Abstract

The variability of heavy metal concentrations (Pb, Cd, Zn, and Cu) of the terrestrial moss *Orthotrichum lyellii* on single trees was evaluated at two sites in the Pacific Northwest, one relatively unpolluted and the other polluted. In addition, a reciprocal transplant of *O. lyellii* between the unpolluted and polluted sites was used to evaluate the response of moss tissue metal concentrations to translocation to sites with higher or lower atmospheric deposition of metals.Transplanted moss samples were collected at 1, 3, 6, 9, and 12 months. The position of moss sampling (interior, middle, exterior) and transplant hanging (interior and exterior) were documented in order to determine the spatial variability of heavy metals within a tree. The starting concentrations of all metals were higher at the SRDP site. In general, heavy metal concentrations in moss transplanted from the unpolluted site to the polluted site increased and reached in situ levels after 3-6 months. In some cases, moss transplant heavy metal levels surpassed in situ moss levels. In contrast, moss concentrations of heavy metals in transplants from the polluted site to the unpolluted site did not decrease substantially in metal levels. Therefore, will mosses can rapidly acquire metals when moved to more polluted sites, they tend to tenaciously hold metals when moved to less polluted sites. Furthermore, this study demonstrates the need for positional consistency and large sample size when collecting moss samples as well as when hanging transplants. We conclude that transplanted moss needs to be in place for at least 6-12 months to accurately represent metal atmospheric deposition at the transplanted site. In addition, metal levels of transplants to less polluted locations are unlikely to be representative of the transplanted site, even after 12 months.

Spatial variability of heavy metals within a tree using the *Othitricum lyellii* moss biomonitor model

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes the release of my thesis to any reader upon request.

Gillette Field, Author

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1. Introduction

Air pollution and pollution patterns have gained much interest over the last several years. Many studies have been undertaken due to increasing concern over air quality. As urban areas grow, exposure to atmospheric heavy metal pollution increases. The world's population is increasing rapidly and moving to cities. Approximately 50% of the world's population now lives in urban areas and 50% of cities in high-income countries do not meet the World Health Organization (WHO) air quality guidelines (World Health Organization n.d.). WHO estimates nine out of every ten people breathe air containing pollutants at high levels. It is now estimated that 7 million deaths globally per year are due to air pollution (World Health Organization n.d.). Atmospheric deposition can be wet or dry. Associated particulate matter (PM) includes heavy metals such as cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). Particulate matter is used as an indicator of air pollution and affects a greater number of people than any other pollutant (World Health Organization n.d.). PM less than 10 microns can lodge inside the lungs while 2.5 microns or less can penetrate lung tissue and enter the bloodstream (World Health Organization n.d.). There is a quantitative relationship between health problems and exposure to PM (World Health Organization n.d.). Poor air quality leads to a higher risk of stroke, heart disease, lung cancer, chronic and acute respiratory diseases (World Health Organization n.d.). Reducing atmospheric heavy metal pollution and associated health issues requires monitoring of pollutant sources, transport, and fate.

Traditional air quality monitors are labor-intensive and expensive. As a result, monitors tend to be widely spaced. This makes fine-scale mapping and monitoring of air pollutants difficult if not impossible. This allows for point sources to go undetected for long periods of

time. A recent study conducted in Portland Oregon used moss as a biomonitor and successfully identified two unknown sources of cadmium pollution that were previously undetected by national air quality monitoring networks (Donovan et al. 2016). Fine-scale monitoring of pollutants with biomonitors such as moss allows for the detection of previously unknown emitters and thus the improvement of air quality at smaller scales.

Lichens and mosses are abundant terrestrial epiphytes that have the capacity to passively monitor air quality. In comparison to traditional monitoring, they are inexpensive, easy to collect and allow for the investigation of high-resolution spatial variations. Moss has been used in air quality studies as early as 1986 when they were evaluated as a biomonitor in Sweden (Ruhling and Tyler 1986). Mosses and lichens are unable to block atmospheric particulates from entering their tissue due to the lack of the protective waxy cuticle and root systems of vascular plants, (Ruhling and Tyler 1986). They obtain all of their nutrients and elements across their entire surface (Ruhling and Tyler 1986). Lacking a typical root system removes the possibility of edaphic contributions and allows tissue concentrations to form a linear relationship with atmospheric metal content (Ruhling and Tyler 1986). As a result, moss and lichen tissue concentrations are indicative of local air quality and making them successful biomonitors.

Orthotrichum lyellii (O. lyellii) has several key characteristics that allow it to be a good biomonitor. *O. lyellii* is an ectohydric moss which lacks a waxy cuticle that is present on endohydric moss. Endohydric moss have water repellent leaves and use rhizoids to absorb water from the substrate to which they are attached. It is thought that this may cause them to be more susceptible to contamination from their substrates (Glime 2017). Ectohydric moss may contain surface ornamentations such as papillae or paraphyllia which allows water to be conducted on

the external surface of the moss by capillary action. Ectohydric mosses may also utilize overlapping concave leaves to collect and direct the movement of water across their surface (B. McCune 2018, pers. comm.). *O. lyellii* has a unique segmental structure in which a new shoot will grow at the apex each year. In this way, each segment is equivalent to one years worth of growth and thus one years worth of atmospheric deposition. *O. lyellii* is successful in moderately polluted environments, making it a good choice for studies of urban areas, such as Portland. In addition, *O. lyellii* is an abundant moss species and can be found in large quantities within a single tree. This allows for large sample sizes and the ability to create a fine-scale map of an area.

A recent study in Portland yielded fine-scale results using a high-density sample collection of moss (Donovan et al. 2016). The study identified sources of specific pollutants, such as cadmium, that were previously unknown spurring an investigation by the state of air quality in specific areas and of specific emitters. Another study recently completed in the Portland and Corvallis areas found significant variability in metal concentration among the moss samples on the same tree (Miller et al., 2017). This preliminary study was limited to assessing variability and did not evaluate environmental and other factors that may contribute to the variability.

This research investigated the spatial variability of metal concentrations of the moss *O. lyellii* model sampled from different positions on a tree branch. In addition, this study examined bioaccumulation and retention rates of various heavy metals in transplanted mosses. This was accomplished through a reciprocal transplant between a relatively polluted site (downwind from Portland) and an unpolluted site (Corvallis, OR). Moss was collected from the two sites and

turned into moss ornaments that were transplanted to the reciprocal site. The transplanted mosses were as hung from either the interior or exterior of the tree branches. This project aimed to improve understanding of the spatial variability of heavy metals among positions in a single tree as well as to examine the response of moss tissue metal levels to changes in atmospheric deposition. In order to do this we posed the following questions:

(1) Does the concentration of heavy metals vary among moss taken from different positions on a single branch?

(2) Will moss transplanted to a less polluted site retain or lose its elevated heavy metal levels and how long will that take?

(3) Will moss transplanted to a more polluted site experience an increase in heavy metals levels and how fast?

(4) Does the position of a transplant on a branch affect its heavy metal concentrations?

2. Materials and methods

2.1. Sampling

Samples of *O. lyellii* were collected from two sites in Oregon, an unpolluted site (Crescent Valley High School) and a polluted site (Sandy River Delta), in August 2018. Moss was collected from an Oregon Ash tree at both sites. Half of each sample was archived for determination of metal concentrations. The other half of each sample was reciprocally transplanted so that samples collected from the unpolluted site were transplanted to the polluted site and samples collected from the polluted site were transplanted to the unpolluted site. Both sites had two transplant positions within them: an interior position close to the trunk and an

exterior position near the end of the branch. The original sample collection consisted of 120 samples (60 from each site). Following the reciprocal transplant, each site contained 60 transplants and each position contained 30 (total of 120 samples). Transplants were collected at three-month intervals, one, three, six, nine, and twelve months after deployment.

2.2. Site Selection

In order to investigate the change in heavy metal accumulation, retention, and loss, two sites were chosen, a polluted site and an unpolluted site. Sites were selected based on proximity to pollution sources, an abundance of *O. lyellii*, and accessibility of the site over the year. Sandy River Delta Park (SRDP) was chosen as the polluted site due to its location downwind from (east of) Portland. Crescent Valley Highschool (CVHS) was chosen as the unpolluted site due to a presumed absence of nearby significant metal pollution sources.

2.3. Moss collection and preparation

Small branches with *O. lyelli* were collected from each tree with pole trimmers. The branch height and position (interior, middle, or exterior) were recorded. Branches were transported in plastic boxes to the lab and air-dried overnight. Once dry, clumps of moss about 1 inch in diameter were collected from the branches and split into two portions by hand using powder-free nitrile gloves. Half of each sample was placed into Whirl-Pak bags to be digested in order to create a baseline of heavy metal concentrations. The other portion was weighed, made into an ornament for transplantation, and weighed again. Transplants were made by utilizing a silicone sealer nylon loop method as described by McCune et al. 1996.

2.4. Transplant Deployment

Transplants were placed into Milli-Q (MQ) washed styrofoam egg cartons and transported to their intended destinations. Transplants were handled with powder-free nitrile gloves and hung on a branch with nylon zip ties. Transplants were hung at random at either the interior branch position near the tree trunk or the exterior branch position near the end.

2.5. Transplants Collection

Wearing powder-free nitrile gloves, transplants were collected by clipping the zip ties holding them to the branch, and placed into MQ washed egg cartons for transport. Transplants were air-dried overnight and weighed then stored in a refrigerator until further analysis. Five transplants were collected at random for each sampling position within each site (total of 10 per site and 20 per sampling interval).

2.6. Reagents and Labware

All labware used for sample preparation and analysis was cleaned successively with 2% Extran, 4M hydrochloric acid (HCl) and 1M nitric acid (HNO_3) to remove all trace metals and organics. All solutions were prepared with Milli-Q water (≥18.2 MΩ cm). Ultrapure HNO_3, HCl, hydrofluoric acid (HF), and hydrogen peroxide (H_2O_2) (Optima, Fisher Chemical) were used in this study to prepare all solutions and to digest cleaned moss samples.

2.7 Preparation and acid digestion

All digestions were carried out at Oregon State University Keck clean lab. Transplants were placed on trays washed in isopropyl alcohol and MQ water. Using powder-free nitrile gloves, the transplants were cleaned of any unwanted materials and trimmed using a titanium scalpel. Transplants were trimmed at the one-year growth segment and trimmings were placed into acid cleaned glass vials, with titanium forceps, and put into a cold room until further analysis. Open vials were placed into MQ cleaned glass pyrex dishes and covered with chem wipes then placed into an oven overnight at 45℃. Trimmings were then weighed and placed into acid clean savillex vessels for further analysis. Trimmings were taken through the following three digestion steps: (1) 5 mL 15M HNO_3 at 120℃, (2) 4 mL 15M HNO_3 and 0.2 mL of 29M HF at 120℃, (3) 4mL 15M HNO_3 and 1mL of H_2O_2 added in 0.5 mL increments.

2.8. ICP-MS Elemental Analysis

Dried and digested samples were diluted with 5 mL of 2% HNO_3 then refluxed at 90℃ for an hour and sonicated for 30 minutes to ensure samples were in solution. A Thermo X-Series II Inductively Coupled Plasma Mass Spectrometer (ICP-MS) at the OSU Keck Collaboratory was used to perform elemental concentration analyses. Masses of 63, 66,111, and 208 were used to determine concentrations of Cu, Zn, Cd, and Pb respectively. Elemental concentrations were quantified using multi-element calibration curves along with indium (In) and rhenium (Re) measured at 115 and 185 respectively, as internal standards. All standards were prepared from 100 or 1000 µg/mL single or multi-element solutions from High Purity Standards. During analyses, quality control was ensured by measuring 1 ppb multi-element solution every 10

samples along with certified reference material BRC-482 (lichen). The root mean square error (RMSE) for Cu, Zn, Cd, and Pb for repeat measurements were as follows: 0.12498, 0.13597, 0.14280, 0.10281.

2.9. Data Analysis

2.9.1. Native concentrations.

The distribution of native sample concentrations was analyzed for skewness. All metals were determined to be right-skewed (> 1) and were log-transformed. A two-way analysis of variance (ANOVA; JMP 14.1.0) was calculated for "source", "source position" and the interaction "source*source position" to test the null hypothesis of no difference in heavy metal concentrations between those factors. Because source*source position was significant in some cases, a one-way ANOVA for each site was calculated for "source position" to test the null hypothesis of no difference in heavy metal concentration between source positions.

2.9.2. Transplants

Because each individual sample was split at the beginning of the experiment, half for initial concentrations and half for transplants, we expressed concentrations in the transplants as the difference from the native concentrations (difflog = log(transplant) - log(native)). This transformation accounts for the individual variation in starting concentrations by expressing the change in concentration as a gain or loss (+ or -).

A two way ANOVA for each source site was calculated for "month", "destination position", and the interaction "month*destination position" to test the null hypothesis of no difference in the change in heavy metal concentrations between those factors. Because

month*destination position was significant in some cases, a one-way ANOVA for each site was calculated for "destination position" to test the null hypothesis of no difference in the change in heavy metal concentrations by destination position.

3. Results

3.1. Native metal concentration variability

There was no consistent difference between position and heavy metal concentrations (Fig. 1 and Table 1). Levels of lead and zinc were significantly higher in the exterior position at SRDP (Table 2). There was a weak difference ($p < 0.065$) in zinc concentration in the exterior position at CVHS (Table 3). Levels of lead, cadmium, and copper did not differ significantly between positions at CVHS (Table 3). There was a consistent significant difference in heavy metal concentrations between source sites (Fig. 1 and Table 1). The concentrations of heavy metals from SRDP were consistently higher compared to those from CVHS though Zn and Cu did not differ as much (Fig 1).

3.1.1. Crescent Valley High School

At CVHS Cu concentrations ranged from 5.50- 21.33 ppm. Zn concentrations ranged from 9.87- 46.06 ppm. Cd concentrations ranged from 0.03-1.13 ppm. Pb concentrations ranged from 0.41- 1.77 ppm. Averages in ppm for Cu, Zn, Cd, and Pb were as follows: 9.82, 20.41, 0.10, 0.76. 2*sd for Cu, Zn, Cd, and Pb are as follows: 7.15, 14.39, 0.33, 0.58.

3.1.2. Sandy River Delta Park

At SRDP Cu concentrations ranged from 5.636-24.428 ppm. Zn concentrations ranged from 14.90- 34.65 ppm. Cd and Pb had significant differences in concentrations by position. Cd concentrations ranged from 0.09 - 0.55, 0.09 - 0.24 ppm in the exterior, 0.12 - 0.51 ppm in the middle, and 0.10 - 0.55 ppm in the interior. Pb concentrations ranged from 1.22- 4.28 ppm, 1.39 - 4.28 ppm in the exterior, 1.22 - 3.61 in the middle, and 1.28- 3.24 in the interior. Averages for Cu, Zn, Cd, and Pb were as follows: 11.88, 23.48, 0.18, 2.52. 2*sd for Cu, Zn, Cd, and Pb are as follows: 7.88, 9.82, 0.20, 1.55.

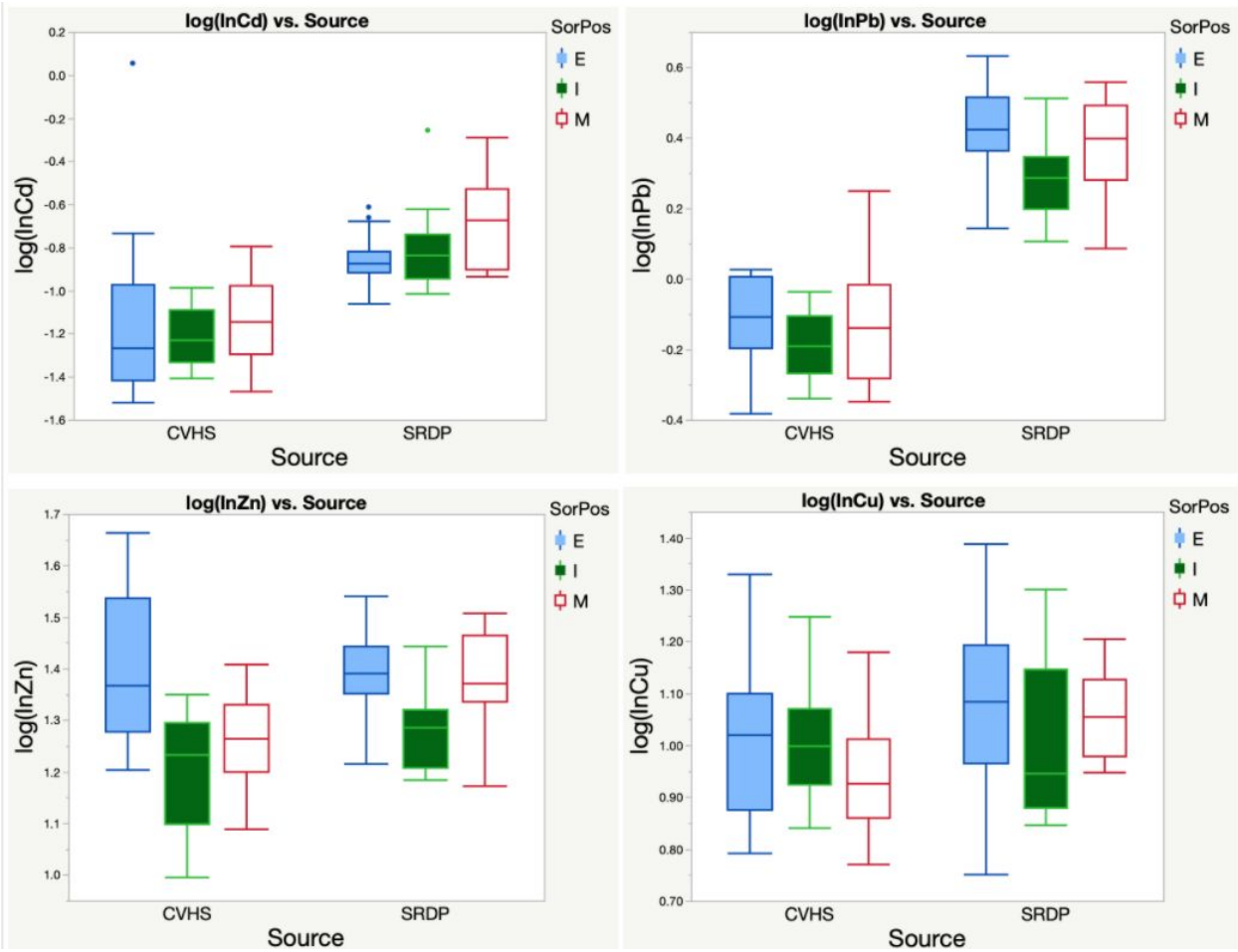

Fig.1. Variations in the concentrations (log($\mu g\ g^{-1}$)) of different heavy metals in the mosses sampled at time zero from SRDP and CVHS. Blue represents the exterior position, red represents the middle position, and green represents the interior position. Boxes represent the interquartile range and whiskers represent max and min excluding outliers. Dots represent outliers.

Table 1

P values from a two way ANOVA comparing the initial heavy metal concentrations of native moss for each source site as well as for source position. This compares source locality to source position on the tree and their interaction.

Element	Source	Source Position	Source*Source Position
Pb	< 0.0001	0.2096	0.0676
Cd	< 0.0001	0.1848	0.5101
Zn	0.0159	0.0006	0.3167
Cu	0.0159	0.9757	0.1647

Table 2

P values from a one way ANOVA comparing the heavy metal concentrations of native SRDP moss to the position the moss was sampled from.

Element	Source Position	Exterior	Middle	Interior
Pb	0.0084	0.0066	0.6633	0.008
Cd	0.0173	0.0193	0.0163	0.7497
Zn	0.0012	0.0079	0.1174	0.0004
Cu	0.3546	0.2973	0.5842	0.1706

Table 3

P values from a one way ANOVA comparing the heavy metal concentrations of native CVHS moss to the position the moss was sampled from.

Element	Source Position	Exterior	Middle	Interior
Pb	0.7582	0.5271	0.5307	0.9417
Cd	0.8955	0.6413	0.8112	0.8002
Zn	0.0636	0.0207	0.4447	0.094
Cu	0.4169	0.5458	0.4323	0.1949

3.2. Transplant Metal Concentration Variability

The averages, 2*sd, max, and min for transplants are shown in Tables 4 and 5. The concentrations of all heavy metals in the moss transplants placed at either site tended to increase through the first six months then decrease slightly for the last six months (Fig. 2). All metals experienced significant change in metal concentrations by month except for Cu in transplants moved to CVHS which had a weak change (Tables 6 and 7). There was no consistent difference between destination position and heavy metal concentrations in transplants moved to either site with the exception of Cu in transplants moved to SRDP (Tables 8 and 9)

3.2.1. Samples transplanted to CVHS

Samples transplanted to CVHS generally experienced no change in heavy metal concentrations with the exception of Cd (Fig 2). Cadmium levels were significantly different by destination position and less variable in the exterior position compared to the interior position (Table 6 and Fig. 2.). Transplants never reached native moss concentrations (Fig 4).

3.2.2. Samples transplanted to SRDP

Samples transplanted to SRDP tended to increase in heavy metal concentrations more quickly and to a greater extent compared to the samples transplanted to CVHS (Figs. 2). Cadmium experienced a weak difference by destination position when transplanted to SRDP (Table 7). Copper levels weakly followed the trend in the exterior position compared to the interior position (Figs. 2.). Transplants reached native concentrations within 3 months of deployment with the exception of Pb which took 6 months (Fig 3).

Table 4

Averages and standard deviations for each month of collection by each heavy metal log($\mu g\ g^{-1}$) for transplants moved to SRDP.

	Cu	Zn	Cd	Pb
Month 1				
AVERAGE	8.16352077	16.1959986	0.07646776	0.84249637
2SD (95%)	6.17772728	8.20008852	0.06264773	0.4774244
Month 3				
AVERAGE	11.3702418	25.3642434	0.26177122	1.41402983
2SD (95%)	6.53799843	11.5362002	0.19519873	0.69562814
Month 6				
AVERAGE	15.1263028	32.5209693	0.43484047	1.91420561
2SD (95%)	11.5773486	25.375695	0.40689307	0.92701924
Month 9				
AVERAGE	13.8924665	76.5169384	0.47673962	1.96950974
2SD (95%)	5.52860589	228.969303	0.45016683	0.62656061
Month 12				
AVERAGE	12.1480336	31.2814118	0.43693019	2.03296232
2SD (95%)	6.42052785	5.08956931	0.57520335	0.84859214

Table 5

Averages and standard deviations for each month of collection by each heavy metal log($\mu g\ g^{-1}$) for transplants moved to CVHS.

	Cu	Zn	Cd	Pb
Month 1				
AVERAGE	12.8827279	25.5818012	0.25289033	1.9959054
2SD (95%)	12.9657708	11.1370712	0.16289191	0.88839648
Month 3				
AVERAGE	10.3729497	27.9072257	0.36993247	2.1572583
2SD (95%)	8.76287704	14.7416078	0.25875318	1.60303237
Month 6				
AVERAGE	12.2043899	30.716556	0.61997786	2.67279148
2SD (95%)	4.82562865	14.1900052	0.9168724	1.16518731
Month 9				
AVERAGE	17.4888393	31.6503573	0.6833836	2.6828477
2SD (95%)	28.164945	14.9177517	0.35024278	2.20987193
Month 12				
AVERAGE	14.1596003	27.5024323	0.77085779	2.32678108
2SD (95%)	10.7642076	15.2722784	0.56959967	1.57234835

Table 6
P values from the two way ANOVA comparing the differences in heavy metal concentrations of transplants moved to CVHS over time. This compares month to destination position and their interaction.

Element	Month	Destination Position	Month* Destination Position
Pb	0.0028	0.6682	0.0597
Cd	<0.0001	0.0452	0.1563
Zn	0.0151	0.0917	0.0959
Cu	0.0591	0.2943	0.1322

Table 7
P values from a two way ANOVA comparing the difference in heavy metal concentrations of transplants moved to SRDP over time. This compares month to destination position and their interaction.

Element	Month	Destination Position	Month* Destination Position
Pb	<0.0001	0.6554	0.2246
Cd	<0.0001	0.0524	0.7756
Zn	0.0007	0.825	0.4267
Cu	0.0009	0.0009	0.0678

Table 8
P values from a one way ANOVA comparing the difference in heavy metal concentrations to the destination position of the transplants moved to CVHS.

Element	Destination Position
Pb	0.789
Cd	0.1001
Zn	0.1567
Cu	0.3572

Table 9

P values from a one way ANOVA comparing the difference in heavy metal concentrations to the destination position of the transplants moved to SRDP.

Element	Destination Position
Pb	0.791
Cd	0.1756
Zn	0.9375
Cu	0.007

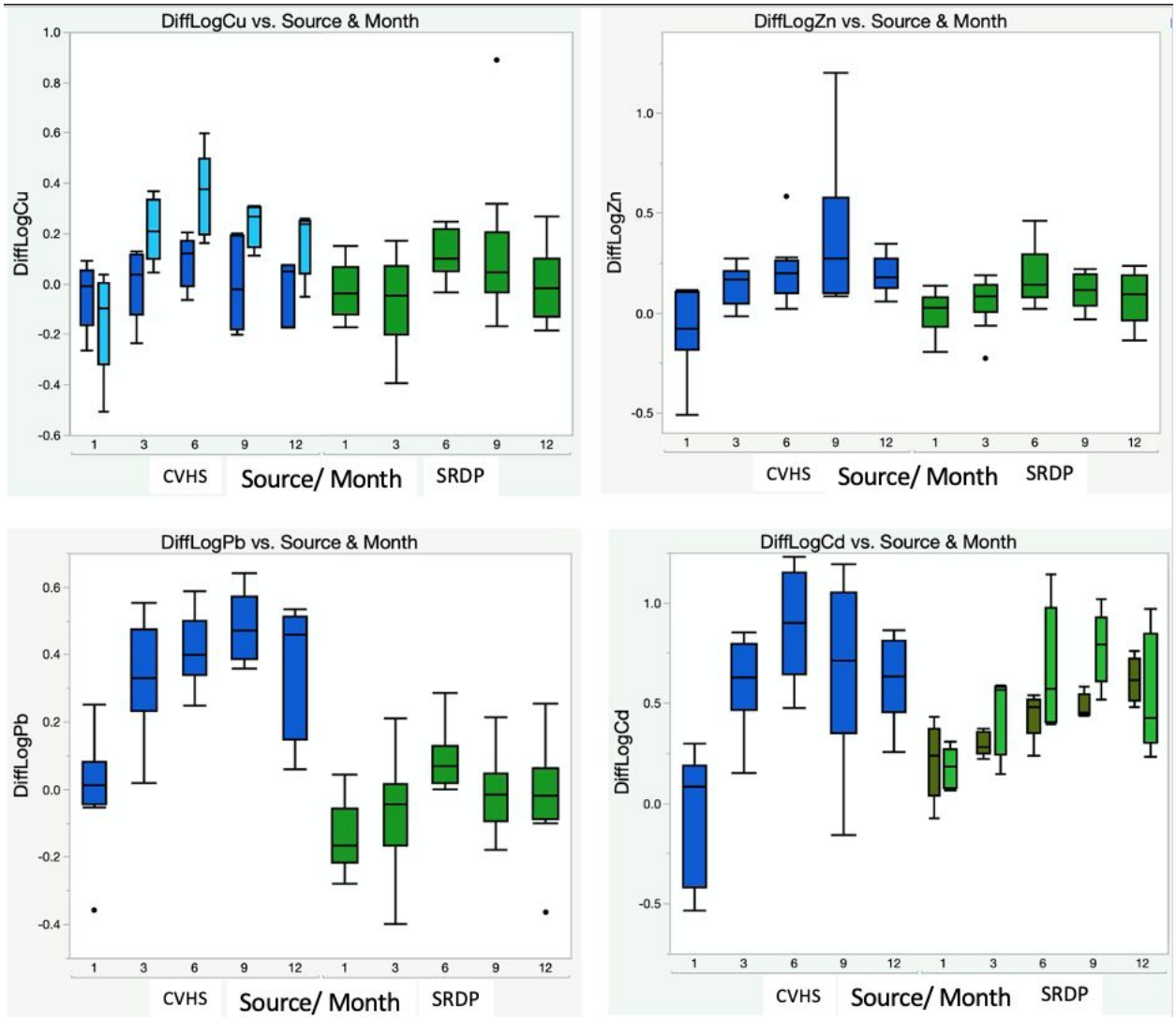

Fig.2. Variations in the difference of concentrations (difflog($\mu g\ g^{-1}$)) of different heavy metals in the moss transplanted reciprocally between CVHS and SRDP over time. Blue graphs represent mosses taken from CVHS and green represent mosses taken from SRDP. Boxes represent the interquartile range and whiskers represent max and min excluding outliers. Dots represent outliers. Dark green boxes represent the exterior position while light green represents the interior position.

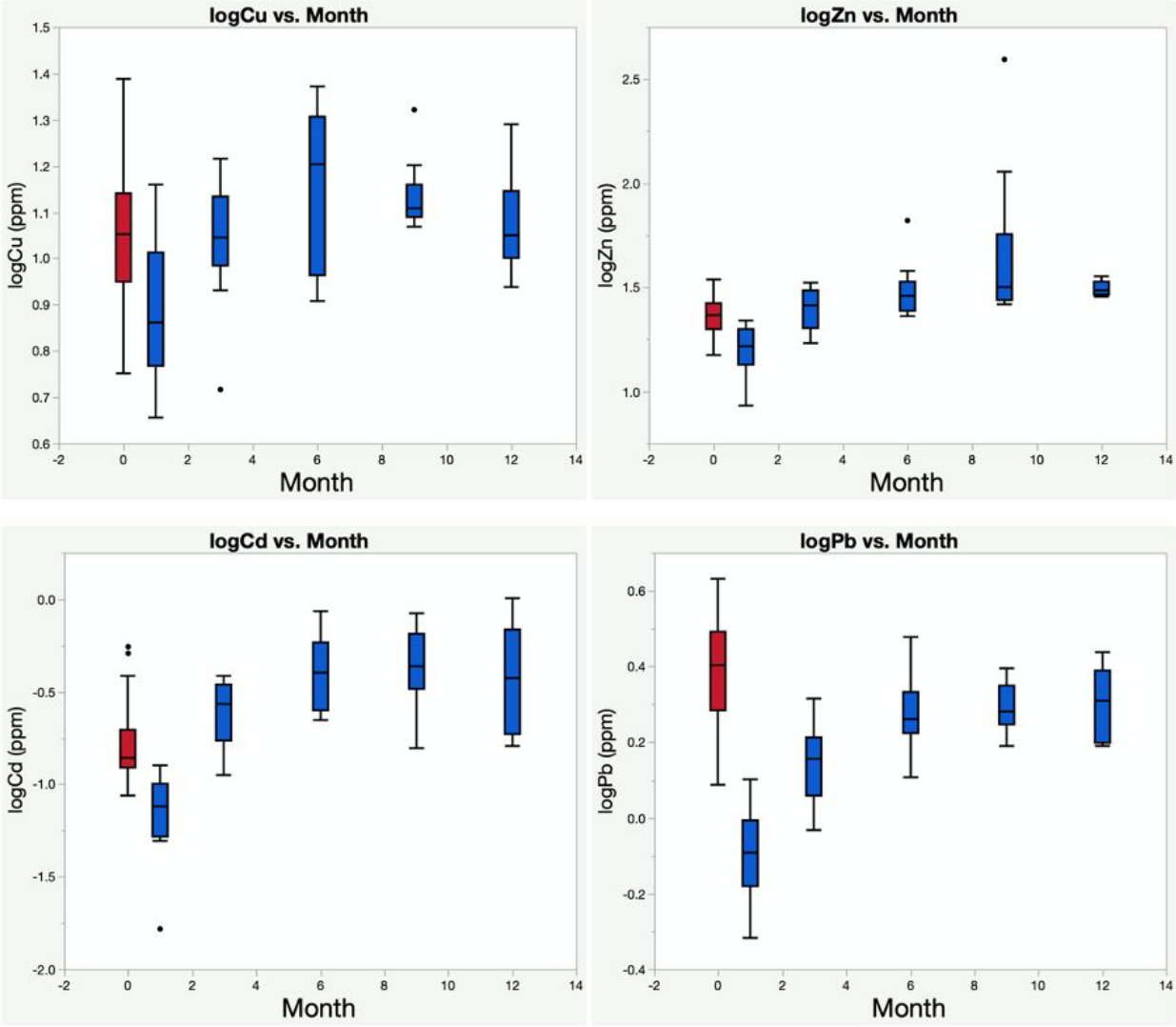

Fig3. Variations in the concentrations (log($\mu g\ g^{-1}$)) of different heavy metals in the moss transplanted to SRDP over time (blue boxes) compared to native SRDP moss (red box). Boxes represent the interquartile range and whiskers represent max and min excluding outliers. Dots represent outliers.

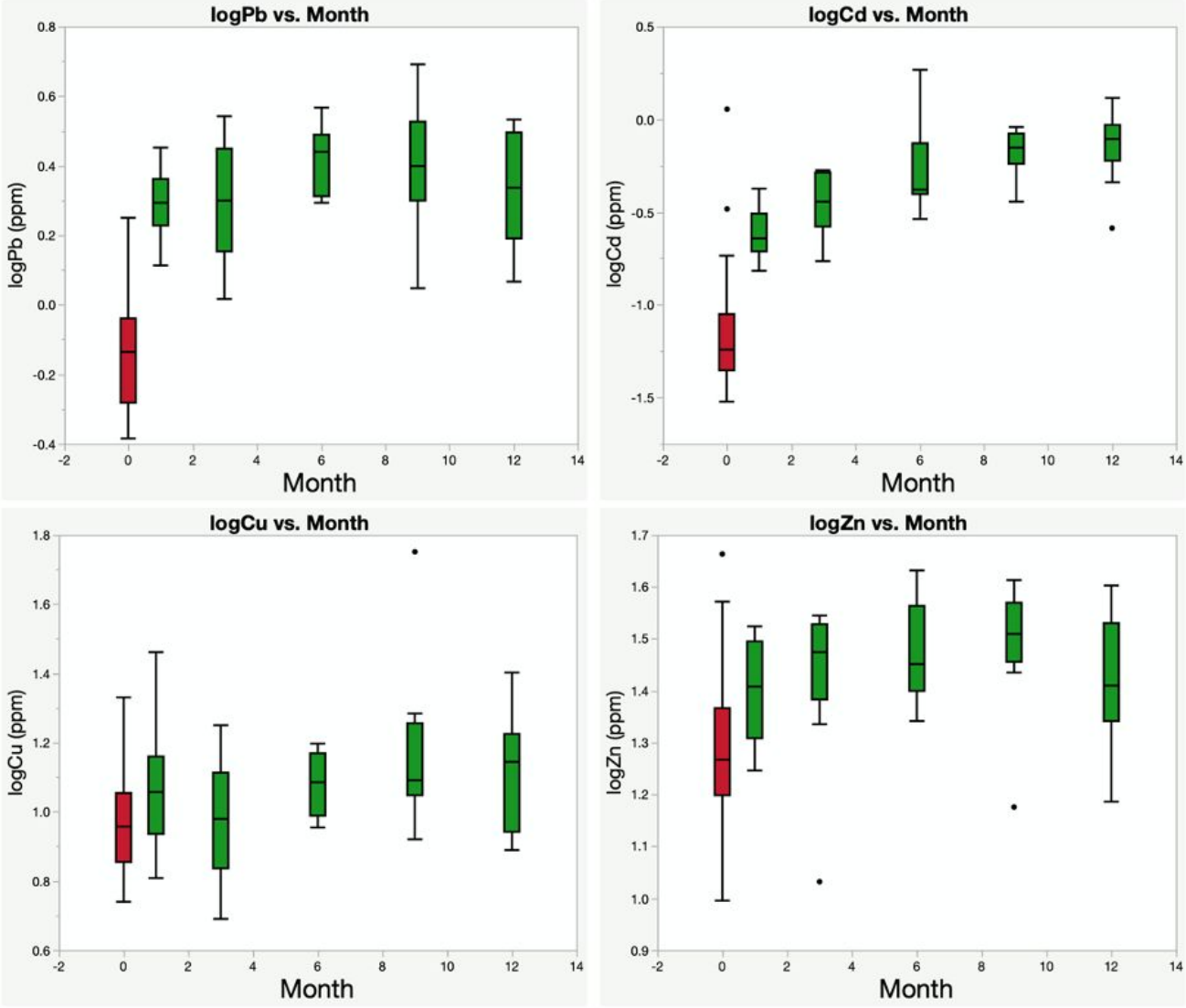

Fig.4. Variations in the concentrations (log($\mu g\ g^{-1}$)) of different heavy metals in the moss transplanted to CVHS over time (green boxes) compared to native CVHS moss (red box). Boxes represent the interquartile range and whiskers represent max and min excluding outliers. Dots represent outliers.

4. Discussion

4.1. Native moss metal concentrations

In the past mosses have been used as a biomonitor to assess air quality and to identify sources of emissions. Many previous studies have focused on large scale spatial variability among sites or at different distances from a point source. However, recent pilot studies in the Shiel group have found high variability of heavy metal concentrations among moss sampled from just one tree (Miller et al., 2017). The present study has gone further by examining 120 samples off single trees at two sites and by documenting the sampled moss position in the tree. We found heavy metal concentrations in native moss tissues to vary by position for some metals. However, transplanted tissue concentrations did not consistently differ in changes in metal content by position. These results are consistent with pilot studies by previous undergraduates in the Shiel group. However, we did not find published work in this area. Clearly more work is needed to investigate the sources of metal level variability among co-located moss. The results of this study highlight the need for consistency in sampling position among trees in a given study or better large sample sizes that will be more representative of the average moss concentration from a site.

4.1.1 Source differences

SRDP was found to differ from CVHS more in lead and cadmium concentrations for native mosses than in zinc and copper. SRDP is exposed to more anthropogenic pollution by being near the city of Portland. Pb and Cd originate almost exclusively from the atmosphere while Cu and Zn exposure can come from many other sources (Aboal et al. 2010). These include

edaphic contributions such as mineral particles from wind-blown soil. These particles may also have contributions from anthropogenic sources. Other contributions can come from live plant leaching or dead plant material. Cu and Zn can be transferred to moss this way (Berg and Steinnes 1997).

4.1.2 Positional effects

Statistical analyses showed that concentrations of heavy metals are determined more by the source location and destination location than by the position of sampling. This may be due to atmospheric deposition being consistent across a branch. Other reasons may be that inputs from plants leaching metals or edaphic contributions are consistent across a branch (Berg and Steinnes 1997).

4.2. Transplants moved to SRDP

Transplants moved to more polluted environments quickly acquire more metals over a 6-9 month period. All metals reached native levels in samples transplanted to SRDP however Zn and Cd transplants surpassed native levels when transplanted to the polluted site. Pb concentrations increased more slowly than the other metals and did not surpass native levels while Cu was quick to reach native levels and also did not surpass native levels. A previous study conducted by Tabors et al. 2004 reported none of the heavy metals in transplants moved to a more polluted site surpassed native levels. This contradicts a study done by Boquete et al. 2013 who reported all heavy metals surpassed autotransplant levels when moved to a more polluted

site. This difference may be because they used autotransplants to compare to transplants while Tabors and this study compared transplants to native moss.

4.3. Transplants moved to CVHS

Transplants moved to less polluted environments tended to retain their elevated levels with the exception of Cd which experienced a large unexplained increase. These results are similar to what Tabors et al. 2004 reported in that the concentrations of transplants moved to a less polluted environment remained almost constant throughout the study. The study conducted by Boquete et al. 2013 reported heavy metal concentrations in transplants moved to a less polluted site experienced a decrease in levels until they reached the same levels as the autotransplants. The difference in results may also be due to the use of native moss versus autotransplants and the lack of a growth dilution correction. These results suggest that particulate matter is held tightly to the moss tissues and does not rinse or leach off easily. This is important for the study of how atmospheric concentrations change over time with the use of moss transplants because it suggests moss that already has high levels of heavy metals will not quickly or accurately reflect decreases in pollutant deposition.

4.3.1 Cadmium increase

There was an unexpected increase in Cd concentration when polluted transplants were moved to the less polluted site at CVHS. This may be due to an unknown source of Cd pollution near the CVHS site.

4.4. Limitations

This study did not utilize autotransplants unlike other similar studies such as Boquete et al. 2013. This would have allowed us to see seasonal changes in heavy metal concentrations. Without autotransplants, we cannot definitively separate a transplant effect from a location effect.

While this study included a large number of samples in total n=200, once broken down into each treatment group there were only 5 samples collected at each position for a total of n=20 each month samples were collected. In order to scale this study up, it would cost more and take a significantly longer time to complete. The silicone sealer and fishing line ornament technique allow for easy transplantation. However, it also allows for significant fragmentation of moss tissue, which prevented consistent and reliable estimates of growth rates of the transplants. With no way to account for the loss of tissue, we were unable to correct for growth dilution.

The chosen polluted and unpolluted sites had similar starting concentrations of Zn and Cu. This limits our ability to detect changes in concentrations in the transplants.

4.5. Future research

Future research may include conducting a larger scaled study with additional metals on the spatial variability of heavy metals within a tree. Samples could be collected from all over the tree and their positions documented. This would allow for a fine-scale map of a tree to be made. With the documentation of additional variables, we may be able to better understand the different processes that affect heavy metal concentrations in moss tissues at different positions.

Repeating the transplant study at sites that have larger starting differences in their Zn and Cu concentrations would also be valuable. Performing lead isotope analysis of transplants would provide turnover rates in the moss tissues by comparing concentrations of old isotopes to new. This would allow us to better understand the uptake and loss rates of Pb in moss. Investigating the source of the unknown Cd pollution at Crescent Valley High School could be performed concurrently with any of the previously mentioned future research projects.

5. Conclusion

Over the course of a year, a reciprocal transplant study was conducted at Sandy River Delta Park and Crescent Valley Highschool. The goals were to better understand the spatial variability of heavy metals within a single tree as well as to document the change in heavy metal uptake, loss and retention in moss tissues. Understanding the variability of moss metals concentrations and sources of variability is key to further developing moss as a biomonitor and developing better sampling protocols. The research questions from the introduction are answered below.

5.1. Does the concentration of heavy metals vary among moss taken from different positions on a single branch?

Yes, native mosses sampled from different positions within a tree were found to vary for some metals. Enough variation was found between interior and exterior positions to recommend consistency in the position of sampling in trees.

5.2. Will moss transplanted to a less polluted site retain or lose its elevated heavy metal levels and how long will that take?

Transplants moved to a less polluted site experienced no significant decrease in their elevated heavy metal levels, even at 12 months. As a result, we recommend the use of transplants with low levels of heavy metals in order to accurately represent atmospheric deposition at the transplant site.

5.3. Will moss transplanted to a more polluted site experience an increase in heavy metals levels and how fast?

Transplants moved to a more polluted site experienced an increase in levels of all heavy metals. For all metals, except Pb, this took at least three months. Lead levels in transplanted moss took longer than six months to reach native levels. We recommend transplants be hung at a site for at least six months, twelve months ideally, to eliminate potential seasonal effects

5.4. Does the position of a transplant on a branch affect its heavy metal concentrations?

All metals examined, except for Cu, did not show any significant difference in concentrations between the interior and exterior positions. There was less variation found at the exterior position for Cd; however, it was not significantly different. From these results, we recommend the position of transplanted moss be taken into consideration.

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