kg}$) in *Usnea* and *Platismatia* against the total mercury concentrations ($\mu\text{g}/\text{kg}$) in the passive dust sampler GFFs collected from the same locations and sampling periods. Data points include sites both at the Montague gold mine tailings site and the reference sites.

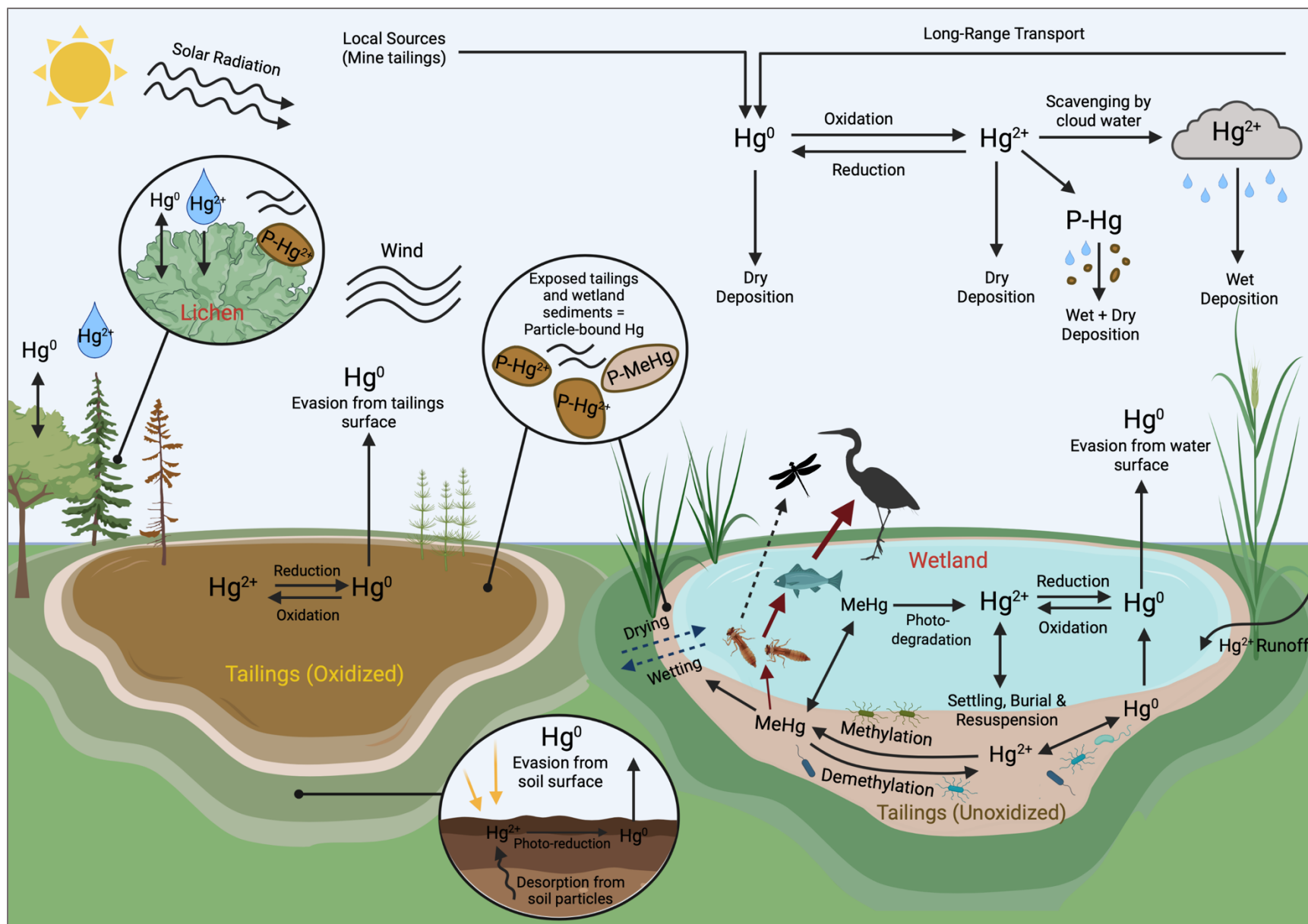


Figure 16. Conceptual diagram of mercury cycling and species transformations at historic gold mine tailings sites in Nova Scotia. Created in BioRender.com.

7.0 Chapter 2 Tables

Table 1. Site names, codes, and coordinates of each passive air sampler and rain catcher.

Sampling Device	Site Name	Site Code	Latitude, Longitude
Air Sampler	Loon Lake	LL	44.71157, -63.51715
Air Sampler	Old Stamp Mill - 1	OSM-1	44.71312, -63.51725
Air Sampler	Old Stamp Mill - 2	OSM-2	44.71315, -63.51766
Air Sampler	Montague - 1	MO-1	44.71462, -63.52308
Air Sampler	Montague - 2	MO-2	44.71483, -63.52258
Air Sampler	Barry's Run	BR	44.71623, -63.54425
Air Sampler	Albro Lake Reference 1	ABL	44.68827, -63.57322
Air Sampler	Saint Mary' University Reference 2	SMU	44.63224, -63.58006
Rain Catcher	Loon Lake	LL	44.71157, -63.51715
Rain Catcher	Montague - 1	MO-1	44.71469, -63.52297
Rain Catcher	Barry's Run	BR	44.71623, -63.54425
Rain Catcher	Saint Mary' University Reference 2	SMU	44.63224, -63.58006

Table 2. Dates of sample collection and replacement to passive air samplers and rain catchers.

Sampling Round	Dates of Sample Collection and Replacement
Installation	June 7 th , July 9 th & 24 th , 2019
Round 1	September 12 th , 2019
Round 2	December 16 th & 17 th , 2019
Round 3	March 16 th & 17 th , 2020
Round 4	July 29 th & 31 st , 2020
Round 5	October 22 nd & 26 th , 2020

Table 3. Species and counts of *Usnea* and *Platismatia* collected in the sampling grids at the Montague gold mine tailings site.

Species	Total Count
<i>Usnea filipendula</i>	54
<i>Usnea subfloridana</i>	28
<i>Usnea scabrata</i>	10
<i>Usnea silesiaca</i>	8
<i>Usnea subscabrosa</i>	4
<i>Usnea fulvoreaegens</i>	2
<i>Usnea merrillii</i>	2
<i>Usnea trichodea</i>	2
<i>Usnea fragilescens</i>	1
<i>Usnea strigosa</i>	1
Grand Total	112
<i>Platismatia glauca</i>	72
<i>Platismatia tuckermanii</i>	41
Grand Total	113

Table 4. Mean, median, and range of total mercury concentrations ($\mu\text{g}/\text{kg}$) measured in *Usnea* and *Platismatia* at historical gold mine tailing sites and reference sites in Nova Scotia. Data are summarized based on site and/or sampling grid.

Lichen	Sampling Location	n	Mean \pm SD ($\mu\text{g}/\text{kg}$)	Median ($\mu\text{g}/\text{kg}$)	Range ($\mu\text{g}/\text{kg}$)
<i>Usnea</i>	Montague – Grid 1	81	151 \pm 46.3	147	69.7 – 319
	Montague – Grid 2	12	138 \pm 18.7	136	100 – 164
	Montague – Grid 3	10	155 \pm 38.0	164	94.7 – 211
	Montague – Grid 4	9	140 \pm 42.2	152	78.1 – 206
	Barry’s Run	1	158*	--	--
	Lake Catcha	1	257*	--	--
	Oldham	1	231*	--	--
	Albro Lake Reference	1	146*	--	--
	SMU Reference	1	115*	--	--
	<i>Platismatia</i>	Montague – Grid 1	81	112 \pm 26.7	110
Montague – Grid 2		12	109 \pm 19.2	105	87.4 – 143
Montague – Grid 3		10	119 \pm 34.8	125	49.9 – 159
Montague – Grid 4		10	111 \pm 28.4	110	63.2 – 160
Barry’s Run		1	89.5*	--	--
Lake Catcha		1	235*	--	--
Oldham		1	271*	--	--
Albro Lake Reference		1	70.1*	--	--
SMU Reference		1	71.0*	--	--

*Single collection.

Table 5. Mean, median, and range of total mercury concentrations ($\mu\text{g}/\text{kg}$) measured in *Usnea* and *Platismatia* at the Montague gold mine tailings site. Data are summarized into categories based on distance to the nearest tailings edge.

Lichen	Distance		Mean \pm SD ($\mu\text{g}/\text{kg}$)	Median ($\mu\text{g}/\text{kg}$)	Range ($\mu\text{g}/\text{kg}$)
	from Nearest Tailings Edge (m)	n			
<i>Usnea</i>	On Tailings	18	187 ± 50.4	179	117 – 319
	0-100	30	153 ± 45.4	143	94.7 – 255
	100-200	28	134 ± 32.9	137	69.7 – 209
	200-300	12	134 ± 33.2	127	91.1 – 211
	>300	24	140 ± 32.1	141	78.1 – 206
<i>Platismatia</i>	On Tailings	16	129 ± 38.4	121	51.9 – 194
	0-100	28	110 ± 26.7	115	49.9 – 159
	100-200	29	108 ± 22.4	105	73.5 – 152
	200-300	14	113 ± 21.9	111	74.2 – 151
	>300	26	108 ± 22.5	105	63.2 – 160

Table 6. Total mercury concentrations ($\mu\text{g}/\text{L}$) measured in seasonal rainwater samples collected from sites at the Montague Gold District and a reference site in Nova Scotia. Note that a rainwater sample from MO-1 (Fall 2019) was the only sample with DHg above MDL ($0.078 \mu\text{g}/\text{L}$).

Site	n	# of non-detects	Sampling Season	THg concentration ($\mu\text{g}/\text{L}$)
Loon Lake (LL)	5	3	Sept-Dec 2019 (Fall)	0.232
			Mar-July 2020 (Spring/Summer)	0.143
Montague (MO-1)	6	2	June-July 2019 (Summer)	0.273
			July-Sept 2019 (Summer)	0.145
			Sept-Dec 2019 (Fall)	0.209
Barry's Run (BR)	4	3	July-Oct 2020 (Summer/Fall)	0.223
SMU Reference (SMU)	5	4	June-Sept 2019 (Summer)	0.162
				0.030

Table 7. Ranges and mean concentrations of mercury ($\mu\text{g}/\text{kg}$) measured in various foliose and fruticose lichens from polluted, unpolluted, and remote areas globally.

Location	Lichen	Sampling Locality	[Hg] Range	[Hg] Mean \pm SD/SE	Reference
Greenland	<i>Flavocetraria nivalis</i> ¹	Former Pb-Zn mine	30-70	--	Hansson et al., 2019
Antarctica	<i>Usnea sphacelata</i> ¹	Active volcanic region	140-240	--	Mão de Ferro et al., 2014
Louisiana, USA	<i>Ramalina stenospora</i> , <i>Parmotrema praesorediosum</i> ¹	Industrial region	<50 - 830	340 \pm 190	Mitchell et al., 2000
British Columbia, CA	<i>Bryoria fuscescens</i> , <i>Alectoria sarmentosa</i> ¹	Former cinnabar mine	240-600	--	Plouffe et al., 2004
Boreal Quebec, CA	<i>Bryoria trichodes</i> , <i>Evernia mesomorpha</i> , <i>Usnea subfloridana</i> ¹	Flooded reservoirs	400-800	--	Zhang et al., 1995
Chile	<i>Usnea</i> sp. ¹	Remote region (Unpolluted)	--	120 \pm 80	Monaci et al., 2012
Nunavut, CA	<i>Thamnolia vermicularis</i> , <i>Flavocetraria cucullata</i> , <i>Vulpiceda tilesii</i> ¹	Coastal and inland sites	36-361	66.8 (median)	St. Pierre et al., 2015
New Brunswick, CA	<i>Usnea</i> spp. ¹	Island, coastal and inland sites	24-635	244 \pm 116	Nasr and Arp, 2015
Nova Scotia, CA	<i>Usnea</i> sp. ¹	Kejimikujik National Park	66-660	--	Rencz et al., 2003
Nova Scotia, CA	<i>Usnea trichodea</i> , <i>Usnea longissima</i> , <i>Usnea strigosa</i> ¹	Province-wide	62-518	160 \pm 75	Klapstein et al., 2020
Nova Scotia, CA	<i>Usnea</i> spp. ¹	Abandoned gold mine tailings site	69-320	149 \pm 43	Current study
Finland	<i>Hypogymnia physodes</i> ²	Chlor-alkali plant	130-870	--	Lodenius & Laaksovirta, 1979
Italy	<i>Parmelia caperata</i> ²	Abandoned mining region	70-580	170 \pm 80	Bargagli et al., 2002
France	<i>Xanthoria parietina</i> ²	Chlor-alkali plant	70-2510	--	Grangeon et al., 2012
Finland	<i>Hypogymnia physodes</i> ²	Unpolluted region (Background)	70-480	223 \pm 76	Lodenius, 1981
Arizona, USA	<i>Xanthoparmelia</i> spp. ²	Grand Canyon region (Unpolluted)	98.9-408	209 \pm 69.7	Sweat et al., 2010
New Brunswick, CA	<i>Hypogymnia physodes</i> ²	Electricity generating plant	180-280	--	Sensen & Richardson, 2002
New Brunswick, CA	<i>Parmelia</i> spp. ²	Island, coastal and inland sites	10-337	124 \pm 74	Nasr and Arp, 2015
Nova Scotia, CA	<i>Platismatia</i> spp. ²	Abandoned gold mine tailings site	49-195	113 \pm 27	Current study

¹Fruticose lichen, ²Foliose lichen.

8.0 Chapter 2 References

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Chapter 3: Distinguishing between particulate and absorbed fractions of mercury in lichen biomonitors at a legacy gold mine tailings site in Nova Scotia

Abstract

Lichens are widely used as biomonitors of dust and air quality, with elemental concentrations in thalli reflecting both surface-deposited and absorbed elements. The objective of this study was to distinguish between these two fractions in lichens to assess the levels of dust originating from a contaminated site. *Usnea* and *Platismatia* spp. were collected from the historical Montague Gold District near Dartmouth, Nova Scotia. As a result of historic gold mining operations in this area, mercury (Hg)-contaminated tailings were deposited directly into the environment and later abandoned, being a potential source of contaminated dust today. Lichen thalli from each site were divided into two subsets, with one half being washed with water and the other remaining unwashed, and then analyzed for total Hg concentrations. Comparable Hg concentrations between the unwashed and washed lichens indicated that most of the Hg was absorbed into lichen thalli. On average, only 13% and 16% of the total Hg loads were washed off the surface of *Usnea* and *Platismatia*, respectively. It was suggested that Hg volatilization is a major process in making Hg available in the atmosphere at these tailings sites today. However, the tailings are likely still a minor source of particulate-bound Hg as more Hg was washed off of lichens that were collected closer to the tailings. Some washed lichen samples proved to have higher Hg concentrations than the paired unwashed samples, reflecting effects from within site elemental variation and lab processing procedures. It was concluded that this washing method can be used to help identify sources and understand the movement of contaminants in the lichens and throughout the environment.

1.0 Introduction

A lichen is a symbiotic association consisting of a fungus and algae or cyanobacteria (Lodenius, 2013; Bargagli, 2016). With the absence of root systems as well as a waxy cuticle that would otherwise control the uptake of elements, essential elements, along with contaminants, are accumulated by lichens via wet and dry atmospheric deposition (Shukla et al., 2014; Bargagli, 2016). Lichens are therefore sensitive to atmospheric pollution and have been successfully used as passive biomonitors in numerous studies, helping to determine the spatial distribution of atmospheric contaminants, the occurrence of contaminant hotspots, and pollutant emission sources (Bargagli et al., 2002; Sensen & Richardson, 2002; Søndergaard et al., 2010).

Lichens accumulate contaminants in various ways. Metals may be deposited or trapped on the surface of lichen thalli in soluble or particulate form (Figure 1) (Tyler, 1989). Metal-containing particulates may then be solubilized by precipitation, acid rain, or the organic acids produced by lichens in walls of the fungal cells (Nash, 1989; Tyler, 1989; Chiarenzelli et al., 1997). These soluble metal ions can bind to extracellular hydroxylic acid exchange sites, displacing ions with a low binding affinity (e.g. hydrogen) (Tyler, 1989; Bargagli, 2016; Vannini et al., 2017). Slowly over time, these extracellularly bound ions can then be incorporated into the cells of the lichen thallus, with the potential to cause toxic effects at the physiological level (Hauck et al., 2002).

Distinguishing and quantifying these surface-deposited versus absorbed elemental fractions in lichens can help to characterize metal pollution. In addition, this approach can provide insights into sources, how elements move through an environment (e.g. during a rain event) along with their potential bioavailability, and the amount and timing

of metal pollution events (i.e. recent versus long-term) (Johnson & Gunnar, 2018, unpublished). Distinguishing between soluble or particulate-bound and absorbed metal contaminants can be accomplished by washing lichen thalli with distilled or deionized water. This has been shown to effectively remove surface-deposited and soluble forms of elements (Gombert et al., 2003; Adamo et al., 2008; Loppi et al., 2014). Washing is also used after lichens are collected from a relatively clean site and before being transplanted to a polluted site (Giordano et al., 2013; Kularatne & de Freitas, 2013; Demková et al., 2017).

Many biomonitoring studies have just analyzed unwashed lichen samples to assess the overall contaminants on the lichens including dust, surface-bound, and absorbed fractions (Bargagli et al., 2002; Frati et al., 2007; Hansson et al., 2019). Other studies have analyzed just washed lichen samples that remove soil and dust particles in order to focus on only bound and absorbed elements (Koch et al., 2000; Plouffe et al., 2004; Doğrul Demiray et al., 2012). A few studies have separated the collected lichen samples into washed and unwashed subsets so to better understand both the particulate and absorbed components of accumulated elements.

Several early studies found no significant differences between washed and unwashed lichen samples for elements such as nitrogen (N), iron (Fe), and zinc (Zn) (Prussia & Killingbeck, 1991), as well as arsenic (As) and vanadium (V) (Saiki et al., 1997). These authors concluded that these elements were not easily leached from the lichens and were collected from sites where dust was not a major component. Other studies have shown that after washing lichens, element concentrations in the lichens decrease substantially, particularly for potassium (K) and aluminum (Al), as found in the

lichen *Parmotrema tinctorum* collected from a national park in Thailand. A decrease of 81% in Al levels was found after washing (Boonpeng et al., 2021). After comparing the washed and unwashed samples of lichens that were exposed to atmospheric lead (Pb) fallout in the northeastern United States, Lawrey and Hale (1981) concluded that the lichens not only trapped Pb in particulate forms, but also actively broke down these particles, enhancing intracellular absorption. Furthermore, Branquinho et al. (1999) found that a copper (Cu) mine in Portugal was a major source of dust after measuring higher concentrations of Cu in the particulate fraction in lichens sampled nearest to the mine in comparison with the extracellular and intracellular fractions. Overall, it is evident that there is a value in trying to distinguish between the particulate versus absorbed fractions of elements in lichens by comparing washed and unwashed samples.

In Nova Scotia, Canada, historic gold mining operations from the 1860s to the mid-1940s resulted in the operation and abandonment of over 360 mines located with 64 formal gold mining districts across the province (Malcolm, 1929). With 1.2 million troy ounces of gold produced over this time also came three million tonnes of mining waste (tailings) deposited at and surrounding these mine sites in both terrestrial and aquatic ecosystems (Parsons et al., 2012). The tailings are contaminated with mercury (Hg) and As due to losses from the Hg amalgamation processing that was used to extract gold, and the presence of arsenopyrite (AsFeS) in milled gold-bearing ore, respectively (Trip & Skilton, 1985; Bates, 1987; Parsons et al., 2012). Elemental Hg (Hg⁰) was used for Hg amalgamation at these Nova Scotian gold mines, which, once lost to the mill tailings or atmosphere, can be oxidized to form divalent Hg (Hg²⁺), allowing for transformations

into organic forms in aquatic systems, such as methylmercury (MeHg) (Kim, 2005; Parsons & Percival, 2005).

Today, the historical gold mine tailings deposits in Nova Scotia remain untreated, many of which are exposed at the land surface. As a result, these deposits are potential sources of Hg to the atmosphere either in particulate-bound forms or from the volatilization of gaseous Hg⁰. Assessing dust and the movement of Hg throughout these mine tailing sites has become increasingly more important with changing climatic conditions that are enhancing dust emissions (Csavina et al., 2012; Bush & Lemmen, 2019). In addition, increasing land use activity at and surrounding many of these sites (e.g. residential developments, recreational activities) may result in more people coming into closer and prolonged contact with these mine wastes. Thus, this study sought to assess the atmospheric contamination originating from these legacy mine sites by using *in situ* epiphytic lichens as dust and air quality biomonitors. The fruticose *Usnea* and the foliose *Platismatia* were sampled at the large Montague gold mine tailings site in Nova Scotia, Canada. The objectives of this study were (1) to determine an estimate of intracellular versus extracellular concentrations of total Hg (THg) in the lichen biomonitors using washed and unwashed samples as well as the wash-water collected from the washing procedure, (2) to assess the different levels of dust (containing Hg) on lichens collected from various distances to the tailings, and (3) to identify adequate lichen sampling, processing, and washing procedures for improved methodologies in future lichen biomonitoring work.

2.0 Methods

2.1 Study Site Description

This study was conducted at the Montague Gold District (44.71541, -63.52332) near Dartmouth, Nova Scotia (Figure 2) (Malcolm, 1929; Bates, 1987). Montague was one of Nova Scotia's most productive former gold mines, producing 65,196.9 troy ounces of gold between 1862 and 1940 (Malcolm, 1929; Trip & Skilton, 1985; Bates, 1987). To extract this amount of gold, approximately 134,278 tonnes of ore were crushed over this time at Montague. The resulting Hg- and As-contaminated tailings were deposited into surrounding terrestrial and aquatic environments, primarily into the Mitchell Brook stream. This stream starts upstream at Loon Lake, extends through the Montague tailings site, and empties downstream into Lake Charles, adjoining multiple wetlands along its course (Figure 2A). Parts of the tailings are entirely bare, exposing the dry and poorly consolidated material to wind action. Other areas across the tailings deposits are only sparsely vegetated with small shrubs and grasses, with some tailings overlapping wetland communities and submerged in water.

2.2 Sample Collection

A total of four sampling grids were delineated over the study area using geographic information systems (GIS), ESRI's ArcGIS (ArcMap 10.7) (Figure 2A). The largest grid, Grid 1, spanned 800,000 m² (1000 x 800 m) and covered the main tailings field at Montague. The remaining three grids were 60,000 m² (300 x 200 m) in area and placed in the direction of the prevailing winds (southerly; Grid 2), near a smaller gold mine tailings deposit (Grid 3), and near a previously used reference site located upstream of the main tailings deposit (Grid 4). There were a total of 138 lichen sampling locations

covering the study area, each of which being at the grid intersection points that are located 100 m apart.

The latitude and longitude of each grid intersection point was extracted remotely in ArcMap 10.7 and a handheld global positioning system (GPS) was used to locate each collection site in the field. During July and August of 2019, both *Usnea* and *Platismatia* spp. were collected at each site from any sides of living or dead, standing or fallen spruce (*Picea*) tree trunks or branches and at heights of approximately 1-2 m above the ground. When possible, lichens were sampled from only one tree at the predetermined sampling site, however, a 25-m sampling radius was extended around each geographically referenced point to allow for sample collection when lichens were sparse, trees were absent, or sites were inaccessible. In such cases, sample collection was only extended until sufficient biomass (~3 g) of lichen were collected. The sampling site was omitted if the entire 25-m sampling radius surrounding the grid point was inaccessible or such area hosted no trees or lichens.

Lichens were collected from trees wearing powder-free nitrile gloves, with all collected thalli from the same genera at a site grouped into one composite sample and placed in a labelled, re-sealable food-grade plastic bag. The GPS coordinates were recorded where the lichen samples were collected. The lichen samples were then temporarily stored in a cooler for transport from Montague to the laboratory at Saint Mary's University in Halifax, Nova Scotia. In addition, two reference sites were selected for this study, which included Saint Mary's University located in Halifax (44.62854, -63.58156) and Albro Lake in Dartmouth, Nova Scotia (44.69082, -63.57547). One lichen

sample of each lichen genera was collected from these sites following this sampling procedure in October of 2020.

2.3 Sample Preparation

Lichens were stored in a freezer in the laboratory until sample preparation. From each site, a small subsample of lichen was separated from each composite sample and used for identification to the species level. This was conducted with a dissecting microscope along with the use of dichotomous keys (Hinds & Hinds, 2007; Brodo, 2016) and chemical tests, such as spot tests and ultra-violet light. For the chemical spot tests, 10% potassium hydroxide, household bleach, and para-phenylenediamine were used.

Following identification, remaining sample preparation was conducted in a clean room laboratory at Saint Mary's University. For each site, the lichen sample was separated by hand from as much extraneous material (e.g. bark, twigs, leaves, invertebrates, etc.) as possible. External (on-thallus) and internal (in-thallus) Hg loads of lichen thalli were then distinguished by splitting each sample into two subsamples of approximate equal mass and leaving one half unwashed and washing the other half with reverse osmosis (RO) water.

The lichen subsample reserved for the washing procedure was transferred to an acid-washed 1 L beaker. The beaker was filled with approximately 200 mL of RO water, where the lichen was then submerged and stirred by hand. Subsamples of the first wash-water rinse from the two most common species of each lichen genus (*Usnea filipendula*, n=54; *Platismatia glauca*, n=72) were collected in order to quantify the amounts of water-soluble, non-bound Hg on the surface of lichen thalli. To determine THg concentrations removed from thalli surfaces, 15 mL of the first wash-water was sampled

by syringe and added to a labelled 15 mL Falcon tube, along with 1% (150 μ L) nitric acid (trace level grade) for preservation. To determine dissolved Hg (DHg) concentrations washed off from thalli surfaces, 15 mL of the first wash-water was sampled by syringe, where a 0.45 μ m syringe filter was then attached and the water dispersed through the filter and into a labelled 15 mL Falcon tube, along with 1% (150 μ L) nitric acid (trace level grade) for preservation. Water samples were stored upright and in the dark at room temperature until analyses.

After the first wash-water samples were collected, the remaining wash-water in the beaker was decanted, and the same washing procedure was repeated for two more washes. The water from the second and third wash were discarded and not kept for analyses. Once washed, each washed lichen sample was placed in a labelled plastic weighing tray and covered loosely with Parafilm to air dry at room temperature for 48 hours. A diagram outlining the workflow and sample numbers for the lichen washing procedures and wash-water collections is shown in Figure 3.

Once air-dried, each washed and unwashed lichen sample was homogenized to a powder in jars using a Retsch mixer mill MM 400 at Saint Mary's University. The jars were thoroughly cleaned in a Citranox and RO water solution between milling of each individual sample. Once individually homogenized, each lichen sample was transferred to a labelled 50 mL Falcon tube and stored in a dark and dry environment (i.e. storage bins) until analysis. Powder-free nitrile gloves were worn while separating, washing, and milling of each lichen sample, being changed between samples.

2.4 Mercury Analyses

The washed and unwashed lichen samples were analyzed for THg concentrations, and the unfiltered and filtered wash-water samples for THg and DHg concentrations, respectively, in acid-washed quartz boats using a Milestone Direct Mercury Analyzer (DMA) 80.3 at Saint Mary's University. The weight or volume of each sample was measured in the boats using a microbalance and then recorded into the DMA software. During each analytical run, Hg standards of varying concentrations and certified reference materials (CRMs) were processed to validate proper calibration of the instrument. The CRMs used with the lichen samples were TORT-3, DORM-4, and BCR-482 Lichen (Willie et al., 2012, 2013; European Commission, 2021). An in-house standard reference material (SRM), a homogenized *Usnea* sample from a previous field season, was also prepared for comparison to the lichen samples and to ensure consistency within and between runs. For the wash-water samples, along with the Hg standards, quality control included a spiked and unspiked matrix and a spiked and unspiked RO water sample. The spiked matrix included lichen wash-water from two unused lichen samples collected from Montague, which were combined to increase the volume for use in all runs as well as to ensure detection of Hg above Method Detection Limits (MDLs). Blank boats were run at the start and end of each analytical run, as well as before and after each set of quality control samples. Within runs, blank boats and duplicate samples were run every 10 and 20 samples, respectively.

2.5 Data Analyses

The Hg datasets for the lichens were first classified to aid in identifying any spatial patterns in subsequent analyses. In ArcMap 10.7, buffer polygons of various

distances (e.g. 100 m, 200 m, 300 m) were created around the tailings layer at Montague using the buffer tool (Figure 4). Lichen sampling locations within each buffer were then classified into that distance category, denoting their approximate distance to the nearest tailings edge (e.g. on tailings, 0-100 m, 100-200 m, 200-300 m, and >300 m). In addition, forest polygon layers from the Nova Scotia Forest Inventory were used to classify the lichen sampling sites into their land type, which included lichens growing on trees near roads, urban areas, wind throws, natural forest stands, treed bogs, and wetlands (Province of Nova Scotia, 2017).

Descriptive statistics for the Hg concentrations within the washed and unwashed groups for each lichen genera were calculated. The difference between the THg concentrations in each paired washed and unwashed lichen sample was also calculated, which represented the extracellular (surface-deposited) fraction of Hg on thalli. In contrast, the THg concentrations measured in the washed samples represented the intracellular (absorbed) Hg fraction.

After determining non-normal distributions of model residuals and/or heterogeneity of variance resulting from applying parametric statistical models, non-parametric analyses were performed on the untransformed washed and unwashed lichen datasets. The paired washed and unwashed lichen samples were plotted on a scatterplot with Spearman-rank correlation analysis and linear regression modelling performed to examine the relationships between Hg concentrations. The Mann-Whitney U test was used to detect significant differences between the mean Hg concentrations in the washed and unwashed lichen groupings. All graphs were generated, and statistical analyses performed, in RStudio.

Concentrations of THg and DHg were measured in only a subsample of the collected wash-water samples from the most common *Usnea* (n=16/108) and *Platismatia* species (n=16/144) due to time constraints and the longer analytical processing times for water samples. Of these 16 wash-water samples for each lichen, eight were unfiltered and analyzed for THg, with the remaining eight being filtered samples and analyzed for DHg concentrations (Figure 3). Thus, for the Hg wash-water datasets, statistical analyses were not performed due to small sample sizes as well as the large number of samples measuring below MDLs (0.001ng) (*Usnea*: n=12/16; *Platismatia*: n=8/16).

3.0 Results

3.1 Mercury Concentrations in Washed vs. Unwashed Lichen Samples

A total of 112 *Usnea* and 113 *Platismatia* samples were collected across the study area, constituting ten and two different species, respectively. The actual lichen sampling locations along the sampling grids are shown in Figure 5. During sample collection in the field, some sites required collection of multiple lichen thalli from various parts of a single tree or from multiple nearby trees, to ensure sufficient biomass for analyses. In the field, all lichen thalli from one site were pooled into one composite sample. During sample processing in the lab, the various thalli samples per site were separated into two subsamples for unwashed and washed procedures. However, the various lichen thalli were not fully homogenized before separating into these two groups. There is potential for variation in elemental concentrations between thalli collected from a single tree or nearby trees due to geographic separation (Sensen and Richardson, 2002; Adams and Gottardo, 2012). Therefore, for data analysis and interpretation purposes, the washed

versus unwashed lichen data presented below are only including samples collected from a single tree (*Usnea*: n=62, *Platismatia*: n=69) in order to reduce concentration variability due to spatial variation (i.e. samples collected from multiple trees).

The unwashed lichen samples represent total Hg loads (absorbed and surface deposits), whereas the washed samples generally represent only the Hg absorbed into, or bound onto, the lichen thalli. When all lichens collected from the sampling grids at Montague were collectively grouped together by lichen genera, there were no significant differences between the washed and unwashed Hg concentrations for either lichen (*Usnea*: $Z = 1993$, p-value = 0.72; *Platismatia*: $Z = 2578$, p-value = 0.60). *Usnea* collected from only one tree across the study area had mean THg concentrations of $152 \pm 46.8 \mu\text{g/kg}$ and $152 \pm 54.9 \mu\text{g/kg}$ in unwashed and washed samples, respectively. *Platismatia* collected from one tree at Montague had lower THg concentrations on average, with a mean concentration of $110 \pm 29.1 \mu\text{g/kg}$ for unwashed and $111 \pm 45.1 \mu\text{g/kg}$ for washed samples. When the data were grouped based on the distance to the nearest tailings edge, there were no significant differences between average washed and unwashed THg concentrations within any of the five distance categories for *Usnea* or *Platismatia* (p-values > 0.05).

Where sampled at the same site, the THg concentrations in the paired unwashed and washed lichen samples showed very similar concentrations, as indicated by the slopes of the linear relationships (*Usnea*: 1.03; *Platismatia*: 0.77; Figure 6). In Figure 6, samples that fall below the reference line with a 1:1 slope (black line) indicate that the unwashed subsample had higher THg concentrations than the corresponding washed subsample (denoted below by positive differences). Thus, samples that showed up at

greater distances from this line were those in which washing displaced greater concentrations of Hg from thalli. Data points above this reference line indicate samples where the washed subsample measured higher in THg than the paired unwashed subsample (denoted below by negative differences). Five unused wash-water samples were analyzed to assess Hg levels in them prior to being used in the washing procedure, all measuring below MDLs for Hg and indicating that Hg was not introduced to the lichens during this washing step.

Most of the *Usnea* samples with the highest THg concentrations that fall below the reference line with a 1:1 slope (black line) in Figure 6A were from sites located on the tailings. Points furthest from this line, and thus with the greatest positive difference in Hg concentration between the paired unwashed and washed samples, were from sites located in wetland or treed bog habitats on the boundaries of the main tailings deposit (+70.4 $\mu\text{g}/\text{kg}$, +48.9 $\mu\text{g}/\text{kg}$) and at the Old Stamp Mill (OSM) tailings deposit in the southeast corner of Grid 1 (+48.6 $\mu\text{g}/\text{kg}$) (Figure 2). These samples had the highest levels of Hg on the lichen surface, ranging from 24-29% of the total Hg load. The majority of other samples with positive differences had less than 13% of the total Hg being washed off the surface, indicating that most was absorbed into lichen thalli. Sites with the smallest positive differences in Hg concentrations between the unwashed and washed samples were located 100-200 m (+0.90 $\mu\text{g}/\text{kg}$, +2.50 $\mu\text{g}/\text{kg}$) and 200-300 m (+2.70 $\mu\text{g}/\text{kg}$) from the tailings edge and the amount washed off was less than 3% of the total Hg load. However, for many sites (n=35/62), higher concentrations of THg were observed in the washed subsample. These greatest negative differences were observed from samples collected on tailings near OSM (-76.2 $\mu\text{g}/\text{kg}$), and at sites located within 100 m of the

nearest tailings edge from natural forest stands and wind throws (-46.8 $\mu\text{g}/\text{kg}$, -42.8 $\mu\text{g}/\text{kg}$).

For *Platismatia*, the slope of the regression line was less and indicates that there was slightly more Hg being displaced by the washing procedure relative to *Usnea* (Figure 6B). As in *Usnea*, the unwashed *Platismatia* samples with highest Hg content were below the reference line with a 1:1 slope (black line) in Figure 6B and were from sites located on the tailings. The sites furthest from this line showing the greatest positive differences in Hg concentrations between the paired unwashed and washed samples were located at a treed bog near the tailings at OSM (+71.3 $\mu\text{g}/\text{kg}$), at the main deposit in a natural forest stand (+66.8 $\mu\text{g}/\text{kg}$), followed by at a wind throw site >300 m from the nearest tailings edge (+33.3 $\mu\text{g}/\text{kg}$). These large differences between corresponding samples represented 26-39% of the total Hg load on the surface. In the majority of other samples, less than 16% of the total Hg was washed off the surface, indicating that most Hg was absorbed into lichen thalli. The smallest positive differences between the THg in unwashed and washed *Platismatia* samples were from sites in natural forest stands or wind throws and located within 0-100 m (+0.60 $\mu\text{g}/\text{kg}$) and >300m from the tailings edge (+0.51 $\mu\text{g}/\text{kg}$, +0.39 $\mu\text{g}/\text{kg}$), which indicated that less than 1% of the total Hg load was removable by washing. As in *Usnea*, some washed *Platismatia* subsamples had higher THg levels than the unwashed subsample (n=28/69). This is indicated by the data points being above the reference line with a 1:1 slope (black line) in Figure 6B. Of these *Platismatia* samples, the greatest negative differences were reported from a wetland site located on the tailings (-105 $\mu\text{g}/\text{kg}$), as well as in natural forest stands located within 0-100 m (-24.7 $\mu\text{g}/\text{kg}$) and 100-200 m of the tailings edge (-21.8 $\mu\text{g}/\text{kg}$).

3.2 Mercury Concentrations in Lichen Wash-water Samples

Concentrations of THg and DHg were measured in a subsample of the collected wash-water samples from the most common *Usnea* (n=16/108) and *Platismatia* species (n=16/144). For DHg, all *Usnea* wash-water samples from both Grids 1 and 2 measured below MDLs (n=8/8). Similarly, for *Platismatia*, only wash-water samples collected from lichens in Grid 1 had DHg concentrations (n=2/5), measuring 0.005 and 0.022 µg/L. For THg, at sampling sites located on tailings or within 100m of the nearest tailings edge (Grid 1), higher THg concentration ranges were measured in wash-water in *Platismatia* (0.011-0.330 µg/L) compared to *Usnea* (0.004-0.009 µg/L) (Table 1). For sites located >300 m from the nearest tailings edge (Grid 2), most THg concentrations in wash-water samples measured below MDLs, but the highest THg concentration in both *Usnea* and *Platismatia* wash-water was found to be 0.005 µg/L (Table 1).

4.0 Discussion

The similar THg concentrations between unwashed and washed lichen samples indicated that most of the THg measured was not easily leached and was absorbed or extracellularly bound to lichen thalli. This was further supported by the fact that many of the wash-water samples had levels of Hg below MDLs. Similar results were found in a large-scale lichen biomonitoring study investigating atmospheric Hg pollution in various provinces of South Africa (Panichev et al., 2019). In the present study, removable dust appeared to be contributing some Hg to the lichens, particularly closer to the tailings, however the contribution to the THg load was generally low overall (<16%), and thus not being a major influence. This also suggests that the lichens are actively taking in Hg

through the absorption of gases or ions, and to a lesser extent, from the breakdown of particles.

This finding is in agreement with the fact that, globally, the predominant form of Hg in the atmosphere is Hg^0 (>95%), and is the main form deposited on lichens as opposed to Hg compounds or particulate-bound Hg, both of which account for <5% of the total airborne Hg (Ferrara et al., 1988; Vannini et al., 2014; Bargagli, 2016; Huang et al., 2020). Mercury in this vapour form can absorb more and rapidly into lichen thalli in comparison to Hg that is bound to particles (Ferrara et al., 1988). Based on experiments with vascular plants (Gaggi et al., 1991) and field studies with lichens (Krishna et al., 2003), it has been hypothesized that lichens can convert (oxidize) this deposited Hg^0 into the water-soluble Hg^{2+} , with the help of the organic acids that lichens produce (Bargagli, 2016; Carvalho, 2017, unpublished). Additionally, acid precipitation can contribute to the oxidation of Hg^0 within lichens (Carvalho, 2017, unpublished). Zvěřina et al. (2014) measured low levels of Hg^0 in lichens relative to the THg load, supporting evidence for an oxidation process transforming Hg^0 to Hg^{2+} . Elemental Hg can also be converted to Hg^{2+} in the atmosphere and deposited onto lichens from precipitation (Krishna et al., 2003).

In the current study, the results suggest that the lichens may be oxidizing intercepted Hg^0 to the ionic form of Hg^{2+} of which is not volatile and has a low mobility. This is because Hg is a Class B metal, exhibiting strong binding preferences to sulphur and nitrogen binding sites that are present in proteins and enzymes in biological molecules and typically becoming irreversibly bound (Nieboer and Richardson, 1980; Pisani et al., 2011). Overall, this property of Hg in combination with the fact that Hg^0 is

the most abundant form of Hg in the atmosphere explains why most of the Hg in our lichens was found to be absorbed and could not be eluted by the washing process. This suggests that Hg volatilization, a process releasing Hg⁰, is likely contributing largely to the atmospheric Hg load at these tailings sites.

Numerous studies have demonstrated the strong binding affinities of Hg. For example, in a laboratory experiment, Vannini et al. (2014) found that lichens exposed to Hg⁰ concentrations showed increasing Hg concentrations over time, however, once the Hg⁰ was removed from the air, Hg in thalli remained. Similar results were found in the moss, *Sphagnum girgensohnii*, in which negligible losses of Hg from evaporation or leaching were observed four weeks after being exposed to Hg⁰ (Lodenius et al., 2003). These works concluded that in lichens and mosses alike, Hg binds strongly and are not easily released back to the atmosphere or removed by precipitation. The results of the present study are consistent with these observations.

As expected, for both *Usnea* and *Platismatia* spp., samples collected closer to the tailings generally had the highest percentages of Hg as dust on their surfaces, and subsequently, higher concentrations of Hg in the wash-water samples. This result was expected because larger contributions of contaminants in particulate forms would originate from closer to this pollution source, which may then deposit on thalli surfaces (Otnyukova, 2007). These deposits on lichen thalli can be more easily washed out than those located intracellularly (Boonpeng et al., 2021). Thus, in the current study, we suggest that the tailings at Montague are the source of this surface-deposited Hg to lichen thalli, most likely originating from windblown tailings and with contributions diminishing with greater distances from the tailings.

For lichen samples where the unwashed measured higher Hg concentrations than the washed, slightly more THg was displaced from the surface of *Platismatia* relative to *Usnea*, which may be explained by the different properties of foliose and fruticose lichens. *Usnea* is a fruticose lichen, which has a three-dimensional, open branching structure, providing a high surface area per unit biomass. Although this open structure allows more air to flow through and to capture airborne particles (St. Clair et al., 2002a, b), this wider surface area allows for more gaseous exchange of Hg⁰ (Monaci et al., 2012). In turn, more Hg can be absorbed or tightly bound to these fruticose thalli.

Also, Bosserman and Hagner (1981) found that the fruticose *Usnea*, being capable of rapid water uptake, was more influenced by wet fall when found to contain higher concentrations of elements from precipitation as compared to the foliose *Parmelia*. The water-soluble Hg²⁺ is the predominant form of Hg in precipitation (O'Driscoll et al., 2003), so more of this Hg likely binds to *Usnea* once deposited from precipitation, owing to its high affinity for binding sites (Nieboer and Richardson, 1980). Foliose lichens on the other hand, like *Platismatia*, have a more continuous and flatter surface area, with its elemental contents suggested to be more influenced by dust fall, such as from the accumulation of particles (Bosserman and Hagner, 1981; St. Clair et al., 2002a). Therefore, *Platismatia* is likely capturing more particles containing Hg that would not bind as rapidly and strongly to thalli as compared to the higher proportions of Hg being incorporated in *Usnea* thalli by ion exchange.

It is important to note that although THg concentrations were generally similar between washed and unwashed lichen samples, some washed samples did measure higher THg than the paired unwashed sample (Figure 6). Similar results were also found for

some lichen samples in other biomonitoring studies with similar washing procedures, such as for Hg (Panichev et al., 2019) and other elements (Saiki et al., 1997; Gombert et al., 2003). In our study, this was likely due to, in part, the sample processing procedures where the individual lichen specimens collected from one site were only mixed broadly and not fully homogenized into one composite sample before separating into the washed and unwashed subsets.

Lichen samples collected from within a site or even from a single tree can differ in elemental contents (Sensen and Richardson, 2002). This is primarily due to varying exposure conditions from different aspects on the trunk, which can affect element accumulation processes in lichens. For example, some thalli may be more exposed to direct rainfall or stemflow running down the trunk which can affect within stand elemental levels in lichens (Ayrault et al., 2007; Asplund et al., 2015; Gauslaa et al., 2020; Loppi et al., 2021). Similarly, different light and moisture gradients exist from the forest floor and up to the canopy (Fanning et al., 2007). Some lichen thalli may be exposed to more direct light and solar radiation which may result in greater amounts of Hg being released to the atmosphere due to photochemical reactions (Saunders, 2017, unpublished). In contrast, more shaded lichens on a tree may be saturated for longer periods of time, and since uptake of ions occurs when wet, this may result in higher element concentrations in these lichens (Hauck, 2011; Adams and Gottardo, 2012). The orientation around the tree trunk may also expose certain lichens to greater wind velocities which may provide greater particulate loadings (Puckett and Finegan, 1980; Loppi et al., 1999). We observed the effect of these varying exposure conditions on the Hg concentrations in our lichen samples collected from a single site (Figure 6). This, in

combination with not fully homogenizing our composite samples, explains the higher THg concentrations in some of the washed lichen samples over the unwashed samples. In addition, lichens may accumulate elements by storing higher concentrations in older parts (inner zones) of the thallus (Ayrault et al., 2007). When splitting the lichen samples into two subsets, some lichen subsamples may have contained more of the inner zones of thalli that potentially have higher concentrations of elements (Adams and Gottardo, 2012).

These findings highlight the importance of sampling and combining specimens into composite samples when conducting lichen biomonitoring studies. It is important to minimize or eliminate within site elemental variation to allow for stronger comparisons of samples between and within sites. This can be accomplished through ensuring nearly identical sampling procedures at each site, which may include sampling from only a single tree, collecting from one or all aspects of the tree and within a defined height range, sampling of only vertically or horizontally exposed thalli, as well as using lichen specimens of similar masses or only analyzing peripheral parts of lichen thalli (Ayrault et al., 2007; Adams and Gottardo, 2012; Boonpeng et al., 2021). Sample processing should also be carried out at one point in time to prevent differences in analytical procedures (Halonen et al., 1993). However, in many areas, lichen abundance may be limited and following consistent sampling procedures may be impractical. In such cases, it is important to record sample site information (e.g. habitat, aspect sampled from, number of trees sampled) and microenvironmental variables (e.g. light exposure, temperature), so that these data can be taken into account when analyzing and comparing the elemental data within and across sites (Fanning et al., 2007).

5.0 Summary and Conclusions

In summary, after splitting our lichen samples and comparing the THg concentrations in the washed and unwashed subsets, most of the Hg measured was found to be absorbed in lichen thalli. These findings were supported by the results from the wash-water samples. It can be concluded that the Hg was bound strongly to thalli, probably in ionic form (Hg^{2+}), with particles only playing a minor role in enriching the lichens with Hg. Thus, with only small amounts of Hg being mobile, precipitation events, for example, may not remove significant quantities of Hg from the lichens and disperse to surrounding environments. However, it should be noted that although precipitation may wash particles and soluble species off and out of lichens, it may also be increasing concentrations of Hg in lichens either by providing more ions (i.e. Hg^{2+}) from the rain itself or by releasing ions from entrapped particles. Yet overall, our results support Hg volatilization as an important and major process in making Hg available in the atmosphere for uptake into lichens at these tailings sites today, as opposed to inputs from dust.

Despite low levels of Hg on the surfaces of the lichens in general, higher levels of Hg were displaced from the washed lichens collected from closer to the tailings deposits compared to those further away. This does suggest that the tailings at Montague are still providing a minor source of Hg to the lichens in the form of dust particles.

Platismatia was found to have slightly greater amounts of Hg displaced from thalli surfaces, whereas *Usnea* had more of the THg load absorbed. Future work assessing atmospheric Hg pollution using lichen biomonitors may therefore benefit from collecting both a foliose and fruticose lichen, which have different morphological forms

and capabilities in accumulating both airborne gaseous and particulate species. This may help to better quantify the contributions of Hg to lichens from different processes occurring across the study area (e.g. Hg from volatilization versus windblown particles).

Some of the washed lichen samples measured higher THg concentrations than the unwashed samples. These were likely effects noticed from within site elemental variation in lichens as well as from the sample processing procedures. Thus, the results of this study can be used to develop appropriate methods for lichen sampling and processing in future biomonitoring work, so to enhance the reliability of the results.

Overall, this study provided insights into the response of lichen biomonitors to Hg pollution. It was demonstrated that this washing method can be used to effectively characterize Hg pollution at contaminated sites. For instance, distinguishing between the particulate and absorbed fractions of Hg in lichens via washing, in combination with the sampling design, helped to confirm atmospheric pollution sources across the study area. These methods also aided in understanding the movement of Hg throughout the study area, including the release from the Montague tailings and uptake into lichens, while also helping to determine the potential of Hg release from lichens and into surrounding environments.

Future lichen biomonitoring work surrounding contaminated sites or pollution sources should continue to separate the target elements into the intracellular and extracellular fractions using a similar washing procedure. This will ensure that the various forms of the target elements are being measured and so additional insights about sources or contaminant mobility/pathways can be obtained. Also, in the current study, only THg concentrations were determined in the washed and unwashed lichen samples.

In future, we recommend investigating the speciation of Hg in lichens which can help to identify pollution sources and assess potential risks to surrounding ecosystems, such as for MeHg and the bioaccumulation in food webs. Furthermore, future biomonitoring research with lichens at similar contaminated sites could include seasonal sampling or lichen transplants. These methods can help to identify when the lichens are receiving more inputs of contaminants from dust particles or gases, for example, as varying meteorological conditions affect the availability and release of elements.

6.0 Chapter 3 Figures

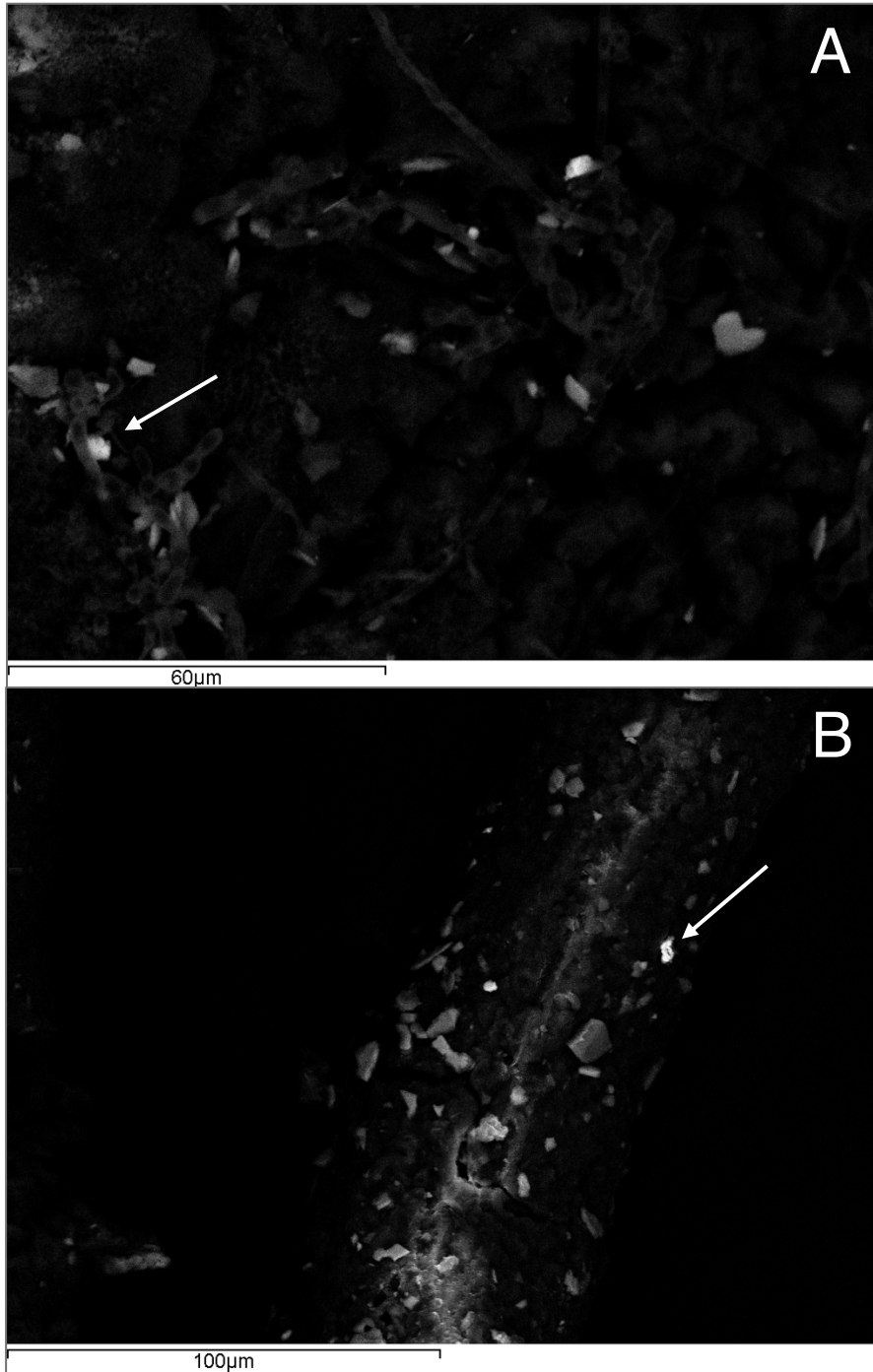


Figure 1. Backscattered electron scans of unwashed *Usnea* branches taken with a scanning electron microscope (SEM). The lichen samples were collected near the main tailings deposit at the Montague Gold District in Nova Scotia. The darker areas indicate organic material while the brighter spots show deposited and trapped particulates. The arrows show trapped particulates containing (A) arsenic and (B) barium and tin.

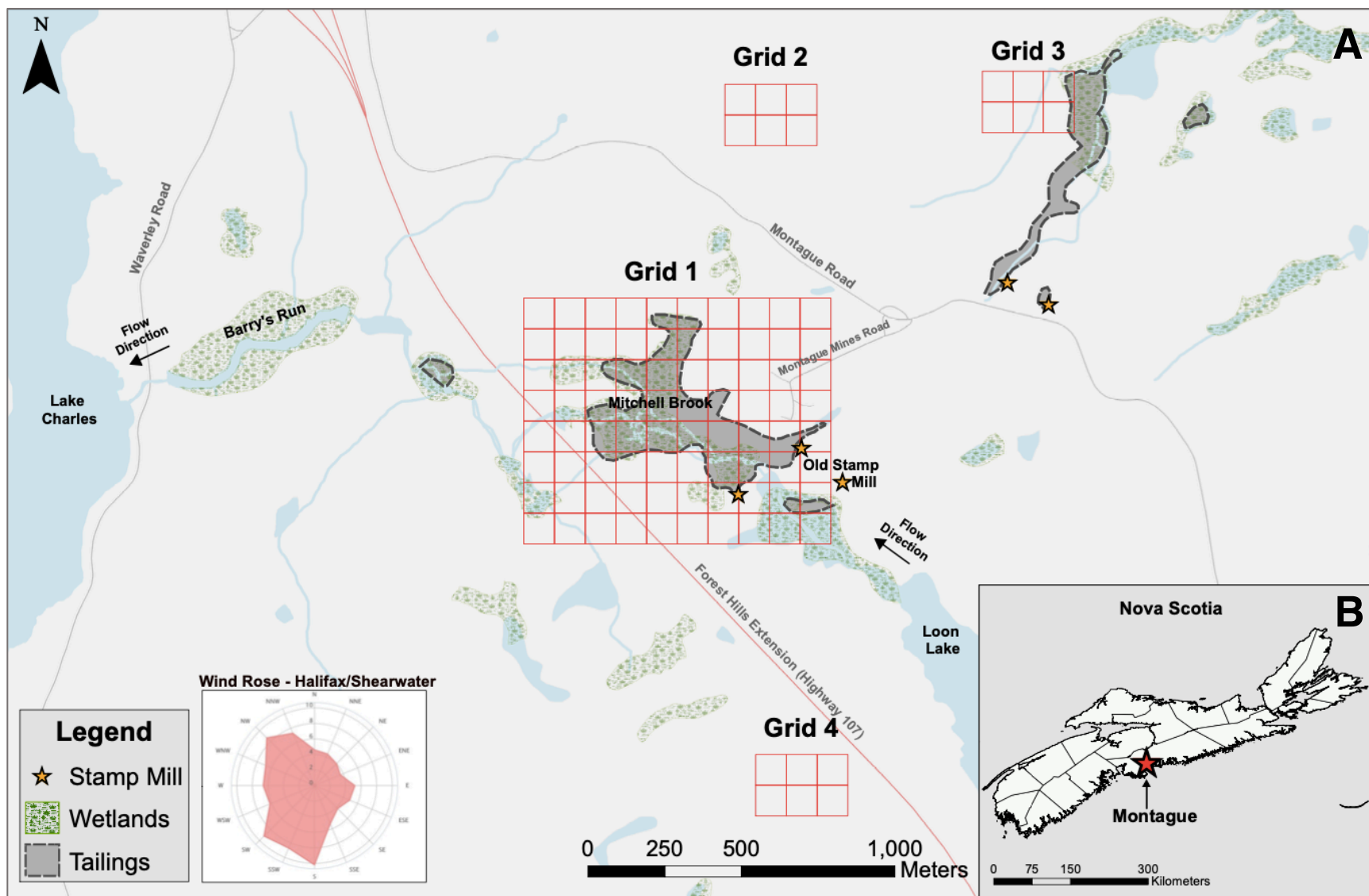


Figure 2. (A) Locations of grids and proposed lichen sampling locations (grid intersections) at the historical Montague gold mine tailings site near Dartmouth, Nova Scotia. (B) The relative location of the Montague gold mine tailings site in Nova Scotia, indicated by a red star.

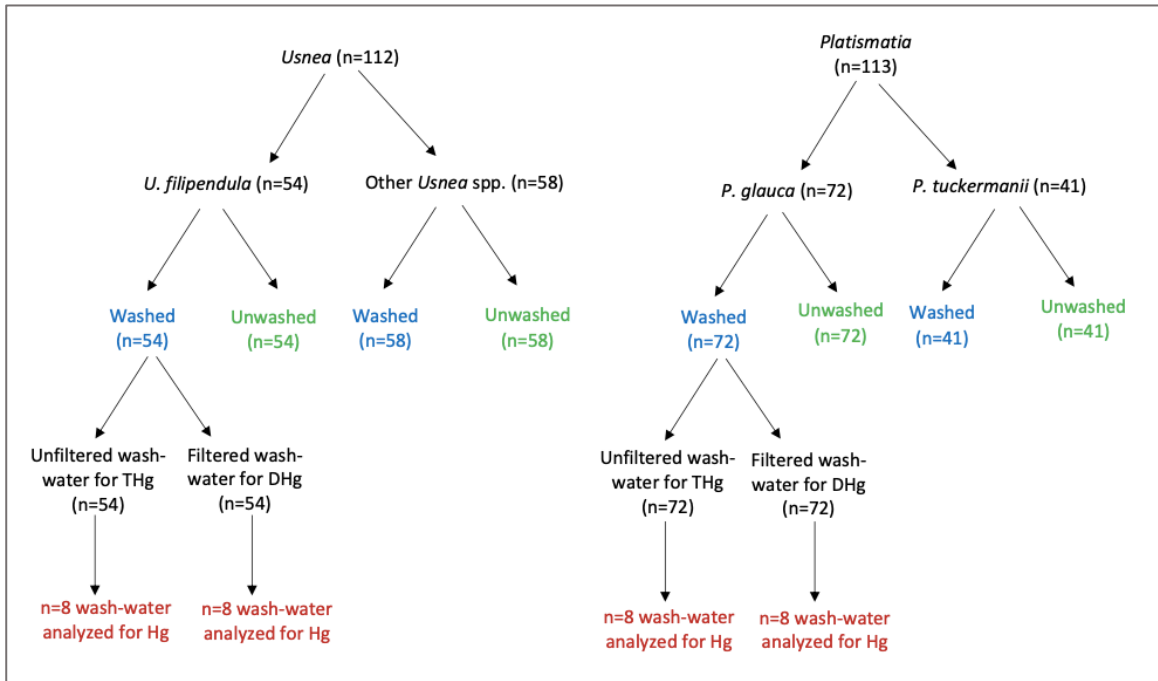


Figure 3. Diagram outlining the workflow and sample numbers for the washing procedures and wash-water collections for both *Usnea* and *Platismatia*.

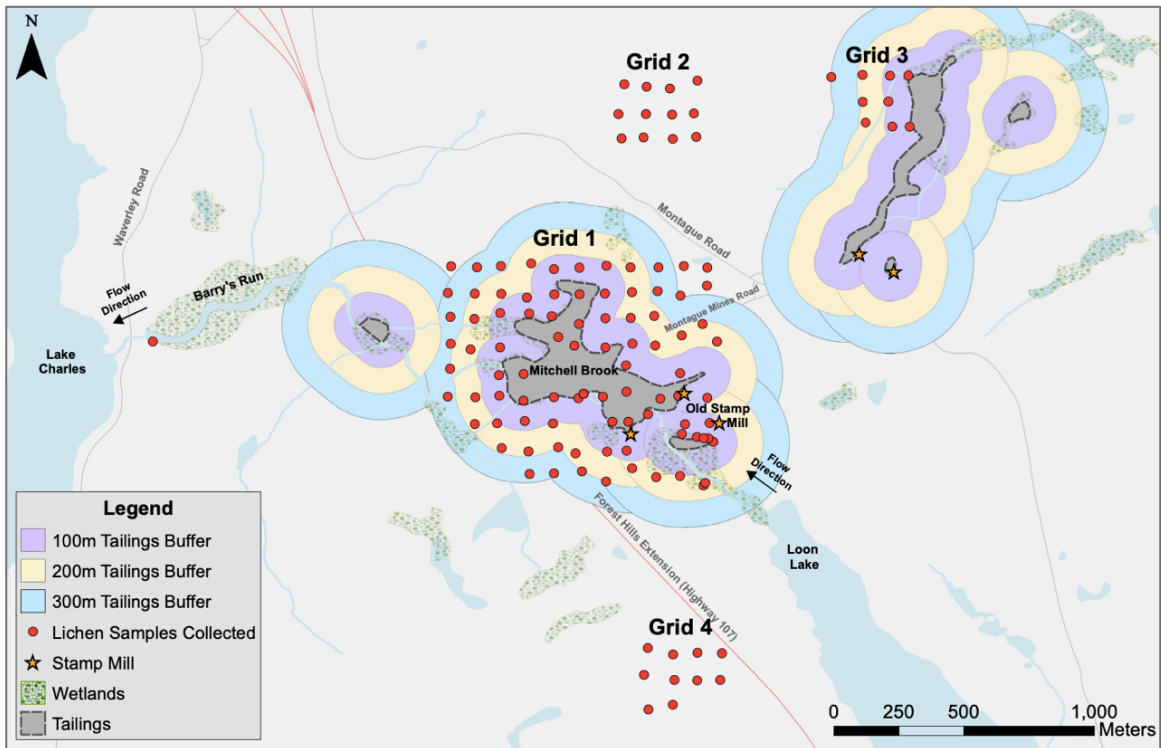


Figure 4. Map of the Montague gold mine tailings site with spatial buffers surrounding the known tailings deposits, used for classifying the lichen sampling sites based on distance to the nearest tailings edge.

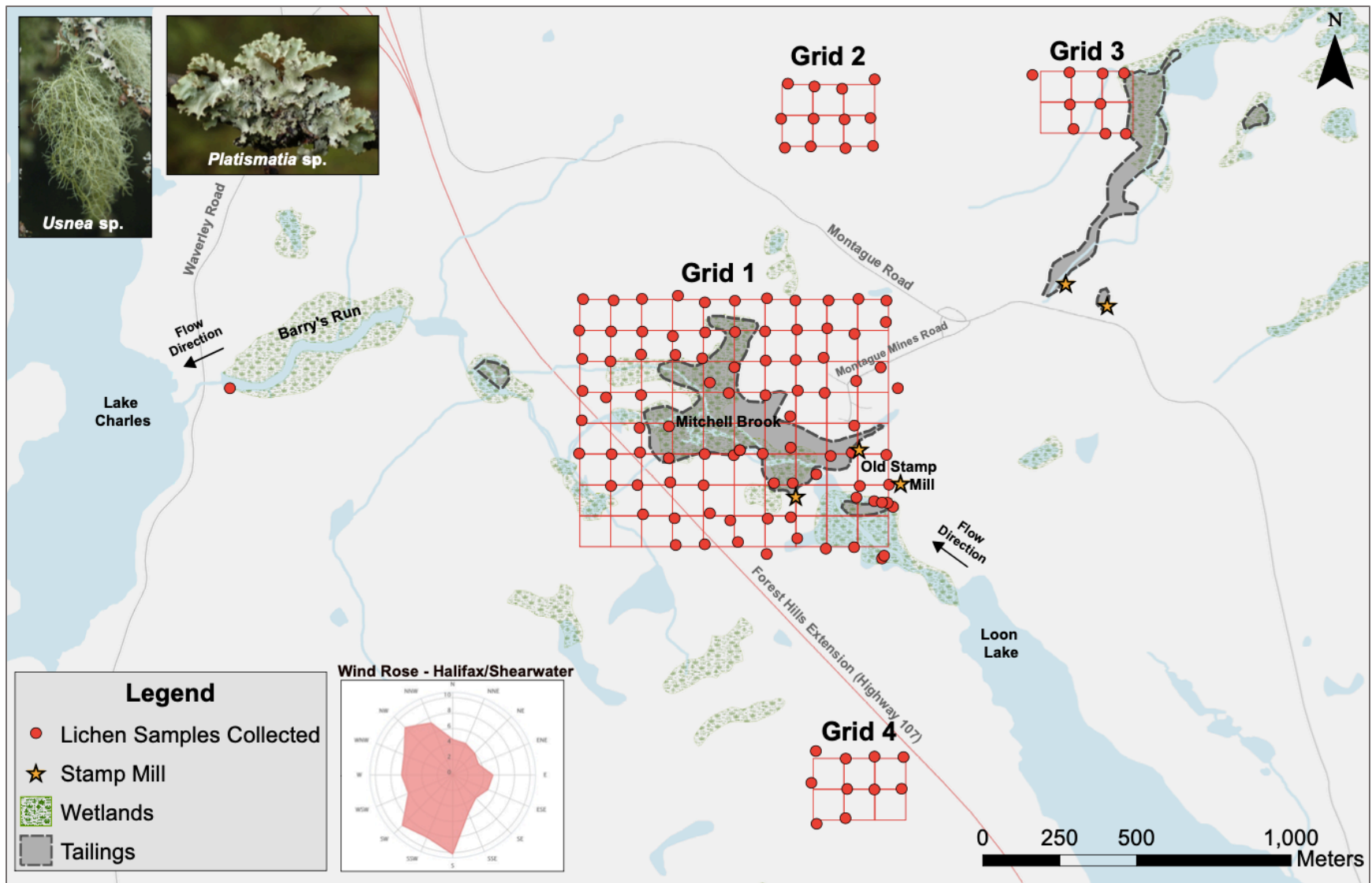


Figure 5. Actual sampling locations of *Usnea* and *Platismatia* in Grids 1, 2, 3, and 4 at the historical Montague gold mine tailings site near Dartmouth, Nova Scotia.

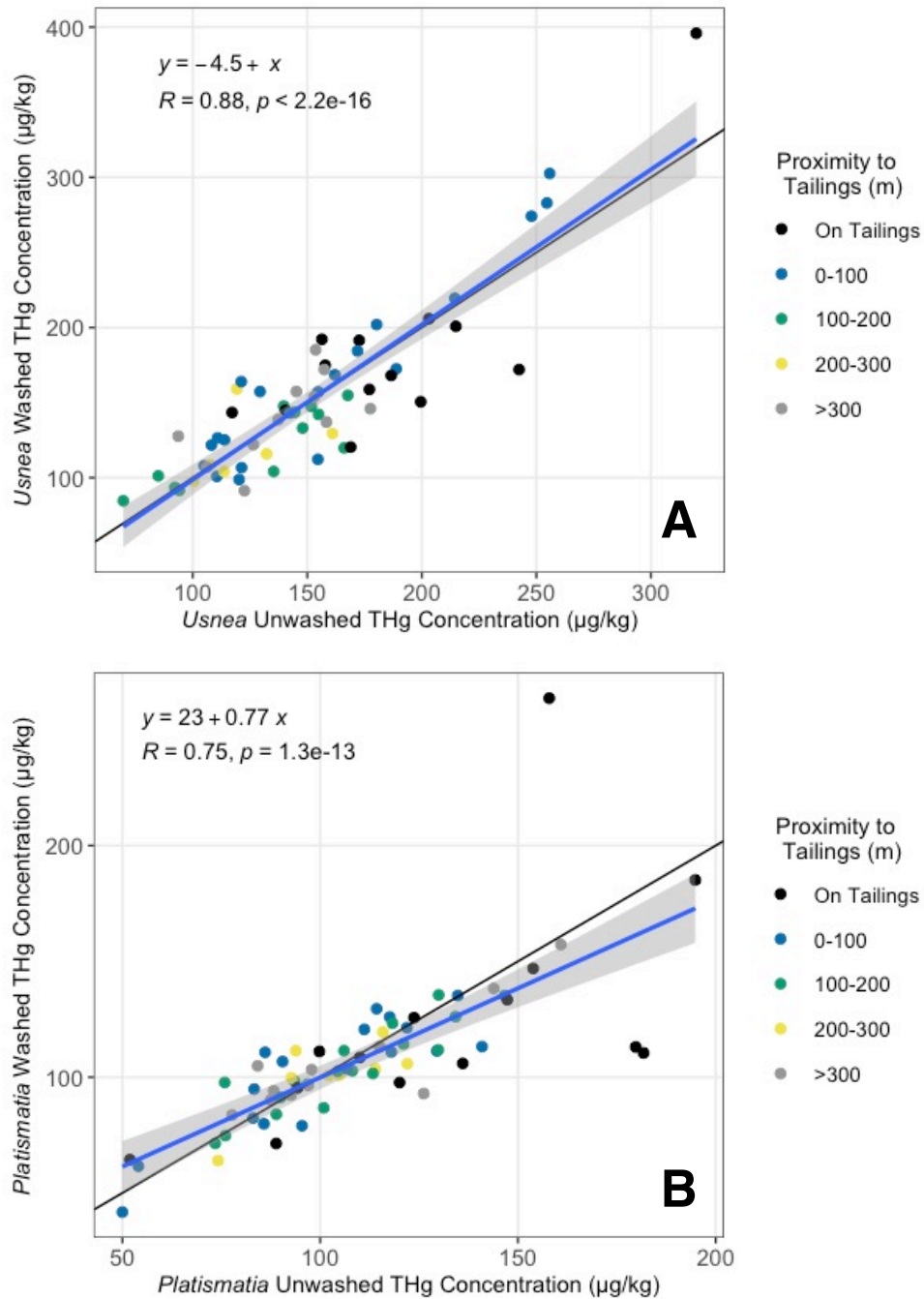


Figure 6. Unwashed versus washed total mercury concentrations ($\mu\text{g}/\text{kg}$) in (A) *Usnea* and (B) *Platismatia* collected from the same sampling site and at sites where lichen thalli were collected from only one tree at the Montague gold mine tailings site. Data points are colour-coded based on their distance to the nearest tailings edge: On tailings, 0-100 m, 100-200 m, 200-300 m, and >300 m. Regression line is plotted in blue and a reference line with a 1:1 slope is plotted in black.

7.0 Chapter 3 Tables

Table 1. Total mercury concentration ranges ($\mu\text{g/L}$) measured in the first wash-water rinse sampled from *Usnea* and *Platismatia* at the Montague gold mine tailings site. Data are summarized based on sampling grid. Range includes those samples that measured above detection only.

Lichen	Sampling Location	n	Number of Non-detects	Range ($\mu\text{g/L}$)
<i>Usnea</i>	Grid 1	5	2	0.0038 - 0.0094
	Grid 2	3	2	0.0054
<i>Platismatia</i>	Grid 1	5	1	0.0110 - 0.3299
	Grid 2	3	1	0.0014 - 0.0054

8.0 Chapter 3 References

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Chapter 4: Summary and Conclusions

1.0 Summary and Conclusions

The Montague Gold District in Nova Scotia contains several mercury (Hg)-contaminated tailings deposits, many of which are exposed at the Earth's surface with sparse vegetation cover. With potential for the remobilization and transport of the tailings into surrounding environments through the air while also posing risks to human and ecosystem health, the spatial distribution of airborne Hg at this site warranted further investigation.

This study used lichens as spatial biomonitors of dust and air quality. In summary, both *Usnea* and *Platismatia* were reliable biomonitors of Hg, showing similar distribution patterns across Montague, and are recommended lichens for future biomonitoring work in Nova Scotia and Atlantic Canada. Interpolation modelling of the Hg data from lichens proved to be a valuable spatial monitoring tool that can be paired with lichen biomonitors in future work, helping to limit sampling, if needed. Main spatial hotspots around this site were on or near certain sections of the tailings deposits, reflecting the higher Hg concentrations in the underlying tailings in such areas, demonstrating the heterogenous nature of these mine wastes. The lichens at the identified hotspots seem to be receiving greater inputs of Hg either directly from windblown tailings and/or volatilization processes, or indirectly from forest canopy interception of Hg and the subsequent release into throughfall or stemflow. Wetland areas were identified as important sources of airborne Hg, despite the tailings being submerged in water. Overall, the results indicate that both the exposed tailings and those overlapping wetland communities are important local sources of atmospheric Hg, with impacts being noticed in lichens up to at least a

few hundred metres from the deposits. This contamination in lichens poses direct risks to wildlife that are in frequent contact with lichens, with the air contamination posing potential risks to human health as well, especially as land development in the vicinity of these sites increases. Therefore, reducing the dispersion of dust should be a priority in future remediation plans for these historic mine sites.

Passive dust samplers and rain catchers were employed across a stream-based transect within Montague. With low concentrations of Hg in the rainwater samples, limited seasonal comparisons were possible and insights into both the soluble and insoluble fractions of dust in wet deposition were not determined. In contrast, the passive dust samplers were effective in determining total and seasonal dust deposition and Hg flux rates at Montague, demonstrating that climatic conditions strongly control the amount and timing of dust dispersal. With a changing climate, dust generation may increase year-round at this tailings site in the future. An important result from this seasonal dust sampling was that, on average, the reference sites accumulated more dust than sites located adjacent to the Montague tailings. Therefore, in future studies, reference sites in more remote areas and less impacted by urban air pollution should be used. Also, from comparison to the lichen biomonitors, it became evident that minor improvements to these sampling methods could be made in future work, including analyzing both the glass fibre filters (GFFs) and the polyurethane foam filters (PUFs), of which have different abilities in capturing elements from dry deposition. In cases where it may be difficult to employ these physical dust samplers for repeated sampling (i.e. in remote areas), *in situ* lichens should be considered for air monitoring, especially when only relative estimates of Hg concentrations are needed. In other cases, the passive dust

samplers can be used as a complementary measurement method to lichen, which would provide a stronger understanding of dust and contaminant mobility.

The washing of the lichen samples allowed for an estimate of absorbed versus surface-deposited Hg loads. Most Hg was found to be absorbed into lichen thalli, owing to this metal's strong binding affinities within biological molecules. This washing procedure helped to confirm pollution sources across Montague, while also providing a stronger understanding of the movement of Hg throughout these tailings sites. It was concluded that low concentrations of Hg may be mobile and released from these lichens in a rain event, for example, likely not being a significant secondary source of metal pollution to the surrounding environments. However, there is still potential for the lichens to transfer Hg throughout the ecosystem, such as from volatilization from thalli as well as from Hg transfer to lichen herbivores and up to higher trophic level species. Improvements to these lichen sampling and processing procedures should be made in future work, such as reducing within-site elemental variation as much as possible and fully homogenizing composite samples. Yet, this washing procedure, in combination with the sampling design and the collection of wash-water samples, are recommended methods for future work with aims of using lichens to characterize Hg pollution at contaminated sites.

In conclusion, this study has demonstrated the air contamination that can originate from abandoned mine sites. With over 10,000 documented abandoned mine sites in Canada (MacKasey, 2001), additional air quality research is needed at other legacy mine sites. In particular, this study has provided baseline data of the concentrations and spatial extent of Hg in lichens at the Montague gold mine tailings site in Nova Scotia. This work

has provided insight into how dust travels and under what environmental conditions (e.g. dry/exposed areas, the importance of ground cover and forested areas, etc.). These results are important for risk management and for informing remediation practices and future development decisions, especially as urbanization increases surrounding these mine sites. This work can also help to inform future mine management practices as implementing climate change adaptation measures will become important for the mining industry moving forward. Finally, it is important to note that in addition to the numerous legacy gold mine tailing sites in the province, Nova Scotia currently has one operating open-pit gold mine with an additional four proposed (St. Barbara Limited, 2019; Anaconda Mining Inc., 2021). Therefore, there is a growing need for monitoring fugitive dust emissions from metal mines and the methods used in this study can provide guidance to the modern-day gold mining industry in the province.

2.0 Chapter 4 References

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