UNIVERSITY OF CALIFORNIA
SANTA CRUZ

Lichen Bioindicators Reveal the Impacts of Atmospheric Mercury in the New Almaden Mining District

A thesis submitted in satisfaction
of the requirements for the degree of

MASTER OF SCIENCE

in

MICROBIOLOGY AND ENVIRONMENTAL TOXICOLOGY

by

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June 2023

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Abstract

Lichen Bioindicators Reveal Atmospheric Mercury's Impacts in the New Almaden Mining District

Brittney Straw

Mercury (Hg) is a highly toxic global environmental pollutant because of its ability to bioaccumulate in living organisms and persist in the environment. Hg has been historically mined because of its unique properties and ability to form a gold amalgam. Although direct Hg mining is not as prevalent today, legacy mine waste still remains in many of the historic mining areas and continues to be a source of contamination to the local environment. Mine waste emits gaseous Hg and, with subsequent deposition, contaminates the local environment. However, this atmospheric contamination pathway remains largely understudied in historic mine sites. Thus, this study aims to determine the atmospheric Hg impact on the local environment in the New Almaden Mining District (NAMD), using lichens as a bioindicator. To accomplish this, we first concluded some properties of using lichens as a bioindicator. Then, we determined the Hg emission sources and their spatial distribution. Furthermore, we gained insight into the overall biogeochemical cycling of Hg in NAMD and how lichens may contribute to the contamination through their litterfall. Our data suggest that atmospheric Hg significantly contributes to the contamination of the local environment and reservoirs in NAMD.

Acknowledgments

Thank you, Santa Clara Valley Water District, for the funding and support

Thank you, Dr. Peter Weiss-Penzias, for sharing your knowledge and mentoring me

Thank you, Santa Clara County Parks District, for the sampling permits

Thank you, USGS Mercury Lab, for equipment and sample analysis

Thank you, Lamborg Lab, for allowing me to use your instruments and equipment

Thank you, undergraduate Michelle Rotham, for your help and willingness to continue this study

Thank you, Jesse Reid, for your constant support and help with experiment setup

Lichen Bioindicators Reveal Atmospheric Mercury's Impacts in the New Almaden Mining District

Brittney Straw

1. Introduction

1.1 Mercury

Mercury (Hg) is a highly toxic global environmental pollutant because of its ability to bioaccumulate in living organisms and persist in the environment[1]. Both natural and anthropogenic processes emit Hg. The natural processes include volcanic eruptions, rock weathering, and forest fires. Anthropogenic activities contributing to elevated Hg emissions include fossil fuel combustion, metal smelting, waste incineration, and direct Hg mining[2]. The atmosphere mainly transports Hg from these emission sources to terrestrial and aquatic ecosystems[3] . Once deposited in aquatic ecosystems, the Hg can become methylated by anaerobic bacteria, resulting in the highly toxic form methylmercury (MeHg). MeHg is a potent neurotoxicant because of its ability to cross the blood-brain barrier and induce neurotoxic effects such as cognitive impairment and ataxia[1]. Furthermore, MeHg bioaccumulates in food webs, so predatory fish often have very high Hg concentrations in their tissues in Hg-contaminated aquatic environments. These Hg-contaminated fish pose an extreme threat to any animal, including humans, that may eat the fish[4].

However, Hg is found in the environment before methylation in several less toxic, inorganic forms, such as elemental mercury (Hg^0), metacinnabar (isometric HgS), and cinnabar. Cinnabar is the chief mineral ore of mercury made up of

hexagonal mercury-sulfur (HgS) compounds[5]. Although direct mercury mining is not as prevalent today as it once was, cinnabar was historically mined and processed using a retort or rotary furnace during calcification. Calcification involves crushing and roasting cinnabar to extract elemental mercury vapor, resulting in the waste rock, calcines. However, this process is inefficient, and the calcines left behind contain unconverted cinnabar, Hg^0, and other mercury byproducts [5,6,7] . Historically, mercury mines tended to generate considerable amounts of calcine that were typically discarded on-site or dumped into waterways, where it was carried downstream in flooding events [4].

Even after these historic mine sites have been inactive for over 50 years, calcines often remain in the area and might be a significant source of mercury emissions into the local environment and atmosphere [8]. Calcines primarily emit Hg to the atmosphere as Hg^0, where it oxidizes to Hg^{2+} with long-range transport through the atmosphere [6]. Once in the atmosphere, Hg can deposit on foliar species, such as leafy plants and lichens, and with subsequent litterfall and decomposition, the Hg can enter local watersheds. With this in mind, fish in reservoirs near mercury-mining districts are often highly contaminated with Hg (typically MeHg)[4,8]. However, relatively little is known about the atmospheric pathway of mercury transport and deposition in mercury-contaminated mining sites. This knowledge gap has led to uncertainty over the true impact of atmospheric mercury depositions on environmental quality in these areas.

1.2 Objectives and Goals

The main objective of this study is to determine the impact of atmospheric Hg emissions in the New Almaden Mining District (NAMD) on the surrounding environment. We used lichens as a bioindicator of atmospheric Hg to accomplish this objective. Although the use of lichens as bioindicators is well established, details and processes remain understudied. Thus, this study's first aims were to better understand using lichen as a bioindicator of atmospheric Hg in contaminated areas. For example, we determined whether the lichen species affect Hg accumulation and what form of Hg the lichens contain. We also aimed to determine the lichens' Hg uptake and release rates when transplanted to a new environment and how this may depend on lichen species and site.

Furthermore, to determine the impact of atmospheric Hg in our study site, we wanted to gain insight into the overall biogeochemical cycling of Hg. So, we first aimed to find the main atmospheric Hg emission areas using total Hg (THg) concentrations and stable-mercury isotopes. Next, we determined these areas' spatial distribution using THg concentrations and a sampling transect. These steps allowed us to determine whether atmospheric Hg emissions from mine waste contribute to the contamination of the local environment in NAMD.

2. Background/ Literature Review

2.1 Historic Mercury Mines

Hg has been mined and used for various applications since the Neolithic Age

(4000-3000 BCE). The first documented use of the mercury ore, cinnabar, was a preservative for human bones and was later used for its red pigment. During the first century, the method of roasting cinnabar to extract elemental Hg quickly spread and became widely used. The extracted elemental Hg also had various uses, such as in alchemy and medicine, and more recently, it has been used with barometers, paint, and batteries [9]. Both historically and presently, Hg has been used for gold amalgamation due to its low boiling point and high density[1,9]. Thus, the gold rushes of the 18th and 19th centuries led to the widespread use of Hg for gold amalgamation, particularly throughout California. As well as the gold being mined in California, most of the Hg used for the amalgamation was obtained from mercury deposits in California. The United States Geological Survey (USGS) has reported approximately 550 abandoned Hg mines throughout California [1]. To this day, significant amounts of Hg-contaminated soils and mine waste remain at the mine sites.

Due to Hg's unique properties, the elemental Hg present in mine waste volatilizes at ambient temperatures and can locally deposit in the nearby environment, contaminating soils and aquatic systems. Thus, the historical mine waste dumps of inefficiently roasted ore are an important source of environmental Hg contamination [10]. This has been demonstrated and studied in historic Hg mining areas like the Almadén mine district (Spain) and the Idrija mercury mine (Slovenia). Almadén mining district was the world's largest Hg mine, and the environmental impacts on the local environment and adjacent ecosystems have been well studied[11,12]. Likewise, the Idrija mine was the 2nd largest mining area, and the atmospheric emissions from the

ore roasting plant have been estimated to have environmental impacts of approximately 160 km2 area [10].

In this study, we chose to analyze the impact of the historic mining practices in the NAMD. NAMD is located approximately 40 miles from the UC Santa Cruz campus and was once the largest mercury mine in North America [4]. Like other historic mining areas, the mine waste continues contaminating the environment around it, making it an important area to study to mitigate these environmental impacts.

2.2 Lichens as Bioindicators of Local Atmospheric Composition

Lichens are a symbiotic relationship between a fungal partner and an algal or cyanobacteria (phycobiont). The fungal partner contributes water, nutrients, and protection, while the phycobiont provides organic carbon [13]. Since lichens lack a root system or any other form of water-absorbing organs, they passively absorb their nutrients and moisture from the atmosphere[14,,15]. As a result, lichens were historically used to monitor pollutants associated with dry deposition, wet deposition, and gaseous emissions,[16,17].

More specifically, the use of lichens as bioindicators of air quality for various heavy metal pollutants is well-established in the literature [16,18,19]. For example, Cayir et al. (2007) conducted a study to assess atmospheric heavy metal pollution (Pb, Zn, Cr, Cu, Cd) in the Canakkale and Balikesir provinces of Turkey[20]. The authors collected *Cladonia rangiformis* lichens at ten stations in the area, and their heavy

metal concentrations were determined. The results showed generally low or background levels of all metals in unpolluted areas, but in an area around an abandoned mine, the concentrations were significantly higher. This data shows that lichens can be used as a bioindicator of the local atmospheric compositions for a whole suite of heavy metals[16].

Furthermore, Berdonces et al. (2017) assessed contamination by atmospheric mercury at two different sites in Spain using lichens as biomonitors in combination with mechanical air collectors [12]. At both locations, as the mercury concentration in the air increased, the concentration in the lichen increased. In addition, the closer to the source, the higher the mercury concentrations within the lichens were. Both studies indicate that lichens can be considered good biomonitors for studies in contaminated regions with trace elements, including mercury[12].

In this study, we used lichen bioindicators to assess the impact of atmospheric Hg emissions on the environment in a historic mine site. Mechanical air collectors can be costly and impractical to bring into the study field[16,17]. Thus, using lichens instead allowed us to sample a much larger area than we would have had the resources to do. Furthermore, using the native lichens provides more insight into the overall Hg cycling in these sites. In all, we used lichens as a bioindicator in this study because it is well established in the literature and is more cost-effective than mechanical air collectors.

2.3 Active Biomonitoring of Atmospheric Mercury at Contaminated Sites with Lichen Transplants

Along with using native lichens as bioindicators of atmospheric Hg, many studies have used the technique of active biomonitoring using lichen transplants. The method of this technique is to transplant lichen from a "clean" uncontaminated environment to the contaminated study area. The initial THg concentration is determined, and subsequent sampling is done at various times. This technique has further advantages over in situ lichens, such as confirming the ongoing release of gaseous Hg and determining the uptake rate since the pre-exposure values are known. It is also easily replicable at different time points, demonstrating reduced emissions or worsening of the situation [21]. Thus, lichen transplantation studies help determine aspects of Hg emissions that in situ lichens cannot provide.

For example, in Mlakar (2010), biomonitoring with lichen transplants was used as a complementary method to instrument analysis in a contaminated area near a cement plant[22]. The main objective of this paper was to evaluate the type and quantity of mercury emissions from a cement plant and to determine the relationship between the amount of mercury emitted from the plant and the concentration in transplanted lichen to quantify this biomonitoring methodology. The main conclusions were that the transplanted lichen methodology was good enough to record and detect a significant response to the polluted air, and a temporal trend was determined. Each site showed that the quantities of emitted mercury were in a good linear correlation

with the mercury concentrations in the transplanted lichen. This study confirms that lichens can be utilized as active bioindicators [22].

Furthermore, in Lopez- Berdonces (2017), the authors transplanted lichen from an uncontaminated area to the Almaden Mining District in Spain to develop a kinetic model for Hg accumulation by lichens [12]. The authors sampled lichens at 10, 45, and 92 days. The ratio of their Hg concentrations at each time point and their initial concentrations versus time were graphed, allowing for the development of a first-order kinetic model dependent on the sampling site[12]. These findings show lichen transplants can provide a temporal factor to Hg emissions from contaminated sites.

These studies demonstrate that lichen transplants are a suitable and complementary tool to using in situ native lichens in contaminated areas. The transplanted lichens provide evidence of the temporal aspect of Hg emissions and accumulation rate by the lichens. Thus, lichen transplants can be important indicators of the spatial and temporal trends of gaseous Hg emissions from contaminated hotspots. So, in this study, we implemented a lichen transplantation experiment to complement our in-situ sampling, allowing us to determine the uptake and release rates of different lichen species at various locations within NAMD.

2.4 Mercury Stable Isotopes

Previous studies have shown that mercury isotopes are valuable for understanding atmospheric mercury transport and fate[23- 26]. Mercury has seven stable

isotopes (Hg-196, Hg-198, Hg-199, Hg-200, Hg-201, Hg-202, and Hg-204). With an overall mass difference of 4% between the lightest and heaviest isotopes, redox chemistry, a volatile elemental form (Hg^0), and a tendency to form covalent bonds, many processes may lead to mercury isotopic fractionation[28]. Moreover, Hg isotopes exhibit mass-dependent fractionation (MDF) and mass-independent fractionation (MIF) in the environment. MDF (typically written as $\delta^{202}Hg$) occurs due to chemical, physical, and biological processes, such as phase changes and diffusion[25].

In contrast, MIF of Hg isotopes is believed to occur via photochemical processes due to the magnetic isotope effect [27]. MIF of Hg isotopes induces both odd-MIF (typically written as $\Delta^{199}Hg$) and even-MIF ($\Delta^{200}Hg$). Studies have reported that MIF results primarily from aqueous and atmospheric photochemical reactions [30] . Thus, mercury isotope measurements may provide insight into the source, Hg emissions from contaminated sites.

Furthermore, studies have shown that lichens can be representative of atmospheric isotopic signatures of Hg [11]. So, determining the stable Hg isotopes in lichens can provide evidence of the source and fate of the atmospheric Hg in contaminated environments. For example, in Jimenez-Moreno et al. (2016), the authors used the Hg isotopic signatures in sediments and lichens from the Almaden mining district in Spain to trace Hg contamination sources [11]. A decreasing trend with distance to the primary Hg mine was displayed by the δ 202 Hg values in the lichens. This isotopic signature depicts a mixing trend between the Hg originating from the mine and the global atmospheric pool. The lichen samples near the mining areas

exhibited δ 202 Hg values similar to those in the cinnabar ores. With distance from the mine, the lighter isotopes became more prevalent. This conclusion is also supported by the correlation between δ 202 Hg values and Hg concentration. Higher Hg concentrations found within the lichens correlated with higher δ202 Hg. Similar results were also depicted in Estrade et al. (2010) when analyzing the Hg isotope composition in lichens throughout various geographical areas [29]. This study also concluded that the δ202 Hg values decreased with distance from an industrial source and with Hg concentrations.

In addition to using MDF to determine the source of Hg emissions, it has been demonstrated that MIF can provide insight into the source. For example, in Estrade et al., (2010), the authors concluded that the Δ^{199}Hg values in lichens collected in industrial areas were slightly negative (-0.15 ± 0.03 ‰), and the lichens collected in urban areas exhibited the largest negative values (-0.5 ± 0.03%) (29). Thus, the Δ^{199}Hg values decreased with increasing distance to the anthropogenic sources. This is supported by Carignan et al. (2009), which demonstrates that negative MIF, particularly Δ^{199}Hg values, is induced by atmospheric photoreduction[30]. So, as the Hg travels through the atmosphere, it is photo reduced and induces increasing negative MIF with the distance traveled.

In lichens, Hg isotopic ratios, particularly δ202 Hg and Δ^{199}Hg values, can be used to identify a contamination source. Furthermore, binary mixing models can attribute the contribution of each source to the contamination in a particular area using stable Hg isotopic ratios[11]. With this, in this study, we analyzed the Hg isotopic

ratios in lichens collected at our sites. Isotopic ratios are important in identifying the Hg sources and determining their relative contributions to the total Hg accumulated in lichens. So, this allowed us to conclude the origins of the Hg contamination and further analyze their spatial distribution.

3. Methods

3.1 Study Areas

3.1.1 New Almaden Mining District

New Almaden Mining District (NAMD) is located in San Jose, California, and was once the largest cinnabar mine in North America from 1845-1971. During its operation, NAMD extracted an estimated 38×10^6 kg of Hg and contained numerous mines, furnace arrays, and mine waste dumps [31]. Although mining operations in New Almaden ceased over fifty years ago, legacy mine waste continues to be a constant source of Hg contamination in the Guadalupe Watershed, which includes the Guadalupe Reservoir (GR), Calero Reservoir (CR), and Almaden Reservoir (AR). Each reservoir is highly contaminated with Hg and contains fish that exceed the regulatory threshold for Hg concentrations [4,32]. Furthermore, the Guadalupe Watershed is connected to San Francisco Bay via the Guadalupe River, so the NAMD is a source of Hg contamination in the bay [32-35].

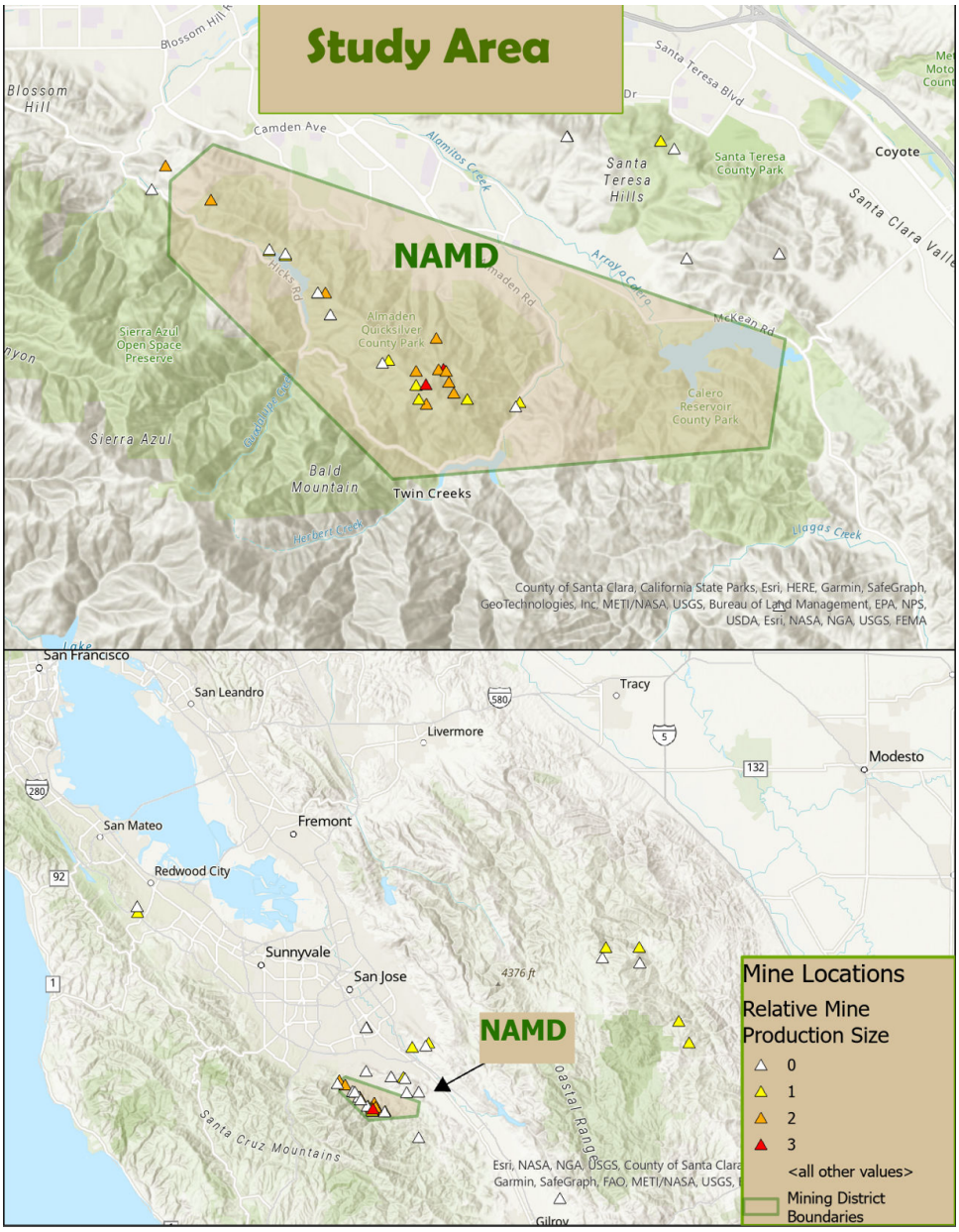

Figure 1. **Study Area**. Map showing the study area, NAMD, with marked Historic mine locations. The mines are colored by relative production size.

3.1.2 Background/Control Sites

Anderson Lake, Coyote Lake, Lexington Reservoir, Santa Teresa, Stevens Creek, and Villa Montalvo are the areas we used as control sites. These areas are all located in

Northern California and were determined not to be impacted by local Hg contamination. So, the Hg concentrations within these lichens are likely all from the background atmospheric Hg pool.

3.2 Sampling Procedure

Lichen samples were collected using a methanol-cleaned 10 m telescoping fruit picker to access lichens from tall tree branches and steep hillsides. Multiple species of lichen were collected to determine if there is a difference in THg sensitivity between different species and morphological characteristics. Using a lichen field guide, we visually identified six lichen species: *Evernia prunastri* (EP), *Ramalina farinacea* (RF), *Ramalina menziesii* (RM), *Ramalina Leptocarpha* (RL), *Flavopunctelia sp.* (FS), and *Usnea sp.* (US)[36]. We recorded the coordinates of each sampling location using Google Maps. Samples were collected using trace-metal clean sampling techniques such as EPA Method 1669 clean hands-dirty hands, cleaning of tools with methanol between samples, and sample storage in two polyethylene storage bags[37]. We stored samples in a climate-controlled environment prior to analysis.

3.3 Sample Preparation

All lichen samples were cleaned by removing foreign materials (bark, bugs, etc.) and separated by species. Initial THg concentration analysis demonstrated no significant differences between species, so subsequent samples were homogenized with their

relative species abundance recorded. A portion of the samples were washed with deionized water or a 1% EDTA solution, as described in Windham-Myers et al. (2014) [38]. Both washed and unwashed samples were homogenized with liquid nitrogen to flash freeze and crushed with an acid-washed mortar and pestle. Samples were stored in a 20 mL glass scintillation vial or a 50 mL falcon tube, then dried in a lyophilizer for >12 hours.

3.4 Total Mercury Analysis

Total Hg (THg) was determined using direct mercury analyzer atomic absorption spectroscopy (DMA-80) (Milestone Corp.) following EPA method 7473 [39]. In summary, approximately 0.01 grams of the dried lichen samples are placed in a sample boat and transferred to the DMA-80. The sample is thermally decomposed, and the mercury released is trapped through gold amalgamation, which is subsequently reheated in a furnace to release the Hg. The Hg then flows to a spectrophotometer, which is quantitatively measured by atomic absorption [40]. We analyzed all samples in duplicate and calibrated them with DORM-4, DOLT-3, or similar fish protein-certified reference material.

3.5 Mercury Speciation Analysis

Subsamples of lyophilized lichens were weighed (0.01-0.03 g), transferred to an acid-cleaned centrifuge tube, and digested with 7mL of 4.57 M HNO3 for >12 hours as described in Hammerschmidt and Fitzgerald (2005)[41]. This method releases MeHg

and inorganic Hg from biological tissue. Inorganic and MeHg were determined from the lichen digests using gas chromatographic cold-vapor atomic fluorescence spectroscopy (GC CVAFS) EPA 1630 [42]. Briefly, 100 µL of the digest was added to ~100 mL of 18.2 MΩ-cm water in a sparging flask. 350 µL of acetate buffer and 50µL of the ethylating reagent, sodium tetraethylborate (NaETB), were added to the flask. The solution was then purged using N_2 gas for 10 minutes and concentrated onto a Tenax trap. The Hg derivatives were then thermally desorbed from the Tenax onto a capillary column GC, followed by pyrolysis and detection with a cold-vapor atomic fluorescence spectrometer (CVAFS).

3.6 Stable Mercury Isotope Analysis

Stable Hg isotope analyses were conducted following standard procedure at the U.S. Geological Survey Mercury Research Laboratory. Approximately 0.1 g of lichen was digested in 5 mL of concentrated nitric acid, oxidized with 5% bromine monochloride, and then heated for 4 hours. Extracts were diluted to a 10% acid concentration and then analyzed for THg stable isotopes. IAEA 407 was used as the isotopic Hg SRM, and UM-Almadén was used as a secondary standard [43].

3.7 Transplantation

A lichen transplantation experiment was conducted in the NAMD to determine the gaseous Hg uptake rate by various lichen species in areas we determined to have elevated Hg emissions. Three lichen species, *Evernia prunastri, Ramalina*

Leptocarpha, and *Usnea,* were collected from the background area, Lexington Reservoir. We then cleaned off the lichens with spring water, pat-dried them with KayDries, and put them into acid-cleaned mesh bags, separated by species. The lichen bags were then transplanted to 8 different sites in NAMD. Figure 3 shows the location of each transplantation site, with 1-3 in the Mine Hill area, 7-9 in Hacienda/Deep Gulch area, and 4-5 near Almaden Reservoir.

Each site had a shield to protect from deposition from rain, with four lichen bags hanging below the shield. The shield and lichen bags were hung approximately 1.5 m from the ground. The four lichen bags were one of each species and one control. The controls were lichens collected from the location of each shield to evaluate any changes in the vitality of the lichens from the collection, washing, and subsequent transplantation (Figure 2).

Furthermore, two reverse transplantation shields were implemented at the background site, Lexington Reservoir. These experiments transplanted contaminated lichens taken from two areas determined to have elevated levels of Hg emissions (Mine Hill and Hacienda) to Lexington Reservoir to determine the release rate of Hg from the lichens. Figure 2 shows shield 6 and 10 as the reverse transplantation sites. The same sampling protocol was used; each shield contained 3 experimental and 1 control lichen bag.

Once the shields were installed, the initial time point was collected by grabbing a small tuft of lichen from each bag. Each lichen bag was sampled approximately every 30 days for three or four months. Samples were collected using

trace-metal clean sampling techniques such as EPA Method 1669 clean hands-dirty hands, cleaning of tools with methanol between samples, and sample storage in two polyethylene storage bags [37] . Samples were stored in a climate-controlled environment prior to analysis.

Figure 2. Transplantation Method. A photo of the method used for the transplantation experiment. Each site had a shield with 4 bags of lichens hanging from it.

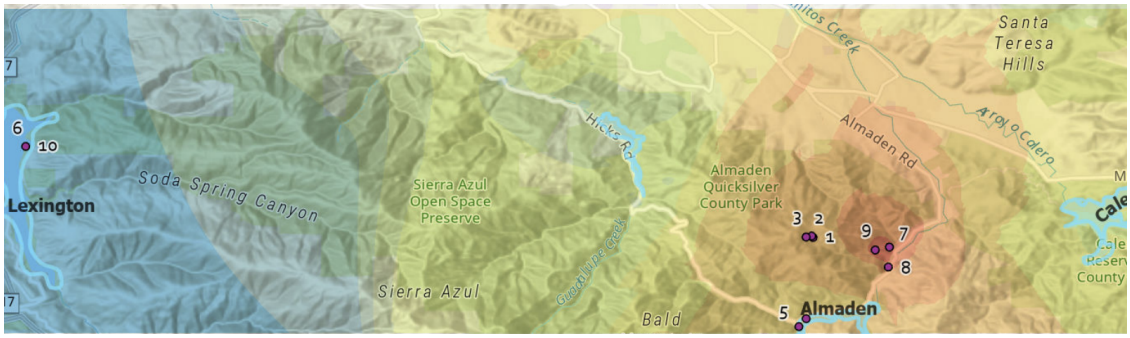

Figure 3. Transplantation Site Locations. A map indicating the location of each

transplantation site in comparison to a heat map. The heatmap shows low average THg levels in blue and high average THg levels in red of the in-situ lichens previously sampled. 1-3 are in Mine Hill, 4-5 are near Almaden Reservoir, 7 and 8 are in Hacienda Furnace Yard, 9 is in the Deep Gulch area, and 6 and 10 are in Lexington Reservoir.

3.8 Mercury Passive Air Samplers

In addition to each lichen transplantation shield set up, two Mercury Passive Air Samplers (MerPas) were installed near each shield (Figure 4). The MerPas were employed in duplicate to ensure replication in analysis. The MerPas were installed approximately 1.5 m from the ground to reflect the same exposure as the transplanted lichens. Each MerPas was left in the field for approximately 60 days. Once uninstalled, the MerPas were mailed to USGS in Madison, WI, for further analysis.

Figure 4. MerPas Methods. A photo showing the setup of the air collectors. Near each shield, 2 collectors were installed approximately 1.5 m from the ground.

3.9 Data Analysis

All statistical analyses were performed on Origin Pro. The analysis for the washed versus unwashed samples used the relative percent difference (RPD) of the washed and unwashed samples (Unwashed-Washed/Average of the two * 100) to determine a difference. The means of the various lichen species in the background were normally distributed, and a Tukey Test was used to compare their means. A Tukey Test was also performed to compare the means of the THg concentrations in the lichens collected near reservoirs with the background samples.

3.10 Geostatistical Analysis

The spatial distribution pattern of atmospheric mercury was determined using ArcGIS pro's Empirical Bayesian Kriging analysis tool. This geostatistical interpolation method automatically calculates parameters through subsetting and simulations to give accurate results[44]. This tool allows for the visualization of Hg emission hotspots and their theoretical spatial extent.

4. Results

4.1 Total Mercury Concentration by Lichen Species

To determine if the lichen species has an effect on THg accumulation, five lichen species were collected from the background locations, and their THg concentrations were compared. The mean THg concentrations of the various lichen

species were; 168 ± 36 ppb (ng/g) in *Flavopunctelia sp*, 176 ± 50 ppb in *Ramalina Meziezii*, 152 ± 52 ppb in *Evernia Prunastri*, 132 ± 46 ppb in *Ramalina Farinacea*, and 166 ± 44 ppb in *Usnea sp.* This data was determined to be normally distributed, and using a Tukey Test, the mean THg of each lichen species was not significantly different (p=0.05) from each other, other than Ramalina Faraniacea and Ramalina Menziezii (p=0.0448). These results indicate that the lichen species used as a bioindicator of atmospheric mercury should not significantly affect identifying areas with elevated emissions. With this in mind, our subsequent samples were homogenized with relative species abundance recorded for ease of sampling.

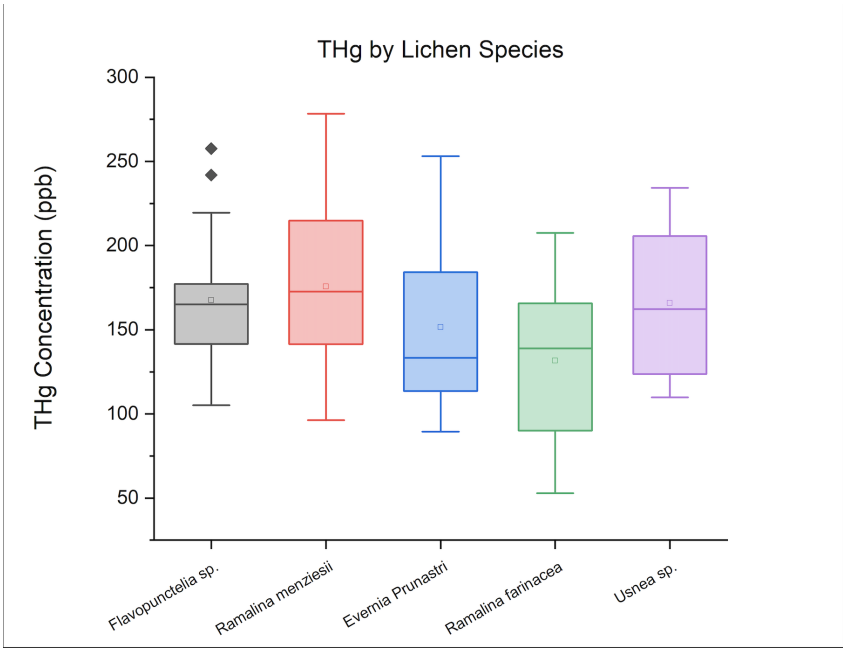

Figure 5. THg by Lichen Species. A box and whiskers plot illustrating the THg concentrations of five lichen species collected at background sites.

4.2 Total Mercury in Washed vs. Unwashed Lichens

Random lichen samples were subsampled into two groups, washed and unwashed, to determine if particulate bound Hg (PBM) was deposited on the surface of the lichens and significantly contributed to the overall THg concentration of the lichens. As stated, the washed lichens were rinsed in either a 1% EDTA solution (N=20) or DI water (N=20). Using a paired sample t-test, there was no significant difference (p=0.05) found between the washed and unwashed samples for both the EDTA-treated group (p=0.4834) and the DI water-treated group (p=0.761). From this, we can assume that PBM is not a significant source of atmospheric Hg in the NAMD, and all the Hg found within the lichens is absorbed into the lichens' tissue.

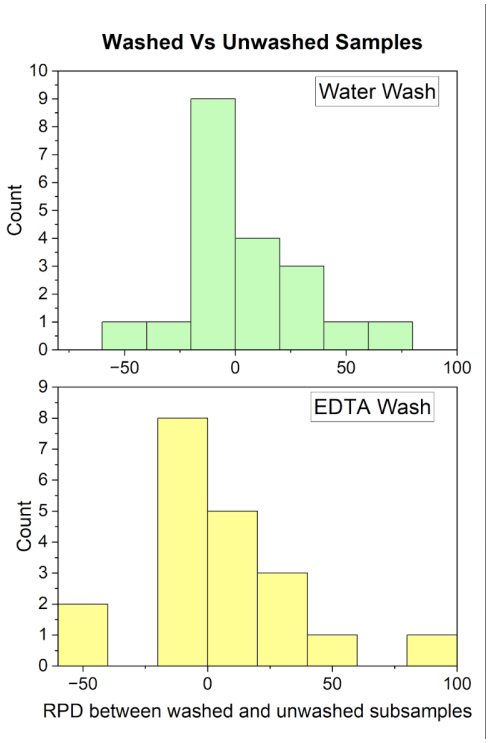

Figure 6. Washed Vs. Unwashed. The relative percent difference (RPD) between unwashed lichens and water-washed subsamples (top). And the RPD between unwashed lichens and EDTA-washed subsamples (bottom).

4.3 Total Mercury Concentrations in Lichens within NAMD and Surrounding Areas

Throughout the NAMD and in the surrounding region out to 30 km distant from NAMD, the THg concentrations within the lichens ranged from 97 to 20,084 ppb (Table 1). Within the NAMD, Hacienda Furnace Yard and the adjacent Deep Gulch area had particularly elevated THg concentrations with mean values of 1681 ppb and 6174 ppb, respectively. These results suggest that these areas are Hg emission hotspots, which was further confirmed using ArcGIS Pro's Empirical Bayesian Kriging analysis tool (Figure 7). This tool gives a visualization of the hotspots using data interpolation methods [44.] Clearly, it shows the highest THg concentrations are located around the Hacienda Furnace Yard and Deep Gulch area.

Site Name	N (samples)	Mean THg (ng/g)	Stdev THg (ng/g)	Max THg (ng/g)	Min THg (ng/g)	Median THg (ng/g)
Background						
Anderson Lake	1	177				
Coyote Lake	21	182	43	278	133	173
Lexington Reservoir	24	152	49	253	53	162
Santa Teresa	3	189	73	242	105	219
Stevens Creek	11	169	50	258	113	145
Villa Montalvo	13	134	33	173	77	148
All Background Sites	*75*	*162*	*47*	*278*	*53*	*159*
Mine Impacted Reservoirs						
Almaden Reservoir	6	341	132	571	211	319
Calero Reservoir	23	247	69	424	147	235
Guadalupe Reservoir	17	280	96	523	162	257
Lake Berryessa	29	234	56	341	115	231
All Mine Impacted Reservoir Sites	*77*	*258*	*79*	*571*	*115*	*241*
Mining District						
Almaden-Calero Transect	6	341	130	699	158	347
Deep Gulch	19	6174	5404	20084	896	3727
Enriquita mine	6	470	139	608	301	505.8
Hacienda Furnace Yard	29	1681	2789	14881	276	851
Hicks flat	6	229	71	350	171	200
Hicks Road	6	221	54	295	158	222
Knoxville	15	366	303	1309	134	255
Mine Hill	57	1742	2035	10541	242	1081
Mockingbird Hill	4	300	98	413	180	303
Providence Mine	4	256	48	319	203	251
San Mateo Mine	6	247	85	383	168	213
Senador Mine	29	503	529	2613	97	324
All Mining District Sites	*204*	*1553*	*2867*	*20084*	*97*	*642*

Table 1. Summary of THg Concentrations

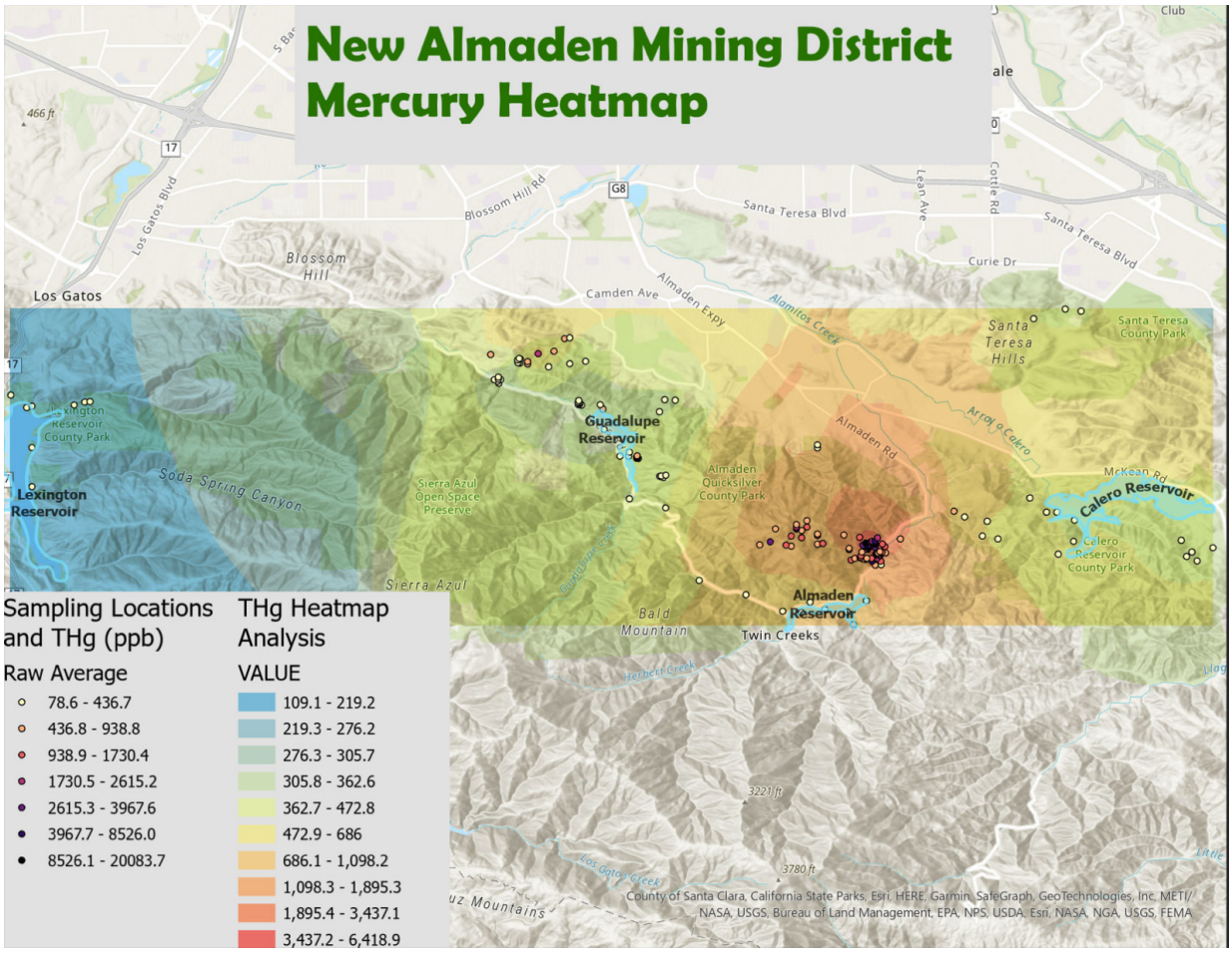

Figure 7. NAMD THg Heatmap. This map was made using ArcGIS's Empirical Bayesian Kriging analysis tool. It indicates each sampling point and its corresponding THg concentration.

Additionally, lichen samples were collected along the shorelines of GR, CR, and AR. The lichens collected along these reservoirs had THg concentrations of 280 ± 96, 247±69, and 341±132 ppb, respectively. Using a Tukey test, each of the reservoir's THg concentrations was significantly higher (p=0.05) than background levels (162 ± 47 ppb) (Figure 8). These results indicate that the atmospheric Hg emissions from historic mining activities impact these reservoirs. To further confirm

this assumption, a transect was sampled starting at the identified hotspot, Hacienda Furnace Yard, to an area with near background concentrations (Calero Reservoir). The lichen's THg concentration was 8,400 ppm at 37 m from the theoretical hotspot; this slowly fell to 176 ppm at 5,860 m from the hotspot. So, as expected, the THg concentration decreased with the distance to the hotspot (Figure 9). Using a power fit analysis between log THg and distance to the hotspot, the curve intersects our average background concentrations (162 ppb) at 7.3 km (Figure 10). This indicates that this hotspot's atmospheric Hg emissions may contaminate the environment up to 7.3 km away.

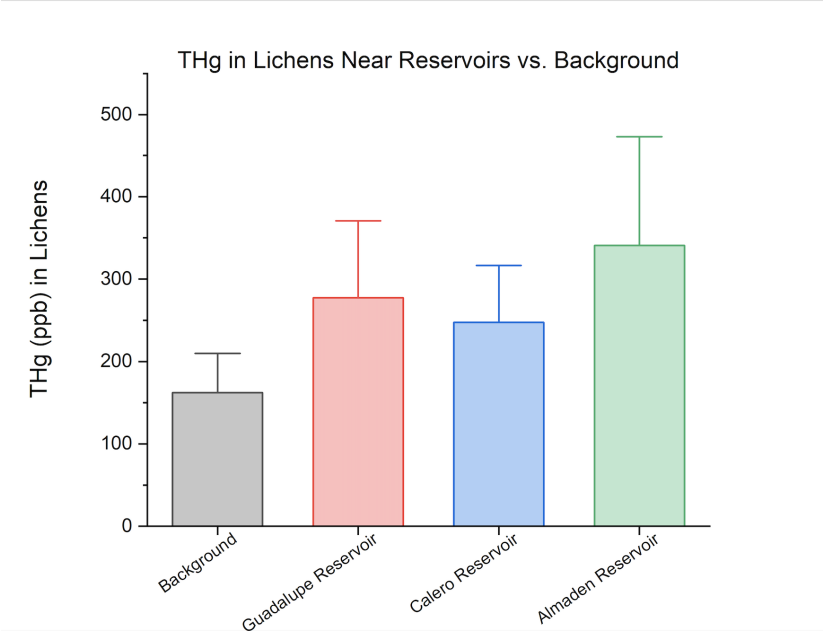

Figure 8. THg in Lichens Near Reservoirs. A column chart comparing the THg concentrations in lichens collected near the reservoirs in NAMD with the concentration of background lichens.

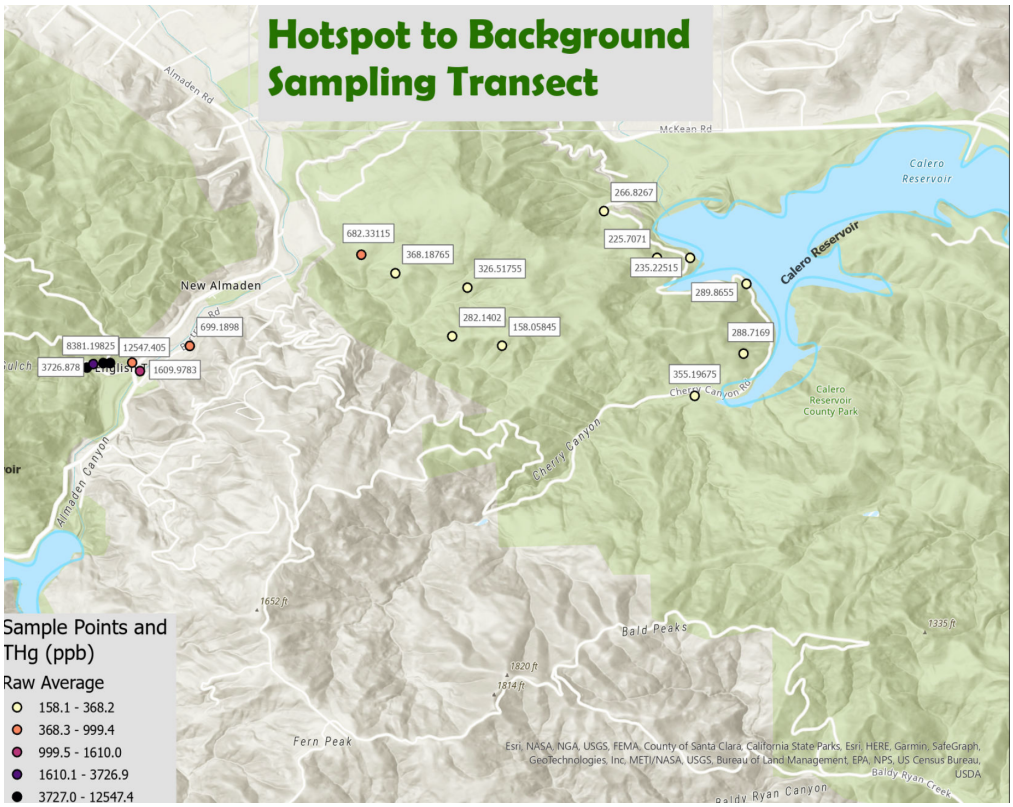

Figure 9. Sampling Transect. A map showing the samples collected in transect starting at the hotspot, Hacienda Furnace Yard, to Calero Reservoir. The THg concentrations are indicated and decrease with distance from the hotspot.

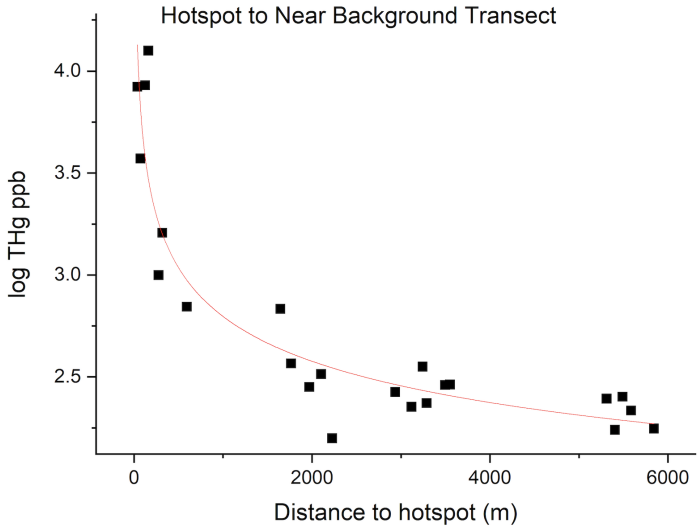

Figure 10. THg and Distance to Hotspot. Power fit analysis between the log THg and the distance to the hotspot. THg was log-transformed for better visualization of the data. The curve intersects the average of our background concentration (162 ppb) at 7.3 km.

4.4 Mercury Speciation in Lichens

Since the chemical form of Hg determines its fate, transport, and bioavailability, random lichen samples (N=68) were selected for Hg speciation analysis. Using the speciated Hg concentration within the lichens as a function of the THg, approximately 79% is Hg^{2+}, 20% is Hg^{0}, and less than 1% is MeHg (Figure 11). These values remained consistent regardless of the THg concentration within the lichens.

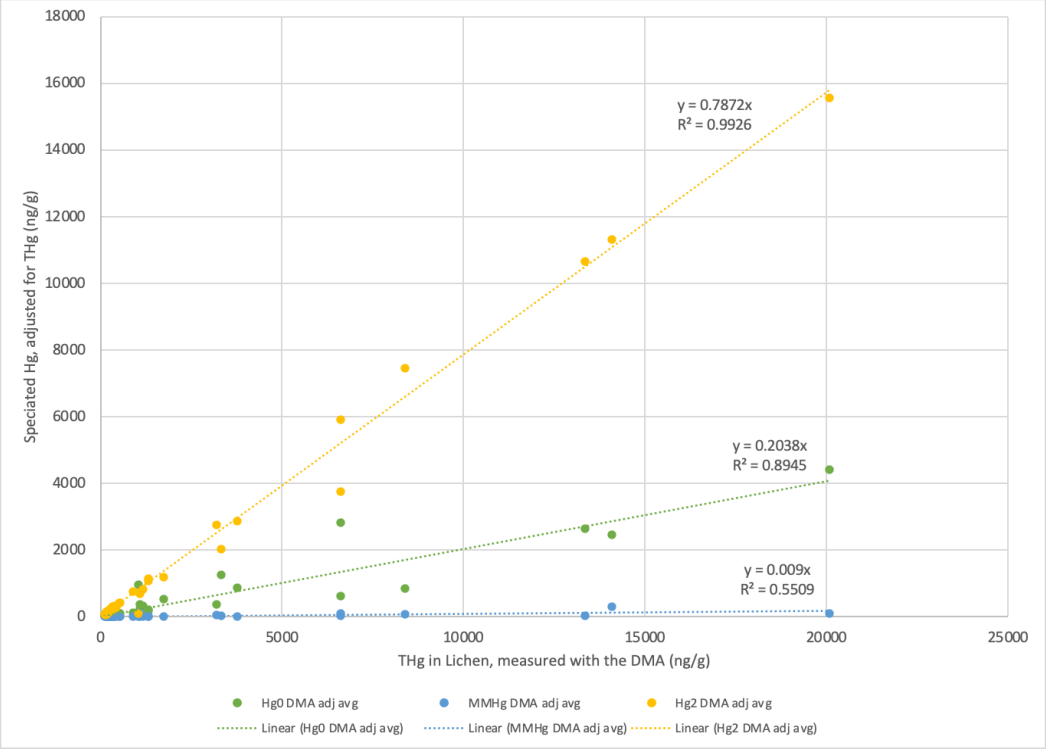

Figure 11. Speciated Form of Hg. Linear regression graph showing the speciated Hg concentrations within the lichens as a function of THg concentrations. Approximately 79% of the total Hg within the lichens is Hg^{2+}, and 20% is Hg^{0}.

4.5 Mercury Isotopes in Lichens

Preliminary stable mercury isotope analysis was performed on lichen samples from background areas (N=3) and within NAMD (N=1). The background lichen samples had THg concentrations of 119-174 ppb, and the mine-impacted lichen sample had a THg concentration of 4882 ppb. The background lichen samples ranged from δ^{202}Hg of -0.06% to -1.49%, while the mine-impacted lichen was -0.69%. The Δ^{199} Hg of the background lichens were -0.42% to -0.69%, and the mine-impacted lichen was 0.00%. The extensive range in δ^{202}Hg values, regardless of THg concentration, is likely due to the isotope fractionation during uptake. Different lichen species from approximately the same area show different δ^{202}Hg values, likely due to different uptake mechanisms, which cause uneven mass-dependent fractionation across lichen species. So, although our stable mercury isotope analysis is only preliminary, our data collected is comparable to other Hg isotopes in lichen data (Figure 12)[11,30]. The lichen data suggests that contaminated sources closer to waste rock and mine tailing do not pick up the negative Δ^{199} Hg signature, making them decipherable from background atmospheric Hg, which has a more negative MIF due to the mercury having undergone atmospheric photoreduction[29].

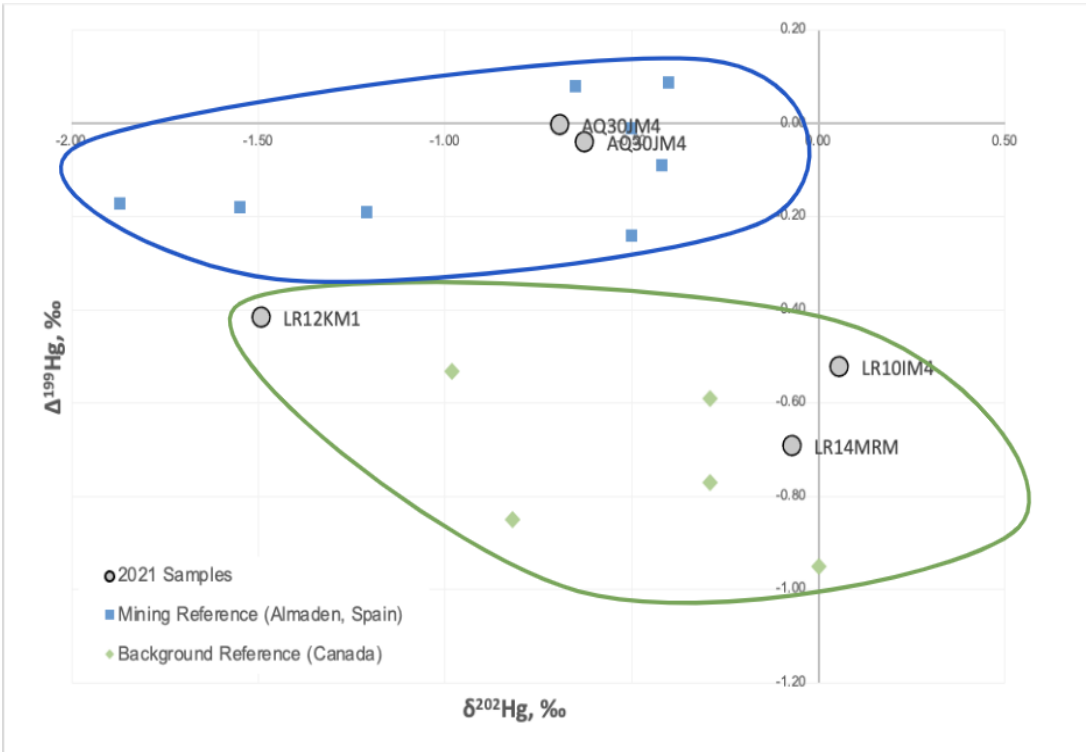

Figure 12. Stable Mercury Isotopes in Lichens. Data and figure by Sarah Janssen at USGS in combination with mining reference data[11] and background reference data [30]. Δ^{199}Hg% values within the lichens are close to 0 near the source, and the values get more negative with distance from the source towards the background locations.

4.6 Transplantation

4.6.1 THg Accumulation by Transplantation Site

Lichen was harvested and put into nylon mesh bags which were suspended from trees at various locations in the NAMD. The transplantation locations included Almaden Reservoir, Mine Hill, and the Hacienda/Deep Gulch area. The lichens were sampled approximately every 30 days (Jan 18th-April 21st, 2023), and their THg concentrations were recorded. Three species of lichen were harvested at Lexington

Reservoir and used for the transplant. The lichen species, *Ramalina Leptocarpha*, was the most abundant and therefore was transplanted to every site, allowing for a comparison of THg accumulation in this species across the various sites. Almaden Reservoir's average THg in the lichens for each sampling time point was; 134 ± 9, 110±8, 102 ±16, and 130 ± 10 ppb, showing no significant increase over time. Mine Hill's transplanted lichens had THg concentrations of; 113 ± 8, 111 ± 16, 106 ± 7, and 135 ± 7 ppb, only showing a large increase (29 ppb) between the March and April sampling. The THg concentrations of the transplanted lichens at Shield 7 in Hacienda were 88 ± 30, 114 ± 16, and 137±11 ppb, respectively, showing a general increase over time. The transplanted lichens at shield 8 in Hacienda's THg concentrations were 115 ± 13, 140 ± 34, and 165 ± 50 ppb, again showing a general increase over time. Shield 9, near the Deep Gulch area, had THg concentrations of 127 ±5, 105 ± 6, and 129 ± 6 ppb, not showing any significant accumulation of Hg (Figure 13).

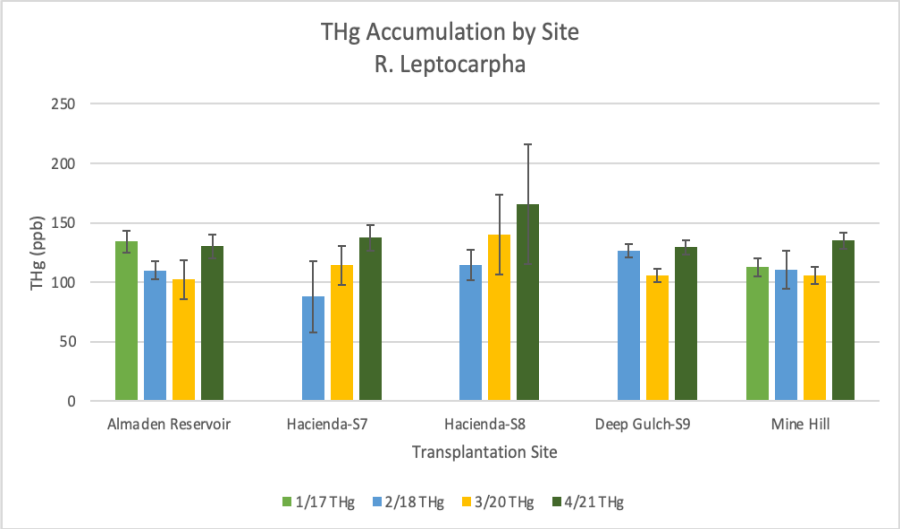

Figure 13. THg Accumulation by Transplantation Site. The THg concentrations at each time point for each site. These lichens were originally collected at Lexington Reservoir and transplanted to the sites indicated. *Ramalina Leptocarpha* was used for this comparison because it was at each transplantation site.

4.6.2 THg Accumulation by Lichen Species

In addition to comparing the THg accumulation by transplantation site for a single species, we aimed to compare the difference in accumulation for different lichen species. Three lichen species, *Evernia Prunasti, Ramalina Leptocarpha,* and *Usnea,* were collected from Lexington Reservoir and transplanted to Mine Hill and Almaden Reservoir. At Mine Hill, *Evernia Prunasti, Ramalina Leptocarpha,* and *Usnea's* THg at each sampling time point were (177±40, 107 ± 45, 99 ± 34, and 156 ± 23 ppb), (113 ± 8, 111±16, 106 ± 7, and 135 ± 6 ppb), and (224 ± 34, 171 ± 72, 160 ±8, and 240 ± 17 ppb), respectively (Figure 14). At Almaden Reservoir, *Evernia Prunasti, Ramalina Leptocarpha,* and *Usnea's* THg at each sampling time point were (172 ±58, 96 ±1, 155 ± 29, and 195 ± 26 ppb), (134 ± 9, 110 ± 8, 102 ± 16, and 130 ±

10 ppb), and (185 ± 27, 193 ±12, 177, and 217 ppb), respectively (Figure 15). Although there is no obvious trend across species for accumulation, *Usnea* had the highest initial and final concentrations at both sites. Furthermore, each lichen species at both sites had THg increases from the March to April sampling. This is consistent with the comparison of *Ramalina Leptocarpha* across all the sites (Figure 13). These results suggest that all species behaved similarly during the transplantation, responding to changing environmental conditions more or less the same.

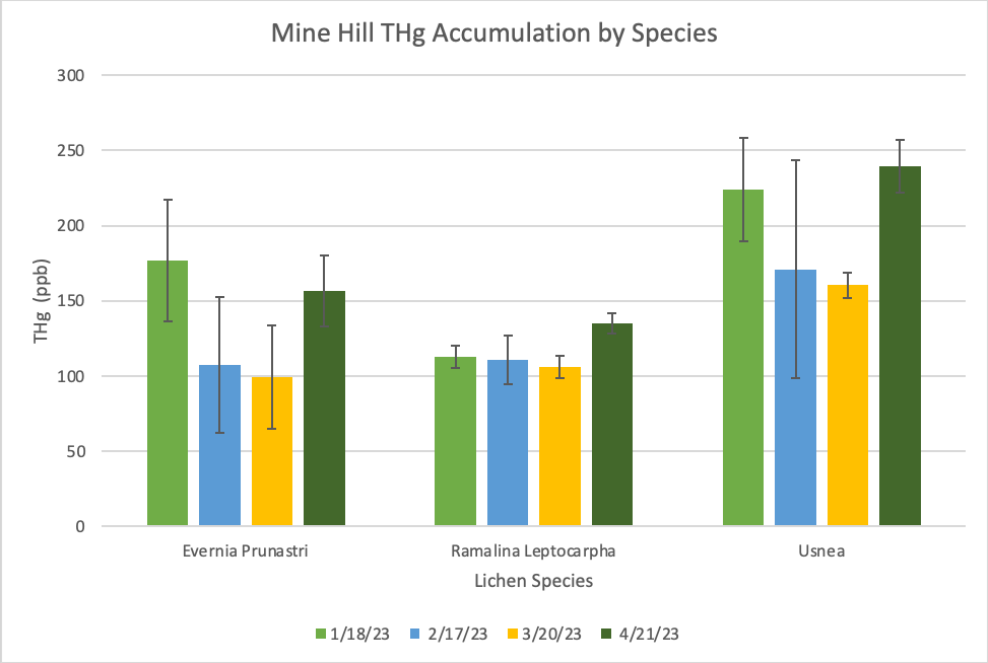

Figure 14. Mine Hill THg Accumulation by Species. Column chart comparing the different lichen species THg accumulation over time. These lichens were originally collected from Lexington Reservoir and transplanted to Mine Hill.

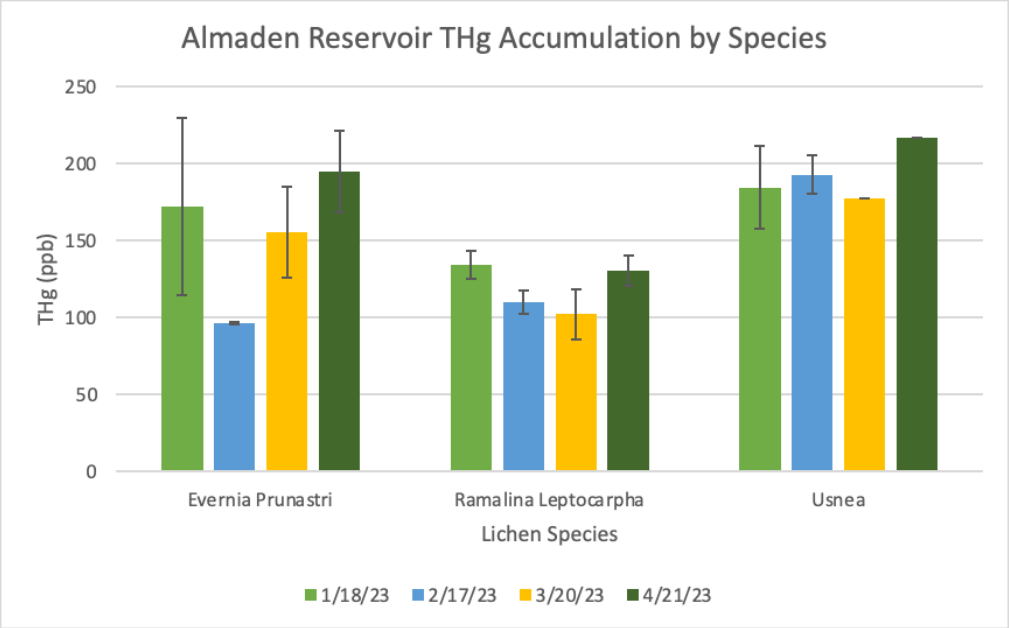

Figure 15. Almaden Reservoir THg Accumulation by Species. Column chart comparing the different lichen species THg accumulation over time. These lichens were originally collected from Lexington Reservoir and transplanted to Almaden Reservoir.

4.6.3 THg Release by Collection Site

As stated previously, contaminated lichens were collected at Mine Hill and the various sites in Hacienda/Deep Gulch area and transplanted at Lexington Reservoir to analyze their release rate. Over time, the lichens originally collected near shield 8 in Hacienda showed a general decrease in THg (820, 732, and 536 ppb). Over time, the lichens collected near Shield 9, close to Deep Gulch, had THg concentrations of (1729, 1502, and 1706 ppb). At shield 7 in Hacienda, there was a general decrease in THg (1569 and 1282 ppb), although only two time points were considered. The lichens originally collected at Mine Hill did not exhibit much of a decreasing trend

and actually slightly increased (225 ± 5, 263 ± 21, 297 ± 26, and 302 ± 50 ppb) with time (Figure 16).

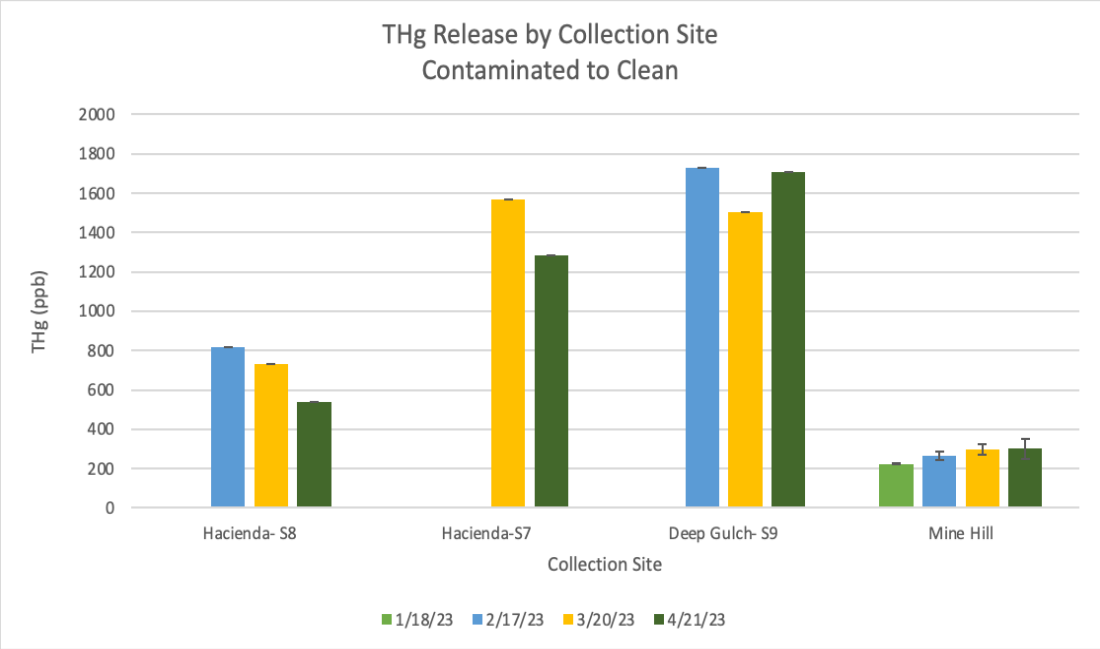

Figure 16. THg Release by Collection Site. Column chart comparing the release of THg of different collection sites. These lichens were collected at the indicated collection site and transplanted to Lexington Reservoir.

5. Discussion

5.1 Lichen as a Bioindicator

As stated before, the main objective of this study was to determine the impact of atmospheric Hg emissions in the NAMD on the surrounding environment, using lichens as a bioindicator. However, before this could be determined, we aimed to gain a better understanding of using lichen as a bioindicator in our study area. We achieved this by analyzing various processes and properties of the lichens.

5.1.1 Effect of Lichen Species

For example, we first sought to determine if the species of lichen used has an effect on the THg accumulation. The 5 species we chose to investigate showed no significant difference in their THg concentrations in background areas, except between Ramalina Faraniacea and Ramalina Menziezii (p=0.0448). These results indicated that the lichen species used as a bioindicator of atmospheric mercury should not significantly affect identifying areas with elevated emissions. With this determined, we continued our study by homogenizing multiple species within a sample. Homogenizing allowed us to control for lichen age and made collections easier because of the higher availability of lichens.

However, this was initially determined by only measuring lichens' THg concentrations in uncontaminated areas because measuring in contaminated sites poses challenges. To determine whether the lichen species influences accumulation, the lichens must be collected at the exact location to control for distance from the source. In the field, the availability of various lichen species can be inconsistent, and thus, finding a location with all 5 species in a small radius would be extremely difficult.

With this, we aimed to restudy the effects of lichen species on Hg accumulation in the transplantation experiment. By transplanting three lichen species, *Evernia prunastri, Ramalina Leptocarpha,* and *Usnea,* we could compare the various species' THg accumulation.

However, our data from this seems to be inconclusive for now (Figures 14 and 15). Since no species exhibited more accumulation than another, this is hard to determine with the data collected thus far. However, we hope this will become clearer with subsequent sampling and more time points.

5.1.2 Form of Hg Absorbed by Lichen*s*

Previous research has suggested that lichens passively absorb gaseous Hg^0 and transform it to Hg^{2+} [45]. Our analysis of the effect on THg concentrations of washing the lichen showed no significant difference between the lichens that were washed or unwashed. These results indicate that particulate-bound Hg is not a significant form of atmospheric Hg in NAMD. However, lichens contain predominantly Hg^{2+}, which is non-volatile, and a smaller portion of Hg^0 which is volatile. Our speciation analysis shows that approximately 79% is Hg^{2+}, 20% is Hg^0, and less than 1% is MeHg. So, we believe the Hg emitted from the contaminated areas is likely gaseous Hg^0 because Hg^0 is extremely volatile compared to the unconverted cinnabar and other Hg byproducts found in the mine waste [6, 46]. The lichen, then, absorbs the Hg as Hg^0 and oxidizes it to Hg^{2+} to be incorporated into their tissues[45].

This is supported by the fact that Hg^0 is extremely volatile and has limited solubility in water, so it is not efficiently scavenged by wet or dry deposition. Thus, the lifetime of gaseous Hg^0 is relatively long, and it is not oxidized to Hg^{2+} until it reacts with atmospheric oxidants such as halogens, ozone, and OH radicals [46]. Since the lichens are in relatively close proximity to the source, the Hg has likely yet to be

oxidized, demonstrating that the lichens absorb gaseous Hg^0 and oxidize it to Hg^{2+}. This is important because it gives us insight into the processes of how lichens act as bioindicators so we can further understand it and have confidence in using it for this study.

5.1.3 THg Accumulation and Release by Transplanted Lichens

With the transplantation experiment, we aimed to determine the uptake rate and release rate of lichens to understand lichens as a bioindicator better. We compared across different sites and with different lichen species. As expected, the two sites in Hacienda Furnace Yard showed a general THg increase with time. However, these two sites seem out of the ordinary compared to the other study sites. Most sites and lichen species showed a decrease in THg concentration from the 1/17 sampling time point until the 3/20 sampling time point, then a sharp increase in THg concentration at the 4/21 sampling time point. This was even consistent across many of the controls (Figure 17). Although this is not what was expected, it correlates very closely with the average solar radiation of the month before collection (Figure 18). This is likely because an increase in solar radiation is known to increase the gaseous Hg^0 emission flux from contaminated soils [47]. An increase in solar radiation not only increases the photochemical reactions in the contaminated soils but also increases the temperature, which increases the thermal reactions, resulting in an increased amount of gaseous Hg^0 being emitted into the atmosphere [47].

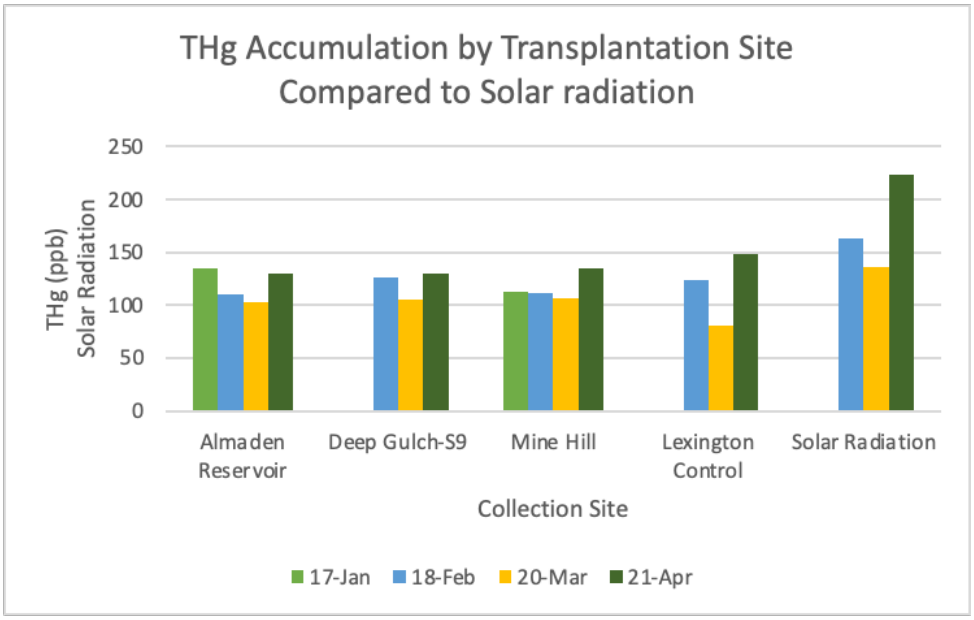

Figure 17. THg Accumulation Compared to Solar Radiation. Column chart comparing the THg accumulation at different sites with the solar radiation. These lichens were collected at Lexington Reservoir and transplanted to the indicated site. The solar radiation indicated on the graph is the average daily solar radiation for the month before collection.

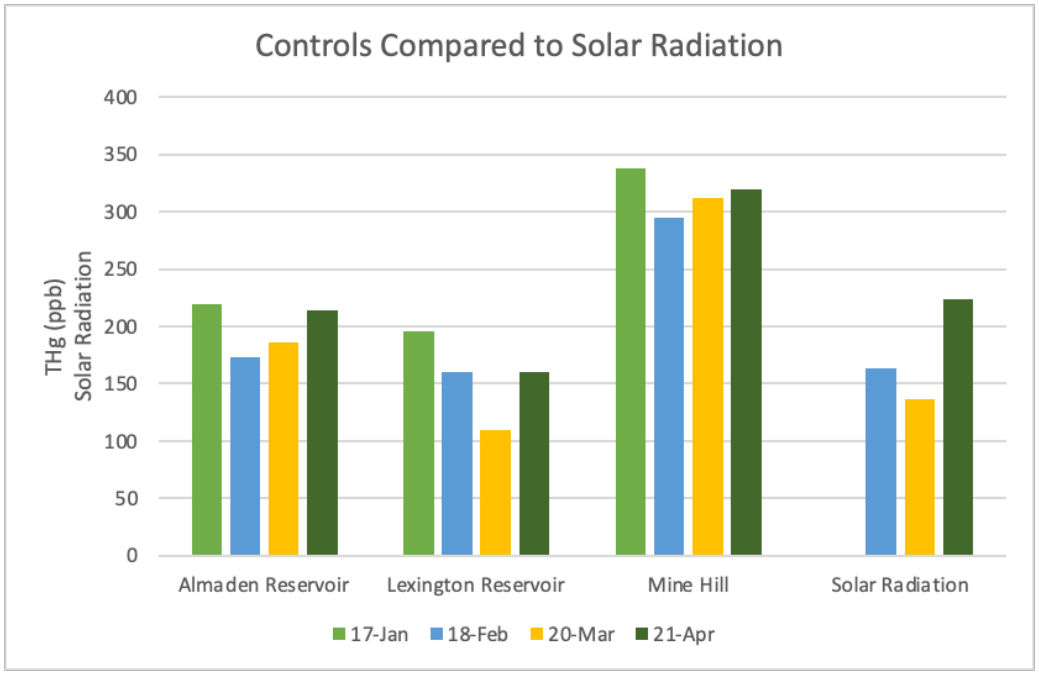

Figure 18. Controls Compared to Solar Radiation. Column chart comparing the THg concentrations of the controls at different sites with the solar radiation. The controls are lichens collected at the indicated site and "transplanted" to the same site. The solar radiation indicated on the graph is the average daily solar radiation for the month before collection.

Furthermore, the lichens collected at the beginning of the transplantation study seemed to have much lower THg concentrations than lichens sampled in the past years (2020-2022) when compared by the collection site (Figure 19). This decrease in THg concentrations is also likely due to the weather. From December 2022 through March 2023, California was hit by numerous atmospheric rivers, with the total precipitation in New Almaden reaching 551 mm [48]. This is almost 3.5 times higher than the previous year's precipitation amounting to 162 mm [49]. With increased precipitation, lichen vitality and growth have been shown to increase [50]. This is important because, with decreased Hg^0 emissions due to decreased solar radiation and increased lichen growth, the THg within the lichens is likely diluted [50,51]. This would explain why we found much lower THg concentrations in the lichens collected in 2023 than those collected in 2020-2022.

So, although our transplantation experiment did not have the expected outcome, it does seem to correlate with the weather conditions during the study period. With such variable weather conditions, our data seemed to vary significantly. However, as the solar radiation increased and precipitation decreased during the last time point, every transplantation site and lichen species showed an increase in THg.

This trend will hopefully continue into the late spring and through the summer, with the dryer and warmer months.

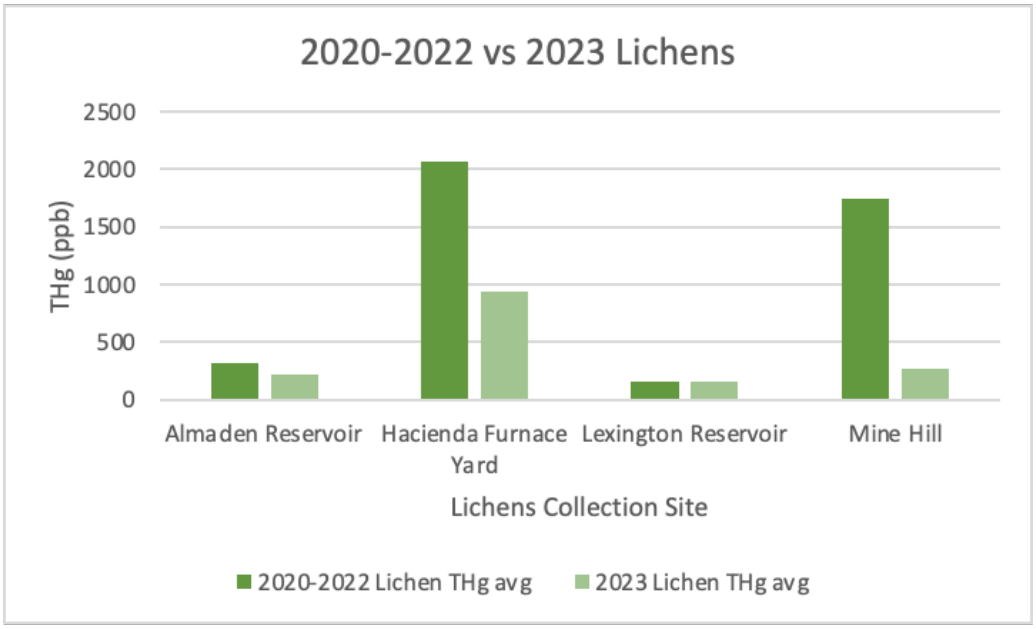

Figure 19. THg Year Comparison. Comparison of the THg concentration in lichens collected in 2020-2022 and those collected in 2023. Concentrations are compared across various collection sites.

5.1.4 Low Cost and Effective

One of the key advantages of using lichens rather than mechanical air collectors is the cost. The MerPas we used for a complementary analysis to the lichen transplantation study cost $71 each (plus shipping and tax)[52]. Throughout this study, we took over 350 lichen samples to spatially cover the NAMD, nearby reservoirs, and background areas. If we had implemented only MerPas instead, that would have surmounted to almost $25,000 compared to the nearly cost-free lichens.

Not only are the lichens a cost-effective alternative, but they have also proven to be effective in identifying Hg emission hotspots. As stated previously, the lichens collected from the Hacienda Furnace Yard and Deep Gulch's average THg concentrations were 10 and 38 times higher than the average background concentrations, respectively. These areas were the most elevated concentrations we found throughout NAMD, indicating these as the emission hotspots. These identified hotspots also correlate with the historical uses of the land. The Hg ore mined throughout NAMD was brought to Hacienda Furnace Yard to be crushed and heated to release the mercury as a vapor to be collected. Furthermore, much of the calcines, or mine waste, that resulted from the extraction were deposited in the Deep Gulch area and still remain there [32]. So, the lichens confirmed that these areas are elevated Hg emission hotspots, which the historical mining uses of these areas can explain. These results show that lichens can be used to identify Hg emission hotspots in the NAMD and, thus, are cost-effective bioindicators.

5.2 Cycling of Hg in Contaminated Mine Sites

To determine the impact of atmospheric Hg emissions in the NAMD on the surrounding environment, we wanted to better understand the overall biogeochemical cycling of Hg and the role lichens may play in it. As mentioned previously, using the THg concentration in lichens allowed us to identify that the main atmospheric Hg sources in NAMD are Hacienda Furnace Yard, Deep Gulch, and Mine Hill. The sources were also confirmed using stable mercury isotopes.

Although our isotope work was only preliminary, the Δ^{199}Hg values can be used to differentiate between Hg sources. The lichen from the mine-impacted area was collected in the Mine Hill area and showed no Δ^{199}Hg fractionation, while the lichens collected from background areas showed significant negative Δ^{199}Hg fractionation. The lack of Δ^{199}Hg fractionation indicates that Mine Hill is one of the sources because Δ^{199}Hg values start out close to zero (the fractionation found in ore) and tend to get more negative with distance from the source due to long-range transport [29,30]. Thus, Δ^{199}Hg is commonly negative in total gaseous Hg in background sites because the main source of Hg to these sites is background atmospheric Hg that has been photo reduced during long transport [53,54,55]. So, the lack of MIF in our sample demonstrates that it was collected very close to the source because it does not pick up the background's negative Δ^{199}Hg signature.

With the sources confirmed, we concluded that these sources' Hg emissions might travel through the atmosphere up to 7.3 km away. From here, the lichens will likely absorb the Hg as Hg^0 and transform it into Hg^{2+}. As Hg^{2+}, the Hg is much less mobile and less likely to be remitted to the atmosphere [3]. So, the Hg is likely not being released back into the environment until the lichens begin decomposing. This is an essential aspect because it provides evidence that the lichens themselves may be contributing to the contamination of the watershed with their litterfall. Other foliage has demonstrated this, with studies suggesting that litterfall significantly contributes to atmospheric Hg deposition [56]. It has been estimated that litterfall deposition

typically exceeds Hg inputs from wet deposition [57]. So, in NAMD, the atmospheric Hg may be deposited in the watershed through lichen litterfall and wet deposition.

5.3 Impact of Atmospheric Hg Emissions in NAMD on the Surrounding Environment

Our study indicates that the atmospheric Hg emissions in NAMD significantly contribute to the contamination of the surrounding environment and, more specifically, the local reservoirs. First, we identified the atmospheric Hg emission hotspots and determined their spatial distribution to be approximately 7.3 km. This is significant because, in the NAMD, each reservoir is within this radius; thus, these emissions are likely contaminating the reservoirs. This is also confirmed by the significantly elevated THg concentrations found in the lichens surrounding the reservoirs. With this, our results indicate that the Hg emitted from these hotspots may be traveling through the atmosphere and contaminating the reservoirs through direct deposition into the reservoirs or depositing on foliar species, such as leafy plants and lichens. The Hg may then enter the local watershed with subsequent litterfall and decomposition of these foliar species.

6. Conclusions

6.1 Impact of Research

The overall impact of my thesis research is that atmospheric Hg emissions from historical mine waste in NAMD contributes to the local reservoirs' contamination. This is impactful because sediment runoff from the mine waste has been the only

analyzed source. Thus, my work demonstrates there are other contamination pathways which is beneficial information for future remediation projects. Since the elevated levels of MeHg in the fish is a regulatory and environmental issue that Santa Clara Valley Water is currently facing, this data may help inform them for future mitigation efforts[4].

6.2 Future Studies

Despite our work advancing the understanding of the atmospheric contamination pathway in NAMD, continuing the study would help solidify some of the conclusions. For example, the transplantation study should and will continue into the dryer and warmer summer months. Hopefully, this will lead to more conclusive data on the lichens' Hg uptake and release rates by lichen species and site location. In addition to the transplantation experiment, we deployed Hg passive air samplers (MerPas) at each site, but we have not collected any data from them due to unseen instrument issues. When the data is collected, the THg concentrations from the air collectors will be help determine the emission rates from the mine waste and how that correlates with the lichens' uptake rates.

Also, determining the Hg isotopic composition within more samples will enhance our understanding of the sources and their fate. Determining the MerPas isotopic ratio will give us insight into the ratios of the gaseous Hg emitted from the sources. We can then compare it to the ratios within the lichens to determine if there is any isotope fractionation during uptake by the lichens. Furthermore, these isotope

analyses could be compared to the isotopic ratios within the local fish. This would determine the source, pathway, and relative THg contribution to the fish.

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