

Examination of Mercury and Arsenic Concentrations in Spruce Tree Cores and Soil
Samples from Historical Gold Mine Tailings Sites in Nova Scotia

by

Brianna L. Bowes

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Approved by the Thesis Supervisor

*(Dr. Nelson O'Driscoll &
Dr. Linda Campbell)*

Date

Approved by the Head or Director of the Department or School

(Dr. Rob Raeside)

Date

Approved by the Chair, Senate Honours Committee

(Dr. Joseph Hayes)

Date

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Abstract

Tree cores and surface soil samples were collected from four historical gold mine tailings sites near the Halifax Regional Municipality (HRM) for mercury (Hg) and arsenic (As) analyses. Five spruce trees (either *Picea glauca* or *Picea mariana*) were cored from each site, and the year of growth estimated from tree ring counts. We found that average surface soil (silt + clay fraction) total Hg and As concentrations from all mine tailings sites exceeded Canadian Health guidelines for acceptable soil Hg (500 µg/kg) and As (12 mg/kg) concentrations in residential areas. We found most tree core samples were below the method detection limit (MDL) for Hg in samples from the Montague, Oldham and Muddy Pond tailings sites (MDL ranging 1.3-4.2 µg/kg), with the notable exception of the Old Stamp Mill site in Montague Mining District. Mercury in tree ring segments for the Old Stamp Mill site ranged from 0.9 to 43.1 µg/kg, with a MDL of 2.0 µg/kg. However, regression analysis for this site did not show a consistent trend between Hg concentration and time between cores. Additionally, using non-parametric Spearman's correlations, no significant correlation ($n = 26$, $p\text{-value} = 0.08$) was found between Hg concentrations in tree bark and Hg in surface soils. Unfortunately, we were not able to measure As in tree cores at the time of this writing due to COVID-19 related delays. The results of this study show that historical gold mine tailings sites in Nova Scotia have soils that are highly contaminated with both Hg and As, and more research is needed to confirm if spruce trees are reliable indicators of mercury bioaccumulation at those sites. This reinforces the need for future studies on the bioaccumulation and effects of both contaminants in living organisms around legacy gold mine tailing sites.

1. Introduction

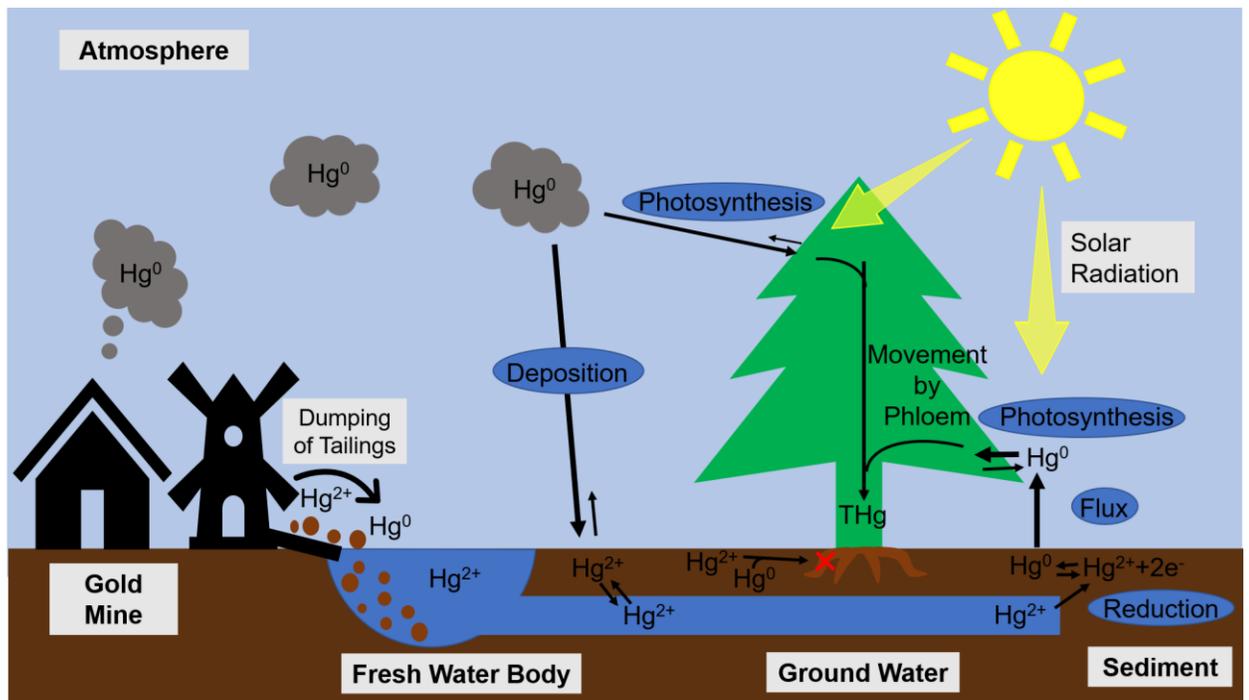
Historically, gold mining was an abundant industry in Nova Scotia over the 19th-20th centuries (Parsons et al. 2012; Doe et al. 2017). Many of the associated mine tailings sites currently remain unreclaimed, with notably high levels of mercury (Hg) and Arsenic (As) contamination that are a known public health risk (Parsons et al. 2012; LeBlanc et al. 2020). However, there is little peer reviewed information on the bioavailability and mobility of contaminants from these sites (Doe et al. 2017; LeBlanc et al. 2020). Trees have been demonstrated to be useful biomonitors for some metals, such as lead (Pb) and zinc (Zn) (Saint-Laurent et al. 2010), and dendro-chemical research regarding trace elements such as Hg and As is an area of growing interest. Elemental mercury (Hg⁰) is a gaseous element and therefore may have different bioaccumulation mechanisms in trees. Several recent studies have shown that uptake by leaf stomata is an important mechanism for gaseous Hg⁰ bioaccumulation in trees (Hojdová et al. 2011; Clackett et al. 2018; Peckham et al. 2019).

Both Hg and As are present in the environment as part of natural surficial geology (Steffen and Morrison, 2016). High concentrations of both elements can be released by both natural and anthropogenic sources (Pannu, et al. 2016; LeBlanc et al. 2020). Both As and Hg exist in the environment in various organic and inorganic forms with different capacities for toxic effects on biota (LeBlanc et al. 2020). Mercury exposure can result in neurotoxic and developmental impacts in animals (O'Driscoll et al. 2005; Bernhoft, 2012) and phytotoxic deficiencies in plants (Azevedo and Rodriguez, 2012). Toxic effects of As include the development of cancers and other chronic health

conditions in animals subjected to long term exposure (Bjørklund et al. 2018) and nutrient uptake and growth deficiencies in affected plants (Smith et. 2010).

The objective of this project is to compare total Hg and As concentrations in segmented tree cores and surface soil samples collected from historical gold mine tailings sites and non-mining sites in Nova Scotia. In addition, this research may help to predict future mobility of Hg and As from historical gold mining sites as annual temperatures increase due to climate change (Wilson et al. 2015).

a)



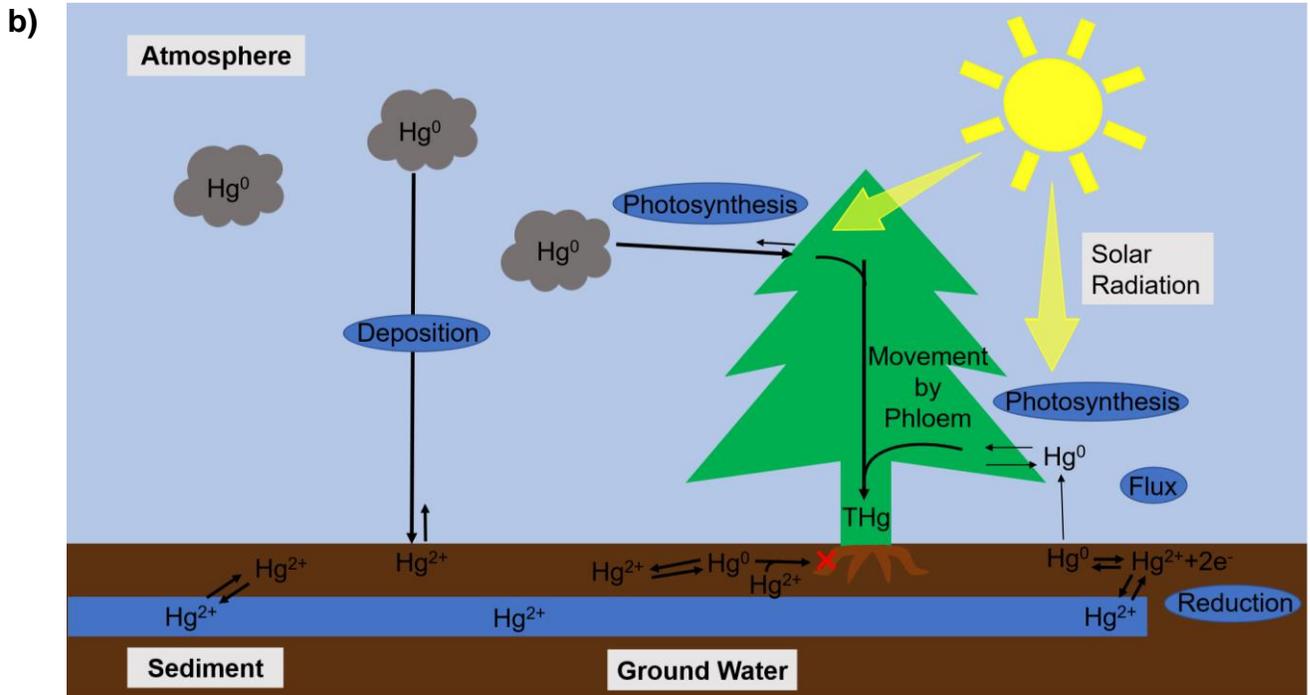


Figure 1: Overview of Hg cycling and uptake by conifer trees in a) a historical gold mine tailings site and, b) an uncontaminated reference site.

1.1. Mercury Speciation

Mercury (Hg) is a highly toxic metal that exists in the environment as both a natural product of geological processes, and an anthropogenic product of human activities (O’Driscoll et al. 2005; Pannu et al. 2016). Mercury is highly mobile and persistent in the atmosphere in its elemental gaseous form (Hg^0), giving it a high capacity for long range transport (Pannu et al. 2016). As a result, the global transport of Hg is common, resulting in its deposition in otherwise pristine environments that are significant distances from the initial site of release (Pannu et al. 2016). Mercury is a global contaminant of concern, resulting in the creation of initiatives such as the United Nations Environment Programme’s Global Mercury Partnership and the Minamata

Convention, in order to address Hg pollution issues on an international scale (O'Driscoll et al. 2005; Pannu et al. 2016; Rimondi et al. 2020b).

Mercury exists in the environment in three main forms (Figure 2); elemental mercury (Hg^0), divalent mercury (Hg^{2+}), and methylmercury (MeHg) (O'Driscoll et al. 2005). Elemental mercury is most commonly found in a gaseous form due to its volatility and low water solubility, making it the most abundant form of Hg in the atmosphere (O'Driscoll et al. 2005). When oxidized, Hg^0 is converted to Hg^{2+} which, due to its higher water solubility and affinity for binding with inorganic particles, is commonly removed from the atmosphere and deposited in aquatic and terrestrial environments (O'Driscoll et al. 2005). Methylmercury is typically considered the most harmful form of Hg to living organisms, due to the compound being highly bioaccumulative and prone to biomagnification in the food-web (O'Driscoll et al. 2005; Doe et al. 2017). Methylmercury is formed when Hg^{2+} in anoxic water or sediment is methylated by anaerobic bacteria, such as sulfate-reducing and iron-reducing bacteria (O'Driscoll et al. 2005). Methylmercury is able to permeate biological membranes including the blood brain barrier, making the compound a potent neurotoxin (O'Driscoll et al. 2005).

Typical background concentrations in of Hg in soils at uncontaminated sites will range between 0.01 to 0.05 mg/kg, although areas enriched by geological stores of Hg can have naturally high concentrations of up to 200 mg/kg (Pannu et al. 2016). Current Canadian soil guidelines recommend Hg concentrations below 6.6 mg/kg for residential and agricultural uses, 24 mg/kg for commercial uses and 50 mg/kg for industrial uses (CCME, 1999; CCME, 2017).

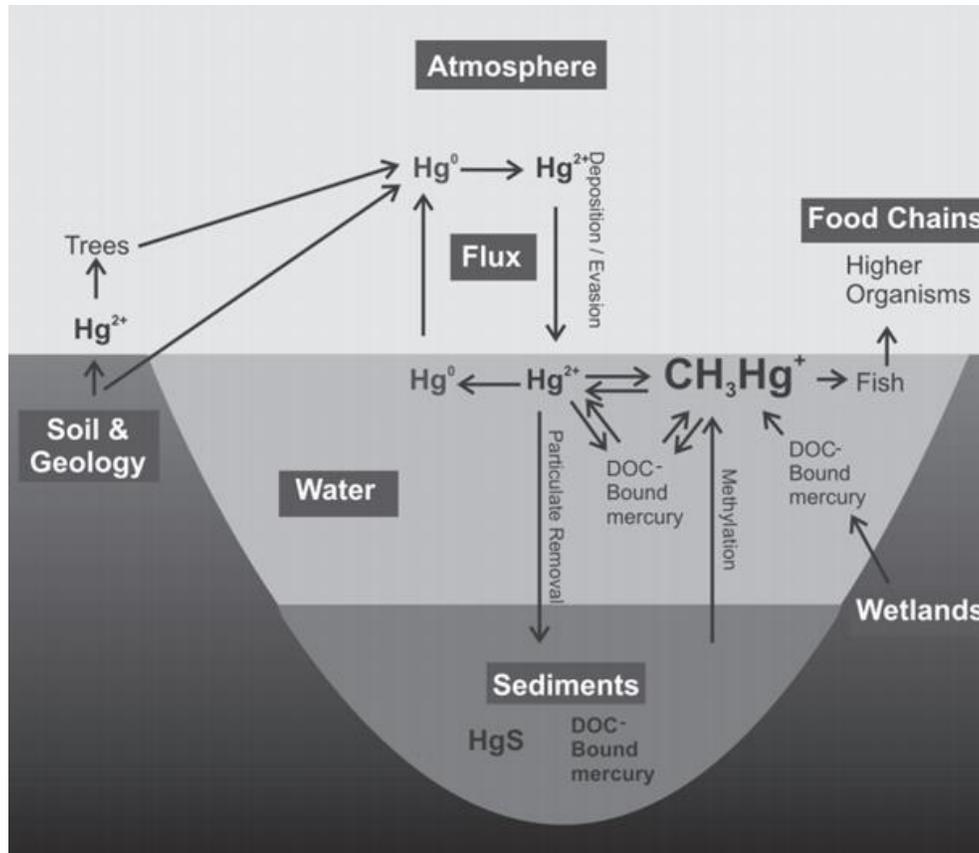


Figure 2: Conceptual diagram of mercury movement and speciation in a freshwater lake environment (taken from O’Driscoll et al. 2005)

1.2. Arsenic Speciation

Arsenic exists in over 50 forms in the environment, originating from natural geological components as well as anthropogenically released gas from smelting and mining activities (Cullen and Reimer, 1989). In Canada, the primary source of anthropogenically released As is mining activities (Wang and Mulligan, 2006). Unlike mercury, it is As’ inorganic forms which have the greatest potential for harm to living organisms and are carcinogenic (Cullen and Reimer, 1989; Leblanc et al. 2020). Arsenate (As(V)) and arsenite (As(III)) are two inorganic forms of As found commonly in association with former gold mining sites (Smith et al. 2010; Beddoe, 2011). However,

all forms of As are considered highly toxic with high potential for harm to human and environmental health; as a result the metalloid is classified as a Group 1 chemical on the Canadian Environmental Protection Act (CEPA)'s Priority Substances List (Wang and Mulligan, 2006).

Similar to Hg, As can be found as a background component of non-contaminated regions within soils, plants and animals. As concentrations in soils from non-contaminated regions, can be as high as 4.8 to 14 mg/kg (Meunier et al. 2011). Canadian soil guidelines state that the highest acceptable total As concentration to occur in residential area soils is 12 mg/kg (CCME, 1999). In plants, a background concentration of approximately 200 µg/kg is considered typical (Cullen and Reimer, 1989).

Plant species affected by arsenic exposure typically exhibit poor seed germination and a reduced capacity for root growth (Smith et al. 2010). Plants can also display secondary toxic effects such as water and nutrient deficiencies due to this reduced root capacity (Smith et al. 2010). Low phosphorus uptake, for example, is often observed in plants studied in areas with high As concentrations, which is particularly concerning due to phosphorus' importance in maintaining metabolic processes (Smith et al. 2010).

1.3. *Historic Gold Mining Industry in Nova Scotia*

Beginning in the mid-1800's and lasting until the mid-1900's, gold mining in Nova Scotia was a thriving industry (Parsons et al. 2012). During this period over 300 individual mines were constructed across the 64 formal gold mining districts established

in the province (Figure 3) (Parsons et al. 2012). In the historic gold removal process, host rocks would be crushed down to sand or silt sized particles before being washed over Hg-coated copper plates, which would form an amalgamation process with any free gold present (Wong et al. 1999; Parsons et al 2012; LeBlanc et al. 2020). The amalgamated Hg and gold would then be boiled to remove most of the Hg⁰ via evaporation. The remaining crushed rock tailings were typically washed directly into local freshwater ecosystems once this process was complete, creating the contaminated tailings sites that are still present in the province (Wong et al. 1999; Parsons et al 2012; LeBlanc et al. 2020).

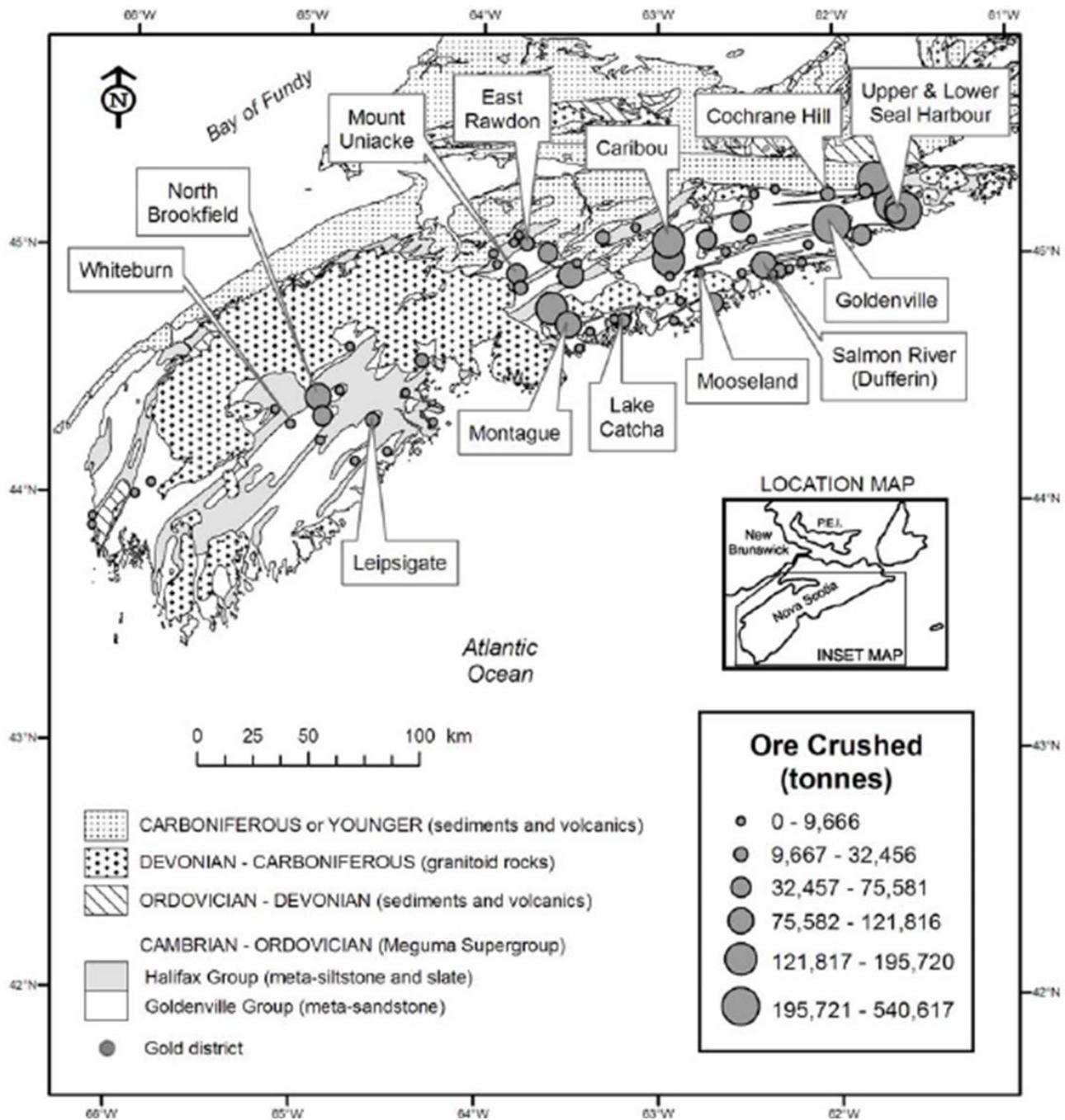


Figure 3: Geological map of Southern Nova Scotia, showing the regions bedrock geological formations and major historical gold mine districts (taken from Parsons et al. 2012)

It is estimated that an average of 10-15% of all total mercury (THg) used in the amalgamation process was released to the environment, either associated with the solid mine tailings, or to the atmosphere as Hg⁰ (Parsons et al. 2012). Over the course of the province's nearly century-long gold rush, a minimum of 3,700-9,100 kg of Hg is estimated to have been released into the environment (Parsons et al. 2012). Typical As contamination in gold mine tailings sites derives from the weathering and oxidizing of arsenopyrite (FeAsS), a sulfide mineral commonly found in association with gold ore deposits (Lengke et al. 2009; LeBlanc et al. 2020). Arsenopyrite would often be crushed along with host rock during the gold extraction process and subsequently released into the environment with the rest of the mine tailings (Wong et al. 1999; LeBlanc et al. 2020).

Previous studies have shown that current soil Hg and As concentrations at historic gold mining sites are typically above Canada's health guidelines (Parsons et al. 2012; Dalziel and Tordon, 2016). Parsons et al. (2012) found the average concentrations of As and Hg from 482 tailings sediment samples taken across 14 historical gold mine sites in Nova Scotia to be 10 mg/kg to 31 wt.% with a median of 2,550 mg/kg, and <5 µg/kg to 350 mg/kg with a median of 1,640 µg/kg, respectively. Greater than 99% of these samples were found to exceed both sediment and soil quality guidelines for As, while Hg concentrations exceeded soil quality guidelines in 20% of samples and sediment quality guidelines in 71% of samples (Parsons et al. 2012). Given the close proximity of many historical mine tailings sites to present day residential developments, high As concentrations are particularly concerning for human exposure and health impacts (Parsons et al. 2012; Parsons and Little 2015). Mercury

concentrations in sediments at these sites are concerning due to the potential for methylation and subsequent bioaccumulation in food webs (Winch et al. 2008). Typical mine tailings sites are low-lying wetlands or lakes and relatively anoxic and reducing in nature. This makes the environment well-suited to sulfate-reducing bacteria, the primary microbial producer of methylmercury (Fortin et al. 2000; Fortin et al. 2002; Winch et al. 2008).

Table 1: Historic gold mines and mining districts in the Halifax Regional Municipality (HRM), listing active years, tonnes of ore crushed and tailings Hg ($\mu\text{g}/\text{kg}$) and As (mg/kg) concentration ranges (where available).

Mine/District	Years Active	Ore Crushed (tonnes)	Tailings [Hg] ($\mu\text{g}/\text{kg}$)	Tailings [As] (mg/kg)	Source
Montague	1863-1940	121,816	166 - 8,390	1,720 - 41,300	Parsons et al. 2012
Old Stamp Mill	1869-1872	300			Malcolm, 1976
Waverley	1862-1940	152,496			Parsons et al. 2012
Oldham	1862-1946	107,080			Parsons et al. 2012

1.3.1. Montague Mine District

Located within the Halifax Regional Municipality (HRM) in the community of Montague Gold Mines, the Montague Mining District was active from 1863 to 1940 (Table 1), over the course of which multiple Stamp Mills were established (Parsons et al. 2012). As was common practice for the time, Hg was used in the gold amalgamation process, after which the remaining tailings, high in Hg and As, would be released directly into the nearby Mitchell Brook (DeSisto et al. 2011; Parsons et al. 2012; Dillon Consultants, 2019). Presently, there are multiple downriver wetland sites along the Mitchell Brook that are known to be contaminated by these tailings (CCME, 1999; Parsons et al. 2012; CCME, 2017; Leblanc et al. 2020). It has been established by

multiple studies conducted over the last decade that the district's tailings sites are well above normal background levels for both Hg and As, as well as some areas that are above environmental and/or human health guideline concentrations (Table 1) (DeSisto et al. 2011; Parsons et al. 2012; Parsons and Little, 2015; DeSisto et al. 2017; Leblanc et al. 2020).

Within the Montague mining area there is an additional site highly contaminated by tailings that is separated from the main tailings site by approximately 500 m, known as the Old Stamp Mill site (Malcolm, 1976). This site was the location for the Taylor and Weir Mill which operated at the site from 1869–1872 (Table 1). Due to the separate origins of the main Montague tailings, and the Old Stamp Mill tailings, they will be sampled as two separate sites in this study.

1.3.2. *Waverley Mine District*

The Waverley gold mine district, located in the Bedford area of the HRM, was in operation from 1862 until its closure in 1940 (Table 1). Based on historical reports, approximately 152,496 tonnes of gold ore are estimated to have been processed using the mercury-gold amalgamation process during this time period (Parsons et al. 2012). Chronic As exposure has been noted in residents of the district since the 1970s, including at least one case of As poisoning caused by contaminated well water (Grantham and Jones, 1977; Hindmarsh et al. 1977; Drage, 2015). It was this case of As poisoning in 1976 which drew attention to the potential for historical gold mining contamination to have a negative impact on human health in Nova Scotia (Parsons et al. 2012; Drage, 2015). Numerous studies conducted since the 1976 case have found elevated levels of Hg and As in fish, water and sediment samples (Mudroch and

Sandilands 1978; Mudroch and Clair, 1985; Mudroch and Clair, 1986), as well as a recent 2019 study which found the average total Hg and As in Waverley sediments to be 10.9 ± 6.5 mg/kg and $17,780.1 \pm 5,155.9$, respectively (LeBlanc, 2019).

1.3.3. Oldham Mine District

The Oldham gold mining district is located within the HRM near the Enfield Municipality, where it operated from 1862 – 1946 (Table 1). Over the 84 years the district was in operation, approximately 107,080 tonnes of gold ore were processed by the Oldham mines (Parsons et al. 2012). Like both the Montague and Waverly mining districts, gold ore was processed using the mercury-gold amalgamation method, and crushed tailings were released into the local river and wetland systems, resulting in multiple areas of high Hg and As contamination within the Municipality (Lane et al. 1988; Lane et al. 1989; Wong et al. 1999; Parsons et al. 2012). Plants, water, invertebrates, fish, and small mammals have been studied most extensively in the district (Lane et al. 1988; Lane et al. 1989; Wong et al. 1999), although Wong et al. 1999 found soil Hg concentrations to be in the range of 500 – 2,000 $\mu\text{g}/\text{kg}$, which is considerably above the Government of Canada's recommended guidelines for environmental and human health (CCME 2017).

1.4. Tree Growth Patterns and Basis for Dendrochronology

Internal tree trunk biology is broken up into two distinct sections: heartwood and sapwood (Cutter and Guyette, 1993; Taylor et al. 2002). Essentially, while the inner, heartwood section of the trunk of the tree is no longer considered to be made up of living tissues, the outer sapwood is where all current growth and nutrient transport

occurs (Cutter and Guyette, 1993; Taylor et al. 2002). To facilitate nutrient and water movement, sapwood contains specialized transport tissues called xylem (aka cambium) and phloem (Bowes, 2010). While these tissues remain present in heartwood, they are no longer functioning tissues (Taylor et al. 2002). Tree growth occurs in distinct growing seasons where new sapwood grows in an outward direction, resulting in the distinguishable rings of growth that allow for dendrochronological studies to be conducted (Cutter and Guyette, 1993; Bowes, 2010). Each seasonal ring grows in a light to dark gradient of colour, with the light portion of the ring representing the earliest seasonal growth, and the dark-coloured wood being produced by all late to end of season growth. It is in the sapwood portion of the tree where, as nutrients and water are transported throughout the tree, so are any contaminants the tree has taken up from its environment, including heavy metals and metalloids (Cutter and Guyette, 1993).

Tree species recommended for dendrochemistry studies in general are old growth with distinct heartwood, low moisture content and a low number of rings (Cutter and Guyette, 1993). Specific conifer trees that meet these criteria and can be found throughout North America (and more specifically, Nova Scotia) are *Pinus* (Pine), *Thuja* (Cedar) (Cutter and Guyette, 1993).and *Picea* (Spruce) species (Clackett et al. 2018).

Several publications indicate that coniferous trees absorb higher levels of atmospheric contaminants than deciduous trees (Cutter and Guyette, 1993; Drenner et al. 2013; Laacouri et al. 2013; Gustin and Weisberg, 2017; Yang et al. 2018). This is hypothesized to be due to conifer needles having higher surface roughness and a greater number of leaf hairs (trichomes), which provide a higher surface area:volume ratio making them better able to filter gaseous elements (Cutter and Guyette, 1993;

Drenner et al. 2013; Laacouri et al. 2013). Also, as most conifers retain their foliage year-round, this allows a greater period of time for Hg uptake to occur (Cutter and Guyette, 1993; Arnold et al. 2018). Conifer leaf cuticles also contain more lipids than those on deciduous leaves; since Hg⁰ is highly lipophilic, this is theorized to be another factor resulting in higher Hg uptake (Laacouri et al. 2013).

1.5. Trees as *Biomonitors of Hg and As*

Uptake of gaseous Hg⁰ by trees occurs primarily through tree foliage via both stomatal and non-stomatal (cuticular) routes (Laacouri et al. 2013; Arnold et al. 2018) and passively through bark (Clackett et al. 2018; Rimondi et al. 2020b). While both foliar routes facilitate Hg absorption, the dominant route appears to be via stomatal uptake (Peckham et al. 2019). Since stomatal opening is controlled by exposure to solar radiation and humidity, Hg uptake is usually greatest during daytime hours when both foliar mechanisms are active (Arnold et al. 2018; Peckham et al. 2019), meanwhile the non-stomatal uptake route, in which Hg passes through the leaf cuticle, dominates during the night (Arnold et al. 2018). However, no significant difference in the quantities of Hg taken up by the two methods has been found (Arnold et al. 2018; Peckham et al. 2019).

Mercury uptake has been strongly linked to the uptake of carbon dioxide (CO₂), during the main growing season (Arnold et al. 2018; Peckham et al. 2019). Indeed, foliar Hg uptake is highest in spring, and lowest in autumn (Peckham et al. 2019). This suggests that the pathways used for both Hg and CO₂ uptake are likely the same, allowing Hg sequestered by tree foliage to be transferred via phloem to the sapwood of the tree where it is stored (Peckham et al. 2019). During this process, Hg⁰ may be

oxidized to Hg^{2+} within the leaf system before binding with proteins and peptides containing thiol groups, allowing the metal to be easily transported by the phloem and stored alongside nutrients in the sapwood (Figure 3) (Chiarantini et al. 2017; Arnold et al. 2018; Peckham et al. 2019). Examples of Hg compounds found in sampled trees include Hg-cysteine compounds, in addition to elemental Hg, mercury (II) sulfide (HgS) and Hg bound to tannic acid (Chiarantini et al. 2017; Gustin et al. 2004; Arnold et al. 2018).

The exact mechanism by which Hg is taken up by the non-stomatal route is not fully understood currently; however, it is likely connected to gaseous and particulate Hg deposition onto the leaf surface, where direct absorption occurs through the cuticle (Stamenkovic and Gustin, 2008; Arnold et al. 2018). Gaseous elemental mercury (GEM) is likely the main form of Hg absorbed from the atmosphere by foliage, due high correlations found between local atmospheric and tree foliage GEM concentrations sampled in the same region (Stamenkovic and Gustin, 2008; Arnold et al. 2018; Peckham et al. 2019). Re-emission of Hg from leaf surfaces after deposition does occur; however, Stamenkovic and Gustin (2008) found that the quantity of Hg re-emitted is significantly below the amount initially deposited on the leaf surface. Additionally, the rate at which Hg is re-emitted decreases significantly over the course of several hours, which suggests that Hg is taken up by the leaf structure and immobilized relatively quickly after deposition (Stamenkovic and Gustin, 2008).

Tree bark sampling is a reliable method of biomonitoring for aerosolized and deposited heavy metal particulates, including Hg (Kang et al. 2019; Rajfur, 2020; Rimondi et al. 2020a; Rimondi et al. 2020b). Mercury accumulates in tree barks when

particulate bound Hg is deposited on the most outer, newest portion of the tree bark, where it is absorbed via passive uptake into the sapwood (Chiarantini et al. 2016; Rimondi et al. 2020b). Tree barks are considered a useful biomonitor in part because metabolism does not occur in this section of the tree, so that little to no contaminants would be lost after uptake by bark (Arnold et al. 2018; Rajfur, 2020). Several studies have observed significant correlations between concentrations of contaminants found in barks and lichens from the same sampling area, which suggests a similar mechanism of uptake between the two (Rajfur, 2020; Rimondi et al. 2020a; Rimondi et al. 2020b). Although the exact mechanism of Hg uptake by tree barks is not fully understood, factors such as site humidity and rainfall likely contribute to higher levels of Hg permeating from the dry, outer layer of bark to the more inward layers where Hg is stored (Chiarantini et al. 2017; Peckham et al. 2019).

While most contaminants, such as As, are absorbed primarily through tree root structures, Hg uptake in this manner is considered negligible (Lindqvist et al. 1991; Wright et al. 2014; Arnold et al. 2018; Clackett et al. 2018). While there is evidence of high Hg uptake from soil into roots, there is little to no transfer of Hg to the tree stem/trunk (Lindqvist et al. 1991; Greger et al. 2004). Chen and Yang (2012) hypothesized that this is due to the cationic Hg^{2+} reacting with anionic compounds within the root structure, such as carbonate, sulfate, or phosphate, to form insoluble products that cannot be easily transported to the shoot of the plant. The paper further hypothesizes that Hg^{2+} could bind to the cell wall of the root due to its high cationic exchange capacity, thereby preventing transport further into the tree (Chen and Yang, 2012). However, no supporting evidence was provided to validate these hypotheses.

Arsenic is typically absorbed from soil or water through the root systems of arbuscular mycorrhizal (AM) plants, a classification which includes all types of trees (Smith et al. 2010). Therefore, tree storage of As is often used to track below surface plumes of the contaminant (Algreen et al. 2012). Both arsenate (As(V)) and arsenite (As(III)), the inorganic forms of As, can be taken up by plants, although evidence shows a typically higher rate of uptake for As(V), which once within the root tissues is rapidly reduced to the As(III) form (Smith et al. 2010). Arsenite is then either transported in its current form to the plant shoots or is converted to organic As (Smith et al. 2010). Arsenate is taken up by plants using similar pathways as inorganic phosphorous, resulting in the two chemicals weakly competing for uptake (Smith et al. 2010). Therefore, trees in areas with particularly high levels of ground As contamination can see reduced phosphorous uptake, resulting in lower overall productivity, in addition to the uptake of harmful levels of As (Smith et al. 2010).

Table 2: Mercury concentrations in tree ring samples from peer reviewed studies

Source	Site Location	Site Description	Tree Type and Sample Size (n)	Sample Dry Weight (mg)	[THg] range (µg/kg)	Mean [THg] (mg/kg) with Standard Deviation	Tree-Ring increment (years)	
Clakett et al. (2018)	Scree Hill, Yukon, USA	Pristine woodland site	White Spruce (55)	20	0.7-1.9	1.22	5	
Peckham et al. (2019)	California and Nevada, USA	Watershed sites	<i>Pinus spp.</i> (20)	> 20	0.75-8.45	1.86±0.54	5	
		Arid sites	<i>Pinus monophyla</i> (30)			1.56±0.83		
		Forest sites	<i>Pinus spp.</i> (3)			3.20±2.36		
Hojdová et al. (2011)	Příbram, Czech Republic	Historical Pb-Ag-Zn ± Sb smelter stack	<i>Picea spp.</i> (3)	30-500	up to 8.4	Not Given	5	
	Jedová Hora, Czech Republic	Historical HgS mining site	<i>Picea spp.</i> (3)		up to 15.0	Not Given		
Jung and Ahn (2017)	Yeosu City, Korea (34°51'10.43"N, 127°45'2.81"E)	Adjacent to Phosphate Fertilizer Plant		500			1	
		4 km	Japanese Cedar		10.05–13.64	11.96 ± 1.02		
		26 km			3.07– 9.43	6.26 ± 1.77		
		40 km			2.69– 5.85	4.45 ± 0.72		
Siwik et al. (2010)	Kingston, Ontario, CA	Reference and potentially contaminated sites	Shoreline (willow and poplar) (21)	70	<0.40-19.00	2.32±1.43	3 or 5 (spp. dependant)	
			Inland (oak and maple) (21)			0.51±0.69		
Wright et al. (2014)	California to Nevada, USA	Adjacent to atmospheric Hg sources	<i>Pinus spp.</i> (26)	Not Given	0.20-32.60	Not Given	5	
Nóvoa-Muñoz et al. (2008)	Galicia, NW Spain	Adjacent to coal-fired power-plant		Not Given	8.30-16.40		Not Specified	
		Xantar, XB	Birch (6)			8.30 ± 0.20		
			Oak			(6)		10.60 ± 0.60
		Piocorto, PS	Pine					11.50 ± 2.30
		Sanche, SB	Birch			(6)		13.40 ± 2.20
Oak	16.40 ± 2.00							
Zhang et al. (1995)	Quebec, CA	Hg contaminated constructed reservoirs	Black Spruce (26)	Not Given	13.00-37.00	Not Given	5	
Navratil et al. (2017)	Central Czech Republic (50°16'29.34"N 14°31'8.71"E)	Adjacent to Chlor-alkali Plant		Not Given	1.80-47.50		5	
		0.5km	Scott's pine (3)			32.70		
		4.1km	Scott's pine (3)			9.00-10.50		
		9.0km	Scott's Pine (3)			4.60-5.80		

Table 3: Arsenic concentrations in tree ring samples from peer reviewed studies

Source	Site Location	Site Description	Tree Type and Sample Size (n)	Sample Dry Weight (mg)	[TAs] Range (µg/kg)	Mean [TAs] (mg/kg)	Tree-Ring Increment Sampled (years)
Cheng et al. (2007)	Vineland, New Jersey, USA	Reference Site	Oak, Pine (30)	20-150	80-990	60.0 ± 60.0	Up to three years
		Former pesticide production site	Oak, Pine, Cedar, Hickory (32)			280.0 ± 15.0	
Algreen, et al. (2012)	Near Hortan, Norway	Reference Site	Birch	500-800	230-1 640	230	0.5-0.8g of one core sample were processed per tree
			Willow			230	
		Møringa, former dump site	Birch			320	
			Willow			470	
Martin, et al. (2000)	Niagara, ON, Canada	Orchard tree treated with lead arsenate	Pear A (1)	100	Not Given	≥50.0	Approximately one year
		Low contamination orchard site	Pear B (1)			63.0 ± 19.0	
			Cherry (2)			46.0 ± 25.0	
			Plum (3)			27.0 ± 3.0	
Jana et al. (2012)	Ouche, Cantal, France	Historic antimony mine site	Scots Pine (12)	100	Not Given	400.0 ± 100.0	Not Specified
			Silver Birch (12)			500.0 ± 200.0	

1.6. Mercury Sediment Flux

Within sediment solids, Hg typically exists in its divalent form in one of three ways; as a mineral component, bound to present organic matter, or adsorbed to the surface of mineral particles (Pannu et al. 2016). Mercury-soil flux, the movement of Hg from storage in soils to the atmosphere, occurs when Hg^{2+} is converted (reduced) to Hg^0 by bacteria or abiotic reactions (Pannu et al. 2016). Since Hg surface adsorption in soils is only maintained for relatively short periods of time, the re-volatilization of soil bound Hg^{2+} to its elemental, gaseous form is considered a significant source of Hg in the atmosphere (Dalziel and Tordon, 2014; Pannu et al. 2016). It is estimated that between 14-24% of the total atmospheric Hg burden is re-emitted from storage in land surfaces (Pannu et al. 2016). Once released, the volatile elemental Hg re-enters the mercury cycle where it can be oxidized back into Hg^{2+} , and eventually re-deposited through precipitation back to storage in soils, where the cycle will continue to repeat (Pannu et al. 2016).

Mercury flux rates are typically highest during summer afternoons, and strong correlations have been shown with increased soil temperatures and solar radiation (Dalziel and Tordon, 2014; Pannu et al. 2016). However, in addition to climactic influences, inherent soil characteristics including soil texture, pH and organic content, can have significant effects on the levels Hg volatilized from soils at different sites (Pannu et al. 2016). For example, higher soil moisture content contributes to an increased reduction of Hg^{2+} to Hg^0 , thus increasing the amount of Hg^0 available to be volatilized (Gustin and Stamenkovic, 2005; O'Driscoll et al. 2005). Meanwhile soils with a greater organic content can see a lowered occurrence of Hg flux because many

organic compounds are able to form very stable bonds with Hg^{2+} , thereby becoming unavailable for reduction by bacteria to Hg^0 , and ultimately preventing volatilization from occurring (O'Driscoll et al. 2005; Pannu et al. 2016). Organic compounds and functional groups which may reduce the availability of Hg species for volatilization include reduced sulfide species, such as thiols, sulfides, disulfides, polysulfides and thiophenes (Pannu et al. 2016). Based on these known interactions, Pannu et al. 2014, proposed a two-step process for Hg soil reduction, in which the first step is a largely irreversible process dominated by the presence of soil micro-organisms which increase the presence of reducible Hg^{2+} . The second step is driven by abiotic processes which quickly reduce Hg^{2+} to Hg^0 , which is available for volatilization to the atmosphere. Collectively, this information suggests that the limiting factor in the volatilization of Hg^0 from soil is the ability for Hg^{2+} to be converted to volatile Hg^0 (Bahlmann and Ebinghaus, 2003; Pannu et al. 2014, 2016).

The impacts of solar radiation and temperature exposure on Hg flux rates are difficult to separate, as the highest levels of the two often occur in relation to the other, if not simultaneously. For example, it is understood that the rate of Hg volatilization from soils on an annual scale peaks during the afternoon, on summer days and is at its lowest at night and in the winter, which coincides with annual radiation and temperature trends (Gustin et al. 2004; Baya and Van Heyst, 2010; Dalziel and Tordon, 2014). Although the influence of soil temperature on Hg flux rates has been observed and quantified by scientific study (Choi and Holsen, 2009), the solar radiation driven mechanism is not fully understood. Studies that have broken down the effects of solar radiation's separate UV-A and UV-B components have determined that UV-A is only a

minor contributor to Hg^0 soil volatilization, meanwhile UV-B is the major contributor to reducing Hg^{2+} to Hg^0 (Bahlmann et al. 2006; Xin and Gustin, 2007). Further, Bahlmann and Ebinghaus (2003) found solar radiation induced volatilization of Hg from dry soils to be 20 hours, and 63 hours from wet soils.

1.7. Gaps in Literature

Although Hg^0 emissions from contaminated soils and sediments are a significant influence of global atmospheric Hg, the amount of elemental Hg released to the atmosphere in this way is not well known (Pannu et al. 2016). Mercury sediment flux determination is further complicated by a lack of set standard procedures for determining Hg flux rates from sediments across the scientific community (Pannu et al 2016). Therefore, more research into this area of study, as well as a scientific consensus on the best procedure for determining Hg flux rates are needed in order to better quantify the impacts Hg flux emissions from sediments.

The potential for translocation of Hg and As between tree rings over time is not well studied (Martin et al. 2000; Rajfur, 2020). The probability and rate at which this may occur is difficult to observe and quantify, and would likely need to involve the repeated sampling of the same tree over a several years of observation. This would be a difficult and expensive project to undertake, and as a result the potential for this phenomenon to occur is not well understood in the scientific community.

Other factors which could affect Hg or As concentration in a tree core sample include the effects of bio-dilution, tree species, tree age and ecosystem micro-climates. All these factors might increase the variation in Hg and As concentrations and growth

patterns in trees within the same site. Therefore, these need to be considered with the results of this study.

1.8. Objective

The objective of this thesis is to conduct dendro-chemical analysis of total mercury and arsenic concentrations in sectioned tree core and surface soil samples collected from 4 sample sites associated with historical gold mining operations and 2 non-contaminated reference sites in Nova Scotia, Canada, with the goal of assessing temporal trends.

1.9. Hypotheses

H1: Mercury and arsenic concentrations in the cores of White Spruce trees from historical gold mine tailings will significantly increase through time, specifically from 1980 to present day. We hypothesize this will be due to increased levels of gaseous mercury released from surface sediments with increasing annual temperatures which is subsequently incorporated through stomatal uptake.

H2: The mean slope of tree core total mercury concentrations over time will positively correlate with adjacent surface soil total mercury concentrations. We hypothesize this will be due to increased Hg volatilization at sites with higher Hg in soils and therefore higher rates of accumulation in tree cores over time.

H3: Tree core arsenic concentrations will be relatively constant in tree cores over time due to root uptake. Average As concentrations in tree cores will positively correlate with adjacent soil As concentrations.

2. Methods

2.1. Field Sampling



Figure 4: Map of the six HRM sampling sites shown in relation to local landmarks.

In June and July of 2020 tree core and soil samples were collected from a total of 6 sample sites, 2 reference sites and 4 contaminated sites connected with historical gold mining processes in the Nova Scotia. All samples were collected while wearing powder-free nitrile gloves to avoid contamination of samples, and all sampling equipment was rinsed with DI water between samples to avoid cross contamination. Spruce trees were selected based on their availability at sample sites and their suitability for dendrochronological study.

A total of 62 cores, 30 cm in length and 5 mm in diameter were collected, with 11 cores sampled from 5 trees at each contaminated site (with one tree sampled in triplicate, and the other 4 sampled in duplicate), and 9 cores from 3 trees at each

reference site (each tree sampled in triplicate). This allowed for both mercury and arsenic testing to be performed on separate cores from the same tree. Cores were collected using a stainless steel Hagl f 3-Thread Increment Borer, which is a standard brand used in dendrochronological studies. Conifer trees, more specifically, White Spruce (*Picea glauca*) and Black Spruce (*Picea mariana*) trees were sampled, based on tree availability at each site. Cores were bored at a height of 1-1.5 m above ground on the trunk (approximately chest height), with vertical intervals of 10-15 cm between each core. Cores were then stored in lab grade plastic straws taped at each end and labelled according to site, tree and sample number and separated by tree in HDPE plastic food storage containers for transport. A small branch with needles was collected, when possible, from each tree for tree species confirmation and potential needle analysis at a later date.

A total of 26 soil samples were collected over the 6 sample sites. For each tree sampled a single soil sample was collected from the top 5 cm of the surrounding ground after removing any covering vegetation. From non-contaminated sites approximately 400-500g of soil was collected, while only approximately 100 g of soil was taken at contaminated sites. Each sample was stored in brown paper bags labelled according to site and tree. Samples were then transported inside coolers to prevent elemental mercury (Hg⁰) loss due to exposure to solar radiation. The trees sampled were photographed and marked with flagging tape for future reference. GPS coordinates for each tree sampled were also recorded.

2.2. Sample Processing and Analysis

Samples were transported to the K.C. Irving Environmental Science Centre (KCIC) Mercury Laboratory and stored in the freezer at a constant temperature of -20°C until processing. Prior to being dried, wet cores were photographed with a ruler for reference, and ring thickness was analyzed using a technique known as skeleton plotting. Using a Microsoft Excel spreadsheet patterns of thin (T), normal (N) and wide (W) rings were recorded. From this, conclusions about a tree's growth rate and specific growing year conditions can be determined. The technique can also be used to identify "false rings", which occur when two or more visibly distinguishable rings are grown in a single growing season. Ring thickness data was then plotted on grouped bar graphs comparing ring thickness against year of growth using Sigma Plot 12.9 software. Ring thickness was plotted as -1, 0 or 1 to denote thin, normal, and wide rings, respectively. Growth trends were examined and compared across 3-5 separate trees per site. This process was confirmed by Alain Belliveau, the curator of the KCIC Herbarium, at which point he also confirmed the species identities of the sampled trees. All except for one tree sampled were confirmed to be either White or Black Spruce (*Picea glauca* and *mariana*, respectively); MMT-T1 was confirmed to be a Balsam Fir (*Abies balsamea*).

Wet cores were then oven dried at 60°C for 2-3 days. Groups of 3-5 cores were placed in the sample drying oven per single plastic tray covered in sheets of aluminum foil that were creased to avoid contact between cores. The aluminum foil sheet was labelled according to site, tree and sample number of each core. After removal from the oven, cores were wrapped in aluminum foil and stored at room temperature in HDPE plastic containers separated by tree sampled, until segmenting was conducted.

Each core was then segmented into groups of 5-year (ring) time increments. All 62 cores collected were oven dried for preservation, however only 26 cores (one core from each tree) were segmented for THg analysis. Of the remaining cores, select samples will be sent for As analysis by Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS) by the Acadia University's Murimboh Lab (summer of 2021, post thesis completion due to COVID-related delays).

Soil samples were air dried in the K.C. Irving Centre greenhouse's phytotron units, which are air flow and temperature-controlled rooms. After drying was completed, soil samples were sieved through size 80 mesh (177 μm) stainless steel sieves to separate the sample into a standard silt-clay texture, subsamples of which were then analyzed for total mercury (THg) and total arsenic (TAs) concentrations. Sieving was done outside while wearing powder-free nitrile gloves and an N95 respirator for the researcher's protection.

2.2.1. Total Mercury Analysis

THg analysis was conducted using the Nippon MA-3000 machine, which determines sample concentrations via thermal degradation-gold amalgamation atomic absorbance (USEPA method 7473). To ensure the accuracy of readings being conducted, the certified reference materials (CRMs), DORM-4 and DOLT-5, as well sample blanks, were analyzed in triplicate after every 10-15 samples. As long as the results are within an acceptable range of the known concentrations (DORM-4 = $410 \pm 55 \mu\text{g/kg}$; DOLT-5 = $440 \pm 180 \mu\text{g/kg}$; MESS-3 = $91 \pm 9 \mu\text{g/kg}$), the Nippon MA-5000

results can be determined to be accurate. To ensure accuracy of mass readings, the Shimadzu 5 decimal balance was used for all samples.

For THg concentrations in soil samples, the MDL was determined to be 7.7 µg/kg for an average 5 mg sample and CRM recovery was 98.1 ± 3.0 % for MESS-3 and 94.5 ± 4.6% for DORM-4. Mean RSD of Hg concentrations between triplicate soil samples were 2.1% (Mitchell Brook reference), 2.3% (Muddy Pond reference), 10.4% (Oldham), 2.3% (Montague), 11.0% (Muddy Pond), 2.2% (Old Stamp Mill).

Total Hg analysis of tree core samples were run in multiple sets. The Mitchell Brook reference cores were analyzed alone; the run MDL was 1.3 µg/kg for an average 50 mg sample and CRM recovery was 99.4 ± 2.9% for DOLT-5 and 89.9 ± 1.0% for DORM-4. The Muddy Pond reference and Old Stamp Mill cores were analyzed in the same analytical run; the MDL was 2.0 µg/kg for an average 50 mg sample and CRM recovery was 87.7 ± 3.0% for DOLT-5 and 95.0 ± 3.2% for DORM-4. The Oldham and Muddy Pond tailings site cores were analyzed in the same analytical run; the MDL was 1.5 µg/kg for an average 50 mg sample and CRM recovery was 86.0 ± 1.2 % for DOLT-5 and 93.0 ± 7.2 % for DORM-4. Tree cores from the Montague tailings site run alone; the MDL was 4.2 µg/kg for an average 50mg sample and CRM recovery was 91.4 ± 2.3 for DOLT-5 and 98.5 ± 1.9 % for DORM-4.

2.2.2. Total Arsenic Analysis

Subsamples of each soil sample were analyzed for As concentrations via x-ray fluorescence spectroscopy (XRF analysis), in collaboration with the Spooner lab of Acadia University. Dunnington et al. 2019, established the reliability of As analysis via

XRF units to be within a relative difference (RD) of $\pm 20\%$, noting that precision can be increased by ensuring greater homogeneity of the sample. The CRM MESS-3 (21.1 ± 1.1 mg/kg) was used to ensure accuracy of As concentration readings. Soil TAs analysis had a CRM recovery of 111.2 ± 10.8 mg/kg for MESS-3, and mean RSD of triplicate samples from all sites was 5.1%.

Total arsenic analysis of tree core samples was delayed due to restrictions and delays caused by the COVID-19 pandemic and could not be completed by the time of thesis submission.

2.2.3. Loss on Ignition

The organic content of soil samples was also determined using a loss on ignition method (LOI) established by Chen et al. (2015). This was also conducted in the KCIC labs using a Shimadzu 5 decimal balance to determine sample masses before and after samples were run for total Hg analysis on the Nippon MA-5000. LOI was determined based on the percentage of each sample that was burned off during the thermal degradation process.

2.3. Statistical Analysis

Statistical analyses were conducted using the Microsoft Excel and SigmaPlot 12.0 programs. The method detection limit (MDL) for each run was determined by multiplying the standard deviation of ng of mercury detected in sample blanks by 3. Quality assurance for Nippon readings were calculated by determining % recovery and % relative standard deviation (RSD) of CRMs used in any single run. Scatter plots of

quantitative results were analyzed using linear regressions, significance of fit and correlation tests.

3. Results

3.1. Hg Concentrations in Surface Soils

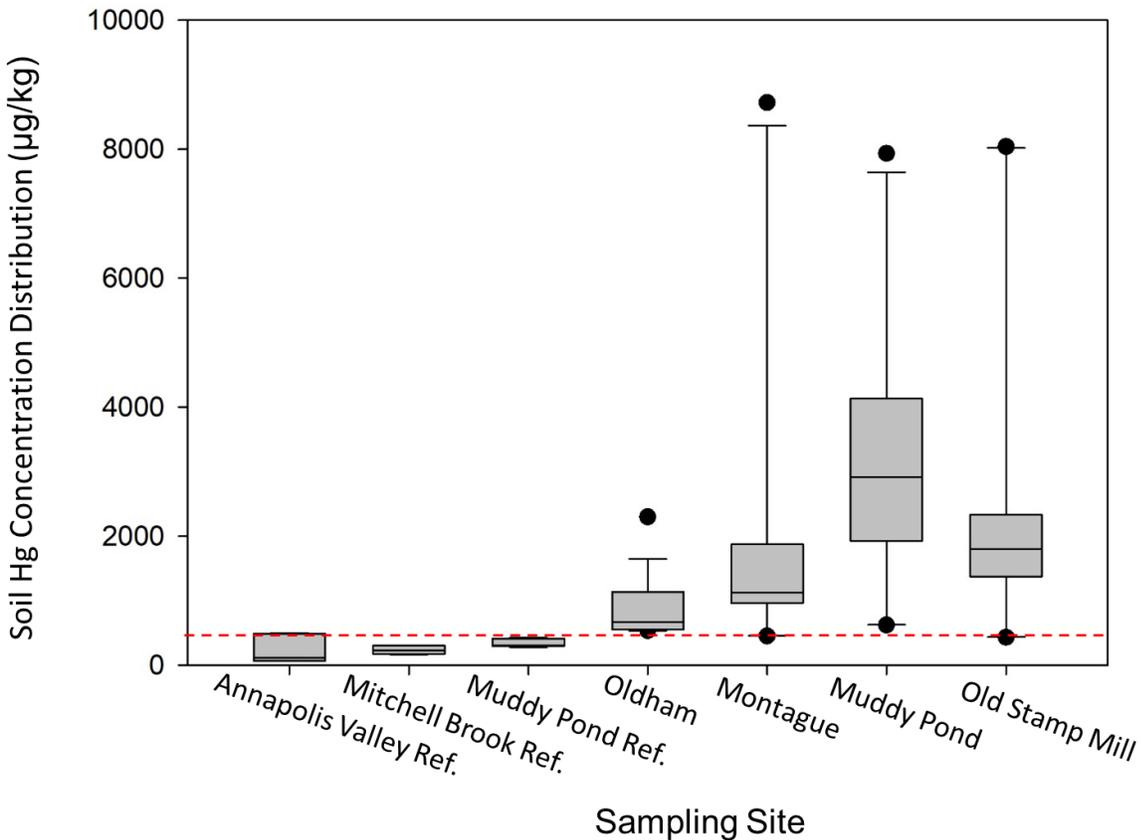


Figure 5: Box plot of mean surface soil total Hg concentrations ($\mu\text{g}/\text{kg}$) adjacent to sampled trees from all sampling sites. Box plots have 25th and 75th percentiles represented as boxes, 5th and 95th percentiles as whiskers, median indicated by solid line, and outliers are denoted by black circles. Maximum acceptable concentration of Hg in residential area soils ($500 \mu\text{g}/\text{kg}$) is denoted by a red dashed line.

All soil Hg concentration data from the Annapolis Valley, Mitchell Brook and Muddy Pond reference sites fall within normal background levels for non-contaminated soil in Canada (Figure 5), for which soils within the range of 100 to 500 $\mu\text{g}/\text{kg}$ are considered normal (Pannu et al. 2016). Consistently low soil Hg concentrations from these background sites supports their use as non-contaminated reference sites in this study. Of all the reference sites, the Black River Lake soil sample from the Annapolis Valley

series was found to have the highest Hg concentration of $491 \pm 6 \mu\text{g}/\text{kg}$. It should be noted that unlike the other reference sites, the Black River Lake location is a human-made lake structure, which are known to accumulate higher levels of contaminants including Hg (Hsu-Kim et al. 2018). Meanwhile the Lumsden Pond soil sample was found to have the lowest Hg concentration of the reference sites, with a concentration of $68 \pm 2 \mu\text{g}/\text{kg}$. Also, as only one soil sample was collected at each location from the Annapolis Valley sites these concentrations are only indicative of the precise area the sample was collected, with no overall site range available. The remaining reference site mean soil Hg concentrations were calculated to be as follows; $231 \pm 54 \mu\text{g}/\text{kg}$ for Mitchell Brook reference site, $337 \pm 57 \mu\text{g}/\text{kg}$ for Muddy Pond Reference, and $114 \pm 5 \mu\text{g}/\text{kg}$ for the Wolfville Nature Preserve Annapolis Valley site (Figure 5).

When compared to reference site soils, surface soils collected from the contaminated sites were consistently found to have higher mean total Hg concentrations (Figure 5). Mean site soil Hg concentrations for each of the four HRM contaminated sites (Oldham, Muddy Pond, Montague, and Old Stamp Mill) were calculated to be $877 \pm 4566 \mu\text{g}/\text{kg}$, $3366 \pm 2280 \mu\text{g}/\text{kg}$, $2525 \pm 2900 \mu\text{g}/\text{kg}$ and $2776 \pm 2672 \mu\text{g}/\text{kg}$, respectively. Therefore, all four contaminated site mean soil Hg concentrations exceed $500 \mu\text{g}/\text{kg}$, making them above the concentration that is expected to occur naturally in Canadian soils (Pannu et al. 2016). The Montague and Old Stamp Mill sites each have one soil sample determined to have a concentration below the $500 \mu\text{g}/\text{kg}$ threshold; however, at $460 \pm 9 \mu\text{g}/\text{kg}$ and $442 \pm 13 \mu\text{g}/\text{kg}$, respectively, the samples would still be

considered quite high to be occurring in a non-contaminated location. Additionally, all other samples collected from the two sites were consistently greater than 500 µg/kg.

Mean Hg concentrations from contaminated sites were determined to be in the following order of increasing concentration; Oldham with the lowest, Montague, Old Stamp Mill, and Muddy Pond with the highest. The Oldham, Montague and Muddy Pond sites all have mean soil Hg concentrations that exceed the the recommended 6.6 mg/kg standard for Hg in residential and agricultural soils as set by the Canadian Council of Ministers of the Environment (CCME, 1999; CCME, 2017). Additionally, all three of the above listed sites are located adjacent to populated residential zones. However, it should be noted that there is a high degree of variability within the contaminated site samples, with site % Relative Standard Deviations (% RSDs) determined to be 52.0% (Oldham), 114.9% (Montague), 96.2% (Old Stamp Mill), and 67.7% (Muddy Pond).

3.2. As Concentrations in Surface Soils

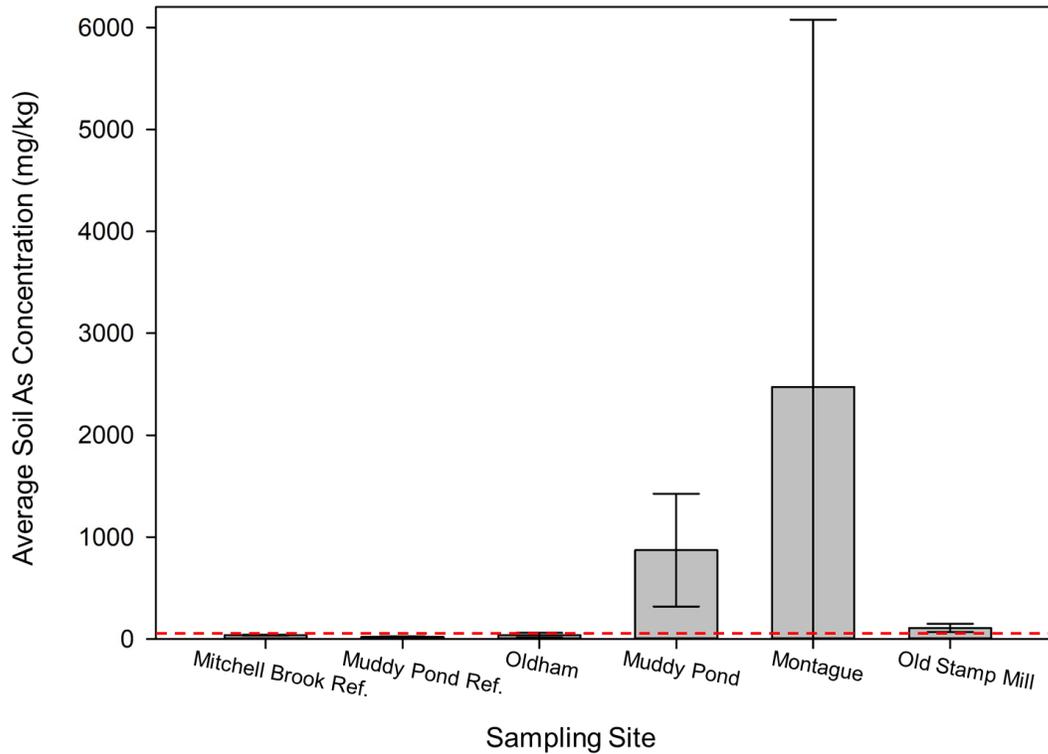


Figure 6: Site mean arsenic (As) concentrations (mg/kg) in soil samples from the Muddy Pond and Mitchell Brook HRM reference sites, as well as the contaminated Oldham, Muddy Pond, Montague and Old Stamp Mill gold mine tailings sites. Soil total Hg concentrations (mg/kg) for 3 sample locations from each site. Standard deviation bars shown are calculated from triplicate analyses of each sample. Maximum acceptable concentration of As in residential area soils (12 mg/kg) is denoted by a red dashed line

Mean surface soil total As concentrations from the Mitchell Brook and Muddy Pond reference sites were found to be 38 ± 5 and 22 ± 4 mg/kg, respectively (Figure 6). Both reference site means are above the Canadian soil guidelines acceptable soil arsenic concentration limit of 12 mg/kg (CCME, 1999); however, it is common for soils in Southern Nova Scotia to have naturally high levels of As, due to underlying bedrock (Dummer et al. 2015).

Of contaminated site soil total As concentrations the lowest site mean was calculated to be from Oldham with a concentrations of 38.1 ± 22.2 mg/kg, which is very close and slightly below the Mitchell Brook site average (Figure 6). The remaining contaminated site mean As soil concentrations were determined to be 109 ± 40 m/kg for Old Stamp Mill, 872 ± 553 mg/kg for Muddy Pond, and highest at the Montague site with a mean of 2474 ± 3603 mg/kg (Figure 6).

3.3. Surface Soil Characteristics

Table 4: Loss on ignition (%) of contaminated site surface soil samples, determined after thermal combustion analysis of triplicate sub-samples was performed, with standard deviation and % relative standard deviation given.

a)	Sample	Mean LOI (%)	% RSD
	Oldham-T1	82.3	2.0
	Oldham-T2	83.5	1.8
	Oldham-T3	93.7	2.1
	Oldham-T4	72.2	7.0
	Oldham-T5	92.6	1.2

b)	Sample	Mean LOI (%)	% RSD
	Muddy Pond-T1	35.2	12.9
	Muddy Pond-T2	28.2	11.9
	Muddy Pond-T3	9.8	10.6
	Muddy Pond-T4	80.8	1.9
	Muddy Pond-T5	11.4	7.3

c)	Sample	Mean LOI (%)	% RSD
	Montague-T1	73.1	5.3
	Montague-T2	43.4	5.8
	Montague-T3	56.6	5.2
	Montague-T4	81.1	2.0
	Montague-T5	9.7	17.1

d)	Sample	Mean LOI (%)	% RSD
	Old Stamp Mill-T1	89.6	2.1
	Old Stamp Mill-T2	93.3	4.8
	Old Stamp Mill-T3	91.7	1.7
	Old Stamp Mill-T4	91.3	2.1
	Old Stamp Mill-T5	74.5	2.0

The majority of surface soil samples were determined to have a relatively high organic content, with 13 out of the twenty samples having a % LOI $\geq 70.0\%$ (Table 4). Notably, the lowest site mean % LOI is 33.1% from the Muddy Pond Tailings site, which

is also the site which had the highest mean Hg concentrations from surface soils (Figure 6). The remaining site mean % LOIs were 52.8%, 84.9%, and 88.1% from the Montague, Oldham, and Old Stamp Mill sites, respectively. Therefore, it can be concluded that on average the Muddy Pond site had the lowest organic content in surface soils, while the Old Stamp Mill site had the on average highest organic content.

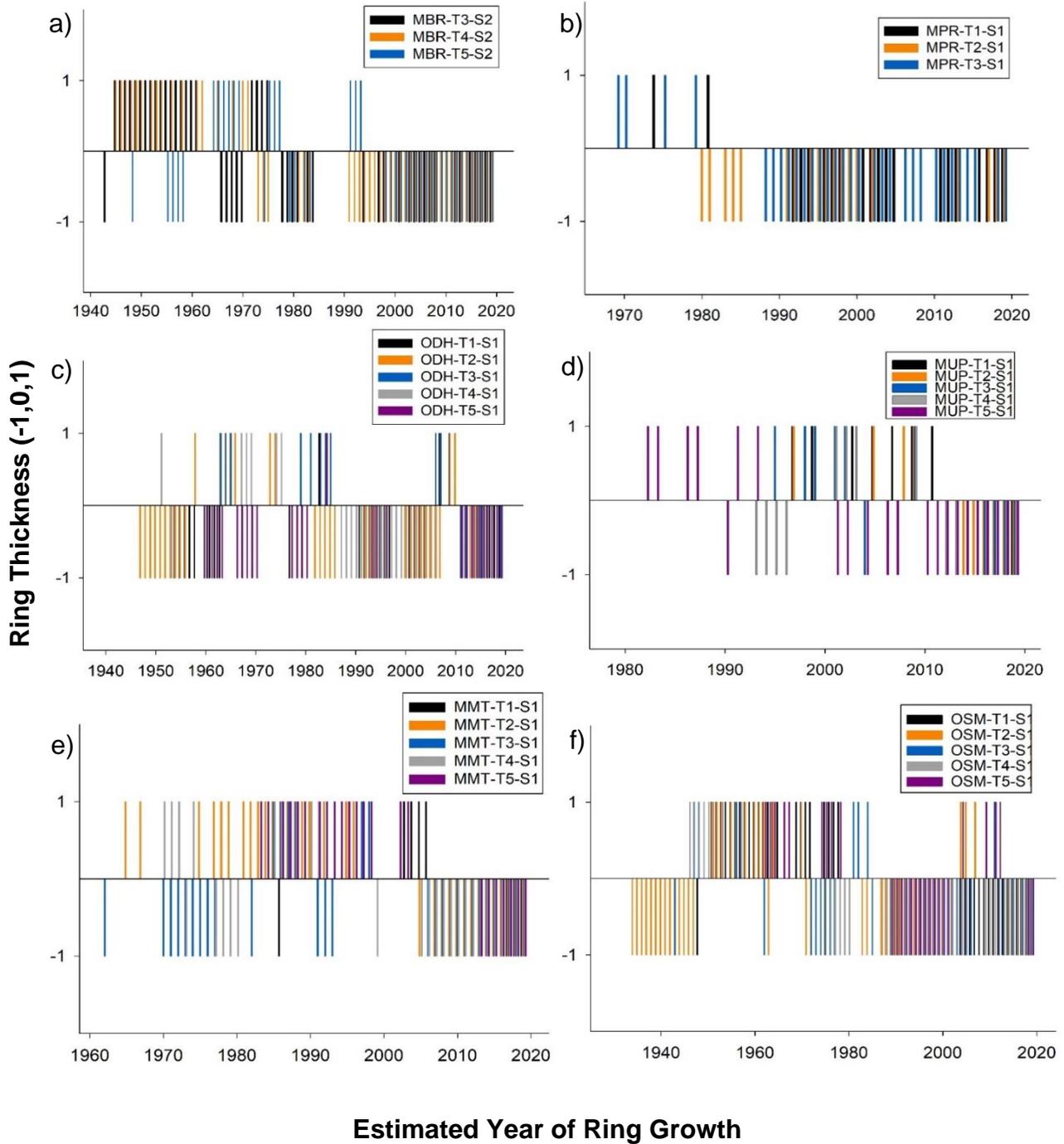


Figure 7: Skeleton plotting ring counting data, showing yearly ring width growth by sampling site; (a) Mitchell Brook Reference (MBR), (b) Muddy Pond Reference (MPR), (c) Oldham Gold Mine Tailings (ODH), (d) Muddy Pond Tailings (MUP), (e) Montague Tailings (MMT), and (f) Old Stamp Mill Tailings. Ring thickness is denoted by a '-1', for 'narrow growth', '0' for 'normal growth' or a '1' for wide growth.

Skeleton plotting data examining one core from each tree sampled shows generally consistent growth trends between sampling sites (Figure 7). It should be noted that “narrow, normal or wide” ring growth, is relative to the average growth of each core. The skeleton plotting method used was verified in part by Alain Belliveau, Collections Manager of the K. C. Irving Centre’s E.C. Smith Herbarium.

3.4. Tree Core Analysis

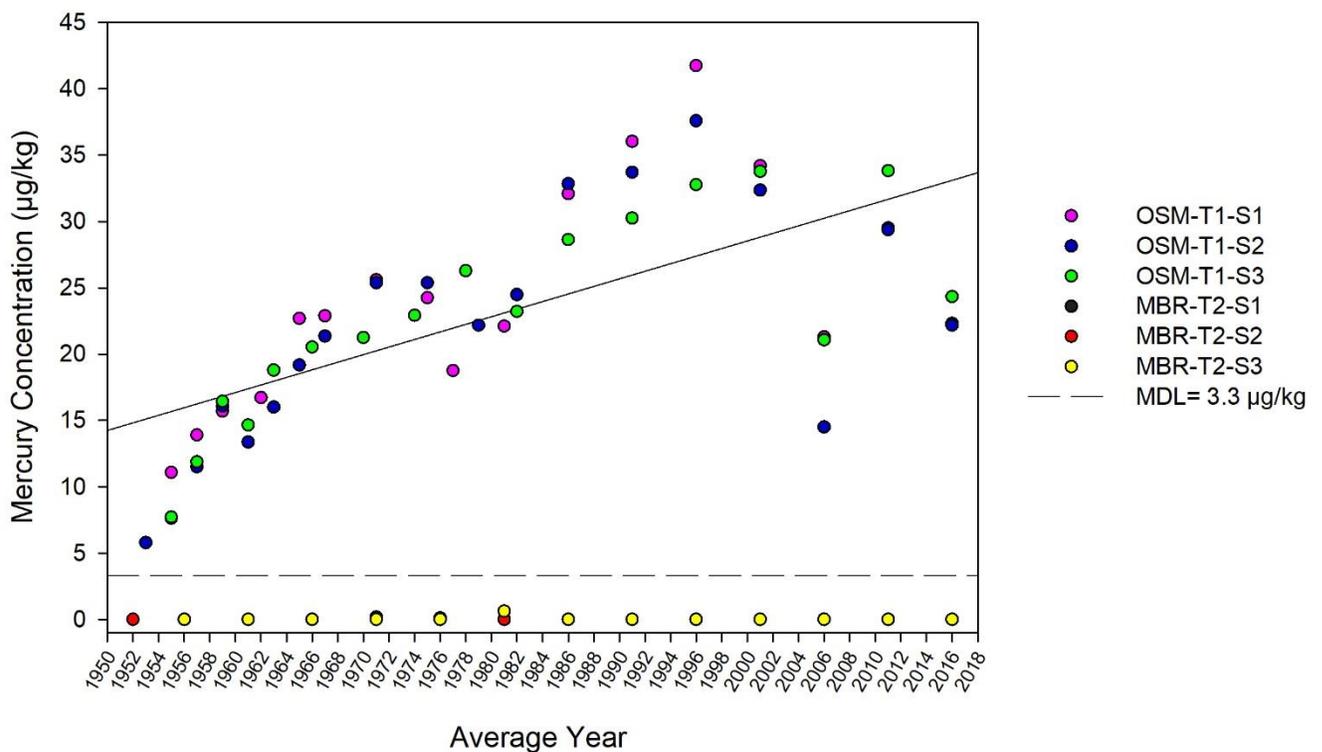


Figure 8: Total Hg concentrations from triplicate tree core samples, one from the Mitchell Brook Reference Site (MBR-T2) and one from the Old Stamp Mill gold mine tailings site (OSM-T1), with a method detection limit of 3.3µg/kg.

Preliminary sampling conducted to test the detection and reproducibility of mercury in tree cores took place in the summer of 2019 at the Old Stamp Mill and Mitchell Brook reference sites (Figure 8). Triplicate tree core samples taken from

Tree 1 at the Old Stamp Mill site showed consistency in Hg concentrations between rings sampled at different heights within the same tree, with an overall RSD of 8.4% between triplicate measurements ($n = 56$). The Old Stamp Mill Tree 1 triplicate measurements also display a significant slope of increasing Hg concentrations over time ($p\text{-value} < 0.05$), from which the hypothesis regarding an observable trend of increasing Hg concentrations through time was developed. Additionally, the consistently low Hg concentrations from the Mitchell Brook reference site triplicates, all of which fell below the MDL of this series (Figure 8), supported the initial idea that trees from highly contaminated sites will have measurable Hg concentrations incorporated into the tree rings. These results also supported the use of the Mitchell Brook site as an example of a non-contaminated background site from the HRM region.

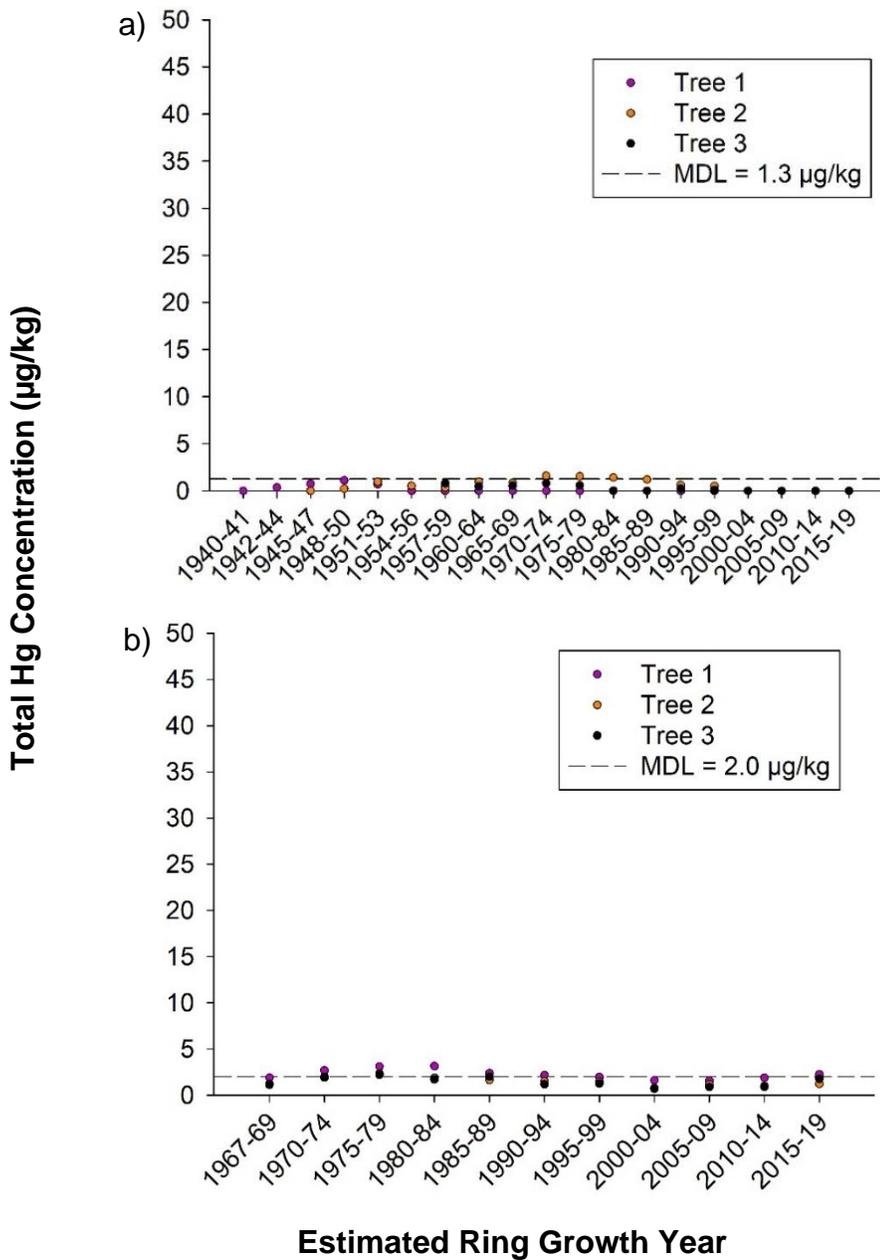


Figure 9: Total Hg concentrations (µg/kg) of 2020 tree core samples through time from the (a) Mitchell Brook and (b) Muddy Pond reference sites, with samples divided into approximately five-year time segments.

All samples from the Mitchell Brook and Muddy Pond non-contaminated reference sites were determined to have total Hg concentrations near or below of the

MDL (Figure 9). This data reinforces the use of these areas as background Hg concentration sites.

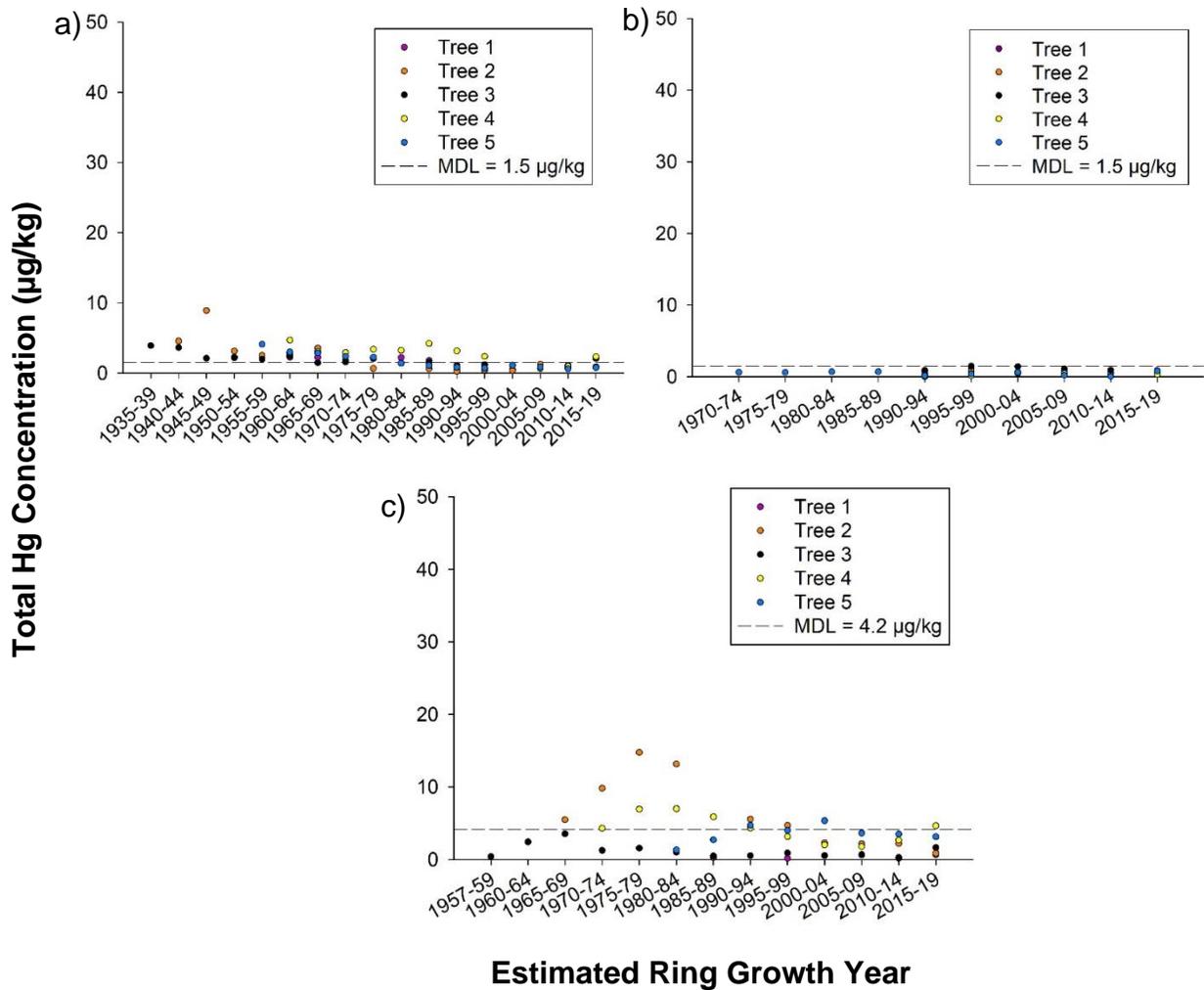


Figure 10: Total Hg concentrations (µg/kg) of 2020 tree core samples displayed through time, from the (a) Oldham (ODH), (b) Muddy Pond (MUP) and (c) Montague (MMT) gold mine sites, with samples divided into five-year time segments.

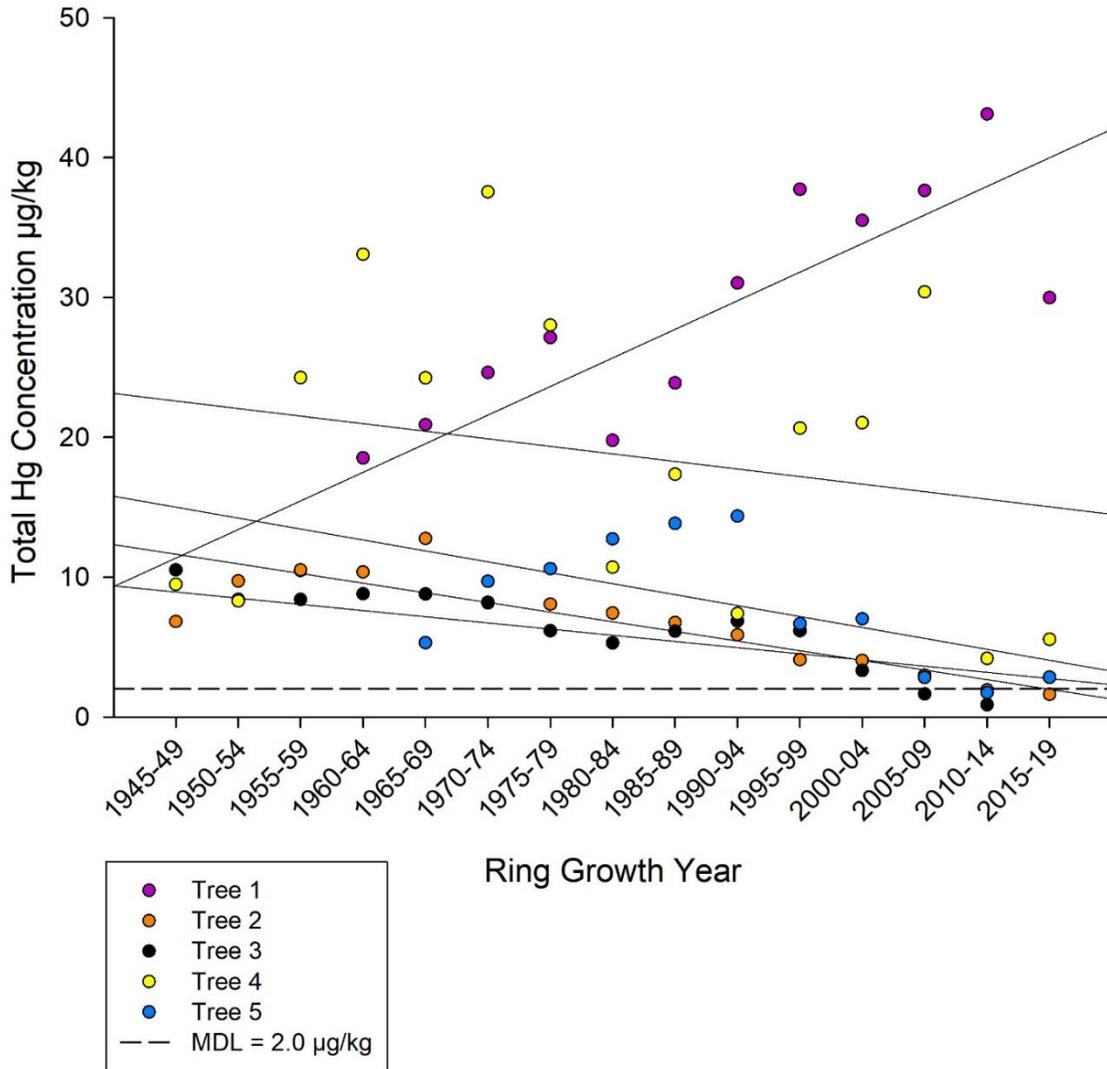


Figure 11: Total Hg concentrations ($\mu\text{g}/\text{kg}$) of 2020 tree core samples through time, from the Old Stamp Mill (OSM) tailings site, with samples divided into five-year segments and linear regression lines displayed.

Total Hg concentrations in tree core samples from the Muddy Pond tailings site were all below or very close to the MDL of $1.5 \mu\text{g}/\text{kg}$ (Figure 10 b). Tree core samples from both the Oldham (Figure 10 a) and Montague (Figure 10 c) tailings sites have some segments with concentrations above the respective MDL (1.5 and $4.2 \mu\text{g}/\text{kg}$).

Of the sites sampled in this study, the only significant linear temporal trends in tree core total Hg concentrations came from the Old Stamp Mill (OSM) tailings site (Figure 11). Total mercury in core segments from Tree 1 at the Old Stamp Mill site were determined to have a significant ($p < 0.05$) positive linear regressions ($p = 1.6E-4$; $r^2 = 0.74$) with mean ring year. Total mercury in Old Stamp Mill Trees 2 and 3 were found to have significant ($p < 0.05$) negative linear regressions, with p-values of $1.5E-6$ and $7.2E-5$, respectively. The total mercury in Old Stamp Mill Trees 4 and 5 had insignificant ($p > 0.05$) decreasing regressions with p-values value of 0.42 and 0.069. It should be noted that Old Stamp Mill Tree 1 is the same tree, which was sampled for preliminary work in the Summer of 2019, from which the hypothesis that a trend of total Hg concentrations in tree core samples increasing through time would be visible across trees sampled from different sites was based.

3.5. Comparison of Hg Concentrations in Surface Soil and Tree Core Samples

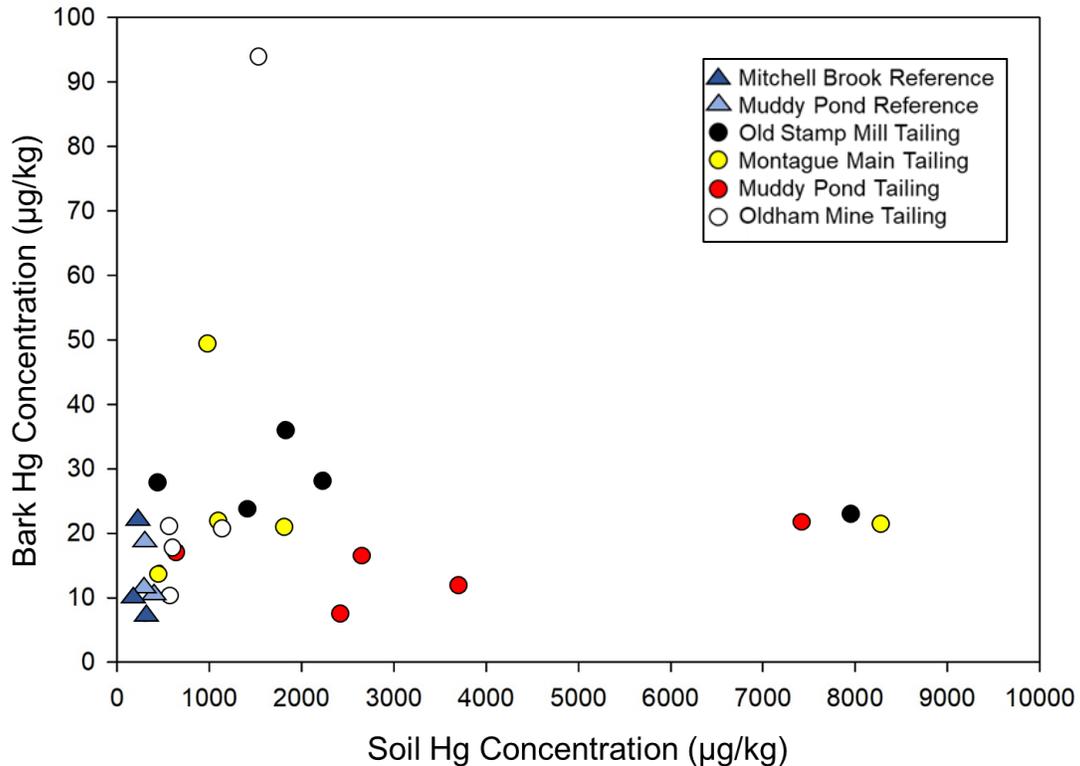


Figure 12: Comparison of bark and soil Hg concentration (µg/kg) from the trees sampled at the six HRM sampling sites; the Mitchell Brook and Muddy Pond Reference sites, Old Stamp Mill, Montague, Oldham and Muddy Pond tailings sites. Linear regression with a 95% confidence interval is displayed.

Bark and soil pairs, sampled from and adjacent to the same tree, were plotted to determine the presence of correlation in total Hg Concentrations in the two media (Figure 12). A Spearman correlation test was conducted, which found no correlation between any of the 26 (n) pairs, with a p-value of 0.08 (p-value > 0.05). A possible explanation for this lack of trend is due to the small number samples of both bark and soil that were collected at each tree.

4. Discussion

4.1. *Distribution of Mercury Concentrations in Surface Soils*

Mean surface soil Hg concentrations from all 5 background reference sites examined in this study were confirmed to be less than 500 µg/kg, reinforcing the use of these sites as reference for background non-contaminated surface soils in Southern Nova Scotia (Pannu et al. 2016). In order of increasing concentrations, the mean Hg values were calculated to be 68 ± 2 µg/kg from Lumsden Pond, 115 ± 5 µg/kg for the Wolfville Nature Preserve, 231 ± 54 µg/kg for Mitchell Brook reference site, 337 ± 57 µg/kg for Muddy Pond Reference, and 491.0 ± 6.1 µg/kg for the Black River Lake site (Figure 5). Soils in this area are underlain by bedrock from the Meguma Supergroup, made up of the meta-sandstone-dominated Goldenville Group, overlain by the slate-dominated Halifax Group (Parsons et al. 2012). Mercury concentrations in these bedrocks typically range between 1.2 – 7.4 µg/kg (O’Driscoll, et al. 2005). Wong et al. (2002) found bedrock Hg concentrations in the Caribou mining district to be in the 20 – 50 µg/kg range, due to natural bedrock composition. As such, bedrock at these sites is likely contributing to a background level of Hg in soils, in addition to deposition from atmospheric Hg.

Kang et al. (2019) found a significant positive linear relationship ($P < 0.001$) between soil organic content (SOC) and soil Hg concentrations at two sites in the Urumqi Valley, China, that were actively being contaminated by emissions from nearby coal-firing plants. In contrast, the Muddy Pond tailings site, which had the highest mean

soil Hg concentration, also had the lowest mean organic content in its soil samples, based on LOI calculations (Figure 5; Table 4).

Mean Hg concentrations in surface soils from the mine tailings contaminated sites were determined to be 877 ± 456 $\mu\text{g}/\text{kg}$ from Oldham, $3,366 \pm 2,280$ $\mu\text{g}/\text{kg}$ from Muddy Pond, $2,525 \pm 2,900$ $\mu\text{g}/\text{kg}$ from Montague, and $2,776 \pm 2,672$ $\mu\text{g}/\text{kg}$ from Old Stamp mill (Figure 5). Based on these mean Hg concentrations, all contaminated sites examined in this study were found to be elevated above the 500 $\mu\text{g}/\text{kg}$ threshold for acceptable Hg concentrations in soils near inhabited residential areas (CCME, 1999, 2017; Pannu et al. 2016). This is especially concerning as nearby residents are known to use the adjacent mine tailings sites as recreational areas, despite repeated warnings that the areas are highly contaminated (Corriveau et al. 2011).

However, the degree to which Hg contamination at these sites presents a risk to human health is dependant on which forms of Hg are present in these environments. Since this study determined total Hg concentrations only, further sampling at these sites would be needed to determine which species of Hg are present, and in what quantities. It is likely, however, that soil Hg concentrations consist largely of divalent Hg (Hg^{2+}), which is most commonly found bound interchangeably with inorganic and/or organic ligands present in sediment (Pannu et al. 2016). Collectively, the high concentrations of THg in soils from these sites was likely introduced by a combination of long-range atmospheric deposition, natural bedrock minerals and wind-blown particles from gold mine tailings.

There is also the potential for substantial methylmercury (MeHg) concentrations to be present within the soil and mine tailings. Winch et al. (2008) collected pore water

samples from the Seal Harbour and Lake Catcha gold mine tailings sites in Nova Scotia to test for the presence of sulfate reducing bacteria (SRBs), which are a key producer of MeHg. The results of their study found SRBs present in the tailings from both sites which, like the sites examined in this study, are low-lying wetlands (Winch et al. 2008). Winch et al. (2008) also noted that the site with the highest MeHg concentration also had the highest SRB presence, and that MeHg accumulation was highest in the more organic rich upper soil horizon. It was also noted that total Hg concentrations seemed to be the limiting factor in MeHg development across all sites (Winch et al. 2008). Based on the similarity and proximity of the Winch et al. (2008) sites it is likely that the sites in this study have SRBs present and similar MeHg production.

The movement of MeHg from these sites into nearby aquatic ecosystems could result in aquatic organisms, including those consumed by people, accumulating high concentrations of MeHg. For example, LeBlanc and Halfyard (2010) found an average Hg concentration of 0.62 mg/kg wet weight in fish collected from 13 gold mine tailings contaminated lakes in Nova Scotia (11 species, n = 172). This concentration in fish tissue is above the current CFIA safety guideline, which sets 0.5 mg/kg wet weight as the maximum acceptable concentration for Hg content in edible fish (CFIA 2011). However, the Hg content in fish from tailings-contaminated lakes (0.62 mg/kg wet weight) was not statistically different than the average concentration in fish from non-contaminated reference lakes, which was calculated to be 0.57 mg/kg wet weight (7 species, n = 45) (LeBlanc and Halfyard, 2010).

4.2. Distribution of Arsenic Concentrations in Surface Soils

Both reference sites (Mitchel Brook and Muddy Pond reference) had surface soil As concentrations greater than the CCME (1999) guidelines for residential areas (12mg/kg). The mean As concentration for Mitchell Brook was 38 ± 5 mg/kg, and 22 ± 4 mg/kg for the Muddy Pond reference site (Figure 6). These As concentrations are likely a result of underlying bedrock at these sites (the Meguma supergroup) (Parsons et al. 2012). It is quite common for soil As concentrations in Southern Nova Scotia to be higher than the Canadian average due to these arsenic containing rock deposits (Dummer et al. 2015).

Of the mine tailings sites examined, the soil with the lowest mean As concentration was from the Oldham tailings site (38.1 ± 22.3 mg/kg) (Figure 6). The Old Stamp Mill, Muddy Pond and Montague tailings sites, had mean soil As concentrations of 108.5 ± 39.6 , 871.8 ± 553.2 and $2,474.0 \pm 3,602.6$ mg/kg, respectively (Figure 6). The lower As concentrations from the Oldham site suggests lower levels of mine tailings contamination (Figures 6 and 8). The site with highest mean As concentration in soil is the Montague tailings site with $2,474.0 \pm 3,602.6$ mg/kg (Figure 6). Parsons et al. (2012) found soil As concentrations at the Montague Gold Mine site ranging between 1,720 – 41,300 mg/kg (Table 1). Here we find mean As concentrations at this site near the lower end of that range (2474.0 ± 3602.6). One explanation for the wider range of concentrations in Parsons et al. (2012) may be due to the collection of samples from multiple depths, while this study was focused on surface soils. Corriveau et al. (2011), found As concentrations ranging from 2,850 – 105,000 mg/kg in the <38 μ m fraction of surface soils (top 5 cm) sampled from three Nova Scotia gold mine tailings

sites. In comparison, the lower range observed in this study could be attributed to sampling from slightly off the main tailings, beneath the tree stands, whereas Corriveau et al. (2011) sampled directly from the uncovered tailings deposits themselves.

The source and speciation of arsenic is a topic that is not directly addressed in this study. However, based on other publications, the high As concentrations in Nova Scotia gold mine tailings are a product of arsenopyrite minerals released from crushed bedrock (Corriveau et al. 2011; LeBlanc et al. 2020). Once crushed these As containing minerals are more easily oxidized and transported by ground water or as wind borne particulate matter (Corriveau et al. 2011). Arsenite [As(III)] and arsenate [As(V)], are the two main oxidation states of arsenic that have been identified (Campbell and Nordstrom, 2014; LeBlanc et al. 2020). Both As(III) and As(V) are inorganic forms of As and are considered more toxic to biota than organic forms (Campbell and Nordstrom, 2014; LeBlanc et al. 2020).

4.3. Tree Core Hg Concentrations

Qualitative observations of skeleton plots developed for each tree sampled (Figure 7) suggest that annual tree growth is consistent within sampling sites. However, tree width and growth between sites was more variable, with trees ranging from an approximate average diameter of 15 cm at the Muddy Pond tailings site, to an average 30 cm diameter at the Old Stamp Mill site. This is likely due to differences in disturbance at each site, resulting in some tree stands being older and more developed than others, and therefore being larger in diameter. It could also be due to differences in nutritional availability at each site, with the trees that have more nutrients readily available growing more in a season than those at less nutritionally rich sites.

The 2019 preliminary sampling from two HRM Spruce trees, one at the Old Stamp Mill site, and one at the Mitchell Brook reference site, support the idea that there is consistency in Hg concentrations within a single tree (Figure 8). This preliminary information supported the decision to sample single cores from Spruce trees to provide a good representation of Hg concentrations within annual ring deposits of the tree. The data from these two trees also supported the hypotheses that tree cores sampled from reference sites associated with gold mining in the HRM would have consistently low or negligible Hg concentrations, while cores sampled from trees directly adjacent to tailings deposits would show a trend of Hg concentrations increasing through time, as are visible in Figure 8.

Both the Mitchell Brook and Muddy Pond Reference sites were found to have tree core Hg concentrations that were consistently at or below the MDL of the respective analytical runs (1.3 and 2.0 $\mu\text{g}/\text{kg}$) (Figure 9). This suggests that Hg is not highly incorporated into trees at these sites, supporting the assumption that trees from reference sites will not have significant Hg accumulation in annual tree rings.

The Oldham, Muddy Pond and Montague tailings sites were all found to have tree core Hg concentrations consistently below the method detection limit (MDL = 1.52 $\mu\text{g}/\text{kg}$ for the Oldham and Muddy Pond samples, and MDL = 4.16 $\mu\text{g}/\text{kg}$ for the Montague samples) (Figure 10; Figure 11). However, tree core samples from the Old Stamp Mill site were determined to have Hg concentrations consistently above the MDL (MDL = 2.01 $\mu\text{g}/\text{kg}$), however the temporal trends between trees had a high degree of variability (Figure 11). In fact, the only tree which displayed the hypothesized trend of increasing Hg concentrations with time was the Old Stamp Mill Tree 1 sample, which

was collected from the same tree that displayed this trend in 2019 preliminary sampling (Figure 8; Figure 11). All other trees sampled at Old Stamp Mill (trees 2-5) instead display varying trends of Hg concentrations generally decreasing through time.

The insignificant Hg content from the trees sampled at the Oldham, Muddy Pond and Montague sites could suggest that re-volatilization of soil-bound-Hg is not significantly occurring at these sites. If this is the case, then it can be assumed that the Hg concentrations consistently above the MDL at the Old Stamp Mill site are because greater levels of re-volatilization of soil-bound-Hg are occurring at higher levels at this site. Low soil Hg volatilization could be a result of the buffering effect tree canopies have on soil temperatures by creating cooler, wetter micro-climates under tree cover (Gaudio et al. 2017). In this scenario, the average local temperature increase would not yet result in an increase in Hg volatilization rates. However, this does not account for the high variability in Hg concentration trends within the Old Stamp Mill site. Therefore, further research quantifying Hg volatilization from soil at historical gold mine tailings sites in the HRM should be conducted to better understand its impact on Hg uptake and incorporation by Spruce trees at these sites.

The variations in tree core Hg concentrations at the Old Stamp Mill site may also be related to the micro-climate variations of individual trees. Micro-climates are defined as changes in conditions, such as temperature, moisture content, pH, and nutrient (and/or contaminant) availability over small distances within a larger ecosystem (Fan et al. 2020). Such changes can be caused by variations in physical landscape such as degree, direction and insolation of a slope, soil properties, vegetation cover, and hydrology (Fan et al. 2020). Therefore, within a single site micro-climatic changes

between trees may result in highly variable conditions that affect Hg volatilization and therefore incorporation into annual ring deposits.

4.4. Comparison of Hg in Tree Barks and Adjacent Surface Soil

No significant correlation was observed between Hg in tree bark and Hg concentrations in soil, which contrasts with the findings of other recent studies (Rimondi et al. 2020b; Chiarantini et al. 2016). Rimondi et al. (2020b) measured total Hg concentrations in the outermost 1.5 cm of sampled tree bark (n = 13) and found Hg concentrations to increase in comparison to total Hg concentrations from adjacent soil samples (< 2 mm fraction). However, Rimondi et al (2020b) did not clarify if the trend was significant. It should also be noted that Rimondi et al. (2020b) used average bark Hg concentrations (n = 8 per tree), as compared to one bark sample per tree in this study. Rimondi et al. (2020b)'s tree bark samples were found to range from 100 to 28,800 µg/kg total Hg, suggesting the study's sampling sites had significantly higher levels of Hg in the air than those examined in this study (THg ranging from 7.0 to 94.3 µg/kg). Chiarantini et al. (2016) also found a significant correlation ($R^2 = 0.95$, $p = 0.003$, $n=18$) between tree bark Hg and soil Hg concentrations for sites with higher mercury concentrations. Correlations between tree bark and soil Hg concentrations were only found at the sites where bark Hg concentrations ranged from 19 to 803 µg/kg (Chiarantini et al. 2016).

There are several explanations for the lack of significant correlation observed in this study. Firstly, it is possible that we did not have sufficient sample sizes to characterize the area. By collecting and analyzing a greater number of bark samples per tree, and reporting means and standard deviations potential outliers can be more

easily identified and eliminated from the total Hg dataset. Secondly, the role of mercury concentrations in air may be a key factor. The sites examined by both Rimondi et al. (2020b) and Chiarantini et al. (2016) were near the area of ongoing mining and geothermal energy activities which contribute to local gaseous elemental mercury (GEM; Hg⁰) concentrations in air peaking at 194 ng/m³, which is highly elevated above background GEM (3 – 5 ng/m³) (Cabassi et al. 2017). The HRM gold mine sites in this study were likely exposed to high levels of GEM in the past but may not be experiencing this in the present.

4.5. Conclusions

The results of this study found that most tree core THg concentrations from the Montague, Muddy Pond and Oldham gold mine tailings sites were below method detection limits (MDL) and did not display significant linear temporal trends. However, Spruce tree cores from the Old Stamp Mill site had THg concentrations consistently above MDL. At this site 4 trees displayed linear decreases in Hg concentrations with time and 1 tree displayed increasing Hg concentrations from ~1945 to present day. However, total Hg concentrations in surface soil at the Old Stamp Mill site was not significantly higher than at the other mine tailings sites studied. In addition, no significant correlation was observed between total mercury concentrations in tree bark and surface soils any of the sampling sites. At each of the 4 gold mine tailings sites examined, mean Hg and As concentrations in surface soils were greater than the Canadian soil guidelines for residential areas. This suggests that historical gold mine tailings sites in Nova Scotia are highly contaminated.

There are many areas for future research which should follow from this work. Specifically, further investigation into the speciation and distribution of Hg and As across different media (i.e. soil, air, plant life) would help to clarify the areas of highest concern these contaminants present to local residents and ecosystems. Additionally, further study into the conditions of gold mine tailings sites that would facilitate the presence of SRBs would be useful in determining the likelihood of MeHg production at these sites. Longer-term studies to assess the role of climate warming in Nova Scotia and the effects on Hg soil flux rates are needed. Site specific studies using Hg flux chambers or mobile monitoring stations (Pannu et al. 2016), would be useful to quantify flux rates at these sites. Further study of the Old Stamp Mill site, with a focus on the impact of tree micro-climates on the uptake and storage of contaminants, is recommended.

It is also recommended that future investigations of Hg and As in tree cores increase the amount of trees sampled per site, and include core, bark and soil samples. Sample collection of other bioindicators, such as lichens, which accumulate up Hg from the atmosphere would also be useful. The results of which would provide another mechanism to observe recent spatial distributions atmospheric Hg concentrations at these sites.

Appendix A – Supplementary Information

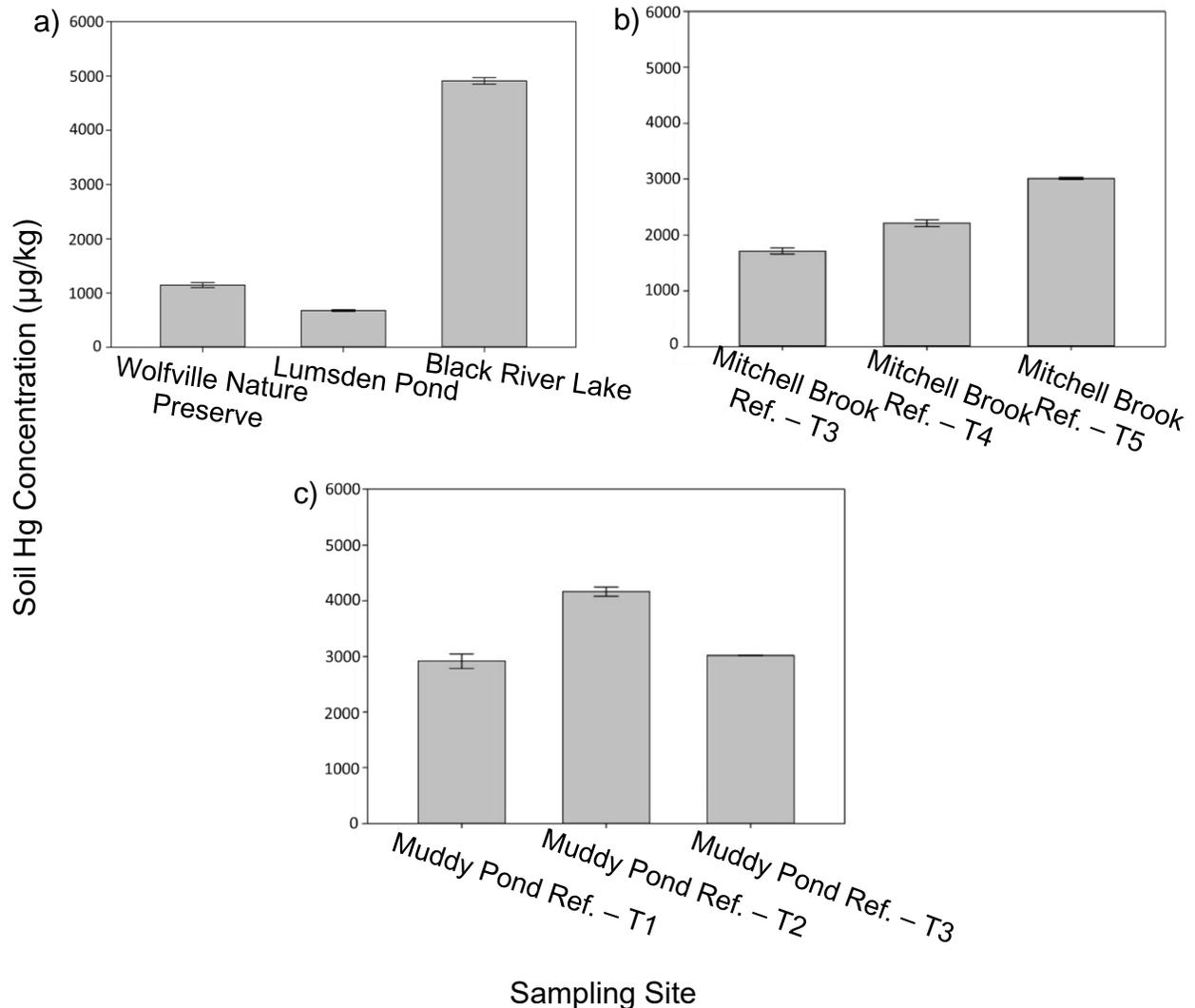


Figure 13: Soil total Hg concentrations ($\mu\text{g}/\text{kg}$) from the (a) Annapolis Valley, (b) Mitchell Brook and (c) Muddy Pond Reference sites, representing background soil Hg concentrations typical for non-contaminated surface soils in Nova Scotia. Soil samples were dried and sieved to the silt-clay fraction ($<177\ \mu\text{m}$). Soil total-Hg concentrations ($\mu\text{g}/\text{kg}$) for 3 sample locations from each site. SD bars shown are calculated from triplicate analyses of each sample. Maximum acceptable concentration of As in residential area soils ($500\ \mu\text{g}/\text{kg}$) is denoted by a red dashed line

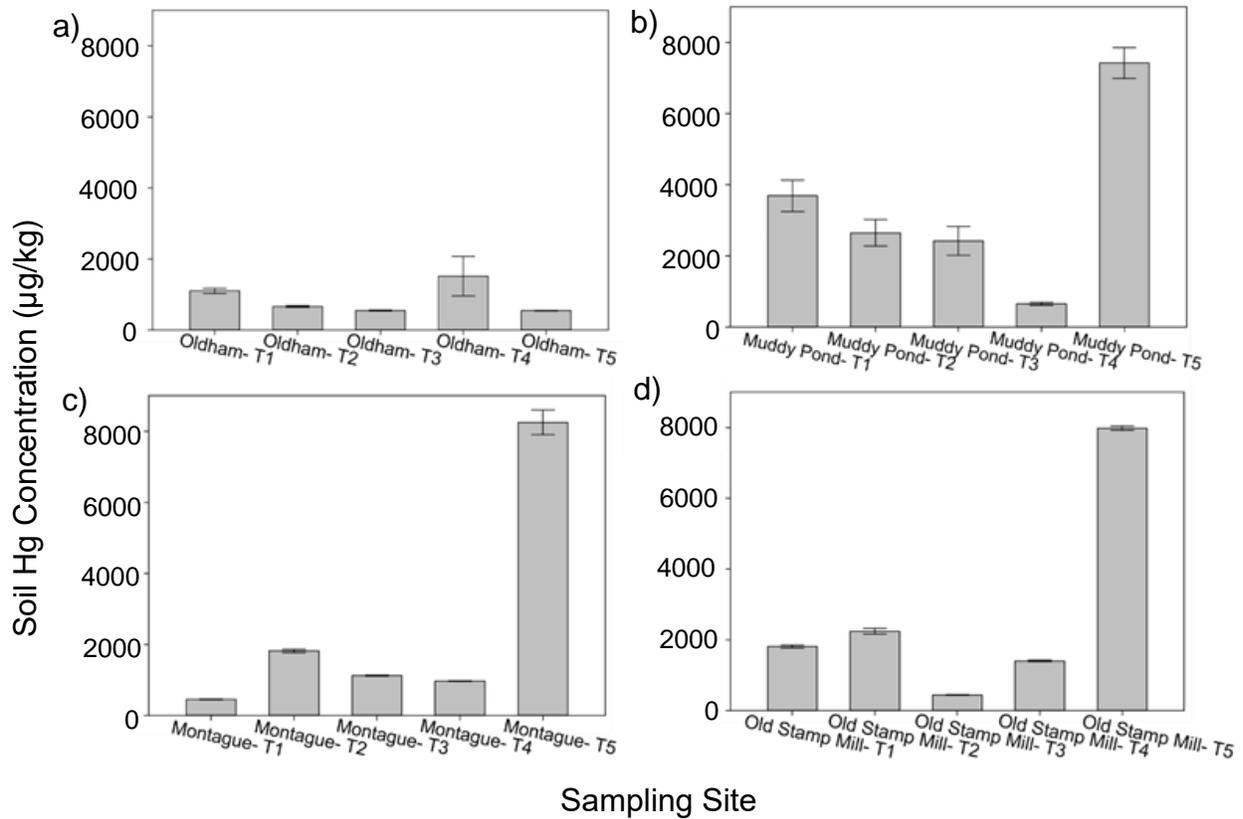


Figure 14: Surface Soil total Hg concentrations (µg/kg) from ground adjacent to sampled trees at the (a) Oldham, (b) Muddy Pond, (c) Montague, and (d) Old Stamp Mill mine tailings sites in the HRM. Soil samples were dried and sieved to the silt-clay fraction (<177 µm). Soil total-Hg concentrations (ug/kg) for 3 sample locations from each site. SD bars shown are calculated from triplicate analyses of each sample. Maximum acceptable concentration of As in residential area soils (12 mg/kg) is denoted by a red dashed line

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