Quicksilver in the Alpine:  
Mercury Transfers through an Alpine Terrestrial Food Web

An Honors Thesis by Clifford Robert Adamchak

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Abstract

Mercury (Hg) is a global pollutant that originates from both natural and anthropogenic sources and cycles through air, land, and water systems. The consequences of Hg movement into the biosphere persist largely because it is dispersed far distances as nanoparticles of inorganic Hg. Within terrestrial ecosystems, atmospherically deposited Hg may be cycled among vegetation, soils, animal tissues, and hydrological flow paths. Past research has documented long-distance transport of Hg to remote regions far from industrial sources, including disproportionately higher levels in high elevation ecosystems. However, knowledge of Hg cycling within mountain ecosystems remains incomplete. Particularly, little is known regarding the mechanisms that underly Hg cycling in the vegetation of high elevation mountain ecosystems or its movement into the food chain. A major concern is the biological transfer of Hg as monomethyl mercury (MMHg), a neurotoxin that bioaccumulates and biomagnifies in successive trophic levels. To better understand the fate of atmospherically deposited Hg in the alpine zone, I used the West Knoll in the Niwot Long-term Ecological Research site as a model system. Specifically, I present total Hg (THg) and MMHg data for dust, lichen, vegetation, pikas, and weasel samples across a snowpack gradient—including both ecosystem pools and a relatively isolated terrestrial food web. At each site, THg was found in all sample types; however, only dust was significantly different from graminoids and forbs (p < 0.05). Data suggest that THg accumulates in vegetation and increases in concentration with the decomposition of plant tissues. Monomethyl mercury was found in high concentrations in the weasels, indicating that animals feeding at higher trophic levels bioaccumulate MMHg in their tissues. However, MMHg concentrations were below detection limits in the dust, vegetation, and pikas, indicating that weasels are getting MMHg from other sources, likely aquatic environments where it can be produced under reducing conditions. This project provides the first investigation of ecosystem Hg cycling within the Niwot Ridge LTER. It will inform future research both locally and at other alpine areas where atmospheric Hg deposition is an increasing concern.
Introduction

Mercury (Hg) is a global pollutant that originates from both natural and anthropogenic sources and cycles through air, land, and water systems (Selin, 2009; Driscoll et al. 2013; Wang et al., 2019). Human activities, such as mining and fossil fuel combustion, have mobilized stable forms of geologic Hg into the biosphere where they can cycle through vegetation, soils, animals, and hydrological flow paths. In addition, Hg has been used for myriad applications, including as a preservative in biological and pharmaceutical products (Magos 2001) and a fungicide in the tanning process (Mikoczy and Hagmar 2005). It is also a common pollutant in industrial wastewater (Wagner-Döbler 2003). Mercury can transform into toxic forms that put animals and people at risk; it has been linked to neurological diseases (Ellingsen et al. 1993; Hsi et al. 2014) cancers (Mikoczy and Hagmar 2005), and behavioral disorders (Evans, Laties, and Weiss 1975; Wolfe, Schwarzbach, and Sulaiman 1998). Thus, it is a global concern that transcends the disciplines of geological science, ecosystem science, wildlife biology, environmental toxicology, and medicine.

The consequences of Hg movement into the biosphere persist largely because it is dispersed as nanoparticles of inorganic Hg. Most industrial sources emit Hg in its reactive form, Hg(II); however, some industrial, and the vast majority of natural sources (e.g., volcanoes and erosion of Hg-enriched rocks), release elemental mercury (Hg(0); Driscoll et al., 2013; Fig. 1). Once in the atmosphere, Hg(0) has a residence time of approximately one year, where it remains until it experiences oxidizing conditions and transforms into Hg(II) (Carignan and Sonke 2010; Driscoll et al. 2013; Selin 2009). Mercury (II) is more soluble in water than Hg(0), and it can bind to atmospheric particulates. Thus, it is the predominant form of Hg deposited on terrestrial ecosystems in both wet and dry deposition.

Within terrestrial ecosystems, atmospherically deposited Hg may be cycled among vegetation, soils, and animal tissues. Previous research in lab settings, montane forests, and tundra ecosystems show that atmospherically deposited Hg can enter plants directly through stomata or remain on plant material until it decomposes into soil (Lindberg et al. 1992; Smith-Downey et al. 2010; Stamenkovic and Gustin 2009). If Hg leached from soils enters downgradient areas with high organic matter (OM), reducing conditions, and elevated sulfate concentrations—conditions typical of wetlands—Hg can be transformed into MMHg (Driscoll et
The production of MMHg is stimulated by sulfur reducing bacteria (SRB), as well as other anaerobic microbes (see Loseto et al. 2004) and can be mobilized into the food web by animals that use the wetlands as breeding grounds and/or refugia (Driscoll et al. 2013). Once MMHg enters the food web, it biomagnifies by ten-fold with each successive trophic level (Benoit et al. 2002; Poste et al. 2015; Selin 2009; Fig. 1). Alternate pathways by which Hg enters the food web include direct uptake of Hg(0) and Hg(II) by plant stomata (Berzas Nevado et al. 2012; Weiss-Penzias et al. 2019). Mercury poisoning due to its mobilization into the environment is an issue broadly within the U.S. and internationally: there are Hg warnings for fish consumption in all U.S. states. Monomethyl mercury also caused widespread poisoning from the 1930s through the late 1960s in Minamata, Japan (Ekino et al. 2007), the result of industrial wastewater release.

Past research has documented long-distance transport of Hg in nanoparticles to remote regions far from sources (Olson et al. 2019). For several reasons, high elevation ecosystems often have higher rates of Hg deposition than lowland areas. First, photochemical transformations of Hg(0) in the atmosphere are elevated in high altitude environments leading to increased levels of Hg(II) reaching these sensitive ecosystems (Murphy et al. 2006; Swartzendruber et al. 2006; Talbot et al. 2008). Second, mountain ecosystems experience higher precipitation and cold condensation, which can contain Hg that is then subsequently deposited (Mast et al., 2005; Faïn et al., 2013). The vast majority of annual precipitation in high elevation environments falls as snow, thus, increases in snowpack depth can lead to higher inputs of atmospheric pollutants, like Hg (Clow et al. 2002; Turk et al. 2001). While dry deposition accounts for a smaller percentage of total atmospheric deposition in the alpine, several investigators have demonstrated that dust is an important input of atmospheric nutrients and pollutants into mountain ecosystems (Heindel et al. 2020; Lawrence and Neff 2009). One study in Alaska observed that areas with higher Hg concentrations in the plants had higher concentrations of elements that are commonly associated with dust (Olson et al. 2019)

Although disproportionately higher levels of atmospheric Hg deposition occur in high elevation ecosystems, knowledge of Hg cycling within these areas remains incomplete. One study in the Colorado Rocky Mountains reported that the majority of Hg does not leave the ecosystem via watershed runoff; only 20% of atmospheric Hg deposition was measured in
streamflow (Mast et al., 2005). These findings suggest that the terrestrial ecosystem may be a net sink for Hg, prompting the important question of where the Hg is being stored.

Little is known regarding the mechanisms that underlie Hg cycling in the vegetation of high elevation mountain ecosystems, including shrubs, grasses, and forbs (Obrist et al. 2016; Walker et al. 1989). However, studies of lichen and litter suggest that plants play an important role in the Hg cycle. Lichen are particularly sensitive to air pollutants as they absorb water and elements from atmospheric deposition (Bargagli 2016; Olson et al. 2019). Pokharel and Obrist (2011) demonstrated in a lab environment that Hg concentrations increase from fresh biomass to litter, concentrations which are augmented by continued atmospheric Hg deposition (Obrist et al. 2016). An important next step is to determine the role that different vegetation functional groups play in the atmosphere-surface transfer of Hg and the relationships among the concentrations of Hg species in living plant tissues, accumulated litter, underlying soil, and animals.

The North American Pika (*Ochotona princeps*) is a generalist mammalian herbivore that lives primarily in alpine talus areas that provide insulation from the elements and protection from predators, like those found in the Colorado Rocky Mountains (Dearing 1997). Pikas’ remote alpine habitats and consumption of shrubs, grasses, and forbs make them desirable subjects for studying Hg pathways and accumulation. Sharing a habitat with the pikas are the carnivorous weasels (*Mustela frenata nevadensis*) whose consumption of pikas could result in bioaccumulation of MMHg within their tissues (Selin, 2009; Benoit et al. 2002).

The West Knoll at the Niwot Ridge Long-term Ecological Research (LTER) site is an ideal environment to determine the fate of atmospherically deposited Hg species in the alpine zone. The climate of Niwot Ridge has been studied extensively, providing a long-term record of snow deposition and prevailing wind patterns (N Molotch 2021, personal communication, 25 March; Jepsen et al. 2012; Kittel et al. 2015; Williams et al. 1996). The exposure of the West Knoll to extreme environmental conditions prevents many plants and animals from inhabiting the area, creating a relatively isolated zone within the larger alpine landscape. Consequently, there are sparse forbs, grasses, and lichen, along with pikas that feed locally, marmots, and weasels that inhabit a wide home range (Jedrzejewski et al. 1995). Several decades of research on the pika population of the West Knoll (see Bhattacharyya and Ray 2015; Benedict et al. 2020; Southwick et al. 1986; Wilkening and Ray 2016; Wilkening et al. 2015; Dearing 1997) have created a substantial dataset of pika behavior, foraging patterns, and preferred habitat features. Thus, it is
possible to identify and study this terrestrial food web with few confounding variables. In this study, I addressed the following questions and hypotheses related to Hg movement and cycling within the alpine zone of the Colorado Rocky Mountains:

Research Questions and Hypotheses

Q1: Does MMHg bioaccumulate and biomagnify with successive trophic levels in a terrestrial alpine food web?

H1: Given several past studies in lowland ecosystems that demonstrate bioaccumulation and biomagnification of MMHg within increases in trophic level, we will observe the same pattern from vegetation to pikas to weasels in the West Knoll.

Q2: Do THg and MMHg concentrations vary across plant functional groups within areas occupied by pikas on the West Knoll?

H2A: The larger leaf area of forbs captures more atmospheric deposition than grasses. Thus, THg concentrations in forb tissues will be greater than those found in grasses. The aboveground tissue of forbs and grasses do not persist for more than a year, and, therefore, prevent THg from significantly accumulating before they start to decompose.

H2B: If MMHg is present in dust, the forbs will have higher concentrations than the grasses.

Q3: Does variation in THg concentrations in pika tissues reflect the spatial variation in the THg content of alpine plants?

H3: There will be a direct relationship between the THg concentrations in the vegetation immediately available to a pika and the THg concentrations in the tissue of that pika. The pika microhabitats with the highest Hg concentrations in the vegetation will have the highest THg concentrations in the pika hairs.

Q4: What is the relationship of snowpack depth to THg concentrations in the vegetation and pika tissue?

H4: Due to atmospheric deposition being the predominant pathway of Hg into alpine ecosystems, areas that historically have received higher snowpack will have accumulated the highest concentrations of Hg within the vegetation. Thus, pikas feeding in areas of higher snowpack will have greater accumulation of THg in their tissues than those living in areas with lower snowpack.
Study site

This project was conducted within the West Knoll area of Niwot Ridge Long-term Ecological Research (LTER) site located in the Front Range of the Colorado Rocky Mountains (40° 03’ N, 105° 36’ W, elevation 3,500 m). Niwot Ridge, and the region more broadly, is part of the traditional territories of the Cheyenne, Ute, and Arapaho Peoples. Based on climate data collected continuously since 1951 from the D1 weather station (40° 3’ 36” N, -105° 37’ 12” W, elevation 3739 m), Niwot Ridge experiences a mean annual temperature of ~−4.1°C and receives ~1,000 mm of precipitation annually, 80% of which falls as snow (Kittel et al. 2015; Williams et al. 1996; Greenland and Losleben, 2001). The West Knoll, as well as the rest of the ridgeline, is subjected to strong westerly winds which disproportionately affect spatial patterns in snow distribution and accumulation (Litaor et al. 2008). Additionally, the south and east-facing aspects of the Ridge, respectively, receive 18% and 14% more net radiation than the northern aspects on summer days with clear skies (Isard 1986). The increased exposure on certain aspects of the West Knoll consequently affects drying and evaporation levels throughout the ridgeline.

The West Knoll ranges from ~3520 to 3620 m in elevation and supports typical pika habitat comprised of alpine meadows and talus regions. The dominant plant species include forbs and grasses, most notably Silene acaulis, Acomastylis rossii, Deschampsia caespitosa, and Carex species (Dearing 1997). Several different species of lichen are present throughout the Knoll, including Xanthoria elegans, Cladonia cariosa, Lecanora epibryon among others. The talus on the West Knoll affords shelter for the pika, which they share with marmots, providing refuge from surface conditions and predators (Wilkening and Ray 2016). During the summer months, each pika builds a haypile from plants foraged within approximately 25 m of the center of its territory (C Ray 2020, personal communication, 22 July; Dearing 1997; Smith and Ivins 1984). During the winter, the talus becomes insulated by snow cover, creating a thermal buffer that helps the pikas to persist into the next year (Wilkening and Ray 2016)


**Methods**

**Study design**

In collaboration with researchers conducting a long-term study of the pika population on the West Knoll, we chose coordinates of three, well-established and active pika territories across an increasing snowpack gradient (Fig. 2). Each of the sample sites along the gradient was selected haphazardly from several available locations in the snow-cover class (C Ray 2021, personal communication, 2 March). Pika hairs, vegetation, lichen, litter, and haypile samples were all collected during Summer 2020. Site 3 is located on the northwestern side of the West Knoll and experiences relatively low levels of snowpack due to strong westerly winds that pick up and deposit large amounts of snow onto sites 1 and 2. Site 1 is located on the eastern side of the Knoll and experiences the highest levels of snow deposition due to the prevailing westerly winds. Lastly, site 2 is situated just below the pinnacle of the West Knoll and experiences a combination of the snow and wind factors described above, and, thus, receives moderate levels of snow compared to the other sites (C Ray 2020, personal communication, 22 July; Table 1). At each of the pika territories, we delineated a 25m radius circle that encompassed each pika’s foraging range (C Ray 2020, personal communication, 22 July; Dearing 1997; Smith and Ivins 1984). We sampled haypiles containing fresh vegetation within the 25m radius at each site (n = 3).

**Field Methods**

At each sampling location, we collected vegetation, litter, lichen, pika haypile contents, and pika hair. We collected vegetation by clipping (with gloved hands) live specimens of the seven plant species most commonly consumed by pikas (Acomastylis rossii, Deschampsia caespitosa and Carex spp, Bistorta bistortoides, Trifolium parryi, Castilleja occidentalis, Erigeron simplex). As demonstrated by Dearing (1997) and Bhattacharyya and Ray (2015), pikas predominantly consume the leaves of Acomastylis rossii and Castilleja occidentalis, the flowers of Erigeron simplex, and the leaves and flowers of Bistorta bistortoides and Trifolium parryi. Pikas often harvest the whole above-ground plant when foraging on Deschampsia caespitosa and Carex spp. Consequently, our sampling of each plant species followed the pikas’ expected foraging patterns. The vegetation, lichen, and litter were sampled on the perimeter at each of the
four cardinal directions and the center of the 25m radius circle. A two-ounce Whirlpak bag was filled to capacity per site with a composite of each plant species. Similar to the vegetation sampling, the litter was sampled with gloved hands and collected from the four points and the center of each circular sampling area until the Whirlpak bag was filled. The three center haypiles (n = 1 per location) were also sampled. Due to the large amounts of plant material in each pile, a quart-sized plastic bag was filled with a representative bulk vegetation sample.

We collected all lichen species found on the West Knoll following the sampling methods used by Weiss-Penzias et al. (2019). In brief, we scraped the lichen with stainless steel instruments cleaned with ethanol. The challenge of scraping lichen from the rocks in high winds resulted in under 4 grams (weighed after samples were ground with liquid nitrogen) of material collected from each site.

The center haypile used in the vegetation sampling was also used to determine the location of pika trapping. Tomahawk Model 201, wire-mesh, “chipmunk” live traps (40.6 x 12.7 x 12.7 centimeters) were set up in close proximity to each haypile, allowing us to sample clumps of hair from the rump of the pika. Pika hair sampling was completed under the guidance of Dr. Chris Ray who holds the required permits to conduct pika trapping and hair sampling. Where the vegetation was sampled within the three radii, the pikas were trapped within each snow band based on other haypiles located within the same snow-cover class (n = 4 per sampling location). Time constraints required that all of the animal trapping be conducted over a two-day period. To maximize the likelihood of trapping success, two traps were set up per haypile for a total of 8 traps per site. Our pika trapping event resulted in capturing one pika in site #1, four in site #2, and three in site #3. Several months prior to this trapping event (July 2020), Dr. Ray sampled an additional pika in site #1. We were able to obtain a sub-sample of the hair collected from this pika, bringing the sampling size up to two in site #1. Dr. Ray used tweezers to extract a clump of hair from each of the pikas’ rumps. All samples were placed in coin envelopes and transported back to the lab on ice.

The solitary behavior and wide range of weasels make them extremely difficult to trap. Therefore, we sub-sampled hair from three different weasel skins present in the vertebrate collection at the Museum of Natural History at the University of Colorado, Boulder. The three weasels were trapped at Niwot Ridge in 1916, 1950, and 1961.
Lastly, three archived, composited dust samples from a study conducted by Heindel et al. (2020) were analyzed for THg and MMHg to determine dust-mediated Hg input into the alpine environment. Dust samples were collected in bulk (wet + dry) deposition during the summer months (June-September).

Due to cost constraints on MMHg analyses, only the vegetation and litter samples in site #1 (high snow, expected endmember), along with all pika, weasel, and dust samples were run for MMHg. The rationale was to establish a baseline in this study for further research on the Hg cycle at Niwot Ridge. The entire sample set was analyzed for THg.

Laboratory Methods

All samples were frozen (-18°C) and freeze-dried following field collection. Frozen samples were ground in liquid nitrogen with a mortar and pestle to create at least 50-100 mg of material that was put into labeled centrifuge tubes. All lab equipment (funnels, mortar, pestle, and funnel) were cleaned with ethanol between each sample. Pika and weasel hairs were placed into small centrifuge tubes with cleaned tweezers. All samples were then sent to the Wisconsin State Hygiene Lab for THg and MMHg analyses using the methods of Hammerschmidt and Fitzgerald (2005). All MMHg samples were analyzed with a Tekran 2700 and all THg samples were analyzed with a Tekran 2600. The analyses were performed using automated Cold Vapor Atomic Fluorescence Spectrophotometers (CVAFS; Tseng et al. 2005). The instrument precision is expressed through the RSD (relative standard deviation) of five calibration factors which must be less than 15% to pass the acceptability threshold. The RSD for both THg and MMHg were all below 15%. To prepare the samples, they were weighed in Teflon digestion vials and then digested in 4.5 M HNO₃ at 65°C for ~16 hours. Monomethyl mercury analysis was conducted from the unmodified digest, while THg analysis was performed after oxidation with bromine monochloride. All internal/external QC passed. However, sample controls (duplicates and spikes) for the hair samples were not prepared due to insufficient mass. Lastly, the detection limit refers to the concentration required for a sample to be considered reliable for the mass analysed.
**MMHg Extraction and Analysis**

Monomethyl mercury was measured in the samples after extraction with dilute HNO$_3$. Subsamples (0.1–0.2 g) of freeze-dried and homogenized biological material were digested with 7.0 mL of 4.57 M HNO$_3$ in a covered 60°C water bath for 12 h (Hammerschmidt and Fitzgerald 2005). This extraction method was used over the traditional KOH/methanol techniques as it allows determination of MMHg and THg in the same extract which reduces random errors (e.g., sample mass determinations, within-sample heterogeneity) associated with the analysis of each Hg species in separate tissue subsamples (Bloom 1992). Polycarbonate filters with microseston were digested similarly with 2 M HNO$_3$. The accuracy of the MMHg measurements was quantified by analyses of 1) blanks and calibration standards taken through the digestion process, 2) certified reference materials from the National Research Council of Canada, lobster hepatopancreas (TORT-2), and dogfish liver (DOLT-2), 3) replicate subsamples of fish and zooplankton, and 4) spiked subsamples (before digestion). The mean measured concentration of MMHg in TORT-2 (certified range, 139–165 ng g$^{-1}$) and DOLT-2 (certified range, 640 – 746 ng g$^{-1}$) were within their respective certified ranges.

**Total Hg Extraction and Analysis**

The 4.57 M HNO$_3$ leachates for MMHg analysis were also used for the determination of THg after treatment with BrCl for 12 h (Hammerschmidt and Fitzgerald 2005). Hydroxylamine hydrochloride (12% wt:vol) was added to digestates as a pre-reductant at least 1 h prior to analysis. Digestates were analyzed for THg by dual-Au amalgamation CVAFS (Fitzgerald and Gill 1979). Total Hg analyses were calibrated with Hg(0) standards removed from the headspace over pure liquid and verified by comparison to analyses of aqueous Hg(II) solutions traceable to the U.S. National Institute of Standards and Technology (NIST). Working standard solutions of MMHg were calibrated, after BrCl oxidation, by comparison to NIST-traceable Hg(II) solutions and Hg(0) standards. Recovery of added Hg(II) averaged 99% (range, 94 – 104%) compared to Hg(0) standards. The mean measured concentration of THg in TORT-2 (certified range, 210 – 330 ng g$^{-1}$) and DOLT-2 (certified range, 1860 – 2420 ng g$^{-1}$) were within their respective certified ranges. The precision of methodically replicated analyses of total Hg was within the acceptable range, 0.1–12%. The estimated detection limit for both MMHg and THg in a 0.1-g sample of lyophilized fish was about 0.1 ng g$^{-1}$.
Data Analysis

I analyzed all data in R (version 1.4.1103). The results violated the assumptions of parametric tests (mostly due to small sample sizes), thus, I used a non-parametric Kruskal-Wallis test to compare differences among the THg concentrations in the dust, lichen, forbs, graminoids, haypiles, and litter. I then ran a Dunn’s Multiple Comparison post hoc test to discern which samples were significantly different. The pika and weasel groups were excluded because, respectively, the concentrations were below the detection limit; in the case of the weasel samples, the THg values may reflect contamination. I observed and compared the pattern of vegetation decomposition from the live vegetation to the litter. I compared the vegetation to the pika across the snowpack gradient, but due to uneven sample sizes, I did not run statistical tests. I report data as means and standard deviation in ng Hg g⁻¹ tissue.

Results

Dust deposition had the highest abiotic THg concentrations (321 ± 36.24 ng g⁻¹), followed by lichen (232 ± 78.88 ng g⁻¹), litter (67.0 ± 5.79 ng g⁻¹), haypiles (17.56 ± 8.41 ng g⁻¹), forbs (6.37 ± 1.32 ng g⁻¹), and graminoids (5.91 ± 2.71 ng g⁻¹) (Fig. 3). Total mercury and MMHg in weasel hairs were 9790 ± 12,923.1 ng g⁻¹ and 625 ± 267.47 ng g⁻¹, respectively. Total Hg concentrations in the pikas and the MMHg concentrations of all samples except weasels were below the detection limit, but greater than their respective mean blanks (Table 2). When a sample concentration is below detection, that indicates that the concentration is definitively above zero, but the exact concentration is unknown. The samples below detection have values because Wisconsin State calculated the estimated concentration that would have been achieved with higher sample mass.

When all sample groups were compared (excluding pikas and weasels), the non-parametric Kruskal-Wallis test reported significance among the group’s THg concentrations (p < 0.05). A Dunn’s post-hoc test revealed that only dust was significantly different from forbs and graminoids (p < 0.05) (Fig. 4). Graminoids and forbs had the lowest THg concentrations out of all the samples (10x lower than the dust) with no significant difference between them (p > 0.05). The haypile THg concentrations are approximately two-fold higher than both forbs and graminoids and did not differ across sites (p > 0.05). The litter concentrations were 3-10x higher.
than the haypiles (p > 0.05). The weasels had 10-80x higher THg concentrations than all other sample groups. The weasel MMHg concentrations were 100x higher than dust (Fig. 5).

Comparison of lichen, plants, and pikas across snow depth bands revealed that graminoids and forbs had similar THg concentrations, between 3 and 10 ng g\(^{-1}\) across all three sites (Fig. 6). Concentrations of THg in lichen were 187 ng g\(^{-1}\) for high snow, 186 ng g\(^{-1}\) for mid snow, and 323 ng g\(^{-1}\) for low snow. Haypile THg concentrations had some variation across sites: high snow had 7.84 ng g\(^{-1}\) and mid and low snow had ~22.5 ng g\(^{-1}\). The litter THg concentrations fell between haypile and lichen with similar concentrations across sites (67.8 to 73.0 ng g\(^{-1}\) from high to low snow).

**Discussion**

This study was designed to evaluate flows of Hg, as THg and MMHg, among terrestrial ecosystems pools, including a relatively isolated food web. The results suggest that Niwot Ridge receives atmospheric deposition of Hg, likely in inorganic forms like Hg(II), but negligible levels of MMHg. Thus, inorganic forms of Hg deposited to the landscape move through ecosystem pools—the focus of the discussion below—but if MMHg is present, it is likely produced in areas with reducing conditions, including wetlands, stream sediments, and lakes. Production of MMHg and its movement within the alpine landscape may be significant and consequential but was outside the scope of this study.

Compared to the THg concentrations in annual bulk deposition collected by Mast et al. (2005) (8.3 to 12.4 μg THg m\(^{-2}\)), the composite dust samples analyzed in this study had a lower concentration (2.18 μg THg m\(^{-2}\)). However, measurements in dust were different from those reported by Mast et al. (2005). Heindel et al. (2020) collected bulk deposition (wet + dry) during the drier summer months whereas Mast et al. (2005) reported annual data. Alpine systems experience enhanced wet deposition, particularly as snow in winter, and although not well constrained, the literature has shown that wet deposition is a significant Hg input, potentially leading to higher rates of Hg deposition in the winter (Bullock et al. 2009; Driscoll et al. 2013; Selin 2009). The samples collected by Mast et al. (2005) are similar to deposition rates in the Midwestern and Northeastern U.S. These results demonstrate the importance of wet deposition as an input of Hg and necessitate further studies assessing Hg inputs in snow and how its distribution varies across the alpine landscape.
I did not observe a pattern of MMHg biomagnification with successive trophic levels from the vegetation to the pikas to the weasels as predicted in H1. However, I measured bioaccumulation of MMHg in the weasels as demonstrated by the very high concentrations relative to the other samples (Fig. 5). Indeed, their level of MMHg relative to dust (~ 100-fold greater) is what I would expect to observe in a multi-trophic level food web. However, MMHg was not present in the vegetation or pika tissues. Instead, the high MMHg concentrations in the weasels have another possible explanation. Although the weasels were collected from either Niwot Ridge or the Green Lakes Valley, weasels have large foraging ranges (see Jedrzejewski et al. 1995), and, thus, high MMHg concentrations could be attributed to feeding from aquatic parts of the landscape where MMHg can be stimulated by sulfate reduction and/or organic matter interactions, then mobilized into biota (Chételat et al. 2020; Driscoll et al. 2013; Loseto, Siciliano, and Lean 2004). If I had sampled aquatic food sources equivalent to the pikas’ trophic level, we may have observed biomagnification with this aquatic food source being ~ 10-fold smaller than the weasels. Regardless, as top predators in this system, my finding that weasels have high MMHg levels in their tissues suggests that they are an important biological Hg reservoir in this alpine system.

The MMHg concentrations (625 ± 267.47 ng g⁻¹) in the weasels were comparable to the concentrations found in organisms from other studies considered to have high MMHg concentrations, such as the work done on a deer and puma food web in Northern California, by Weiss-Penzias et al. (2019). They measured 491.8 ± 503.1 ng g⁻¹ of THg in inland adult puma and 15.5 ± 9.6 ng g⁻¹ of THg in inland deer, indicating bioaccumulation with higher trophic levels (Fig. 7). Their research also highlights the relationship between MMHg concentrations and an organism’s interaction with aquatic environments. The THg concentrations for the coastal adult puma (used in that system as a proxy for MMHg), who are disproportionately affected by marine fog (a source of MMHg), were 1544 ± 1462 ng g⁻¹, compared with 491.8 ± 503.1 ng g⁻¹ in inland adult pumas. They concluded that increased exposure to aquatic systems will lead to increased MMHg concentrations in the animal tissue. Further, a study examining MMHg concentrations in the liver (and other tissue) of beluga whales in the Canadian Arctic found a mean MMHg concentration of 1830 ± 630 ng g⁻¹ (Lemes et al. 2011). Beluga whales live in completely aquatic environments and are predominantly carnivores, further illustrating the role of aquatic interaction and higher trophic levels in MMHg bioaccumulation. Lastly, When the
Weasel MMHg concentrations (0.625 ± 0.27 mg kg\(^{-1}\)) are compared to the EPA standards, the concentrations are above the levels considered safe (0.3 mg MMHg/kg fish) (USEPA 2010), illustrating that the weasels are accumulating dangerous levels of MMHg in relation to human consumption.

There are, however, several possible confounding factors to consider. The THg concentrations in the weasels are possibly elevated due to Hg preservatives, as well as greater air pollution during the early to mid-20\(^{th}\) century when they were collected (1916-1961). Several regulations on atmospheric pollution were passed soon after the last weasel sample was collected, and, since then, there have been acute observations of atmospheric deposition of trace metals decreasing with time (Pratte et al. 2013). Thus, the concentrations in the weasels may be reflecting elevated Hg deposition a century ago rather than bioaccumulation in the present day. Additionally, because it has been observed that inorganic Hg will pass through organisms without being completely absorbed by the gut, it seems improbable that THg concentrations in the weasels naturally reached these levels (Chételat et al. 2020). However, although potentially contaminated, the highest THg concentration in the weasels (24,710 ng g\(^{-1}\)) is comparable to the highest THg concentration for coastal adult puma (22,052 ng g\(^{-1}\)) from Weiss-Penzias et al. (2019), indicating that THg concentrations can be elevated in predators. These last two contradictory considerations indicate the need for future research on Hg accumulation in top predators.

Contrary to H\(^2\) there was no clear difference between the THg concentrations in forb and graminoid plant functional groups despite the larger leaf surface area of the forbs (p > 0.05) (Fig. 4). This result could be attributed to the small sample size or because leaf area is less important in atmospheric Hg uptake than other characteristics of plants or factors affecting atmospheric deposition patterns. Additionally, the vegetation on the West Knoll is small due to the harsh environment, which likely contributes to low THg concentrations. Several studies have indicated that although less important than direct uptake of atmospheric deposition, uptake of Hg via roots is a factor to consider (Beauford et al. 1977; Obrist 2007; Olson et al. 2019). Further, MMHg concentrations were below detection in the forbs and graminoids, consistent with low concentrations in the dust. It may be the case that plants living in or near alpine wetlands have higher levels of MMHg, if they are taking it up via roots. Without clear differences between the
graminoids and forbs, the knowledge gap identified by Obrist et al (2016), pertaining to the role of different plant functional groups in alpine Hg cycling remains open.

Concerning **H3**, there is no clear relationship between the variation in the THg accumulation in pika tissues and the spatial variation in the THg content of alpine plants. The concentrations of both THg and MMHg were below the detection limit in pika tissue, which prevents any strong conclusions. I did observe a pattern of average THg concentrations in the pika tissue declining with decreasing snowpack, yet results were highly variable, and due to uneven sample sizes, I did not statistically test the relationship among groups (**Fig. 6**). The THg concentrations in the plants do not reflect the concentrations in the pikas and do not follow the snowpack gradient as predicted in **H4**. This pattern could be attributed to snow deposition being a less significant input of atmospheric Hg than predicted, the sites not perfectly reflecting a snowpack depth gradient, or, more likely, to the small sample size. The absence of measurable MMHg concentrations in the vegetation and pikas is likely due to the lack of reducing conditions on the West Knoll and MMHg being a negligible ecosystem input in atmospheric deposition. Further, the pikas are likely not reflecting accumulation of Hg in their tissue due to inorganic Hg (Hg(0) and Hg(II)) not being efficiently absorbed by the organism (Chételat et al. 2020). The very low THg and MMHg concentrations in the forbs, graminoids, and pikas indicate that Hg is not immediately threatening the health of this localized terrestrial food web.

An interesting positive correlation between THg concentrations and the degree of vegetation decomposition was observed in our dataset, with THg concentrations increasing from live plants (graminoids and forbs) to haypiles, and to litter (p > 0.05) (**Fig. 3**). Although not a part of my original research questions, this was an exciting observation, as it points to litter acting as an important pathway for THg into the underlying soils and groundwater (Obrist et al. 2016; Walker et al. 1989). Inorganic Hg is soluble in water, unlike organic species, and once inputted into ground water could be transported to other parts of the landscape (Driscoll et al. 2013). Other studies of temperate forested ecosystems have made the same observation of Hg concentration in litter (Demers et al. 2007; Obrist et al. 2012; Pokharel and Obrist 2011), but this is the first evidence from an alpine system dominated by herbaceous plant species and warrants follow-up investigation.

The haypile concentrations are notably higher than the forb and graminoids which follows the logic that a composite of fresh biomass that has started decomposition will have
higher concentrations than live plant tissue (p > 0.05). A study conducted by Weis and Weis (2004) observed that metal concentrations (Hg included) in plant tissue rose by orders of magnitude (10- to 100-fold) over two years of decomposition. When compared to the litter that has been decomposing uninterrupted for several years (p > 0.05) (for litter decomposition rates of different functional groups, see Ebert-May et al. 2002), the haypiles may have lower concentrations because they are regularly augmented with fresh biomass by the pikas. Pika are selective with respect to the plant tissues that they bring back to their haypiles (flowers, stems, or the whole individual, see Bhattacharyya and Ray 2015 and Dearing 1997), which may also explain why the haypile concentrations are lower than the litter; I did not measure Hg concentrations in different plant parts. Pika have also been shown to manipulate the chemistry of their haypiles by collecting plants with toxins that act as preservatives and then caching these plants until the toxins have degraded to levels low enough for consumption (Dearing 1997). Even if Hg is concentrating in the haypiles, my data suggest that it is present in inorganic forms that can pass through the animal (Chételat et al. 2020), and, therefore, are probably not a health threat.

Although unassociated with my research questions, The THg content of the lichen reflects the levels of atmospheric deposition of Hg likely due to their sensitivity to atmospheric pollutants and their long lifespan (see Bargagli 2016 and Weiss-Penzias et al. 2019), thus connecting Hg in the atmosphere to the biosphere (Fig. 4). When the two groups are compared, the dust and lichen are not significantly different (p > 0.05). Briefly, the average lichen concentrations in this study (232 ± 78.88 ng g⁻¹) are comparable to the lichen concentrations in the study conducted by Weiss-Penzias et al. (2019), who observed 138.1 ± 44.4 ng g⁻¹ of THg in the ocean-facing lichen and 333.6 ± 345.1 ng g⁻¹ THg in the bay-facing lichen in Northern California. The THg concentrations in the lichen were not positively correlated with greater snowpack depth as predicted in H4 (correlation between the samples and snowpack). One possible explanation for why the lichen does not follow the predicted snowpack gradient is that the strong westerly winds that disproportionately affect WK3 (low snow) subject the lichen on that slope aspect to increased dust deposition. However, the concentrations across sites are similar enough that I would need a larger sample size to be more confident in my interpretations.
Limitations of the Study

Due to the limited time frame of this thesis project and the high cost of MMHg analyses, the sample size was low. This increased the margin of error and heavily restricted the available statistical analyses due to uneven distributions. Due to MMHg being insignificant within my focal study area (the West Knoll), with more time and funding, increased THg measurements for all of the samples would greatly increase the statistical power of my dataset and potentially illustrate new patterns with greater confidence. The project was conducted during the COVID-19 epidemic which further delayed the process, particularly for laboratory analyses. However, the dataset still provides an important baseline for further study of Hg cycling and pathways at Niwot Ridge.

Next Steps

This project demonstrated that there is Hg mobilization within remote alpine ecosystems and that atmospheric deposition, vegetation, and animals play important roles in facilitating its movement. My thesis research also illustrates a set of key outstanding questions that can help guide future research. In particular, evidence of high MMHg concentrations in weasels, but not in plants or pikas of the West Knoll, suggests that aquatic parts of the landscape, and the food webs that they support, may be more important for mobilizing MMHg in animals. As sites of potential MMHg production, there is a need to investigate Hg cycling within the wetlands, stream sediments, and lakes of Niwot Ridge. Similarly, my research showed an interesting pattern in THg accumulation within litter, suggesting that decomposition of plant tissues may concentrate Hg. The dynamics of Hg cycling within vegetation and its ultimate fates are outstanding, yet my dataset for THg accumulation in alpine herbaceous species relate to a broader interest within the biogeochemistry community to address the relationship between plants and Hg inputs to terrestrial ecosystems (Demers et al. 2007; Obrist, Johnson, and Edmonds 2012; Pokharel and Obrist 2011). In Summer 2021, I will continue work on this project as a research assistant with Dr. Eve-Lyn Hinckley and Ph.D. student Hannah Miller to follow up on these questions.
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Mountains, USA.” *Limnology and Oceanography.*

### Tables and Figures

**Table 1. Site Soil Characteristics and Plant Composition.**

<table>
<thead>
<tr>
<th>Site</th>
<th>Snow Persistence</th>
<th>Slope*</th>
<th>Soil Type†</th>
<th>Soil pH‡</th>
<th>BD (g cm⁻³)§</th>
<th>Species Comprising Majority of Pika Foraging**</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Knoll 1</td>
<td>High</td>
<td>18.80%</td>
<td>Moran family and 60% similar soils. Lithic cryorthents and 20% similar soils.</td>
<td>5.0 ± 0.3</td>
<td>0.76 ± 0.07</td>
<td>Acomastylis rossii, Deschampsia caespitosa and Carex spp, Bistorta bistortoides, Trifolium parryi, Castilleja occidentalis, Erigeron simplex</td>
</tr>
<tr>
<td>West Knoll 2</td>
<td>Mid</td>
<td>21.50%</td>
<td>Moran family and 60% similar soils. Lithic cryorthents and 20% similar soils.</td>
<td>5.0 ± 0.3</td>
<td>0.76 ± 0.07</td>
<td>Acomastylis rossii, Deschampsia caespitosa and Carex spp, Bistorta bistortoides, Trifolium parryi, Castilleja occidentalis, Erigeron simplex</td>
</tr>
<tr>
<td>West Knoll 3</td>
<td>Low</td>
<td>9.50%</td>
<td>Moran family and 60% similar soils. Lithic cryorthents and 20% similar soils.</td>
<td>5.0 ± 0.3</td>
<td>0.76 ± 0.07</td>
<td>Acomastylis rossii, Deschampsia caespitosa and Carex spp, Bistorta bistortoides, Trifolium parryi, Castilleja occidentalis, Erigeron simplex</td>
</tr>
</tbody>
</table>

**Footnotes:**

* Approximate Calculation from Google Earth Pro.
† Derived from the USDA Web Soil Survey.
‡ Unpublished Data Summer 2020, Hannah Miller.
§ (Chen et al. 2020), Table 1 values of dry alpine meadow sites which is representative of West Knoll soils.
** (Dearing, MD 1997).
Table 2. The mean THg and MMHg concentrations and mean detection limits of all samples.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Mean THg Concentration (ng g⁻¹)</th>
<th>Mean THg Method Detection limit (ng g⁻¹)</th>
<th>Mean MMHg Concentration (ng g⁻¹)</th>
<th>Mean MMHg Method Detection limit (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust (n=3)</td>
<td>321±36.24</td>
<td>11.86±0.62</td>
<td>7.03±1.66</td>
<td>23.73±1.24</td>
</tr>
<tr>
<td>Lichen (n=3)</td>
<td>232±78.88</td>
<td>1.16±0.08</td>
<td>1.39</td>
<td>2.40</td>
</tr>
<tr>
<td>Forb (n=3)</td>
<td>6.37±1.32</td>
<td>1.20±0.02</td>
<td>0.014</td>
<td>2.44</td>
</tr>
<tr>
<td>Graminoid (n=3)</td>
<td>5.91±2.71</td>
<td>1.07±0.08</td>
<td>0.0243</td>
<td>2.47</td>
</tr>
<tr>
<td>Litter (n=3)</td>
<td>67.0±5.79</td>
<td>1.04±0.11</td>
<td>0.152</td>
<td>1.89</td>
</tr>
<tr>
<td>Haypile (n=3)</td>
<td>17.56±8.41</td>
<td>1.08±1.17</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pika (n=9)</td>
<td>71.04±58.84</td>
<td>433.54±385.46</td>
<td>62.83±102.27</td>
<td>433.54±385.46</td>
</tr>
<tr>
<td>Weasel (n=3)</td>
<td>9790±12,923.09</td>
<td>171.74±73.3</td>
<td>625±267.47</td>
<td>171.74±73.3</td>
</tr>
</tbody>
</table>

Note. The mean THg and MMHg concentrations and mean method detection limits for each sample type group (e.g., all 3 dust samples). Red values indicate that the concentrations for the sample group were below the mean detection limit. The method detection limit is based on the mass digested for each sample and is thus different for each sample. The detection limit for each of the sample groups is similar enough to be averaged. If no standard deviation is included, the sample only had one measurement for MMHg (this sample will always come from West Knoll 1).
Figure 1: Conceptual model of Hg cycling in the alpine ecosystem. Orange arrows indicate aspects of the Hg cycle addressed in this study. Questions marks indicate uncertainties in Hg pathways. Dashed arrows indicate mobilization of THg or MMHg into the food web.
Figure 2: The West Knoll of Niwot Ridge study area showing haypile (center of each snow band) and pika sample locations. The dashed lines indicate the three different snow bands with increasing snow depth from left to right.
Figure 3: Mean THg concentrations of each sample type. Error bars show standard deviation. Black dashed line indicate the analytical detection limit for pikas as they were the only samples with THg concentrations below detection. Each sample has a different detection limit because these limits are based on the sample’s mass. The samples to the right of the vertical grey dashed line were not included in the statistical analysis (p < 0.05).
Figure 4: Mean THg in sample types that were included in a Kruskal Wallis test. Error bars are standard deviation. Lowercase letters indicate significant statistical differences among groups (p < 0.05).
Figure 5: Mean MMHg concentrations of each sample type. Error bars are standard deviation. All samples were below detection except for the weasels. Black dashed line indicate the analytical detection limit for each respective sample type. Sample types without data were below detection and had negligible MMHg concentrations, except for the haypiles which were not run for MMHg.
Figure 6: Variation in THg accumulation in pika tissues compared to the spatial variation in the THg content of alpine plants. Error bars show standard deviation. Black dashed line indicate the analytical detection limit for pikas as they were the only samples with THg concentrations below detection. Per site, (n=1) for each sample except for the pikas. Pikas in WK1, high snow (n=2), in WK2, mid snow (n=4), and in WK3, low snow (n=3).
Figure 7: Mean MMHg concentrations across animal types in different trophic levels and with different levels of interaction with aquatic environments. Error bars show standard deviation. Weasel data are from this study. Deer and puma data are from Weiss-Penzias et al. (2019); beluga data are from Lemes et al. (2011).