Assessment of the Suitability of Tree Rings as Archives of Atmospheric Mercury Pollution using Tree Cores and Results of a controlled field experiment to assess the use of tree tissue concentrations as bioindicators of air Hg

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Hydrology

By

Matthew A. Peckham

Dr. Mae S. Gustin/Thesis Advisor

May, 2018
We recommend that the thesis prepared under our supervision by

MATTHEW A. PECKHAM

Entitled

Assessment Of The Suitability Of Tree Rings As Archives Of Atmospheric Mercury Pollution Using Tree Cores And Results Of A Controlled Field Experiment To Assess The Use Of Tree Tissue Concentrations As Bioindicators Of Air Hg

be accepted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Mae S. Gustin, Ph.D., Advisor

Peter J. Weisberg, Ph.D., Committee Member

Adam Z. Csank, Ph.D., Graduate School Representative

David W. Zeh, Ph.D., Dean, Graduate School

May, 2018
Abstract

Mercury (Hg), a toxic metal and known neurotoxin, is a widespread environmental contaminant. The element, and its speciated compounds, are released naturally through geothermal and volcanic activity, but global concentrations have increased due to anthropogenic sources such as coal-fired power plants and mining activity. Because of its ability to stay aloft in the atmosphere, Hg may travel vast distances from point sources, contaminating aquatic and terrestrial ecosystems. Atmospheric Hg may be assimilated via biological processes and sequestered within the tissues of trees.

The work presented in Chapter 1 explored whether tree rings are suitable archives for atmospheric Hg concentrations. Methodology, and the utility of dendrochemistry for the spatial and temporal assessment of atmospheric mercury were explored. We analyzed tree cores from California and Nevada, USA, choosing locations based on proximity to, or remoteness from, known past and present sources of Hg emissions. By revisiting National Park and BLM lands that were previously sampled, stability of Hg concentration over time was investigated through a dataset comparison. For the sites where sampling was successfully duplicated, similar historical trends were observed, suggesting that Hg concentrations are temporally consistent for the upland tree species; *Pinus jeffreyi*, *Pinus ponderosa* and *Pinus monticola*. Other tree species that occur in arid regions with low primary productivity (*Pinus monophylla*) are less suitable for use as temporal Hg archives.
The work presented in Chapter 2 examines the effect of local ambient air Hg concentrations on accumulation in tree tissue, and was investigated using a 2-year field experiment that entailed movement of nursery trees (Pinus nigra) from Oregon, to strategically chosen locations in Nevada and California. Needles, bark, and tree rings were sampled at regular intervals and analyzed for THg to survey concentration differences between growth occurring pre-experimental placement and growth occurring at each discrete location.

Needle Hg concentrations increased significantly relative to the control. Concentrations for spring needles were low and increased during the growing season, indicating potential resorption. In the first and second years, both inner and outer bark concentrations increased significantly, as did the outermost tree rings. HgBr₂ root spiking treatments produced no significant impacts for above ground tree tissues, following. The results from this study highlight the potential for Pinus nigra needles, bark, and tree rings to serve as widespread and cost-effective proxies for atmospheric mercury concentrations, also aiding in the scientific comprehension of tree Hg uptake and behavior, post assimilation.
Acknowledgements

Financial support for this study was provided by the National Science Foundation (NSF), grant number 1461314. The opinions presented within are not representative of the NSF. The authors wish to thank the permitting branches of the National Park Service and the Bureau of Land Management, as well as the University of California- Santa Cruz (UCSC) Arboretum. We are grateful for the assistance provided by Thomas Dilts and by graduate students Miranda Redmond and Alexandra Urza at the Weisberg Landscape Ecology lab at the University of Nevada, and the careful work of UNR students; Jennifer Arnold, Cameron Bennet, Logan DiStefano, Madeleine Lohmn, Addie Luippold, Maggie Vargas-Estrada, Natasha Wesely, and Nicholas Wong who assisted with field sampling, tree care and sample analysis at the UNR Gustin Biogeochemical lab. Additionally, Jaycee Martinez and Scott Conrad from UCSC are thanked for their assistance with tree care and laboratory analysis, along with David Metz for his help addressing challenging site logistics.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abstract</strong></td>
<td>i</td>
</tr>
<tr>
<td><strong>Acknowledgements</strong></td>
<td>iii</td>
</tr>
<tr>
<td><strong>List of Tables and Figures</strong></td>
<td>vi</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>vi</td>
</tr>
<tr>
<td>Supplemental Information, Chapter 1</td>
<td>vii</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>viii</td>
</tr>
<tr>
<td>Supplemental Information, Chapter 2</td>
<td>viii</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td>Overview</td>
<td>1</td>
</tr>
<tr>
<td>Mercury</td>
<td>2</td>
</tr>
<tr>
<td>Records of Hg Pollution</td>
<td>5</td>
</tr>
<tr>
<td>Ice cores</td>
<td>5</td>
</tr>
<tr>
<td>Lake sediments</td>
<td>6</td>
</tr>
<tr>
<td>Peat bogs</td>
<td>7</td>
</tr>
<tr>
<td>Soil Records</td>
<td>9</td>
</tr>
<tr>
<td>Dendrochronology /Chemistry</td>
<td>11</td>
</tr>
<tr>
<td>Tree Growth and Physiology</td>
<td>14</td>
</tr>
<tr>
<td>Pathways to Assimilation</td>
<td>17</td>
</tr>
<tr>
<td>Foliage</td>
<td>21</td>
</tr>
<tr>
<td>Bark</td>
<td>22</td>
</tr>
<tr>
<td>Tree rings</td>
<td>24</td>
</tr>
<tr>
<td>Literature cited</td>
<td>28</td>
</tr>
<tr>
<td><strong>CHAPTER ONE</strong></td>
<td>42</td>
</tr>
<tr>
<td>Abstract</td>
<td>43</td>
</tr>
<tr>
<td>Introduction</td>
<td>44</td>
</tr>
<tr>
<td>Methods</td>
<td>48</td>
</tr>
<tr>
<td>Results</td>
<td>57</td>
</tr>
<tr>
<td>Discussion</td>
<td>67</td>
</tr>
<tr>
<td>Conclusions</td>
<td>72</td>
</tr>
</tbody>
</table>
List of Tables and Figures

Chapter 1

Table 1-1: Summary of mercury concentrations in tree-rings and other woody tissue modified and updated from Wright et al. 2014.

Table 1-2: Statistical comparisons for trees in this study and for Wright et al. 2014.

Figure 1-1: Map of all sampling locations for chapter 1.

Figure 1-2: Intra-tree linear regression of tree core mercury concentrations of 2 arbitrarily chosen trees, illustrating variable correlative nature of tree cores.

Figure 1-3a: Mean UTC sample core concentrations for the complete historical period.

Figure 1-3b: Mean LTC sample core concentrations for the complete historical period.

Figure 1-4: Mean Hg concentration comparisons for UTC, LTC with standard error. UTC is shown in blue, LTC is dashed red line. Labels indicate total tree cores analyzed.

Figure 1-5: Tree ring mercury profiles in 5 year increments, for 2 arbitrarily chosen trees. Upper and lower watershed sites; top row shows untreated data, row 2 is normalized by ring width, and row 3 is normalized by full core mean concentration. Note differing y-axis scales. Same trees used in Figure 1-2 regression plots.

Figure 1-6: Arid site profiles showing mean values for 2015 collection (n= 10 trees) versus cores collected by Wright (n= 3). Error bars (representing standard deviation).
Figure 1-7: Mean National Park tree core Hg concentration profiles for this study in ng g⁻¹, compared to values of Wright et al. 2014. *Error bars* denote standard deviation.

*Supplemental Information, Chapter 1*

**SI Table 1-1**: Mean Hg concentrations for 5-year time increments at all locations for this study.

**SI Table 1-2**: Pearson correlation test values for the Thomas Creek location, between cores of the same trees.

**SI Table 1-3**: Pearson correlations between cores of 3 arbitrarily chosen trees at UTC and LTC sites, normalized by ring width and by mean Hg concentration value.

**SI Figure 1-1**: Correlation (Pearson) plots between cores for each TC tree. UTC top, LTC below.

**SI Figure 1-2a**: UTC sample cores averaged for each tree within the high elevation stand. *N* = 10 trees, *n* = 3 cores per tree. UTC data gap resulted from selective focus on specific time spans. Dotted lines represent trees cored from multiple directions; solid lines are trees cored from one direction.

**SI Figure 1-2b**: LTC sample cores averaged for each tree within the lower elevation stand. *N* = 10 trees each site, *n* = 3 cores per tree. Break in LTC data resulted from selective focus on specific time spans. Note differing time scales between UTC and LTC.
Chapter 2

Figure 2-1: Map of all sampling locations for chapter 2.

Figure 2-2: Post HgBr₂ spiking concentration data distributions for (in order) new needles, growth ring 2017, inner bark and outer bark (grouped by color, alternating from non-spiked to spiked).

Figure 2-3: 2016, 2017 needles (including spring 2017) compared to initial control concentration.

Figure 2-4: Concentration values by site for 2016 and 2017 sampling year (outer growth ring only), shown with 2015 control concentration data (all growth rings included).

Figure 2-5: 2016 and 2017 concentration data for each site compared to the initial 2015 control tree rings by year.

Figure 2-6: Inner and outer bark layer concentration distribution.

Supplemental Information, Chapter 2

SI Figure 2-1: Sampling method depiction for selecting needles for Hg analysis.

SI Figure 2-2: Sampling method depiction for bark layers. A. denotes the outer bark layer from a harvested cross-sectional trunk disk. B. indicates the inner bark layer.
Introduction

Overview

Tree components, such as foliage, bark and tree-rings, are receiving increased attention as naturally occurring, widespread environmental biomonitoring tools for quantification of mercury (Hg) pollution (Chiarantini et al. 2016, Jung and Ahn 2017, Maillard et al. 2016, Naavrátíl et al. 2017, Wright et al. 2014). Trees can provide insight into current and historical patterns of air pollution by acting as stationary proxies, assimilating atmospheric constituents both passively and actively, depending on tissue type (Abreu et al. 2008, Arnold et al. 2018, Baes and McLaughlin 1984, Blackwell and Driscoll 2015, Cocozza et al. 2016).

Estimation of atmospheric mercury (Hg) is deserving of attention, due to its propensity for long-range transport, potential toxicity, and its bioaccumulative tendency within aquatic food webs (Boening 2000, Schroeder and Munthe 1998, Selin et al. 2009). Monitoring equipment for atmospheric Hg is costly and requires highly trained technicians and frequent maintenance. Thus, complex atmospheric Hg dispersal patterns remain poorly characterized (Eagles-Smith et al. 2016). Given their spatial coverage, trees may offer a simple and cost-effective solution for many atmospheric monitoring applications, offering samples in the form of tree-rings, foliage and bark layers.

This project focused on refining sampling methods for various tree tissue types, examining the efficacy of using tree ring Hg concentrations as a record of historic and
spatial atmospheric conditions, and studying the Hg assimilation response of young
trees placed in strategic locations of known atmospheric Hg concentrations. The
objective was to build and expand upon current understanding of tree Hg assimilation,
sequestration and behavior once incorporated into tree tissues. Numerous, interrelated
projects have converged on the idea that most tree Hg is of atmospheric origin, as
opposed to soil (Arnold et al. 2018, Chiarantini et al. 2016, Fleck et al. 1999, Obrist et al.
2016 and many more). In this work, tree ring Hg was analyzed to determine the
consistency of intra and inter-tree temporal concentration patterns. Trees from a
former study were resampled in an effort to quantify the degree Hg ring permanence
once assimilation has taken place. Additionally an effort was made to characterize
uptake rates and mechanisms within small artificial “stands” of young trees placed in
areas experiencing unique environmental conditions, and varying concentrations and Hg
species. Specifically, how do atmospheric Hg and environmental conditions
synergistically influence tree tissue concentrations? This document discusses these
research questions in 2 chapters contained herein.

Mercury

Mercury (Hg) is a complex and extraordinary transition metal that maintains
liquid phase at ambient temperatures and pressures. Three oxidation states exist; Hg⁰,
Hg⁺¹ and Hg⁺² (Schroeder and Munthe 1998). It is considered a soft acid and forms
stable bonds with soft bases, including sulfides, thiols and various sulfur containing
ligands. Cinnabar (HgS), is the most prevalent mineralized form of Hg (Boening 2000).
Elemental mercury (Hg⁰) is minimally water soluble and fairly inert; however, its high vapor pressure (0.18 Pa) makes the monoatomic gaseous form significant (Lehnherr 2014). A relatively long atmospheric residence time of ~1.25 years facilitates dispersal, often at great distances from the original source, contributing to challenges in understanding its biogeochemical cycles (Fitzgerald and Lamborg 2014). Elemental Hg and gaseous oxidized Hg compounds are released to the atmosphere naturally through volcanic and geothermal activity, but concentrations are thought to have increased several-fold since the Industrial Revolution, primarily due to sources such as coal-fired power plants, cement production and artisanal mining activity (Futsaeter and Wilson, 2013). The atmosphere is the primary vehicle for Hg dispersion (Schroeder and Munthe 1998). The +1 (rare) and +2 oxidation states form salts that if soluble, are bioavailable and toxic (Boening 2000). In the natural environment, Hg⁺² has potential for biological methylation, the rate of which is controlled by microbial communities of mostly sulfur-reducing bacteria. Other abiotic methylation pathways exist within the environment, but are not well characterized. Other Hg compounds may be human-induced, and used for agricultural and/ or industrial applications (Boening 2000). Monomethylmercury (MMHg) if formed in aquatic environments or wetlands, can bioaccumulate up aquatic food webs from algae, to zooplankton, fish, and finally the apex predator level, which includes humans (Lehnherr 2014).

As a known neurotoxin and widespread environmental contaminant, Hg began receiving greater attention after widespread monomethylmercury (MMHg) poisoning in
Minamata and Niigata, Japan. Factory wastewater containing MMHg was discharged into the ocean, where the toxic compound bioaccumulated in fish and other aquatic organisms - major food sources to the local population. Many deaths, poisonings, and other health concerns were reported during this catastrophic event, resulting from what is now called Minamata disease. Paired with increasing global emissions, the mass poisonings in Japan and an incident in Iraq involving ingestion of grain coated with a Hg-based pesticide, resulted in the composition of an agreement called the Minamata Convention. Spearheaded by Norway and Switzerland, the convention specifically seeks to address and limit the long-range transport and bioaccumulation of mercury through comprehensive and legally binding policy. As of 2018, 128 countries signed the agreement for future compliance with the Minamata Convention in an increasingly globalized effort to curb the spread of this contaminant (http://www.mercuryconvention.org/).

Recent studies have increased the understanding of Hg emission and deposition within the scientific community, however many uncertainties still exist within the realm of Hg biogeochemical cycling (Fitzgerald and Lamborg 2014, Jaffe et al. 2014, Obrist et al. 2016, Zhang et al. 1995). In gaseous form, ~95-99% of Hg in the atmosphere was thought to be elemental (GEM). However, current work demonstrates that gaseous oxidized mercury (GOM) can make up 25% of Hg in the air depending on location (Gustin et al. 2015). Currently, science lacks an effective means of tracking atmospheric Hg concentrations through time.
Records of Hg Pollution

Detailed analytical work with sediment, lacustrian, peat bog, and ice-cores have yielded important paleoenvironmental results concerning historical Hg pollution (Beal et al. 2015, Chellman et al. 2017, Enrico et al. 2017, Heyvaert et al. 2000, Schuster et al. 2002, Nóvoa-Muñoz et al. 2008, Qiu et al. 2006). It is necessary to use all available techniques in order to increase understanding and constrain the data surrounding past emissions. Existing uncertainties in historical pollution propagate into modern estimations of Hg in the environment and also future predictions (Beal et al. 2015).

Ice cores

Glacial layers have considerable utility in elucidating Hg deposition trends through time. Although glacially deposited Hg may be subject to photoreduction, revolatilization and/ or meltwater processes altering chemical composition (Beal et al. 2015, Schuster et al. 2002). Work by Schuster et al. (2002) provided compelling fine-scale deposition data from the Upper Fremont Glacier of Wyoming, USA. Ice-cores dated from 1720 to 1993 were collected and 97 samples were analyzed for total mercury (THg). Data resulting from this work suggested Hg deposition was from both regional and global sources, and that the THg signal had anthropogenic as well as natural origin, however over the last 100 years, 70% of THg was from anthropogenic sources. Unlike the results of sediment-core records which indicate a 2-7 fold increase in THg input during the Industrial Revolution, the Fremont ice-core showed a 20 fold
increase in this study. Schuster et al. also suggested that increases after ~1850 may have been caused by increased Hg volitization due to the California Gold Rush, however most sediment-core studies do not note this increase (Drevnick et al. 2016). The last 10 years for both ice and sediment-cores indicate a decline resulting from decreased atmospheric deposition.

Chellman et al. (2017) revisited the Upper Fremont Glacier ice-cores in an attempt to improve upon Schuster’s seminal work and to also resolve dating discrepancies by synchronizing glacier water isotope ratios to a nearby dated tree-ring chronology. Comparisons were also made with lake sediment and other ice-core records from North America and Greenland. Chellman et al.’s (2017) work resulted in a revised chronology with time-scale discrepancies of up to 80 years, while remaining congruous with established age controls present within the core strata. As a result of the 80 year shift, the elevated Hg noted by Schuster et al. (2002) following the onset of the California Gold Rush, became consistent instead with industrial emissions during the 1900’s. This work also strengthened correlations with ice-cores from Greenland and North America as well as lake sediment-cores.

Lake sediments

Similar to ice-cores, records of historical pollution have been reconstructed using lake sediments (Drevnick et al. 2009, 2016, Fitzgerald et al. 2005, Heyvaert et al. 2000). Lake sediments are clearly not affected by factors confounding ice core chronologies,
and may be less susceptible to the effects of photoreduction, particularly in deeper lakes. However losses in Hg may result from the cultural eutrophication and warming of lakes. Enhanced regional oxidation of GEM may also factor largely in increased Hg evasion from the lake surface (Drevnick et al. 2009).

Drevnick et al. (2009) reported Hg flux approximations for the sediments of Lake Tahoe, California-Nevada, USA that were lower than other global estimates. Flux between preindustrial and modern sediment layers were 2 and 15-20 µg m⁻² y⁻¹ (respectively), for a ratio of 7.5-10; far higher than the widely accepted global average of ~3. The modern sediment Hg flux found by Drevnick et al. (2009), while high by global standards, was only ~1/3 of what was found in Heyvaert et al. (2000). These wide discrepancies, both found in studies of Lake Tahoe, are illustrative of the problems still existing for these types of records, and were hypothesized to result from imprecise sedimentation rate modeling, faulty data treatments and possible RGM enhancements (Drevnick et al. 2009).

**Peat bogs**

It is widely accepted that peatbogs and other wetlands facilitate MMHg production due to anaerobic conditions and the presence of methylating bacteria, specifically sulfate (SO₄) reducing bacteria (SRB). Because of the health risks associated with MMHg, it is therefore important to examine SO₄ additions as related to Hg sequestration and evasion from wetland areas. Fritsche et al. (2014) studied peatbog
elemental Hg (Hg⁰) evasion, as influenced by sulfate additions. Analysis indicated that Hg⁰ evasion is suppressed with elevated SO₄ inputs, and that Hg binding to S in organic matter may result in decreased Hg⁰ volatilization or increased transport by alternative means, such as runoff.

Peatbogs offer other unique insights into Hg cycling, concerning long-term sequestration of Hg within the peat layers, providing historical insight into atmospheric Hg concentrations. Enrico et al. (2017) used isotopic signatures of Hg⁰ to reconstruct a millennial-scale concentration record by anchoring historic dry deposition inputs to those detected in more modern peat layers, which were in accord with monitoring data. Enrico et al. (2017) not only detected the same Industrial Revolution-related increase as shown in other studies, but hypothesized that isotopic shifts aligning with the medieval and Renaissance period were related to biomass burning and deforestation.

Clearly, only specific geographical locations maintain the inundated, anoxic conditions needed for peatbogs to be reliable archives for Hg deposition. Fritsche et al. (2014) recorded significant mercury evasion from peat bogs in Sweden, possibly diminishing their utility for the reconstruction of historical Hg deposition and perhaps other elements. Hg concentrations within the peatbogs are sensitive to the spatially and temporally variable nature of seasonal water inputs and atmospheric chemistry of depositional inputs. Additionally, human-caused disturbances and the activity of soil microorganisms also have a negative effect on the analysis of peat for trace metals (Lepp 1975).
Soil Records

Sedimentary layers in the earth offer another tool for understanding Hg deposition and distribution patterns (Jung and Ahn 2017, Nóvoa-Muñoz et al. 2008, Obrist et al. 2015, Qiu et al. 2006). Globally, for terrestrial ecosystems—soil, in particular harbors over 90% of the total Hg load (Engle et al. 2006, Grigal 2003, Schwesig and Matzner 2000). Although soils tend to be high in Hg, (both Hg and Pb bind easily to organic material) sediment horizons tend to be less useful for historical reconstructions, due to factors like leaching, differing chemical affinities for each horizon, human disturbance, and mixing by burrowing animals (Klaminder et al. 2008). In Klaminder et al. (2008), minimally disturbed, organic, podzolized soil horizons (having many similarities with peat) were investigated in boreal forests of northern Sweden and were found to be suitable “semi-archives”. Lead isotope ratios were determined from ombrotrophic peat cores and varved lake sediments, and were bracketed with the boreal soil data set in order to verify vertically stratified concentration fluxes. This study did not reconstruct extended historical records but rather, demonstrated preservation of a significant heavy metal pulse (1950-1980) near a smelter and a decreasing concentration gradient with distance. The methods and findings of this study align with those of Jung and Ahn (2017), Nóvoa-Muñoz et al. (2008) and Qiu et al. (2006), who tested soils near a fertilizer plant, coal-fired power plant and an area with extensive cinnabar mining (respectively).
Soil horizons have the greatest utility in tracking point pollution from past and present sources, and are of less use for finer scale, long term monitoring of historical Hg pollution. Obrist et al. (2016) found that western United States soils were generally high in Hg, although admitting to a bias toward Hg-enriched sites. They also noted land cover and vegetation types and found soils from forested land were higher in Hg than other land cover types.

The ice-core, peatbog, lake sediment and soil horizon methods discussed above may be constrained by one or more factors including latitude, elevation, climate, soil pH, leaching rates, geologic parent material, photoreduction rates, topography, hydrogeology and others (Enrico et al. 2016, Fritsche et al. 2014, Klaminder et al. 2008, Schuster et al. 2002, Watmough 1997). Additionally, these mediums accumulate Hg slowly and may be difficult to accurately date. Further, many mechanisms exist that facilitate re-emission of the metal from terrestrial and aquatic ecosystems back into the atmosphere (Fitzgerald and Lamborg 2014, Fritsche et al. 2014, Gustin 2011, Obrist et al. 2016, Zhang et al. 1995). The passive nature of Hg collection, and the potential for re-emission once deposited also confound concentration data accuracy for several of these methods. Living organisms like trees- actively assimilating, and sequestering constituents of the ambient local environment may help to more completely quantify historical Hg concentration pattern.
**Dendrochronology /Chemistry**

The science of tree ring dating, or dendrochronology, was originally developed by an astronomer named A.E. Douglass (Fritts and Swetnam 1989). The practice is based upon the comparative analysis of tree-ring characteristics, through a practice called cross-dating. By counting rings and matching patterns of narrow (usually considered drought years) and wide rings, it becomes relatively easy to determine the exact year that each ring was formed. Cross-dating was initially developed to determine the age of supporting beams and charcoal pieces left in abandoned Navajo hogans (Stokes and Smiley 1996). The technique has since been applied to detecting past forest disturbances such as fires, floods, rockslides and drought (Fritts and Swetnam 1989). Dendrochronologists operate under the assumption that trees from the same location will have similar ring patterns due to the unique environmental conditions found there.

By matching ring growth trends between multiple samples, it is possible to identify and overlap patterns, creating a “master chronology” that is a general growth pattern record for a specific species at a location, based on shared pattern attributes (Stokes and Smiley 1996).

Dendrochemistry is the measurement and environmental interpretation of elements in tree rings, expanding upon the chronological dating practices described above. Dendrochemists operate under the pretense that constituents of each growth ring represent the environmental conditions at the site during a specified year (Watmough 1997). By isolating and analyzing specific growth years, ion concentration
data may be amassed for specific years or time periods. This specialized branch of
science has helped to shape scientific comprehension of past climate and pollution
trends, and is still being developed today. The study of dendrochemistry has proven
useful in tracking changes in both atmospheric and soil deposition of elements (ex: Al,
Ba, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Zn) (Baes and McLaughlin 1984, Berish and

There are uncertainties associated with dendrochemical pollution trend
analyses. Trees are not passive recorders of environmental change (Smith and Shortle
1996) like other mediums. It is important to note that an understanding of tree biology
is needed to interpret inter-annual tree-ring and foliar concentration trends. Dendrochemists assume that chemical constituents of individual tree-rings represent
the ambient environmental conditions during each ring’s formation. Unfortunately,
physiological differences between tree species and the behavior and affinities of
individual pollutants being tracked can greatly confound the objectivity of a tree-ring
study (Watmough 1999). For instance, elemental movement within a tree is generally
confined to the physiologically active sapwood that is the most recent growth where
active water transport still occurs. Also, within the sapwood, elements have widely
varying behavior. For instance, As, Na, and Mg are believed to have greater tendency for
movement, and elements like Pb, Cd, and Al have far less (Padilla and Anderson 2002,
Cutter and Guyette 1993). Ice and sediment cores, being passively deposited, lack several complications that tree uptake mechanisms present; however, with continued study, these issues can be reconciled and it may be argued that disadvantages are outweighed by the following benefits.

Trees have several advantages over other methods of studying past pollution. The wide environmental tolerance and spatial extent of trees, in many cases, give them advantages over geographically limited, ice and sediment-cores. In numerous studies, analyses of tree ring constituents has been used to draw conclusions regarding diverse historical phenomena such as volcanic eruptions, groundwater quality, and acid rain (Baes and McLaughlin 1984, Frelich et al. 1989, Sheppard et al. 2008). Periods of both natural and anthropogenically-caused atmospheric pollutant emissions are actively assimilated into tree tissues as they grow, capturing information regarding mean yearly air concentrations rather than simple deposition as noted for other mediums. Dendroanalysis has also led to a greater understanding of historical trace-metal deposition caused by combustion of coal and petroleum products, mining practices, ore refining, and other anthropogenic factors (Baes and McLaughlin 1984, Hagemeyer et al. 1992, Kirchner et al. 2008, Sheppard et al. 2008, Watmough 1997, 1999, Zhang et al. 1995).

Trees also archive long historical records, making them useful environmental proxies (Berish and Ragsdale 1985, Padilla and Anderson 2002, Sheppard and Funk 1975, Watmough 1999, Zhang et al. 1995). Additionally, removing core samples from a tree
using an increment boring tool has very little, if any effect on tree health if done properly (Mantgem and Stephenson, 2004). Healthy trees fill the holes with sap and regrow a protective bark layer in order to minimize the chance of infection or insect damage. This may increase the difficulty in locating and resampling the exact same trees within a dense stand.

Lepp (1975) cites desirable organismal characteristics for the study of past trace-metal pollution as; being long-living, stationary, in possession of quantifiable uptake pathways, having seasonally accumulating growth that is easily dated, and can be non-destructively sampled. While uptake pathways are not satisfactorily quantified, trees align well with the remaining characteristics making them useful spatial and temporal proxies of air concentration.

**Tree Growth and Physiology**

Trees can be divided into several categories; 1) the palms and bamboos that are perennial grasses with woody stems, are monocotyledons; 2) the hardwoods, that are typically deciduous with net-veined leaves, and are dicotyledonous angiosperms bearing fruit-covered seeds; and 3) the softwood conifers, which bear exposed seed cones and are typically evergreen, having scaled, or narrow needle-shaped leaves. Conifers are gymnosperms and have a more primitive vascular structure than the angiosperms (Higuchi, 1997).
Photosynthetic processes in the leaves of the crown draw CO\(_2\) gas into the leaf, through stomata, regulated by guard cells embedded in a waxy cuticle. These openings regulate gas exchange with the environment and are called stomata (Taiz and Zeiger 2002). Within the *Pinus* genus, stomata are located on the top and bottom of the leaf, while most deciduous species have them on the top of the leaf only. Atmospheric exchange includes CO\(_2\) assimilation and the expulsion of excess water vapor. CO\(_2\), once drawn into the leaf structure, is photosynthetically synthesized into sugars (primarily in the sucrose form) and transported around the tree via the phloem, a vascular tissue layer beneath the outer bark. These sugars are used for energy to produce lignin, cellulose, proteins and others minor compounds (Higuchi 1997, Taiz and Zeiger 2002). Photosynthetic processes in the leaves (involving transpiration) induce water transfer from the roots upward, within the xylem; a supportive structure that transports minerals and water from the root system upward to the rest of the plant-thus, enabling photosynthesis. The Soil, Plant, Atmospheric Continuum (SPAC) represents, in effect a continually flowing ion-exchange column within a tree, being driven by atmospheric water potential (Smith and Shortle 1996, Van De Geijn and Petit 1979). Beyond water and nutrient transport, these important vascular tissues also protect the tree exterior from injury and give rigid support to the stem and crown (phloem and xylem, respectively)(Higuchi 1997).

Stems of woody plants simultaneously stretch upward and expand concentrically outward from the center pith; which is a remnant of the embryonic stem. Growth
occurs at the thin layer of meristematic cells called the cambium, where cells divide tangentially, creating phloem tissue on the outside margins and xylem tissue on the inside. As the cambium grows and expands, it increases radially to cover the xylem as it thickens. Trees in temperate regions experience a yearly fluctuation between growth of large, thin-walled xylem cells (earlywood) that form in spring and summer, and smaller cells with thicker walls (latewood) that form in late summer to fall. The alternation of these cell types produces visible annual rings in the wood. Wood sampled from tropical trees generally does not have annual rings. As a tree ages, the center rings become physiologically inactive and fill with resin. This is called heartwood. In general the water content decreases and the primary function of heartwood becomes support of the stem and crown of the tree. The remaining, actively conducting layers closer to the outside of the tree are called the sapwood. Within the xylem cell matrix, more primitive conifers (gymnosperms) have longitudinally arranged tracheids that offer mechanical support to the tree and also transport water from the roots to growing tissues like branch tips and leaves. Latewood tracheids tend to be more supportive in function whereas the earlywood tracheids do the majority of water transport. Conifers also have ray cells that transport water and nutrients horizontally (across yearly growth rings); some conifers also use ray cells to excrete resin. Ray cells are more numerous in the hardwood angiosperm (deciduous) classification, making lateral fluid transfer (radial permeability) more prevalent. Additionally, angiosperms have vessels rather than tracheids for water transfer (Higuchi 1997, Taiz and Zeiger 2002). Because the less-evolved conifers have fewer ray cells that are shorter than those in hardwood species, and have tracheids
instead of vessels, conifers more effectively isolate the flow of xylem fluids containing water, nutrients, and often pollutants, such as trace-metals within the sapwood, limiting lateral “bleed” of fluid between growth years (Watmough 1997).

Appropriate trees for use in dendrochemical pursuits share a few key features, including high geographical distribution and adaptability, long life spans, well-defined heartwood with few rings in the sapwood, low radial permeability, and a low heartwood moisture content (Cutter and Guyette 1993). The majority of conifers meet the aforementioned recommendations and are therefore widely used in tree-ring studies for dendrochemistry.

Pathways to Assimilation

There are 3 uptake pathways that trace-metals may follow before deposition in the growth rings of a tree (see Fig. 1, below); uptake by the roots, foliar absorption-through the cuticle and/or by way of stomatal pores, and lastly direct stem uptake (Lepp 1975, Cutter and Guyette 1993, Watmough 1997). Trees are important in the biogeochemical cycling of Hg and have three potential assimilatory pathways; 1) root uptake of Hg derived from the soil; 2) uptake by way of foliage, through stomatal openings during photosynthetic gas exchange or direct absorption through the cuticle; and 3) passive infiltration of gaseous Hg into bark (Lepp 1975, Cutter and Guyette 1993, Watmough 1997, Arnold et al. 2017).
The root system is an important pathway for many trace metals in terms of ability to increase the elemental concentrations within tree biomass; however, translocation lag time between roots and the tree bole may be significant (Geijn and Petit 1979). Hg in soil is less bioavailable and if assimilated by roots, likely stays there. Concentrations can be significantly higher in the roots than in the rest of the tree, and cations, such as Hg, as suggested by Frescholtz et al. (2003), may not migrate up to the trunk or stem, which correlates with Bishop et al. 1998, Chiarantini et al. 2016, Fleck et al. 1999, Hojdova et al. 2010, Jung and Ahn et al. 2017, and others. Smith and Shortle (1996) suggest that cation binding capacity wanes as a tree ages. Trace-metal cations, if
absorbed through roots are incorporated into the sap flow and may bind to ligands within the xylem wall (Cutter and Guyette 1993).

Foliar uptake is a significant sink for atmospheric Hg. Gaseous metals can enter leaves through stomatal pores (Lindberg et al. 1992), or become sorbed on the surface of leaves or needles of a tree. These atmospheric constituents can also traverse the waxy cuticle of a leaf, reaching the epidermis, as suggested by Stamenkovic and Gustin (2009). The pollutant is then relocated by metabolites and ultimately assimilated by the phloem (Strzałka and Prasad 2002). Wet deposition onto foliar surfaces may also be a significant factor; however, most pollutants are washed from the foliage and are transported to the soil surface via throughfall (Schroeder and Munthe 1998, Rea et al. 1996). Studies with quaking aspen (Populus tremuloides) and other deciduous species, have suggested that deciduous leaf senescence, after deposition and/or uptake of atmospheric trace metals through stomatal pores, is also an important transport mechanism in pollutant (Hg) inputs to the soil (Erickson et al. 2003, Frescholtz et al. 2003). This is also true of trees bearing needle leaves although conifers transpire at a lower rate than deciduous trees, so stomatal uptake may be lessened (Frescholtz and Gustin 2004). Conversely, most coniferous species keep needles between 2 and 7 years, sometimes much longer. Because the needles are exposed to the atmosphere year-round over multiple seasons, depositional accumulation may be enhanced (Fay and Gustin 2006). Conifers also possess greater surface roughness, slowing air flow and increasing Hg scavenging ability (Laacouri et al. 2013).
Metal absorption through bark surfaces is the third, and least likely uptake pathway, whereby trace metals become deposited on the exterior surface of the bark and translocate to the phloem and then into the xylem of a tree. The physical characteristics of the bark, namely the fact that it is composed of 2 layers divided by a barrier called the periderm that is impervious to moisture, suggest that metal absorption would be difficult (Cutter and Guyette 1993). Although seemingly unlikely, element uptake through the bark has been demonstrated by several studies (Martin and Coughtrey 1982, Fay and Gustin 2006). Conversely, elemental components of the xylem may be translocated to the bark. It is also important to note that very small percentages of an element's total concentration can essentially become "lost" at each sequential step of translocation (Robitaille 1981).

Once inside the tree, the absorbed cations may be found in the sap or within cellular structures; their concentrations and distribution influenced primarily by sap pH and the elemental properties of each trace-metal (Cutter and Guyette 1993). Metals bind to ray cells, phloem or the xylem. In some cases they can also remain free in the sap, being redistributed vertically or radially (Hagemeyer 1993). Lepp (1975) demonstrated that toxins and metabolites can be compartmentalized in specific areas between sapwood and heartwood, which may influence trace metal distribution. Seasonal changes in spatial carbon allocation due to nutrient availability, temperature and pH may also have a role to play in where metals accumulate within a tree (Kagawa et al. 2005). Not all elements have equal mobility once entering plant xylem, and careful
consideration must be given to the choice of tree species used to study more mobile elements such as Mg, P, or K. For instance, P is an essential component of many metabolic compounds within plant tissues; it follows that it would be readily redistributed throughout the plant to a much greater extent than a relatively inert element such as Pb or Hg, both of which have high density and low solubility (Cutter and Guyette 1993).

**Foliage**

In a 2014 field study, Hutnik et al. (2014) tested the utility of Austrian pine (*Pinus nigra*) foliage as an environmental proxy for multi-year THg concentrations. The species was originally farmed as a Christmas tree and now occurs widely across Pennsylvania, USA. Needles were collected in autumn over 7 years at 15-21 locations throughout a 5000 km² area. Current year, previous year and 3rd year samples, which were analyzed for THg. Data showed that 3rd year needles had the highest concentrations and the first year needles, the lowest. Atmospheric Hg was implicated as the primary driver in the Hg sequestration within needles. Also noted was the fact that the lowest needle concentrations consistently came from trees furthest downwind from industrial pollution sources. Hutnik et al. (2014) suggest that any biomonitoring study involving conifers should consider needle age when sampling. Additionally, higher concentrations within older needles correlated strongly with the widely held hypothesis that senescent foliage drop is a significant THg contributor to soil load. Because *Pinus nigra* is
taxonomically similar to many North American conifer species, it follows that it may be a suitable proxy for spatiotemporal atmospheric Hg dispersal patterns.

Fleck et al. (1999) also included coniferous needles in a study relating red pine (Pinus resinosa Ait.) Hg tissue concentration to soil and atmospheric Hg sources. Within 2 pine plantations, 3 areas were sampled, each site having 2 discrete soil Hg concentrations. Data from the study indicated that foliar Hg was not related to wood tissue or soil Hg concentration; in several instances showed an inverse relationship. Older needles (2 years) had approximately double the Hg of younger (1 year) needles. Fleck et al. concluded that the Hg present within plant tissue was of atmospheric origin, rather than soil. Additionally, tissue Hg concentrations for foliar and wood were found to be very correlated to growing season length. Other similar factors like growing degree days and actual evapotranspiration closely followed this trend. This indicates the importance of physiological activity and photosynthesis in the active, biologically-based assimilation of atmospheric Hg.

**Bark**

In Schulz et al. (1999), bark from Scots pine (Pinus sylvestris L.) was sampled at two locations in eastern Germany and tested for atmospherically transmitted organic and inorganic contaminants including benzo[a]pyrene, fluoranthene, pyrene, α-hexachlorocyclohexane (α-HCH) dichlorodiphenyl-trichloroethane (DDT), Al, As, B, Ca, Cd, Ce, Cr, Cu, Fe, Hg, Mo, NH₄⁺, Ni, NO₃⁻, PO₄³⁻, Pb, Sr, SO₄²⁻, Ti, V, W, Zr and Zn.
Collections took place between 1987 and 1996 and it was found that after 1991, concentrations of most of the substances listed above declined, reflecting significant changes in infra-structure and industrial practices for the locations sampled. The porous structure of bark retains constituents of the atmosphere, and the lack of metabolic activity renders it nearly inert, making it useful and potentially less biased toward preferential assimilation. Schulz et al. (1999) additionally stated that the use of bark could aid researchers by enabling high-density, low-cost biomonitoring networks.

Chiarantini et al. (2016) showed that bark was a useful, low-cost biomonitor for Hg, retaining airborne particles and elements at the porous, plant/ atmosphere interface. Bark from *Pinus nigra* in the Mt. Amiata district in Italy, where Hg was mined in the past and geothermal power plants operate presently. Bark from the most heavily polluted areas showed concentrations up to 8.6 mg/kg, the highest ever reported in scientific literature. Sample concentrations decreased at locations further away from polluted sites. Outer bark concentrations were highest and decreased inward to the inner bark layers. Results from the study indicated that bark is comparable to lichen concentrations in the same area, supporting the case of atmospheric Hg assimilation in bark and suggesting its reliability. As a follow-up, to the 2016 study, Chiarantini et al. (2017) also determined the speciation of the Mt. Amiata district bark, finding that organic Hg species were higher in the inner layers and may be a good proxy for long-term exposure, while outer bark layers are more useful proxies for short-term events.
**Tree rings**

In addition to bark and foliage, the annual growth rings of trees offer one more tool for reconstructing historical pollution records, because a distinct ring is generally formed each year and can be accurately dated with the proper protocol. Trees have wide environmental tolerance and high spatial coverage. It is also likely that once assimilated in a living tree, Hg is permanently sequestered within the woody biomass and is not subject to further concentration, volatilization or evasion into the surrounding environment (Cutter and Guyette 1993).

The work of S.A. Watmough has contributed significantly to dendrochemical analysis techniques for historical trace-metal monitoring. In Watmough (1999), the wide suitability of tree-rings for biomonitoring is discussed, along with drawbacks like varying lateral mobilization tendencies for different ions. Sapwood ring number and permeability differs between species, and hinges largely upon ray cells within the xylem. Variation in radial ion distribution may be up to 100% for some tree species as well. Additional differences may also be present in late and earlywood. This highlights the need for an adequate knowledge of tree biology and species-specific differences. Tree-ring Hg has not been adequately studied, with early studies focusing primarily on Cd and Pb. If tree species are carefully evaluated and chosen, tree-ring chemical analysis can provide records of historical pollution that are not available elsewhere.
Hojdová et al. (2010) was the first study to report time-dependent Hg concentrations for tree-rings near historically contaminated sites, acknowledging point source production. They measured Hg in spruce and beech trees (core samples cut into 5-year increments) in a heavily contaminated area of the Czech Republic, affected by metal mining and smelting. Spruce trees (coniferous species) located nearest to a HgS smelting site registered the highest concentrations in the study, up to 15 ng g⁻¹. Concentration increases and decreases were noted within the dendrochronological record and relationships to increased HgS processing and technological advancements in flue gas cleaning were inferred using historical Hg concentration profiles. The study demonstrated the utility of tree rings for the temporal tracking of heavily polluted areas but called for further investigation to fully interpret historical records.

Dendrochemical analysis by Wright et al. (2014) included a wider geographical region in California and Nevada, USA. Sites influenced by past Hg emissions were included, as well as sites not proximate to known sources. Coniferous species were exclusively used for this study, and both arid and upland species were represented. Wright reported decreasing concentrations from coastal California eastward (inland), and evidence for locally-caused Hg concentration increases and those of a global nature, influenced by events such as the Industrial Revolution onset (1900’s) and potentially the California gold rush (~1850). Parallel trends with ice and sediment-cores were noted. Acknowledging that further advancements and technique development was needed,
Wright et al. (2014) concluded that tree-cores had significant potential as proxies of temporal Hg concentrations.

Obrist et al. (2016) synthesized spatial Hg trends over terrestrial surfaces in the western USA. Land cover was shown to strongly influence Hg concentration. Soil carbon, vegetative productivity and precipitation appeared to be primary drivers for higher Hg. Water-limited productivity was linked to lower soil Hg. Higher soil Hg was strongly linked to higher primary production. In an analysis of Hg concentrations within vegetation types; foliage ≈ branches, > bark > woody tissue. This pattern reinforces other studies and heightens the importance of atmospheric transport and the foliar uptake mechanism, as opposed to pathways like root system uptake or adsorption through the bark and into woody xylem tissues.

Scientific knowledge is lacking where the quantification of time-dependent chemical concentration in trees is concerned. There is a clear need within the scientific community to increase the understanding of elemental behavior in trees and how the physiology of different tree species assert influence on specific elements (Padilla and Anderson 2002, Cutter and Guyette 1993, Watmough 1999, Wright et al. 2014). Continued study of the mechanisms and relationships involved is needed. Tree species selection is a significant concern in dendrochemical studies and data deficiencies exist regarding suitability of tree types for examining specific element uptake. The temporal stability of tree ring Hg concentration and radial concentration variance have not been adequately addressed. Additionally there are many studies focused on heavily
contaminated sites and a notable lack of those exploring more subtle, long term trends over larger regions.

In this study, sampling protocols for wood, bark and foliar tissues were addressed. Operating on the widely accepted hypothesis that vegetative Hg is primarily of atmospheric origin, differing environmental conditions and influences on THg accumulation within the various tissue types were investigated through the strategic relocation of young nursery stock trees from the Pinus genus. There remains a need for more complete speciation of Hg within plant tissue types. Temporal stability was also investigated by way of comparison and expansion upon the work done by Wright et al. (2014). Additionally, an elevation-influenced Hg gradient was examined, along with radial core sampling effects on THg, and inter/ intra-tree THg variability in the Thomas Creek watershed adjacent to Reno, NV, USA.
Literature cited


doi:10.1016/s0048-9697(99)00109-6


CHAPTER ONE

Assessment of the Suitability of Tree Rings as Archives of Atmospheric Mercury Pollution using Tree Cores

(To be submitted to Biogeochemistry)

Matthew A. Peckham‡†, Mae Sexauer Gustin‡*, Peter J. Weisberg‡

‡Department of Natural Resources and Environmental Science

†Graduate Program of Hydrologic Sciences

University of Nevada-Reno, Reno, Nevada, USA 89557

*Corresponding author: mgustin@cabnr.unr.edu 001-775-784-4203
Abstract

This study investigated the methodology and utility of dendrochemistry for the spatial and temporal assessment of atmospheric mercury. We analyzed tree cores from California and Nevada, USA, choosing locations based on proximity to, or remoteness from, known past and present sources of Hg emissions. A Milestone™ DMA-80 instrument was used to thermally decompose samples in 5-year growth increments and measure total mercury vapor. Results suggest using tree cores from ~10 or more trees whenever possible, to attain the most robust mean concentration for a population of trees. This is especially important for monitoring atmospheric mercury in areas unaffected by pollution. For trace metal studies, 4 sides of a tree should be cored to account for radially asymmetric variations associated with environment or injury, which remains poorly characterized within literature. Our results build upon previous research suggesting tree rings are suitable proxies for historical air mercury concentrations, but indicate that temporal consistency may vary widely with geographic location and differing biotic and abiotic influences.
Introduction

Mercury (Hg) is a long-lived environmental contaminant that, in its gaseous elemental form (Hg⁰), may be transported thousands of kilometers from the original source. Oxidation of Hg⁰ to Hg²⁺ compounds facilitates both wet and dry deposition to terrestrial and marine ecosystems. Because of the importance of the atmosphere as a transport pathway, Hg contamination can occur even in isolated regions (Fitzgerald and Lamborg 2014, Schroeder and Munthe, 1998). Approximately 1/3rd of the Hg in the atmosphere is taken up by vegetation each year (Arnold et al., 2018).

Trees possess three main pathways for assimilation of environmental chemicals: (1) the root system, taking up dissolved soil constituents, (2) direct absorption through the bark, and (3) foliar assimilation by way of stomata or cuticle (Lepp 1975). After entering the cell structure or sap of the tree, trace metal behavior and distribution may vary widely (Cutter and Guyette 1993, Smith and Shortle 1996). Arnold et al. (2017) recently demonstrated that mercury was incorporated into tree rings as a function of air exposure concentration, and stomatal uptake was the main driver for concentrations observed indicating that daytime concentrations should be correlated with those in the wood. Thus, trees are not passive recorders (Smith and Shortle 1996), and uptake is controlled by active biological functions.

Tree rings have been used to understand atmospheric Hg exposures associated with contamination (Table 1). Wright et al. (2014) suggested that tree rings could be
used as biomonitors for changes in global concentrations. However, their utility for monitoring changes in atmospheric concentrations has not been fully demonstrated.

### Table 1-3: Mercury concentrations in tree-rings and other woody tissue modified and updated from Wright et al. 2014

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Conc. range: (Hg, ng g⁻¹)</th>
<th>Location</th>
<th>Species:</th>
<th>Tree-ring increment sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abreu et al. (2008)</td>
<td>20-280</td>
<td>Ria de Aveiro, Portugal</td>
<td>Black Poplar (<em>Populus nigra</em>)</td>
<td>~2 years</td>
</tr>
<tr>
<td>Fleck et al. (1999)</td>
<td>1.2-4.5</td>
<td>Minnesota, USA</td>
<td>Red Pine (<em>Pinus resinosa</em>)</td>
<td>most recent 5-10 years</td>
</tr>
<tr>
<td>Friedli et al. (2007)</td>
<td>1.9-2.0</td>
<td>Saskatchewan, Canada</td>
<td>Trembling Aspen (<em>Populus tremuloides</em>), White Pine (<em>Pinus strobus</em>)</td>
<td>sampled at 39 and 133 years after fire</td>
</tr>
<tr>
<td>Hojdová et al. (2010)</td>
<td>up to 15.0</td>
<td>central Czech Republic</td>
<td>Norway Spruce (<em>Picea abies</em>), European Beech (<em>Fagus sylvatica</em>)</td>
<td>5 year segments</td>
</tr>
<tr>
<td>Jung and Ahn (2017)</td>
<td>1.8-13.6</td>
<td>Yeosu City, Korea</td>
<td>Japanese Cedar (<em>Cryptomeria japonica</em>)</td>
<td>1 year segments</td>
</tr>
<tr>
<td>Mailard et al. (2016)</td>
<td>1.7-39</td>
<td>France (location specifics not listed)</td>
<td>Poplar (<em>Populus sp.</em>), Willow (<em>Salix spp.</em>), Black Locust (<em>Robinia pseudoacacia</em>), Common Buckthorn (<em>Rhamnus catharticus</em>), European Ash (<em>Fraxinus excelsior</em>)</td>
<td>1 year segments</td>
</tr>
<tr>
<td>Navrátil et al. (2017)</td>
<td>1.8-47.5</td>
<td>Neratovice, Czech Republic</td>
<td>Scots Pine (<em>Pinus sylvestris</em>)</td>
<td>5 year segments</td>
</tr>
<tr>
<td>Nóvoa-Muñoz et al. (2008)</td>
<td>8.3-16.4</td>
<td>Galicia, Spain</td>
<td>Birch (<em>Betula alba</em>), Oak (<em>Quercus robur</em>) and Pine (<em>Pinus sylvestris</em>)</td>
<td>not specified</td>
</tr>
<tr>
<td>Reimann et al. (2008)</td>
<td>1.0-5.0</td>
<td>Oslo, Norway</td>
<td>Birch (<em>Betula pubescens</em>)</td>
<td>by weight (stripped twigs collected fall 2005)</td>
</tr>
<tr>
<td>Siwik et al. (2010)</td>
<td>&lt;0.4-19</td>
<td>Kingston, Ontario, Canada</td>
<td>Red Oak (<em>Quercus rubra</em>), Poplar (<em>Populus deltoides</em>), Willow (<em>Salix spp.</em>), Sugar Maple (<em>Acer saccharum</em>), Silver Maple (<em>Acer saccharinum</em>),</td>
<td>3 or 5 years, depending on species (1906-2006)</td>
</tr>
</tbody>
</table>
Wide dispersion of Hg in the atmosphere can facilitate contamination of remote areas. Because tree rings are widely distributed, sampling and analyses could provide a simple method for recording atmospheric concentrations that do not require sophisticated instrumentation or upkeep. Use of tree rings to monitor global pollution may ultimately lead to a global network of tree-ring Hg data and could provide for understanding air concentrations over time. A greater knowledge of historical atmospheric mercury concentrations will facilitate improved policy decisions regarding control of industrial emissions, and help to build awareness of Hg contamination and bioaccumulation pathways. This is particularly important for high-risk communities dependent on consumption of fish and other seafood.
Our project goal was to refine and validate methods of Hg detection in trees through the investigation of 3 interrelated research questions, expanding upon the work of Wright et al. (2014). We investigated whether 1) spatial and temporal Hg consistency exists across tree populations; 2) differing air Hg concentrations are accurately reflected in tree-ring Hg concentrations; and 3) tree-ring concentrations remain constant over time, or if degradation or losses occur after the Hg is assimilated in the rings. Additionally, due to the higher concentrations of gaseous oxidized Hg (GOM) in the free-troposphere in California and Nevada (demonstrated by Huang and Gustin 2014), the potential impact of Hg gradient in GOM concentration for trees growing in the same area at two different elevations (1860 m, 2311m above sea level) was investigated. Recommendations for future dendrochemical studies involving mercury are provided, regarding choice of species and tree selection, sampling methods and confounding factors.

Arnold et al. (2018) stressed the importance of stomatal uptake of gaseous elemental mercury (GEM) with subsequent transport to the growth rings of the tree, and a lack of influence for soil Hg uptake through the roots. Wright et al. (2014) asserted that tree rings could be used for proxies of global air Hg concentrations. Increasing air concentrations would be associated with increased deposition that has been recorded by natural archives such as lacustrine sediment, peat bog, and ice cores. These studies generally show that deposition has increased ~3-fold from Pre-Industrial Revolution levels (Beal et al. 2015, Chellman et al. 2017, Enrico et al. 2017, Heyvaert et al. 2000,
Schuster et al. 2002). If trees are suitable proxies for background air concentrations, one would expect tree-ring Hg concentrations to also have increased.

Methods

Species sampled in this study included *Pinus monophylla, Pinus jeffreyi, Pinus ponderosa, Pinus monticola* and *Pinus flexillis*. These species were chosen based on traits specified by Cutter and Guyette (1993) as desirable for tree ring studies including long lifespans, wide geographic distribution, distinct heartwood with low moisture content, few sapwood rings, and low radial permeability.

Field collection sites

Resampled areas

Areas sampled by Wright et al. (2014) over 3 years (2009-2011), were revisited during the summers of 2015 and 2016 to investigate Hg concentration measurement reliability (repeatability) and stability over time. Fourteen trees at 7 areas initially sampled by Wright et al. (2014) were cored. Ten trees were sampled at each of 3 areas regulated by the Bureau of Land Management, and are referred to as the Arid sites – Curtz Lake (CL), Bald Mountain (BM), and Virginia City (VC)(3 cores from each tree, for *n* = 30 cores at each site). Three trees were sampled at each of 4 National Park areas; these locations are referred to as the Upland sites (*n* = 9 cores at each site). The dataset was divided into tree cores for which it was precisely known that the same trees from
Wright et al. (2014) were resampled, and those for which there was uncertainty due to GPS imprecision and high stand density.

Arid sites

The Curtz Lake (CL) site (38.731N, -119.788W, elev. 1860 m asl) is located on a gently sloping southwest-facing hillside, ~800 m from State Highway 89 in California along Airport Road, a quiet thoroughfare receiving very limited use. This location is, in general, isolated from anthropogenic sources of Hg; however, approximately 5 km downwind from CL is a geothermal site that has undergone commercial development. Airflow at the CL site is primarily from the southwest. Natural Hg enrichment has been reported in the area (Fischer and Gustin 2002). The Bald Mountain (BM) site (39.907N, -115.467W, elev. 2130 m asl) is located in east-central Nevada, downwind from an active open-pit gold mine owned by the Kinross Gold Corporation. The mine utilizes heap leach processing at multiple pads, and covers 600 km², making it the largest mine site by area in the United States (www.kinross.com). Prevailing winds are from the west. The Virginia City (VC) site (39.323N, -119.733W, elev. 1790 m asl) was chosen to test for the historical effects of mining-related Hg emissions. The mountains surrounding the site experienced a period of very intense gold mining prior to 1910. Using mercury for ore amalgamation was standard practice during this period. Airflow at the VC site is typically west to east.
**Upland sites**

The Upland locations sampled by Wright et al. (2014) were revisited at Yosemite (YNP, 37.730N, -119.574W, elev. 2195 m), Sequoia (SNP, 36.566N, -118.777W, elev. 1930 m) and Great Basin (GBNP, 39.005N, -114.219W, elev. 2081 m asl) National Parks. These locations do not have known local or regional sources of Hg pollution.

**Thomas Creek**

Samples were collected during summer, 2017 from the Thomas Creek watershed (TC), a west-to-east oriented drainage basin in the eastern Sierra Range just southwest of Reno, NV, USA on US Forest Service land (Figure 1). Average slope steepness is estimated to be 25-40° within the watershed. Soils are composed primarily of weathered granite material.

A high elevation site (UTC) 39.3971N, -119.9004W, elev. 2311m) and a low elevation site (LTC) 39.3929N, -119.8429W, elev. 1860m) were chosen for core sampling. The sites were separated horizontally (east-to-west oriented) by ~5 km, and a 450 m difference in elevation. Three cores were obtained from each of 10 trees in each area (total n= 60 cores). UTC and LTC mean tree diameters were 42 cm (~120 years old) and 54 cm (~100 years old) respectively (n= 10 each site).

To investigate the potential for minimizing concentration variability due to ring width variation and reduce counting errors (sampling bias), UTC trees 1-3 were each sampled from random directions. UTC trees 4-10 were all sampled from the south facing
side of the tree. At LTC, all 3 cores were taken from the same side of each tree. The
directional orientation for coring LTC trees was almost exclusively from the north facing
side.

![Figure 1-2: Circular icons denote National Parks (shown in green) and other sampling locations. Panel A shows locations of sites within California and Nevada, USA. Inset panel B shows details regarding sampling locations in the vicinity of Reno, Nevada. GBNP is Great Basin (National Park), SNP is Sequoia and YNP is Yosemite.](image)

**Sampling and Analysis**

Tree cores were taken by inserting a Haglöf® PTFE resin-coated increment borer
perpendicularly into the trunk of each tree, ~1.5 m above the soil surface. A 5.1 mm
diameter borer with a 60 cm bit was used at all sites except for trees 1-3 at UTC, where
a 12 mm diameter borer with a 45.7 cm bit length was used. Discolored and sap-containing cores were discarded in the field.

The borer barrel was wiped clean with new Kimtech Science “Kimwipes” and isopropyl alcohol after each sample. Latex gloves were worn at all times while handling the samples. Most tree cores were stored in Starbucks® straws (tested and shown to have negligible Hg concentrations (0.05 ± 0.06 ng g⁻¹)) sealed with Teflon® tape to avoid contamination, and organized in separate Ziploc® freezer bags by tree. TC samples taken using the 12 mm diameter borer were stored in pre-cut sections of Microsoap™ cleaned, ¾” PVC pipe sealed with end caps and Teflon tape. The tubes were stored in re-sealable plastic bags and labeled by tree number. All sample material was contained by site group in Ziploc® freezer bags stored as quickly as possible after reaching the laboratory in a scientific grade, Revco® freezer set to -20°C. Samples were dried while still housed in straws (or PVC tubes) using a Tekran® model 1100 zero air generator to cycle clean air over the cores for a minimum of 72 hours.

Dried samples were placed in a standard, slotted mounting board over a layer of scientific-grade Teflon tape. The cores were planed length-wise with an alcohol-cleaned stainless steel razor blade or X-acto® knife to create a flat, uniform surface; therefore aiding visual distinction of finer growth increments. For some samples, fine and super fine (600, 1200 diamond grit) Eze-lap® hand sharpeners were used to smooth the planed surface of the tree core, facilitating greater surface uniformity and visual clarity (cf. Wright et al., 2014). Small bead of 18 mΩ MilliQ® (Millipore®) water were often smoothed over the wood surface to further enhance growth ring resolution. The rings of
each tree core were counted a minimum of twice by individual technicians using a Meiji Techno® stereo-microscope. Following technician count agreement, tiny notches along the top, planed surface of the tree core were cut with X-acto® blades to “bracket” 10 year time intervals. This was done to facilitate further dissection, because trees were ultimately analyzed in 5-year segments.

Skeleton-plots, which are a means of reducing growth ring width information for an entire tree core to a strip of graph paper using a series of lines, were generated for each individual core to manually crossdate cores within trees and across trees within stands. The skeleton plot ring measurements were visually compared to tree-ring “master chronology” datasets for the same species within the NOAA’s paleoclimatology database (https://www.ncdc.noaa.gov/dataaccess/paleoclimatologydata/datasets/tree-ring).

Tree-core ring measurement was performed with the aforementioned stereo-microscope. The core was advanced on a stage beneath the lens, using a Velmex® manual crank paired to a Velmex® manual clicker. By advancing the core beneath the cross hairs in the ocular, distances between the first earlywood of a ring to the outermost latewood were precisely recorded by pressing a button after each full ring. Measurements were noted on a Metronics® digital display, model #QC10-V, and recorded in the MeasureJ2X® program. Cross-dating was done using standard dendrochronological methods along with manual skeleton plots confirmed by statistical cross-dating with COFECHA (Stokes and Smiley 2008). All cores were correlated by $r \geq 0.3$, using COFECHA. Greater than 95% of measured cores fell within correlation range
of \( r = 0.5 \) to 0.8. Upon adequate resolution of COFECHA’s statistical “flags” by remeasuring and retesting, cores were cut into 5 year growth increments, enclosed in labeled 7.4 ml borosilicate vials, sealed with laboratory Parafilm® (vented to compensate for negative pressure), and lyophilized using a Bench Top 5 VirTis™ freeze dryer to remove water weight.

Analysis was performed using a Milestone® DMA-80 (Direct Mercury Analyzer) that has a detection limit of 0.02 ng Hg based on “blank” sample boat testing \((n = 790)\). Quartz, rather than tin boats, were used due to the statistically lower memory effects of quartz (Milestone manual). Individual samples were analyzed in sets of ten, after which a triplicate set of sample material, one NIST standard of known concentration (NIST 1575a, 39.9 ng g\(^{-1}\) Hg ± 0.7 with mean returns of ~93%, \((n = 128)\), which was compensated for using a calibration factor automatically calculated for each sample run day), and a blank were tested as control. This calibration process was performed before and midway through each analytical batch of samples (typically \(n = 30\)). For Milestone analyses, sample boats containing a pre-recorded mass of material are lifted and inserted into a cylindrical chamber. Tree core samples were thermally decomposed under a continuous flow of ultra-pure oxygen. Volatilized sample material was transported via “ultrapure” O\(_2\) gas into a catalyst bed (maintained at 850°C). Hg vapor was collected on a gold trap that amalgamates Hg. Hg on the trap was desorbed and measured by an atomic absorption spectrophotometer. Light is generated at a wavelength of 253.7 nm. Hg atoms are optimally excited at 253.7 nm and emit energy. Total Hg content measured and the initial sample mass were used to calculate a final
concentration (Milestone® manual). Concentration data are recorded in mass of Hg per unit mass of sample material (ng g⁻¹). Five-year core increments were used throughout the study to provide sufficient mass to minimize the signal to noise ratio and to be consistent with Wright et al. (2014).

Analytical variability in triplicate samples tested with the Milestone DMA-80 had a coefficient of variation (CV) mean value of 0.24 ± 0.18 \((n= 180\) sets).

**Statistical Analysis**

Three cores from each of three trees at each TC area were fully analyzed \((n= 9\) per site at \(n= 2\) sites). Specific time periods with distinct Hg concentration trends were identified. The remaining 7 trees in each area were then analyzed for those time spans \((n= 21\) cores). At UTC, growth segments from 1995 to the present, as well as from the earliest ring forward to 1955 were analyzed. At LTC, growth segments from 1975-2015 growth were tested. Correlation analysis was performed to examine intra-tree correlation of Hg concentration. This was done between the first and second core, first and third core, and the second and third core, for each tree, for \(n= 60\) total correlation comparisons for the TC sites. Coefficient of variance (CV) between cores of each tree was determined for all TC cores and was done for high and low elevation sites for tree core records of 40 years \((1975-2015, n= 8)\) and for records +100 years \((1915 \text{ or older until 2015, } n= 5)\).

For BM, CL and VC, the three cores from each of the trees \((Pinus monophylla)\) were combined, given the low concentrations and desired minimum mass requirement
of ≥ 0.02 grams of sample material and to ensure sufficient sample based on concentrations similar to Wright et al. (2014).

Outlier data points were identified using the relative percent difference equation (with O as a potential outlier, and A as the mean):

$$\text{RPD} = \frac{100 - |O - A|}{A}$$

(Equation 1)

Two cores from each Upland site tree were analyzed separately in the requisite 5 year spans. Intra-tree linear regressions were performed between cores using average Hg concentration from our 2 trees, (for each 5-year segment) and mean of Wright et al. (2014), across all sites, to compare Hg concentration across trees and locations. Coefficients of variation (CV) values were also calculated across trees. To compare trends across sites and studies, tree Hg concentrations were normalized by total mean Hg concentration of the tree core. Normalizing by width was also tested for n= 6 tree cores. Regression plots and accompanying $r^2$ values were considered (Table 4, supplemental materials).
Results

*Thomas Creek tree cores*

Hg concentrations were variable even for the same tree rings on different sides of the tree, and intra-tree correlations ranged from \( r = 0.002 \), to over \( r = 0.94 \). Figure 3 shows an example of the core comparisons with one randomly chosen tree from each site (see Supplemental Information for full documentation). Significant correlations (\( p \leq 0.05 \)) were found for 57% of the \( n = 60 \) total TC cores, (60% at UTC and 53% at LTC).

Cores taken from random points around the tree trunk at UTC (\( n = 9 \)) had lower correlations (\( r = 0.39 \pm 0.4 \)) than the 21 UTC cores from trees (\( r = 0.55 \pm 0.3 \)) that were all cored from the south-facing side of the trunk. The LTC cores, all taken from one side of the tree, had correlation of \( r = 0.48 \pm 0.2 \) (\( n = 30 \)).

Cores recording 100 years of growth or more had a CV of 0.18 \pm 0.1 between concentrations, whereas the 40 year records had a CV of 0.22 \pm 0.1 in corresponding growth ring increments. All mean values and relevant statistics for this study and from Wright et al. (2014) were compiled in Table 2 for reference and ease of comparison.
Table 1-4: The top panel statistics represent the full temporal record for trees in this study. Mean concentration of Hg (ng g⁻¹) ± SD, slope representing the linear rate of change, \( r^2 \), p value, coefficient of variance (CV), maximum (Max.), minimum (Min.), median and \( n \) (number of data points) from a time (x-axis) versus concentration (y-axis) linear regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>GBMP</th>
<th>SKPG</th>
<th>YGP</th>
<th>YTBD</th>
<th>BM</th>
<th>CL</th>
<th>VC</th>
<th>UTC</th>
<th>LTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± Std</td>
<td>2.8 ± 1.0</td>
<td>3.32 ± 1.2</td>
<td>3.2 ± 1.4</td>
<td>3.5 ± 1.0</td>
<td>1.5 ± 0.7</td>
<td>1.67 ± 0.3</td>
<td>1.54 ± 0.3</td>
<td>2.0 ± 0.4</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0090</td>
<td>0.0134</td>
<td>-0.0152</td>
<td>-0.0017</td>
<td>0.0052</td>
<td>0.0040</td>
<td>0.0120</td>
<td>-0.0037</td>
<td>-0.0005</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.61</td>
<td>0.63</td>
<td>0.26</td>
<td>0.005</td>
<td>0.3</td>
<td>0.4</td>
<td>0.68</td>
<td>0.09</td>
<td>0.003</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>0.73</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.14</td>
<td>0.82</td>
</tr>
<tr>
<td>CV</td>
<td>0.34</td>
<td>0.37</td>
<td>0.44</td>
<td>0.30</td>
<td>0.47</td>
<td>0.20</td>
<td>0.19</td>
<td>0.32</td>
<td>0.17</td>
</tr>
<tr>
<td>Max.</td>
<td>5.6</td>
<td>6.0</td>
<td>8.4</td>
<td>7.0</td>
<td>5.1</td>
<td>2.4</td>
<td>2.3</td>
<td>2.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Min.</td>
<td>0.8</td>
<td>0.9</td>
<td>1.5</td>
<td>1.8</td>
<td>0.8</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Median</td>
<td>2.7</td>
<td>3.3</td>
<td>3.0</td>
<td>3.4</td>
<td>1.3</td>
<td>1.7</td>
<td>1.6</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>( n )</td>
<td>55</td>
<td>50</td>
<td>32</td>
<td>29</td>
<td>52</td>
<td>37</td>
<td>14</td>
<td>23</td>
<td>22</td>
</tr>
</tbody>
</table>

Bottom panel presents statistical results from Wright et al. 2014, for the full time record of all tree-core data.

*GBMP Great Basin Mather Point location, SKPG Sequoia Panther Gap, YGP Yosemite Glacier Point, YTBD Yosemite Turtleback Dome, BM Bald Mountain, CL Curtz Lake, VC Virginia City, UTC Upper Thomas Creek, LTC Lower Thomas Creek.*
Upper Thomas Creek:

![Graphs showing intra-tree linear regression of tree core mercury concentrations for arbitrarily chosen trees.](image)

Lower Thomas Creek:

![Graphs showing intra-tree linear regression of tree core mercury concentrations for arbitrarily chosen trees.](image)

Figure 1-2: Intra-tree linear regression of tree core mercury concentrations of 2 arbitrarily chosen trees, illustrating variable correlative nature of tree cores. Upper and lower Thomas Creek watershed sites. In this instance, the UTC cores were more highly correlated than the LTC, which was not a consistent occurrence for these sites. Cores were consistently compared with the 1st and 2nd letter of the sample code as the x and y-axis. For all site regression plots, see SI Figure 1-1.

Despite the variation in concentrations, trends within stands of trees were similar (Figure 4 below). For UTC, Hg concentrations declined from 1935 until ~1955 period when concentrations stabilized. All trees showed this general trend, despite a range in concentrations from 4 to 2 ng. From 1995 to 2015, more variability existed, particularly for 2005-2015, however many of the cores have a similar, gentle decline between the years of 1995 and 2005. LTC cores showed a decline from 1975 to 2010 and a slight increase to 2015.
Figure 1-3a: Mean UTC sample core concentrations for the complete historical period. Because coring depth varied between trees, identical records were not captured for all trees; thus, earliest trend lines may only represent $n=1$ or 2 cores for the tree. Gaps in data for several trees represent intentional selective time span analysis.

Figure 1-3b: Mean LTC sample core concentrations for the complete historical period. Because coring depth varied between trees, identical records were not captured for all trees; thus, earliest trend lines may only represent $n=1$ or 2 cores for the tree. Break in data after 1975 represents intentional selective time span analysis.

Mean Hg concentrations across all years were 2.0 and 1.7 ng g$^{-1}$ Hg for UTC and LTC. Combining and averaging all tree means for UTC and for LTC (Figure 5) as well as
regression comparison of corresponding concentrations showed that the upper and lower elevation sites are not closely related over the full measured period ($r^2 = 0.003$, $p = 0.82$). Early in the record, concentrations differed significantly, by up to 1.5 ng g$^{-1}$ until ~1955.

The sites become much more closely related from 1955 to 2015 ($r^2 = 0.8$, $p = 4.7E-05$). From 1975 to 2010 mean concentration decreased by 1.8 ng g$^{-1}$. A small spike in concentration occurred ~1975 at both locations (2.3 ± 0.3 at UTC and 2.5 ± 0.1 LTC). A slight increase in mean concentrations was observed between 2010 and 2015. UTC and LTC standard deviations ranged from 0.3 to 0.4.
In order to investigate potential methods for reducing variability, data was separately normalized by width, and by the mean Hg concentration of all the rings in each tree core. Overall, trend lines retained general patterns (Figure 6). Normalizing by the width reduced statistical correlation, for example in the case of TCL1, A-B shown in figure 4, \( r = 0.15 \) and \( p = 0.10 \) became \( r = 0.01 \) and \( p = 0.70 \). Regression analysis was performed for 3 trees at both UTC and LTC. For these 6 randomly chosen trees, the mean correlation value for normalizing by ring width was \( r = 0.29 \). Using the same 6 trees, correlation was statistically improved to \( r = 0.41 \) when normalizing by the mean Hg concentration value (Table 3, SI).

**Upper and Lower Thomas Creek**

![Graphs showing Hg concentration over time for UTC 2 and LTC 1 trees.](image-url)
Overall, Hg TC concentrations were low due a lack of point sources for air pollution and no mining activity proximate to the catchment area. Other studies, adjacent to point source pollution and contaminated with legacy Hg, have measured Hg concentrations up to 280 and 644 ng g⁻¹ in wood (Abreu et al. 2008, Becnel et al. 2004).

Arid site tree cores

The tree cores from BM, CL and VC display similar trends to the cores taken by Wright et al (2014), although for these locations, our sample set mean is 0.67 ng g⁻¹ higher than the grouped mean concentrations reported by Wright et al. 2014. Mean
values ranged from 1.67 ± 0.3 to 1.5 ± 0.7 ng g⁻¹ compared to those previously taken (1.2 ± 0.3 to 0.4 ± 0.3) (Table 2). Mean Hg concentrations for overlapping time periods were correlated with the Wright et al. (2014) study by $r$ values of 0.59, 0.12, and 0.39 ($p < 0.001$). Standard deviations for this study ranged from 0.3 to 0.7 and were 0.3 to 0.4 for Wright et al. (2014).
Figure 1-6: Arid site profiles showing mean values for 2015 collection (n = 10 trees) versus cores collected by Wright (n = 3). Error bars (representing standard deviation). Note differing scales and missing 2005 data point for Wright’s Curtz Lake profile. Scatter plots show the relationship between this study’s data (x-axis) and Wright et al. (y-axis).

Upland tree cores

Two cores were analyzed for each tree at the Upland National Park locations. Mercury concentration profiles between studies showed greater correlations at Panther Gap and Glacier Point, where the same trees were cored. Statistics for these sites were $r = 0.45$, $p = 0.00$ and $r = 0.51$, $p = 0.003$ (Panther Gap and Glacier Point respectively). Again, similar trends were observed. At Mather Point an increase in concentration is seen at ~1755 and again at ~1770 before a decrease in 1800, after which mean concentrations are fairly constant at ~ 2.5 ng g⁻¹. Slight peaks are observed in 1920s and then ~1965, with a dip in both data sets in 1995, followed by an increase. The other locations show similar qualitative trends.
Figure 1-7: Mean National Park tree core Hg concentration profiles for this study in ng g⁻¹, compared to values of Wright et al. 2014. Error bars denote standard deviation. Note differing axis values. Scatter plots show correlation between this study’s data (x-axis) and Wright et al. (y-axis).
**Discussion**

*Hg consistency within trees, between trees, and between tree populations*

Despite variability within the large data set, consistent trends in concentrations were observed. Thomas Creek tree cores \( (n = 60) \) showed a wide range of intra-tree correlative variability as well as inter-tree variability, indicating the need for multiple cores for trees from areas with low Hg concentrations. Correlations improved when trees were cored from one side of the tree stem only.

Radial tree-ring characteristics can be influenced by age, surface area of the tree, past and present injuries, prevailing wind direction, lean direction (hill slope), and likely more. Tree ring width varies greatly over the full surface area of the tree. Robust annual growth present on one side of the tree may be very thin, or completely absent on other parts of the tree.

Tree stands at UTC and LTC were homogenous in regard to positioning, hillslope, aspect, canopy characteristics, and other relevant environmental parameters; however, UTC trees were older and smaller in stature (mean diameters were 42 cm and 54 cm, UTC and LTC respectively) (oldest dated tree-ring for UTC was from 1895, for LTC: 1920). The early disparity between concentrations at the TC sites may be related to tree height and canopy dynamics, rather than differing air Hg concentrations influenced by elevation. The older, UTC stand would have been approximately 20 years old when the LTC trees were seedling age, possibly utilizing greater foliar surface area to slow, capture and assimilate atmospheric Hg.
The authors speculate that greater initial stand height at UTC may have allowed the trees better access to less-impeded airflow over the terrain. As the UTC trees reached a mature height, their stomatal conductance rates slowed and Hg concentrations declined to meet those of the younger LTC trees, which were gradually increasing due to greater stomatal conductance as influenced by lower height. Once the faster-growing LTC trees reached the upper canopy, their Hg concentrations became more temporally consistent with the UTC trees and were statistically well-correlated \( r = 0.8 \).

**Consistency of tree-ring Hg concentrations over time**

Repeatability was investigated by returning to stands and specific trees, when possible, sampled by Wright et al. (2014). In general, mean concentration profiles correspond to general trends established by Wright (2014). At all 3 arid sites, regression analysis showed statistically significant differences in the concentration profiles (overlapping time spans only). Arid site samples for this study showed consistently higher Hg than the concentrations reported by Wright. The reason for the concentration difference is unknown. NIST reference materials as well as aqueous standards were used to calibrate the DMA-80 for this study. Wright et al. used exclusively solid NIST reference materials to build calibration curves. Clean handling of samples, identical storage and drying techniques, as well as the consistency of the higher concentrations made contamination-induced differences unlikely.
The steady increase in tree-ring Hg concentrations over time at the Arid sites and several Upland sites (GBMP, SKPG) for this study may correspond with rising Hg concentrations globally. Similar concentration peaks were recorded for both studies at BM ~1900, corresponding with the onset of the Industrial Revolution. Paired increases were also noted for the BM tree cores, and to a lesser extent at CL ~1980, when open-pit mining practices began in Nevada.

Profiles for SKPG and YGP showed good correlation with Wright’s data. These are the locations where the same trees were sampled. This indicates a degree of temporal consistency for assimilated tree-ring Hg, and that degradation or translocation of Hg in upland *Pinus* in particular, is not significant under field conditions. This is consistent with Peckham et al. 2018 (in review), which did not report any evidence of lateral translocation between rings for 6-7 year-old *Pinus nigra* in outdoor locations. Instances of uncertainty concerning exact locations for Wrights’ trees resulted in poorly correlated data. Offset data peaks and other notable features on the profile trend lines were likely caused by minor cross-dating errors from using multiple technicians.

*Regional trends of Hg concentration*

Despite historically elevated Hg, Arid site tree-ring concentrations were low relative to the Upland trees. This may be related to species-specific adaptations for arid environments. All Arid site trees from this study compared to Wright et al. (2014) were *Pinus monophylla* versus *Pinus jeffreyi*, *Pinus ponderosa* and *Pinus monticola* at the other locations. The Arid sites were located on the much drier, eastern side of the Sierra
Nevada range. The TC trees, all *Pinus jeffreyi*, were also located in the eastern Sierras and had lower Hg concentrations than trees at the Upland sites, but were higher than the Arid sites. The TC location experiences greater precipitation, runoff and general water availability than the Arid sites.

Upland site cores from California, located along the wetter, western side of the Sierra Nevada had higher Hg concentrations than those from the Arid sites (all mean concentrations ≥ 3.2 ng g⁻¹), consistent with Wright et al. (Table 2). These observations align with the findings of Obrist et al. (2015), who stated that greenness, organic carbon and plant productivity (all influenced by water availability) had an “overwhelming” effect on soil Hg. The higher soil Hg was strongly linked with atmospheric deposition to, and stomatal adsorption of, atmospheric Hg to foliage, which when senescent, returns to the soil Hg pool. Nearly equal concentrations in the needles and the branches suggested that atmospheric Hg is being assimilated to woody tree tissues through foliage (facilitated by photosynthetic gas exchange processes) (Obrist et al. 2015). Therefore, these trees may have interacted with enhanced concentrations of long-range pollution in the free troposphere, potentially influencing Hg assimilation. Additionally, being located on the western side of the Sierra Nevada range, proximate to the Central Valley and major population centers, anthropogenic Hg emissions are another likely source for the California Upland sites, with prevailing winds moving west to east.

Dimethyl Hg is a toxic and volatile form produced in marine environments which easily passes the human skin barrier. Weiss-Penzias et al. (2012) highlighted significant concentrations of dimethyl Hg within coastal fog. Potential therefore exists, as
suggested in Wright et al. (2014), for this form to spread inland, assimilating into tree tissues at a greater rate than other Hg species.

Similar peaks were seen in both data sets just after 1849 (commonly accepted as the beginning of the California Gold Rush) at SKPG and GBMP. Peaks are also observed ~1900 at YGP and to a lesser extent at YTBD, which corresponds with the onset of the Industrial Revolution and global distribution of atmospheric pollution. A small increase was also recorded at GBMP, which is located in eastern Nevada, and isolated from urban development. Similar to Wright’s study, GBMP concentrations were ~0.5 ng g⁻¹ lower than the other NPS sites. Based on the work of Huang and Gustin (2014), higher elevation (~2750 m asl) makes long-range pollution the most likely Hg source for this site.

**Methodological recommendations for Hg Dendrochemistry**

Along with the standard dendrochronological preferences of a straight trunk without forking, or excessive branches around the coring target area (Stokes and Smiley 2008), when coring from a group of trees for the purpose of trace metal analyses, the authors suggest that: 1.) the stand be well-spaced, to minimize the potential effects of resource competition. An increased equalization of resource access should, in theory, minimize differences in ion access and absorption through physiological and metabolic processes like soil moisture uptake and photosynthetic activity which likely influences the foliar assimilation of atmospheric constituents; 2.) Consideration should be given to an equal degree of atmospheric circulation around the stand canopy, as well as stand
age and height when comparing concentrations between separate stands.

3.) Because trees do not produce a radially symmetrical ring each season, it is also recommended that trees being cored for dendrochemical analysis be sampled from 4 sides of the trunk to account for variations in concentration.

Conclusions

Following Wright et al., (2014) all Arid and Upland trees for this study were cored from different sides. Directional orientation experiments within the TC watershed showed that Hg (and likely other trace metals) trends in tree cores were better correlated when sampled from a single side of a tree. Due to high radial variability, and the potential for error, increased sample sizes and coring trees from 4 standardized directions are preferred. Data normalization by mean Hg concentration for each core was more useful than normalizing by tree width.

Matching peaks between studies indicates that trees are sensitive recorders of anthropogenic perturbation. The most consistently matching increases are all \( \sim 1900 \), when the rise of large scale industry triggered a \( \geq 3 \)-fold increase in atmospheric pollution. Between 1900 and the present, concentrations at most sites increased. Although tree-ring Hg concentrations observed by Wright et al. could not be precisely duplicated and several of our correlations with that study were weak, similar trends were observed, for the Upland sites that were successfully re-cored. This suggests that for the tree species sampled, *Pinus jeffreyi*, *Pinus ponderosa* and *Pinus monticola*, Hg
concentrations remain stable over time. Trees in arid regions with low primary productivity may be less suited for historic Hg record reconstruction. Due to analytical mass constraints dictating the use of 5-year growth increments, fine-scale temporal Hg consistency could not be adequately assessed. Advancements in non-destructive and precise, low concentration sample analysis technology are needed. The efficient analysis of individual radial growth rings from tree cross-sections will significantly advance the current understanding of Hg behavior following wood assimilation. Developing dendrochronological practices around the suggestions made here, along with emerging analytical techniques will increase the utility of trees as atmospheric Hg recorders, decreasing the need for deploying field equipment.
Literature cited


### SI Table 1: Mean Hg concentrations for 5-year time increments at all locations for this study.

<table>
<thead>
<tr>
<th>Year</th>
<th>UTC</th>
<th>LTC</th>
<th>BM</th>
<th>CL</th>
<th>VC</th>
<th>YGP</th>
<th>YTBD</th>
<th>SKPG</th>
<th>GBMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1745</td>
<td>0.99</td>
<td>1.05</td>
<td>1.36</td>
<td>1.54</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>1750</td>
<td>1.08</td>
<td>1.53</td>
<td>2.18</td>
<td>2.63</td>
<td>1.47</td>
<td>1.92</td>
<td>2.06</td>
<td>1.06</td>
<td>1.80</td>
</tr>
<tr>
<td>1755</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1760</td>
<td>2.13</td>
<td>1.82</td>
<td>2.00</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1765</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1770</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1775</td>
<td>1.14</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1780</td>
<td>1.25</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1785</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1790</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1795</td>
<td>1.14</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1800</td>
<td>1.25</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1805</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1810</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1815</td>
<td>1.14</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1820</td>
<td>1.25</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1825</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1830</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1835</td>
<td>1.14</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1840</td>
<td>1.25</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1845</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1850</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1855</td>
<td>1.14</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1860</td>
<td>1.25</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1865</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1870</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1875</td>
<td>1.14</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1880</td>
<td>1.25</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1885</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1890</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1895</td>
<td>1.14</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1900</td>
<td>1.25</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1905</td>
<td>1.41</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>1910</td>
<td>1.27</td>
<td>1.82</td>
<td>2.30</td>
<td>1.79</td>
<td>2.06</td>
<td>1.53</td>
<td>2.04</td>
<td>2.86</td>
<td>1.82</td>
</tr>
<tr>
<td>Year</td>
<td>Mean conc.</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
<td>----</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>2.00 1.73 1.48 1.67 1.54</td>
<td>3.19 3.50 3.32 2.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>1.64 1.47 5.13 2.18 2.25</td>
<td>1.90 2.90 5.78 3.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>1.52 1.43 2.69 2.36 1.66</td>
<td>2.00 3.68 5.22 3.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.57 1.49 2.27 2.10 1.65</td>
<td>1.52 5.18 6.01 2.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>1.64 1.62 2.67 2.03 1.61</td>
<td>1.71 4.71 4.50 2.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>1.78 1.77 2.29 1.69 1.57</td>
<td>2.26 2.90 3.78 3.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1985</td>
<td>2.07 1.91 1.75 1.65 1.52</td>
<td>2.64 2.89 3.12 3.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1980</td>
<td>2.06 2.06 1.79 1.88 1.51</td>
<td>3.08 3.58 2.93 3.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1975</td>
<td>2.26 2.45 1.74 1.82 1.59</td>
<td>3.01 3.48 2.91 3.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1970</td>
<td>1.83 1.84 1.46 1.66 1.47</td>
<td>3.11 3.80 3.22 5.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>1.74 1.99 1.34 1.72 1.60</td>
<td>2.70 3.61 3.59 4.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1960</td>
<td>1.87 2.01 1.40 1.52 1.35</td>
<td>3.15 3.11 3.76 3.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1955</td>
<td>1.87 2.06 1.58 1.79 0.92</td>
<td>3.04 3.99 3.77 4.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1950</td>
<td>1.97 1.70 1.66 1.96 1.15</td>
<td>2.85 2.67 3.45 4.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1945</td>
<td>2.29 1.60 1.33 1.75</td>
<td>2.83 2.94 4.49 3.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1940</td>
<td>2.62 1.88 1.21 1.52</td>
<td>2.68 3.00 3.68 3.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1935</td>
<td>2.94 1.39 1.31 1.45</td>
<td>3.36 3.40 4.59 4.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1930</td>
<td>2.69 1.36 1.43 1.63</td>
<td>3.17 2.83 4.39 4.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1925</td>
<td>2.51 1.47 1.36 1.45</td>
<td>3.32 3.03 3.85 3.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1920</td>
<td>2.66 1.60 1.31 1.45</td>
<td>3.36 3.40 4.96 3.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1915</td>
<td>2.23 2.05 1.35 1.50</td>
<td>2.62 2.99 4.72 3.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SI Table 1-2: Pearson correlation test values for the Thomas Creek location, between cores of the same trees. \( N \) = number of 5 year increments in the sample, not the total number of years.

<table>
<thead>
<tr>
<th>Lower Thomas Creek</th>
<th></th>
<th></th>
<th></th>
<th>Upper Thomas Creek</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>tree</td>
<td>( r^2 )</td>
<td>( p ) value</td>
<td>( n )</td>
<td>tree</td>
<td>( r^2 )</td>
<td>( p ) value</td>
<td>( n )</td>
</tr>
<tr>
<td>1 A-B</td>
<td>0.15</td>
<td>0.10</td>
<td>19</td>
<td>1 A-B</td>
<td>0.29</td>
<td>0.03</td>
<td>16</td>
</tr>
<tr>
<td>1 A-C</td>
<td>0.34</td>
<td>0.01</td>
<td>19</td>
<td>1 A-C</td>
<td>0.01</td>
<td>0.77</td>
<td>17</td>
</tr>
<tr>
<td>1 B-C</td>
<td>0.08</td>
<td>0.24</td>
<td>19</td>
<td>1 B-C</td>
<td>0.01</td>
<td>0.69</td>
<td>16</td>
</tr>
<tr>
<td>2 A-B</td>
<td>0.54</td>
<td>0.00</td>
<td>15</td>
<td>2 A-B</td>
<td>0.70</td>
<td>0.00</td>
<td>16</td>
</tr>
<tr>
<td>2 A-C</td>
<td>0.33</td>
<td>0.03</td>
<td>15</td>
<td>2 A-C</td>
<td>0.76</td>
<td>0.00</td>
<td>19</td>
</tr>
<tr>
<td>2 B-C</td>
<td>0.86</td>
<td>0.00</td>
<td>15</td>
<td>2 B-C</td>
<td>0.85</td>
<td>0.00</td>
<td>16</td>
</tr>
<tr>
<td>3 A-B</td>
<td>0.89</td>
<td>0.00</td>
<td>9</td>
<td>3 A-B</td>
<td>0.08</td>
<td>0.28</td>
<td>17</td>
</tr>
<tr>
<td>3 A-C</td>
<td>0.45</td>
<td>0.05</td>
<td>9</td>
<td>3 A-C</td>
<td>0.82</td>
<td>0.00</td>
<td>17</td>
</tr>
<tr>
<td>3 B-C</td>
<td>0.66</td>
<td>0.01</td>
<td>9</td>
<td>3 B-C</td>
<td>0.01</td>
<td>0.64</td>
<td>18</td>
</tr>
<tr>
<td>4 A-B</td>
<td>0.46</td>
<td>0.04</td>
<td>9</td>
<td>4 A-B</td>
<td>0.60</td>
<td>0.00</td>
<td>13</td>
</tr>
<tr>
<td>4 A-C</td>
<td>0.79</td>
<td>0.00</td>
<td>9</td>
<td>4 A-C</td>
<td>0.63</td>
<td>0.00</td>
<td>13</td>
</tr>
<tr>
<td>4 B-C</td>
<td>0.67</td>
<td>0.01</td>
<td>9</td>
<td>4 B-C</td>
<td>0.75</td>
<td>0.00</td>
<td>13</td>
</tr>
<tr>
<td>5 A-B</td>
<td>0.14</td>
<td>0.32</td>
<td>9</td>
<td>5 A-B</td>
<td>0.34</td>
<td>0.06</td>
<td>11</td>
</tr>
<tr>
<td>5 A-C</td>
<td>0.65</td>
<td>0.01</td>
<td>9</td>
<td>5 A-C</td>
<td>0.00</td>
<td>0.90</td>
<td>11</td>
</tr>
<tr>
<td>5 B-C</td>
<td>0.44</td>
<td>0.05</td>
<td>9</td>
<td>5 B-C</td>
<td>0.04</td>
<td>0.55</td>
<td>11</td>
</tr>
<tr>
<td>6 A-B</td>
<td>0.82</td>
<td>0.00</td>
<td>18</td>
<td>6 A-B</td>
<td>0.81</td>
<td>0.00</td>
<td>17</td>
</tr>
<tr>
<td>6 A-C</td>
<td>0.26</td>
<td>0.16</td>
<td>9</td>
<td>6 A-C</td>
<td>0.20</td>
<td>0.07</td>
<td>17</td>
</tr>
<tr>
<td>6 B-C</td>
<td>0.40</td>
<td>0.07</td>
<td>9</td>
<td>6 B-C</td>
<td>0.20</td>
<td>0.06</td>
<td>18</td>
</tr>
<tr>
<td>7 A-B</td>
<td>0.42</td>
<td>0.06</td>
<td>9</td>
<td>7 A-B</td>
<td>0.84</td>
<td>0.01</td>
<td>6</td>
</tr>
<tr>
<td>7 A-C</td>
<td>0.37</td>
<td>0.08</td>
<td>9</td>
<td>7 A-C</td>
<td>0.28</td>
<td>0.22</td>
<td>7</td>
</tr>
<tr>
<td>7 B-C</td>
<td>0.75</td>
<td>0.00</td>
<td>9</td>
<td>7 B-C</td>
<td>0.26</td>
<td>0.31</td>
<td>6</td>
</tr>
<tr>
<td>8 A-B</td>
<td>0.61</td>
<td>0.01</td>
<td>9</td>
<td>8 A-B</td>
<td>0.95</td>
<td>0.00</td>
<td>7</td>
</tr>
<tr>
<td>8 A-C</td>
<td>0.61</td>
<td>0.01</td>
<td>9</td>
<td>8 A-C</td>
<td>0.95</td>
<td>0.00</td>
<td>7</td>
</tr>
<tr>
<td>8 B-C</td>
<td>0.78</td>
<td>0.00</td>
<td>9</td>
<td>8 B-C</td>
<td>0.94</td>
<td>0.00</td>
<td>7</td>
</tr>
<tr>
<td>9 A-B</td>
<td>0.18</td>
<td>0.26</td>
<td>9</td>
<td>9 A-B</td>
<td>0.76</td>
<td>0.00</td>
<td>9</td>
</tr>
<tr>
<td>9 A-C</td>
<td>0.15</td>
<td>0.30</td>
<td>9</td>
<td>9 A-C</td>
<td>0.69</td>
<td>0.01</td>
<td>9</td>
</tr>
<tr>
<td>9 B-C</td>
<td>0.50</td>
<td>0.03</td>
<td>9</td>
<td>9 B-C</td>
<td>0.83</td>
<td>0.00</td>
<td>9</td>
</tr>
<tr>
<td>10 A-B</td>
<td>0.57</td>
<td>0.02</td>
<td>9</td>
<td>10 A-B</td>
<td>0.69</td>
<td>0.08</td>
<td>5</td>
</tr>
<tr>
<td>10 A-C</td>
<td>0.20</td>
<td>0.22</td>
<td>9</td>
<td>10 A-C</td>
<td>0.49</td>
<td>0.19</td>
<td>5</td>
</tr>
<tr>
<td>10 B-C</td>
<td>0.30</td>
<td>0.13</td>
<td>9</td>
<td>10 B-C</td>
<td>0.42</td>
<td>0.23</td>
<td>5</td>
</tr>
</tbody>
</table>
**SI Table 1-3:** Pearson correlations between cores of 3 arbitrarily chosen trees at UTC and LTC sites, normalized by ring width and by mean Hg concentration value.

<table>
<thead>
<tr>
<th>Samples</th>
<th>n=</th>
<th>(r^2)</th>
<th>(p) value</th>
<th>(r^2)</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTC2</td>
<td>A-B 16</td>
<td>0.04</td>
<td>4.45E-01</td>
<td>0.70</td>
<td>5.84E-05</td>
</tr>
<tr>
<td></td>
<td>A-C 19</td>
<td>0.84</td>
<td>4.14E-08</td>
<td>0.76</td>
<td>1.18E-06</td>
</tr>
<tr>
<td></td>
<td>B-C 16</td>
<td>0.15</td>
<td>1.32E-01</td>
<td>0.85</td>
<td>4.04E-07</td>
</tr>
<tr>
<td>UTC3</td>
<td>A-B 9</td>
<td>0.07</td>
<td>4.94E-01</td>
<td>0.23</td>
<td>1.93E-01</td>
</tr>
<tr>
<td></td>
<td>A-C 9</td>
<td>0.05</td>
<td>5.77E-01</td>
<td>0.58</td>
<td>1.69E-02</td>
</tr>
<tr>
<td></td>
<td>B-C 9</td>
<td>0.00</td>
<td>9.82E-01</td>
<td>0.26</td>
<td>1.57E-01</td>
</tr>
<tr>
<td>UTC4</td>
<td>A-B 9</td>
<td>0.44</td>
<td>5.11E-02</td>
<td>0.08</td>
<td>4.64E-01</td>
</tr>
<tr>
<td></td>
<td>A-C 5</td>
<td>0.22</td>
<td>4.31E-01</td>
<td>0.29</td>
<td>3.52E-01</td>
</tr>
<tr>
<td></td>
<td>B-C 5</td>
<td>0.11</td>
<td>5.86E-01</td>
<td>0.01</td>
<td>8.78E-01</td>
</tr>
<tr>
<td>LTC1</td>
<td>A-B 19</td>
<td>0.01</td>
<td>7.05E-01</td>
<td>0.15</td>
<td>1.03E-01</td>
</tr>
<tr>
<td></td>
<td>A-C 19</td>
<td>0.64</td>
<td>4.00E-05</td>
<td>0.34</td>
<td>9.24E-03</td>
</tr>
<tr>
<td></td>
<td>B-C 19</td>
<td>0.02</td>
<td>5.65E-01</td>
<td>0.08</td>
<td>2.37E-01</td>
</tr>
<tr>
<td>LTC7</td>
<td>A-B 9</td>
<td>0.12</td>
<td>3.67E-01</td>
<td>0.42</td>
<td>5.85E-02</td>
</tr>
<tr>
<td></td>
<td>A-C 9</td>
<td>0.10</td>
<td>3.95E-01</td>
<td>0.37</td>
<td>8.33E-02</td>
</tr>
<tr>
<td></td>
<td>B-C 9</td>
<td>0.63</td>
<td>1.07E-02</td>
<td>0.31</td>
<td>1.22E-01</td>
</tr>
<tr>
<td>LTC8</td>
<td>A-B 9</td>
<td>0.36</td>
<td>8.56E-02</td>
<td>0.61</td>
<td>1.31E-02</td>
</tr>
<tr>
<td></td>
<td>A-C 9</td>
<td>0.75</td>
<td>2.59E-03</td>
<td>0.61</td>
<td>1.26E-02</td>
</tr>
<tr>
<td></td>
<td>B-C 9</td>
<td>0.70</td>
<td>4.83E-03</td>
<td>0.78</td>
<td>1.69E-03</td>
</tr>
<tr>
<td>means:</td>
<td></td>
<td>0.29</td>
<td>3.24E-01</td>
<td>0.41</td>
<td>1.50E-01</td>
</tr>
</tbody>
</table>

**SI Figure 1-1:** Correlation (Pearson) plots between cores for each TC tree. UTC top, LTC below.
LTC1 A-B
$r^2 = 0.15$

LTC1 A-C
$r^2 = 0.34$

LTC1 B-C
$r^2 = 0.08$

LTC2 A-B
$r^2 = 0.54$

LTC2 A-C
$r^2 = 0.33$

LTC2 B-C
$r^2 = 0.86$

LTC3 A-B
$r^2 = 0.86$

LTC3 A-C
$r^2 = 0.45$

LTC3 B-C
$r^2 = 0.66$

LTC4 A-B
$r^2 = 0.46$

LTC4 A-C
$r^2 = 0.79$

LTC4 B-C
$r^2 = 0.67$

LTC5 A-B
$r^2 = 0.14$

LTC5 A-C
$r^2 = 0.65$

LTC5 B-C
$r^2 = 0.44$

LTC6 A-B
$r^2 = 0.28$

LTC6 A-C
$r^2 = 0.26$

LTC6 B-C
$r^2 = 0.40$

LTC7 A-B
$r^2 = 0.42$

LTC7 A-C
$r^2 = 0.37$

LTC7 B-C
$r^2 = 0.75$

LTC8 A-B
$r^2 = 0.61$

LTC8 A-C
$r^2 = 0.61$

LTC8 B-C
$r^2 = 0.78$
SI Figure 1-2a: UTC sample cores averaged for each tree within the high elevation stand. \( N = 10 \) trees, \( n = 3 \) cores per tree. UTC data gap resulted from selective focus on specific time spans. Dotted lines represent trees cored from multiple directions; solid lines are trees cored from one direction.
SI Figure 1-2b: LTC sample cores averaged for each tree within the lower elevation stand. \( N \) = 10 trees each site, \( n \) = 3 cores per tree. Break in LTC data resulted from selective focus on specific time spans. Note differing time scales between UTC and LTC.
Results of a controlled field experiment to assess the use of tree tissue concentrations as bioindicators of air Hg

(To be submitted to Science of the Total Environment)

Matthew A. Peckham‡‡, Mae Sexauer Gustin‡*, Peter J. Weisberg‡, Peter Weiss-Penzias¥

‡Department of Natural Resources and Environmental Science, and †Graduate Program of Hydrologic Sciences, University of Nevada-Reno, Reno, Nevada, USA 89557

¥Chemistry & Biochemistry Department, University of California-Santa Cruz, Santa Cruz California, USA 95064

*Corresponding author: mgustin@cabnr.unr.edu 001-775-784-4203
Abstract

The potential utility of trees as bioindicators of atmospheric mercury depends upon how accurately tree tissue concentrations reflect air-mercury concentrations at a given location and time. Air-mercury concentration influence on tree components was investigated using 6 to 7 year-old, potted Pinus nigra, or Austrian pines obtained from a tree farm. Trees were positioned in 3 experimental locations with different weather and background concentrations of atmospheric Hg to examine the efficacy of trees as Hg recorders. The effect of differing environments with their unique forms of ambient gaseous oxidized mercury (GOM), along with the effects of an aqueous mercury bromide root spike were also investigated. Over 2 growing seasons, needles, bark, and tree rings were sampled and analyzed for THg to survey concentration differences between growth occurring prior to experimental placement, which served as the experimental control, and growth occurring at each discrete location. Overall, foliar Hg concentrations increased significantly relative to pre-placement growth. Spring sample concentrations were low for needles and increased over the growing season, possibly indicating resorption. Root treatment with HgBr₂ had no significant impact on above ground plant tissues. All trees had higher mean Hg concentrations in the outermost tree rings relative to the control. Inner bark concentrations increased significantly by 28% in the first year and 50% in the second year relative to the control, whereas outer bark increased 32% and 17% for the same periods. The results from this study demonstrate
that *Pinus nigra* needles, bark, and tree rings can serve as effective bioindicators for atmospheric Hg, reflecting ambient concentrations under unique growing conditions.

**Introduction**

Coal-fired energy production, base-metal ore smelting, cement production, and artisanal gold mining are among the major contributors to a rise in atmospheric mercury (Hg) concentrations on a global scale (UNEP 2013). Studies have demonstrated a 2-to-3 fold increase in Hg deposition since the beginning of the industrial revolution through historical records such as lacustrine sediment cores, soil, and ice and tree core records (Beal et al. 2015, Biester et al. 2007, Boyle et al. 2015, Chellman et al. 2017, Fitzgerald and Lamborg 2014, Wright et al. 2014). Gaseous elemental mercury (GEM) is considered the predominant form in the atmosphere, and is widely dispersed due to a long residence time and its relative inertness (Selin et al. 2009, Gustin et al. 2015). Gaseous oxidized Hg (GOM) may contribute up to 25% of the total atmospheric Hg load (Gustin et al. 2015). Deposition of divalent Hg(II) specifically, may lead to the contamination of surface waters through biological methylation within anoxic sediments, yielding methylmercury (MeHg) (Cesário et al. 2017, Lehn herr 2014, Ullrich et al. 2001). MeHg bioaccumulates in aquatic organisms, and bioconcentrates in food webs. At high concentrations, MeHg is neurotoxic, and is known to induce brain irregularities, deformities, birth defects, organ malfunction, and death. Subtle neurological impacts
occur at low concentration (Ha et al. 2017). It is important to monitor spatial concentration trends in atmospheric Hg to improve environmental policy and protect the public, particularly populations with greater seafood consumption. Recent work has suggested that quantifying Hg in the woody and foliar tissues of trees may serve as a method for tracking local, regional, and global sources of pollution (Chiarantini et al. 2016, Jung and Ahn 2017, Maillard et al. 2016, Navrátil et al. 2017, Wright et al. 2014).

It has long been accepted that the physical characteristics of tree rings are influenced by the environmental factors endured during the lifespan of the tree (Fritts 1976). A careful study of ring patterns can play a key role in reconstructing long-term temporal records of climate change, drought patterns, stand dynamics, and hydrologic regime (Conte et al. 2018, Jones 2004, Luthardt and Rößler 2017, Meko et al. 2007). There is a growing body of work indicating that tree rings and foliage can provide useful information for studying historical atmospheric pollution. Seasonal growth can be used as a temporal proxy because trees in temperate climates generally produce a new ring annually (Fritts 1976). Dendrochemists operate under the premise that the chemical composition of tree rings is related in large part to the environmental conditions present during each growing season (Amato, 1988). Dendrochemistry has proven useful in tracking changes in atmospheric deposition of elements to soils that are taken up by the roots (Baes and McLaughlin 1984, Berish and Ragsdale 1985, Frelich et al. 1988, Hagemeyer et al. 1992, Kagawa et al. 2006, Kirchner et al. 2008, Maillard 2016, Odabasi et al. 2016, Padilla and Anderson 2002, Smith and Shortle 1996, Watmough 1995, 1997).
Trees have three potential pathways for assimilating Hg: 1) root uptake of Hg derived from the soil, 2) uptake by way of foliage, through stomatal openings during photosynthetic gas exchange or direct absorption through the cuticle, and 3) passive infiltration of gaseous Hg into bark (Lepp 1975, Cutter and Guyette 1993, Watmough 1997, Kirchner 2008, Stamenkovic and Gustin 2008). Previous studies indicate that Hg uptake from the soil into the trunk and foliage is negligible (Bishop et al. 1998, Fleck et al. 1999, Frescholtz et al. 2003). Recent work (Arnold et al. 2017) has reinforced the case for nonstomatal uptake of gaseous Hg into foliage demonstrated previously by Stamenkovic and Gustin (2008). Chiarantini et al. (2016) reported that bark Hg concentrations were consistent with lichens from the same polluted area, suggesting that bark can be a simple and inexpensive indicator of atmospheric Hg pollution. The porous structure and lack of physiological activity may aid in efficient adsorption of airborne particles. Siwik et al. (2010) found Hg in bark and wood were highly correlated. The outer, drier and more porous bark, referred to as the rhytidome, is essentially dead tissue. The secondary phloem, or inner bark, is a thinner and more fibrous layer under the rhytidome that transports nutrients and hormones and forms a moisture-resistant barrier to keep the tree from drying out or absorbing too much moisture from the environment (Panshin and de Zeeuw, 1980).

This study involved relocating Pinus nigra (Austrian pine) saplings of common genetic stock from a tree farm near Canby, Oregon, USA. Potted trees (approximately 6 to 7 years old) were transported to a high elevation site in Nevada (Peavine Peak, PV),
an urban site near downtown Reno, Nevada (VR), and a coastal site in California (Santa Cruz, SC) (Figure 1). Each site is exposed to unique forms of gaseous oxidized Hg (GOM) (Gustin et al. 2016, Huang et al. 2013, Zhang et al. 2016). The objectives of this study were to assess the efficacy of trees as spatial and temporal proxies for the air Hg, and the impact of different growing environments with their unique air chemistries on needles, bark, and tree rings over 2 years. During year 2, aqueous HgBr₂ spike treatments were administered to the soil of a subset of trees to test the impact of root exposure during the growing season.

Using novel experimental design, coupled with measurement of air Hg concentrations and the compounds of GOM present, we addressed the following research hypothesis: trees moved from one location to another will exhibit changes in concentration of foliage, bark, and tree rings that reflect the different Hg exposures of ambient air. This was based on previous work that showed that the atmosphere is the primary pathway by which Hg is accumulated in foliage (Blackwell et al. 2014, Ericksen et al. 2003, Rea et al. 2002). Here this was determined for the 3 tissue types.

Quantifying pre- and post-experimental treatment tree Hg concentrations also improves scientific understanding of Hg assimilation pathways and the efficacy of using strategic tree sampling for spatiotemporal data collection. Spiking with HgBr₂ solution served to broaden the current base of knowledge concerning the tree root Hg uptake pathway. The potential for radial translocation of Hg between tree rings was also investigated.
Methods

Experimental Settings

Ninety-five 6-to-7 year old *Pinus nigra* nursery stock trees were purchased from a tree farm near Canby, Oregon, USA. *Pinus nigra* was chosen for this study on the basis of availability, hardiness, and taxonomic affinity with naturally occurring ponderosa pines (*Pinus ponderosa*), and Jeffrey pines (*Pinus jeffreyi*), dominant over much of the Sierra Nevada mountain range, USA. Mean 24-hour TGM exposure concentrations for the Oregon trees were likely 1.5 ± 0.2 ng m\(^{-3}\) based on those measured by the Jaffe group at the Mount Bachelor Observatory (elevation 9068 m asl, lat 43.977419°, lon -121.6861139°) ~250 km south of the tree farm location (Weiss-Penzias et al. 2016, (data measured 2004-2010)). Nursery trees (~2 m tall) with *in situ* soil and roots bound in burlap material in 56.8 L plastic pots, along with additional filler soil from the same Oregon tree plantation (mean Hg 46 ± 8 ng g\(^{-1}\), \(n=5\)) were delivered (September 24\(^{th}\) 2015) in one shipment to the University of Nevada, College of Biotechnology and Natural Resources Nevada Agricultural Experiment Station (NAES), located in central Reno, Nevada (elevation 1371 m, lat 39.537421°, lon -119.804674°). Upon arrival, foliar material, trunk wood, bark, and soil were collected from 5 trees to serve as controls. Subsets of nursery trees were then transported to locations of previous atmospheric Hg research where air Hg concentrations and chemical composition were known (Huang and Gustin 2015, Wright et al. 2014a), (Figure 1).
Thirty trees, arranged for ample air circulation, were positioned on a gradual hillside (≥ 1 m apart) October 2\textsuperscript{nd}, 2015 ~4 km inland from the Pacific Ocean, at the University Arboretum in Santa Cruz, California (SC), (elevation 140 m asl, lat 36.985253°, lon -122.060670°). The SC nursery stock, were exposed to the marine boundary layer at this location. Huang et al. (2013) measured atmospheric GOM at Elkhorn slough, ~30 km southeast of Santa Cruz, CA. Compounds reported in the study were HgCl\textsubscript{2} and HgBr\textsubscript{2}, with mean atmospheric concentrations estimated at 22 pg m\textsuperscript{-3}. Reported TGM concentrations were 1.48 ng m\textsuperscript{-3} for this location (Zhang et al. 2016).

Concentrations of monomethylmercury (MMHg) in fog water have been reported for SC and locations in and around California’s Monterey Bay ranging from 0.07 to 9.8 ng L\textsuperscript{-1} (mean = 3.4 ± 3.8 ng L\textsuperscript{-1}). The fog water THg mean was 10.7 ± 6.8 ng L\textsuperscript{-1}. Total Hg and MMHg deposition via fog is estimated to be between 42-4600 and 14-1500 ng m\textsuperscript{-2} y\textsuperscript{-1} (respectively) along the central California coast near Santa Cruz. These inputs represent 7-42% Total Hg, and 61-99% of total MMHg deposition for that area (Weiss-Penzias et al. 2012). Conaway et al. (2010) reported THg in rain for this area, ranging from 421.9 – 3712.7 ng L\textsuperscript{-1}, with a sample mean of 1392.3 ± 928.2 ng L\textsuperscript{-1}. MMHg makes up 0.3-22% (median 1.9%) of THg in rain water for the Monterey Bay area (Conaway et al. 2010). MMHg rain concentrations ranged from 5 to 97 ng L\textsuperscript{-1} with a sample mean of 29.5 ± 21 ng L\textsuperscript{-1}. 
Ten of the trees were moved to the top of Peavine Peak (PV), (elevation 2519 m asl, (lat 39.589570°, lon -119.928507°) on October 9th, 2015, ~12 km northwest of downtown Reno, Nevada (Figure 2-1). This mountaintop location has been demonstrated to be impacted by long-range Hg pollution transported in the free troposphere (Huang and Gustin 2015, Weiss-Penzias et al. 2014). The site is accessed via a gravel road on the south side of the mountain. Radio relay tower service trucks make up most of the sparse traffic, along with occasional off-road recreational vehicles. The experimental trees were located in a secure enclosure, adjacent to a privately-owned radio communications building, and were tethered upright, ~1 m apart using 1.3 cm rebar anchoring shafts and wire. Wire guy-lines were passed through sections of garden
hose (~20 cm in length) to avoid wire damage to the tree trunks. Gravel-filled sandbags were placed around the pots to provide additional anchoring from the wind and to buffer the tree roots against rapid temperature changes. Continuous Tekran TGM measurements were unavailable for the site, however, active system capture of GOM on CEM filters indicated a mean GOM value of 147 ± 73 pg m⁻³ with the chemical form dominated by halogenated compounds (Gustin et al. 2016). The remaining 50 trees were located at the College of Agriculture Biotechnology and Natural Resources Nevada Agricultural Experiment Station on Valley Road (VR) in Reno, NV, elevation 1371 m asl, where they were positioned along a chain-link fence (spaced ~2.5 m apart). The fence parallels U.S. Interstate 80 spanning from east to west, (at a distance of 50 m) and at the corner of the property, forms a 90 degree angle to the north. Half of the Austrian pines were positioned facing the highway to the south, and the remaining half along the north/south section of fence facing east. VR GOM is primarily HgSO₄ and Hg(NO₃)₂ at concentrations of 86 ± 48 pg m⁻³. Gustin et al. (2016) reported a 24 hr. mean TGM concentration of 2.0 ng m⁻³.

A map showing the location of each tree at the three field locations was generated at the time of placement. The orientation of all trees with respect to cardinal direction was noted when sampling. During the fall, winter, and spring, the trees were watered based on weather and soil moisture. In the summer, the trees were watered once a week, and during periods of very high temperatures, the VR and PV trees were watered twice weekly.
Weather

Mean temperature during the experiment was 14°C at Santa Cruz, with ranges from 3.1 to 26.3 °C. The majority of precipitation occurs over the winter months, with dry summers. SC received 292 cm of rain during the experiment (123 cm, 169 cm for first and second seasons respectively). Fog water deposition was estimated to be between 10-30 mm y⁻¹ in the coastal Santa Cruz Mountain range, ~70 km north of SC (Chung et al. 2017). Winds are typically from the south at 5-10 knots. Reno mean temperature during the experiment was 9.4 °C, ranging from -7.3 to 32.1 °C. The Reno area received a total of 88 cm precipitation during the experiment (39 and 49 cm for first and second seasons, respectively). Winds in the Reno area are generally from the southwest ranging from 5-15 knots, and highest in the afternoons (www.noaa.gov/, http://prism.oregonstate.edu). Temperatures at PV are typically at least 5 °C cooler than the valley floor and rainfall totals are typically four times greater (Klieforth 1992). Wind speeds at PV are typically 10-15 knots greater than the valley floor (OHara, 2007). Precipitation is seasonally distributed more evenly in Reno than it is in Santa Cruz; however, the wettest months are still in the winter (November-March).
Root spike treatments

During the summer of 2017, soil from 3 of the VR trees was spiked with HgBr₂ aqueous solution 5 times at regularly spaced intervals. The 18.8 μg L⁻¹ HgBr₂ solution was prepared by adding HgBr₂ (Sigma-Aldrich 99.996%) to 18.2 mΩ water. For each tree, 10 mL of spike solution was added to 1 L of tap water to create a 188 ng L⁻¹ solution which was then poured evenly over the soil surface. This concentration represented a doubling of the theoretical concentration reaching the terrestrial environment during an average Nevada rain event (Huang and Gustin 2012). All VR trees not receiving the experimental solution were given 1 L of tap water on days that the spike was administered. Tap water from the VR greenhouse complex was tested and Hg content proved negligible (<1 ng L⁻¹) (Arnold et al. 2018). Water from the SC Arboretum was also used for periodic watering and concentrations measured, using the method described in Arnold et al. (2018), were less than 1 ng L⁻¹.

Sampling Procedures

Five trees with in situ soil were sampled immediately after arrival in fall of 2015. Mercury concentrations of soil and tree tissues in these samples are considered as the control or baseline concentration derived from growth in the Oregon nursery. All samples were enclosed in clean 7.4 ml glass vials, within 2 sealed Ziploc® bags and stored in a scientific-grade upright freezer set at -23°C.
A professional-grade 1.9 cm ID (inside diameter) LaMotte™ tubular auger was used to remove 3 soil samples from equidistant points around each of 5 sample tree pots. Soil plugs from each pot were combined, air dried, homogenized with a sieve, and stored.

The needles for the control data set were taken from midway up the tree, approximately 0.75 m above the soil surface. These samples were taken randomly in triplicate and likely represented a range of needle ages. Foliar tissue was removed from the trees using ceramic-bladed scissors, and placed in Ziploc bags. The scissors were cleaned using isopropyl alcohol and Kim wipes™ between each tree. All control needles were rinsed in 18.2 mΩ Millipore™ water. Latex gloves were worn at all times and needles were stored at -23 °C as soon as possible after sampling. Needles were later processed for analysis by cutting to ≤ 3 cm with ceramic scissors and stored in vials.

Needles were again sampled in triplicate for each of 5 trees in the fall of 2016, spring of 2017 and the fall of 2017. Unlike the control, none of these needles were rinsed in Millipore™ water. Previous work has demonstrated that Hg dry deposition is not an important pathway by which Hg accumulates in trees (Fay and Gustin 2006). Additionally, work by Graydon et al. (2006) suggested that a significant percentage of Hg, once deposited to foliage, is photoreduced and re-emitted as Hg⁰. Therefore needle rinsing during the primary sampling period was not performed. Each round of sampling included \( n = 5 \) trees at each of the 3 exposure locations, except fall of 2017 at VR when \( n = 6 \) trees were sampled. Scissors were always wiped with alcohol between each sample
type and each tree. The PV and the VR locations were sampled in triplicate, on two sides of the tree to test for Hg concentration gradients related to direction. Old versus young growth, and directional effects (North/South) were investigated at SC in the first year only. This yielded $n = 75$ samples for fall 2016 and spring 2017 and $n = 81$ for fall 2017. In 2016, needles were taken from each whorl, near the trunk, at PV and VR. New needles at these locations were taken from the uppermost whorl of the tree. The lower whorls were labeled as old. At SC needle ages were separated by taking older needles from 1-2 branch “forkings” away from the tip, and taking new needles from the buds at the end of the branch. After 2016, the sampling at PV and VR was changed to utilize the “branch forking” sampling method used at SC. (Sampling methods depicted in Figure 1, SI).

Tree-ring samples were taken from 5 control trees at the onset of the study, and at the end of each growing season from each of the 3 field locations. Trees were sampled at ~10 cm from the soil surface. Ten-to-fifteen cm sections were cut from the trunk, using a stainless steel Fiskars® bow saw, that was thoroughly wiped with isopropyl alcohol prior to use, and between the cutting of each tree. These were then cut using a stainless steel-bladed band saw to produce cross-sectional disks, or "cookies" ~1.5 cm thick, from each tree bole (Figure 2, SI). These disks were taken close to the center of each removed trunk segment. Care was taken to avoid areas with past and present shoots or branches, the growth of which distorts the wood, and can confound ring counting. To enable triplicate analysis of each control tree growth year, a band saw was used to make 3 equally-spaced cuts from the outside bark surface of the sample
cookie toward the pith. These cuts were discontinued just outside the third year tree ring and the blade was backed out. Prior to collecting samples for analyses, surfaces contacting the band saw were sheared away using clean razor blades to avoid any possible contamination. Due to mass-dependent sample requirements of laboratory instruments, growth years 1-3 for each sample tree were combined for analysis. From growth year 4 outward to the most recent ring, sufficient biomass was present, enabling discrete sampling from each of the 3 sections created by the radial saw cuts. This resulted in \( n = 3 \) samples from each tree. Wood was cut into small cubes, sized to fit the sample boats of a Milestone™ DMA-80. Samples were place in 7.4 ml vials and frozen until lyophilized.

Bark was removed from the same cross-sectional disks from which rings were sampled. Inner and outer bark samples were separated from the trunk at the end of the outermost ring using alcohol-cleaned stainless steel blades. Inner bark was delineated based on its lighter rust color and fibrous texture, and was carefully separated from the darker, dryer, flaking texture of outer layer bark (Figure 2, SI). Stainless steel blades used for dissections were cleaned between each type of material, and were stored in clean glass vials. Inner and outer bark material was sampled in triplicate.

Bark grows outward from the interior of the tree, and conifers generally produce stratified bark sheets. These layers increase in age towards the outermost layers. Being porous and directly exposed to ambient air, bark has been shown to passively accumulate Hg in areas of high air Hg concentration (Chiarantini et al. 2016). The same
A study showed decreasing Hg concentrations with increased distance from a source of pollution, and showed that Hg is highest in the oldest, outer bark layers. There is no established protocol for sampling bark for Hg analysis. Species specific variation in bark morphology limits sampling methods with regard to obtaining layers of uniform age. For this reason, it is likely not feasible to obtain a resolution finer than our designation of inner and outer bark layers.

In the fall of 2016 and late summer 2017, trees were sampled from the side facing the interstate (south), and the opposing side (north) of the VR site, in order to investigate any existence of a directional gradient. The same sampling principle was applied to the PV summit trees; however, given the effect of wind on this location, needles, bark and wood from each tree were sampled from the side facing the most prevalent wind currents (from the southwest) and the opposing side (northeast). This was done in triplicate for needles and bark for each tree on 2 sides for \( n = 6 \) for each material type. Wood was sampled on two sides of the tree and was not done in triplicate due to the moderate homogenizing effect of cutting the wood into small pieces and analyzing multiple pieces in the same sample boat. The same format was used for the SC tree needles during the first year (North vs South), but directionality was not addressed in the tree ring or bark samples from that location.

All soil and tree tissue samples were subject to 48 h of lyophilization using a Bench Top 5 VirTis™ and a Harvest Right™ freeze dryer prior to analysis.
Analyses

Mercury concentrations in all samples were analyzed using a Milestone™ DMA-80 instrument (EPA Method 7473) that operates on the principals of thermal decomposition, amalgamation, and atomic absorption spectrophotometry (λ 253.65 nm). Blanks averaged 0.02 ng g⁻¹. Before and after every 12 samples are analyzed, accuracy was determined using National Institute of Science and Technology (NIST) reference material (Pine Needles NIST 1575a or Tomato Leaves NIST 1573a) (± 10% considered passing). Triplicate analyses were performed every 10 samples to check instrument precision. Elemental Hg content is reported as a concentration, in ng/g based fundamentally on total sample mass and the nanograms of elemental Hg present, as derived by the internal spectrophotometer. All tree-ring and bark samples were analyzed by students in the Gustin biogeochemical laboratory, along with the majority of needles. Two rounds of needle sample analysis were performed by students in the Weiss-Penzias laboratory at the University of California, Santa Cruz during the first year of the experiment for needles sampled at the SC location only using the same protocol and NIST standards.

QA/QC

Total mean coefficient of variance (CV) for all needle samples analyzed in triplicate (n = 25) was 0.07 ± 0.03. Mean triplicate CV for all tree ring samples was 0.17 ± 0.11 (n= 24) CV for inner and outer bark layers was 0.23 ± 0.14 (n= 5). After all data was
compiled and checked for normalcy and homogeneity of variance, appropriate tests were assigned to each data set.

*Statistical methods*

ANOVA and Kruskal-Wallis tests were used to test for statistically different mean concentrations. Multiple comparison testing was performed using the Tukey HSD test or Dunn (1964) Kruskal-Wallis test, adjusting P-values (Dunn only) using the Bonferroni method to prevent false positives. Data for needles was split to better examine significant differences between control and new needle concentrations and for control and old needle concentration comparisons. Data for the bark was analyzed to determine if there were statistically different concentrations between inner and outer bark. We also examined differences in inner and outer bark samples relative to the corresponding inner or outer bark control concentration distributions. All analyses were implemented using R.

*Results and Discussion*

*Baseline concentrations*

Mean foliar Hg concentration of control trees was $6.5 \pm 1 \text{ ng g}^{-1}$ ($n=5$ trees). Mean concentration of the youngest ring wood (2015) was $2.0 \pm 0.4$, and the mean for
all rings across all 5 control trees was $1.6 \pm 0.4 \text{ ng g}^{-1}$. Bark Hg concentration means for inner and outer layers were $4.2 \pm 2.1$ and $9.0 \pm 2.4$, respectively.

**Spike testing at VR**

The spiking treatment did not result in significant Hg concentration differences for needles, bark, or wood ($p > 0.05$; Figure 2-2).

![Figure 2-2: Concentration data distributions for (in order) new needles, growth ring 2017, inner bark and outer bark (grouped by color, alternating from non-spiked to spiked). N= new needles, 17= 2017 growth, IB=inner bark, OB=outer bark. Bold lines in each box represent the median. Whiskers denote upper and lower quartiles.](image)

The lack of significant differences in Hg concentrations for any of the plant tissues between spiked VR trees and the non-spiked trees strongly suggests that in the locations and climates tested, the root pathway for coniferous species does not play a

Foliar mercury

2016

After one growing season (fall 2016), needles did not display any preferential concentration relative to the direction trees were facing at the sites where this was tested (PV, VR, and SC). Therefore samples taken from different directions were aggregated prior to analysis. For old and new needles, concentrations were PV > VR > SC (Figure 2-3 below). At all three sites, foliar Hg concentrations were significantly higher in the new foliage than in the control ((Dunn Kruskal-Wallis test, $p<< 0.05$). Mean (new) foliar Hg for PV = 20 ± 5, VR= 14 ± 2.6 and SC= 9.9 ± 3.5 ng g$^{-1}$). Old foliage was also higher ($p<< 0.05$). Mean (old) foliar Hg for PV= 19.8 ± 3.3, VR= 17.1 ± 4.3 and SC= 12.7 ± 5.2 ng g$^{-1}$) relative to the control concentration. At PV, foliage concentrations in fall 2016 were not significantly different for new and old foliage (19.8 ± 3.3 and 20.1 ± 5 ng g$^{-1}$ respectively). At SC new and old 2016 foliage had different mean concentrations (Old > New, $p= 0.005$). The old 2016 VR foliage mean was greater than the new foliage, but the differences were not statistically significant.
In spring 2017, no new foliage was significantly different from the control. Older spring 2017 foliage was significantly greater than the control at all sites (14 ± 2.6, 12.1 ± 3.2 and 12.9 ± 1.9 ng g⁻¹, \( p = 0.028, p = 6.2E-04, p = 0.001 \) (PV, SC and VR respectively)).

No new foliage in the fall of 2017 was statistically significantly different than the control. However, old foliage for this sampling was much greater than the control at PV and SC (PV: 24.9 ± 3.2, \( p = 1E-06 \), SC: 27 ± 3.6 ng g⁻¹, \( p = 1.9E-12 \)) and quite significant at VR (16.4 ± 3.8 ng g⁻¹, \( p = 9.5E-05 \)).

Figure 2-3: 2016, 2017 needles (including spring 2017) compared to initial control concentration. Sites are grouped by color. Within site groupings, sampling times are ordered chronologically: fall 2016, spring 2017 and fall 2017. N= new, O= old. Solid center bar within each box represents the median. Whiskers represent the upper and lower quartiles.
After 2 full seasons of atmospheric exposure, mean new needle concentrations were higher than the experimental control at all locations, but differences were not statistically significant (PV: 9 ± 1.5 ng g⁻¹, p = 1.0, SC: 7.3 ± 2.9 ng g⁻¹, p = 1.0, VR: 8.3 ± 1.6 ng g⁻¹, p = 1.0). Both new and old needles from year one showed similar concentrations while foliar samples from 2017 exhibited greater disparity between new and old, likely due in part to altered sampling protocol (details in methods section). A potential contributing factor is that the old 2017 needles had an extra year to assimilate Hg than the older needles in 2016. After the first season, the whorl sampling method was set aside in favor of sampling needles at discrete branch “forkings”. Using this sampling protocol, clearer trends emerged across all sites, showing that older needles were predictably higher in Hg than the newer needles (p << 0.05, both years and across sites) that typically emerged in late winter and early spring. Older needles, being physiologically active (to varying degrees) year-round, assimilate Hg over multiple growing seasons.

As hypothesized, across all sites older needles had greater Hg concentrations (mean = 15 ± 4.5 ng g⁻¹) than new needles (mean = 11 ± 5.5 ng g⁻¹) with this difference increasing throughout the duration of the experiment (Figure 2-3). The lowest mean concentration values at each site were found for new, spring 2017 needles sampled at PV and VR. This trend was absent at SC. In fall 2017 only the old needles were
statistically higher than the control. Hg concentrations for older fall foliage for 2016 and 2017 were similar at PV and VR but not at SC.

**Evidence for Foliar Resorption**

Heerwaarden et al. (2003) calculated average real resorption efficiency (RRE) for nitrogen and phosphorus to be 56% (leaf area) and 62% (leaf mass), establishing the importance of resorption for plant nutrient retention. The same study also suggested that foliar mass reduction and shrinkage may have led to underestimation of resorption importance in previous studies (Heerwaarden et al. 2003). Killingbeck (1996) showed that evergreen species as a group tend to decrease foliar nitrogen (N) and phosphorus (P) via resorption in senescing foliage significantly more than deciduous species, although resorption rates have wide temporal variance from year to year. Reduction efficiency of P in senescing leaves was significantly correlated with reduction of N (Killingbeck 1996). This earlier work suggests an increased probability of Hg being drawn out of foliage along with other nutrient and water resources, although precise mechanisms concerning Hg have not been studied. At VR, concentrations of the old needles and young needles were lower in the spring suggesting Hg was being resorbed. In the fall they were higher, but not as much, possibly due to growth restriction. This pattern was also observed at PV. In spring of 2017, mean concentration of new foliage at PV was 7.8 ± 2.3 ng g⁻¹ and in the fall of the same year, new foliage was 9 ± 1.5 ng g⁻¹; neither significantly different from the control. Old needle Hg concentrations during the spring and fall periods were both higher \( p < 0.05 \) than the new needles. The
concentration decreased in the spring and increased during the growing season. The disparity between new and old needle concentrations was least dramatic at SC were the climate is more temperate. Lower than predicted concentrations in 2017 may be attributed to the trees becoming more pot-bound, slowing growth and photosynthetic rates.

Tree ring mercury

2016

Mean tree ring Hg concentration was $1.6 \pm 0.4$ ng g$^{-1}$ across all control data. Outermost tree ring Hg concentrations for year one (2016) were $2.44 \pm 0.5$, $2.25 \pm 0.9$ and $2.25 \pm 0.7$ ng g$^{-1}$ (PV, SC, and VR respectively, Figure 2-4). Between the 3 locations, there were no significant differences in 2016 ring Hg concentration. The 2016 ring concentrations for the PV and VR trees were significantly higher than the control, but SC was not ($p=0.00015$, $p=0.01$ and $p=0.26$ respectively); however all means were higher.
Figure 2-4: Concentration values by site for 2016 and 2017 sampling year (outer growth ring only), shown with 2015 control concentration data (all growth rings included). Asterisks denote statistical significance. Bold lines in each box represent the median, whiskers denote upper and lower quartiles.

*2017*

After two years at each exposure site, mean Hg concentrations in the outermost tree ring (2017) were 1.7 ± 0.3, 2.4 ± 1, and 2.3 ± 0.6 ng g⁻¹ at PV, SC, and VR respectively. Concentrations for the 2016 tree rings (from the same trees sampled in 2017) were 1.8 ± 0.3, 1.6 ± 0.3 and 1.7 ± 0.5 ng g⁻¹ Hg at PV, SC and VR. Significant Hg increases in 2017 concentration relative to the control were found at the VR site only (p= 0.0003). Although significant differences were not seen across all sites in 2017, Hg concentrations for 2016 and 2017 consistently exceeded those of the 2015 control, across the three experimental locations (Figure 2-5 below).
Figure 2-5: 2016 and 2017 concentration data for each site compared to the initial 2015 control tree rings by year. Clockwise from upper left: PV, SC and VR. For each year, $n=5$ except at PV in 2017, $n=2$. Also, $n=3$ for VR 2017 (only non-spiked).

2016 and 2017

For all sites, 2016 ring concentrations increased relative to those associated with the control location. This is likely due to higher atmospheric GEM concentrations at the NV location relative to the OR location and the potential influence of methylmercury at SC. Ring concentrations for 2016 were not statistically significantly different across locations; however SC had the lowest mean concentration likely due to lower
atmospheric TGM. Concentrations at PV were higher in 2016 relative to 2017, which probably reflect ongoing stress impacts of high winds and colder temperature at the location. Lastly it is noteworthy that for each exposure location, the mean 2016 tree ring concentration as measured during our 2017 sample analysis is lower than the 2016 concentration as measured for the same year.

*Bark layer Hg*

**2016**

Inner and outer bark concentrations for the control were significantly different (Tukey test, $p = 0.0003$, 4.7 ± 1.6 ng g$^{-1}$ for inner bark, 9.0 ± 2.4 ng g$^{-1}$ for outer bark). Mean 2016 inner bark concentrations were 5.4 ± 2.3, 7 ± 1.4, and 7.2 ± 1.3 ng g$^{-1}$ (PV, SC and VR respectively). Inner bark in 2016 was not significantly different from the control at PV ($p = 0.94$), but was significantly higher for SC and VR ($p = 0.03$, both sites). Mean Hg concentrations for the outer bark were 17.7 ± 7.4, 9.3 ± 2.7, and 12.7 ± 3.9 ng g$^{-1}$ (PV, SC, and VR). PV was statistically higher ($p = 0.014$).

**2017**

In 2017, inner bark Hg increased relative to the control and the previous year at all sites, ($p = 0.06$, $p = 0.00$, $p = 0.00007$) and means were 7.6 ± 1.0, 11.7 ± 1.5, and 9.2 ± 1.8 ng g$^{-1}$ for PV, SC, and VR, respectively (Figure 2-6). Outer bark 2017 concentrations were 13.4 ± 0.9, 8.3 ± 1.4, and 10.8 ± 3.3 ng g$^{-1}$ (PV, SC and VR respectively).
The general trend of outer > inner bark mean values was apparent across years and sites with a notable exception being SC in 2017 where the general trend was reversed. Divergence from this trend may have been due to the climate at SC, where trees and bark were wetted more frequently over the year by maritime fog and rain, as opposed the PV and VR, both experiencing much drier conditions. Additionally, mean 24-hour TGM concentrations at SC were more similar the Oregon air concentrations where the trees were grown, thus decreasing the magnitude of Hg gain at the SC site.

Figure 2-6: Inner and outer bark layer concentration distribution. N= 5 trees for all sampling except n= 2 for PV 2017. Triplicate testing performed for all samples; this was doubled for all PV and VR samples. Asterisk denotes statistically significant difference from the inner and outer bark control samples taken in 2015. Control shown in red, with sites grouped by color. I= inner, O= outer. Bold lines in each box represent the median, whiskers denote upper and lower quartiles.
Implications

Tree biomonitor utility has been widely assessed with respect to air Hg concentrations (Chiarantini et al. 2016, Hojdová et al. 2010, Nóvoa-Muñoz et al. 2008, Peckham et al. 2018 (in review), Wright et al. 2014); however, the viability of this approach and underlying mechanisms for Hg uptake and movement within the tree have not been fully quantified. In this study, relocating trees from one site having atmospheric mean 24-hour TGM concentrations of 1.5 ± 0.2 ng m⁻³ to three different areas, (two with greater and one with comparable Hg concentrations) resulted in significantly higher concentrations in tree tissues. Our findings correspond with previous work suggesting that Hg concentrations of evergreen plant constituents increase along with local air Hg concentrations (Arnold et al. 2018, Fay and Gustin 2006, Fleck et al. 1999, Frescholtz et al. 2003, Hutnik at al. 2014, Jung and Ahn 2017, Navrátil et al. 2017, Odabasi et al. 2016, Zhang et al. 1995).

For HgBr₂, roots are not a significant pathway for Hg into the above ground portions of Pinus nigra, which supports the findings of Arnold et al. 2018. The conclusion that the root uptake pathway is not significant has also emerged from previous experimental studies that grew coniferous and deciduous trees in soils spiked with HgCl₂ (Millhollen et al. 2006, Stamenkovic and Gustin 2009) and with HgS (Fay and Gustin 2007).
Foliar Hg concentrations in spring were less than in the fall, particularly for the PV and VR sites, suggesting readsoption. Readsoption is an important mechanism for nutrient conservation in trees occurring in foliage prior to leaf senescence. Changes in the foliage concentrations at PV and VR suggest that Hg may be reabsorbed. Relative to fall 2016, old and new foliage concentrations decreased in spring 2017 and increased in fall of 2017. Nieminen and Helmisaari (1996) reported on the pre-senescence retranslocation of mobile nutrients (N, P, and K) within coniferous forests, showing a 67-88% overall decrease in needle concentration of NPK and a dry mass reduction of 31-43%. The data from Nieminen and Helmisaari suggests that in this study, Hg, being relatively inert, was drawn, along with the NPK and water readsoption, into other needles and potentially, woody tissues of the plant in the fall prior to needle senescence. In our study, this pattern was observed at PV and VR, where the trees likely experienced greater physiological stress and lost more foliage due to the semi-arid Great Basin climate. The lack of effect at SC may be due to the more temperate climate.

When using trees as environmental biomonitors, needles are an important component for studying short-term, cumulative impacts of Hg pollution. Under typical conditions, needles remain on Pinus nigra for 3 years (Hutnik et al. 2014) and it would be expected that Hg concentrations would increase each year due to continued assimilation. In this study, older needles always had higher concentrations than younger needles; averaged across sites, the old needle Hg concentrations were 27% higher than the new. How needle samples are collected likely has an effect on old versus new
needle concentration ratios. When sampling using the whorl method, needles sampled on the inner branches may have been older than the expected 3 year life span of *Pinus nigra* foliage. These inner needles, no longer physiologically active may remain attached due to a lack of disturbance or mechanical abrasion. Upper whorls may not have reliably captured the newest growth on the branch tips. Instead, by sampling the outermost tips of branches at a standardized height on the tree, as well as needles from one or more “forking” removed from the tip, greater resolution between foliage age classes may be attained in the concentration data.

Increased foliage concentrations would be expected to lead to higher concentrations in outer wood growth rings from 2016 and 2017 relative to the control. Arnold et al (2018) demonstrated that stomatal uptake of GEM by foliage was an important pathway by which Hg is subsequently transported to rings. Consistent with expectations, all trees in our study had higher mean concentrations for 2016 and 2017 rings relative to the control. Despite the lower concentration of atmospheric Hg at SC, the higher concentrations could reflect uptake of some form of methylmercury.

TGM concentrations varied minimally between sites; however as a passive sampler, bark concentrations reflected the differences in air GOM concentrations as understood (control < SC < VR < PV). Across sites, inner bark concentrations increased each year, with mean concentration increasing 28% from the control in 2016 and 50% from the control in 2017. Outer bark Hg concentrations increased by 32% in 2016 relative to the control, but only 17% relative to the control in 2017. Greater differences
in mean Hg concentration relative to its variability suggest that relocating the trees to their experimental locations influenced the inner bark layers to a greater degree than the outer layers.

Prasetia et al. (2018) suggested that the porous structure of bark can be a good bioindicator for atmospheric Hg and data from that study suggested that there was a pathway for THg collected by the outer bark to become assimilated into the inner, vascular layers of bark. There is a lack of research surrounding specific Hg species interactions with bark and the actual pathway mechanisms of Hg infiltration and assimilation. The inner bark at the wetter SC location for 2017 exhibited the greatest change relative to the other experimental sites and the control. This may be due to the movement of mercury species from precipitation or fog into bark or assimilation by the leaves and translocation. More frequent wetting of the porous outer bark layers with frequent rain and fog water at SC may have increased the accumulation of THg in the inner bark. The potential for Hg movement into inner bark layers was suggested by Prasetia et al. (2018) and Chiarantini et al. (2017), and in this case may have occurred (possibly to a greater extent) by way of “soaking.” A lack of literature in this area prevents any hard conclusion.

This study indicates that Pinus nigra and likely other conifers may serve as effective biomonitor of ambient Hg. Needles are most effective over 1-3 year spans and accumulate Hg actively and passively until they senesce. The use of tree rings is suitable mainly for trend observations on a macro-scale. Fine scale variations may be more
readily captured through passive assimilation within porous bark structures. The tendency for potted plants to become root-bound may hamper above-ground tissue growth and affect tree health, thus limiting full assimilatory potential. Research opportunities surrounding dendrochemistry and the unique relationships between various tree tissues and atmospheric Hg species are abundant. Future work should investigate weather and physiological stress effects on tree tissue Hg concentrations as well as standardizing a bark protocol and investigating the mechanistic differences in outer and inner bark, potentially address topical moisture effects on bark Hg assimilation. There is need for precise quantification of specific Hg compounds within plant tissues, especially at lower concentrations.
Literature cited


Adjacent Valley and High Elevation Locations. Environmental Science & Technology, 50(22), 12225-12231. doi:10.1021/acs.est.6b03339


SI Figure 2-1: Sampling methods for selecting needles for Hg analysis. Depicted to the left, fall of 2016 sampling protocol (Used at PV and VR) focused on sampling by whorl. Under healthy growing conditions, trees generally add 1 whorl per season. Needles taken from the branch, closer to the trunk represented the year of whorl formation. To the right is an *Pinus nigra* branch, showing the branch-fork sampling method (Used both years at SC and at all sites in 2017). Samples were removed at approximately breast-height for this method. For both sampling protocols, older needles were removed from the areas of the tree shown with red circle and oval shapes. New needle areas are in blue.
Figure 2-2: A. denotes the outer bark layer from a harvested cross-sectional trunk disk. B. indicates the inner bark layer. Photo shows the two distinct layers beginning to separate.
Summary and Recommendations

This project succeeded in enhancing scientific comprehension of atmospheric Hg assimilation and behavior once integrated into genus *Pinus* tissues, including needles, bark and wood. Through the placement of young trees at environmentally distinct experimental locations, root-spiking with a HgBr₂ solution, retesting mature trees from a previous study, and the use of a field sampling plan more extensive than has been documented in other Hg-related dendrochemistry studies, we were able to elucidate some fundamental questions surrounding the fate of Hg within trees and whether they are suitable spatial or temporal biomonitors for atmospheric Hg.

To answer the initial research questions in the most complete manner, research was divided into 2 “sub-projects,” each of which resulted in a thesis chapter and a manuscript for publication. In the first, the effects of differing TGM and GOM species, and environmental growing conditions on coniferous trees were examined by relocating small groups of *Pinus nigra* from one site having atmospheric mean 24-hour TGM concentrations of 1.5 ± 0.2 ng m⁻³ to three different areas, (2 with greater Hg, 1 with ≈ Hg (1.48 ng m⁻³). Tree relocation resulted in significantly higher concentrations in the tree parts. Spiking the soil with HgBr₂ solution had no significant effect on the aboveground tissues of *Pinus nigra*. Additionally, changes in the foliage concentrations at PV and VR suggest that Hg may be reabsorbed. Readsoption is an important mechanism for nutrient conservation in trees occurring in foliage prior to leaf
senescence and a portion of foliar Hg may be transferred with nutrient and fluid reallocation.

In the second project, following Wright et al. (2014), western region arid, and upland tree species (Pinus genus) that were sampled in 2009-2011 were re-sampled to examine the degree of annual Hg consistency within trees. Although tree-ring Hg concentrations observed by Wright et al. could not be precisely duplicated and several of our correlations with that study were weak, similar trends were observed, for the Upland sites that were successfully re-cored. This suggests that for the tree species sampled; Pinus jeffreyi, Pinus ponderosa and Pinus monticola, Hg concentrations remain stable over time. Trees in arid regions with low primary productivity may be less suited for historic Hg record reconstruction. Due to analytical mass constraints dictating the use of 5-year growth increments, fine-scale temporal Hg consistency could not be adequately assessed. Matching peaks between studies indicate that trees are sensitive recorders of anthropogenic perturbation. The most consistently matching increases are all ~1900, when the rise of large scale industry triggered a ≥ 3-fold increase in atmospheric pollution. Between 1900 and the present, concentrations at most sites increased. Directional orientation experiments within the TC watershed showed that Hg (and likely other trace metals) trends in tree cores were better correlated when sampled from a single side of a tree. Due to high radial variability, and the potential for error, increased sample sizes are preferred. Data normalization by mean Hg concentration for each core was more useful than normalizing by tree width.
In an attempt to refine temporal resolution of tree-ring Hg concentrations, the use of laser ablation was explored in cooperation with the University of Victoria, in Victoria BC, Canada. Although the small laser shots made testing each ring’s early and latewood possible, it was determined that the detection limits were too low for the Hg concentrations found in trees grown in at outdoor locations with ambient baseline atmospheric Hg. As such, temporal resolution for this study was limited.

This study indicates that genus *Pinus* and potentially other conifers may serve as effective biomonitors of ambient Hg. Needles are most effective over 1-3 year spans and accumulate Hg actively and passively until they senesce. Tree rings are primarily suitable for trend observations on a macro-scale. Fine-scale variations may be more readily captured through passive assimilation within porous bark structures. Total primary productivity is limited when potted plants become root-bound; therefore these conditions should be monitored and mitigated. Research opportunities surrounding dendrochemistry and the unique relationships between various tree tissues and atmospheric Hg species are abundant. Future work should investigate weather and physiological stress effects on tree tissue Hg concentrations as well as standardizing a bark protocol and investigating the outer and inner bark mechanistic differences in assimilation, potentially addressing topical moisture effects on bark Hg assimilation. There is need for precise quantification of specific Hg compounds within plant tissues, especially at lower concentrations.
Additionally, advancements in precise, non-destructive, low-concentration sample analysis technology are needed. The efficient analysis of individual radial growth rings from tree cross-sections will significantly advance the current understanding of Hg behavior following wood assimilation. Developing dendrochronological practices around the suggestions made here, along with emerging analytical techniques will increase the utility of trees as atmospheric Hg recorders, potentially enabling inter-annual concentration measurements, decreasing the need for deploying costly field equipment.