Local Variation of Trace Element Concentrations in *Usnea subfloridana*.

This thesis is submitted to the Faculty of Graduate Studies to partially fulfill the requirements for a Master of Environmental Studies degree offered through the Northern Environments and Cultures Program at Lakehead University

By

Matthew David Adams

Permission to Use

In submitting this dissertation as a component to fulfill the requirements of a Master of Environmental Studies at Lakehead University, I agree that this document be made available for apprehension and interpretation at Lakehead University Library. Further, I consent to the replication of this document, entire or in part, for academic intent following permission from one of my advisory committee members, specifically Dr. Christine Gottardo, Dr. Philip Fralick or Dr. Robert Stewart, or the Coordinator of the NECU MES program at Lakehead University. The scholarly use of information taken from this dissertation should be duly accredited to me, as well as Lakehead University.

Requests for use and permission to copy this document can be made to:

Coordinator of the MES in NECU Lakehead University
955 Oliver Road
Thunder Bay, Ontario
P7B 5E1

Abstract

A mensurative study into the local variability of trace element concentrations in the lichen *Usnea subfloridana* was conducted. Samples were collected from an *Abies balsamea* located in the Thunder Bay region away from any known point sources of pollution. One-thousand and thirty-seven samples were collected and individually weighed. These samples were subsequently grouped into 97 grouped samples, based on their aspect, height on the tree, and weight. Elemental concentrations were determined by inductively coupled plasma atomic emission spectroscopy. Data analyses were conducted on Al, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, S, Sr, and Zn concentrations. Two main wind directions were present at the study site; lichens were grouped according to which pattern they were nearest when creating the grouped samples. All elements except Mn were significantly different in concentration at $p < 0.001$ between the two aspect groups. Comparison of the variation around the mean for each element's experimental results and the literature values, indicate local variation can be equal to or greater than what has been found in regional studies. Linear regression models suggest that strict guidelines can reduce this variation for some elements. In conclusion it is found that the common practice of low sampling density over large regions may not be a suitable technique because of the large local variation which is seen in this study. Researchers must know the local variability before making quantitative comparisons at a regional level with few samples.

Acknowledgements

I would first like to thank my supervisor on this project, Dr. Christine Gottardo. Without her endless support and guidance this project would not have been possible. I would like to thank my committee members for their review and suggestions on the document, which added greatly to the final product, Drs. Philip Fralick, Daniel McCarthy, and Robert Stewart. My fellow colleagues in the program for their support during our time together. Saara Rizzo for her extensive help in the field. Erika North for her education on lichens. The Lakehead University Environmental Lab, particularly Johane Joncas for her guidance on analytical work. Dave Challen and Beth Kuiper for providing a place to conduct the research. And my deepest apologies to anyone I may have missed in this list as there were numerous people who helped see this project through.

Finally, I would like to express my thanks to my parents for their endless support and love, David and Judi Adams.

Table of Contents

List of Figures

List of Tables

CHAPTER 1

INTRODUCTION

Biomonitoring, the process of using biological organisms to monitor ecosystem health, is used to evaluate many facets of the environment. Some examples of biomonitoring applications include:

1) Using sentinel species as indicators of dramatic environmental change, such as when toxic diatom (algae) blooms were identified because of the death of 160 brown pelicans at Cabo San Lucas (Beltrán et al. 1997);

2) Evaluating community changes in species composition in response to a stressor, such as examining the response of benthic macro-invertebrates to metal concentrations in water (LaPoint et al. 1984; Clements 1994); and;

3) Laboratory experiments examining direct toxicity by immersing the organism in chemical containing solutions and evaluating rates of lethal toxicity (Könemann 1981).

Biomonitoring has been extensively used for the evaluation of air pollution. This monitoring has been conducted by looking at biological organisms' community responses to air pollution, commonly using lichens (Loppi et al. 2004; Loppi et al. 2002). A second method determines the concentration of the pollutant in biological tissue with two commonly used types of tissue being moss (Barclay-Estrup and Rinne 1979) and lichen tissue (Bennett and Wetmore 2000). Studies are conducted which use both lichen and moss tissue (Mendil et al. 2009). The latter method is the focus of this thesis, which is the evaluation of trace element concentrations in and on lichen tissue.

1.1 Lichens

Lichens are symbiotic organisms commonly composed of two organisms: a fungus which gives the lichen its structure and at least one species of algae or cyanobacterium, which is responsible for the production of energy in the lichen using photosynthesis (Nash III 2008). This symbiotic relationship is not limited to two species, and can occur with three or more species, from up to three kingdoms (Nash III 2008). For all intents and purposes in this work lichens will be treated as an individual unit.

Growth forms, the appearance of the lichen thallus, are normally divided into three morphological groups: crustose, foliose, and fruticose. Crustose lichens grow closely appressed to the substrate and in some cases can look as if they are painted on. They lack a defined lower surface. These lichens are quite challenging to remove from many surfaces without damage, but are very resilient to anthropogenic disturbance (McMillan and Larson 2002). Foliose lichens give the appearance of being leaf-like; they have a distinctive upper and lower surface and commonly grow attached to a substrate with rhizines. Fruticose lichens are pendent and have a three-dimensional form. Reindeer lichen, which is in the genera *Cladonia* is a well known fruticose lichen genus. Fruticose lichens easily detach from their substrate, sometimes they can be simply lifted off.

Lichens as a qualitative indicator of pollution can be traced back over 200 years to Erasmus Darwin, who noted that near the copper smelters in Wales, lichens failed to grow (Nimis and Purvis 2002). In the 1960s a proliferation of lichen biomonitoring research began with the determination of sulphur dioxide as a limiting factor on lichen growth. A paper by Nimis and Purvis (2002) indicated that well over 1500 papers have been published on lichen biomonitoring, as well as a number of books. This number has undoubtedly increased. Geographic distribution of lichens is almost a full terrestrial coverage (Baffi et al. 2002), which allows for lichen biomonitoring studies to be wide spread (Yenisoy-Karakaş and Tuncel 2004a; Wolterbeek and Bode 1995).

Nutrients necessary for lichen growth are derived primarily from the air, via wet and dry deposition. Dry deposition includes gas or particulate matter settling out of the atmosphere, while large particles settle out close to the source. Finer particulate matter (0.01 µm to 100 µm) continuously settles out of the environment and can travel great distances, including high-arctic deposition (Garty 2001). Wet deposition comes in the form of water runoff, rain, snowfall, fog, and dew which saturates the lichen and then is transported into the thalli (Bargagli and Mikhailova 2002).

Not all lichen species are as equally useful as biomonitors. The amount of particulate matter that will be trapped inside lichen is dependent on several factors, including thallus type**.** Thallus type is related to lichen growth form, such as fruticose, foliose, or crustose. Changes also develop in individual species, which include branching, wrinkling, and roughness, thereby creating changes in surface area. Lichens also have different sized pores in the epicortex, which can control the size of the particulate matter able to enter (Bargagli and Mikhailova 2002).

Lichen sensitivity to air pollution is caused by the lack of a developed outer cuticle (Nimis and Purvis 2002). Because of the lack of an outer cuticle, lichens are ectohydric organisms. These are organisms with no specialized structure for transferring gases or water containing dissolved substances (Bargagli and Mikhailova 2002). Thus, lichens require many nutrients from air deposition for growth, but they lack the ability to sort out particular elements, those that would be beneficial and those that are potentially harmful.

1.2 Air Pollution

Air pollution is deleterious to most biological organisms. Acute exposure is linked to angina, myocardial infarction, and heart failure in humans. Long-term exposure can increase the risk of coronary heart disease leading to death. Organic and transition metal nanoparticles, in this context are the main drivers behind potential cardiovascular health issues (Mills et al. 2009). Both long- and short-term studies agree that air pollution has severely impacted the human population. In the Netherlands it is expected that air pollution deaths correlated to elevated PM10 (particulate matter 10 µm or smaller) are greater than the number of deaths caused by traffic accidents (Brunekreef and Holgate 2002). Deaths are correlated with PM10 because particles of that size can penetrate into the deepest part of the lungs, with higher levels (150 micrograms/m^3) being correlated to decreased lung function (3-6%) in patients with asthma between the ages of 8 and 72 years of age (Pope et al. 1991). A 10 micrograms/m^3 increase in two-day mean PM2.5 (particulate matter 2.5 µm or smaller) was correlated with a 1.5% increase in total daily mortality. PM10 and 2.5 are not a pollutant themselves but a measure of particulate size which has been found to correlate with increased health risk (Schwartz et al. 1996).

Variability in the concentrations of trace elements in and on lichen tissue is the focus of this research. Common sources can include: fossil fuel combustions, which are a major source of Cr, Hg, Mn, Sb, Se, Sn, and Ti with coal combustion; Ni and V with oil combustion. Gasoline, including low- and un-leaded gasoline is the major source of atmospheric Pb. Another major trace metal source is non-ferrous metal production which accounts for the largest source of As, Cd, Cu, In, and Zn (Pacyna and Pacyna 2001). Natural sources (local geology) of trace elements include particulate matter derived from soil and local geology and often correlate to Al, Ti and Si concentrations (Bargagli and Mikhailova 2002).

Short- and long-term air pollution studies all indicate a continued need to research and manage air pollution because health effects are increasingly being found at lower concentrations (Brunekreef and Holgate 2002).

1.3 Research Objectives

The intent of this thesis is to better understand the very-local trace elemental accumulation variation in lichens. With the well understood potential for variability from field sampling, analytical methods (Tuncel et al. 2004; Moreira et al. 2005) or different digestion techniques (Baffi et al. 2002), it appears one of the main components which may cause variation has been overlooked. This potential variation is local variation or within site variation. To understand this variation highly dense sampled, small spatial extent studies are required, these have not currently occurred. By better understanding local variation, it will be possible to make better quantitative conclusions between sampling locations separated by large distances. Formulas used to calculate a suitable sampling density require *a priori* knowledge of the expected variation in results (Ferretti and Erhardt 2002). Reviews of the field also suggest the importance of studying environmental situations which have not been compromised (Conti and Cecchetti 2001). As well it is noted that design and quality assurance are the two least understood concepts in biomonitoring programs (Ferretti and Erhardt 2002).

One main reason to use lichens for biomonitoring is that they have large and ubiquitous ranges (Brodo et al. 2001). These large ranges have allowed very large scale projects to occur using lichen biomonitors. Unfortunately the local variation is poorly understood because samples are typically lumped together or very few samples are collected, such as 1 site per 256 km^2 (Jeran et al. 2007); 47 sites in 65.9 km^2 (Bennett and Wetmore 2003); and 8 sites to cover 10,000 km^2 (Helena et al. 2004).

This research project is designed to better understand what, if any, variation is occurring in the accumulation of trace elements in lichens. The literature review will highlight general information about lichens as trace element biomonitors; focus in on boreal forest biomonitors; examine current sampling designs; look at the potential for local variation; explore field sampling, analytical, and data analysis methods; and then the placement of this study within the current literature. The methodology will borrow from other studies in sample handling, analytical methods and data interpretation, but will be focused on a new approach for sampling design. Results are going to be analyzed with univariate and multivariate, exploratory and significance based statistical analysis procedures. Conclusions will be drawn upon the results and followed with discussion on how they apply within the current literature.

This research is mensurative in nature, and there is very little information which can be used to help design the sampling approach because of the use of high density sampling in a small site. With this research being mensurative, the research question of “What are the very-local variations in trace elemental concentrations in *Usnea subfloridana*?” is applied. This research is a baseline study and further exploration into this topic in other regions, with other species, and at different spatial scales should occur.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Nimis and Purvis (2002) produced an extensive list which outlined the major benefits of using lichen based biomonitoring programs for air pollution. These benefits can be summarized as: 1) Lichens are widely distributed and are often increasing in diversity; 2) They absorb nutrients and pollutants over their entire surface; 3) If either symbiotic partner is damaged, the lichen will die; 4) Year round availability; 5) Ability to accumulate high toxic elemental concentrations and still survive; and; 6) Air pollution monitoring instruments can be vandalised or stolen. These benefits have allowed lichens to become the most widely used organism for biomonitoring of the terrestrial environment (Nimis and Purvis 2002).

The benefits of using lichens as biomonitors do come with some concerns that must be acknowledged during the development of such a monitoring program. The natural environment is very complex; this complexity means that researchers need to be well aware of the design and methodological issues that are important for implementing a robust sampling design, to correctly represent the environmental conditions. Similar studies which use plant species for biomonitoring have found that sampling in the field can be a major source of error, up to 1000%. The subsequent steps in lab based processing, including the drying of samples, homogenization, and chemical decomposition are known to account for errors between 100-300% (Ferretti and Erhardt 2002).

With such a large potential for errors caused by field sampling, and the extensive literature evaluating different analytical methods such as different digestion techniques (Tuncel et al. 2004; Moreira et al. 2005), or different instruments for analysis (Baffi et al. 2002), it is important to better understand the type of variation which may occur in a localized site, such as a single sampling location as this appears to have not been conducted in much detail. Highly dense small scoping studies are likely not occurring because they offer little reward to a funding body; a common funder may be the public health authorities who are often concerned with the spatial distribution of a pollutant over their region (Ferretti and Erhardt 2002).

2.2 Lichen as Biomonitors

Research has shown, in both laboratory (Puckett et al. 1973) and in field experiments (Adamo et al. 2003; Barclay-Estrup and Rinne 1979; Bennett and Wetmore 2003), that lichens are capable of accumulating elements that they do not require for their metabolic processes. This uptake of unnecessary elements is likely due to lichens lacking any type of cuticle or barrier to control inputs through their upper cortex, in addition to the different sized pores in the epicortex *vide supra*; because of this lichens are a suitable biomonitoring organism (Nimis and Purvis 2002). Lichens have a significant amount of intercellular space. In this intercellular space they can store accumulates such as trace elements (St. Clair et al. 2002; Di Lella et al. 2003). In one of the few species which has been quantitatively examined, *Xanthoria parietina,* the intercellular space composes up to 18% of the lichen (Collins and Farrar 1978).

Lichen biomonitoring is done both as an independent analysis (Adamo et al. 2003; Adamo et al. 2004; Aslan et al. 2004; Barclay-Estrup and Rinne 1979; Loppi et al. 1994; Loppiet al. 1998a) and as a complement to instrumental air pollution monitoring stations (Godinho et al. 2008; Purvis et al. 2004).

Accumulation capacity in lichens is species-dependent (Folkeson 1979; Cercasov et al. 2002; Chiarenzelli et al. 1997; Sloof and Wolterbeek 1993; Yenisoy-Karakaş and Tuncel 2004b). Why individual species accumulate more or less is poorly understood, but it may be due to differences in species' morphology (Bargagli and Mikhailova 2002). Success in correlating the tissue concentration of trace elements between species in the same region for a biomonitoring program has been mixed (Cercasov et al. 2002; Folkeson 1979; Sloof and Wolterbeek 1993; Yenisoy-Karakaş and Tuncel 2004b). The issues with correlating trace element concentrations in different lichen species leads to the need to pick a single species of lichen that is ubiquitous and well distributed within the studies' spatial extent. Comparative studies in localized areas have found that species growing closely appressed to their substrate (crustose) accumulate the highest concentrations and can be considered the most efficient bioaccumulators, followed by species growing slightly removed (foliose), and species growing away from the substrate (fruticose) accumulating the least (Bačkor and Loppi 2009).

Lichen biomonitors are especially valuable because many species have large ranges and can be ubiquitous over their range (Brodo et al. 2001). Biomonitoring techniques need to be examined very critically because if they are not capturing the correct environmental gradients, environmental managers and policy makers will not have a solid foundation on which to base their decisions. This research project is heavily focused on understanding local variation in lichen biomonitoring research because this author believes that there is a significant local variation in accumulation, which has yet to be well studied.

2.3 Review of Previous Studies

The United States Forest Service has been conducting lichen biomonitoring studies measuring concentrations of trace elements in and on lichen tissue. These data are available online in a digital clearing house (USFS 2011a). Data in the database were not divided by study. I will first review reports from research that was conducted in the Eastern Region, which is the area nearest Thunder Bay, ON, Canada. The USFS research uses aggregated samples (Lawrey and Hale 1998; Wetmore 1990, 1993), which takes the approach of collecting a bulk sample of lichen tissue from a site, and then analysing a portion of it. There are a few potential issues with this technique because it is not possible to establish an exposure time if lichens vary in size or location of collection (aspect, height, etc). With unknown exposure times this is a potential issue when comparing values at different sites. Even if collections are made with lichen tissue from similarly sized lichens, it cannot be safely assumed that the exposure time is similar because of microclimate variation that may affect lichen growth rates. Microclimatic conditions include branches above blocking rain or light, aspect, predation by insects, among many other known and unknown variables that could affect lichen growth.

Lawrey and Hale (1998) analyzed lichen tissue from the species *Flavoparmelia caperata* for concentrations of elements in the Dolly Sods and Otter Creek Wildernesses of Monogahela National Forest in West Virginia. This elemental analysis was one of three methods which was employed for monitoring air quality, the other two included the characterization of lichen flora to determine if patterns were consistent with patterns where air pollution is known to occur, and the other was to establish plots for photographic analysis. *Flavoparmelia caperata* is a foliose lichen which occurs on trees and sometimes rock; this species was sampled between June and September of 1987. A total of 169 samples were collected, 121 each from one square kilometre sections and 4 replicates placed in every tenth quadrat. Samples were analysed for P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn, B, Ni, Cr, Pb, Cd, Al, Mo, Sr, Ba, V, Ti, Be, Sn, and Co with an inductively coupled plasma spectrograph. Samples were washed in distilled water after having extraneous material removed. Citrus leaf SRM No. 1572 was used as the reference but information regarding purity of acids was not included. The community analysis found species which are not commonly seen in polluted areas (*Lobaria* spp., *Pseudevernia consocians, Usnea* spp.). The floristic data and the elemental data all indicated that the study region was not being adversely affected by air pollution. Summation of the elemental analysis results is included in Table 2.1.

Table 2.1 Elemental Concentrations from Lawrey and Hale (1998)
Concentrations detected in *Flavoparmelia caperata* from the two study regions. Otter Creek (N=112) and Dolly Sods (N=57). All values are reported in µg/g except S which is reported in percent. Standard Error was reported but converted to standard deviation by S.E. * SQRT(n)

Element	Minimum	Maximum	Otter Cr. Mean	S.D.	Dolly Sods Mean	S.D.
Al	293.3	2927	668.77	263.94	954.59	538.45
Ba	8.25	337.6	64.6	62.02	67.7	60.70
Cr	0.967	6.392	2.87	1.16	2.9	0.98
Cu	4.057	27.94	8.46	2.33	9.74	0.98
Fe	101	1586	400.5	167.21	471.7	280.33
K	1277	5458	2588.28	721.55	2926.16	733.09
Mg	137.3	648.8	268.61	103.40	336.95	118.23
Mn	19.84	920	159.56	110.27	223.16	161.94
Na	7.85	148.7	28.42	16.83	50.06	31.63
P	330.1	1996	786.65	289.87	854.39	367.75
Pb	13.06	103.2	33.67	15.35	40.92	16.91
S	0.078	0.2	0.124	0.02	0.147	0.02
Sr	2.924	69.32	14.14	10.05	12.18	9.51
Ti	4.59	98.94	22.79	11.85	23.47	14.34
Zn	16.07	227.5	41.14	24.13	64.24	37.67

Wetmore (1990) examined lichen tissue elemental concentrations in the Boundary Waters Canoe Area of the Superior National Forest, which is located less than 100 km from Thunder Bay, ON, Canada. Three species were collected for trace element analysis, *Cladina rangiferina* (soil substrate), *Evernia mesomorpha* (tree substrate), and *Hypogymnia physodes* (tree substrate). Only the two species growing on trees are going to be reviewed. Six regions were chosen in the study areas which were to represent the geographical extremes of the area. At each location a bag of 10 to 20 g of each species was collected. The samples were air dried and cleaned of extraneous material, but not washed. Sulphur was measured by dry combustion and evolved sulphur dioxide by infra red absorption. Other elements were tested with ICP-AES. One gram of material was used for the ICP analysis, no mention of reference material or quality of the acids is included. The analyses were conducted at the Research Analytical Laboratory at the University of Minnesota. Table 2.2 are the results for *Evernia mesomorpha* and Table 2.3 includes the results for *Hypogymnia physodes*.

Table 2.2 Results for *E. mesomorpha* from Wetmore (1990) Results from BWCA elemental concentration testing, ten samples were collected in the study region. Results were reported as ppm.

Element	Minimum	Maximum	S.D.	Range	Mean
Al	371	966	165.63	595	608.7
Cr	0.7	1.5	0.21	0.8	1.03
Cu	1.8	3.3	0.46	1.5	2.64
Fe	296	1037	214.15	741	614.8
K	1540	2746	359.10	1206	2248.1
Mg	246	479	65.28	233	335.4
Mn	23.8	93.1	24.55	69.3	44.01
Na	22.7	52.3	9.79	29.6	37.22
P	319	603	90.93	284	470.4
Pb	4.4	7.5	1.07	3.1	5.46
S	910	1373	155.10	463	1091.5
Zn	21.5	34.7	3.80	13.2	29.21

Table 2.3 Results for *H. physodes* from Wetmore (1990) Results from BWCA elemental concentration testing, ten samples were collected in the study region. Results were reported as ppm.

Element	Minimum	Maximum	S.D.	Range	Mean
Al	306	640	101.23	334	480.4
Cr	0.6	1.1	0.17	0.5	0.87
Cu	2.7	4.2	0.51	1.5	3.38
Fe	257	607	122.66	350	489.7
K	2575	3775	345.60	1200	3300.8
Mg	553	905	120.24	352	695.1
Mn	76.5	340.3	94.42	263.8	205.27
Na	20.7	41.7	6.71	21	31.21
P	447	929	136.05	482	702.7
Pb	13.8	29.9	4.70	16.1	19.53
S	770	1118	104.88	348	951.2
Zn	47.3	105.7	15.85	58.4	66.49

Wetmore (1993) conducted a study located in the Rainbow Lake Wilderness, which is less than 300 km Euclidean distance from Thunder Bay, ON, Canada. Species collected included *Cladina rangiferina*, *Evernia mesomorpha*, *Hypogymnia physodes*, and *Parmelia sulcata.* Three locations were selected and 20 grams of each species were collected at each location. Samples were air dried and cleaned of extraneous material and not washed. Each sample had three replicates tested. Elemental analyses were conducted with the same methods as in the previously mentioned study (Wetmore 1990). Replicate samples showed a lower variance between replicates than between samples, this method was only tested to examine instrument error and the sampling design was not designed to determine local vacation. Only 9 of the 16 elements were statistically significantly (α = 0.05) higher in variability between samples than sub-samples. This suggests a potential for the other 7 elements to have variation equal to that between samples when specimens of *Cladina rangiferina* are combined as a sample and then analysed through sub-samples, which were derived prior to grinding. This region shows no significant results that lichens are being damaged by any of the elements studied or sulphur dioxide. Results from elemental concentration testing for the epiphytic species are included in Tables 2.4-2.6.

Table 2.4 Results for *E. mesomorpha* from Wetmore (1993)
Results from Rainbow Lake Wilderness elemental concentration testing, nice samples were collected in the study region. Results were reported as ppm.

Element	Minimum	Maximum	S.D.	Range	Mean
Al	410	811	142.60	401	597.22
Cd	0.2	0.2	0.00	0	0.20
Cr	0.9	1.6	0.24	0.7	1.20
Cu	2.7	4.5	0.65	1.8	3.60
Fe	436	932	175.52	496	651.89
K	1836	2726	391.31	890	2178.11
Mg	273	444	62.33	171	343.11
Mn	26	73.1	18.54	47.1	43.23
Na	26.4	36	3.59	9.6	31.40
P	440	716	110.70	276	536.00
Pb	4.8	8.6	1.29	3.8	6.34
S	940	1150	77.15	210	1061.78
Zn	34.2	49.1	5.4	14.9	43

Table 2.5 Results for *H. physodes* from Wetmore (1993)
Results from Rainbow Lake Wilderness elemental concentration testing, nice samples were collected in the study region. Results were reported as ppm.

Element	Minimum	Maximum	S.D.	Range	Mean
Al	409	674	102.38	265	535.11
Cd	0.5	0.8	0.12	0.3	0.64
Cr	0.9	1.4	0.16	0.5	1.12
Cu	4.7	5.4	0.23	0.7	5.08
Fe	446	751	110.11	305	614.78
K	2489	3550	311.94	1061	3125.56
Mg	636	801	55.53	165	724.56
Mn	191.6	389.5	72.93	197.9	302.09
Na	22.6	39.1	4.73	16.5	29.06
P	591	1003	147.75	412	797.78
Pb	10.3	16.5	1.97	6.2	14.29
S	917	1110	61.61	193	1002.78
Zn	65.9	90.5	8.41	24.6	78.43

Table 2.6 Results for *P. sulcata* from Wetmore (1993)
Results from Rainbow Lake Wilderness elemental concentration testing, nice samples were collected in the study region. Results were reported as ppm.

Element	Minimum	Maximum	S.D.	Range	Mean
Al	543.00	859.00	127.28	316.00	670.78
Cd	0.30	0.40	0.05	0.10	0.34
Cr	0.80	1.50	0.27	0.70	1.20
Cu	4.20	7.20	1.00	3.00	5.84
Fe	564.00	861.00	130.44	297.00	704.89
K	3206.00	3910.00	235.93	704.00	3600.11
Mg	522.00	638.00	43.65	116.00	577.89
Mn	188.10	351.20	50.82	163.10	254.58
Na	19.70	30.60	4.04	10.90	25.42
P	1104.00	1625.00	170.60	521.00	1371.22
Pb	8.90	18.10	3.01	9.20	13.48
S	995.00	1340.00	108.12	345.00	1109.44
Zn	79.50	95.20	5.01	15.70	84.49

The USFS has developed values for what they consider to be clean sites, these are tissues concentrations for ten lichen taxon (USFS 2011b), and the values for *Usena* spp. are presented in Table 2.7. All data which have been collected by the United States Forest Service are available online and available for download (USFS 2011c). All available data available for *Usnea subfloridana* were downloaded and summarized in Table 2.8.

Table 2.7 Provisional Element Analysis Thresholds
Values defined by the United States Forest Service as element concentrations which are representative of a natural condition for *Usnea* spp. from 40 sites within Oregon, Washing and Alaska National Forests. Units are in parts per million dry weight.

Al	Ba	Ca	Cd	Cr	Cu	Fe	K
499	30.1	8202	0.3	4.1	25.6	272	3674

Mg	Mn	Na	P	Pb	Sr	Ti	Zn
2280	572	934	1174	13.3	31.4	40.6	65.8

Table 2.8 Results from USFS Elemental Analysis for *Usnea subfloridana*
Results from the testing of samples which were collected between 1993 and 2001, in the states of Colorado, Idaho and Wyoming. Values are ppm dry weight.

	Minimum	Maximum	Average	S.D.	Samples Tested	Range
Al	142	600	354	230.89	3	458
Ba	31.3	64.8	43.92	11.51	6	33.5
Ca	0.389	1.21	0.71	0.33	9	0.821
Cr	1.7	2.89	2.36	0.53	6	1.19
Cu	2.63	15.6	5.61	4.36	9	12.97
Fe	176	694	489.22	182.56	9	518
Pb	3.6	18.1	8.59	4.66	8	14.5
Mg	43	199	125.51	48.73	9	156
P	600	2070	1252.71	495.79	7	1470
K	0.234	0.643	0.41	0.13	9	0.409
Sr	10.9	54.5	30.4	17.48	7	43.6
S	0.051	0.164	0.09	0.04	9	0.113
Ti	29.1	102	66.9	25.14	9	72.9
Zn	16.2	55.9	32	11.62	9	39.7

Table 2.9 lists results from previous lichen biomonitoring that were not conducted by the United States Forest Service and tested for at least five similar elements to this study. All these studies used *in-situ* species for analysis, the table includes both the mean value and the range that was found over the study region. All studies use *in-situ* species and not transplants. When available, QA/QC results were included. Variation in the concentration is seen to be within one-order of magnitude around the mean depending on the particular study and element chosen. For example, a study covering over 20,000 km^2, the range for Cr in 1992 was 2.33-21.8 μg/g with a mean of 5.94 μg/g dry weight (Jeran et al. 2007). Another large scale study (2250 km^2) using lichen species *Parmelia caperata* had a range of 1.19-5.66 μg/g with a mean of 2.48 μg/g dry weight. A small scale study (50 km^2) with the same species also had a similar range of 0.8 – 5.3 with a mean of 3.3 μg/g dry weight. These studies should not be directly compared but it is

interesting that two very different spatial scales using the same species found a very similar range in concentrations.

Table 2.9 Trace Element Concentrations Review
Trace elemental concentration results from multiple studies that used *in situ* specimens.

Species Location (spatial size km^2); Samples (n) Notes		Al	Ba	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	P	Pb	S	Sr	Ti	Zn
Parmelia caperata Italy (2250); 90 composite samples. Whole thalli, collected from 5-7 trees in 50 x 50 m plot, only outermost 3-4 mm used and combined to get 150 mg. 1 plot per 5x5 km grid. Does not mention the use of trace metal grade acids. Standard Recovery 91-103%. Research Aim: Establish is moss and lichen produce similar results (Bargagli et al. 2002)	Mean	649	8.10	0.26	2.48	5.77	541			65.5			3.88	892		20.1	34.7
	S.D.	395	3.53	0.11	1.13	1.29	368			39.4			2.48	177		8.72	6.53
	Range	216 2333	3.73 17.7	0.06 0.69	1.19 5.66	3.94 9.17	161 2503			18.8 170			0.68 11.20	619 1387		7.20 52.3	25.9 57.7
Umbilicaria decussata Antarctica (N/A);37 Composite Samples Sampled on rock outcrops. 1999 Data Used. Standard Recovery 92-105%. Replicate Variation (n=5) 5.5 to 19.4% Research Aim: Establish Baseline Values (Bargagli et al. 2000)	Mean	1030		0.19	1.86	4.9	1829	2296	608	25	175	789	0.77				21
	S.D.	898		0.18	3.94	3.2	1046	1387	500	14	88	507	1.73				6
	Range	101 5254		0.03 0.79	0.40 3.94	1.2 18	224 4927	982 6500	30 2898	6 80	56 422	184 1921	0.06- 1.73				7 43
Parmelia caperata Italy (50); 39 Composite Samples from 1km^2 areas. Does not mention the use of trace metal grade acids. Replicate Variation (n=5) 3.4 to 13.8% NBS Reference Material 1572 (Standard Values in Parentheses) Al=105±21 (92±15); Cd=0.04±0.01 (0.03±0.01); Cr = 1.0 ± 0.2 (0.8±0.2); Cu = 15.2 ± 2.8 (16.5 ± 1.0); Fe=98±12 (90± 10); Mn = 19 ±4 (23±2); Pb=17.4±3.3 (13.3±2.4); Zn=27±3 (29±2). Research Aim: Effects of pollutants on a damaged ecosystem, and comparison between lichen and pine needle results. (Bargagli et al. 1987)	Mean	985		0.45	3.3	8.1	734			29.1			23.5				48.5
	S.D.	340		0.14	1.4	2.6	234			14.6			8.2				11.3
	Range	480 1680		0.10 0.92	0.8 5.3	4.7 16.6	277 1149			15.1 57.4			5.0 60.0				29.5 77.6

Species Location (spatial size km^2); Samples (n) Notes			Al	Ba	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	P	Pb	S	Sr	Ti	Zn
Hypogymnia physodes United States (2310); 18 locations composite samples. 5 – 15 g of material were collected per site. 3 collections over 9 years. Research Aims: Determine temporal changes; compare two species; and overall pattern. (Bennett 1995)		Mean	565		0.64	1.17	7.91	710	3070	779	164	18.6	824	29.7				89
		S.D.																
		Range	270 1356		0.09 1.4	0.4 1.86	3.7 14.9	362 1523	2313 4061	401 1474	62 639	9.3 28.1	558 1597	12.2 62.6				64.3 135.4
Hypogymnia physodes Slovenia (20,200) Composite Samples from 3-5 trees in 16 km^2 grids. Overall uncertainty is 3.5% IAEA Reference Material 336 results within 95% confidence interval. Research Aim: Examine relationship between trace element, N and S concentrations. (Jeran et al. 2007)	76 [1992]	Mean			1.01	5.94			4150						0.19			91.71
		Range			0.21 5.42	2.33 21.80			1652 8644						0.10 0.37			47.26 181.60
	77 [2001]	Mean			0.75	3.67			3878						0.13			95.33
		Range			<0.2 2.45	1.11 35.85			2304 6188						0.07			45.63 182.52
Hypogymnia physodes Slovenia (20,200) 82- 86 (depending on element) Composite Samples from 3-5 trees in 16 km^2 grids. Standards were run, but not reported. (Jeran et al. 1996)		Mean		28.3	1.05	5.78		1253	4094			181				22.2		90.2
		S.D.		26.7	0.65	3.84		665	1208			99.3				14.5		24.4
		Range		7.13 212	0.31 5.42	2.33 21.8		492 3756	1652 8644			64.8 474				5.17 77.8		47.3 151

2.4 Boreal Region Biomonitoring

Common species that are often utilized for biomonitoring studies and which naturally occur within the boreal region include: 1) *Hypogymnia physodes* (Białońska and Dayan 2005; Pfeiffer and Barclay-Estrup 1992; Purvis et al. 2006; Hauck 2008; Rusu et al. 2006; Williamson et al. 2003; Helena et al. 2004; Sensen and Richardson 2002; Jeran et al. 2007; Kubin et al. 1997) ; 2) *Evernia mesomorpha* (Bennett and Wetmore 2003; Bennett 1995; Wetmore 1987; McCarthy et al. 2009) ; and 3) *Parmelia sulcata* (Bennett and Wetmore 2003; Bennett and Wetmore 2000; Kirchner and Daillant 2002; Horvat et al. 2000; Daillant et al. 2004) . *H. physodes* and *P. sulcata* are foliose species, but *P. sulcata* grows much more closely appressed to the substrate. *E. mesomorpha* is a fruticose species with a much textured surface.

Lichen biomonitoring has occurred in the Thunder Bay region with a five transect study using *H. physodes;* samples were collected in the summer of 1987. When the authors compared their results to other studies' ranges of concentrations, the concentrations they found were within those ranges, but values for Hg and Pb were high (Pfeiffer and Barclay-Estrup 1992). This study indicates with the high Pb values, when compared with values in Tables 2.1-2.9, that air pollution monitoring in the Thunder Bay region should be continued.

2.5 Sampling Design

Sampling design is important when trying to characterize a region's environmental pollution gradient (Ellis and Schneider 1997). Lichen biomonitoring programs often utilize a composite sample, as seen with all studies in section 2.3 Review of Previous Studies. This has been a successful technique for monitoring general trends (Jeran 1996). A composite sample technique must often be employed by the researchers because lichen do not typically grow very large (Brodo et al. 2001), and are very slow growing; research of biomass growth using *Alectoria sarmentosa*, *Evernia prunastri*, *Lobaria oregano*, *Lobaria pulmonaria*, *Pseudocyphellaria rainierensis*, *and Usnea longissima* found absolute annual biomass gains of about 0.01 – 0.1 g per sample testing samples weighing between 0.05 and 0.25 g (McCune et al. 1996).

Slow growth rates and small biomass create a problem with most lichen species that they do not meet the critical mass required for analysis. This is because when the samples are put into solution, the concentrations of elements in and on the lichen tissue must be enough that when the tissue is in solution the elemental concentrations are above detection limits; this varies depending on the type of analysis. With ICP-AES, which is being used in this study, critical mass is commonly at least 150 mg; 150 mg (Bargagli et al. 2000), 200 mg (Yenisoy-Karakaş and Tuncel, 2004). Critical mass for instrumental analysis is highly dependent on the method and the instrument, so it is superfluous to review all different options.

Lichen biomonitoring research commonly examines large scale environmental pollution gradients using low density sampling designs, such as 1 site per 256 km^2(Jeran et al. 2007); 47 sites in 65.9 km^2(Bennett and Wetmore 2003); and 8 sites to cover 10,000 km^2(Helena et al. 2004). These designs depend upon the specific research goals which can include: 1) Correlation of different tissue types (Bargagli et al. 2002; Bargagli et al. 1987), 2) Establishing baseline values (Bargagli et al. 2000), and 3) Looking at temporal changes (Bennett 1995), among others. No study has yet had a research goal of understanding very local variability, which is being defined by this study as a single tree.

Sampling site placement commonly uses one of three methods. 1) Grid method: section the sampling region into grid cells and collect a sample from each grid cell (Kubin et al. 1997; Jeran et al. 2007; Bargagli et al. 1987). 2) Transects from a point source: determine the most likely pollution source and sample along transects from this central point (Sensen and Richardson 2002; Barclay-Estrup and Rinne 1979). 3) Random sampling pattern (Bennett and Wetmore 2000; Bennett 1995; Bennett and Wetmore 2003; Bargagli et al. 2000). Each sampling pattern will have its benefits and draw backs, but they are typically designed to cover regional areas and not a small study area such as this.

Current literature has rarely examined the small scale variability within a local area (Rossbach and Lambrecht 2006). This is likely because lichen biomonitoring programs are paired with other pollution analysis techniques such as floristic studies (Wetmore 1990, 1993), so they are not the only determinant on the health of the environment. As well the economics behind biomonitoring programs will always be a concern; lichen biomonitoring has two main costs including the financial costs of analyses, and the cost of people to conduct the collection, preparation, interpretation, etc.

Researchers must choose a balance of spatial coverage and sampling density to fit their intended goal. To accomplish this, researchers choose to use composite samples of lichen tissue from many specimens to produce a sample. This approach has been successful at identifying trends. Sensen and Richardson (2002) were able to identify that a chlor-alkali plant had a 2.4 – 3.4 km sphere of influence for elevated levels of mercury deposition using *Hypogymnia physodes*. Bennett and Wetmore (2003) analyzed data collected from 4 lichen species over a 15 year period and found that Al, Cr, Fe, Na, Ni, and S had increased in tissue concentration during the study period, Cu, K, P, Pb, and Zn had decreased, and Ca, Cd, Mg, and Mn were constant. Research using *Xanthoria parietina* in Veneto (NE Italy) involving the collection of 200 composite samples over an 18 364 km^2 study area was suitable for the selection of high-risk areas that should be monitored by instrumental monitoring (Nimis et al. 2000). Countless more studies exist where general patterns have been successfully determined both spatially and temporally using the above mentioned approaches.

This work is intended to try and help researchers understand what very local variation can occur to help improve accuracy and design for monitoring programs. By understanding the local site variation in tissue concentration it may lead to the potential of more quantitative comparisons between locations with lower margins of error.

2.6 Potential for Local Variation

Lichens are of course biological organisms, and as with all organisms we tend to see variation among specimens. Researchers recognize this and choose to use a technique of picking samples and bulking them together. The relevant question which is not being addressed though is what type of variation can be expected because of the biological variability in lichens. In this section a few possible causes for a variation in elemental accumulation or concentrations within lichens are identified.

Lichens growing under stressing conditions such as poor nutrient availability will have a reduced ability to photosynthesize the available light. It is suggested that this may have an effect on trace element accumulation (Adamo et al. 2007). Furthermore, depending on where samples are collected, either branches or the trunk of a tree, there are statistically significant differences in Ca, Cr, Mg, Pb, Hg, and K tissue concentrations (Adamo et al. 2008).

Naturally occurring lichen substances play a role in the ability for uptake of different elements. When acetone was used to extract the natural lichen substances from *Hypogymnia physodes* a significant increase was seen when those samples were exposed to Cu^{2+} and Mn^{2+} compared to a control group. No differences were seen for the uptake of Fe^{2+} and Zn^{2+}(Hauck 2008).

Lichens are well documented as having a good correlation between elemental concentration in the lichen and the concentration of that element within the substrate (Prussia and Killingbeck 1991). It was shown that N, K, Ca, Cu, Fe, and Zn concentrations were significantly different within a single species of lichen when comparing thalli from two different trees of the same genus, *Quercus alba* and *Q. borealis*(Prussia and Killingbeck 1991).

It is suggested that species growing attached to their substrate with rhizines need particular care in which section is sampled and that it be consistent, as they only uptake elements in dissolved form and on the exposed surface (Rossbach and Lambrecht 2006). As well it is found that zones of element concentrations occur within the lichen and that the section sampled must be the same between sites, i.e. if the outermost 3 mm are sampled at one site, it should not be compared to studies which sampled the entire thallus (Adamo et al. 2008; Ayrault et al. 2007).

When a lichen transplant study conducted with *Evernia prunasti* had tissue concentrations of Ti, V, Cr, Co, Cu, Zn, Rb, Cd, Sb and Pb tested, it was found that when transplants exposed to rain were compared to those that were not exposed to rain Cr, Cu, Rb, Ti V, and Zn concentrations were significantly higher and Pb was significantly lower. The angle at which samples were positioned was found to be statistically significantly different for all elements, but Pb was determined be the only one with biological significance, with a difference of tissue concentrations greater than an order of magnitude between the horizontal and vertical positions(Ayrault et al. 2007).

Little data are available for concentrations of trace elements in lichens collected at different heights in a local area. A study conducted in the urban environment with transplants of *Psedudevernia furfuracea* at heights of 3, 6, 9, and 12 m above the ground, found that no significant difference occurred for Cd, Cu, Ni, and Zn, but that a statistically significant increase in Pb tissue concentration occurred in the transplanted lichens with increased height (Pirintsos et al. 2006).

Local variation has been identified as a concern with biomonitoring programs (H Wolterbeek and Bode 1995), and the need for further study is apparent by the lack of success in species correlation studies (Folkeson 1979; Yenisoy-Karakaş and Tuncel 2004a; Sloof and Wolterbeek 1993). The above literature suggests the potential for very local (<10 m) variation, but no study has quantified the variation.

2.7 Field Sampling Method

Field sampling technique is very important to ensure that lichen samples are not contaminated. Standard protocol in the field is to utilize plastic or ceramic tools as they should not contain any trace metals (Bergamaschi et al. 2004; Bergamaschi et al. 2002). After the collection, samples are stored in polyethylene baggies with a sample number indicated on them (Lupsina et al. 1992; Richardson et al. 1995; Poblet et al. 1997). Researchers do often use stainless steel tools (Sloof and Wolterbeek 1993; Bennett 1995; Sensen and Richardson 2002; Bargagli et al. 2000). These tools contain iron and may contain chromium, manganese, titanium, molybdenum, and other metals that could cause contamination issues. Following field collection, lichens are stored in a freezer until further processing occurs (Rossbach and Lambrecht 2006; Pfeiffer and Barclay-Estrup 1992)

Lichen size is a variable often measured as a surrogate for lichen age (Zschau et al. 2003; Samecka-Cymerman et al. 2006; Yenisoy-Karakaş and Tuncel 2004b) and therefore similarly sized specimens will be collected. Lichens have been found to exhibit growth patterns which can be highly variable and correlated with climatic conditions (Armstrong 2009), which suggests comparing equally sized lichens from different regions with different climatic regimes may be ineffective. Lichen age is considered important because it may be a factor in the accumulation potential as older lichen parts have been shown to have higher concentrations of elements (Adamo et al. 2008).

Bargagli and Nimis (2002) produced a ten part list for taking samples and it is summarized into:

1. Use fruticose or broad-lobed foliose species only;
2. Samples should be collected from tree bark because the substrate should be homogeneous, epiphytic species;
3. Use one species;
4. Minimize sampling period and not following heavy precipitation;
5. Trees must have an inclination less than 10°, no signs of disturbance, no surface flow tracks, no sampling near back wounds, and minimal bryophyte growth;
6. Sample all aspects, unless study is scoped to a particular aspect;
7. Sample 1 m above ground, reduce terrigenous and animal contamination;
8. Detach with a steel knife, and use metal-free filter paper;
9. Use six individual thalli from three different trees to get an average condition; and;
10. Record location, tree and lichen species, diameter at breast height, diameter of lichen, lichens visual health, and soil type and land use.

2.8 Trace Metal Analysis

Lichens must be cleaned of extraneous material prior to any chemical analysis; the extraneous material often includes bark, other lichens, seeds, and soil particulate matter. This step is commonly done by either removing the material under a microscope with tweezers (Di Lella et al. 2003; Loppi et al. 2003; Frati et al. 2007), or by washing the lichen in water (Adamo et al. 2007; Cercasov et al. 2002). Studies have found that washing can leach out the entire concentration of certain elements when pre- and post-washing tissue concentrations were analysed (Adamo et al. 2008; Adamo et al. 2007).

Lichen dry weight must be taken in a reliable manner because lichens rapidly absorb moisture that is in the air (Quevauviller et al. 1996).

Sample preparation for elemental analysis is similar between studies. First the samples are dried, either by air drying (Bargagli and Nimis 2002), or by oven drying

(Adamo et al. 2007; Adamo et al. 2008). The oven dried samples are powdered, by either using a mortar and pestle (Brunialti and Frati 2007), a Wiley mill (Chiarenzelli et al. 1997) or a ceramic knife (Adamo et al. 2007). The powdered lichen material is weighed and digested in acid with heat applied. Acid combinations vary in concentration but often include all or parts of concentrated HNO_3 (Bargagliet al.1987), HCl (Bermudez et al.2009), HF (Baptista et al. 2008) and H_2O_2 (Baffi et al. 2002). The samples are sometimes heated in a heating block (Garty et al. 2002), or more commonly in a microwave (Purvis et al. 2004). The cooled samples will be brought up to volume with distilled deionised water to the concentration necessary for the particular instrument being used.

Analysis of trace elements, when focused on metal in particular, relies on spectroscopic techniques. All spectrometers work similarly; they assess either the concentration or presence of a given chemical species by examining the radiation emission (Kealy and Haines 2005). Each spectrometer must be coupled with a heat source which excites the ions. Resolution of the instrument is the most important factor when examining trace metals because common tissue concentrations are a few µg/g or lower (Conti and Cecchetti 2001).

Three very common spectrometers for trace element analyses include: 1) Atomic absorption spectroscopy (AAS) (Bermudez et al.2009; Rossbach and Lambrecht 2006; Scerbo et al. 2002; Quevauviller et al. 1996; Bergamaschi et al. 2007), 2) Atomic emission spectroscopy (AES) (Purvis et al. 2004; Rossbach and Lambrecht 2006; Rusu et al. 2006; Garty et al. 2002; Berlekamp et al. 1998), and, 3) Mass spectrometry(MS)

(Basile et al. 2008; Kylander et al. 2007; Frati and Brunialti 2007; Rossbach and Lambrecht 2006).

AAS can examine 50 elements and is coupled with a flame or graphite furnace to volatilize the sample. The heat source is used to separate the molecules into their atoms, but not ions. Detection limits are sufficient, e.g., parts per billion or better for most elements. AAS requires a reference sample of the element to be examined, and can only examine for one element at a time (Kealy and Haines 2005). The inability to test for multiple elements at one time reduces the value of this spectrometer for trace metal analysis, because studies commonly examine 10 or more elements (Yildiz et al. 2008; Bargagli et al. 2000; Bennett and Wetmore 2003).

AES and MS techniques for trace metal analysis are coupled with an inductively coupled plasma (ICP) torch. A gas is heated to temperature up to 10000 K and will form plasma (a gas containing a high proportion of electrons and ions). The plasma breaks apart the material being examined into its elemental form. If the ionic state of the elements is to be determined a MS must be used for the analysis, because AES only determines the elemental concentration. Detection limits for both AES and MS are in the parts per billion range, with MS having average lower detection limits. MS and AES can both simultaneously test for up to 70 elements in one sample. ICP-MS tends to be more costly than ICP-AES, but has the ability to detect ionic state (Kealy and Haines 2005). The ability of AES and MS to determine multiple elements per sample is possibly the greatest benefit over AAS.

Lichen certified reference material has been developed as a quality assurance tool for biomonitoring research to establish the accuracy and precision of analytical techniques; this material is collected in large bulk samples of around 40 kg and powdered. The fine powder is mixed for multiple weeks to create one homogenous powder. This homogenous powder is highly reproducible in elemental concentrations when tested with an array of techniques and instruments (Stone et al. 1995; Quevauviller et al. 1996; Freitas et al. 1993). Researchers can compare their analytical results of the lichen reference material to the certified values to establish percent recovery, precision and accuracy.

Analytical errors which can occur include contamination, errors in result reporting and improper analytical procedures; errors will always be present to some degree. Minimizing these errors can be done through the use of non-contaminating equipment such as plastics and ceramic tools (Bergamaschi et al. 2004; Rizzio et al. 2001), handling material with disposable gloves (Bargagli et al. 2002; Frontasyeva et al. 2004), and working in a clean and organized facility. These principles, when followed, have been shown to significantly reduce any analytical errors that can occur (Stone et al. 1995).

2.9 Data Analysis

Data analysis commonly uses two general methods. One is the mapping of the concentrations to explore patterns (Yenisoy-Karakaş and Tuncel 2004a). The maps can be easily interpreted to see where the highest concentrations were found. These can be effective visual aids, but are really limited to one element per map. The second method is statistical analysis of the dataset, which is often used as the primary method for interpretation. Some of the methods used include regression modelling (Sensen and Richardson 2002; Bennett and Benson 2005), to try and understand an explanatory relationship between variables and concentrations. Many different methods of significance testing are often applied, which include analysis of variance tests (ANOVA) (Bermudez et al. 2009; Helena et al. 2004; Carreras et al. 1998) to examine differences between conditions, e.g. do the lichens transplanted in the city have a higher concentration in *x* than the control group? An ANOVA is an extension of the Student's T Test, which is used for testing of multiple groups; the T test is often applied when only two groups (conditions) occur (Moreira et al. 2005; Godinho et al. 2009). Correlation statistics are often applied when trying to relate concentrations between lichens and other organisms such as the substrate, moss or lichen species (Gombert et al. 2003; Ugur et al. 2004; Carreras et al. 2009; Frati et al. 2007).

Multi-element biomonitoring programs produce large sets of data points, commonly greater than 1000 (Bennett and Wetmore 2000; Bennett and Wetmore 2003). These large datasets need to be treated as multivariate because each unit has multiple observations, e.g. each elemental concentration, position, size. To properly handle this type of data, multivariate statistical approaches need to be applied along with univariate ones because they can reveal information about the data that testing only one observation at a time cannot, such as common origins of elements. Cluster analysis and principle components analysis are common methods employed to define groups of elements in a set of data which can then be traced back to common origins that can include anthropogenic, and local geologic conditions (Zschau et al. 2003).

2.10 Placement within the Current Literature

This research project is designed to better understand what, if any, variation is occurring in accumulation of trace toxic metals in lichens. There is thought to be local variation because within the lichenological literature there are many suggestions of biological processes that may be causing variability on a small scale, as was highlighted in Section 2.6 Potential for Local Variation. If successful, this research will help guide future studies in choosing the suitable number of samples required to capture this variation at a single sampling site.

In conclusion, lichen biomonitoring is currently an inarguably valuable tool for the qualitative identification of toxic metals in the air, i.e., if they are present in the air you will find them concentrated in lichens. This research will help build the framework to allow quantitative analysis between different locations by understanding what type of variability will occur within a localized area, and allow better judgement if other sites are significantly different because of an environmental gradient, and not just natural variation.

CHAPTER 3

METHODOLOGY

Note:
Specimen refers to individual lichen removed from the tree.
Sample refers to specimens that have been grouped in order to have enough material for the analytical procedure.

3.0 Introduction

Trees are the most common lichen substrate used in lichen biomonitoring studies, but as noted in section 2.6, little research has occurred to determine the trace elemental accumulation variability between samples collected in a small area. This methodological approach is designed to better understand the trace elemental accumulation variability in a relatively unpolluted sampling site on a very local scale, an individual tree.

A census approach was used in the field collection of the lichen specimens, opposed to a sampling approach. A census removes one major concern in sampling design; that each sample has the same chance of being selected (Bargagli and Nimis 2002).

Usnea subfloridana Stirt. is used as the lichen biomonitor species. This species was chosen after numerous preliminary field studies did not turn up enough *Hypogymnia physodes* (L) Nyl., which was used in a previous regional survey (Pfeiffer and Barclay-Estrup 1992). Following the preliminary field surveys a sample site was chosen northeast of the City of Thunder Bay, Ontario, Canada. The sampling site was about 25 km Euclidean distance from the city. Universal Transverse Mercator coordinates are Zone 16 N, 345805 m E and 5385358 m N. See Appendix I for map. *U. subfloridana* fits the suggested criteria by Bargagli and Nimis (2002) to use either fruticose or broad-leafed foliose species; *U. subfloridana* is a fruticose species and was highly abundant in the study area. The study area was dominated by *Abies balsamea* (L.) Mill. (balsam fir), those were very similar in size to the study tree, which was around 60 years of age. All trees had a similar growth pattern of lichen on them, with *U. subfloridana* being a dominate species. The study tree chosen appeared upon investigation to be typical for this area, with *U. subfloridana* typically ranging up to about 15 cm in un-stretched length. Most specimens in the area were about 3-7 cm. Other common lichen species were *Parmelia sulcata*, *Evernia mesomorpha*, and *Bryoria* spp. At the time of year when sampling occurred there was little undergrowth with most trees far exceeding 10 m.

The four main stages which occur in the methodology are: 1) Conduct field work and collect samples; 2) Prepare samples for analytical analysis; 3) Conduct analytical analysis; and 4) Statistical analysis of trace element concentrations.

3.1 Field Collection

3.1.1 Pre-Sampling Conditions

Weather data collection is highly dispersed in the study region, with no known data collected near the sampling site. A PortLog Weather Station was set-up in the field on April 21, 2010. The weather station was three metres from the study tree. The main sensor unit is about two metres from the group when set-up. It is a self-contained unit which is pole mounted and subsequently mounted to a tripod. The station was placed in the center of a treeless gap that was about 2-5 m from the nearest tree. This data should be representative of the conditions that would be affect the lichen at a similar height on the sampled tree as there were no major obstacles around the area and the forest was not dense at this level as all trees were mature with no low branches.

All of the PortLog sensors are traceable to NIST. Data used for analysis of weather conditions began at 12:00am on April 22, 2010. Data were recorded every 20 minutes. Some variables were logged as an average, max, or cumulative value during each 20 minute interval; explanation of each variable measured is located in Table 3.1. Logging occurred until May 31st, 2010 at 12:00pm. Data for this same period was downloaded from Environment Canada's Historical Weather Database, Thunder Bay Station A (Environment Canada 2011). The important weather parameter recorded was wind direction. The data indicate that at our study site wind blows from two primary directions, the northwest and the southeast. The mean direction is from the northwest, see Figure 3.1.

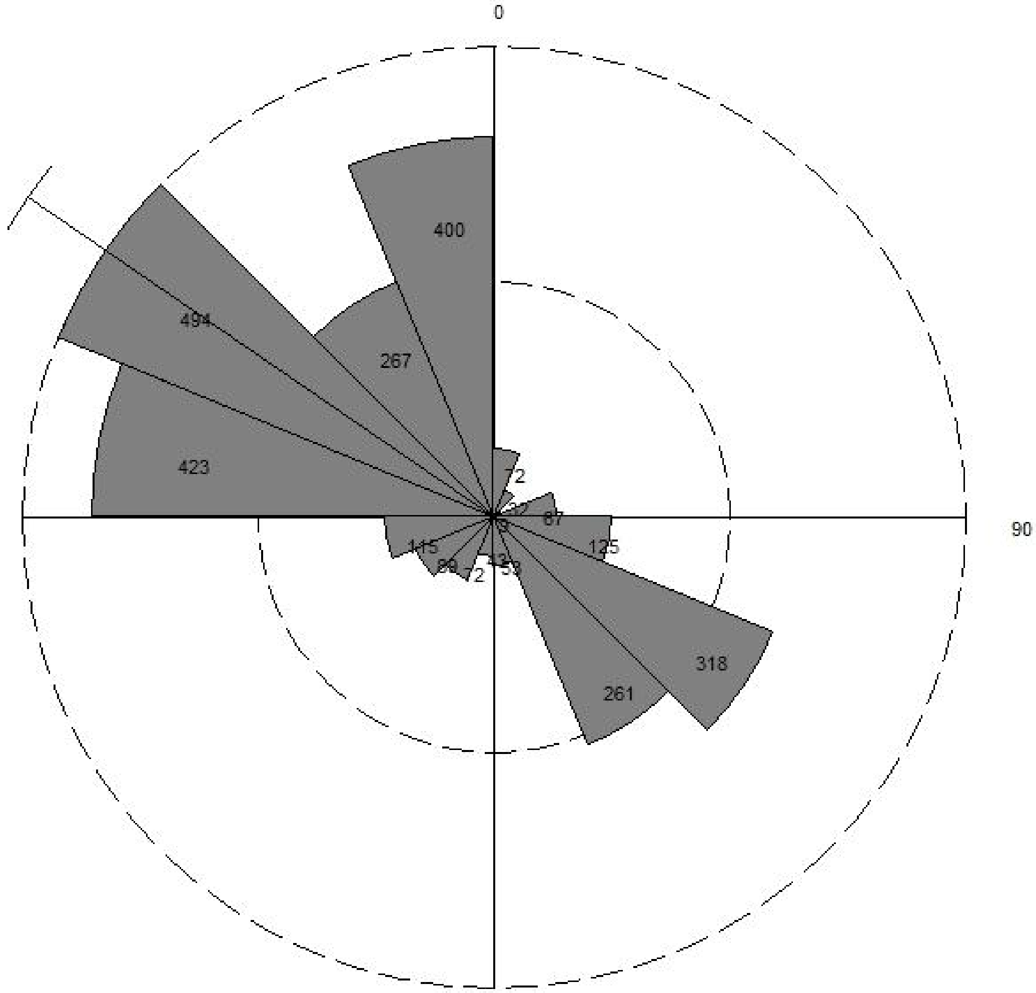


Figure 3.1 Field Site Wind Direction Plot
Plot of wind direction data collected at study site. Numbers within each section indicate the number of observations within that range. The line with a T top indicates the mean direction. North is located at the 0 mark of the plot.

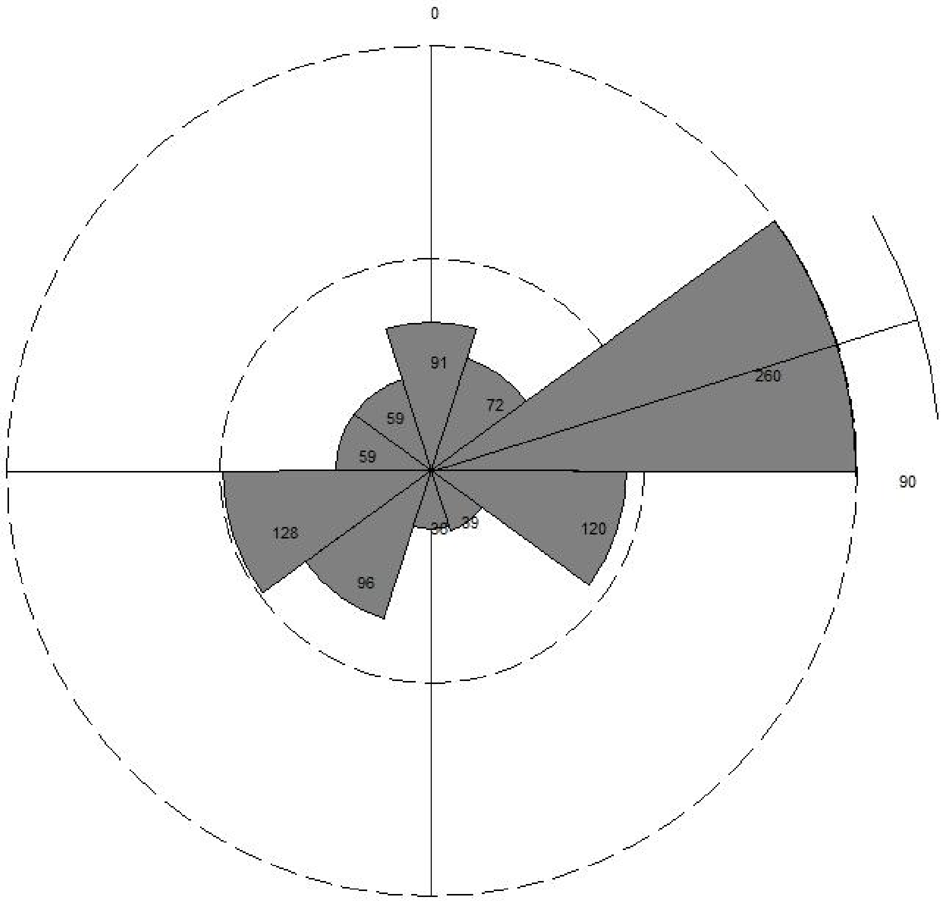


Figure 3.2 Plot of Environment Canada Wind Direction Data
Plot of wind direction data collected by Environment Canada for Thunder Bay, ON, Canada. Numbers within each section indicate the number of observations within that range. The line with a T top indicates the mean direction. North is located at the 0 mark of the plot.

Table 3.1 Weather Parameters Collected

Variable Measured	Type of Logging	
Air Temperature	Average	Range: -54° to + 65° C. Accuracy: ± 1.0 °C.
Humidity	Average	Range: 0 – 100% Accuracy: ± 2% from –40° to +65° C.
Dew Point	Average	Range: -40° to +43° C. Accuracy: ± 2° C.
Barometric Pressure	Average	Range: 300 to 1100 millibars (hPa) Accuracy: ± 0.5 millibars (hPa) @ 25°C *temperature compensated from –40° to +85° C.
Wind Direction	Average	Range: 0-360 with 1 degree resolution. Accuracy: ± 3°
Wind Speed	Average	Range: 0 – 67 meters per second. Resolution: 1.0 unit. Threshold: 0.5 meters per second Accuracy: ± 2% of full scale
Maximum Wind Speed	Maximum	Same as Wind Speed.
Solar Radiation	Average	Range: 0 to 2, 000 watts per square meter Response: 400 to 1, 200 nanometers Accuracy: Maximum ±5.0% - typical ±3.0%
Solar Radiation Sum	Cumulative	Same as Solar Radiation
Rainfall	Cumulative	Range: Unlimited tipping bucket with203.2 mm dia. collector. Resolution: 0.25 mm. Accuracy: ± 2% @ 1.0 inches per hour.

3.1.2 Field Sampling

Field collection was conducted on May 4, 2010. All specimens were collected within 12 hours. Specimens were collected from an *Abies balsamea* (L.) Mill. (balsam fir). Nomenclature for tree identification was Trees of Ontario (Kershaw 2001). The tree, which the land owner had slotted for fire wood was cut down the previous day. To safely fell the tree a guide rope was necessary. To attach the rope we used a ladder against the tree. This position was marked and there was a non-collection buffer of 20 cm above and below that position. The tree was guided to rest perpendicular to a previously felled tree; this was beneficial so lichen specimens did not touch the ground. Tree height was about

17 m. North aspect was indicated on the tree prior to cutting it down at both the base and at 3 m. On the ground four guide lines were run along the tree, representative of each cardinal direction. These lines were run the entire length of the tree.

Lichens were collected from the tree trunk only; branch samples were not included in the collection. Sampling started on the section of the tree which was 1 m above the ground on the erect tree.

Each collected specimen's height and aspect were recorded. Height was measured in 1 dm collars. Lichen aspect was recorded to the nearest of 16 directions, see Figure 3.2 for the 4 cardinal directions, 4 ordinal directions, and 8 further divisions used.

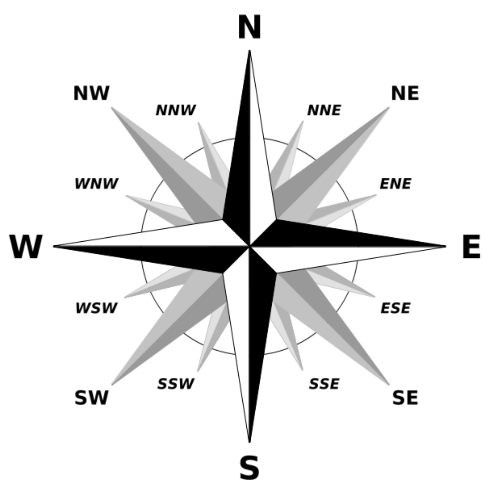


Figure 3.3 Compass Rose
Compass rose with the 16 directions used when recording lichen aspect.

Any specimen similar in appearance, in the field, to the target species of *Usnea subfloridana* (Brodo et al. 2001) was collected. Specimens were collected by inverting a polyethylene bag over the collector's hand without touching any part of the inside of the bag. With the bag over top of the collector's hand, the specimen was grabbed firmly at the base and with a quick pull removed from the bark substrate. The bag was inverted to its normal configuration and sealed. Bags were labelled with a Sample ID written in permanent marker, and specimens were stored in larger polyethylene bags in groups of one hundred. Specimens were placed into a freezer for storage at the end of the field collection day until laboratory processing.

3.1.3 Data Recording

In the field, data were recorded on field sheets. These data were transferred into a Microsoft Excel© workbook. Each datum input was cross checked twice after input.

3.2 Sample Preparation

3.2.1 Lichen Cleaning

During all laboratory procedures FischerBrand™ Nitrile gloves were worn and samples were handled with plastic tweezers. In the laboratory each sample was examined using a dissection microscope. Each lichen specimen was examined for a blackened base, which is one of the important characteristics of *U. subfloridana*. Also, each sample was examined for a positive reaction to a UV light on the medulla tissue which is reaction that occurs because of squamatic acid in the lichen medulla (Brodo et al. 2001). All work performed under the microscope was done in a glass dish, which was wiped out between samples. Chemical spot tests for appropriate acids were not conducted because of the increased risk of contamination and deterioration of the lichen material.

Lichen specimens collected from the research tree, but above the collection area on the tree, were identified to species by myself and then verified by Erika North, Curator of the Claude E Garton Herbarium at Lakehead University. These samples were all identified to be *Usnea subfloridana*.

Extraneous material was removed from each sample, which included parts of other lichens; commonly *Bryoria sp., Evernia mesomorpha, Hypogymnia physodes* and *Parmelia sulcata*, and other material such as seeds, small plant needles, and bark. Dead lichen material was removed from the specimens at this step as well. This dead material was easily identifiable as it was a much darker colour and easily pulled off the specimen.

During the cleaning procedure it was common to find that multiple specimens had been collected simultaneously. These multiple-specimen samples were divided and adjusted in the data set; since they were collected at the same location it did not cause any problems for data loss, as the data could just be duplicated. Cleaned samples were frozen until weighing.

3.2.2 Lichen Weighing

Each lichen specimen was air dried in open plastic sorting boxes for at least 24 hours. After the air-drying, the specimens were put in desiccators for 24 hours. Samples were processed in 48 sample batches (batch), each processing step occurred in under one hour. Loading four sorting boxes with 48 samples took about 30 minutes. Loading sample boxes into desiccators was performed in approximately one minute. Weighing samples lasted about one hour; 15 minutes per sorting box. The sorting box that was loaded first was weighed first to minimize differences in drying times. Specimen weight was measured with an Ohaus Adventurer® Analytical Balance with a reliability of 0.1

mg. A Petri dish was placed on the scale to eliminate the lichen touching the balance directly, and wiped clean between uses.

During the weighing process relative humidity (RH) and temperature (T°) of the laboratory were recorded often during the processing with a Fischer Scientific Mason's Hygrometer. No major fluctuations in humidity occurred during the processing period.

3.2.3 Sample Group Delineation

Following drying it was determined that most specimens did not have the critical mass to be individually tested. Average specimen weight was well below the required analytical procedure material weight of 250 mg. To alleviate this issue, specimens were combined into groups (Samples). A method was employed to try and minimized the difference in what were determined to be critical factors that were able to be measured. It must be noted that ideally individual specimens be used but the low tissue weight for the specimens prevented this. Prior to group delineation, all specimens weighing over 250 mg were removed from the data set because they could be individually tested; see Appendix II for a list of the specimens which were tested individually.

Research indicates that lichens selected in biomonitoring should be collected within a one metre height, because there is a potential variability due to height on the tree. As well, wind direction is thought to influence accumulation (Bargagli and Nimis 2002). Finally it is said that lichen age can play a significant role in accumulation, and that older lichens, commonly determined by size, are likely to have the highest accumulation (Nimis et al. 2001).

With those concerns under consideration, specimens were divided by first splitting the data set into two groups based on the wind data collected at the site. Even though the data set is shorter temporally compared to available data from Environment Canada, it has a poor agreement to that data collected for the Thunder Bay region (Figure 3.1 vs. Figure 3.2). Since there is poor agreement it would not make sense to utilize historic data for the Thunder Bay region when it is shown that the site, for at least the time measured, is not affected by the same wind patterns. These data are not representative of the historic wind patterns for the study site, but they are the only data which are known to be correct for the area in the timeframe leading up to sampling, so they were utilized. This decision was made because this work is not concerned with where potential trace elements are sourced, but is focused on trying to determine the potential variability with concentrations of trace elements in and on lichen tissue.

The two groups included: a) Group south which included all lichens specimens with an aspect between 67.5º and 255º; b) Group north included all lichens specimens with an aspect between 247.5º – 45º. This division of groups was based on the two main wind directions from the local weather station data. Next specimens were sorted into 5 dm collar groups; i.e. collars 1-5, 6-10, and 11-15 were each a respective group. Within each 5 dm collar groups the lichens were sorted by weight, and starting with the heaviest specimens, and were combined until a sample's total weight reached at least 250 mg. Preliminary tests found 250 mg to be the minimal amount of material required for the next steps.

This method produced samples from specimens which were similar to each other based on two major aspect groups based on wind data collection at the site, minimized the variation in height within samples to less than 50 cm, and finally within the 50 cm regions minimized weight differences. As well any sample which had the critical mass for analytical analysis was individually tested, and by grouping starting with the heaviest samples it reduced the number of heavier samples which were required to reach the critical mass.

3.2.4 Sample Preparation

Lichens were cut into small particles by hand with a ceramic knife, a process which takes about 10 – 15 minutes per sample. Commonly, researchers use a Wiley mill (Chiarenzelli et al. 1997; France and Coquery 1996; Bennett and Wetmore 2000; DHS Richardson et al. 1995; Bennett and Benson 2005), but that was not possible because of the low initial lichen material and too much lichen material was lost in the milling process. The ceramic knife technique is suitable as it produces a homogenized powder (Chiarenzelli et al. 1997; Loppiet al. 1998b), which is important in many studies that digest only a portion of one more thalli (Loppi and Bargagli 1996). This was not a concern because each sample was almost completely used in the digestion.

3.3 Analytical Method for Trace Metal Analysis

3.3.1 Preparation

Analytical procedures were performed in the Lakehead University Environmental Laboratory (LUEL), which is ISO-17025 accredited for Water [Alkalinity, pH, Conductivity, Nitrogen, Phosphorus, and Total Suspended Solids] and Soil Nutrient Analysis [pH, Ca, K, Mg, Na, P, NO3-N, Mn, and Zn]. LUEL uses a custom designed Laboratory Information Management System (LIMS), which stores all data in a database, and data are input into the system through the instruments, opposed to transcribing results and then entering them into the computer database. This methodology is followed to reduce transcription errors.

Inductively coupled plasma – atomic emission spectroscopy with a microwave assisted digestion was used for trace metal analysis of total recoverable Al, As, Ba, Be, Ca, Cd, Co, Cr Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sr, Ti, Tl, V, and Zn. Microwave digestions appear to be the current standard with most analyses because of the benefit of efficiency (Baffi et al. 2002; Moreira et al. 2005; Mendil et al. 2009; Scerbo et al. 2002; M Bettinelli et al. 2002; Adamo et al. 2007).

Cut samples were dried in a 100 °C oven for at least 12 hours to assure dry weight and were weighed into the vessel and reported in the final values. After drying, lichens were kept in a desiccator until just prior to weighing.

Each sample was weighed to about 200 mg using a Denver P403 balance with a reliability of 0.1 mg, the material was weighed onto FisherBrand^{TM} Polystyrene Antistatic Weighing Dishes. The weight was recorded directly into LIMS from the balance.

Weighted samples were poured into CEM MARSXpress™ PFA Teflon® vessels, which are pressure vessels and designed for temperatures up to 260ºC.

In each vessel 3 mL of FischerBrand^{TM} Trace Metal Grade concentrated HNO_3 was added with a Brand Tech Scientific Dispensette III Bottle-Top Dispenser, and was left to sit overnight for at least 18 hours. Then 1 mL of FischerBrand^{TM} Trace Metal Grade concentrated HCl was added with a Brand Tech Scientific Dispensette III Bottle-Top Dispenser to the vessels and left for three hours.

Microwave digestions were done with the 3 mL HNO_3 and 1 mL HCl solution, and 200 mg lichen sample. A CEM MARSXpress, closed vessel Acid Digestion – MARS System was used. This instrument processes up to 40 samples simultaneous and rapidly monitors the temperature of each vessel using two highly sensitive IR internal temperature sensors. These two sensors are NIST traceable.

Microwave program:

Step 1 – 25 minutes ramping to 180°C

Step 2 – Hold at 180°C for 25 minutes

Step 3 – Cooling cycle.

During most runs the MARS system was run at full power, 1600 watts. On the final run, which was about half the normal number of samples, the power was decreased to 800 watts. The program ran in the same manner and reached 180 °C in the same manner as the full power method.

When the vessels cooled down below 40 °C they were opened and 1.5 mL of H_2O_2 were added with a FischerBrand™ Digital Single Channel Finnpippette. This addition caused a reaction lasting about 10 minutes and left the samples clear, opposed to the red-yellow colour they were following digestion.

When the H_2O_2 reaction was completed the samples were transferred to 50 mL Fischer Scientific Centrifuge Tubes and brought up to 25 mL with Type I distilled deionised water (Barnstead E-Pure Ultrapure Water Purification System with 18MΩ cm specific resistivity capability).

Vessels were cleaned in accordance to the manufacture's recommendations by first using a liquid detergent, followed with an acid rinse, and then by a triple rinse with Type I distilled deionised water.

3.3.2 ICP-AES Analysis

Trace element analysis was conducted on a Varian Vista Pro CCD Simultaneous ICP-AES with a CETAC ASX-510 Auto Sampler. This process was performed by a technician from the Lakehead University Environmental Laboratory. All instrument parameters are found in Appendix III.

Raw data results were recorded and entered in LIMS. These raw results were processed with the following calculation to determine sample concentration:

$$concentraion\ \left(\frac{mg}{kg}\right) = raw\ conc. \left(\frac{mg}{L}\right)\left(\frac{final\ volume\ (mL)}{sample\ weight\ (g)}\right)(dilution\ factor)$$

Final results are reported as mg/kg dry weight to 3 decimal places.

3.3.3 Quality Control and Assurance

Blanks and standards were run after every 11 lichen samples. Two standards were used to test for recovery, accuracy and precision. 1) Certified Reference Material BCR® - 482, a lichen powder certified by the Community Bureau of Reference, and 2) Standard Reference Material 1570a from the National Institute of Standards and Technology. Standards and blanks were processed in the same manner as all other samples. Accuracy, precision, and recovery were determined by following the methods outlined in ISO 5725-1,-2,-4.

Accuracy % Error = $\frac{MeanoftheStandard}{ExpectedValue} * 100$

Precision (Relative Deviation) = $\frac{StandardDeviationoftheStandard}{MeanoftheStandard}$

Recovery % = $\frac{ObservedStandardValue}{ExpectedStandardValue} x\ 100$

3.4 Statistical Analysis

Statistical analyses were conducted in PAST (Hammer et al. 2001) and R(R Development Core Team 2010).

Data were tested for normality with multivariate normality tests which included Mardia's multivariate skewness and kurtosis, and the Doornik and Hansen omnibus test.

Mardia's multivariate skewness and kurtosis test was developed on the *t*- statistic, but with extended studies on robustness (Mardia 1970). The Omnibus test is found to be a powerful test, which controls well for sample size in determining multivariate normality (Doornik and Hansen 2008). Since both tests are known to be powerful, any results indicating a shift from normality should be assumed to have a non-normal distribution. Both tests assume a normal distribution. A 95% confidence level is used; α = 0.05.

Shapiro-Wilk test was used to test each element individually for a normal distribution. A 95% confidence level is used; α = 0.05. The Shapiro-Wilk test's W test statistic is small when the data-set is not normally distributed (Shapiro and Wilk 1965).

A Two-Group multivariate permutation test was used to determine if the means of the two aspect groups had equality of means for elemental concentrations. This is a nonparametric alternative to the Hotelling's test, which is a multivariate analogue to the *t*-test. A 95% confidence level is used; $\alpha = 0.05$.

Mann-Whitney-Wilcoxon rank sum test with continuity correction test was used to determine which individual elements were significantly different between the two aspect groupings. A 95% confidence level is used; $\alpha = 0.05$. This test assumes the null hypothesis that both sample medians are from the same population.

Cluster analyses were performed using the paired group algorithm and the Euclidean similarity measure. Three cluster analyses were performed on the dataset, the first included clustering on the element data, the second on the attribute data and third included both attribute and element data.

Stepwise linear regressions were applied to evaluate if explanatory relationships exist between height on the tree and mean specimen weight, and trace elemental accumulation. Any variable significant at $p < 0.15$ was kept in the model. Models were created for both the north and south aspect groupings.

Spearman's Rho was used to explore correlations between all elements. This was done because correlations in elemental accumulation are suggestive to indicate a similar origin (Bargagli and Nimis 2002).

Principal components analyses were conducted to explore the relationships between the different elements when reduced to the major components. Data were normalized for the tests because of the magnitudes of difference between elements. Biplots were used to see how the element vectors aligned to the different axes.

CHAPTER 4

RESULTS

4.1 Qualitative information from sample processing

During the sample processing few samples required removal of dead material. Many samples were split into two, because two specimens had been collected simultaneously. Samples ranged from sterile in appearance to heavily sexually reproductive. In general, the lichens were longer than wide. Visually, samples appeared to all be *Usnea subfloridana* and were positive under UV light.

4.2 Weather Data Analysis

Weather parameter data measured between April 22 12:00 am and May 3 at 12:00 pm are displayed in Table 4.1. Basic descriptive statistics are included. Sampling length prior to collection was restricted by the ability of getting to the site and being able to set the equipment up so that it was not on ice or snow.

Table 4.1 Study Site Weather Data Descriptive Statistics
Descriptive statistics on the weather parameters collected at the field site prior to the collection of the lichen specimens.

	Air Temp	Humidity	Dew Point	Barometric Pressure	Wind Speed Avg.	Wind Speed Max.	Solar Radiation	Solar Radiation Sum	Rain Fall (mm)
Min.	-4.8	16	-13.4	936.50	0.00	0.00	0.00	0.00	0.00
Max.	21.2	96	9.90	960.50	5.80	19.80	1145.00	1350000.00	1.50
Mean	7.08	54.45	-3.01	950.56	1.96	5.81	93.06	113934.00	0.01
Std. Dev.	5.55	23.57	5.27	6.94	1.69	4.43	215.60	254717.00	0.08
Sum	N/A	N/A	N/A	N/A	N/A	N/A	N/A	9.84E+07	9.7

4.3 Lichen Non-Grouped Descriptive Statistics

Descriptive statistics of the attribute data for the ungrouped samples are presented in Table 4.2. Histograms were plotted for aspect (Figure 4.1), weight (Figure 4.2) and collar height (Figure 4.3).The weights of specimens were plotted against the height at which they were collected (Figure 4.4).

Table 4.2 Descriptive Statistics for Individual Lichen Specimens

	Number	Mean	S.D.	Median	Min.	Max.	Range	Skew.	Kurtosis	S.E.
Collar	1037	47.31	19.75	49	1	80	79	-0.18	-1.04	0.61
North	1037	0.08	0.72	0.11	-0.95	1	1.95	-0.06	-1.48	0.02
East	1037	-0.08	0.69	-0.23	-1	0.94	1.94	0.13	-1.36	0.02
Weight	1037	0.03	0.03	0.02	0	0.48	0.47	5.02	43.79	0

Lichen aspect mean, taking circularity into account, was 331º (95% Confidence 320º, 343º; Bootstrap 5000 mean replicates 319 º, 344 º). Rayleigh's spread *R* value was 0.1948 with a *p*-value of 5.6931×10^{-18}, indication of a non-normal or unimodal distribution.

Lichen Aspects

Aspect	Count
0	113
22.5	50
45	77
67.5	47
90	89
112.5	24
135	48
157.5	24
180	62
202.5	37
225	77
247.5	33
270	113
292.5	46
315	115
337.5	82

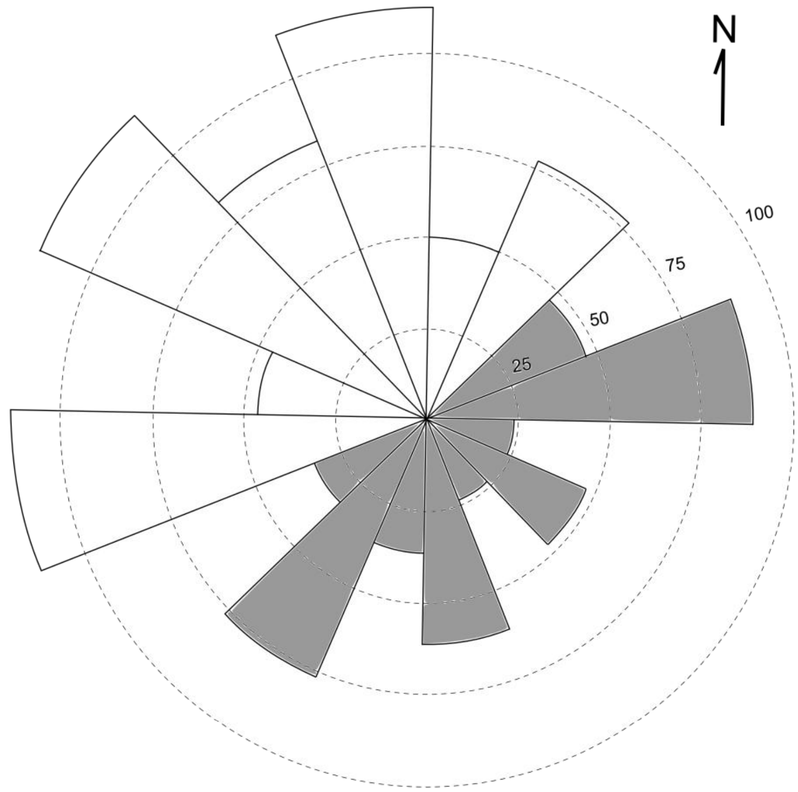


Figure 4.1 Circular Histogram of lichen aspects
A circular plot of the distribution of the individual lichen specimens collected in the field. The black background boxes are the specimens which were put in aspect group south. Lichens were well dispersed between the two aspect groups: South n = 408, North n = 629. Numbers located on the dotted circles indicate the count for each group. The groupings which are in the south group are shaded on the circular plot.

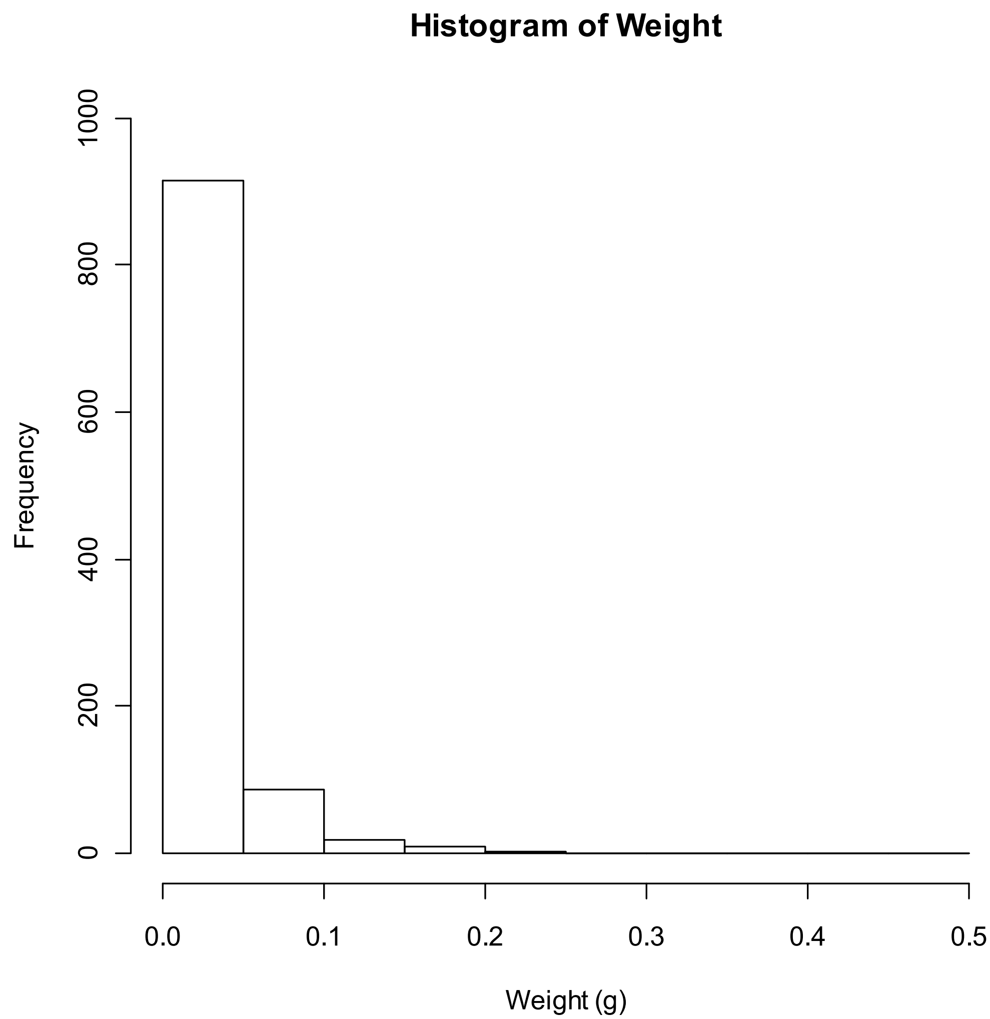


Figure 4.2 Histogram of lichen dry weight
Histogram of the lichen dry weight of the samples prior to them being grouped (n = 1037). Only three samples had enough material to be analysed individually.

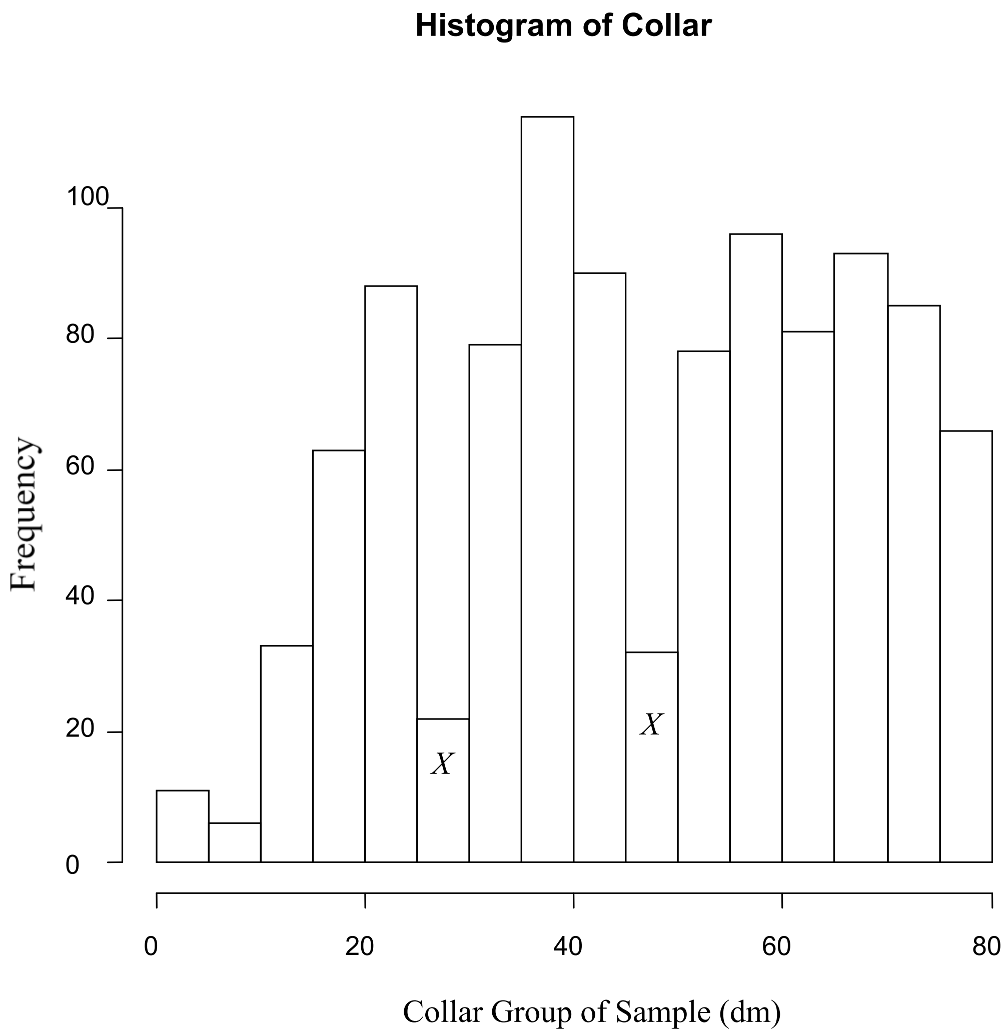


Figure 4.3 Histogram of Samples per Collar Group
The two lower bins marked with Xs correspond to the two areas on the tree which were not sampled because of inaccessibility.

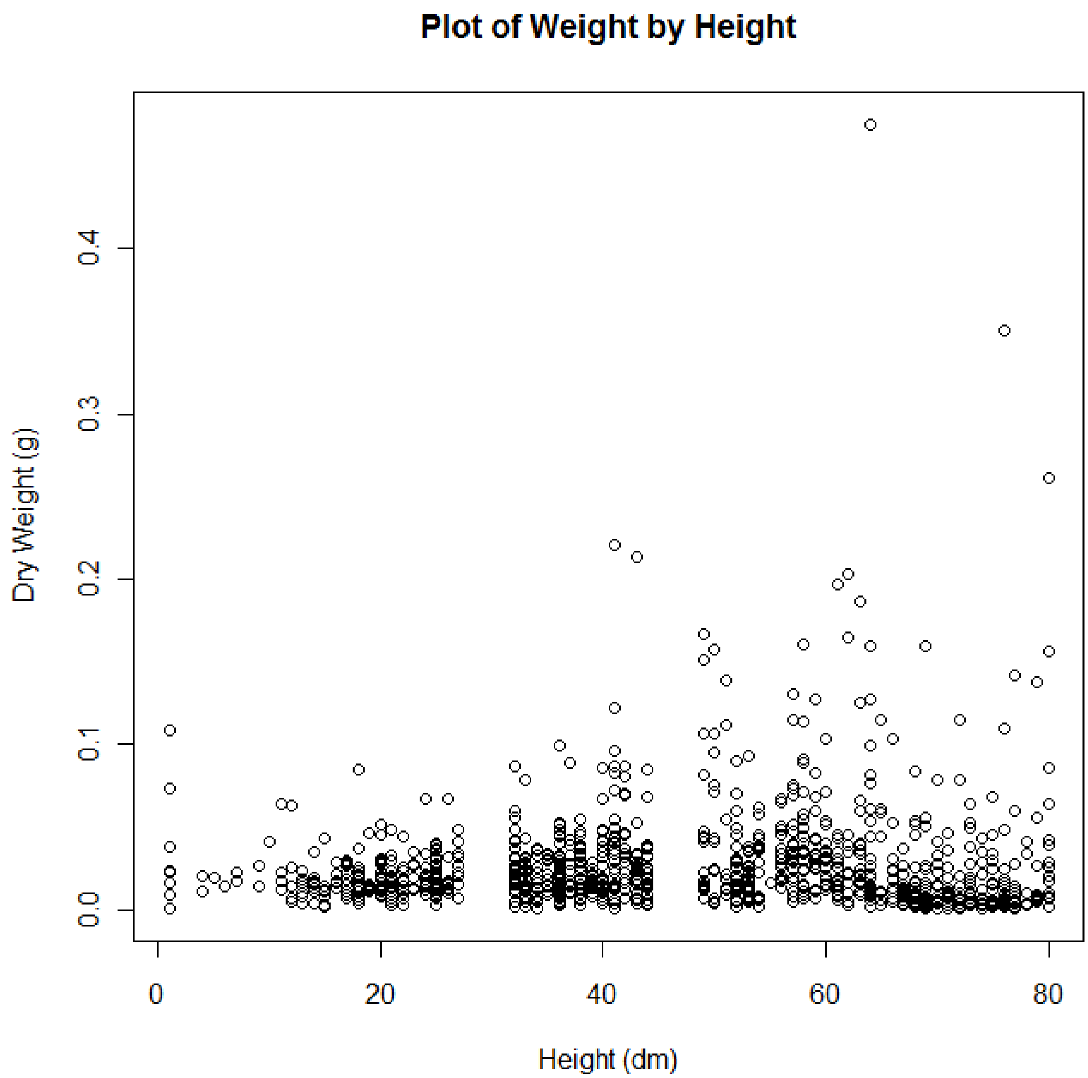


Figure 4.4 Plot of Specimens' Weights by Height of Collection
A linear regression model was insignificant ($r^2 = 0.003262$) for any relationship in changes in weight due to increased or decreased height on the tree. No non-linear relationships appear in the data. A trend appears in which specimens with greater biomass (>0.2 g) appear to occur only above 40 dm.

4.4 Quality Assurance

Accuracy, precision and contamination assessment are essential in lichen biomonitoring programs. To evaluate the quality of the analytical procedures blanks and standards were run every 11 samples. Blanks and standards were processed the same as the tested samples. All sample blanks were below detection limits for all elements analyzed.

Two standards were used to test for recovery, accuracy and precision. Certified Reference Material BCR® - 482, a lichen powder certified by the Community Bureau of Reference, and Standard Reference Material 1570a from the National Institute of Standards and Technology, a spinach powder. Results from analysis of the lichen reference material are in Table 4.3. Results from analysis of the spinach reference material are in Table 4.4.

The lichen reference material results had an average accuracy of 7.9%, precision of 4.79% and recovery of 92.07%. The spinach reference material results had an average accuracy of 2.96%, precision of 4.27% and recovery of 96.59%. Precision in the spinach standard would have been better but one sample (Sample 129) was much higher than the average of the others for all but one element. There was no reason which could be well supported to remove it from the data, but it does suggest this sample may have had an error in processing.

Table 4.3 Lichen Reference Material's Accuracy and Precision
As, Be, Co, Mo and Tl were present in the standard but values were not above detection limits. Si had very poor precision and was also removed, precision = 20.19%.

Element (Total Recoverable)	Mean	Std. Dev.	Certified Value	Precision (%)	Accuracy (%)	Recovery (%)
Aluminum	916.7	53.0	1103	5.78	16.9	83.11
Barium	10.7	0.9		8.84		
Calcium	2235.8	19.9		0.89		
Cadmium	0.5	0.0	0.56	4.72	8.0	91.96
Chromium	4.0	0.1	4.12	2.16	3.6	96.40
Copper	6.8	0.2	7.03	3.14	4.0	96.02
Iron	770.0	12.5		1.62		
Potassium	3533.8	28.7		0.81		
Magnesium	541.0	4.2		0.78		
Manganese	28.4	0.2		0.84		
Sodium	47.2	1.9		4.06		
Nickel	2.4	0.2	2.47	10.41	3.9	96.09
Phosphorus	637.9	10.7		1.68		
Lead	37.0	1.3	40.9	3.56	9.5	90.52
Sulfur	1718.6	79.3		4.61		
Strotium	9.2	0.1		1.19		
Titanium	21.7	2.4		11.18		
Vanadium	3.0	0.1		4.71		
Zinc	90.9	4.2	100.6	4.63	9.6	90.37
Average (n= 6)				3.98	7.9	92.07

Table 4.4 Spinach Reference Material's Accuracy and Precision
Standard Reference Material 1570a NIST has been used extensively in the Lakehead University Environmental Laboratory and those records have been stored. The stored data mean was used for the non-certified values in the column Certified Value Source. As, Be, Co, Mo, Tl and V were present in the standard but values were not above detection limits. Si had very poor precision and was also removed, precision = 27.22%.

Element (Total Recoverable)	Mean	Std. Dev.	Certified Value	Source	Precision (%)	Accuracy (%)	Recovery (%)
Aluminum	292.2	5.5	310.00	SRM Value	1.89	5.7	94.26
Barium	5.7	0.2	5.38	non-certified	2.72	-5.6	105.56
Calcium	14116.7	297.3	15,300.00	SRM Value	2.11	7.7	92.27
Cadmium	2.6	0.1	2.89	SRM Value	3.85	8.8	91.23
Chromium	1.1	0.1	0.96	non-certified	10.79	-14.2	114.17
Copper	11.2	0.2	12.20	SRM Value	2.16	8.6	91.41
Iron	260.6	7.2	247.33	non-certified	2.77	-5.3	105.35
Potassium	26600.0	562.7	27,500.00	non-certified	2.12	3.3	96.73
Magnesium	8183.5	186.1	8,606.66	non-certified	2.27	4.9	95.08
Manganese	68.5	1.5	75.90	SRM Value	2.24	9.7	90.31
Sodium	16820.6	298.8	18,200.00	SRM Value	1.78	7.6	92.42
Nickel	2.0	0.1	2.14	SRM Value	5.12	4.4	95.64
Phosphorus	5090.5	86.7	5,180.00	SRM Value	1.70	1.7	98.27
Lead	1.3	0					
Sulfur	4565.6	267.3	4,400.30	non-certified	5.85	-3.8	103.76
Strotium	51.3	0.9	55.60	SRM Value	1.75	7.8	92.24
Titanium	8.4	1.6			19.40		
Zinc	76.1	3.1	82.00	SRM Value	4.10	7.2	92.84
Average (n= 6)					4.27	2.96	96.59

4.5 Grouped Lichen Samples Statistical Analysis

4.5.1 Introduction

Data for seven of the elements tested were below detection limits (DL) for most samples, Ni (65), Co (92), Mo (96), As (97), Be (97), Ti (97), and V (97) and were removed. Pb was included in analysis but 15 samples were below the detection limit. Cr and Ti both had 2 samples below DL, and all other elements were above DL for all samples (Al, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, and Zn). Data were not adjusted for those samples below the DL and 0 was kept as the value. All tissue concentration results and other measured variables for each sample are included in Appendix IV.

4.5.2 Descriptive Statistics

Descriptive statistics were generated for the entire dataset used in the following analyses and are shown in Table 4.5.

4.5.3 Distribution Analysis

The dataset was found to not be drawn from a normally distributed population. The two multivariate normal distribution tests indicated large shifts in multivariate normality.

The Mardia's multivariate skewness results were a coefficient of 265.6, a test statistics of 4294 with 1771 DF and a p-value of 5.178E-210. The kurtosis results were a coefficient of 616 a test statistic of 21.07 and a p-value of 0.

The Doorknik and Hansen Omnibus test has an Ep of 1510 and a p-value of 2.452e-289. Both tests reject (α= 0.05) their null hypotheses (H_o) of: Samples are drawn from a normally distributed population.

The Shapiro-Wilk test applied to individual elements indicated many of the elements to not be drawn from a normally distributed population when α = 0.05. These results are included in Table 4.6.

Table 4.5 Descriptive Statistics for Variables used in Statistical Analysis
These data are from the grouped samples which were tested (n = 97). All element values are reported in (mg/kg).

	Mean	S.D.	Median	Min.	Max.	Range	Skew.	Kurtosis	S.E.
Mean Weight	0.06	0.07	0.04	0.01	0.48	0.47	3.2	14.19	0.01
Mean Collar Height	48.67	17.76	49.62	1	80	79	-0.23	-0.62	1.8
Mean North	0.11	0.34	0.1	-0.52	1	1.52	0.57	0.13	0.03
Mean East	-0.09	0.41	-0.09	-0.98	0.94	1.92	0.27	0.03	0.04
Al	197.3	45.34	184.16	92.56	337.5	244.94	0.84	0.66	4.6
Ba	31.75	4.23	31.58	20.42	44.43	24.01	0.13	0.21	0.43
Ca	6598.31	1924.92	6118.2	3146.3	13100	9953.7	1.16	1.83	195.45
Cd	0.44	0.1	0.42	0.23	0.78	0.55	0.61	0.61	0.01
Cr	0.33	0.17	0.3	0	0.81	0.81	0.58	-0.09	0.02
Cu	3.01	1.48	2.67	1.76	13.27	11.51	4.52	27.08	0.15
Fe	241.53	66.97	224.63	109.34	528.54	419.2	1.24	2.73	6.8
K	2521.89	340.02	2453.9	1871.3	3451.5	1580.2	0.66	0.01	34.52
Mg	932.99	129.49	906.6	628.4	1289.7	661.3	0.65	0.6	13.15
Mn	365.82	45.77	365.53	254.85	475.47	220.62	0.02	-0.03	4.65
Na	70.79	26.56	64.8	31.3	235.6	204.3	2.59	14.24	2.7
P	375.69	55.89	356.9	276.6	523.1	246.5	0.84	0.01	5.67
Pb	1.92	1.19	2.15	0	4.37	4.37	-0.21	-0.94	0.12
S	1129.84	136.73	1121.3	858.9	1532.8	673.9	0.48	-0.01	13.88
Sr	12.51	1.95	12.1	8.8	18.3	9.5	0.61	0.25	0.2
Ti	9.1	2.65	8.6	0	16.1	16.1	-0.09	2.21	0.27
Zn	53.79	7.95	53.1	29.24	71.49	42.25	-0.15	0.02	0.81

Table 4.6 Shapiro-Wilk Test Results for Element Concentrations
Results from the Shapiro-Wilk test on the element concentration data in the grouped samples. H_o: Samples came from a normally distributed population.

	N	Shapiro-Wilk W	*p*(normal)
Al	97	0.9412	0.000286
Ba	97	0.9932	0.9101
Ca	97	0.9181	1.47E-05
Cd	97	0.9739	0.05013
Co	97	0.2336	3.45E-20
Cr	97	0.9648	0.01061
Cu	97	0.5304	4.33E-16
Fe	97	0.9166	1.24E-05
K	97	0.9582	0.003577
Mg	97	0.9594	0.004396
Mn	97	0.9939	0.9443
Na	97	0.8073	6.32E-10
Ni	97	0.4304	1.21E-17
P	97	0.9247	3.31E-05
Pb	97	0.9427	0.000355
S	97	0.9761	0.07316
Sr	97	0.9647	0.01031
Ti	97	0.9338	0.000106
Zn	97	0.9831	0.2461

4.5.4 Difference of Trace Element Means Analysis

The analysis of the wind data showed two main directions. To explore this directionality the samples were split into two sample groups based on aspect. The Two-Group Permutation Test resulted after 2000 permutations, with a Mahalanobis distance of 0.607 and $p < 0.0005$. This is well below the α value of 0.05, so the null hypothesis (Equality of means) was rejected, and it was determined that the two aspect groups are different in their elemental accumulation.

The Mann-Whitney-Wilcoxon rank sum test with continuity correction was used *post hoc* to determine which particular elements between samples of Group South and Group North were significantly different. Results can be found in Table 4.7.

Table 4.7 Mann-Whitney-Wilcoxon rank sum test results with continuity correction
Mann-Whitney-Wilcoxon rank sum tests to examine if differences occur between the aspect groupings in element concentration distribution.

Element	W	*p*-value	Group South Mean	Group North Mean
Al	499	4.368e-06	178.95	225.79
Ba	615	0.0001871	30.47	33.75
Ca	298	1.213e-09	5675.72	8030.74
Cd	615.5	0.0001864	0.41	0.49
Cr	239.5	7.381e-11	0.24	0.46
Cu	440	4.918e-07	2.76	3.4
Fe	497	4.069e-06	216.5	280.39
K	1721	9.403e-06	2645.33	2330.25
Mg	424	2.642e-07	877.34	1019.38
Mn	885	0.08179	360.02	374.83
Na	1957	6.63e-10	79.43	57.37
P	1484.5	0.007303	390.95	351.99
Pb	715	0.002679	1.63	2.36
S	364	2.261e-08	1068.85	1224.54
Sr	385.5	5.521e-08	11.65	13.86
Ti	511	6.62e-06	8.08	10.68
Zn	297	1.158e-09	49.98	59.7

4.5.5 Clustering Analysis

Three cluster analyses were performed on the dataset, the first included clustering on the element data (Figure 4.5), the second on the attribute data (Figure 4.6) and third included both attribute and element data (Figure 4.7).

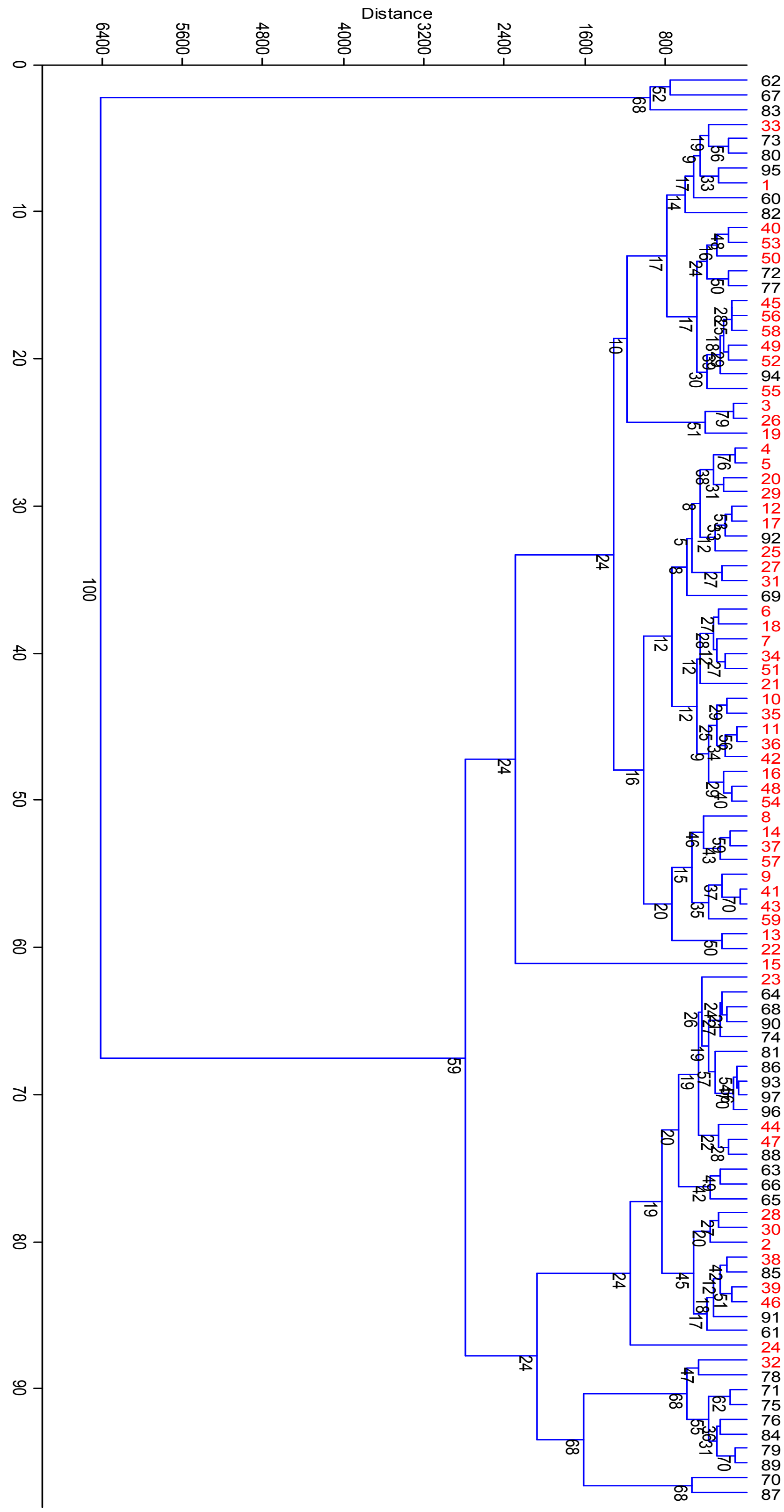


Figure 4.5 Element Data Clustering Analysis
Clustering of the samples based on their element concentration data. Group South (Red) and Group North (Black). Bootstrapped 100 times, values at each cluster indicate number of bootstrap successes for that cluster.

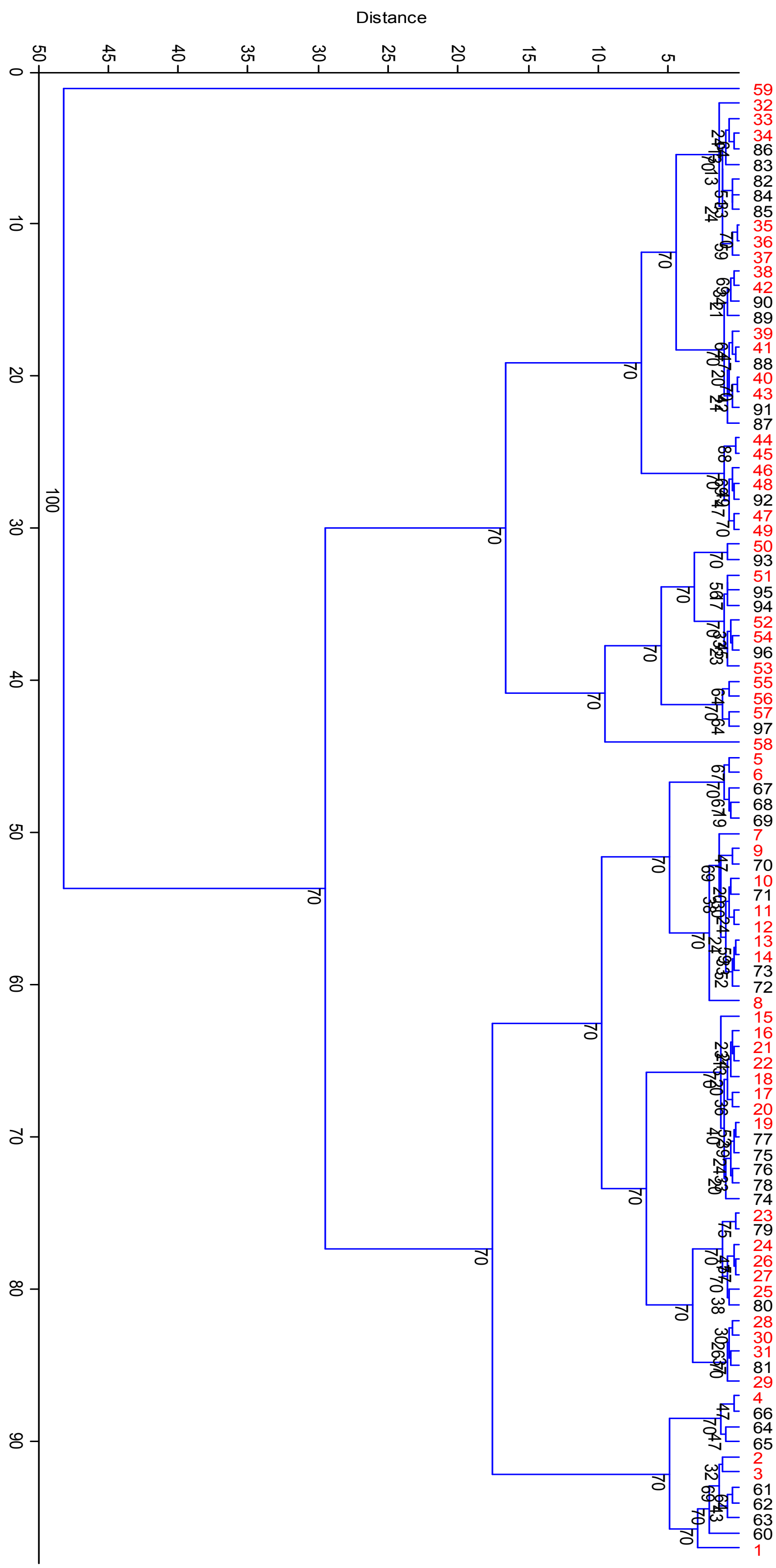


Figure 4.6 Attribute Data Clustering Analysis Plot
Clustering of the samples based on their attribute data. Group South (Red) and Group North (Black). Bootstrapped 100 times, values at each cluster indicate number of bootstrap successes for that cluster.

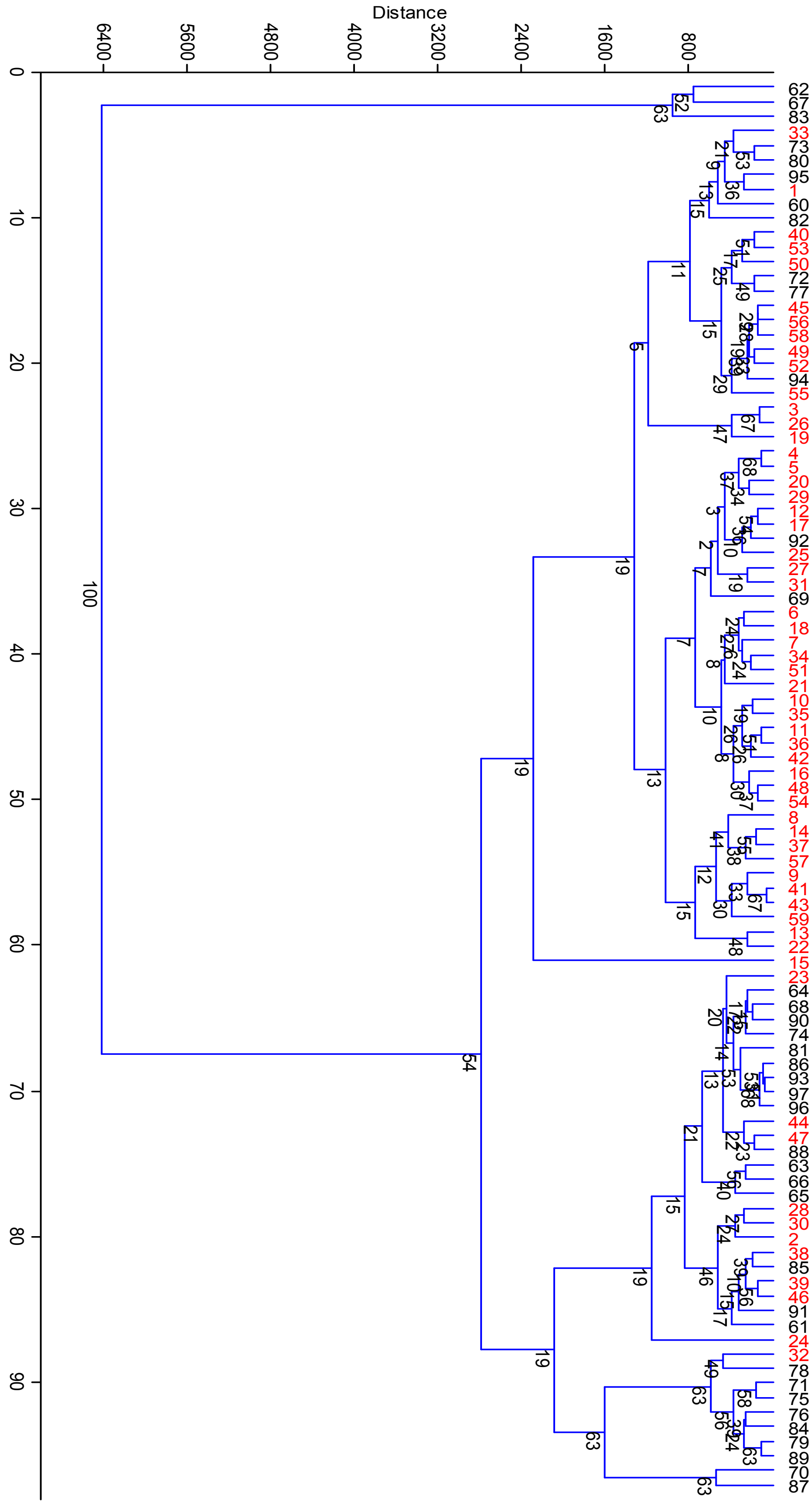


Figure 4.7 Attribute and Element Data Clustering Analysis
Clustering of the samples based on their element concentration and attribute data. Group South (Red) and Group North (Black). Bootstrapped 100 times, values at each cluster indicate number of bootstrap successes for that cluster.

4.5.6 Stepwise Linear Regression

Both Ways Stepwise Linear regression models were produced to examine any explanatory relationships occurring on the lichen element concentrations from the attribute data collected for the lichen samples.

Table 4.8 Al South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
69.270	15.388	3.522	16.148	65.736
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	154.5376	10.3464	14.936	<2e-16
collar_mean	0.482	0.2115	2.279	0.0265
east_mean	20.0881	11.2135	1.791	0.0786
Residual standard error:	27.77 on 56 degrees of freedom			
Multiple R-squared:	0.1531	Adjusted R-squared:	0.1229	
F-statistic:		5.062 on 2 and 56 DF	*p*-value:	0.009534

Table 4.9 Al North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-68.592	-20.573	-3.644	26.247	68.842
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	139.165	18.9456	7.346	1.64E-08
collar_mean	1.8568	0.3482	5.332	6.36E-06
east_mean	-41.8969	16.8537	-2.486	0.017998
weight_mean	-431.849	117.6979	-3.669	0.000826
Residual standard error:	36.16 on 34 degrees of freedom			
Multiple R-squared:	0.5352	Adjusted R-squared:	0.4942	
F-statistic:	13.05 on 3 and 34 DF		*p*-value:	7.857e-06

Table 4.10 Ba South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-8.727	-2.325	-0.599	2.597	8.539
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	31.1213	0.6605	47.115	<2e-16
east_mean	2.7489	1.5639	1.758	0.0843
Residual standard error:	3.853 on 56 degrees of freedom			
Multiple R-squared:	0.1039	Adjusted R-squared:	0.07187	
F-statistic:	3.246 on 2 and 56 DF		*p*-value:	0.04638

Table 4.11 Ba North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-6.37	-2.6788	0.2766	2.0689	9.4571
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)				
(Intercept)	38.15642	1.80028	21.195	<2e-16
collar_mean	-0.08452	0.03274	-2.582	0.0141
Residual standard error:	3.553 on 36 degrees of freedom			
Multiple R-squared:	0.1562	Adjusted R-squared:	0.1328	
F-statistic:	6.665 on 1 and 36 DF		*p*-value:	0.01405

Table 4.12 Ca South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-2529.4	-861.4	-234.2	454.9	3054.6
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5675.7	160.4	35.39	<2e-16
Residual standard error:	1232 on 58 degrees of freedom			
Multiple R-squared:	N/A	Adjusted R-squared:	N/A	
F-statistic:			*p*-value:	N/A

Table 4.13 Ca North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-2350.9	-1215.1	-280.3	693.1	5143
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	8207	520.7	15.761	<2e-16
east_mean	1931.3	852.2	2.266	0.0297
weight_mean	9213.3	5722.1	1.61	0.1164
Residual standard error:	1830 on 35 degrees of freedom			
Multiple R-squared:	0.1598	Adjusted R-squared:	0.1118	
F-statistic:	3.328 on 2 and 35 DF		*p*-value:	0.04751

Table 4.14 Cd South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-0.151687	-0.060805	-0.006197	0.041501	0.220115
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.4691956	0.0308376	15.215	<2e-16
collar_mean	-0.0013727	0.0006218	-2.208	0.0313
Residual standard error:	0.08288 on 57 degrees of freedom			
Multiple R-squared:	0.07876	Adjusted R-squared:	0.0626	
F-statistic:	4.873 on 1 and 57 DF		*p*-value:	0.03132

Table 4.15 Cd North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-0.11195	-0.06802	-0.02837	0.04117	0.30092
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.616119	0.048422	12.724	1.09E-14
collar_mean	-0.00198	0.000856	-2.315	0.0266
east_mean	0.072386	0.042612	1.699	0.0982
Residual standard error:	0.09255 on 35 degrees of freedom			
Multiple R-squared:	0.205	Adjusted R-squared:	0.1596	
F-statistic:	4.513 on 2 and 35 DF		*p*-value:	0.01804

Table 4.16 Cr South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-0.27074	-0.08914	-0.01152	0.05016	0.40382
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.1381122	0.0472519	2.923	0.00497
collar_mean	0.0022588	0.0009528	2.371	0.02116
Residual standard error:	0.127 on 57 degrees of freedom			
Multiple R-squared:	0.08975	Adjusted R-squared:	0.07378	
F-statistic:	5.62 on 1 and 57 DF		*p*-value:	0.02116

Table 4.17 Cr North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-0.27125	-0.07686	-0.0028	0.09205	0.311074
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.287457	0.06164	4.663	4.4146E-05
collar_mean	0.004122	0.001165	3.537	0.00116
weight_mean	-0.72484	0.389388	-1.861	0.07109
Residual standard error:	0.1211 on 35 degrees of freedom			
Multiple R-squared:	0.2752	Adjusted R-squared:	0.2337	
F-statistic:	6.643 on 2 and 35 DF		*p*-value:	0.003583

Table 4.18 Cu South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-1.4856	-0.5808	-0.2201	0.0546	10.2997
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.51168	0.56385	6.228	6.07e-08
collar_mean	-0.01609	0.01137	-1.415	0.162
Residual standard error:	1.516 on 57 degrees of freedom			
Multiple R-squared:	0.03394	Adjusted R-squared:	0.01699	
F-statistic:	2.002 on 1 and 57 DF		*p*-value:	0.1625

Table 4.19 Cu North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-1.2006	-0.7193	-0.3458	0.124	5.8493
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	3.8608	0.3181	12.14	2.77E-14
weight_mean	-7.687	3.9821	-1.93	0.0615
Residual standard error:	1.293 on 36 degrees of freedom			
Multiple R-squared:	0.0938	Adjusted R-squared:	0.06863	
F-statistic:	3.726 on 1 and 36 DF		*p*-value:	0.06146

Table 4.20 Fe South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-78.114	-27.835	-2.503	24.628	160.275
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	174.8710	16.4890	10.605	5.2e-15
collar_mean	1.0076	0.3464	2.909	0.0052
north_mean	-31.0983	17.6754	-1.759	0.0840
Residual standard error:	44.02 on 56 degrees of freedom			
Multiple R-squared:	0.1426	Adjusted R-squared:	0.112	
F-statistic:	4.657 on 2 and 56 DF		*p*-value:	0.01347

Table 4.21 Fe North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-72.605	-31.738	-6.583	14.153	154.44
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	127.893	25.408	5.034	1.55E-05
collar_mean	2.906	0.467	6.223	4.42E-07
east_mean	-83.565	22.602	-3.697	0.000764
weight_mean	-507.464	157.844	-3.215	0.002858
Residual standard error:	48.49 on 34 degrees of freedom			
Multiple R-squared:	0.6188	Adjusted R-squared:	0.5851	
F-statistic:	18.4 on 3 and 34 DF		*p*-value:	2.885e-07

Table 4.22 K South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-403.82	-204.63	29.35	180.34	616.73
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2057.181	94.499	21.769	< 2e-16
collar_mean	15.067	2.085	7.227	1.60e-09
east_mean	-190.498	103.111	-1.848	0.07005
weight_mean	-1387.174	459.737	-3.017	0.00386
Residual standard error:	252.7 on 55 degrees of freedom			
Multiple R-squared:	0.494	Adjusted R-squared:		0.4663
F-statistic:	17.9 on 3 and 55 DF,		*p*-value:	3.146e-08

Table 4.23 K North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-293.46	-107.75	14.38	117.69	342.22
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2299.044	91.791	25.046	<2e-16
collar_mean	3.672	1.74	2.111	4.22E-02
north_mean	-140.979	92.893	-1.518	0.1383
weight_mean	-2614.94	566.817	-4.613	5.42E-05
Residual standard error:	175.7 on 34 degrees of freedom			
Multiple R-squared:	0.437	Adjusted R-squared:	0.3873	
F-statistic:	8.796 on 3 and 34 DF		*p*-value:	0.0001867

Table 4.24 Mg South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-160.087	-48.324	2.636	33.950	182.558
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	768.0890	26.9015	28.552	< 2e-16
collar_mean	3.1064	0.5967	5.206	2.96E-06
north_mean	-67.6039	30.3828	-2.225	0.0302
weight_mean	-356.303	136.2458	-2.615	0.0115
Residual standard error:	71.7 on 55 degrees of freedom			
Multiple R-squared:	0.3661	Adjusted R-squared:	0.3315	
F-statistic:	10.59 on 3 and 55 DF		*p*-value:	1.342e-05

Table 4.25 Mg North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-214.977	-42.886	7.317	76.15	147.051
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	750.0042	52.2078	14.366	<2e-16
collar_mean	5.1708	0.9494	5.446	3.82E-06
Residual standard error:	103 on 36 degrees of freedom			
Multiple R-squared:	0.4518	Adjusted R-squared:	0.4365	
F-statistic:	29.66 on 1 and 36 DF		*p*-value:	3.819e-06

Table 4.26 Mn South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-98.173	-23.007	-6.812	23.057	112.245
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	342.3048	15.9286	21.490	<2e-16
collar_mean	0.5722	0.3499	1.636	0.1075
weight_mean	-132.726	76.767	-1.729	0.0893
Residual standard error:	42.63 on 56 degrees of freedom			
Multiple R-squared:	0.0672	Adjusted R-squared:	0.03389	
F-statistic:	2.017 on 2 and 56 DF		*p*-value:	0.1426

Table 4.27 Mn North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-95.723	-22.038	2.752	33.369	100.637
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	374.833	7.865	47.66	<2e-16
Residual standard error:	48.48 on 37 degrees of freedom			
Multiple R-squared:	N/A	Adjusted R-squared:	N/A	
F-statistic:	N/A		*p*-value:	N/A

Table 4.28 Na South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-30.784	-8.196	-1.403	9.086	28.909
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	49.2965	4.9258	10.008	5.43E-14
collar_mean	0.7221	0.1093	6.608	1.65E-08
north_mean	18.0722	5.5633	3.248	0.00198
weight_mean	-96.1623	24.9473	-3.855	0.000306
Residual standard error:	13.13 on 55 degrees of freedom			
Multiple R-squared:	0.5344	Adjusted R-squared:	0.509	
F-statistic:	21.04 on 3 and 55 DF		*p*-value:	3.296e-09

Table 4.29 Na North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-38.108	-11.772	-3.207	2.95	154.012
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	30.4484	15.516	1.962	5.77E-02
collar_mean	0.5039	0.2815	1.79	8.21E-02
north_mean	30.3705	15.6935	1.935	0.0611
Residual standard error:	29.78 on 35 degrees of freedom			
Multiple R-squared:	0.1398	Adjusted R-squared:	0.09064	
F-statistic:	2.844 on 2 and 35 DF		*p*-value:	0.0717

Table 4.30 P South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-69.21	-34.29	0.87	29.42	111.57
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	281.6548	17.2279	16.349	<
collar_mean	2.6281	0.3784	6.945	4.27E-09
weight_mean	-191.604	83.0284	-2.308	0.0247
Residual standard error:	46.11 on 56 degrees of freedom			
Multiple R-squared:	0.4643	Adjusted R-squared:	0.4452	
F-statistic:	24.27 on 2 and 56 DF		*p*-value:	2.565e-08

Table 4.31 P North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-42.008	-21.478	1.193	20.012	48.685
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	312.729	13.167	23.751	<2e-16
collar_mean	1.049	0.242	4.336	1.23E-04
east_mean	-19.831	11.713	-1.693	0.09959
weight_mean	-381.291	81.8	-4.661	4.70E-05
Residual standard error:	25.13 on 34 degrees of freedom			
Multiple R-squared:	0.4952	Adjusted R-squared:	0.4507	
F-statistic:	11.12 on 3 and 34 DF		*p*-value:	3.086e-05

Table 4.32 S South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-189.768	-59.404	6.334	59.783	249.394
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	884.5037	32.993	26.809	<2e-16
collar_mean	4.5706	0.7319	6.245	6.45E-08
north_mean	-58.9675	37.2626	-1.582	0.119
weight_mean	-271.979	167.0972	-1.628	0.109
Residual standard error:	87.93 on 55 degrees of freedom			
Multiple R-squared:	0.4171	Adjusted R-squared:	0.3853	
F-statistic:	13.12 on 3 and 55 DF		*p*-value:	1.420e-06

Table 4.33 S North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-174.38	-35.5	4.39	38.51	210.55
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1098.628	41.7539	26.312	<2e-16
collar_mean	3.9734	0.7893	5.034	1.45E-05
weight_mean	-1350.46	263.7633	-5.12	1.12E-05
Residual standard error:	82.04 on 35 degrees of freedom			
Multiple R-squared:	0.5335	Adjusted R-squared:	0.5068	
F-statistic:	20.01 on 2 and 35 DF		*p*-value:	1.605e-06

Table 4.34 Sr South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-3.1468	-0.8579	-0.2773	0.6522	3.6789
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	11.8018	0.2142	55.088	<2e-16
north_mean	-0.9267	0.5657	-1.638	1.07E-01

Residual standard error:	1.478 on 57 degrees of freedom			
Multiple R-squared:	0.04496	Adjusted R-squared:	0.02821	
F-statistic:	2.684 on 1 and 57 DF		*p*-value:	0.1069

Table 4.35 Sr North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-2.8753	-1.4103	-0.0161	0.824	4.7261
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	13.9476	0.4869	28.646	<2e-16
east_mean	1.6525	0.7969	2.074	4.55E-02

Residual standard error:	1.711 on 35 degrees of freedom			
Multiple R-squared:	0.1473	Adjusted R-squared:	0.09858	
F-statistic:	3.023 on 2 and 35 DF		*p*-value:	0.0615

Table 4.36 Ti South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-6.9551	-0.7882	0.2663	1.3136	2.9932
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.99106	0.72328	8.283	2.65E-11
collar_mean	0.04153	0.01479	2.809	6.84E-03
east_mean	1.63262	0.78389	2.083	0.04186
Residual standard error:	1.941 on 56 degrees of freedom			
Multiple R-squared:	0.2079	Adjusted R-squared:	0.1796	
F-statistic:	7.349 on 2 and 56 DF		*p*-value:	0.001465

Table 4.37 Ti North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.88175	0.9731	6.044	7.54E-07
collar_mean	0.09934	0.01789	5.554	3.27E-06
east_mean	-2.00365	0.86566	-2.315	2.68E-02
weight_mean	-18.8145	6.04532	-3.112	3.75E-03
Residual standard error:	1.857 on 34 degrees of freedom			
Multiple R-squared:	0.533	Adjusted R-squared:	0.4918	
F-statistic:	12.94 on 3 and 34 DF		*p*-value:	8.48e-06

Table 4.38 Zn South Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-20.7375	-3.6575	0.1425	3.0275	17.0225
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	49.9775	0.8916	56.06	<2e-16
Residual standard error:	6.848 on 58 degrees of freedom			
Multiple R-squared:	N/A	Adjusted R-squared:	N/A	
F-statistic:	N/A		*p*-value:	N/A

Table 4.39 Zn North Linear Regression

Residuals:				
Min.	1Q	Median	3Q	Max
-12.8671	-4.2421	0.6329	4.1429	11.7929
Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	59.6971	0.9123	65.44	<2e-16
Residual standard error:	5.624 on 37 degrees of freedom			
Multiple R-squared:	N/A	Adjusted R-squared:	N/A	
F-statistic:	N/A		*p*-value:	N/A

4.5.7 Common Origin Tests

Spearman's Rho was used to explore correlations between all elements. These results are shown in Table 4.40 as a matrix. As well a correlogram, Figure 4.8, was plotted that includes confidence intervals around smooth lines and scatter plots for all combinations of elements.

Table 4.40 Spearman's Rank Sum Element Correlation Analysis Results
Correlation values are in the lower left of the matrix. Correlation significance is in the upper right of the matrix. Toxic metals are italicized. Primary, secondary macronutrients and micronutrients are underlined.

	Al	*Ba*	Ca	*Cd*	*Cr*	*Cu*	*Fe*	K	Mg	Mn	Na	P	*Pb*	S	Sr	Ti	*Zn*
Al		0.039	<.000	0.049	<.000	<.000	<.000	0.079	<.000	0.441	0.047	0.959	0.015	<.000	<.000	<.000	<.000
Ba	0.21		<.000	<.000	0.003	0.061	0.054	0.005	<.000	<.000	<.000	0.002	0.645	0.643	<.000	0.111	<.000
Ca	0.46	0.67		<.000	<.000	0.001	<.000	<.000	<.000	<.000	<.000	0.004	0.053	0.002	<.000	<.000	<.000
Cd	0.20	0.65	0.65		0.002	0.016	0.071	0.005	0.001	<.000	<.000	0.014	0.330	0.363	<.000	0.103	<.000
Cr	0.63	0.30	0.57	0.30		<.000	<.000	0.005	<.000	0.104	0.002	0.446	0.001	<.000	<.000	<.000	<.000
Cu	0.50	0.19	0.34	0.24	0.48		<.000	0.875	<.000	0.115	0.367	0.084	0.193	<.000	0.006	<.000	<.000
Fe	**0.95**	0.20	0.46	0.18	0.62	0.53		0.098	<.000	0.331	0.059	0.621	0.004	<.000	<.000	<.000	<.000
K	-0.18	-0.28	-0.43	-0.28	-0.28	0.02	-0.17		0.499	0.240	<.000	<.000	0.712	0.037	0.003	0.066	0.087
Mg	0.49	0.52	0.57	0.34	0.51	0.46	0.51	0.07		<.000	0.206	0.036	0.075	<.000	<.000	<.000	<.000
Mn	0.08	0.68	0.48	0.46	0.17	0.16	0.10	0.12	**0.76**		0.840	0.424	0.489	0.501	<.000	0.684	<.000
Na	-0.20	-0.43	-0.52	-0.35	-0.32	-0.09	-0.19	**0.77**	-0.13	-0.02		<.000	0.271	0.984	<.000	0.053	<.000
P	0.01	-0.31	-0.29	-0.25	-0.08	0.18	0.05	**0.87**	0.21	0.08	0.69		0.019	<.000	0.038	0.990	0.210
Pb	0.25	-0.05	0.20	0.10	0.34	0.13	0.29	0.04	0.18	-0.07	-0.11	0.24		<.000	0.074	0.006	0.198
S	0.57	0.05	0.31	0.09	0.50	0.62	0.61	0.21	0.54	0.07	0.00	0.42	0.45		0.004	<.000	<.000
Sr	0.39	**0.83**	**0.92**	0.68	0.47	0.28	0.41	-0.30	0.68	0.69	-0.43	-0.21	0.18	0.29		<.000	<.000
Ti	**0.97**	0.16	0.47	0.17	0.61	0.46	0.94	-0.19	0.47	0.04	-0.20	0.00	0.28	0.60	0.39		<.000
Zn	0.43	0.78	0.74	0.56	0.54	0.43	0.41	-0.17	**0.75**	**0.75**	-0.36	-0.13	0.13	0.36	0.83	0.38	

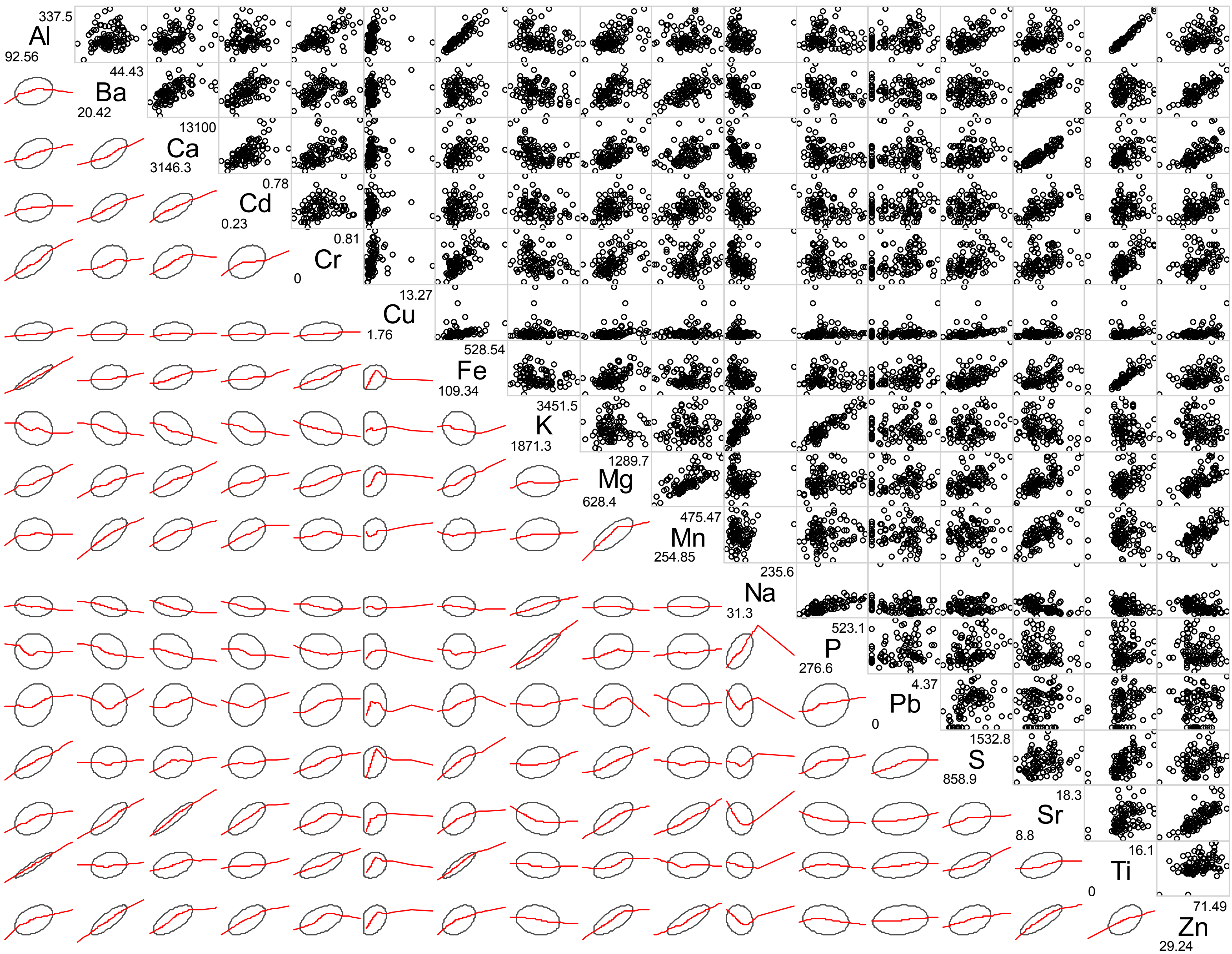


Figure 4.8 Correlogram of Element Data
Bottom Panel: Smoothed Line Plots with Confidence Ellipse and, Top Panel: Scatter Plots.

4.5.8 PCA Analysis

Principal components analysis (PCA) was conducted on the elemental data. The PCA will allow for the analysis of the data through major components which represent most of the variation in the multivariate dataset. For the PCA analysis, there is: 1) Table of the Components and their Eigenvalues and % Variance explained. 2) A broken stick plot indicating which components are significant. 3) Plots and Biplots of the significant components. 4) Loading graphs of the load of each variable for each significant component. PCA Loadings are in Appendix V.

Table 4.41 Element Data PCA
Results from a Principal Components Analysis conducted on the samples with only the element data.

Principal Component	Eigenvalue	% Variance
1	160.54	31.47
2	135.02	34.14
3	224.88	25.26
4	192.04	29.56
5	221.95	34.26
6	207.68	30.2
7	170.02	20.42
8	214.68	24.3
9	192.28	30.7
10	212.87	31.81
11	191.16	27.46
12	174.75	29.22
13	162.01	25.85
14	147.54	25.55
15	177.56	26.9
16	177.55	25.49
17	146.26	26.22

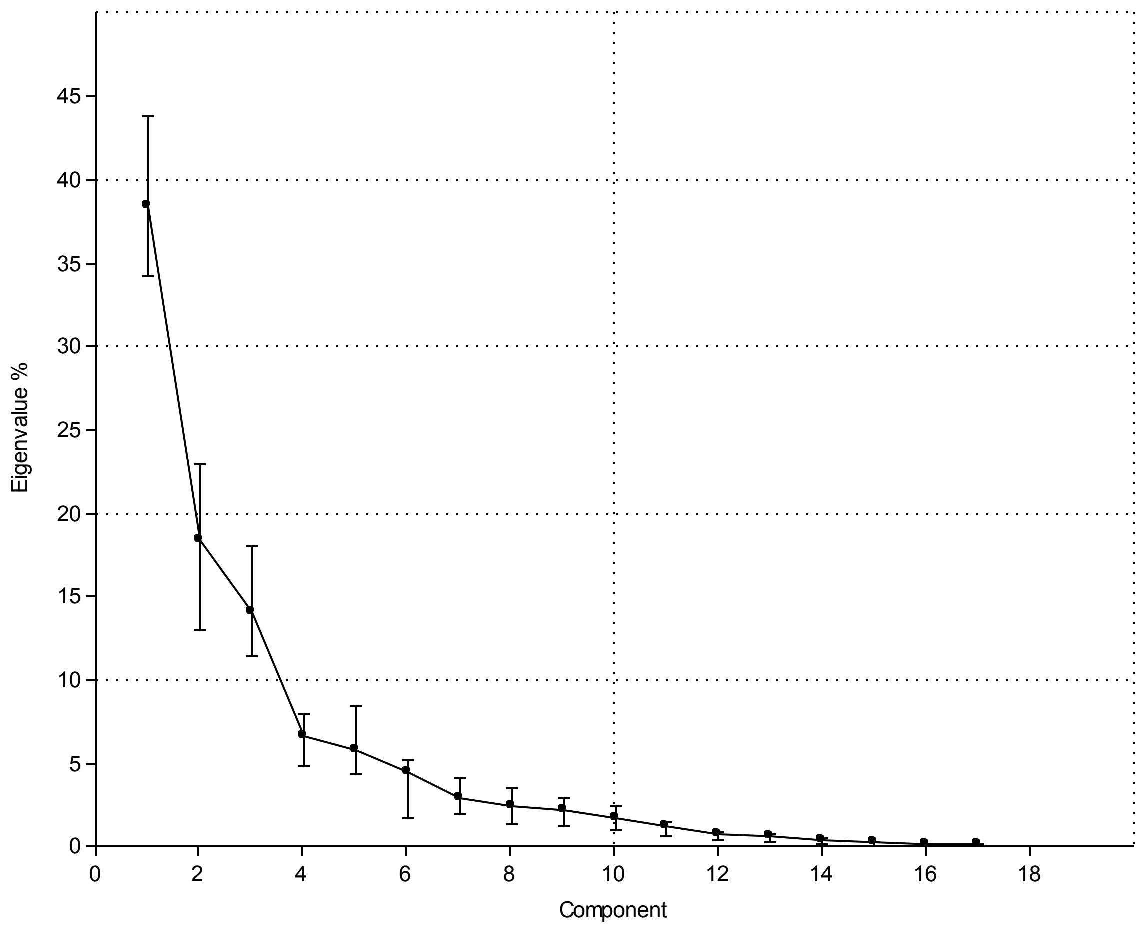


Figure 4.9 Element Data PCA Scree Plot
Scree Plot of the components and their respective eigenvalue % in the solid line. Error bars are the 95% confidence intervals following boot strapping (n= 1000).

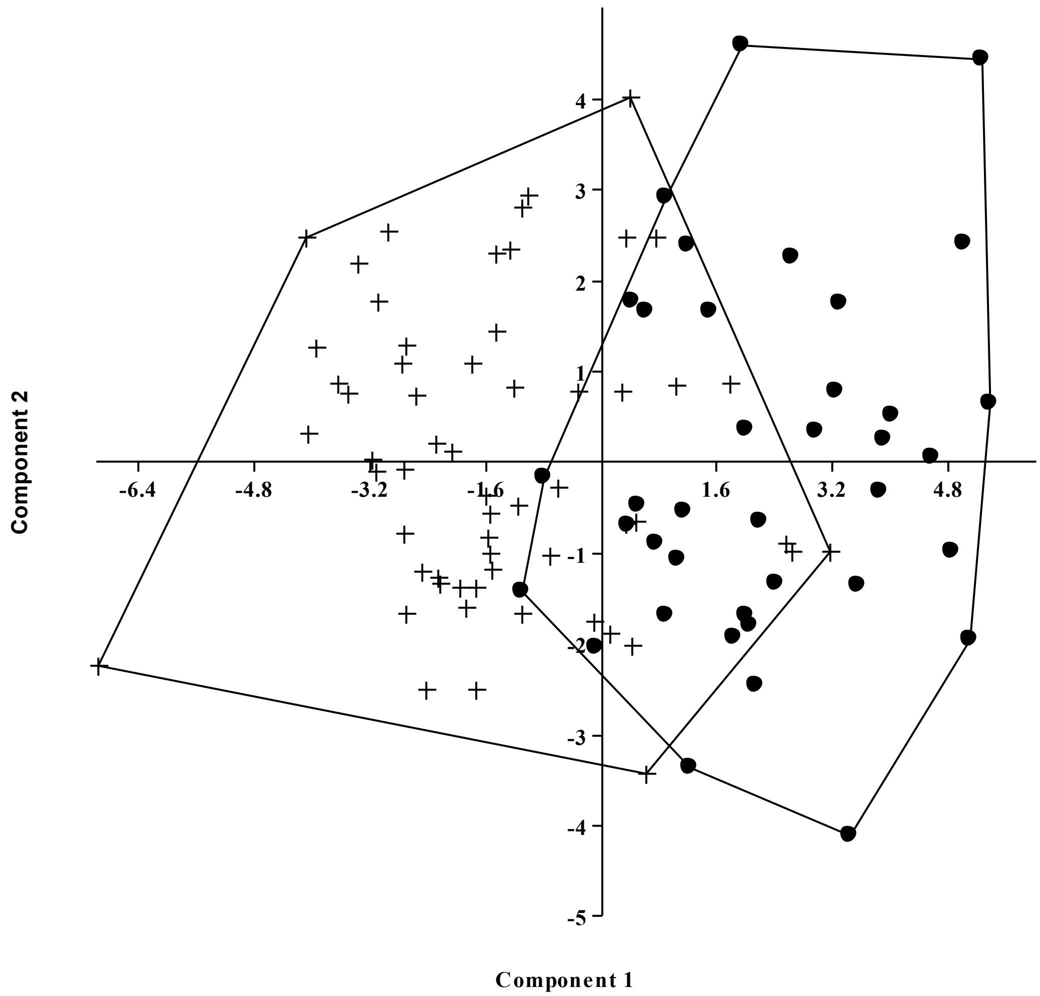


Figure 4.10 Plot of Components 1 and 2 from Element PCA
Plot of Component 1 and Component 2 from the Element PCA. South and North Aspect Groups are encapsulated with Complex Hulls. Group South (Plus) and Group North (Circle).

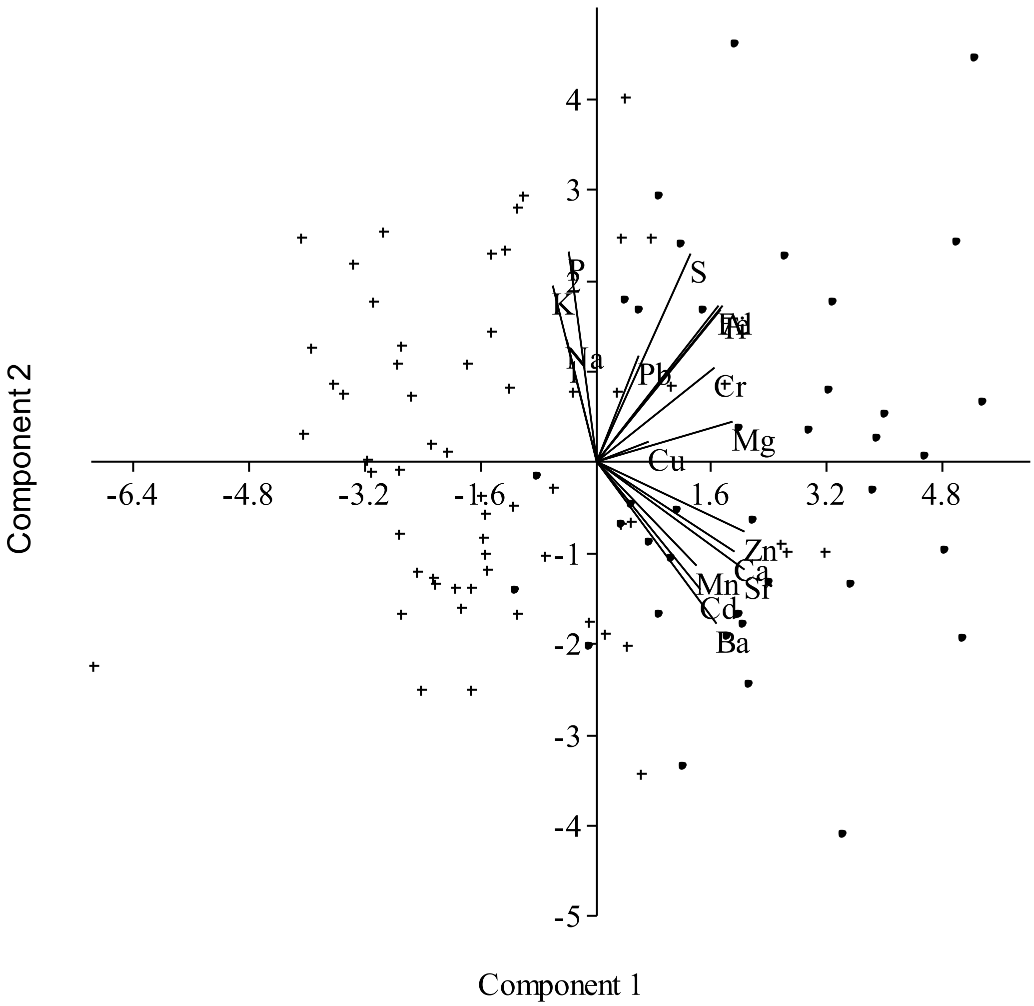


Figure 4.11 Biplot of Component 1 and 2 from Element Data PCA
Biplot of the element vectors laid onto the plot of Component 1 and 2 from the element data PCA. Group South (Plus) and Group North (Circle).

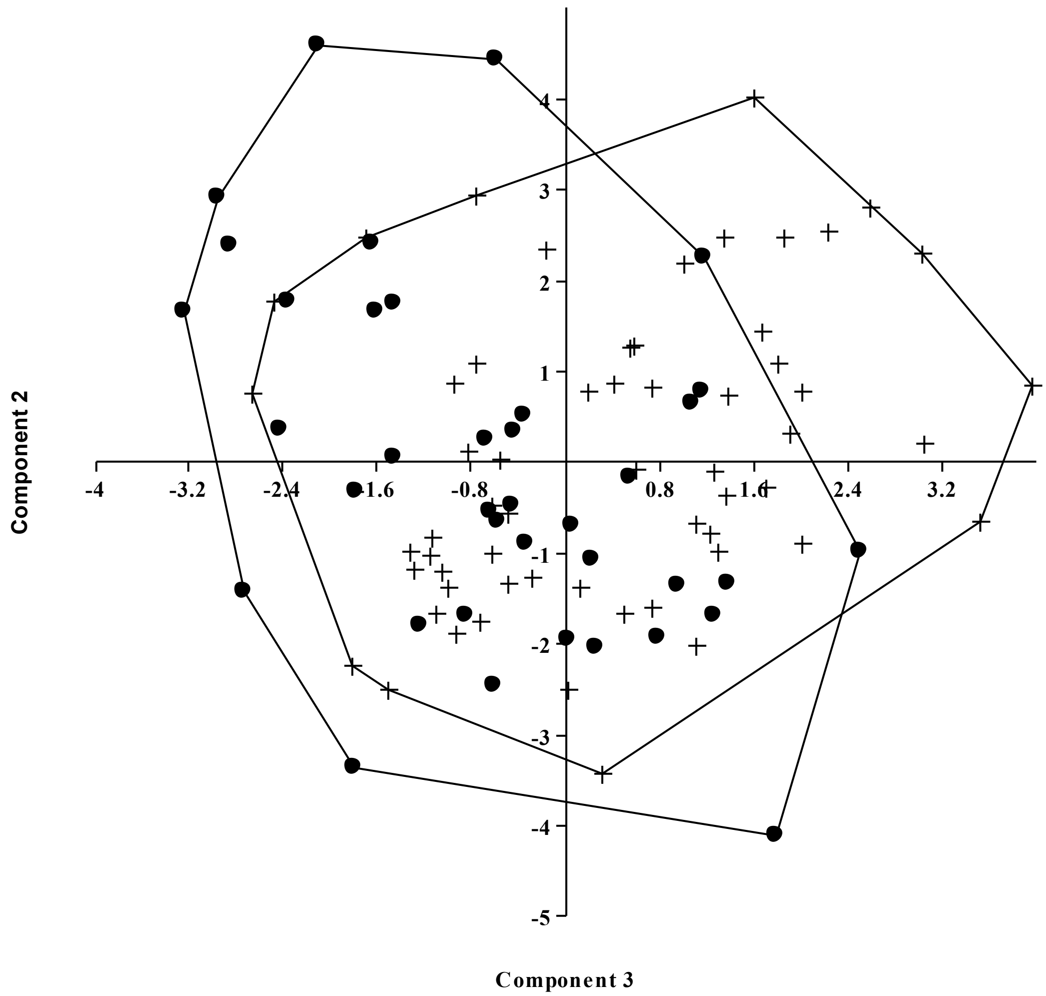


Figure 4.12 Plot of Components 2 and 3 from Element Data PCA
Plot of Component 2 and Component 3 from the Element PCA. South and North Aspect Groups are encapsulated with Complex Hulls. Group South (Plus) and Group North (Circle).

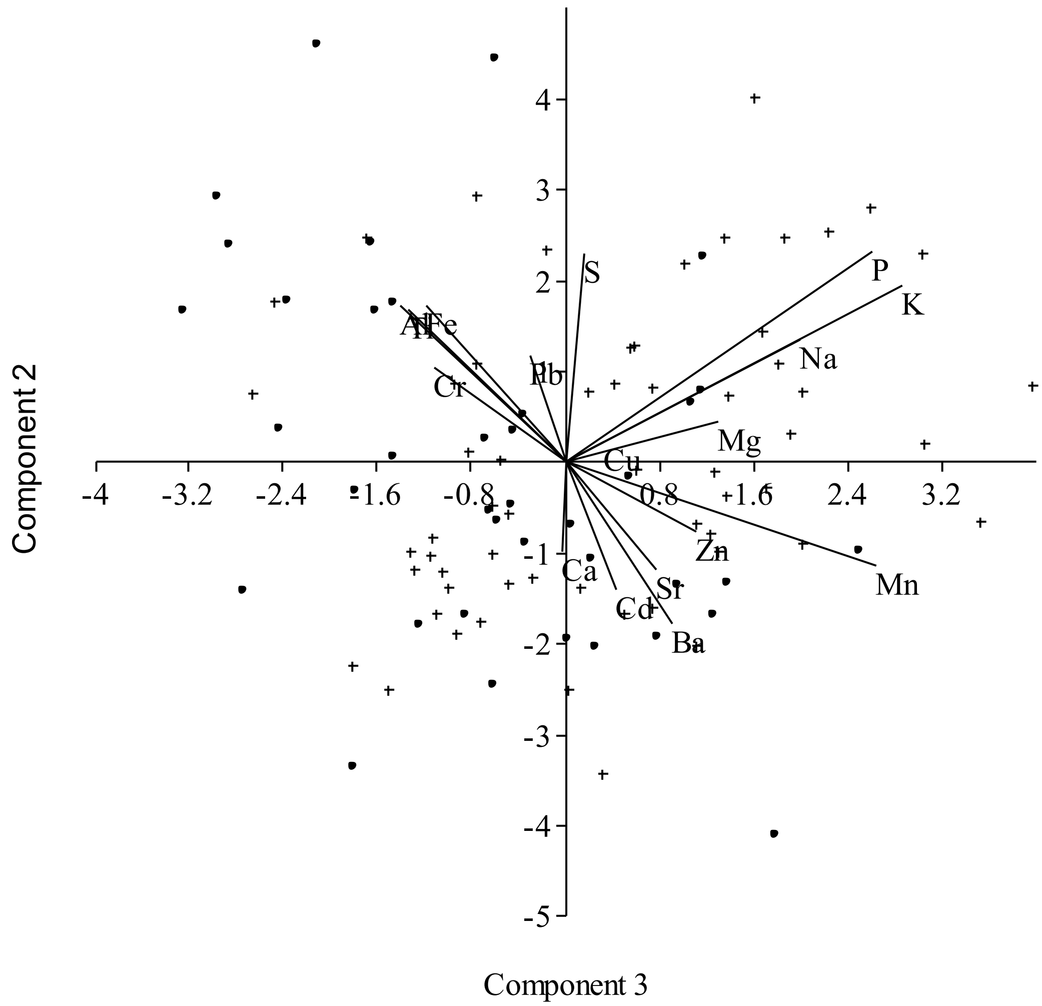


Figure 4.13 Biplot of Components 2 and 3 from Elemental PCA
Biplot of the element vectors laid onto the plot of Component 2 and 3 from the element data PCA. Group South (Plus) and Group North (Circle).

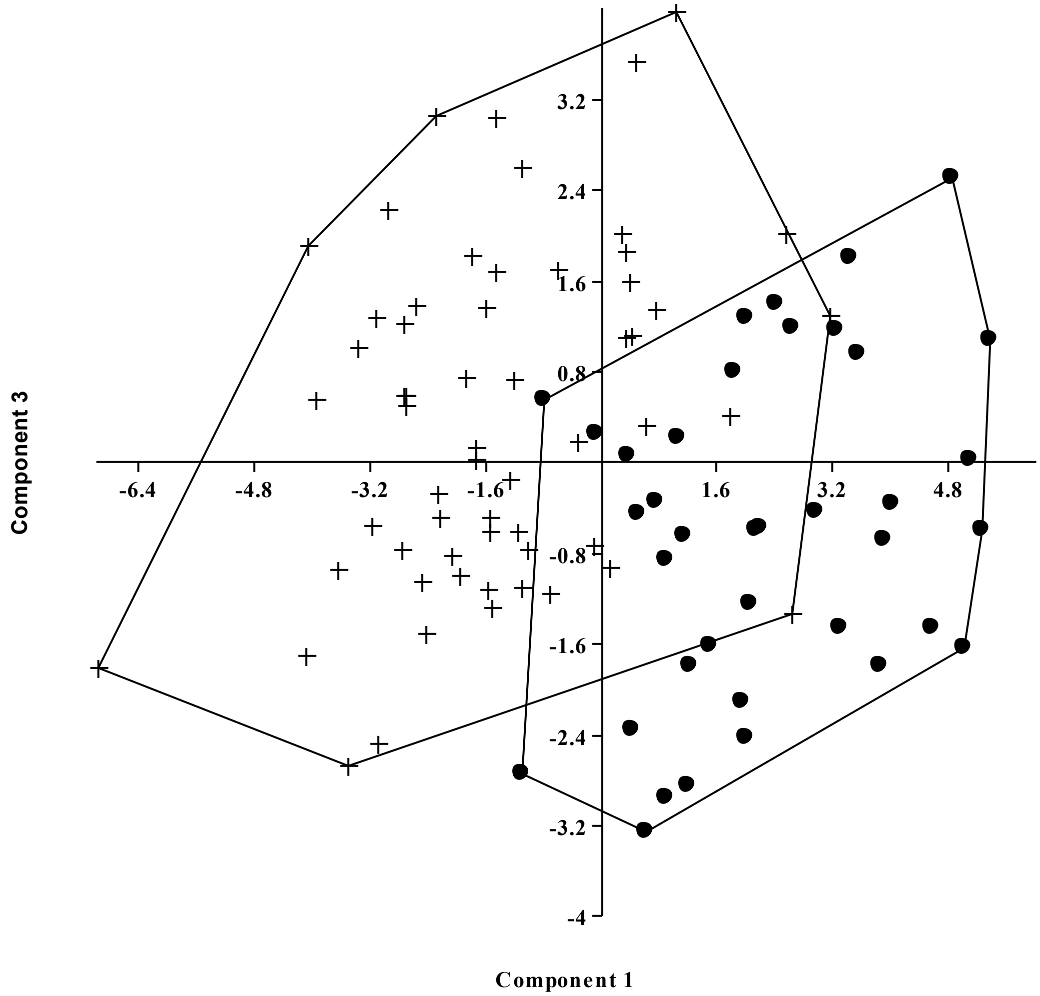


Figure 4.14 Plot of Components 1 and 3 from Elemental PCA
Plot of Component 1 and Component 3 from the Element PCA. South and North Aspect Groups are encapsulated with Complex Hulls. Group South (Plus) and Group North (Circle).

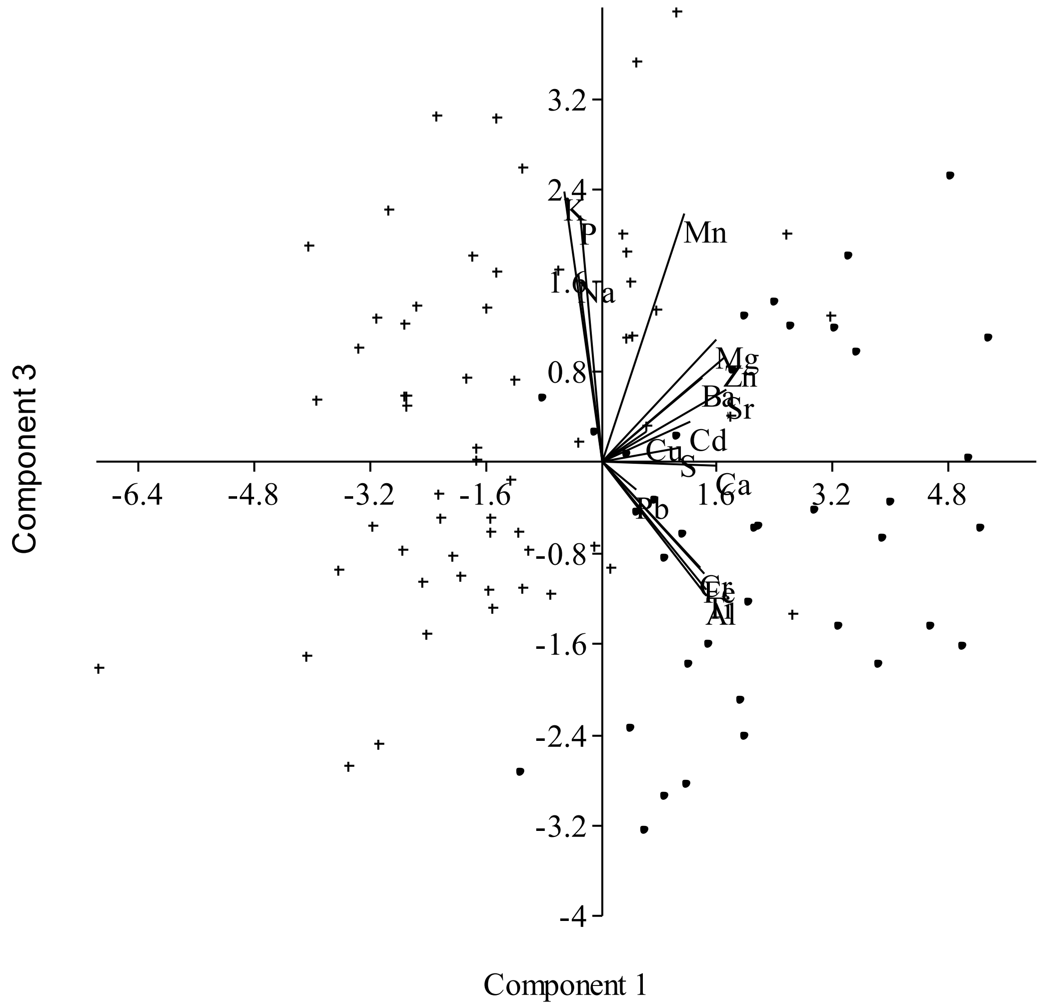


Figure 4.15 Biplot of Components 1 and 3 from Elemental PCA
Biplot of the element vectors laid onto the plot of Component 1 and 3 from the element data PCA. Group South (Plus) and Group North (Circle).

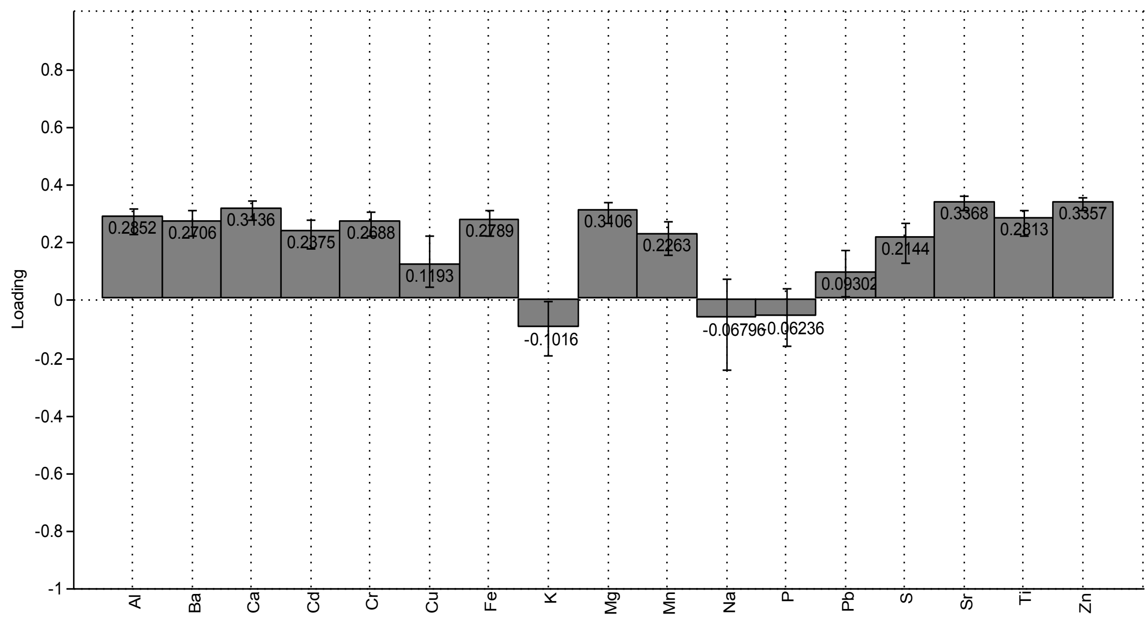


Figure 4.16 Component 1 loadings from Elemental PCA
Component loadings for each element, based on coefficients. Error bars are the 95% confidence intervals following boot strapping (n= 1000).

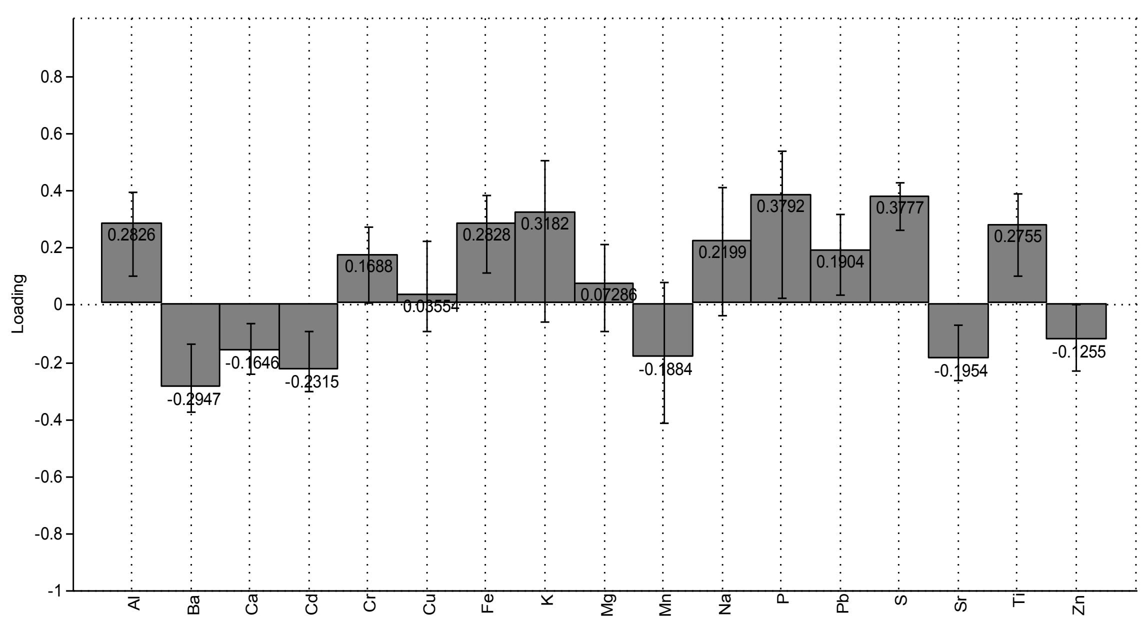


Figure 4.17 Component 2 loadings from Elemental PCA
Component loadings for each element, based on coefficients. Error bars are the 95% confidence intervals following boot strapping (n= 1000).

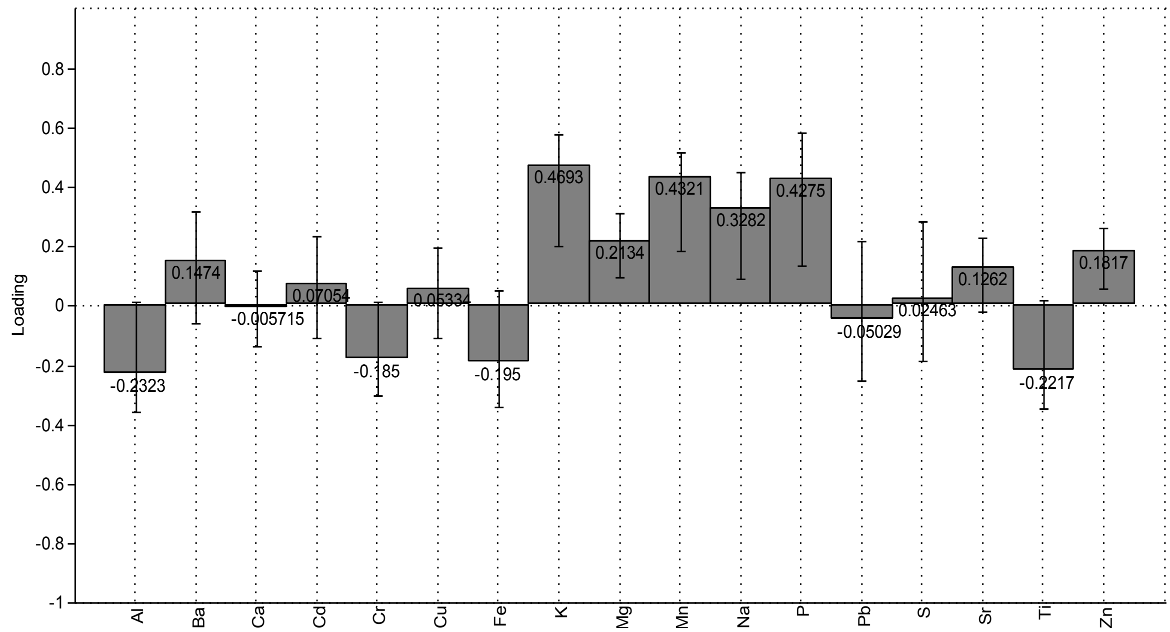


Figure 4.18 Component 3 loadings from Elemental PCA
Component loadings for each element, based on coefficients. Error bars are the 95% confidence intervals following boot strapping (n= 1000).

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Introduction

An exploratory design was used for this mensurative study. The study goals will lay the foundation for a better understanding of the local variability of the concentration of trace elements which can occur in and on lichen tissue at a single site. Typically in biomonitoring programs a sampling population has been composed of trees within a state or large region, with sampling units being individual trees using either an individual or bulk sample of lichen. The goal of this research is to shrink the sampling population and move it to an individual tree with sampling units being individual lichen specimens. Because of analytical requirements for material, specimens were often grouped into samples to get a minimum material weight based on position on tree (aspect and height) and their dry weight (used as a surrogate for age). Three major themes were explored: 1) How much variation can occur between samples on an individual tree for elemental concentration, and how does this coincide with the literature; 2) Does differing aspect show significant differences in elemental concentrations; and 3) Are there abiotic factors which can be used to explain any variability that occurs in concentration.

5.2 Data Quality

Reference samples were analyzed to show that the analytical technique was suitable with good results being obtained. The lichen reference material's (BCR® - 482) overall recovery was 92% with a precision of 3.98 % and an accuracy of 7.9 %. Two elements were notable in their poor quality of precision: silicon (20.9%) and titanium (11.2%). A similar pattern was seen with the spinach reference material (NIST 1570a). Its overall recovery was 96.6 %, with a precision of 4.27% and an accuracy of 2.96%. Silicon's precision was 27.2 % and titanium was 19.4%.

Silicon's poor precision is likely due to an interaction within the column of the ICP-AES which is glass containing silica and the reactions of HNO_3/HCl with glassware. With this poor precision Si was removed from all analysis. Ti was still included because it was decided that an 11.2% precision for the lichen reference material would suffice; however, it is important to note that Ti results may be less accurate.

5.3 Local Variation and Comparison

The primary question at hand was in regards to the extent of variation which can be seen at the local level, and how this compares to other studies. If high variability occurs at the local level, then corresponding higher variation should be required between sites located further apart to make a definitive biologically significant comparison. Most current studies have not examined what local variation does occur. A comparison will be made with the results from other studies located around the world; a comparison will be made using the mean and standard deviation. Comparisons will be made by element. Data which are being used as a baseline are located in Section 2.3.

The question which spawned this research was, —Do the current sampling strategies which use few samples spread over a large region really reveal the environmental gradients?" In this section a comparison has been made to other research. This has been by no means an all inclusive review, but was focused on establishing a baseline with which to compare future studies.

This intensive investigation into a very local site, a single tree, indicates that sampling strategies must be more intensive if quantitative conclusions are to be drawn. In comparison to these studies there were many elements with standard deviations equal to standard deviations of studies which cover large areas >1000 km^2 with few samples. Being able to find a similar variation on a single tree can only suggest that if a researcher took individual samples from trees that are spread out, and if they find significant differences in trace elemental concentrations in tissue between sites it could likely be the natural variability of the organism.

Table 5.1 Element Variation Discussion
Comparison of experimental results to other studies for variability around the mean. *Study did not mention use of Trace Metal Grade Acids, but recoveries were suitable. [USFS] Provisional Element Analysis Thresholds for Unpolluted Locations.

	Experimental Mean (S.D.) [USFS]	Concentration mg/kg (std. dev.) North American Studies International Studies	Discussion
Al	197.3 (45.34) [499]	669 (264) *F. caperata* OC (Lawrey and Hale 1998)* 955 (538) *F. caperata* DS (Lawrey and Hale 1998)* 608 (165) *E. mesomorpha* (Wetmore 1990)* 480 (101) *H. physodes* (Wetmore 1990)* 597 (142) *E. mesomorpha* (Wetmore 1993)* 535 (102) *H. physodes* (Wetmore 1993)* 671 (127) *P. sulcata* (Wetmore 1993)* 649 (395) *P. caperata* (Bargagli et al. 2002)* 1030 (898) *U. decussata* (Bargagli et al. 2000) 985 (340) *P. caperata* (Bargagli et al. 1987)*	Al concentrations were low relative to other research, but the standard deviation is almost ¼ of the mean. This large variability suggests Al may be prone to large fluctuations at the local level. Compared to the other studies they all have a standard deviation of about the same relative value to the mean. Al should be further investigated as per the amount of variability that can occur at local scales if quantitative analyses are to be done.
Ba	31.75 (4.23) [30.1]	64.6 (62) *F. caperata* OC (Lawrey and Hale 1998)* 68 (61) *F. caperata* DS (Lawrey and Hale 1998)* 28.3 (26.7) *H. physodes* (Jeran et al. 1996) 8.1 (3.53) *P. caperata* (Bargagli et al. 2002)*	Ba is comparable in concentration to other studies, but low when compared to the North American Studies which were above USFS Thresholds. When compared to the results of the Jeran et al. (1996) study there is a similar mean with a much smaller standard deviation. Based on this data set, Ba does not appear to be prone to local scale fluctuations.

Cd	0.44 (0.1) [0.3]	0.20 (0.00) *E. mesomorpha* (Wetmore 1993)* 0.64 (0.12) *H. physodes* (Wetmore 1993)* 0.34 (0.05) *P. sulcata* (Wetmore 1993)* 0.26 (0.11) *P. caperata* (Bargagli et al. 2002)* 0.19 (0.18) *U. decussata* (Bargagli et al. 2000) 0.45 (0.14) *P. caperata* (Bargagli et al.1987)* 1.05 (0.65) *H. physodes* (Jeran et al. 1996)	Cd concentrations compared to the North American studies is about in the middle of the range. This concentration is above USFS Thresholds suggesting impairment. As well it has a relatively large standard deviation. Cd results show a similar variation in concentrations to a study which covered 2250 km^2 (Bargagli et al. 2002). As well, similar values are seen in studies which cover smaller regions (Bargagli et al. 2000;1987). Cd appears to be an element which can show a high variation at a local site when compared to some of the variation seen in other studies. It should be noted a larger study (>1,000 km^2) shows a larger variation (Jeran et al. 1996).
Cr	0.33 (0.17) [4.1]	2.87 (1.16) *F. caperata* OC (Lawrey and Hale 1998)* 2.9 (0.98) *F. caperata* DS (Lawrey and Hale 1998)* 1.03 (0.21) *E. mesomorpha* (Wetmore 1990)* 0.87 (0.17) *H. physodes* (Wetmore 1990)* 1.20 (0.24) *E. mesomorpha* (Wetmore 1993)* 1.12 (0.16) *H. physodes* (Wetmore 1993)* 1.20 (0.27) *P. sulcata* (Wetmore 1993)*	Cr concentrations were low compared to all other studies, but a relatively large standard deviation of about half the mean occurred. Compared to all studies except Lawrey and Hale (1998) the variation seen around the mean is equal (absolute) to the others and is suggestive that Cr may not be greatly affected by local scale variation.
Cu	3.01 (1.48) [25.6]	8.46 (2.33) *F. caperata* OC (Lawrey and Hale 1998)* 9.74 (0.98) *F. caperata* DS (Lawrey and Hale 1998)* 2.64 (0.46) *E. mesomorpha* (Wetmore 1990)* 3.38 (0.51) *H. physodes* (Wetmore 1990)* 3.6 (0.65) *E. mesomorpha* (Wetmore 1993) 5.08 (0.23) *H. physodes* (Wetmore 1993)* 5.84 (1.00) *P. sulcata* (Wetmore 1993)* 5.77 (1.29) *P. caperata* (Bargagli et al. 2002)* 8.1 (2.6) *P. caperata* (Bargagli et al. 1987)*	Cu is on the lower end compared to North American studies in concentration with a higher variability when compared to other studies. This indicates that Cu may not be a highly suitable element for making quantitative comparisons between sites unless the very local variability is first determined.

Fe	241.53 (66.97) [272]	401 (167) *F. caperata* OC (Lawrey and Hale 1998)* 472 (280) *F. caperata* DS (Lawrey and Hale 1998)* 615 (214) *E. mesomorpha* (Wetmore 1990)* 490 (123) *H. physodes* (Wetmore 1990)* 652 (176) *E. mesomorpha* (Wetmore 1993)* 614 (110) *H. physodes* (Wetmore 1993)* 705 (130) *P. sulcata* (Wetmore 1993)* 1829 (1046) *U. decussata* (Bargagli et al. 2000) 734 (234) *P. caperata* (Bargagli et al. 1987)*	Fe results were low but showed a relatively high variability at this local site when compared with the variation that is seen in other studies. The mean value is about half the value of the next smallest in the studies reviewed. This result suggests from the large variation around a small mean and a relatively equal variation to other studies, if variation is scalable, that Fe should be further investigated at the local scale for variability of tissue concentration if better quantitative comparisons are to be made.
K	2521.89 (340.02) [3674]	2588 (822) *F. caperata* OC (Lawrey and Hale 1998)* 2926 (733) *F. caperata* DS (Lawrey and Hale 1998)* 2248 (359) *E. mesomorpha* (Wetmore 1990)* 3300 (346) *H. physodes* (Wetmore 1990)* 2178 (391) *E. mesomorpha* (Wetmore 1993)* 3125 (312) *H. physodes* (Wetmore 1993)* 3600 (236) *P. sulcata* (Wetmore 1993)* 4094 (1208) *H. physodes* (Jeran et al. 1996)	K results were typical compared to other studies for the mean: however a low standard deviation occurred, which suggests at the local level in this region, K would be a good quantitative indicator.
Mg	932.99 (129.49) [2280]	269 (103) *F. caperata* OC (Lawrey and Hale 1998)* 337 (118) *F. caperata* DS (Lawrey and Hale 1998)* 335 (65) *E. mesomorpha* (Wetmore 1990)* 695 (120) *H. physodes* (Wetmore 1990)* 343 (62) *E. mesomorpha* (Wetmore 1993)* 725 (56) *H. physodes* (Wetmore 1993)* 578 (44) *P. sulcata* (Wetmore 1993)* 608 (500) *U. decussata* (Bargagli et al. 2000)	Mg tissue concentrations were higher than any other study reviewed. This study's result is high but shows a relatively small standard deviation, which is still greater than all but one other study.

Mn	365.82 (45.77) [572]	160 (110) *F. caperata* OC (Lawrey and Hale 1998)* 223 (162) *F. caperata* DS (Lawrey and Hale 1998)* 44 (25) *E. mesomorpha* (Wetmore 1990)* 205 (94) *H. physodes* (Wetmore 1990)* 43 (19) *E. mesomorpha* (Wetmore 1993)* 302 (73) *H. physodes* (Wetmore 1993)* 255 (51) *P. sulcata* (Wetmore 1993)* 65.5 (39.4) *P. caperata* (Bargagli et al. 2002)* 25 (14) *U. decussata* (Bargagli et al. 2000)	Mn results were higher than any other study reviewed. The variability in the dataset is not very high and is lower than all but two North American studies. This suggests that Mn does not vary highly on the small scale.
Na	70.79 (26.56) [934]	28 (17) *F. caperata* OC (Lawrey and Hale 1998)* 50 (32) *F. caperata* DS (Lawrey and Hale 1998)* 37(10) *E. mesomorpha* (Wetmore 1990)* 31 (7) *H. physodes* (Wetmore 1990)* 31 (4) *E. mesomorpha* (Wetmore 1993)* 29 (5) *H. physodes* (Wetmore 1993)* 25 (4) *P. sulcata* (Wetmore 1993)* 181(99.3) *H. physodes* (Jeran et al. 1996) 175 (88) *U. decussata* (Bargagli et al. 2000)	Na values were higher than North American results but lower than the international studies and well under the USFS Thresholds. The variability is high at about 37 % of the mean. Which is equal to most other studies, Na appears to vary similarly on all scales of study.
P	375.69 (55.89) [1174]	787 (290) *F. caperata* OC (Lawrey and Hale 1998)* 854 (368) *F. caperata* DS(Lawrey and Hale 1998)* 470 (91) *E. mesomorpha* (Wetmore 1990)* 702 (136) *H. physodes* (Wetmore 1990)* 536(111) *E. mesomorpha* (Wetmore 1993)* 798 (148) *H. physodes* (Wetmore 1993)* 1371 (171) *P. sulcata* (Wetmore 1993)* 789 (507) *U. decussata* (Bargagli et al. 2000)	P results are lower with a low variability around the mean. P in this study site shows little variability at the local scale and does not indicate issues in large scale studies. Typically the variation except Lawrey and Hale (1998) is between 10 and 20%.

Pb	1.92 (1.19) [13.3]	34 (15) *F. caperata* OC (Lawrey and Hale 1998)* 41 (17) *F. caperata* DS (Lawrey and Hale 1998)* 5.46(1.07) *E. mesomorpha* (Wetmore 1990)* 20 (5) *H. physodes* (Wetmore 1990)* 6.43 (1.29) *E. mesomorpha* (Wetmore 1993)* 14 (2) *H. physodes* (Wetmore 1993)* 13 (3) *P. sulcata* (Wetmore 1993)* 3.88 (2.48) *P. caperata* (Bargagli et al. 2002)* 0.77 (1.73) *U. decussata* (Bargagli et al. 2000) 23.5(8.2) *P. caperata* (Bargagli et al.1987)*	Pb results were very low compared to other studies. The Pb variability is very high, but with such low values it is potentially not an issue. Especially because most of the reviewed studies have a very low variability around the mean.
S	1129.84 (136.73) [N.A.]	1092 (155) *E. mesomorpha* (Wetmore 1990)* 9521 (105) *H. physodes* (Wetmore 1990)* 1062(77) *E. mesomorpha* (Wetmore 1993)* 1003 (61) *H. physodes* (Wetmore 1993)* 1109 (108) *P. sulcata* (Wetmore 1993)* 892 (177) *P. caperata* (Bargagli et al. 2002)*	S values are similar to values in North America for both mean and variability. This suggests the potential that large scale studies which do not see a greater variance than this are not biologically significant between locations.
Sr	12.51 (1.95) [31.4]	14 (10) *F. caperata* OC (Lawrey and Hale 1998)* 12 (10) *F. caperata* DS (Lawrey and Hale 1998)* 22.2 (14.5) *H. physodes* (Jeran et al. 1996)	Sr results are within the typical range for North American studies but with a very small standard deviation. The results indicate Sr as a potential good element for quantitative analysis between sites.
Ti	9.1 (2.65) [40.6]	23 (12) *F. caperata* OC (Lawrey and Hale 1998)* 23 (14) *F. caperata* DS (Lawrey and Hale 1998)* 20.1 (8.2) *P. caperata* (Bargagli et al. 2002)*	Ti results have a somewhat smaller variation compared to other studies, 30% compared to 40 – 60%. The standard deviation is high and not that far off from the Lawrey and Hale (1998) study which has consistently high standard deviations, and did not report the use of trace element grade acids. Ti should be investigated further for variation at higher concentrations.

Zn	53.79 (7.95) [65.8]	41 (24) *F. caperata* OC (Lawrey and Hale 1998)* 64 (38) *F. caperata* DS (Lawrey and Hale 1998)* 29(4) *E. mesomorpha* (Wetmore 1990)* 66 (16) *H. physodes* (Wetmore 1990)* 43(5.4) *E. mesomorpha* (Wetmore 1993)* 78 (8) *H. physodes* (Wetmore 1993)* 85 (5) *P. sulcata* (Wetmore 1993)* 34.7 (6.53) *P. caperata* (Bargagli et al. 2002)* 21 (6) *U. decussatea* (Bargagli et al. 2000) 48.5 (11.3) *P. caperata* (Bargagli et al. 1987)*	Zn results were large in mean but common in standard deviation to other studies. This smaller standard deviation relative to the mean indicates Zn may be a suitable element for quantitative analysis when small variation occurs between sites.

I conclude this section with the concept that all biomonitoring studies which intend to make quantitative conclusions must utilize a sampling density which can determine local variation. Sampling density must be able to determine local variation in each region, and only then determine if statistical and biological significance occur. If the research is primarily concerned with the absence or presence, or general trends of pollutants then the current methods are effective.

5.4 Aspect

This research was conducted at a location with two distinct wind source directions (during the period prior to sampling): one which was primarily from the south and one which was primarily from the north. Analysis was conducted with a multivariate approach using a Two-Group Multivariate Permutation Test which indicated the two groups were statistically significantly different ($p = <0.0005$). *Post hoc* analysis was conducted with the Mann-Whitney-Wilcoxon rank sum test (Table 4.7) for all elements. All elements except Mn were significantly different ($p = <0.001$).

The first cluster analysis (Figure 4.5) of the elements indicated three major clusters. Cluster 1, the lowest 36 values, in which every node was placed into that cluster 24/100 times following the bootstrapping, had 25/36 values from group north. Cluster 2, the middle cluster, as well 24/100 following bootstrapping, had 48/58 from group south. Cluster 3, the top cluster, was 66/100 bootstrapped, had three group south samples. These were likely outliers in the data set because they were split from the entire 94 other samples at the first hierarchy level. This information does not present results that are different from the Two-Group Multivariate Permutation Test and *post hoc* tests indicated.

The abiotic data cluster analysis (Figure 4.6) is distinctly different from the first cluster analysis. South and north group samples are interspersed within the groupings. At a similar hierarchy level, the ratios of group south to north are different from the element clustering. Twenty-two samples from the north group are in the first cluster and 16 in the second. The aspect group samples are spread very evenly between the two groupings.

When all data are clustered (Figure 4.6) a similar pattern as the element only analysis occurs.

These data show that aspect plays a significant role for differences in trace elemental concentrations in and on lichen tissue. This may be due to the wind patterns identified but may also be due to difference in growth rates, or any other variation which can affect the accumulation of trace elements in and on lichen tissue, that is different between the two aspect groups. It is not likely any of the variables which were measured in this study aside from wind because of the completely different pattern when samples were clustered based on the abiotic variables to the clustering based on element data.

This data set which was taken from a small region highlights that aspect can cause significant difference between samples. This has been recognized in the literature and has been confirmed to exist at the very small scale.

5.5 Linear Models

Stepwise linear regression models were used to explore explanatory relationships between the abiotic variables and the element concentrations in the lichen samples. Each element had two regressions because of the significant difference between the south and north aspect groups.

Many of the elements' models did not produce results with much explanatory power. Cu, Mn, and Zn models were all below 10% variation explained by the abiotic variables. Ba, Ca, Cd, and Sr models were all below 20%, which is not very significant because over 80% of the variation in the data was unexplained by the model. Cr's north model was marginal with 23% of the variability in the data explained. Still this is not exceptional or very useful.

The other elements had either their south or north model explain at least 39% of the variability within the data by the abiotic variables measured.

Al for the southern group showed little explanatory power; only about 12% of the variation could be explained with the model. The north group model was much better with about 50% of the variance explained by the model. In this model, weight and east value are negatively related to concentration, collar height is positively related to concentration.

Fe's south model was not a good model as only 11% of the variability was explained, but Fe's north model was excellent with 58% of the variation seen in the data due to the abiotic variables. In this model, north value and east value are negatively related to concentration, height is positively related to concentration.

K models were both strong with the south model explaining 46% of the variation. In this model, weight and east value are negatively related to concentration, height is positively related to concentration. The north model explained 39% of the variability in the data set. North and weight were negatively related to concentration. Height was positively related.

Mg's south model explained 33% of the variation within the data set. Height was positively related to concentration. North aspect and weight were negatively related. The north model was better with 44% of the variability explained by the model. This was explained only with height positively related to concentration.

Na's south model was excellent with 51% of the variability explained by the model. Height and north were positively related to concentration. Weight was negatively related to concentration. The north model explained very little, only 9% of the variability.

P's south model explained 45% of the variability. Height was positively related to concentration. Weight was negatively related. The north model explained 45% of the variability. Height was positively related to concentration. East and weight were negatively related to concentration.

S's south model explained 39% of the variability. North and weight were negatively related to concentration. Height was positively related to concentration. The north model was excellent with an explanatory power of 51% of the variation. Height was positively related to concentration. Weight was negatively related to concentration.

Ti's south model was poor in explaining the concentrations (17%), but the north model was successful in explaining 49% of the variability. Height was positively related to concentration. East and weight were negatively related to concentration.

The ability to explain the variation seen within the data set was very strong for many of the elements particularly for K, Mg, P, and S. Models for these elements all explained greater than 39% of the variation in the dataset, for both the south and north models with the abiotic factors measured. It is interesting because these can all be considered macronutrients.

Increased height when significant ($\alpha = 0.05$) in models always showed an increase in concentration of elements for the lichen sampled; this could be due to the settling out of particulate matter higher on the tree. More likely, the higher up the tree the sample was collected, the closer it was to branches which would restrict rain water from directly hitting the samples, which may decrease rates of leaching. This is not always going to be the case in all trees, but with this particular tree all samples were collected below the biologically active region of the tree with foliage covered branches, see Figures 5.1 and 5.2. Since there were no branches with foliage lower on the tree, rain was more likely to directly hit the trunk. The biologically active region of the tree, which had large branches with foliage to block the rain, was the upper portion of the tree; no samples were collected from this region.

Figure 5.1 Felled Sample Tree
Lichen thalli were sampled on this 2 m long section of the tree. This section of the tree has no living branches. Compare with Figure 5.2

Figure 5.2 Transition Area of Tree
A transition zone of dead branches separates the lower section of trunk from the uppermost part of the tree that has live branches. No lichen thalli were collected from the transition zone.

5.6 Common Origins

It is suggested that correlation analysis can reveal elements with common origins. Some extremely strong correlations existed (Table 4.40) in the data set between Al and Fe (0.95, p <.000), Al and Ti (0.97, p <.000). Sr is strongly correlated to Ba (0.83, p <.000) and Ca (0.92, p <.000). K is strongly correlated to Na (0.77, p <.000) and P (0.87, p <.000). Among the other elements many correlations greater than 0.3 exist. Potential sources are hard to determine because this study is only examining samples from 1 m^2, though it should be noted that this area is not near any known sources of air pollution and is located in a relatively natural part of the world. With no known sources of pollution it is expected that the elemental concentrations will be similar to those outline by the United States Forest Service's Provisional Element Analysis Thresholds (clean sites) which were reviewed in Table 2.7. Wind normals (1971-2000) from Environment Canada (Environment Canada 2011b) for the region indicate that for 5 months the prevailing wind direction is from the west, 3 months it is from the southwest and the other 4 months is from the east. With this wind activity the region should not be affected by pollution from the major cities of the United States of America as occurs in Southern Ontario. When the wind is coming from the east it must pass over Lake Superior. When wind is blowing from the west and south west; there are large protected areas of Quetico Provincial Park and Superior National Forest/Kabetogama State Forest/Chippewa National Forest. With all of these protected areas any elevated sources would most likely be very long distance transport > 800 km or from more localized industry in the City of Thunder Bay.

Examining the Biplots of Figures 4.10, 4.12, and 4.14, K, P and Na, vectors are contained within the same space; this indicates that along with the correlation, these particular elements are from the same source. As well, Al, Fe, Ti, and Cr's vectors are also within the same space, allowing the conclusion to be drawn they are also from a similar source.

Looking at the Component 1 loadings (Figure 4.15), K, Na and P are the only negatively loaded variables, indicating a separation from the rest of the data set. In component 3 (Figure 4.17) Al, Cr, Fe, and Ti show high negative loads onto this component while all others show positive or very minimal negative loads.

In conclusion there appear to be three groupings: a) K, P and Na; b) Al, Cr, Fe, and Ti; c) other elements indicate no significant pattern. As this study was spatially small, drawing conclusions about sources would be premature until these same correlations and relationships could be seen over a larger region.

5.7 Conclusions and Suggestions

In current biomonitoring literature, as indicated in the literature review section, a common sampling technique uses single or a few samples to cover very large spatial extents. These results indicate that this is not a suitable technique because many of the variances seen in larger regional data were paralleled here with samples collected from a single tree.

Large standard deviations occurred for many elements. If our tree had been used as a sampling site in a method which only collected one sample, there would be a 64 percent chance of collecting a sample with a Pb concentration between 0.73 to 3.11 mg/kg. If the study region only has a total range of 0.68-11.20 as seen with Bargagli et al (2002), how would one know which samples are significantly different in real world significance? These results can only indicate that biomonitoring needs to move from a method where a single sample or few samples are collected to permanent stations that have been densely sampled to determine the local variability.

The division of the two samples groups based on the wind data collected from the site showed a statistically significant difference ($\alpha = 0.05$) in lichen tissue concentrations in multivariate space (Mahalanobis distance of 0.607, $p < 0.01$), which allowed *post hoc* testing of individual elements. Al, Ba, Ca, Cd, Cr, Cu, Fe, k, Mg, Na, P, Pb, S, Sr, Ti, and Zn were all significantly ($p < 0.01$) different between the two aspect groupings. The only element that had the same concentration between aspect groups was Mn. No causality was tested and since it was only one location, it may be due to difference in solar radiation, precipitation, wind or other climatic or biological controls. These results should be further explored in a research project which is designed to control for that many factors. Nonetheless there is a significant difference between the two aspect groups which were defined based on wind direction in this study on this tree.

Height of collection variation in samples was minimized to be equal to or less than 50 cm in the sample groups, by the grouping method used. The mean height for each sample, averaged over all specimens in the sample, was included in the linear regression models. It was a significant coefficient in many of the models. Each element had two models constructed, one for each of the aspect groups. Two models were constructed because aspect could only be included as a dummy variable (north or south) and could potentially inflate the significance of the model. Height is a significant coefficient ($p < 0.05$) in 21 of 32 models and mildly significant in 2 more models ($p < 0.15$). These data indicate that, at least in this situation, understanding at which height lichen tissue is collected can be an important factor. If this were a study site, a minimization in the range at which lichen could be collected from would have reduced variability in the data for many elements. Height has a positive effect on elemental concentrations in 19 of the 23 models. The only models in which there was negative effect were only able to predict less than 17% of the variability in the data set. Elements with height as a significant coefficient are included in Table 5.2.

Table 5.2 Linear regression models of which height was a significant coefficient
Any models which included height (Collar Mean) as a significant factor are included in this table. The r^2 or explanatory power of the model to predict the tissue concentration of the element in and on the lichen is also included. r^2 can be multiplied by 100 and expressed as the percentage of variation in the data set explained, i.e. Al (N) is able to explain 49% of the variation in the dataset based on the model. Table is sorted in descending order by explanatory power.

Element (S or N)	Effect on Concentration	P value of Height Coefficient	r^2 of model
Fe (N)	+	4.42E-07	0.5851
Na (S)	+	1.65E-08	0.509
S (N)	+	1.45E-05	0.5068
Al (N)	+	6.36E-06	0.4942
Ti (N)	+	3.27E-06	0.4918
K (S)	+	1.60e-09	0.4663
P (N)	+	1.23E-04	0.4507
P (S)	+	4.27E-09	0.4452
Mg (N)	+	3.82E-06	0.4365
K (N)	+	4.22E-02	0.3873
S (S)	+	6.45E-08	0.3853
Mg (S)	+	2.96E-06	0.3315
Cr (N)	+	0.00116	0.2337
Ti (S)	+	6.84E-03	0.1796
Cd (N)	-	0.0266	0.1596
Ba (N)	-	0.0141	0.1328
Fe (S)	+	0.0052	0.112
Na (N)	+	8.21E-02	0.09064
Cr (S)	+	02116	0.07378
Cd (S)	-	0.0313	0.0626
Mn (S)	+	0.1075*	0.03389
Cu (S)	-	0.162*	0.01699
Al (S)	+	0.0265	0.009

Specimen dry weights were all individually recorded and then a minimization approach was used which is outlined in 3.2.3 Sample Group Delineation. For each sample the mean weight of all specimens was used in producing linear regression models. This factor was significant in many of the models. Table 5.3 outlines the models which included weight as a significant factor, including weight's effect on concentration, the significance of the factor, and explanatory power of the model. All models except for Ca (N) had increased elemental concentrations in the lichen tissue with increased average weight of specimens in the sample. This is likely due to an increased exposure time, which was not unexpected, and by quantifying the weight there is a potential to decrease variability at sampling locations. Weight was significant at $p < 0.05$ for 10 models, $p < 0.1$ for 3 and mildly significant for two others $p = 0.116$ (Ca N) and 0.109 (S S).

Table 5.3 Linear regression models of which weight was a significant coefficient
Any models which included mean weight as a significant factor are included in this table. The r^2 or explanatory power of the model to predict the tissue concentration of the element in and on the lichen is also included. r^2 can be multiplied by 100 and expressed as the percentage of variation in the data set explained, i.e. Fe (N) is able to explain 59% of the variation in the dataset based on the model. Table is sorted in descending order by explanatory power.

Element (S or N)	Effect on Concentration	P value of Weight Coefficient	r^2 of model
Fe (N)	-	0.002858	0.5851
Na (S)	-	0.000306	0.509
S (N)	-	1.12E-05	0.5068
Al (N)	-	0.00826	0.4942
Ti (N)	-	3.75E-03	0.4918
K (S)	-	0.00386	0.4663
P (N)	-	4.70E-05	0.4507
P (S)	-	0.0247	0.4452
K (N)	-	5.42E-05	0.3873
S (S)	-	0.109	0.3853
Mg (S)	-	0.0115	0.3315
Cr (N)	-	0.07109	0.2337
Ca (N)	+	0.1164	0.1118
Cu (N)	-	0.0615	0.06863
Mn(S)	-	0.0893	0.03389

5.8 Further Research

Results from this research show relatively high variations in element concentrations based solely on one tree sampled. If time and resources were unlimited, this work would have sampled hundreds of trees with the same intensity to draw conclusions over a greater spatial distribution. This was not possible. Processing more than 1000 individual specimens collected was exhaustive. But now with this knowledge the next appropriate study should have a very detailed sampling protocol, including limited height, weight and aspect at which the samples are collected and focus on a slightly larger region, preferably about 1 km^2.

This research only examined one species of lichen, but there are many species used for biomonitoring purposes. Local variability should be tested for those other species if they are to be used for quantitative analysis.

This study has provided a cornerstone into a better methodology of lichen biomonitoring programs. Further research will need to occur to further develop appropriate methods which can be used for quantitative comparison between locations.

REFERENCES

Adamo, P., M. Arienzo, M. Pugliese, V. Roca, and P. Violante. 2004. Accumulation history of radionuclides in the lichen *Stereocaulon vesuvianum* from Mt. Vesuvius (south Italy). *Environmental Pollution* 127 (3), 455-461.

Adamo, P., R. Bargagli, S. Giordano, P. Modenesi, F. Monaci, E. Pittao, V. Spagnuolo, and M. Tretiach. 2008. Natural and pre-treatments induced variability in the chemical composition and morphology of lichens and mosses selected for active monitoring of airborne elements. *Environmental Pollution* 152 (1), 11-9.

Adamo, P., P. Crisafulli, S. Giordano, V. Minganti, P. Modenesi, F. Monaci, E. Pittao, M. Tretiach, and R. Bargagli. 2007. Lichen and moss bags as monitoring devices in urban areas. Part II: trace element content in living and dead biomonitors and comparison with synthetic materials. *Environmental Pollution* 146 (2), 392-9.

Adamo, P., S. Giordano, S. Vingiani, R. Castaldo Cobianchi, and P. Violante. 2003. Trace element accumulation by moss and lichen exposed in bags in the city of Naples (Italy). *Environmental Pollution* 122 (1), 91-103.

Armstrong, R.A. 2009. Monthly fluctuations in radial growth of individual lobes of the lichen *Parmelia conspersa* (Erhr. ex Ach.)Ach. *Symbiosis* 47 (1), 9-15.

Aslan, A., G. Budak, and A. Karabulut. 2004. The amounts Fe, Ba, Sr, K, Ca and Ti in some lichens growing in Erzurum province (Turkey). *Journal of Quantitative Spectroscopy and Radiative Transfer* 88 (4), 423-431.

Ayrault, S., R. Clochiatti, F. Carrot, L. Daudin, and J.P. Bennett. 2007. Factors to consider for trace element deposition biomonitoring surveys with lichen transplants. *The Science of the Total Environment* 372 (2-3), 717-27.

Baffi, C., M Bettinelli, G.M. Beone, and S. Spezia. 2002. Comparison of different analytical procedures in the determination of trace elements in lichens. *Chemosphere* 48 (3), 299-306.

Baptista, M.S., M.T.S.D. Vasconcelos, J.P. Cabral, M.C. Freitas, and A.M.G. Pacheco. 2008. Copper, nickel and lead in lichen and tree bark transplants over different periods of time. *Environmental Pollution* 151(2), 408-413.

Barclay-Estrup, P., and R.J.K. Rinne. 1979. Trace Element Accumulation in a Feather Moss and in Soil near a Kraft Paper Mill in Ontario. *The Bryologist* 82 (4), 599–602.

Bargagli, R., F. Borghini, and C. Celesti. 2000. Elemental composition of the lichen *Umbilicaria decussata*. *Italian Journal of Zoology* 67 (Supp. 1) 157-162.

Bargagli, R., M.L. D'Amato, and F.P. Iosco. 1987. Lichen biomonitoring of metals in the San Rossore park: Contrast with previous pine needle data. *Environmental Monitoring and Assessment* 9 (3), 285–294.

Bargagli, R., and I. Mikhailova. 2002. Accumulation of Inorganic Contaminants. In *Monitoring with Lichens - Monitoring Lichens*, ed. P. Nimis, C. Scheidegger, and P. Wolseley, 65-84. Dordrecht / Boston / London: Kluwer Academic Publishers.

Bargagli, R., F. Monaci, F. Borghini, F. Bravi, and C. Agnorelli. 2002. Mosses and lichens as biomonitors of trace metals. A comparison study on *Hypnum cupressiforme* and *Parmelia caperata* in a former mining district in Italy.*Environmental Pollution* 116 (2), 279-287.

Bargagli, R., and P.L. Nimis. 2002. Guidelines for the use of epiphytic lichens as biomonitors of atmospheric deposition of trace elements. In *Monitoring with Lichens - Monitoring Lichens*, 295-299. Dordrecht / Boston / London: Kluwer Academic Publishers.

Basile, A., S. Sorbo, G. Aprile, B. Conte, and R. Castaldo Cobianchi. 2008. Comparison of the heavy metal bioaccumulation capacity of an epiphytic moss and an epiphytic lichen. *Environmental Pollution* 151 (2), 401-407.

Bačkor, M., and S. Loppi. 2009. Interactions of lichens with heavy metals. *Biologia Plantarum* 53 (2), 214-222.

Beltrán, A.S., M. Palafox-Uribe, J. Grajales-Montiel, A. Cruz-Villacorta, and J.L. Ochoa. 1997. Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. *Toxicon* 35 (3), 447–453.

Bennett, J.P. 1995. Abnormal chemical element concentrations in lichens of Isle Royale National Park.*Environmental and Experimental Botany* 35 (3): 259–277.

Bennett, J.P., and S. Benson. 2005. Elemental content of lichens of the Point Reyes Peninsula, northern California. *The Science of the Total Environment* 343 (1-3), 199-206.

Bennett, J.P., and C.M. Wetmore. 2000. 16-year trends in elements of lichens at Theodore Roosevelt National Park, North Dakota. *The Science of the Total Environment* 263 (1-3), 231-241.

Bennett, J.P., and C.M. Wetmore 2003.Elemental chemistry of four lichen species from the Apostle Islands, Wisconsin, 1987, 1995 and 2001.*The Science of the Total Environment* 305 (1-3), 77-86.

Bergamaschi L., E. Rizzio, M.G. Valcuvia, G. Verza, A. Profumo, and M. Gallorini. 2002. Determination of trace elements and evaluation of their enrichment factors in Himalayan lichens. *Environmental Pollution* 120 (1), 137-144.

Bergamaschi, L., E. Rizzio, G. Giaveri, A. Profumo, S. Loppi, and M. Gallorini. 2004. Determination of baseline element composition of lichens using samples from high elevations. *Chemosphere* 55 (7), 933-9.

Bergamaschi, L., E. Rizzio, G. Giaveri, S. Loppi, and M. Gallorini. 2007. Comparison between the accumulation capacity of four lichen species transplanted to a urban site. *Environmental Pollution* 148 (2), 468-476.

Berlekamp, J., U. Herpin, M. Matthies, H. Lieth, B. Markert, V. Weckert, B Wolterbeek, T. Verburg, H.J. Zinner, and U. Siewers. 1998. Geographic classification of heavy metal concentrations in mosses and stream sediments in the Federal Republic of Germany. *Water, Air, & Soil Pollution* 101, 177-195.

Bermudez, G.M.A., J.H. Rodriguez, and M.L. Pignata. 2009. Comparison of the air pollution biomonitoring ability of three *Tillandsia* species and the lichen *Ramalina celastri* in Argentina. *Environmental research* 109 (1), 6-14.

Bettinelli, M, M. Perotti, S. Spezia, C. Baffi, G.M. Beone, F. Alberici, S. Bergonzi, C. Bettinelli, P. Cantarini, and L. Mascetti. 2002. The role of analytical methods for the determination of trace elements in environmental biomonitors. *Microchemical Journal* 73 (1-2), 131-152.

Białońska, D., and F.E. Dayan. 2005. Chemistry of the lichen *Hypogymnia physodes* transplanted to an industrial region. *Journal of Chemical Ecology* 31 (12), 2975-2991.

Brodo, I., S.D. Sharnoff, and S. Sharnoff. 2001. *Lichens of North America*. New Haven and London: Yale University Press.

Brunekreef, B., and S. Holgate. 2002. Air pollution and health. *The Lancet* 360 (9341), 1233-1242.

Brunialti, G., and L. Frati. 2007. Biomonitoring of nine elements by the lichen *Xanthoria parietina* in Adriatic Italy: a retrospective study over a 7-year time span. *The Science of the Total Environment* 387, 289-300.

Carreras, H.A., G.L. Gudiño, and M.L. Pignata. 1998. Comparative biomonitoring of atmospheric quality in five zones of Córdoba city (Argentina) employing the transplanted lichen *Usnea* sp. *Environmental Pollution* 103 (2-3), 317-325.

Carreras, HA, E.D. Wannaz, and M.L. Pignata. 2009. Assessment of human health risk related to metals by the use of biomonitors in the province of Córdoba, Argentina. *Environmental Pollution* 157 (1), 117-22.

Cercasov, V., A. Pantelică, M. Sălăgean, G. Caniglia, and A. Scarlat. 2002. Comparative study of the suitability of three lichen species to trace-element air monitoring. *Environmental Pollution* 119 (1), 129-39.

Chiarenzelli, J.R., L.B. Aspler, D.L. Ozarko, G.E.M. Hall, K.B. Powis, and J.A. Donaldson. 1997. Heavy metals in lichens, southern district of Keewatin, Northwest Territories, Canada. *Chemosphere* 35 (6), 1329-1341.

Clements, W.H. 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River Basin, Colorado.*Journal of the North American Benthological Society* 13 (1), 30–44.

Collins, C.R., and J.F. Farrar. 1978. Structural resistances to mass transfer in the lichen *Xanthoria parietina*. *New Phytologist* 81 (1), 71–83.

Conti, M.E., and G. Cecchetti. 2001. Biological monitoring: lichens as bioindicators of air pollution assessment—a review. *Environmental Pollution* 114 (3), 471–492.

Daillant, O., G. Kirchner, G. Pigrée, and J. Porstendörfer. 2004. Lichens as indicators of tritium and radiocarbon contamination. *The Science of the Total Environment* 323 (1-3), 253-262.

Di Lella, L.A., L. Frati, S. Loppi, G. Protano, and F. Riccobono. 2003. Lichens as biomonitors of uranium and other trace elements in an area of Kosovo heavily shelled with depleted uranium rounds. *Atmospheric Environment* 37 (38), 5445-5449.

Doornik, J.A., and H. Hansen. 2008. An Omnibus Test for Univariate and Multivariate Normality. *Oxford Bulletin of Economics and Statistics* 70, 927-939.

Ellis, J.I., and D.C. Schneider. 1997. Evaluation of a gradient sampling design for environmental impact assessment. *Environmental Monitoring and Assessment*, 48, 157-172.

Environment Canada.2011a. National Climate Data and Information Archive. Data retrieved for April and May 2010 from http://www.climate.weatheroffice.gc.ca/Welcome_e.html.

Environment Canada.2011b. Thunder Bay, ON, Canada Climate Normals. Data Retrieved May 2011 from http://www.climate.weatheroffice.gc.ca/climate_normals/results_e.html?Province=ALL&StationName=Thunder%20Bay&SearchType=BeginsWith&LocateBy=Province&Proximity=25&ProximityFrom=City&StationNumber=&IDType=MSC&CityName=&ParkName=&LatitudeDegrees=&LatitudeMinutes=&LongitudeDegrees=&LongitudeMinutes=&NormalsClass=A&SelNormals=&StnId=4055&.

Ferretti, M., and W. Erhardt. 2002. Key Issues in Designing Biomonitoring Programs. In *Monitoring with Lichens - Monitoring Lichens*, ed. P.L. Nimis, C. Scheidegger, and P. Wolseley, 111-139. Dordrecht / Boston / London: Kluwer Academic Publishers.

Folkeson, L. 1979. Interspecies calibration of heavy-metal concentrations in nine mosses and lichens: -Applicability to deposition measurements. *Water, Air, and Soil Pollution* 11(2), 253-260.

France, R., and M. Coquery. 1996. Lead concentrations in lichens from the Canadian high Arctic in relation to the latitudinal pollution gradient. *Water, Air, & Soil Pollution* 90, 469-474.

Frati, L., S. Santoni, V. Nicolardi, C. Gaggi, G. Brunialti, A Guttova, S. Gaudino, A. Pati, S.A. Pirintsos, and S. Loppi. 2007. Lichen biomonitoring of ammonia emission andnitrogen deposition around a pig stockfarm. *Environmental Pollution* 146 (2), 311-6.

Freitas, M.C., F. M. Catarino, C. Branquinho, and C. Maguas. 1993. Preparation of a lichen reference material. *Journal of Radioanalytical and Nuclear Chemistry Articles* 169(1), 47-55.

Frontasyeva, M.V., L.I. Smirnov, E. Steinnes, S.M. Lyapunov, and V.D. Cherchintsev. 2004. Heavy metal atmospheric deposition study in the South Ural Mountains. *Journal of Radioanalytical and Nuclear Chemistry* 259 (1), 19–26.

Garty, J. 2001. Biomonitoring atmospheric heavy metals with lichens: theory and application. *Critical Reviews in Plant Sciences* 20 (4), 309–371.

Garty, J., T. Levin, Y. Cohen, and H. Lehr. 2002. Biomonitoring air pollution with the desert lichen *Ramalina maciformis.Physiologia Plantarum* 115 (2), 267–275.

Godinho, R.M., T.G. Verburg, M.C. Freitas, and H.Th. Wolterbeek. 2009. Accumulation of trace elements in the peripheral and central parts of two species of epiphytic lichens transplanted to a polluted site in Portugal. *Environmental Pollution* 157 (1), 102-109.

Godinho, R.M., H.Th. Wolterbeek, T. Verburg, and M.C. Freitas. 2008. Bioaccumulation behaviour of transplants of the lichen *Flavoparmelia caperata* in relation to total deposition at a polluted location in Portugal. *Environmental Pollution* 151 (2), 318-325.

Gombert, S., J. Asta, and M.R.D. Seaward. 2003. Correlation between the nitrogen concentration of two epiphytic lichens and the traffic density in an urban area. *Environmental Pollution* 123 (2), 281-290.

Hammer, Ø., D.A.T. Harper, and P. D. Ryan. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4 (1),1-9.

Hauck, M. 2008. Metal homeostasis in *Hypogymnia physodes* is controlled by lichen substances. *Environmental Pollution* 153 (2), 304-308.

Helena, P., B. Franc, and R.L. Cvetka. 2004. Monitoring of Short-Term Heavy Metal Deposition by Accumulation in Epiphytic Lichens (*Hypogymnia Physodes* (L.) Nyl.).*Journal of Atmospheric Chemistry* 49 (1-3), 223-230.

Horvat, M., Z. Jeran, Z. Špirič, R. Jaćimović, and V. Miklavčič. 2000. Mercury and other elements in lichens near the INA Naftaplin gas treatment plant, Molve, Croatia. *Journal of Environmental Monitoring* 2 (2), 139-144.

Jeran, Z., R. Jaćimović, F. Batič, B. Smodiš, and H.T. Wolterbeek. 1996. Atmospheric heavy metal pollution in Slovenia derived from results for epiphytic lichens. *Fresenius' Journal of Analytical Chemistry* 354 (5), 681–687.

Jeran, Z., T. Mrak, R. Jaćimović, F. Batic, D. Kastelec, R. Mavsar, and P. Simoncic. 2007. Epiphytic lichens as biomonitors of atmospheric pollution in Slovenian forests. *Environmental Pollution* 146 (2), 324-31.

Kealy, D., and P.J. Haines. 2005. *Analytical Chemistry*. Oxford: BIOS Scientific Publishers Ltd.

Kershaw, L. 2001. *Trees of Ontario Including Tall Shrubs*. Aburn, WA: Lone Pine Publishing.

Kirchner, G., and O. Daillant. 2002. The potential of lichens as long-term biomonitors of natural and artificial radionuclides. *Environmental Pollution* 120 (1), 145–150.

Kubin, E., H. Lippo, J. Karhu, and J. Poikolainen. 1997. Environmental specimen banking of nationwide biomonitoring samples in Finland. *Chemosphere* 34 (9-10), 1939-1944.

Kylander, M.E., D.J. Weiss, T.E. Jeffries, B. Kober, A. Dolgopolova, R. Garcia-Sanchez, and B.J. Coles. 2007. A rapid and reliable method for Pb isotopic analysis of peat and lichens by laser ablation-quadrupole-inductively coupled plasma-mass spectrometry for biomonitoring and sample screening. *Analytica Chimica Acta*.582 (1), 116-124.

Könemann, H. 1981. Quantitative structure-activity relationships in fish toxicity studies Part 1: Relationship for 50 industrial pollutants. *Toxicology* 19 (3), 209-221.

LaPoint, T.W., S.M. Melancon, and M.K. Morris. 1984. Relationships among observed metal concentrations, criteria, and benthic community structural responses in 15 streams. *Journal* (*Water Pollution Control Federation*)56 (9), 1030-1038.

Lawrey, J.D., and M.E. Hale, Jr. 1988.Lichens as Indicators of Atmospheric Quality in the Dolly Sods and Otter Creek Wildernesses of the Monongahela National Forest, West Virginia. Final Report Submitted to Forest Supervisor, Monongahela National Forest, USDA Forest Service.

Loppi, S., and R. Bargagli. 1996. Lichen biomonitoring of trace elements in a geothermal area (Central Italy). *Water, Air, and Soil Pollution* 88, 177-187.

Loppi, S., E. Cenni, F. Bussotti, and M. Ferretti.1998a. Biomonitoring of geothermal air pollution by epiphytic lichens and forest trees.*Chemosphere* 36 (4-5), 1079–1082.

Loppi, S., F. Chiti, A. Corsini, and L. Bernardi. 1994. Lichen biomonitoring of trace metals in the Pistoia area (central northern Italy). *Environmental Monitoring and Assessment* 29 (1), 17-27.

Loppi, S., L. Frati, L. Paoli, V. Bigagli, C. Rossetti, C. Bruscoli, and A. Corsini. 2004. Biodiversity of epiphytic lichens and heavy metal contents of *Flavoparmelia caperata* thalli as indicators of temporal variations of air pollution in the town of Montecatini Terme (central Italy). *The Science of the Total Environment* 326 (1-3), 113-122.

Loppi, S., D. Ivanov, and R. Boccardi. 2002. Biodiversity of epiphytic lichens and air pollution in the town of Siena (Central Italy). *Environmental Pollution* 116 (1),123-128.

Loppi, S., G. Pacioni, N. Olivieri, and F. Di Giacomo. 1998b. accumulation of trace metals in the lichen *Evernia prunastri*transplanted at Biomonitoring Sites in Central Italy. *The Bryologist* 101 (3), 451-454.

Loppi, S., F. Riccobono, Z.H. Zhang, S. Savic, D. Ivanov, and S.A. Pirintsos. 2003. Lichens as biomonitors of uranium in the Balkan area. *Environmental Pollution* 125 (2), 277-280.

Lupsina, V., M. Horvat, Z. Jeran, and P. Stegnar. 1992. Investigation of mercury speciation in lichens. *The Analyst* 117 (3), 673-5.

Mardia, K. V. 1970. Measures of multivariate skewness and kurtosis with applications.*Biometrika* 57 (3), 519-530.

McCarthy, D.P., Craig, B. and U. Brand 2009: Lichen monitoring of urban air quality, Hamilton, Ontario. Legge, A.H., (ed.), *Air Quality and Ecological Impacts: relating sources to effects.* Developments in Environmental Science, Volume 9, Elsevier Science.Oxford, UK. p. 247-267.

McCune, B., C.C. Derr, P.S. Muir, A. Shirazi, S.C. Sillett, and W.J. Daly.1996. Lichen pendants for transplant and growth experiments. *Lichenologist* 28 (2), 161-169.

McMillan, M.A., and D.W. Larson. 2002. Effects of rock climbing on the vegetation of the Niagara Escarpment in Southern Ontario, Canada. *Conservation Biology* 16 (2), 389-398.

Mendil, D., F. Çelik, M. Tuzen, and M. Soylak. 2009. Assessment of trace metal levels in some moss and lichen samples collected from near the motorway in Turkey. *Journal of hazardous materials* 166 (2-3), 1344-1350.

Mills, N.L, K. Donaldson, P.W. Hadoke, N.A. Boon, W. MacNee, F.R. Cassee, T. Sandström, A. Blomberg, and D.E. Newby. 2009. Adverse cardiovascular effects of air pollution. *Nature clinical practice.Cardiovascular medicine* 6 (1), 36-44.

Moreira, F., R. Borges, and R. Oliveira. 2005. Comparison of two digestion procedures for the determination of lead in lichens by electrothermal atomic absorption spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy* 60 (5), 755-758.

Nash III, T.H. 2008. Introduction.In *Lichen Biology*, ed. T. H. Nash III, 1-28.Second Edition. Cambridge: Cambridge University Press.

Nimis, P.L., G. Lazzarin, A. Lazzarin, N. Skert. 2000. Biomonitoring of trace elements with lichens in Veneto (NE Italy). *The Science of the Total Environment* 255, 97-111.

Nimis, P.L, S. Andreussi, and E. Pittao. 2001. The performance of two lichen species as bioaccumulators of trace metals. *The Science of the Total Environment* 275 (1-3), 43-51.

Nimis, P.L, and O. Purvis. 2002. Monitoring lichens as indicators of pollution. In *Monitoring with Lichens - Monitoring Lichens*, ed. P.L. Nimis, C. Scheidegger, and P. Wolseley, 7 - 10. Dordrecht / Boston / London: Kluwer Academic Publishers.

Pacyna, J.M., and E.G. Pacyna. 2001. An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. *Environmental Reviews* 9 (4), 269-298.

Pfeiffer, H.N, and P. Barclay-Estrup. 1992. The use of a single lichen species, *Hypogymnia physodes*, as an Indicator of Air Quality in Northwestern Ontario. *The Bryologist* 95 (1), 38-41.

Pirintsos, S.A., T. Matsi, D. Vokou, C. Gaggi, and S. Loppi. 2006. Vertical distribution patterns of trace elements in an urban environment as reflected by their accumulation in lichen transplants. *Journal of Atmospheric Chemistry* 54 (2), 121-131.

Poblet, A., S. Andradeb, M. Scagliola, C. Vodopivezd, A. Curtosid, and A. Puccib. 1997. The use of epilithic Antarctic lichens (*Usnea aurantiacoatra* and *U. antartica*) to determine deposition patterns of heavy metals in the Shetland Islands, Antarctic. *The Science of the Total Environment* 207, 187-194.

Pope, C.A. 3rd, Dockery, D.W., Spengler, J.D., and Raizenne, M.E. 1991.Respiratory health and PM10 pollution.A daily time series.*American Review of Respiratory Disease*. 144 (3), 668-674.

Prussia, C.M., and K.T. Killingbeck. 1991. Concentrations of ten elements in two common foliose lichens: leachability, seasonality, and the influence of rock and tree bark substrates. *The Bryologist* 94 (2), 135-142.

Puckett, K.J., E. Nieboer, M.J. Gorzynski, and D.H.S. Richardson. 1973. The uptake of metal ions by lichens: a modified ion-exchange process. *New Phytologist* 72 (2), 329–342.

Purvis, O.W., P.J.Chimonides, G.C. Jones, I.N. Mikhailova, B. Spiro, D.J. Weiss, and B.J. Williamson. 2004. Lichen biomonitoring near Karabash Smelter Town, Ural Mountains, Russia, one of the most polluted areas in the world. *Proceedings.Biological sciences / The Royal Society* 271 (1536), 221-226.

Purvis, O.W., J. Longden, G. Shaw, P.D.J. Chimonides, T.E. Jeffries, G.C. Jones, I.N. Mikhailova, and B.J. Williamson. 2006. Biogeochemical signatures in the lichen *Hypogymnia physodes* in the mid Urals. *Journal of environmental radioactivity* 90 (2), 151-162.

Quevauviller, Ph., R. Herzig, and H. Muntau. 1996. Certified reference material of lichen (CRM 482) for the quality control of trace element biomonitoring. *Science of The Total Environment* 187 (2), 143-152.

R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org/.

Richardson, D.H.S., M. Shore, R. Hartree, and R.M. Richardson. 1995. The use of X-ray fluorescence spectrometry for the analysis of plants, especially lichens, employed in biological monitoring. *The Science of the Total Environment* 176, 97-105.

Rizzio, E., L. Bergamaschi, M.G. Valcuvia, A. Profumo, and M. Gallorini. 2001. Trace elements determination in lichens and in the airborne particulate matter for the evaluation of the atmospheric pollution in a region of northern Italy. *Environment International* 26, 543-549.

Rossbach, M., and S. Lambrecht. 2006. Lichens as biomonitors: global, regional and local aspects. *Croatica Chemica Acta* 79 (1), 119-124.

Rusu, A.-M., G.C. Jones, P.D.J., Chimonides, and O.W. Purvis. 2006. Biomonitoring using the lichen *Hypogymnia physodes* and bark samples near Zlatna, Romania immediately following closure of a copper ore-processing plant. *Environmental Pollution* 143 (1), 81-8.

Samecka-Cymerman, A., G. Kosior, and A.J. Kempers. 2006. Comparison of the moss *Pleurozium schreberi* with needles and bark of *Pinus sylvestris* as biomonitors of pollution by industry in Stalowa Wola (southeast Poland). *Ecotoxicology and Environmental Safety* 65 (1), 108-117.

Scerbo, R., T. Ristori, L. Possenti, L. Lampugnani, R. Barale, and C. Barghigiani. 2002. Lichen (*Xanthoria parietina*) biomonitoring of trace element contamination and air quality assessment in Pisa Province (Tuscany, Italy). *The Science of the Total Environment* 286, 27-40.

Schwartz, J., D.W. Dockery, L.M. Neas, 1996. Is daily mortality associated specifically with fine particles? *Journal of the Air & Waste Management Association*. 45(10), 927-939.

Sensen, M., and D.H.S. Richardson. 2002. Mercury levels in lichens from different host trees around a chlor-alkali plant in New Brunswick, Canada. *The Science of the Total Environment* 293 (1-3), 31-45.

Shapiro, S.S., and M.B. Wilk. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52 (3-4), 591-611.

Sloof, J., and B.T. Wolterbeek. 1993. Interspecies comparison of lichens as biomonitors of trace-element air pollution. *Environmental Monitoring and Assessment* 25 (2), 149-157.

St. Clair, SB, L.L. St. Clair, N.F. Mangelson, and D.J. Weber. 2002. Influence of growth form on the accumulation of airborne copper by lichens. *Atmospheric Environment* 36 (36-37), 5637-5644.

Stone, S.F., M.C. Freitas, R.M. Parr, and R. Zeisler. 1995. Elemental characterization of a candidate lichen research material—IAEA-336. *Fresenius' Journal of Analytical Chemistry* 352 (1), 227–231.

Tuncel, S.G., S. Yenisoy-Karakaş, and A. Dogangün. 2004. Determination of metal concentrations in lichen samples by inductively coupled plasma atomic emission spectroscopy technique after applying different digestion procedures. *Talanta* 63 (2), 273–277.

Ugur, A., B. Özden, M.M. Saç, G. Yener, Ü. Altinbaş, Y. Kurucu, and M. Bolca. 2004. Lichens and mosses for correlation between trace elements and 210 Po in the areas near coal-fired power plant at Yatağan, Turkey. *Journal of Radioanalytical and Nuclear Chemistry* 259, (1), 87-92.

USFS 2011a.2011. United States Forest Service National Lichens & Air Quality Database and Clearinghouse.Retrieved April 23, 2011 from http://gis.nacse.org/lichenair/index.php.

USFS 2011b.2011. United States Forest Service National Lichens & Air Quality Database and Clearinghouse. Provisional Element Analysis Thresholds Retrieved April 23, 2011 from http://gis.nacse.org/lichenair/index.php?page=cleansite.

USFS 2011c.2011. United States Forest Service National Lichens & Air Quality Database and Clearinghouse.Elemental Analysis Database Queries.Retrieved April 23, 2011 from http://gis.nacse.org/lichenair/index.php?page=query&type=analysis.

Wetmore, C.M. 1987. *Lichens and Air Quality in Boundary Water Canoe Area of Superior National Forest.Sulphur*. St. Paul. St. Paul. 27pp.

Wetmore, C.M. 1990. Lichens and Air Quality in Boundary Waters Canoe Area of the Superior National Forest. Final Report submitted to USDA Forest Service, Duluth, MN. 27 pp.

Wetmore, C.M. 1993. Lichens and Air Quality in Rainbow Lake Wilderness of The Chequamegon National Forest. Final Report submitted to USDA Forest Service. 27 pp.

Williamson, B, I. Mikhailova, O.W. Purvis, and V. Udachin. 2004. SEM-EDX analysis in the source apportionment of particulate matter on *Hypogymnia physodes* lichen transplants around the Cu smelter and former mining town of Karabash, South Urals, Russia. *The Science of the Total Environment* 322 (1-3), 139-154.

Wolterbeek, H, and P. Bode. 1995. Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution. *Science of The Total Environment* 176 (1-3), 33-43.

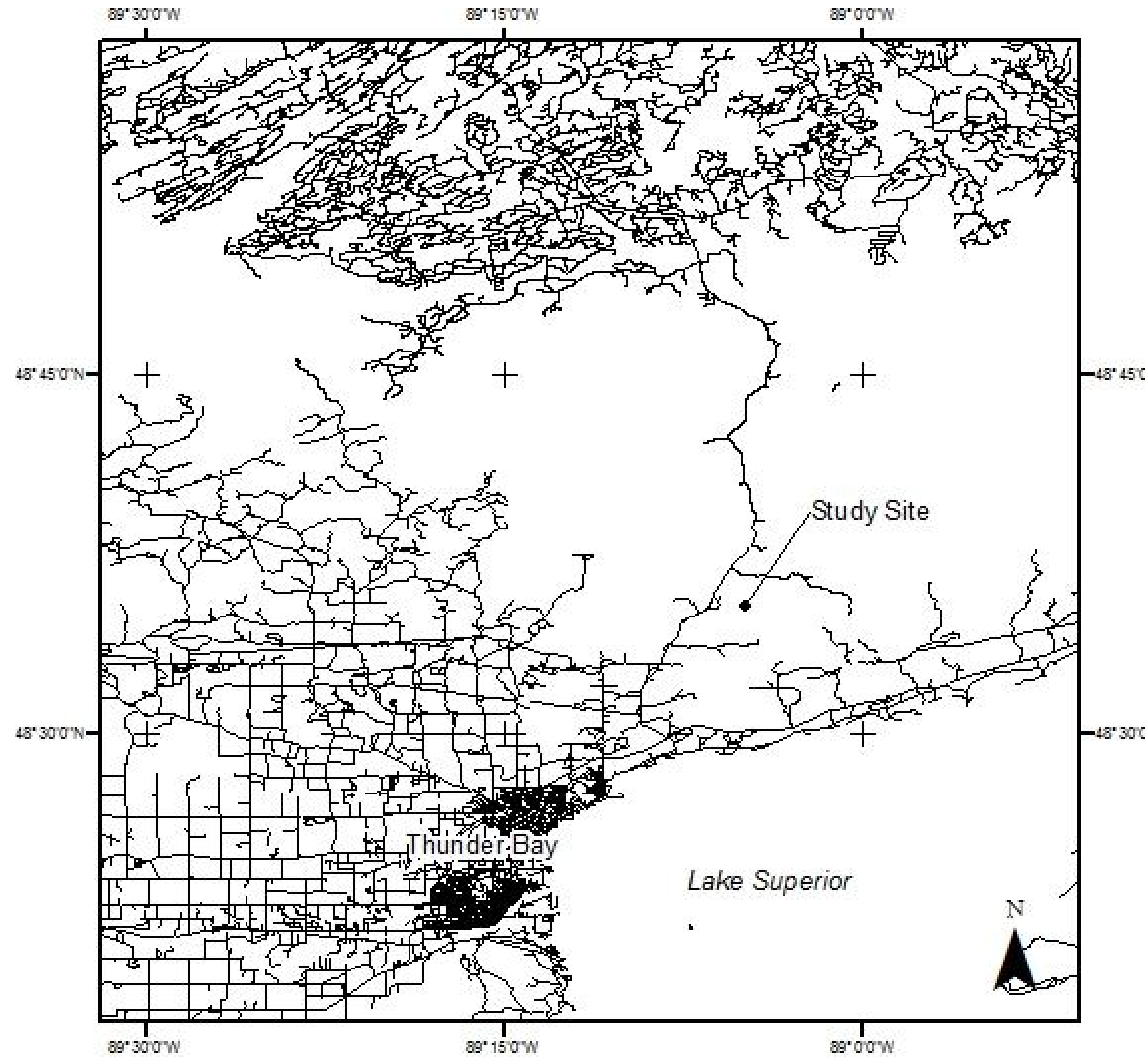
Yenisoy-Karakaş, S., and S.G. Tuncel.2004a. Geographic patterns of elemental deposition in the Aegean region of Turkey indicated by the lichen, *Xanthoria parietina* (L.) Th. Fr. *The Science of the Total Environment* 329 (1-3), 43-60.

Yenisoy-Karakaş, S., and S.G. Tuncel.2004b. Comparison of accumulation capacities of two lichen species analyzed by instrumental neutron activation analysis.*Journal of Radioanalytical and Nuclear Chemistry* 259 (1), 113-118.

Yildiz, A., A. Aksoy, G.N. Tug, C. Islek, and D. Demirezen. 2008. Biomonitoring of heavy metals by *Pseudevernia furfuracea* (L.) Zopf in Ankara (Turkey).*Journal of Atmospheric Chemistry* 60 (1), 71-81.

Zschau, T., S. Getty, C. Gries, Y. Ameron, A. Zambrano, and T.H. Nash III.2003. Historical and current atmospheric deposition to the epilithic lichen *Xanthoparmelia* in Maricopa County, Arizona. *Environmental Pollution* 125 (1), 21-30.

Appendix I: Study Site Map



Data Soure: Ontario Basic Mapping

Appendix II: Group Delineation Information

SamID	Weight	Collar	North	East	Meter	Group Number	Individual Specimen Sample
788b	0.3501	76	0.33	0.94	8.5	1	Yes
825	0.1375	79	1.00	0.00	8.5	2	No
803b	0.1422	77	1.00	0.00	8.5	2	No
826	0.0076	79	1.00	0.00	8.5	3	No
827	0.0085	79	-0.62	0.79	8.5	3	No
822	0.0089	79	0.54	0.84	8.5	3	No
818	0.0097	78	-0.78	0.62	8.5	3	No
831	0.0097	80	-0.62	0.79	8.5	3	No
803a	0.0123	77	1.00	0.00	8.5	3	No
789	0.0166	76	1.00	0.00	8.5	3	No
841	0.0171	80	-0.01	-1.00	8.5	3	No
834b	0.0211	80	1.00	0.00	8.5	3	No
829	0.0254	80	-0.62	0.79	8.5	3	No
817	0.0342	78	-0.01	-1.00	8.5	3	No
834a	0.0863	80	1.00	0.00	8.5	3	No
739	0.0151	73	-0.62	0.79	8	4	No
782b	0.0166	75	0.33	0.94	8	4	No
738a	0.0179	73	-0.62	0.79	8	4	No
755	0.0286	74	-0.62	0.79	8	4	No
771	0.0295	75	0.54	0.84	8	4	No
770	0.0337	75	-0.62	0.79	8	4	No
709	0.0365	71	0.94	-0.34	8	4	No
743	0.0382	73	1.00	0.00	8	4	No
708	0.0469	71	0.94	-0.34	8	4	No
658	0.0538	68	0.54	0.84	7.5	5	No
679	0.056	69	1.00	0.00	7.5	5	No
666	0.0843	68	1.00	0.00	7.5	5	No
634	0.1032	66	1.00	0.00	7.5	5	No
663	0.0177	68	-0.01	-1.00	7.5	6	No
670	0.0178	68	0.33	0.94	7.5	6	No
636	0.0183	66	0.33	0.94	7.5	6	No
648	0.0221	67	1.00	0.00	7.5	6	No
641	0.0376	67	0.54	0.84	7.5	6	No
705	0.0418	70	0.33	0.94	7.5	6	No
662b	0.045	68	-0.01	-1.00	7.5	6	No
637	0.0524	66	-0.78	0.62	7.5	6	No
610a	0.4754	64	0.94	-0.34	7	7	Yes
567	0.1965	61	0.33	0.94	7	8	No

579a	0.2031	62	0.33	0.94	7	8	No
616	0.1597	64	0.33	0.94	7	9	No
589	0.1864	63	0.33	0.94	7	9	No
582a	0.1253	63	-0.01	-1.00	7	10	No
617	0.1277	64	-0.78	0.62	7	10	No
618	0.0609	64	-0.78	0.62	7	11	No
583	0.0664	63	-0.01	-1.00	7	11	No
611	0.0822	64	1.00	0.00	7	11	No
614	0.0997	64	1.00	0.00	7	11	No
555	0.0459	61	-0.01	-1.00	7	12	No
579b	0.0459	62	0.33	0.94	7	12	No
612	0.0542	64	0.94	-0.34	7	12	No
623	0.059	65	-0.78	0.62	7	12	No
622	0.0608	65	-0.01	-1.00	7	12	No
554a	0.0288	61	-0.95	-0.32	7	13	No
560	0.0307	61	-0.01	-1.00	7	13	No
585	0.0337	63	1.00	0.00	7	13	No
590	0.0363	63	-0.78	0.62	7	13	No
556	0.0437	61	-0.01	-1.00	7	13	No
613	0.0441	64	0.94	-0.34	7	13	No
620	0.0443	65	-0.62	0.79	7	13	No
624b	0.0102	65	-0.78	0.62	7	14	No
600	0.0128	64	0.54	0.84	7	14	No
619b	0.013	64	-0.78	0.62	7	14	No
610b	0.0139	64	0.94	-0.34	7	14	No
565	0.0147	61	1.00	0.00	7	14	No
563	0.0152	61	0.94	-0.34	7	14	No
562	0.0155	61	-0.01	-1.00	7	14	No
561	0.0175	61	-0.01	-1.00	7	14	No
582b	0.0204	63	-0.01	-1.00	7	14	No
581	0.0213	63	-0.62	0.79	7	14	No
599a	0.0216	64	-0.95	-0.32	7	14	No
588	0.0251	63	0.94	-0.34	7	14	No
557	0.026	61	-0.01	-1.00	7	14	No
566b	0.0284	61	1.00	0.00	7	14	No
495	0.1304	57	1.00	0.00	6.5	15	No
513	0.1603	58	1.00	0.00	6.5	15	No
520	0.1139	58	-0.78	0.62	6.5	16	No
497	0.1153	57	0.33	0.94	6.5	16	No
537	0.1277	59	1.00	0.00	6.5	16	No
488	0.0734	57	0.94	-0.34	6.5	17	No
505	0.0907	58	-0.62	0.79	6.5	17	No
544b	0.1035	60	1.00	0.00	6.5	17	No

481	0.051	56	-0.78	0.62	6.5	18	No
548b	0.052	60	-0.78	0.62	6.5	18	No
511	0.0584	58	0.94	-0.34	6.5	18	No
526a	0.0602	59	-0.62	0.79	6.5	18	No
486	0.0693	57	-0.62	0.79	6.5	18	No
529	0.0428	59	-0.01	-1.00	6.5	19	No
530	0.0434	59	-0.01	-1.00	6.5	19	No
474	0.0452	56	0.54	0.84	6.5	19	No
487	0.0475	57	-0.01	-1.00	6.5	19	No
473	0.0482	56	-0.95	-0.32	6.5	19	No
507	0.0497	58	0.54	0.84	6.5	19	No
519	0.0345	58	1.00	0.00	6.5	20	No
510b	0.0347	58	-0.01	-1.00	6.5	20	No
545	0.0355	60	0.94	-0.34	6.5	20	No
548a	0.0361	60	-0.78	0.62	6.5	20	No
509	0.0398	58	-0.01	-1.00	6.5	20	No
496	0.0399	57	1.00	0.00	6.5	20	No
539b	0.0407	60	-0.62	0.79	6.5	20	No
479	0.0278	56	1.00	0.00	6.5	21	No
494	0.0292	57	1.00	0.00	6.5	21	No
498a	0.0296	57	0.33	0.94	6.5	21	No
475	0.0305	56	-0.62	0.79	6.5	21	No
541	0.0307	60	-0.95	-0.32	6.5	21	No
482	0.0314	56	-0.78	0.62	6.5	21	No
527	0.0314	59	-0.95	-0.32	6.5	21	No
543	0.0322	60	1.00	0.00	6.5	21	No
532	0.033	59	1.00	0.00	6.5	21	No
542	0.0152	60	1.00	0.00	6.5	22	No
540	0.0154	60	-0.62	0.79	6.5	22	No
549	0.0182	60	-0.78	0.62	6.5	22	No
480	0.0183	56	0.94	-0.34	6.5	22	No
500	0.0197	57	1.00	0.00	6.5	22	No
472	0.0201	56	-0.95	-0.32	6.5	22	No
506	0.0238	58	-0.62	0.79	6.5	22	No
533	0.0248	59	1.00	0.00	6.5	22	No
489	0.0249	57	-0.01	-1.00	6.5	22	No
471	0.0256	56	-0.62	0.79	6.5	22	No
539a	0.026	60	-0.62	0.79	6.5	22	No
531	0.0275	59	0.94	-0.34	6.5	22	No
437	0.0903	52	1.00	0.00	6	23	No
450b	0.0928	53	0.94	-0.34	6	23	No
423	0.1121	51	-0.78	0.62	6	23	No
463	0.043	54	0.94	-0.34	6	24	No

459a	0.0453	54	0.54	0.84	6	24	No
425a	0.0601	52	-0.62	0.79	6	24	No
460b	0.0623	54	-0.95	-0.32	6	24	No
432	0.0704	52	-0.78	0.62	6	24	No
454	0.0378	53	1.00	0.00	6	25	No
462b	0.038	54	-0.01	-1.00	6	25	No
462a	0.0384	54	-0.01	-1.00	6	25	No
426b	0.0386	52	-0.01	-1.00	6	25	No
431	0.0387	52	1.00	0.00	6	25	No
451a	0.0387	53	0.33	0.94	6	25	No
464	0.0393	54	1.00	0.00	6	25	No
452	0.0273	53	0.94	-0.34	6	26	No
425b	0.0287	52	-0.62	0.79	6	26	No
424	0.0303	52	-0.62	0.79	6	26	No
429	0.031	52	1.00	0.00	6	26	No
447	0.0347	53	-0.62	0.79	6	26	No
445	0.036	53	-0.62	0.79	6	26	No
465	0.0361	54	1.00	0.00	6	26	No
461a	0.0371	54	-0.62	0.79	6	26	No
470	0.0167	55	-0.62	0.79	6	27	No
450a	0.019	53	0.94	-0.34	6	27	No
453	0.02	53	1.00	0.00	6	27	No
443a	0.0214	53	-0.62	0.79	6	27	No
458a	0.0217	53	-0.78	0.62	6	27	No
418	0.0218	51	-0.62	0.79	6	27	No
448	0.0221	53	-0.62	0.79	6	27	No
457	0.0223	53	1.00	0.00	6	27	No
466b	0.0226	54	0.33	0.94	6	27	No
455b	0.0242	53	-0.78	0.62	6	27	No
427	0.0253	52	-0.01	-1.00	6	27	No
449	0.026	53	-0.01	-1.00	6	27	No
405	0.158	50	1.00	0.00	5.5	28	No
403	0.1671	49	-0.78	0.62	5.5	28	No
408	0.1064	50	-0.01	-1.00	5.5	29	No
398b	0.1516	49	0.94	-0.34	5.5	29	No
404a	0.0472	49	-0.78	0.62	5.5	30	No
406a	0.0714	50	-0.01	-1.00	5.5	30	No
411	0.0756	50	1.00	0.00	5.5	30	No
399b	0.0813	49	1.00	0.00	5.5	30	No
395	0.0228	49	0.54	0.84	5.5	31	No
416	0.0233	50	-0.62	0.79	5.5	31	No
407	0.0234	50	-0.01	-1.00	5.5	31	No
409	0.025	50	-0.95	-0.32	5.5	31	No

406b	0.041	50	-0.01	-1.00	5.5	31	No
410	0.0429	50	-0.62	0.79	5.5	31	No
404b	0.043	49	-0.78	0.62	5.5	31	No
397	0.044	49	-0.01	-1.00	5.5	31	No
355	0.0869	42	0.33	0.94	5	32	No
347b	0.0872	41	-0.78	0.62	5	32	No
348	0.1222	41	-0.78	0.62	5	32	No
330	0.0543	41	0.54	0.84	5	33	No
357a	0.0689	42	-0.78	0.62	5	33	No
356	0.0707	42	-0.78	0.62	5	33	No
386b	0.0854	44	0.94	-0.34	5	33	No
343	0.0361	41	0.33	0.94	5	34	No
341a	0.0365	41	1.00	0.00	5	34	No
386c	0.0367	44	0.94	-0.34	5	34	No
389a	0.0392	44	0.94	-0.34	5	34	No
342b	0.0406	41	1.00	0.00	5	34	No
347a	0.0437	41	-0.78	0.62	5	34	No
354	0.0458	42	1.00	0.00	5	34	No
370	0.0254	43	-0.01	-1.00	5	35	No
380	0.0254	44	-0.95	-0.32	5	35	No
364	0.0271	43	-0.62	0.79	5	35	No
371	0.0292	43	-0.95	-0.32	5	35	No
387	0.032	44	0.94	-0.34	5	35	No
366	0.0329	43	-0.62	0.79	5	35	No
390	0.0331	44	-0.78	0.62	5	35	No
344	0.0339	41	0.33	0.94	5	35	No
373	0.0345	43	0.94	-0.34	5	35	No
389b	0.0155	44	0.94	-0.34	5	36	No
333a	0.0156	41	-0.01	-1.00	5	36	No
392a	0.0169	44	-0.78	0.62	5	36	No
367	0.0172	43	-0.62	0.79	5	36	No
374a	0.0172	43	0.94	-0.34	5	36	No
392b	0.0185	44	-0.78	0.62	5	36	No
353	0.0186	42	1.00	0.00	5	36	No
369	0.0189	43	-0.95	-0.32	5	36	No
384	0.0198	44	-0.62	0.79	5	36	No
360	0.0231	42	-0.78	0.62	5	36	No
383	0.0231	44	-0.95	-0.32	5	36	No
372b	0.0239	43	0.94	-0.34	5	36	No
388	0.0249	44	0.94	-0.34	5	36	No
332a	0.0013	41	-0.01	-1.00	5	37	No
381c	0.0027	44	-0.62	0.79	5	37	No
381b	0.0028	44	-0.62	0.79	5	37	No

374b	0.0038	43	0.94	-0.34	5	37	No
385a	0.0041	44	-0.62	0.79	5	37	No
333b	0.0045	41	-0.01	-1.00	5	37	No
341b	0.0058	41	1.00	0.00	5	37	No
391b	0.0059	44	-0.78	0.62	5	37	No
386a	0.0063	44	0.94	-0.34	5	37	No
372a	0.0069	43	0.94	-0.34	5	37	No
391a	0.0092	44	-0.78	0.62	5	37	No
375	0.0099	43	0.94	-0.34	5	37	No
365	0.0106	43	-0.62	0.79	5	37	No
333c	0.0119	41	-0.01	-1.00	5	37	No
357b	0.0124	42	-0.78	0.62	5	37	No
352	0.0128	42	0.94	-0.34	5	37	No
385b	0.0132	44	-0.62	0.79	5	37	No
332b	0.0136	41	-0.01	-1.00	5	37	No
385c	0.014	44	-0.62	0.79	5	37	No
350	0.0143	41	1.00	0.00	5	37	No
342a	0.0144	41	1.00	0.00	5	37	No
378	0.0151	43	-0.01	-1.00	5	37	No
382	0.0151	44	0.94	-0.34	5	37	No
329	0.0152	41	-0.62	0.79	5	37	No
381a	0.0153	44	-0.62	0.79	5	37	No
257	0.0481	36	-0.62	0.79	4.5	38	No
318	0.0484	40	-0.78	0.62	4.5	38	No
314	0.0487	40	1.00	0.00	4.5	38	No
291	0.0548	38	-0.01	-1.00	4.5	38	No
288	0.055	38	-0.01	-1.00	4.5	38	No
281	0.0344	38	0.94	-0.34	4.5	39	No
289	0.0387	38	-0.01	-1.00	4.5	39	No
260	0.0398	36	-0.62	0.79	4.5	39	No
259b	0.0444	36	0.54	0.84	4.5	39	No
246a	0.0454	36	0.33	0.94	4.5	39	No
295	0.048	38	-0.95	-0.32	4.5	39	No
312b	0.0265	40	0.94	-0.34	4.5	40	No
254	0.0281	36	1.00	0.00	4.5	40	No
294c	0.0289	38	-0.62	0.79	4.5	40	No
293	0.0293	38	-0.01	-1.00	4.5	40	No
258	0.03	36	-0.62	0.79	4.5	40	No
259a	0.0302	36	0.54	0.84	4.5	40	No
317	0.0315	40	-0.78	0.62	4.5	40	No
282	0.0321	38	0.94	-0.34	4.5	40	No
285a	0.0326	38	1.00	0.00	4.5	40	No
284b	0.0233	38	-0.01	-1.00	4.5	41	No

284c	0.0233	38	-0.01	-1.00	4.5	41	No
284d	0.0233	38	-0.01	-1.00	4.5	41	No
273	0.0235	37	0.33	0.94	4.5	41	No
255b	0.0246	36	0.94	-0.34	4.5	41	No
245b	0.0248	36	0.33	0.94	4.5	41	No
251	0.0248	36	1.00	0.00	4.5	41	No
253	0.025	36	1.00	0.00	4.5	41	No
286	0.0253	38	-0.01	-1.00	4.5	41	No
287	0.0255	38	-0.01	-1.00	4.5	41	No
247	0.0262	36	-0.78	0.62	4.5	41	No
322	0.0148	40	-0.78	0.62	4.5	42	No
283	0.0152	38	1.00	0.00	4.5	42	No
274	0.0155	37	0.94	-0.34	4.5	42	No
308	0.0174	39	-0.01	-1.00	4.5	42	No
316	0.0178	40	0.94	-0.34	4.5	42	No
310	0.0182	39	1.00	0.00	4.5	42	No
292	0.0186	38	-0.95	-0.32	4.5	42	No
313	0.0188	40	0.94	-0.34	4.5	42	No
244	0.0191	36	-0.78	0.62	4.5	42	No
319b	0.0194	40	-0.78	0.62	4.5	42	No
300	0.0201	39	-0.01	-1.00	4.5	42	No
252	0.0203	36	1.00	0.00	4.5	42	No
245a	0.0215	36	0.33	0.94	4.5	42	No
284a	0.0233	38	-0.01	-1.00	4.5	42	No
255a	0.0034	36	0.94	-0.34	4.5	43	No
312a	0.0037	40	0.94	-0.34	4.5	43	No
248b	0.0045	36	-0.78	0.62	4.5	43	No
256a	0.0048	36	0.94	-0.34	4.5	43	No
312c	0.006	40	0.94	-0.34	4.5	43	No
246b	0.0068	36	0.33	0.94	4.5	43	No
294b	0.0072	38	-0.62	0.79	4.5	43	No
315b	0.0084	40	0.33	0.94	4.5	43	No
243	0.0098	36	-0.78	0.62	4.5	43	No
294a	0.0103	38	-0.62	0.79	4.5	43	No
309	0.0104	39	-0.95	-0.32	4.5	43	No
315a	0.0104	40	0.33	0.94	4.5	43	No
275	0.0107	37	0.94	-0.34	4.5	43	No
311	0.0113	40	-0.01	-1.00	4.5	43	No
280	0.0116	37	-0.78	0.62	4.5	43	No
319a	0.0116	40	-0.78	0.62	4.5	43	No
290	0.0126	38	-0.01	-1.00	4.5	43	No
256b	0.0128	36	0.94	-0.34	4.5	43	No
307	0.0135	39	1.00	0.00	4.5	43	No

245c	0.0136	36	0.33	0.94	4.5	43	No
285b	0.0137	38	1.00	0.00	4.5	43	No
276	0.0139	37	1.00	0.00	4.5	43	No
248a	0.0142	36	-0.78	0.62	4.5	43	No
177	0.0556	32	-0.01	-1.00	4	44	No
196	0.0603	32	0.54	0.84	4	44	No
205a	0.0786	33	0.94	-0.34	4	44	No
187	0.0869	32	0.94	-0.34	4	44	No
225	0.0375	34	-0.95	-0.32	4	45	No
178	0.0414	32	0.94	-0.34	4	45	No
189	0.0428	32	0.94	-0.34	4	45	No
213	0.0434	33	0.94	-0.34	4	45	No
179	0.0481	32	0.94	-0.34	4	45	No
180	0.0491	32	1.00	0.00	4	45	No
199a	0.031	32	-0.62	0.79	4	46	No
181	0.0312	32	1.00	0.00	4	46	No
214	0.0315	33	-0.62	0.79	4	46	No
186	0.032	32	-0.01	-1.00	4	46	No
222	0.0333	34	-0.01	-1.00	4	46	No
241	0.0339	35	-0.01	-1.00	4	46	No
228	0.0366	34	-0.62	0.79	4	46	No
193	0.0374	32	-0.62	0.79	4	46	No
223	0.0255	34	0.94	-0.34	4	47	No
226	0.026	34	-0.95	-0.32	4	47	No
209	0.0267	33	-0.95	-0.32	4	47	No
215	0.028	33	-0.62	0.79	4	47	No
210	0.029	33	-0.95	-0.32	4	47	No
208	0.0294	33	0.94	-0.34	4	47	No
237	0.0296	35	0.94	-0.34	4	47	No
202	0.0307	33	0.94	-0.34	4	47	No
240	0.0308	35	-0.01	-1.00	4	47	No
201	0.0158	33	0.94	-0.34	4	48	No
221	0.0166	34	-0.95	-0.32	4	48	No
190	0.0167	32	0.94	-0.34	4	48	No
188	0.0173	32	1.00	0.00	4	48	No
192	0.0188	32	-0.01	-1.00	4	48	No
206b	0.0194	33	1.00	0.00	4	48	No
207	0.0198	33	1.00	0.00	4	48	No
242	0.0221	35	-0.95	-0.32	4	48	No
218	0.0225	33	-0.62	0.79	4	48	No
231	0.0226	34	0.54	0.84	4	48	No
184	0.0237	32	-0.78	0.62	4	48	No
211	0.0237	33	-0.95	-0.32	4	48	No

203	0.0247	33	1.00	0.00	4	48	No
224a	0.0011	34	-0.01	-1.00	4	49	No
199c	0.0023	32	-0.62	0.79	4	49	No
220b	0.0048	34	-0.62	0.79	4	49	No
199b	0.005	32	-0.62	0.79	4	49	No
205b	0.0051	33	0.94	-0.34	4	49	No
227b	0.0062	34	-0.95	-0.32	4	49	No
224b	0.0079	34	-0.01	-1.00	4	49	No
235b	0.0091	35	0.54	0.84	4	49	No
182	0.0103	32	1.00	0.00	4	49	No
235a	0.0112	35	0.54	0.84	4	49	No
206a	0.0118	33	1.00	0.00	4	49	No
220a	0.012	34	-0.62	0.79	4	49	No
219	0.0126	34	-0.62	0.79	4	49	No
227a	0.0126	34	-0.95	-0.32	4	49	No
204	0.0129	33	1.00	0.00	4	49	No
236	0.0146	35	-0.62	0.79	4	49	No
212	0.0148	33	-0.01	-1.00	4	49	No
238	0.0151	35	0.94	-0.34	4	49	No
239	0.0157	35	0.94	-0.34	4	49	No
191	0.0158	32	1.00	0.00	4	49	No
160	0.012	26	1.00	0.00	3.5	50	No
162	0.0138	26	-0.62	0.79	3.5	50	No
158	0.0172	26	-0.78	0.62	3.5	50	No
161	0.0174	26	1.00	0.00	3.5	50	No
171	0.0208	27	-0.62	0.79	3.5	50	No
170	0.0222	27	-0.62	0.79	3.5	50	No
174	0.0265	27	-0.62	0.79	3.5	50	No
172	0.0267	27	-0.62	0.79	3.5	50	No
169	0.0342	27	-0.01	-1.00	3.5	50	No
173	0.0413	27	-0.62	0.79	3.5	50	No
138b	0.0308	25	1.00	0.00	3	51	No
95b	0.0335	21	1.00	0.00	3	51	No
138c	0.0359	25	1.00	0.00	3	51	No
147	0.0363	25	-0.01	-1.00	3	51	No
139	0.0391	25	1.00	0.00	3	51	No
140a	0.0402	25	1.00	0.00	3	51	No
111	0.0448	22	0.33	0.94	3	51	No
127	0.0219	24	-0.78	0.62	3	52	No
149	0.0225	25	-0.01	-1.00	3	52	No
118	0.0235	22	-0.62	0.79	3	52	No
104	0.0237	21	-0.62	0.79	3	52	No
116	0.0241	22	-0.62	0.79	3	52	No

145	0.0249	25	-0.95	-0.32	3	52	No
136	0.0271	25	0.33	0.94	3	52	No
126	0.0275	23	-0.62	0.79	3	52	No
144	0.0275	25	-0.01	-1.00	3	52	No
105a	0.0295	21	0.54	0.84	3	52	No
100	0.0164	21	-0.01	-1.00	3	53	No
128	0.0166	24	0.33	0.94	3	53	No
112	0.0179	22	1.00	0.00	3	53	No
105b	0.0185	21	0.54	0.84	3	53	No
117a	0.0185	22	-0.62	0.79	3	53	No
137	0.0185	25	0.33	0.94	3	53	No
148	0.0187	25	-0.01	-1.00	3	53	No
103	0.0188	21	-0.95	-0.32	3	53	No
94	0.0196	21	1.00	0.00	3	53	No
122	0.0196	23	1.00	0.00	3	53	No
146	0.0196	25	-0.62	0.79	3	53	No
101	0.0197	21	0.94	-0.34	3	53	No
102	0.0203	21	0.94	-0.34	3	53	No
143b	0.0206	25	-0.01	-1.00	3	53	No
143d	0.0033	25	-0.01	-1.00	3	54	No
95e	0.0052	21	1.00	0.00	3	54	No
95c	0.0071	21	1.00	0.00	3	54	No
115	0.0073	22	-0.01	-1.00	3	54	No
138e	0.0074	25	1.00	0.00	3	54	No
95d	0.0075	21	1.00	0.00	3	54	No
95a	0.0076	21	1.00	0.00	3	54	No
138d	0.0086	25	1.00	0.00	3	54	No
131b	0.0092	24	-0.95	-0.32	3	54	No
138a	0.0102	25	1.00	0.00	3	54	No
140b	0.0106	25	1.00	0.00	3	54	No
121	0.0121	23	1.00	0.00	3	54	No
129	0.0123	24	-0.78	0.62	3	54	No
113	0.0125	22	-0.01	-1.00	3	54	No
143a	0.013	25	-0.01	-1.00	3	54	No
143c	0.013	25	-0.01	-1.00	3	54	No
96	0.0135	21	-0.78	0.62	3	54	No
114	0.014	22	-0.01	-1.00	3	54	No
117b	0.0147	22	-0.62	0.79	3	54	No
131c	0.0148	24	-0.95	-0.32	3	54	No
135	0.015	25	-0.78	0.62	3	54	No
131a	0.0159	24	-0.95	-0.32	3	54	No
83	0.0305	20	-0.01	-1.00	2.5	55	No
63	0.0368	18	-0.01	-1.00	2.5	55	No

90	0.0459	20	0.54	0.84	2.5	55	No
81	0.0522	20	0.94	-0.34	2.5	55	No
64	0.0854	18	-0.01	-1.00	2.5	55	No
92b	0.0223	20	0.54	0.84	2.5	56	No
92a	0.0225	20	0.54	0.84	2.5	56	No
82	0.0229	20	-0.01	-1.00	2.5	56	No
65	0.0237	18	-0.95	-0.32	2.5	56	No
48a	0.0266	17	-0.01	-1.00	2.5	56	No
88	0.0275	20	-0.62	0.79	2.5	56	No
84	0.028	20	-0.01	-1.00	2.5	56	No
50	0.0282	17	-0.62	0.79	2.5	56	No
44	0.0285	16	1.00	0.00	2.5	56	No
78	0.0303	20	0.33	0.94	2.5	56	No
91	0.0146	20	-0.62	0.79	2.5	57	No
73	0.0147	19	1.00	0.00	2.5	57	No
48b	0.0154	17	-0.01	-1.00	2.5	57	No
71	0.0154	19	-0.78	0.62	2.5	57	No
58b	0.0172	18	-0.78	0.62	2.5	57	No
59	0.0178	18	-0.78	0.62	2.5	57	No
45a	0.0179	16	0.94	-0.34	2.5	57	No
86	0.0185	20	-0.95	-0.32	2.5	57	No
49	0.0188	17	0.94	-0.34	2.5	57	No
68a	0.0206	18	-0.62	0.79	2.5	57	No
87	0.0208	20	-0.95	-0.32	2.5	57	No
66	0.0212	18	-0.62	0.79	2.5	57	No
46	0.0216	16	-0.62	0.79	2.5	57	No
53	0.0223	17	0.54	0.84	2.5	57	No
34	0.0152	14	0.54	0.84	2	58	No
32b	0.0169	14	-0.62	0.79	2	58	No
17	0.0173	11	0.94	-0.34	2	58	No
31	0.0179	14	-0.95	-0.32	2	58	No
19	0.0225	11	-0.62	0.79	2	58	No
41	0.0431	15	-0.62	0.79	2	58	No
22a	0.0632	12	-0.01	-1.00	2	58	No
18	0.0639	11	0.94	-0.34	2	58	No
1b	0.0225	1	-0.62	0.79	1	59	No
5	0.024	1	0.94	-0.34	1	59	No
4	0.0386	1	-0.62	0.79	1	59	No
1a	0.073	1	-0.62	0.79	1	59	No
2b	0.1092	1	-0.95	-0.32	1	59	No
837	0.2617	80	0.22	-0.98	8.5	60	Yes
787b	0.1093	76	0.22	-0.98	8.5	61	No
836	0.1568	80	-0.85	-0.53	8.5	61	No

838	0.0425	80	0.22	-0.98	8.5	62	No
786	0.0488	76	-0.90	-0.43	8.5	62	No
821	0.0563	79	0.97	-0.23	8.5	62	No
809a	0.0603	77	-0.85	-0.53	8.5	62	No
832	0.0638	80	-0.90	-0.43	8.5	62	No
798	0.0096	76	0.22	-0.98	8.5	63	No
813	0.0106	77	0.44	0.90	8.5	63	No
809b	0.0143	77	-0.85	-0.53	8.5	63	No
796	0.0147	76	-0.85	-0.53	8.5	63	No
785	0.0187	76	-0.70	0.71	8.5	63	No
797	0.0243	76	-0.85	-0.53	8.5	63	No
823	0.0272	79	0.11	-0.99	8.5	63	No
810	0.028	77	0.22	-0.98	8.5	63	No
840	0.0284	80	0.22	-0.98	8.5	63	No
835	0.0392	80	-0.85	-0.53	8.5	63	No
815	0.041	78	0.11	-0.99	8.5	63	No
784	0.0684	75	0.22	-0.98	8	64	No
731a	0.0791	72	0.22	-0.98	8	64	No
729	0.1148	72	0.22	-0.98	8	64	No
760	0.0435	74	0.11	-0.99	8	65	No
780	0.0457	75	0.11	-0.99	8	65	No
746	0.0495	73	-0.85	-0.53	8	65	No
747	0.0523	73	-0.85	-0.53	8	65	No
751	0.0638	73	-0.90	-0.43	8	65	No
727	0.0148	72	-0.70	0.71	8	66	No
781	0.0151	75	-0.90	-0.43	8	66	No
753	0.0191	73	0.44	0.90	8	66	No
725	0.0198	71	-0.70	0.71	8	66	No
730	0.0208	72	0.99	-0.11	8	66	No
764	0.0212	74	0.99	-0.11	8	66	No
772a	0.0248	75	0.97	-0.23	8	66	No
719	0.0262	71	-0.85	-0.53	8	66	No
748	0.0279	73	0.97	-0.23	8	66	No
763	0.0288	74	0.44	0.90	8	66	No
762	0.0354	74	0.44	0.90	8	66	No
630	0.0525	66	0.22	-0.98	7.5	67	No
703	0.0789	70	0.44	0.90	7.5	67	No
684	0.1592	69	0.99	-0.11	7.5	67	No
675	0.0261	68	-0.85	-0.53	7.5	68	No
652	0.0268	67	0.99	-0.11	7.5	68	No
688	0.0271	69	0.22	-0.98	7.5	68	No
702	0.0283	70	-0.70	0.71	7.5	68	No
638	0.0313	66	-0.85	-0.53	7.5	68	No

697	0.033	70	0.97	-0.23	7.5	68	No
691	0.051	69	-0.90	-0.43	7.5	68	No
665	0.0522	68	-0.90	-0.43	7.5	68	No
656	0.0116	67	0.44	0.90	7.5	69	No
644b	0.012	67	0.97	-0.23	7.5	69	No
692	0.012	69	-0.90	-0.43	7.5	69	No
690	0.0125	69	0.97	-0.23	7.5	69	No
650	0.0135	67	0.22	-0.98	7.5	69	No
653	0.0136	67	-0.90	-0.43	7.5	69	No
699	0.0143	70	0.97	-0.23	7.5	69	No
700	0.0153	70	0.11	-0.99	7.5	69	No
649	0.0158	67	0.22	-0.98	7.5	69	No
689	0.0162	69	-0.85	-0.53	7.5	69	No
698	0.0222	70	0.11	-0.99	7.5	69	No
632	0.0233	66	0.22	-0.98	7.5	69	No
627	0.0245	66	0.97	-0.23	7.5	69	No
687	0.0245	69	0.44	0.90	7.5	69	No
660	0.0261	68	0.11	-0.99	7.5	69	No
626	0.1151	65	0.44	0.90	7	70	No
572	0.1646	62	-0.70	0.71	7	70	No
595	0.0393	63	0.99	-0.11	7	71	No
592	0.0408	63	0.22	-0.98	7	71	No
593	0.0595	63	-0.90	-0.43	7	71	No
594	0.0599	63	-0.90	-0.43	7	71	No
604	0.0761	64	0.11	-0.99	7	71	No
574	0.0213	62	-0.90	-0.43	7	72	No
580b	0.0231	62	0.22	-0.98	7	72	No
597	0.0248	63	0.99	-0.11	7	72	No
609	0.0267	64	-0.90	-0.43	7	72	No
601	0.0312	64	0.97	-0.23	7	72	No
605	0.0314	64	0.97	-0.23	7	72	No
558	0.0352	61	0.11	-0.99	7	72	No
580a	0.0356	62	0.22	-0.98	7	72	No
570	0.0388	62	0.11	-0.99	7	72	No
608	0.0024	64	0.44	0.90	7	73	No
573	0.0033	62	-0.70	0.71	7	73	No
621	0.0062	65	0.97	-0.23	7	73	No
559	0.008	61	-0.90	-0.43	7	73	No
603	0.0095	64	0.97	-0.23	7	73	No
602	0.0098	64	0.97	-0.23	7	73	No
571	0.0106	62	0.11	-0.99	7	73	No
575	0.0107	62	0.22	-0.98	7	73	No
625	0.011	65	-0.90	-0.43	7	73	No

606	0.0111	64	-0.70	0.71	7	73	No
598	0.0112	63	-0.70	0.71	7	73	No
568	0.0128	61	0.22	-0.98	7	73	No
591	0.0152	63	-0.85	-0.53	7	73	No
576	0.0169	62	-0.70	0.71	7	73	No
596	0.0169	63	0.99	-0.11	7	73	No
586	0.0173	63	0.97	-0.23	7	73	No
569	0.0187	61	0.44	0.90	7	73	No
607	0.0198	64	0.99	-0.11	7	73	No
551	0.0718	60	-0.70	0.71	6.5	74	No
493	0.0751	57	-0.90	-0.43	6.5	74	No
528	0.0831	59	0.11	-0.99	6.5	74	No
515	0.0893	58	0.11	-0.99	6.5	74	No
477	0.0649	56	-0.70	0.71	6.5	75	No
476a	0.0675	56	-0.90	-0.43	6.5	75	No
535	0.068	59	0.99	-0.11	6.5	75	No
516	0.0711	58	0.11	-0.99	6.5	75	No
485	0.0372	56	-0.85	-0.53	6.5	76	No
492b	0.0392	57	0.97	-0.23	6.5	76	No
521	0.0441	58	0.22	-0.98	6.5	76	No
504	0.0515	57	0.22	-0.98	6.5	76	No
552	0.0541	60	0.22	-0.98	6.5	76	No
518	0.0546	58	-0.90	-0.43	6.5	76	No
550b	0.0301	60	-0.90	-0.43	6.5	77	No
491	0.0323	57	0.11	-0.99	6.5	77	No
483	0.0336	56	-0.85	-0.53	6.5	77	No
484	0.0344	56	0.22	-0.98	6.5	77	No
490a	0.0345	57	0.97	-0.23	6.5	77	No
536	0.0345	59	-0.85	-0.53	6.5	77	No
503	0.0349	57	0.44	0.90	6.5	77	No
517	0.0361	58	-0.90	-0.43	6.5	77	No
512	0.0108	58	0.97	-0.23	6.5	78	No
490b	0.011	57	0.97	-0.23	6.5	78	No
502	0.0114	57	0.97	-0.23	6.5	78	No
522b	0.0116	58	-0.85	-0.53	6.5	78	No
534b	0.0118	59	-0.90	-0.43	6.5	78	No
522a	0.0156	58	-0.85	-0.53	6.5	78	No
476b	0.017	56	-0.90	-0.43	6.5	78	No
499	0.0178	57	0.22	-0.98	6.5	78	No
553	0.0207	60	0.22	-0.98	6.5	78	No
524a	0.0237	58	0.22	-0.98	6.5	78	No
524b	0.0245	58	0.22	-0.98	6.5	78	No
534a	0.0255	59	-0.90	-0.43	6.5	78	No

550a	0.0262	60	-0.90	-0.43	6.5	78	No
523	0.03	58	-0.90	-0.43	6.5	78	No
422	0.0545	51	0.44	0.90	6	79	No
469a	0.0579	54	0.22	-0.98	6	79	No
421	0.1387	51	0.44	0.90	6	79	No
439	0.0209	52	0.44	0.90	6	80	No
435	0.0288	52	-0.85	-0.53	6	80	No
469b	0.0294	54	0.22	-0.98	6	80	No
444	0.0347	53	0.97	-0.23	6	80	No
468	0.045	54	-0.85	-0.53	6	80	No
433	0.0452	52	0.22	-0.98	6	80	No
434	0.05	52	-0.85	-0.53	6	80	No
414	0.0412	50	-0.85	-0.53	5.5	81	No
402	0.0418	49	0.22	-0.98	5.5	81	No
415	0.0949	50	-0.85	-0.53	5.5	81	No
401	0.1071	49	0.99	-0.11	5.5	81	No
379a	0.2136	43	0.22	-0.98	5	82	No
345	0.2209	41	-0.85	-0.53	5	82	No
361a	0.0807	42	0.44	0.90	5	83	No
337b	0.0833	41	0.99	-0.11	5	83	No
336	0.0961	41	0.99	-0.11	5	83	No
362a	0.047	42	-0.90	-0.43	5	84	No
334	0.0495	41	-0.90	-0.43	5	84	No
368	0.0531	43	-0.90	-0.43	5	84	No
393	0.0688	44	-0.90	-0.43	5	84	No
337a	0.0727	41	0.99	-0.11	5	84	No
335	0.0295	41	-0.70	0.71	5	85	No
363	0.0318	42	0.22	-0.98	5	85	No
351	0.0345	42	0.11	-0.99	5	85	No
358	0.0367	42	-0.85	-0.53	5	85	No
394	0.0378	44	0.44	0.90	5	85	No
346	0.0414	41	-0.85	-0.53	5	85	No
349	0.0442	41	0.22	-0.98	5	85	No
331b	0.0036	41	0.97	-0.23	5	86	No
377a	0.0061	43	0.99	-0.11	5	86	No
362b	0.0074	42	-0.90	-0.43	5	86	No
340	0.0142	41	-0.90	-0.43	5	86	No
331a	0.0148	41	0.97	-0.23	5	86	No
361b	0.016	42	0.44	0.90	5	86	No
379b	0.0176	43	0.22	-0.98	5	86	No
377b	0.0221	43	0.99	-0.11	5	86	No
339	0.0227	41	0.44	0.90	5	86	No
338	0.0245	41	0.44	0.90	5	86	No

376	0.0251	43	0.44	0.90	5	86	No
359	0.029	42	0.22	-0.98	5	86	No
328	0.0856	40	0.22	-0.98	4.5	87	No
277	0.0888	37	0.22	-0.98	4.5	87	No
267	0.0996	36	0.99	-0.11	4.5	87	No
298a	0.0476	38	0.22	-0.98	4.5	88	No
266	0.0481	36	-0.70	0.71	4.5	88	No
249	0.0517	36	0.22	-0.98	4.5	88	No
265a	0.0524	36	0.44	0.90	4.5	88	No
323a	0.067	40	0.22	-0.98	4.5	88	No
296a	0.0396	38	0.99	-0.11	4.5	89	No
324a	0.0416	40	0.22	-0.98	4.5	89	No
279	0.0424	37	0.22	-0.98	4.5	89	No
320	0.0427	40	-0.85	-0.53	4.5	89	No
298b	0.0459	38	0.22	-0.98	4.5	89	No
321	0.0475	40	0.22	-0.98	4.5	89	No
323b	0.0321	40	0.22	-0.98	4.5	90	No
268	0.0322	36	0.99	-0.11	4.5	90	No
263a	0.0326	36	-0.90	-0.43	4.5	90	No
278b	0.0331	37	0.22	-0.98	4.5	90	No
326	0.0353	40	-0.90	-0.43	4.5	90	No
296b	0.0367	38	0.99	-0.11	4.5	90	No
325	0.0382	40	0.22	-0.98	4.5	90	No
271	0.0394	37	-0.85	-0.53	4.5	90	No
301	0.0187	39	0.11	-0.99	4.5	91	No
297	0.019	38	-0.90	-0.43	4.5	91	No
299b	0.0193	38	-0.85	-0.53	4.5	91	No
250	0.0212	36	0.22	-0.98	4.5	91	No
265b	0.022	36	0.44	0.90	4.5	91	No
304a	0.022	39	0.22	-0.98	4.5	91	No
262	0.0223	36	0.44	0.90	4.5	91	No
302	0.0248	39	0.44	0.90	4.5	91	No
264b	0.0264	36	0.44	0.90	4.5	91	No
272	0.0274	37	-0.90	-0.43	4.5	91	No
305	0.0286	39	0.22	-0.98	4.5	91	No
232	0.0128	34	-0.90	-0.43	4	92	No
229	0.0133	34	0.99	-0.11	4	92	No
198	0.017	32	0.97	-0.23	4	92	No
234	0.0183	35	0.22	-0.98	4	92	No
230	0.0185	34	-0.70	0.71	4	92	No
183	0.0197	32	-0.85	-0.53	4	92	No
195b	0.0218	32	0.97	-0.23	4	92	No
197b	0.0245	32	0.97	-0.23	4	92	No

185	0.0251	32	0.22	-0.98	4	92	No
200	0.0255	32	0.97	-0.23	4	92	No
195a	0.0272	32	0.97	-0.23	4	92	No
233	0.0331	35	0.99	-0.11	4	92	No
163	0.0118	26	0.22	-0.98	3.5	93	No
168	0.013	26	-0.90	-0.43	3.5	93	No
164	0.0156	26	-0.90	-0.43	3.5	93	No
175a	0.0157	27	0.44	0.90	3.5	93	No
166	0.0168	26	-0.90	-0.43	3.5	93	No
176a	0.0317	27	0.97	-0.23	3.5	93	No
157	0.0323	26	-0.85	-0.53	3.5	93	No
176b	0.0484	27	0.97	-0.23	3.5	93	No
167	0.0669	26	-0.90	-0.43	3.5	93	No
124	0.0346	23	0.97	-0.23	3	94	No
156	0.0376	25	0.97	-0.23	3	94	No
154	0.0401	25	0.97	-0.23	3	94	No
97	0.0484	21	0.22	-0.98	3	94	No
107a	0.0488	21	0.97	-0.23	3	94	No
132	0.0675	24	0.22	-0.98	3	94	No
152	0.0195	25	-0.90	-0.43	3	95	No
98	0.0201	21	0.22	-0.98	3	95	No
133b	0.0205	24	0.22	-0.98	3	95	No
142	0.0214	25	0.22	-0.98	3	95	No
133a	0.0255	24	0.22	-0.98	3	95	No
120c	0.0258	22	0.22	-0.98	3	95	No
125b	0.0273	23	0.97	-0.23	3	95	No
153	0.0278	25	0.97	-0.23	3	95	No
150	0.0325	25	0.44	0.90	3	95	No
134b	0.0338	24	0.22	-0.98	3	95	No
108	0.0115	21	-0.70	0.71	3	96	No
155	0.012	25	0.11	-0.99	3	96	No
123	0.0132	23	-0.90	-0.43	3	96	No
130b	0.0139	24	0.44	0.90	3	96	No
106	0.0143	21	-0.90	-0.43	3	96	No
134a	0.0147	24	0.22	-0.98	3	96	No
130a	0.0161	24	0.44	0.90	3	96	No
151b	0.0162	25	-0.90	-0.43	3	96	No
99	0.0163	21	-0.85	-0.53	3	96	No
110	0.0166	21	-0.70	0.71	3	96	No
119	0.0177	22	-0.90	-0.43	3	96	No
109	0.0182	21	0.97	-0.23	3	96	No
134c	0.0186	24	0.22	-0.98	3	96	No
141	0.0192	25	-0.85	-0.53	3	96	No

151a	0.0193	25	-0.90	-0.43	3	96	No
125a	0.0195	23	0.97	-0.23	3	96	No
43a	0.0178	16	-0.90	-0.43	2.5	97	No
47	0.0181	17	-0.85	-0.53	2.5	97	No
61	0.0194	18	0.22	-0.98	2.5	97	No
89a	0.0197	20	0.11	-0.99	2.5	97	No
62	0.0255	18	0.97	-0.23	2.5	97	No
55	0.028	17	-0.90	-0.43	2.5	97	No
54	0.0292	17	0.97	-0.23	2.5	97	No
89b	0.0292	20	0.11	-0.99	2.5	97	No
56b	0.0303	17	0.44	0.90	2.5	97	No
74	0.0461	19	-0.90	-0.43	2.5	97	No

Appendix III: ICP-AES Method Parameters

Method Report 12/13/2010 1:54:31 PM Vista PRO ELSEPA. Page 1 of 7

Method Parameters

Analysis Lines

Label	El	Wavelen.	Type	I/S	Bkg Mode	PPP	OBCL	OBCR
Al-hi	Al	237.312	Analyte	-	Fitted	2		
Al	Al	396.152	Analyte	-	Fitted	2		
As	As	188.980	Analyte	-	OPBC	2		0.020
B	B	249.772	Analyte	-	Fitted	2		
Ba	Ba	493.408	Analyte	-	Fitted	1		
Be	Be	234.861	Analyte	-	Fitted	2		
Ca-hi	Ca	317.933	Analyte	-	Fitted	2		
Ca	Ca	422.673	Analyte	-	Fitted	2		
Cd214	Cd	214.439	Analyte	-	Fitted	2		
Cd	Cd	228.802	Analyte	-	Fitted	2		
Co	Co	228.615	Analyte	-	Fitted	3		
Cr	Cr	267.716	Analyte	-	Fitted	1		
Cu	Cu	327.395	Analyte	-	Fitted	2		
Fe	Fe	259.940	Analyte	-	Fitted	2		
Fe-hi	Fe	261.187	Analyte	-	Fitted	2		
Hg	Hg	194.164	Analyte	-	Fitted	2		
K	K	766.491	Analyte	-	OPBC	2		0.093
Li	Li	670.783	Analyte	-	Fitted	2		
Mg-hi	Mg	279.800	Analyte	-	Fitted	2		
Mg	Mg	280.270	Analyte	-	Fitted	2		
Mn	Mn	257.610	Analyte	-	Fitted	2		
Mo	Mo	202.032	Analyte	-	Fitted	2		
Mo 204.598	Mo	204.598	Analyte	-	Fitted	2		
Na-hi	Na	330.237	Analyte	-	Fitted	2		
Na 568.821	Na	568.821	Analyte	-	Fitted	2		
Na	Na	588.995	Analyte	-	Fitted	1		
Ni 230.299	Ni	230.299	Analyte	-	Fitted	2		
Ni	Ni	231.604	Analyte	-	Fitted	2		
P 177.434	P	177.434	Analyte	-	OPBC	2		0.011
P	P	213.618	Analyte	-	OPBC	2		0.020
Pb 217.000	Pb	217.000	Analyte	-	Fitted	2		
Pb	Pb	220.353	Analyte	-	Fitted	2		
S	S	180.669	Analyte	-	Fitted	2		
Se	Se	196.026	Analyte	-	Fitted	2		
Se 203.985	Se	203.985	Analyte	-	Fitted	2		
Si	Si	251.611	Analyte	-	Fitted	2		
Sr	Sr	421.552	Analyte	-	Fitted	1		
Ti	Ti	334.941	Analyte	-	Fitted	2		
Ti 336.122	Ti	336.122	Analyte	-	Fitted	2		
Tl	Tl	190.794	Analyte	-	Fitted	2		
U 385.957	U	385.957	Analyte	-	Fitted	2		
V	V	292.401	Analyte	-	Fitted	2		
W 239.708	W	239.708	Analyte	-	Fitted	2		
Zn 202.548	Zn	202.548	Analyte	-	Fitted	2		
Zn	Zn	213.857	Analyte	-	OPBC	3	0.012	0.013

Conditions Sets (All lines share a single condition set)

Pwr(kW)	PlasFlow(L/min)	AuxFlow(L/min)	NebFlow(L/min)	Replicate Time(s)	Stab Time(s)	ViewHeight(mm)
1.10	15.0	1.50	0.75	15.000	20	11

Sample Uptake(s)	Rinse Time(s)	Pump Rate(rpm)	Fast Pump
35	60	15	On

Appendix IV: Sample Group Information

ID	Aspect Group	Collar	Weight	Number of Specimens	Weight Mean	Weight S.D.	Weight Range	Collar Mean	Collar S.D.	Collar Range	North Mean	North S.D.	North Range	East Mean	East S.D.	East Range
1	1	8.5	0.3501	1	0.3501	0	0	76	0	0	0.330747	0	0	0.943719	0	0
2	1	8.5	0.2797	2	0.13985	0.003323	0.0047	78	1.414214	2	1	0	0	0	0	0
3	1	8.5	0.2574	12	0.02145	0.021957	0.0787	78.83333	1.337116	4	0.240423	0.759997	1.781211	0.15206	0.651943	1.84378
4	1	8	0.263	9	0.029222	0.010918	0.0318	73.33333	1.581139	4	0.141657	0.751748	1.61882	0.472784	0.534635	1.281019
5	1	7.5	0.2973	4	0.074325	0.023737	0.0494	67.75	1.258306	3	0.884162	0.231676	0.463351	0.210951	0.421903	0.843805
6	1	7.5	0.2527	8	0.031588	0.014157	0.0347	67.5	1.309307	4	0.216722	0.5142	1.781211	0.28741	0.854617	1.943694
7	1	7	0.4754	1	0.4754	0	0	64	0	0	0.941397	0	0	-0.3373	0	0
8	1	7	0.3996	2	0.1998	0.004667	0.0066	61.5	0.707107	1	0.330747	0	0	0.943719	0	0
9	1	7	0.3461	2	0.17305	0.01888	0.0267	63.5	0.707107	1	0.330747	0	0	0.943719	0	0
10	1	7	0.253	2	0.1265	0.001697	0.0024	63.5	0.707107	1	-0.39408	0.547485	0.77426	-0.18786	1.148511	1.62424
11	1	7	0.3092	4	0.0773	0.01745	0.0388	63.75	0.5	1	0.30296	0.864716	1.781211	-0.09393	0.671905	1.62424
12	1	7	0.2658	5	0.05316	0.007053	0.0149	63.4	1.81659	4	0.095406	0.624599	1.722608	-0.15385	0.904935	1.943694
13	1	7	0.2616	7	0.037371	0.006658	0.0155	62.57143	1.618347	4	-0.05979	0.79051	1.945995	-0.1788	0.707311	1.785507
14	1	7	0.2556	14	0.018257	0.00559	0.0182	62.57143	1.504572	4	0.157557	0.738906	1.945995	-0.17558	0.6863	1.84378
15	1	6.5	0.2907	2	0.14535	0.021142	0.0299	57.5	0.707107	1	1	0	0	0	0	0
16	1	6.5	0.3569	3	0.118967	0.007596	0.0138	58	1	2	0.183179	0.899728	1.781211	0.522661	0.479994	0.943719
17	1	6.5	0.2676	3	0.0892	0.015106	0.0301	58.33333	1.527525	3	0.440859	0.918177	1.61882	0.149411	0.576134	1.122832
18	1	6.5	0.2909	5	0.05818	0.007374	0.0183	58	1.581139	4	-0.37173	0.738539	1.722608	0.496459	0.473009	1.122832
19	1	6.5	0.2768	6	0.046133	0.002768	0.0069	57.5	1.378405	3	0.017742	0.54206	1.482644	-0.27275	0.903615	1.84378
20	1	6.5	0.2612	7	0.037314	0.002703	0.0062	58.71429	1.253566	3	0.218209	0.768792	1.781211	-0.13249	0.706819	1.785507
21	1	6.5	0.2758	9	0.030644	0.001597	0.0052	57.77778	1.715938	4	0.115414	0.920092	1.945995	0.189462	0.472294	1.267898
22	1	6.5	0.2595	12	0.021625	0.004305	0.0123	58.16667	1.696699	4	0.056113	0.840545	1.945995	0.147303	0.597735	1.785507
23	1	6	0.2952	3	0.0984	0.01193	0.0218	52	1	2	0.386729	1.01189	1.781211	0.095655	0.487867	0.961565

24	1	6	0.2811	5	0.05622	0.011695	0.0274	53.2	1.095445	2	-0.1736	0.853182	1.887392	0.318425	0.598052	1.181105
25	1	6	0.2695	7	0.0385	0.000497	0.0015	53.14286	0.899735	2	0.472842	0.507359	1.006951	-0.29374	0.740097	1.943694
26	1	6	0.2612	8	0.03265	0.003772	0.0098	52.875	0.834523	2	-0.01909	0.827907	1.61882	0.448795	0.476256	1.122832
27	1	6	0.2631	12	0.021925	0.002588	0.0093	53	0.953463	4	-0.06496	0.719686	1.781211	0.249761	0.707323	1.943694
28	1	5.5	0.3251	2	0.16255	0.006435	0.0091	49.5	0.707107	1	0.109395	1.259506	1.781211	0.312133	0.441422	0.624265
29	1	5.5	0.258	2	0.129	0.031961	0.0452	49.5	0.707107	1	0.467223	0.670583	0.948348	-0.66864	0.468582	0.662675
30	1	5.5	0.2755	4	0.068875	0.015009	0.0341	49.5	0.57735	1	0.30296	0.864716	1.781211	-0.09393	0.671905	1.62424
31	1	5.5	0.2654	8	0.033175	0.010261	0.0212	49.625	0.517549	1	-0.30613	0.508426	1.482644	-0.03562	0.880797	1.84378
32	1	5	0.2963	3	0.098767	0.020294	0.0353	41.33333	0.57735	1	-0.41056	0.641989	1.111958	0.73075	0.184437	0.319454
33	1	5	0.2793	4	0.069825	0.012718	0.0311	42.25	1.258306	3	-0.02109	0.893126	1.722608	0.438759	0.527622	1.181105
34	1	5	0.2786	7	0.0398	0.003795	0.0097	42	1.414214	3	0.63319	0.669033	1.781211	0.127626	0.481981	1.281019
35	1	5	0.2735	9	0.030389	0.003666	0.0091	43.11111	0.927961	3	-0.18936	0.768534	1.887392	0.090679	0.696249	1.943694
36	1	5	0.2532	13	0.019477	0.003231	0.0094	43.15385	0.987096	3	-0.05497	0.860825	1.945995	0.034333	0.581173	1.785507
37	1	5	0.2411	25	0.009644	0.004749	0.014	42.68	1.314027	3	0.052777	0.739999	1.781211	0.045335	0.699303	1.785507
38	1	4.5	0.255	5	0.051	0.003567	0.0069	38.4	1.67332	4	-0.08279	0.699832	1.781211	-0.11803	0.856886	1.785507
39	1	4.5	0.2507	6	0.041783	0.00503	0.0136	37	1.095445	2	0.039505	0.714443	1.887392	0.151934	0.812402	1.943694
40	1	4.5	0.2692	9	0.029911	0.001967	0.0061	37.77778	1.563472	4	0.26596	0.774601	1.781211	0.151618	0.64773	1.84378
41	1	4.5	0.2696	11	0.024509	0.001014	0.0029	37	1	2	0.253357	0.548586	1.781211	-0.25686	0.809857	1.943694
42	1	4.5	0.26	14	0.018571	0.002392	0.0085	38.28571	1.540658	4	0.203176	0.780339	1.945995	-0.10854	0.640977	1.943694
43	1	4.5	0.2252	23	0.009791	0.003505	0.0108	37.78261	1.650249	4	0.168164	0.763272	1.945995	0.179102	0.632273	1.943694
44	1	4	0.2814	4	0.07035	0.014838	0.0313	32.25	0.5	1	0.603123	0.449247	0.948348	-0.20769	0.767454	1.84378
45	1	4	0.2623	6	0.043717	0.004316	0.0116	32.5	0.83666	2	0.636599	0.775664	1.945995	-0.2789	0.136732	0.3373
46	1	4	0.2669	8	0.033363	0.002469	0.0064	33	1.195229	3	-0.18702	0.567211	1.61882	0.017775	0.883626	1.785507
47	1	4	0.2557	9	0.028411	0.001971	0.0053	33.66667	0.866025	2	0.033537	0.908467	1.887392	-0.2818	0.457258	1.785507
48	1	4	0.2637	13	0.020285	0.003096	0.0089	33	0.912871	3	0.167267	0.883871	1.945995	-0.03027	0.520387	1.84378
49	1	4	0.2009	20	0.010045	0.004595	0.0147	33.65	1.089423	3	0.113586	0.763104	1.945995	0.087031	0.669888	1.84378
50	1	3.5	0.2321	10	0.02321	0.009201	0.0293	26.6	0.516398	1	-0.25011	0.689801	1.781211	0.433748	0.597276	1.785507
51	1	3	0.2606	7	0.037229	0.00461	0.014	24	1.732051	4	0.760542	0.420411	1.006951	-0.00804	0.561264	1.943694

52	1	3	0.2522	10	0.02522	0.002523	0.0076	23.3	1.702939	4	-0.3349	0.506936	1.482644	0.322979	0.783326	1.943694
53	1	3	0.2633	14	0.018807	0.001238	0.0042	22.64286	1.780542	4	0.276889	0.671784	1.945995	0.021686	0.736585	1.943694
54	1	3	0.2348	22	0.010673	0.003536	0.0126	23.27273	1.6671	4	0.143539	0.800165	1.945995	-0.19609	0.58778	1.785507
55	1	2.5	0.2508	5	0.05016	0.021384	0.0549	19.2	1.095445	2	0.291439	0.432921	0.948348	-0.49868	0.803461	1.84378
56	1	2.5	0.2605	10	0.02605	0.002925	0.008	18.8	1.619328	4	0.019956	0.609401	1.945995	0.087829	0.854826	1.943694
57	1	2.5	0.2568	14	0.018343	0.002663	0.0077	18.07143	1.384768	4	-0.2356	0.756298	1.945995	0.252557	0.613568	1.84378
58	1	2	0.26	8	0.0325	0.021138	0.0487	12.75	1.669046	4	-0.04875	0.763938	1.887392	0.150206	0.728529	1.84378
59	1	1	0.2673	5	0.05346	0.037202	0.0867	1	0	0	-0.37221	0.747871	1.887392	0.339023	0.611425	1.122832
60	2	8.5	0.2617	1	0.2617	0	0	80	0	0	0.220584	0	0	-0.97537	0	0
61	2	8.5	0.2661	2	0.13305	0.033588	0.0475	78	2.828427	4	-0.31347	0.75526	1.068099	-0.75307	0.314378	0.444597
62	2	8.5	0.2717	5	0.05434	0.008656	0.0213	78.4	1.81659	4	-0.2917	0.854325	1.876495	-0.51882	0.278028	0.748008
63	2	8.5	0.256	11	0.023273	0.010749	0.0314	77.45455	1.572491	4	-0.25285	0.550098	1.284081	-0.49349	0.67763	1.893874
64	2	8	0.2623	3	0.087433	0.024297	0.0464	73	1.732051	3	0.220584	0	0	-0.97537	0	0
65	2	8	0.2548	5	0.05096	0.007939	0.0203	73.6	0.894427	2	-0.47653	0.533643	1.010207	-0.69605	0.275248	0.563901
66	2	8	0.2539	11	0.023082	0.006229	0.0206	73.09091	1.445998	4	0.189517	0.810757	1.896116	0.224862	0.589528	1.430442
67	2	7.5	0.2906	3	0.096867	0.055573	0.1067	68.33333	2.081666	4	0.550194	0.398756	0.772847	-0.06338	0.938562	1.875039
68	2	7.5	0.2758	8	0.034475	0.01083	0.0261	68.375	1.407886	4	-0.25215	0.848359	1.896116	-0.31622	0.486005	1.684927
69	2	7.5	0.2574	15	0.01716	0.005326	0.0145	68.06667	1.437591	4	0.206654	0.658051	1.876495	-0.42734	0.630057	1.893874
70	2	7	0.2797	2	0.13985	0.035002	0.0495	63.5	2.12132	3	-0.13404	0.806957	1.14121	0.804616	0.13443	0.190112
71	2	7	0.2756	5	0.05512	0.01531	0.0368	63.2	0.447214	1	-0.09677	0.810861	1.896116	-0.58892	0.38375	0.879771
72	2	7	0.2681	9	0.029789	0.006124	0.0175	62.66667	1.118034	3	0.199099	0.731169	1.896116	-0.59654	0.381376	0.879771
73	2	7	0.2114	18	0.011744	0.005007	0.0174	62.94444	1.304843	4	0.101804	0.782327	1.896116	-0.0465	0.658426	1.893874
74	2	6.5	0.3193	4	0.079825	0.0079	0.0175	58.5	1.290994	3	-0.34807	0.53225	1.010207	-0.42729	0.803164	1.703762
75	2	6.5	0.2715	4	0.067875	0.002543	0.0062	57.25	1.5	3	-0.12659	0.865202	1.896116	-0.20734	0.711384	1.703762
76	2	6.5	0.2807	6	0.046783	0.007661	0.0174	57.66667	1.36626	4	-0.01911	0.724598	1.876495	-0.68576	0.331973	0.748008
77	2	6.5	0.2704	8	0.0338	0.001845	0.006	57.5	1.414214	4	-0.22024	0.744246	1.876495	-0.40242	0.590286	1.893874
78	2	6.5	0.2576	14	0.0184	0.006667	0.0192	58.07143	1.141139	4	-0.17176	0.791166	1.876495	-0.5569	0.291911	0.748008
79	2	6	0.2511	3	0.0837	0.047662	0.0842	52	1.732051	3	0.364572	0.124697	0.215982	0.274659	1.082554	1.875039

80	2	6	0.254	7	0.036286	0.010694	0.0291	52.71429	0.95119	2	-0.09871	0.744182	1.821325	-0.4101	0.63661	1.875039
81	2	5.5	0.285	4	0.07125	0.034712	0.0659	49.5	0.57735	1	-0.12025	0.897085	1.840946	-0.53783	0.35157	0.860936
82	2	5	0.4345	2	0.21725	0.005162	0.0073	42	1.414214	2	-0.31347	0.75526	1.068099	-0.75307	0.314378	0.444597
83	2	5	0.2601	3	0.0867	0.008244	0.0154	41.33333	0.57735	1	0.807809	0.321506	0.556865	0.223603	0.585493	1.014103
84	2	5	0.2911	5	0.05822	0.011723	0.0257	42.2	1.303841	3	-0.52346	0.847969	1.896116	-0.36713	0.141261	0.31587
85	2	5	0.2559	7	0.036557	0.005166	0.0147	41.85714	1.069045	3	-0.20206	0.569613	1.284081	-0.34246	0.811015	1.893874
86	2	5	0.2031	12	0.016925	0.00814	0.0254	41.91667	0.900337	2	0.359712	0.661064	1.896116	0.008648	0.716006	1.875039
87	2	4.5	0.274	3	0.091333	0.007336	0.014	37.66667	2.081666	4	0.4782	0.446203	0.772847	-0.68839	0.497062	0.860936
88	2	4.5	0.2668	5	0.05336	0.007915	0.0194	37.2	1.788854	4	0.078735	0.447797	1.14121	-0.26337	0.977251	1.875039
89	2	4.5	0.2597	6	0.043283	0.002903	0.0079	38.83333	1.32916	3	0.171375	0.587128	1.840946	-0.75778	0.361886	0.860936
90	2	4.5	0.2796	8	0.03495	0.002883	0.0073	38	1.772811	4	-0.00053	0.798905	1.896116	-0.56829	0.368123	0.860936
91	2	4.5	0.2517	11	0.022882	0.003454	0.0099	37.54545	1.368476	3	-0.01249	0.571502	1.339251	-0.15573	0.864024	1.893874
92	2	4	0.2568	12	0.0214	0.005959	0.0203	33	1.279204	3	0.40352	0.791337	1.896116	-0.29733	0.436604	1.684927
93	2	3.5	0.2522	9	0.028022	0.018972	0.0551	26.33333	0.5	1	-0.20594	0.846448	1.876495	-0.30915	0.503277	1.875039
94	2	3	0.277	6	0.046167	0.011925	0.0329	23.16667	1.834848	4	0.722735	0.388964	0.753226	-0.4767	0.38627	0.748008
95	2	3	0.2542	10	0.02542	0.005093	0.0143	23.8	1.398412	4	0.280501	0.51717	1.876495	-0.58375	0.614483	1.875039
96	2	3	0.2573	16	0.016081	0.002599	0.008	23.0625	1.652019	4	-0.26552	0.719374	1.876495	-0.21214	0.652659	1.893874
97	2	2.5	0.2633	10	0.02633	0.008557	0.0283	17.9	1.37032	4	-0.07358	0.765045	1.876495	-0.43405	0.557197	1.893874

ID	Al	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	P	Pb	S	Si	Sr	Ti	Zn
1	160.54	31.47	6398.3	0.4	0.33	2.11	172.92	2566.2	995.8	428.06	68.3	450.7	3.052	1051.2	190.4	11.8	7.4	55.91
2	135.02	34.14	7943.2	0.41	0.6	2.2	148.19	2873.2	1016.1	447.95	126.6	442	2.512	1030.6	165.2	14.1	5.8	65.31
3	224.88	25.26	6143	0.36	0.72	2.82	274.25	3267.4	1061.9	392.16	100.2	506.8	2.948	1250	248	11.3	11	51.34
4	192.04	29.56	5483.8	0.37	0.25	2.85	231.84	3202.7	986.9	366.67	111.2	523.1	1.617	1281.1	218.3	11	9.2	53.1
5	221.95	34.26	5441.5	0.37	0.35	2.77	268.9	3168.3	921.7	384.76	113.6	450.5	2.268	1295.1	242.3	12	11.1	57.12
6	207.68	30.2	4615.9	0.35	0.3	2.73	282.68	2854.9	866.2	320.49	96.3	403.7	2.902	1223.2	224.1	10.7	10.1	48.13
7	170.02	20.42	4739.1	0.35	0.29	2.18	241.02	2717.2	628.4	254.85	87.8	401.7	2.282	1183.6	204.4	8.8	8.7	39.78
8	214.68	24.3	3996.4	0.32	0.23	2.08	258.37	2353.2	815.5	283.98	84.3	343.2	3.07	1042.4	219.5	9.4	10.4	35.06
9	192.28	30.7	4663	0.49	0.27	1.88	243.75	2389.7	850.5	348.16	53.7	350.6	0.919	918.4	209.6	11.1	9.4	45.87
10	212.87	31.81	5163	0.32	0.29	2.27	261.64	2714.5	964	388.36	58.3	393.3	2.169	1111.4	211.6	12.2	9.9	52.51
11	191.16	27.46	5166.7	0.23	0.16	2.35	214.52	2641.4	819.9	323.74	74.6	372.6	2.437	1087.9	219.2	10.7	8.9	44.1
12	174.75	29.22	5374.4	0.54	0.18	2.44	202.71	2732.8	892	358.25	95.7	401.8	4.126	1176.1	208.6	11.8	8.9	46.19
13	162.01	25.85	4291.3	0.28	0.17	2.51	194.05	3065.5	885.7	323.79	88.4	455	2.815	1168.9	173.9	10	7.2	44.05
14	147.54	25.55	4124.4	0.28	0.12	2.34	173.65	2764.8	842.2	331.65	92.6	423.2	2.315	1113.9	170	10	6.8	40.7
15	177.56	26.9	3146.3	0.28	0.13	2.22	218.05	2428	750.7	306.95	90.8	374.3	2.11	1037.3	170.9	9.4	8	47.13
16	177.55	25.49	4956.3	0.28	0.28	2.44	226.34	2299.8	733.4	261.53	59.7	343.1	3.01	1012.4	187.9	10	8.2	37.61
17	146.26	26.22	5380.4	0.4	0.14	2.44	188.77	2851.4	906.6	360.51	105.2	413.3	2.548	1107.2	156.8	11.3	6.8	46.7
18	238.53	26.15	4751.2	0.33	0.24	2.87	308.57	2618.4	946.3	327.05	82.7	435	2.126	1222.2	218.8	10.8	12.1	44.12
19	172.51	27.4	6455.2	0.32	0.15	2.55	204.35	3451.5	961.1	388.18	93.3	505.1	2.152	1260	189.7	12.6	8.2	51.33
20	142.72	31.65	5402.8	0.4	0	2	164.27	3168.7	958.3	404.96	68.2	499	1.964	1078.4	174.7	12	6.5	48.04
21	175.75	28.62	4987.6	0.61	0.3	2.31	206.09	3038.6	902.1	348.01	98.3	479.1	2.923	1149	180.3	11.4	8	46.13
22	154.98	26.82	4428.4	0.34	0.24	2.96	181.19	3252.4	884.1	342.84	99.2	512.1	3.083	1210.4	180.5	10.4	6.6	46.4
23	247.26	36.44	7171.4	0.42	0.31	2.61	292.86	2721.4	984.6	384.29	70.8	403.3	3.941	1140.8	241.3	14.2	11.3	56.7
24	217.56	29.68	8196.3	0.57	0.28	2.76	262.56	3213.4	856.7	342.56	93.7	470	3.781	1372	231.3	13.8	11	51.49
25	169.98	27.33	5647.1	0.32	0.12	2.46	203.31	2840.7	795.5	331.25	88	438.5	2.402	1121.3	183.5	11.3	8.4	46.1
26	199.51	39.78	6057	0.47	0.17	2.94	245.39	3339.8	1026.3	421	112.9	482.3	0	1291.3	205	13.9	9.3	62.31
27	155.46	29.76	5998.8	0.4	0.11	2.79	204.13	2851.9	882.9	355.46	95.7	478.9	4.017	1140.7	160.4	12.5	6.9	48.69

28	233.29	37.24	8126.2	0.53	0.26	2.67	270.79	2727.2	1039.1	461.3	83.7	405.5	2.548	963.5	220.6	15.1	10.9	65.34
29	127.6	25.54	5360.1	0.35	0.41	3.08	164.48	3065.6	795.3	338.86	93.1	464.1	1.658	987.6	79.7	10.5	0	47.03
30	220.77	38.44	7981.9	0.57	0.36	2.57	375.6	2520.5	979.2	454.11	87.7	381.6	1.836	1022.9	195.4	15.2	10.2	67
31	186.04	31.02	5804.6	0.47	0.33	2.63	249.39	2883.5	1004.4	430.46	92.9	431.4	2.864	1149.2	183	12.6	8.6	56.07
32	231.77	37.16	8730.3	0.47	0.47	2.61	334.24	2097.3	902.8	384.36	52.3	332.8	3.461	1055.8	198.6	15.1	10.5	59.03
33	175.37	35.06	6267.4	0.36	0.2	2.25	216.04	2109.5	873	353.48	64.5	310.1	1.43	1003.2	190.9	12.7	8.5	46.38
34	194.81	30	4877.4	0.34	0.34	2.43	210.14	2640.3	786.3	317.81	83.1	337	0	1049.4	259.8	10.4	9.2	52.71
35	180	31.9	5080	0.4	0.21	2.23	193	2847.5	915.8	385.75	87.9	396.9	0	1015.8	236.9	11.7	8.2	52.91
36	176.88	27.66	5228.3	0.32	0.22	2.18	185.55	2608.4	795.1	323.84	79.3	360.7	0.708	1019.4	217.3	10.2	7.4	44.54
37	165.57	29.04	4094.3	0.28	0.09	2.23	188.47	2775.4	890.7	375.3	105.5	385.3	0	974.9	211.2	10.5	6.9	51.15
38	211.14	32.54	7790.2	0.49	0.23	2.45	238.99	2183.9	878	371.11	58.9	326.9	0	951.8	259.1	13.3	10	53.23
39	165.58	37.11	8014.4	0.52	0.31	2.19	186.39	2201.6	947.9	409.03	53.1	319.8	0	937.4	204.5	14.5	7.5	58.36
40	152.63	33.94	5995	0.38	0.13	1.98	170	2318.8	904	375.88	58.7	322.3	0	930	187.9	12.3	7	51.73
41	167.84	30.82	4481	0.35	0.35	2.13	184.21	2467.8	859.8	368.27	74.8	323.1	0	929.8	199.3	10.8	6.9	51.3
42	176.91	31.58	5204.1	0.4	0.22	2.14	194.13	2470.7	864.4	377.3	72.2	349.7	0.587	925.4	213.1	11.7	7.4	52.64
43	157.04	29.9	4432.6	0.39	0.06	2.16	176.5	2488	886.5	385.93	82	329.8	0	924.1	191.9	11	6.7	50.39
44	196.45	28.54	6877.5	0.45	0.2	2.7	224.63	2536.8	799.6	344.61	63.9	353.8	1.385	1039.2	226.6	11.8	9	53.1
45	178.06	28.2	5632.7	0.43	0.2	3.1	192.09	2330.4	786	336.22	73.5	326.4	0	925.1	223.2	10.7	8.4	46.26
46	199.48	33.75	7971.6	0.45	0.19	2.4	240.34	2273.2	896.9	361.98	55.6	330.3	0	1007.7	240.9	13.2	9.3	51.86
47	187.76	31.8	6918.8	0.44	0.36	2.56	264.43	2287.4	836	347.16	63.4	332.5	0	1042	204.4	12.1	8.3	48.72
48	183.54	31.55	5013.6	0.45	0.2	2.4	215.22	2394.8	849.3	351.24	72.2	323.9	0.842	1028.2	215	11.2	8.3	50.53
49	174.1	33.77	5573.8	0.35	0.26	13.27	218.07	2450.3	945.5	410.39	91.5	339.5	0.693	1064.6	213.7	12.3	8.6	56.82
50	172.55	38.27	6118.2	0.57	0.22	5.48	205.43	2536.7	949.6	397.83	59.5	357.9	0	1094	213.6	12.7	7.7	56.7
51	145.24	30.19	4744	0.42	0.14	5.5	164.29	2734.5	847.3	361.9	81.2	396.1	0	996.8	180.2	10.3	6	50.32
52	154.6	31.06	5639.3	0.47	0.1	2.57	188.06	2462.7	814.4	329.6	53.1	349.4	0.92	1034.6	196.5	11.1	7.5	48.31
53	160.1	31.61	5908.9	0.42	0.59	2.88	189.16	2392.9	817.7	342	81.1	371.6	0.85	1030.2	204.2	11.6	6.9	45.79
54	151.95	31.95	4934.9	0.51	0.14	2.33	176.56	2500	869	392.45	74.5	356.9	1.328	1000.1	193.9	11.3	6.5	50.12
55	153.59	31.74	5813.1	0.42	0.16	2.36	184.41	2054.5	773.3	335.4	50.1	296.7	0.52	926.5	179.2	11.4	6.6	46.55

56	164.8	31.34	5718.1	0.46	0.24	2.44	190.69	2339.3	820	331.12	54.7	328.6	0.906	1034.7	199.2	11.1	7.2	46.84
57	152.99	30.6	4252.5	0.42	0.17	3.43	181.09	2579.6	839.7	340.3	67.3	437.2	1.791	1045.5	187.2	10.3	6.4	47.66
58	184.16	29.1	5653.5	0.51	0.25	2.85	217.45	2210.4	804.7	346.41	58.8	328.8	0.928	1017.2	221	11	8.3	48.12
59	92.56	22.58	4506.4	0.32	0	2.01	109.34	2197	656.4	272.46	46.1	307.2	1.377	858.9	121.1	9	0	29.24
60	179.98	29.78	6559.7	0.32	0.21	2.99	265.55	2028.6	1245	436.07	49	335.6	1.107	1143.7	197.1	13.2	8.5	61.11
61	201.49	31.75	8056	0.45	0.62	2.84	289.05	2337.1	1189.9	398.76	50.6	326.7	1.468	1054.5	233.2	14.2	10.3	54.98
62	214.42	35.94	12848.6	0.55	0.3	3.17	298.92	2692.3	1289.7	469.47	62	407.9	0.829	1307.7	240.7	18.3	10	62.37
63	240.05	33.35	7428.6	0.42	0.38	3.76	343.24	2900.5	1204.3	382.27	64.8	443.6	0.957	1451.5	261.6	13.3	11.1	59.62
64	274.64	34.09	7206.9	0.37	0.55	4.93	410.05	2291.9	1102	369.26	54.5	375.5	2.584	1216.5	226.8	13.9	13.3	55.98
65	337.5	32.18	7242.4	0.4	0.59	4.96	528.54	2787.9	1241.7	389.02	66.4	421	0.96	1532.8	310.2	13.5	16	62.37
66	234.42	34.94	7500	0.45	0.45	3.4	303.77	2663.3	1260.1	423.62	59	396.7	0	1397	280.2	14.3	11.1	60.87
67	272	32.4	13100	0.65	0.81	3.28	326.75	2030	1098.3	409.5	235.6	345	0.65	1207.9	290.9	17.5	13.5	63.89
68	260.38	28.3	7170	0.36	0.71	3.05	315.5	2241.3	955.9	296.75	62.4	355.9	2.925	1287.5	305.1	12.2	12.5	46.83
69	303.66	27.44	5556.8	0.35	0.67	3.79	367.17	2596	993.3	305.05	70.7	410.6	3.144	1468.4	360.1	11.1	14.7	54.34
70	143.9	36.51	11046.9	0.62	0.39	1.76	152.11	2212.4	1225.4	475.47	46.6	312.3	2.301	1049.6	184	17.7	7.3	68.04
71	326.23	30.49	9285.7	0.46	0.58	3.46	401.6	2346.1	1137.4	389.78	51.6	366.7	3.177	1362.1	342	14.3	16.1	64.31
72	271.5	26.49	6097.8	0.42	0.64	3.24	323.31	2340.6	910.9	279.11	56.3	376.1	2.597	1311.6	321.3	10.9	13.5	50.91
73	238.59	27.13	6430.7	0.46	0.52	3.44	282.88	2202.4	939.7	293.34	64.9	352.6	1.929	1353.9	290.6	11.1	11.2	49.04
74	236.33	35.74	7001.2	0.44	0.51	3.18	280.96	2293.2	1098.5	390.3	50.7	354.6	2.488	1329.4	272.4	13.7	11.4	64.42
75	267.63	34.71	9330	0.51	0.48	3.64	315.38	2385	1100	385.5	43.5	358.5	2.588	1251.3	307.9	15	12.4	62.99
76	286.7	34.7	9452	0.53	0.59	3.14	339.66	2226.6	1098.5	404.31	47.2	327.3	3.374	1096.2	325.1	14.9	14.8	63.85
77	254.17	30.5	6117.6	0.37	0.54	3.1	302.94	2339.5	1067	344.12	49.4	365.8	2.402	1257.4	287.9	11.9	12.2	55.28
78	297.63	35.69	8947.5	0.59	0.44	6.35	281.13	2437.5	1043.4	377.5	70.6	356.4	2.488	1271.3	293	14.3	13	63.15
79	254.48	31.48	9256.2	0.45	0.61	2.51	286.44	2160.4	892.4	320.65	44.1	349.4	2.91	1154.1	294.5	13.9	11.9	55.15
80	260.71	28.83	6486.5	0.37	0.48	2.91	305.54	2118.2	831	292.49	46.4	340.8	3.153	1241.4	286.1	11.4	13	53.3
81	263.85	35.42	7441.2	0.78	0.6	2.99	378.92	2220.6	985	360.78	44	332.4	2.892	1128.8	251.3	13.7	12.4	60.64
82	172.48	30.09	6807.8	0.39	0.34	2.33	199.58	1919.1	752.2	304.1	31.3	294.7	2.605	1079.8	196.7	11.9	8.6	53.47
83	161.76	34.12	12414.2	0.58	0.28	2.34	187.01	1871.3	788.5	333.46	37.7	276.6	2.574	1134.3	188.8	16.4	7.8	50.98

84	191.63	38.67	9516.5	0.47	0.33	2.69	235.85	2096.7	935.4	380.66	38.6	311.6	2.512	1120.6	210.3	15.2	8.4	63.87
85	194.15	36.95	7761.2	0.44	0.34	2.67	220.4	2248.8	891.4	359.83	46	312.8	1.555	1128	221.9	13.5	9.2	59.48
86	196.75	41.66	7405.8	0.5	0.37	9.58	233.28	2417.2	1051	418.83	48.5	327.3	4.367	1212.8	227.3	14.3	9	71.49
87	245.81	44.43	10580	0.58	0.49	2.48	302.96	2213.1	1038.2	432.88	42.8	338.5	3.251	1154.7	238.9	16.6	11.4	68.52
88	143.15	36.06	7024	0.49	0.3	2.25	176.68	2332.9	925.2	370.91	48.5	354	3.798	1035.7	155.2	13.6	6.4	56.65
89	207.65	36.84	9285.2	0.52	0.39	2.59	248.42	2100.7	921.2	356.8	43.2	311.7	1.833	1166.7	223.8	15.2	9.8	56.88
90	182.28	35.46	7142	0.4	0.39	2.86	221.72	2354.4	928.2	365.53	50.2	339.4	2.039	1218.4	191.9	13.2	8.4	60.42
91	194.17	34.41	7845.9	0.46	0.38	3.01	236.04	2400.5	897.9	351.46	46.2	342.1	2.755	1253.6	210.8	13.4	9.1	60.24
92	164.98	31.41	5479	0.43	0.32	2.93	193.32	2634.9	902.4	362.87	69.4	371.3	2.005	1211.6	167.8	11.7	7.1	57.76
93	179.07	36.24	7356.5	0.57	0.36	4.64	213.64	2459.3	1010.4	429.07	58.1	356	2.129	1170	185.3	13.9	7.9	66.77
94	186.49	33.36	5590	0.5	0.45	3.13	224.88	2287.9	909.6	366.47	54.2	358.4	2.784	1199.1	188.3	12	8.3	60.11
95	174.88	32.23	6542.5	0.51	0.36	2.94	219.54	2453.9	892.8	377.67	64.4	348.3	2.718	1183.4	194.8	12.9	8.3	58.63
96	182.65	36.84	7467.2	0.61	0.39	4.14	226.09	2508.5	1016.3	427.43	61.1	368.9	2.779	1220.9	205.7	14.4	8.1	65.97
97	181.97	36	7388	0.68	0.27	2.7	215.98	2398.9	966.4	412.57	49.4	352.6	3.21	1168.4	189.3	14.1	8	63.81

Appendix V: PCA Loadings

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Al	0.300804	-0.27878	0.137898	-0.07649	0.064016	-0.0431	0.156017	-0.18458	0.095103
Ba	0.239966	0.33806	-0.08898	-0.04909	-0.14288	-0.10093	0.232227	-0.23425	0.023654
Ca	0.292673	0.196624	0.017608	0.189217	0.223368	0.160259	-0.15898	0.408638	0.379627
Cd	0.213398	0.26331	-0.03254	0.21576	0.108331	0.307482	0.632331	0.145043	-0.39286
Cr	0.272701	-0.15411	0.109548	0.095747	0.110623	0.291054	-0.46225	-0.05464	-0.63124
Cu	0.115471	-0.01142	-0.06467	-0.42394	-0.7047	0.470724	-0.01631	0.096946	0.12447
Fe	0.28874	-0.26364	0.093439	-0.0755	0.043951	-0.11439	0.089298	-0.19661	0.091952
K	-0.09811	-0.23085	-0.51202	0.005705	-0.01457	-0.11942	0.22262	0.054096	-0.07097
Mg	0.296151	0.004917	-0.23898	-0.14936	0.005767	-0.29482	-0.33968	0.101327	-0.07617
Mn	0.193449	0.279491	-0.37668	-0.15098	0.036439	-0.16445	-0.11875	-0.26434	-0.02744
Na	-0.06375	-0.16436	-0.35034	-0.16089	0.428007	0.620598	-0.06209	-0.21551	0.211487
P	-0.05822	-0.28468	-0.49696	0.131339	0.010781	-0.08916	0.085629	0.057282	-0.05826
Pb	0.089584	-0.1472	-0.04002	0.739817	-0.36558	0.126667	-0.10973	-0.35972	0.177975
S	0.222923	-0.31938	-0.13699	0.124108	-0.18925	-0.06531	0.032516	0.558353	-0.04393
Si	0.265473	-0.26051	0.199315	-0.20232	0.112426	-0.03701	0.156203	0.061116	-0.02121
Sr	0.307809	0.253347	-0.10276	0.131459	0.129408	0.024999	-0.0838	0.145778	0.321024
Ti	0.297178	-0.2725	0.131598	-0.06221	0.079156	-0.05676	0.195046	-0.2035	0.196819
Zn	0.310165	0.192744	-0.16718	-0.04131	-0.1144	-0.03883	-0.04362	-0.17789	-0.18431

	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18
Al	-0.02721	0.063586	-0.10609	0.093148	-0.02526	0.398236	-0.13721	-0.72553	-0.06601
Ba	0.504844	0.219461	-0.03948	-0.5637	-0.03205	0.095819	0.041738	0.024541	0.207213
Ca	0.024863	0.026987	-0.32733	0.180734	-0.05791	0.044019	0.011637	-0.00076	0.530122
Cd	-0.3781	-0.04775	0.087096	-0.02335	-0.00013	0.016392	-0.04687	0.008394	0.010721
Cr	0.136669	0.147972	-0.27113	-0.12178	0.085177	-0.1676	0.019425	-0.05705	-0.0274
Cu	-0.15886	0.012949	-0.1766	0.002606	-0.01858	-0.03403	-0.02613	0.004829	-0.02195
Fe	-0.27104	0.632592	0.051808	0.155251	0.076336	0.096569	0.276356	0.410614	0.045174
K	0.130173	0.004124	-0.34872	0.120529	0.647354	0.009081	-0.14709	0.045342	0.083415
Mg	-0.42678	-0.16599	0.216496	-0.34989	0.060615	0.253459	-0.36826	0.119914	0.132459
Mn	-0.21755	-0.17514	0.008803	0.141621	0.031878	-0.29542	0.570699	-0.29957	0.069422
Na	0.103344	-0.02428	0.341738	-0.11164	0.020227	0.113966	0.008857	0.044205	0.031405
P	-0.0378	0.08313	-0.32238	-0.07992	-0.7056	-0.00608	-0.00543	0.056126	-0.09763
Pb	-0.09178	-0.22098	0.059446	-0.05935	0.094616	0.110581	0.081382	0.06507	0.047038
S	0.264644	0.081826	0.521582	-0.0316	0.03526	-0.13117	0.242795	-0.17904	-0.00866
Si	0.149527	-0.59952	-0.19743	-0.08216	-0.00613	0.240923	0.364897	0.329855	-0.10421
Sr	0.02045	0.093514	-0.13189	-0.07676	0.134145	-0.08126	-0.07095	0.045284	-0.77485
Ti	-0.00142	-0.15633	0.026765	-0.05387	-0.04887	-0.71509	-0.36306	0.031995	0.11803
Zn	0.351672	-0.09312	0.207685	0.636778	-0.17	0.14628	-0.28468	0.203968	-0.03125