DETERMINATION OF CALCIUM, MAGNESIUM, AND ALUMINUM IN FRASER FIR (*Abies fraseri*) FOLIAGE AND SURROUNDING SOIL IN THE GREAT SMOKY MOUNTAINS, BALSAM MOUNTAINS, AND BLACK MOUNTAINS USING INDUCTIVELY-COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY

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ABSTRACT

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The Fraser fir (*Abies Fraseri*) is a conifer commonly found in the Eastern United States. In the Southern Appalachian Mountains Fraser fir share an ecosystem with Red Spruce (*Picea rubens*) in island-like stands typically above 1500 m. The Balsam Wooly Adelgid is recognized to be the primary reason for Fraser fir decline in the Southern Appalachian Mountains, but atmospheric deposition may also be involved. Acid deposition allows nutrients calcium (Ca^{2+}) and magnesium (Mg^{2+}) to be leached from soil and foliage, and allows for mobilization of toxic metals like aluminum (Al^{3+}) to become available for interaction with the fir. Investigation of these effects could summarize the intensity of acidic deposition in the Southern Appalachian Mountain ranges studied.

Samples of Fraser fir foliage and surrounding soil were gathered from 8 sites in the Great Smoky Mountains, in the Balsam Mountains, and in the Black Mountains. 30 samples were collected from each site, divided into 3 classes of life stage (10 seedlings, 10 saplings, and 10 mature trees). Using an acid digestion method for foliage and a soil extraction method for exchangeable metals in soil, concentrations of calcium, magnesium, and aluminum were found using Inductively-Coupled Plasma Optical

Emission Spectroscopy. Student's t-test, analysis of variance, and linear regression analysis were used to statistically compare the data.

There was a considerable amount of correlation between foliar and exchangeable soil metal concentrations as a result of elevation or longitude. The 8 sites were divided in half based on elevation (4 sites above 1900 m, 4 sites below 1900 m), and comparisons were made. Western sites are closer in proximity to coal-burning power plants in Tennessee, so they were expected to exhibit increased effects of acid deposition. Foliar and exchangeable soil metal concentrations were tested against soil pH, and very little correlation was found. Three life stage classes of samples were acquired (seedlings, saplings, and mature trees) and expected to all have statistically similar concentrations of metals in both foliage and soil, but almost all were different. No correlation was found in soil exchangeable metal concentrations and foliar metal concentrations, but a trend existed in soil exchangeable aluminum and foliar calcium concentrations.

The data from this experiment was also compared to previous studies from 1969, 1994, and 1996 at two different sites. The comparison to the 1996 study at Clingmans Dome showed differences in foliar magnesium and aluminum concentrations, with decreased toxic metal and increased nutrient concentrations as expected. Differences also existed when comparing foliar nutrient concentrations to the 1969 and 1994 studies at Richland Balsam. Since 1994, a decline in acid deposition related effects was observed, which could show success of the 1990 Clean Air Act Amendments.

1. INTRODUCTION

1.1 Fraser Fir Background

The Fraser fir (*Abies fraseri*) is a medium-sized conifer that is native to the Southern Appalachian Mountains, and is found within a unique ecosystem shared with the red spruce (*Picea rubens*). Spruce-fir forests encompass a broad region of North America, ranging from Alaska to eastern Canada, and along the Appalachian Mountain chain. The southern-most spruce-fir forests in eastern North America are located among the high peaks of the Southern Appalachian Mountains in North Carolina (NC) and Tennessee (TN) in island-like stands above 4500 ft. Between 1880 and 1930, a total of 50% of the Southern Appalachian spruce-fir was removed by logging operations or fire (White 1984). According to Weaver (1972), the present-day spruce-fir forests result from regeneration of uncut timber or from reestablished seedlings following the logging operations. Deforestation can result in calcium depletion from the soil due to the high content of calcium present in fir bark and wood (Schaberg *et al.* 2001). Most recently, a decline has been observed in the populations of spruce-fir trees in the high elevation Southern Appalachian Mountains. Possible causes for the decline include long term climate change, pest infestation, such as the Balsam Woolly Adelgid (BWA), disease, gaseous pollution, or acid deposition. The latter is focused on in this paper. Eastern North America's location, topography, and other characteristics make the high elevation spruce-fir ecosystems of Southern Appalachia high receptors of acidic deposition. Johnson (1983) claims no other major forested area in the United States is at more risk.

1.2 Balsam Woolly Adelgid

The BWA is the primary cause for Fraser fir decline. Native to Europe, its first appearance in the United States was in 1908 in Maine, on Balsam fir (*Abies balsamea*). It was first noticed in the Southern Appalachian Mountains inhabiting Fraser fir at Mount Mitchell in 1957, from which it spread further into other fir forests due to Mitchell's central location with respect to other spruce-fir populations. The BWA travels efficiently in the high velocity wind of the high elevation Southern Appalachian Mountains, or could be transported passively by humans or other animals (Eagar 1984).

Upon hatching from an egg, the BWA finds a suitable host, a mature fir tree. It inserts its stylet into the trunk and begins to feed, where it will remain for life. BWA infestation on a fir results in an abnormal type of wood called rotholz, and this inhibits nutrient transport from the roots to the crown of a fir. A Fraser fir can be easily killed by the BWA within 3 to 9 years following infestation. A comparison of nutrient concentrations in soil and foliage of mature trees could represent adelgid damage (Eagar 1984) (Sutton 1997).

1.3 Acid Deposition - Pollutants and Transformation

Introduction of acid into an ecosystem begins with emission of pollutants sulfur dioxide (SO_2) and nitric oxides (NO_x), mainly from industries or automobiles. When a fuel is burnt (such as coal or fossil fuels), chemical reactions occur in the combustion process combining oxygen with the constituents of the fuel, which produces gaseous oxides such as H_2O, CO_2, SO_2, or NO_x. SO_2 and NO_x forms from nitrogen and sulfur in the fuel. For instance, crude oil contains 0.1 to 3% sulfur, and coal contains 2 to 3%

sulfur, which becomes a pollutant when burnt. Attempts to curb pollution via SO_2 and NO_x include increasing the height of smokestacks at coal burning power plants, but this only distributes the pollutants over a wider range (Park 1987). Industrial and vehicular emissions have been regulated by laws such as the Clean Air Act and its amendments (EPA, 1990), but with predicted increases in power generation and vehicular use NO_x emissions are not likely to decrease substantially without more regulation (Aber *et al.* 2003).

Once the pollutants are in the atmosphere, a series of complex reactions involving sunlight occur transforming the pollutants into acids. There are many ways for transformation to occur, which depend on the initial concentration of pollutants, wind speed, sunlight intensity, humidity, temperature, rainfall frequency, and others. Presence and concentration of other chemicals such as reactive hydrocarbons (RHC) or very reactive hydroxyl radicals (•OH) influence the transformation as well. RHC comes from petroleum refining and storage, or industrial and vehicular emission. •OH concentration depends on concentration of ozone (O_3), NO_x, RHC, and sunlight, so all are considered together because concentration of one will affect concentration of another (OTA 1984).

During intense periods of sunlight, •OH is formed through a chain reaction and, as a result, gaseous SO_2 is oxidized into sulfate (SO_4^{-2}), at a rate of 1 - 4% oxidized per hour. Aqueous oxidation of SO_2 occurs as well, when the pollutant is dissolved into cloud water. The oxidizing agent is unknown, but probably hydrogen peroxide (H_2O_2). The route taken in SO_2 oxidation depends on atmospheric conditions (OTA 1984).

Oxidation of NO_x into nitrate (NO_3^-) is mostly a gaseous process. This transformation utilizes RHC and •OH, and the process is similar to that above, except the rate is ten times as rapid. Atmospheric acid preparation is complete when the anions sulfate and nitrate combine with water in the atmosphere, forming dilute solutions of sulfuric acid and nitric acid (OTA 1984).

1.4 Deposition Processes

Acids in the atmosphere are deposited into ecosystems, and can be divided into two main categories: wet deposition and dry deposition. Wet deposition involves deposition of acids by precipitation. Once the acids are scavenged by water in clouds, they remain dissolved until the water falls as rain or snow. The rate of wet deposition is controlled by the ability of acids being scavenged and the ability for precipitation to occur. In high elevations, an increase in rainfall of nearly 50% is seen when comparing to an elevation 1,000 meters lower (Lovett 1984). Acidic rain can remove calcium directly from the foliage of a tree and, if the rate of removal is greater than the rate of uptake, tree nutrient deficiency will occur (OTA 1984). Also, winters bring heavy snow to the Southern Appalachian highlands frequently. Snow is better at scavenging acids and holding them within its crystal structures. For these reasons, higher elevations are hypothesized to have an increase in the effects of acid deposition (Lovett 1984).

Dry deposition involves acids not saturated in solution being deposited to the ecosystem by wind. The needle-like foliage of Fraser fir is at increased risk of effect from dry deposition with its higher surface area than deciduous trees, and its year round presence on the tree. At higher elevations, an increase in wind speed is observed. This gives acidic pollutants greater momentum when colliding with the foliar surfaces of trees and mountain soils (Lovett 1984).

Cloud deposition cannot fit well into either wet or dry deposition categories. Pollutants dissolved in cloud water interact with soil and foliar surfaces as they are blown by the high wind speeds present at higher elevation. The high elevation mountains of Southern Appalachia are frequently immersed in clouds, which may significantly contribute to acid deposition (Lovett 1984).

1.5 Effects of Acid Deposition on Fir Populations

Acid deposition results in vital nutrients in soil to be mobilized. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations are replaced by hydrogen ions (H^+) under acidic conditions and, when mobilized, can be leached through soil water solution accompanied by anions that were introduced with the H^+ (Park 1987). McNulty *et al.* (1996) fertilized a forest with increased nitrate and studied changes over a 7-year period. Results showed a decrease in tree growth due to nutrient imbalance.

In addition to nutrient leaching, toxic metals are also of concern for Fraser fir damage. Increased soil acidity results in an increased amount of toxic heavy metals including aluminum, cadmium, zinc, lead, mercury, or iron. Some toxic metals are acceptable at low levels, but can become toxic at elevated levels. These toxic metals are soluble in acidic soil water solutions and free to deposit in above ground water systems or absorbed by root osmosis (Park 1987). Elemental aluminum comes from rock weathering, of which its solubility is pH dependent. The solubility of aluminum drops in the range of 5-7, but organic ligands in soil enhance solubility, with the formation of organic-aluminum complexes (Cronan *et al.* 1979). Aluminum mobilized by acidic deposition has the ability to reduce soil storage of calcium, and disables root uptake of calcium (Dehayes *et al.* 1999). By analyzing concentrations of exchangeable aluminum in soil, conclusions can be drawn regarding nutrient deficiency in Fraser fir.

Between the two above consequences of acid deposition, the most important effects of deposition and possibly the most probable reason for decline would be the collaboration of toxic metal mobilization and nutrient deficiency. Mature trees require an increasing amount of calcium from the roots and, as aluminum is mobilized in soil, calcium becomes less available. This results in a decrease of sapwood area, which is the section of wood in the fir that is used for transporting water and nutrients across the tree. Due to changes in the wood (either from BWA or soil aluminum), the crown of the fir is usually the first place foliar shedding occurs. When less than 25% of the sapwood has been damaged, vulnerability to death becomes drastic due to insects, the BWA, or extreme temperatures (Shortle *et al.* 1988).

1.6 Previous Studies of Fraser Fir Decline in the Southern Appalachian Mountains

Three similar previous studies were conducted on Fraser fir foliage in the region. A 1969 study by Weaver provided concentrations of calcium, magnesium, potassium, and phosphorous in a number of high elevation plants, including Fraser fir sapling foliage. The data were gathered from Richland Balsam, where samples were collected during this study (Weaver, 1972). Another study performed by Shepard *et al.* (1994) used flame atomic absorption spectroscopy to acquire Fraser fir foliar concentrations of calcium and magnesium in saplings at Richland Balsam.

A third project by Lee (1996) consisted of sampling Fraser fir saplings at Clingmans Dome in the Great Smoky Mountains National Park (GSMNP), and analyzing foliage and surrounding soil for calcium, magnesium, and aluminum. Clingmans Dome was also sampled in this study.

Due to the Clean Air Act Amendments of 1990, it is estimated that soil and foliar nutrient levels would have increased since the dates of the previous work, and that toxic metal levels would have decreased. The Clean Air Act Amendments of 1990 attempted to reduce sulfur dioxide and nitric oxide by a considerable amount (EPA 1990). Concentrations of foliar calcium and magnesium from Richland Balsam will be compared to the 1969 and 1994 studies, and foliar calcium, magnesium, and aluminum concentrations from Clingmans Dome will be compared to the 1996 study, to determine if the Amendments have had a positive effect.

1.7 Goals and Hypothesis

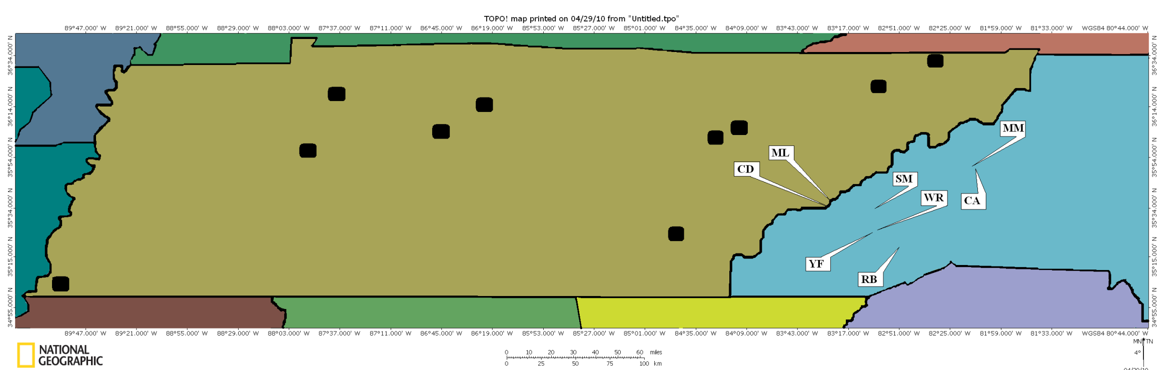
The concentrations of calcium, magnesium, and aluminum in Fraser fir foliage and surrounding soil were measured to provide evidence that acid deposition may be a factor in Fraser fir decline. Using these concentrations, statistical tests were utilized to form a number of major conclusions based on elevation, longitude, and other potential factors.

To compare and contrast metal concentrations with respect to elevation, four of eight sites sampled were high elevation sites above 1900 m (6,000 ft), and four were lower elevation sites below 1900 m (6,000 ft). Two sets of sites in the extreme west and east sample range were close in proximity but differed in elevation by roughly 300 m

(1,000 ft). It was hypothesized that higher elevation sites, being more subject to acid deposition than lower sites, would exhibit decreased levels of calcium and magnesium, along with increased levels of aluminum.

Two sites were chosen in the extreme western portion of the sample range to compare deposition effects to other sites in order to conclude if the coal burning power plants in Tennessee affected the western mountains more than the central and eastern mountains sampled. Locations of major coal burning power plants of Tennessee are shown in Figure 1.1 (CGD, 2010). It was hypothesized that the western sites would have lower concentrations of calcium and magnesium and higher concentrations of aluminum than other sites.

Figure 1.1: Locations of Major Coal Burning Power Plants and Their Proximity to All Samples Sites



Soil pH was measured and compared to soil and foliar toxic metal levels, to confirm a hypothesized increase in aluminum concentrations under acidic conditions. pH data were also analyzed with respect to soil and foliage nutrient levels.

The 30 samples at each site were divided into three age classes: seedling, sapling, and mature (10 samples each). It is hypothesized that soil and foliage metal concentrations in each class would be the same.

Another comparison involved finding a possible trend in foliar concentrations of metals as a function of exchangeable soil metal concentrations. It was hypothesized that there would be a positive, linear correlation between each element in soil and foliage. In addition, it was predicted that as aluminum increased, the foliar calcium concentration would decrease.

Previous studies were conducted at two of the sites sampled in this study. In 1969 and 1994, Fraser fir foliar nutrients were determined at Richland Balsam. A decrease in foliar calcium and magnesium was observed when comparing the two studies. In 1996, foliar calcium, magnesium, and aluminum were determined at Clingmans Dome. When comparing this data to previous studies, it was hypothesized that the Clean Air Act of 1990 would result in somewhat of an increase in foliar calcium and magnesium levels, and decrease in foliar aluminum levels when comparing to previous studies at Richland Balsam and Clingmans Dome.

2. EXPERIMENTAL

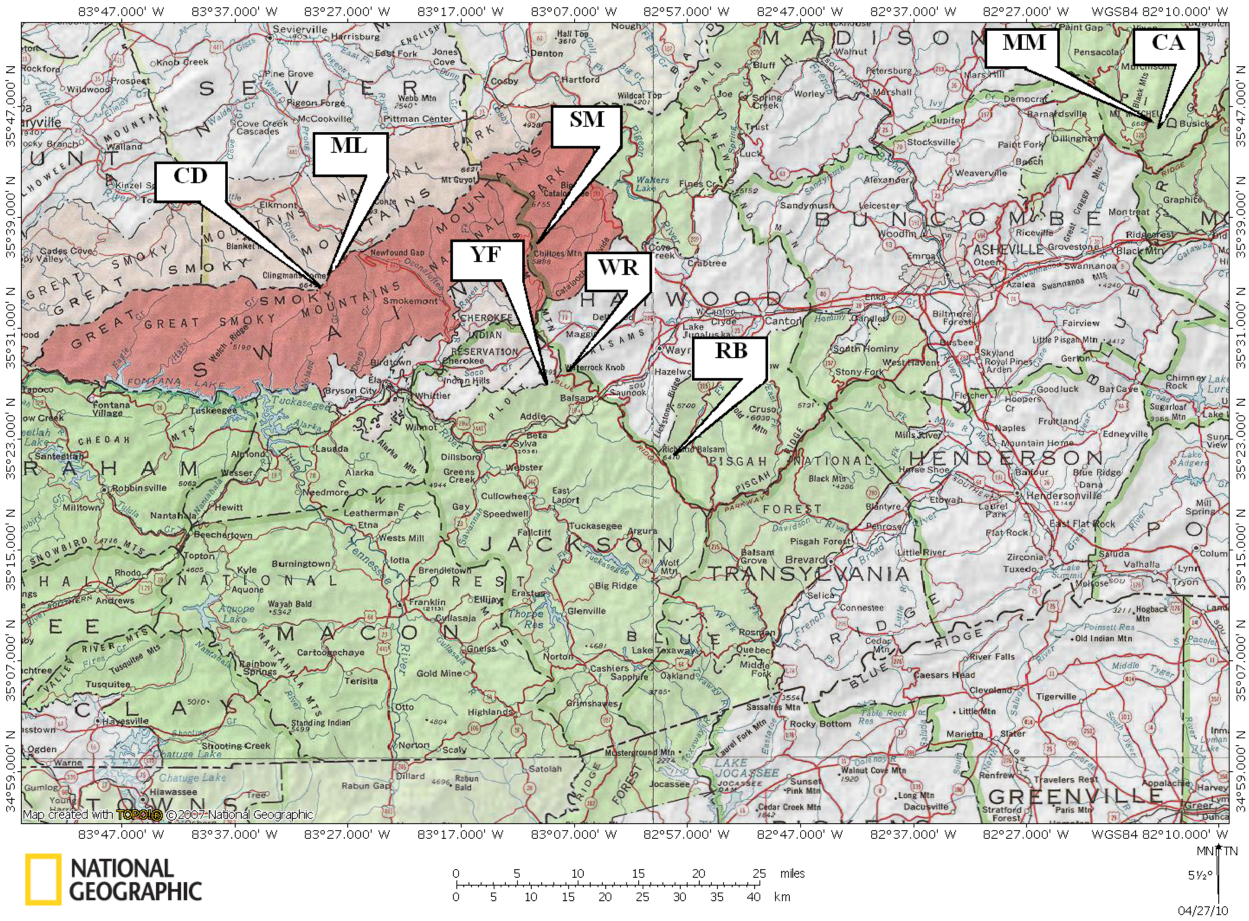
2.1 Sample Sites

Samples were acquired from eight sites located within the Great Smoky Mountains, the Balsam Mountains, and the Black Mountains. All sample sites are listed in Table 2.1. Criteria for selection of a site included: a spruce-fir ecosystem, a broad distribution of Fraser fir samples, and elevation above 1370 m (4500 ft). The location and elevation of each sample was recorded using a Garmin Global Positioning System (GPS) 76CSx receiver unit. Table 2.1 contains GPS average data for each site, and Figure 2.1 is a map locating all sample sites in this study. For maps of all sample areas, see Appendix A.

Table 2.1: Sample sites, their site initials, latitude/longitude, and elevation above sea level

Site	**Site Initial**	**North Latitude**	**West Longitude**	**Elevation**
Water Rock Knob	WR	35° 27.846'	83° 08.269'	1907 m
Richland Balsam	RB	35° 22.894'	82° 59.373'	1908 m
Clingmans Dome	CD	35° 33.744'	83° 29.895'	2016 m
Mingus Lead	ML	35° 36.725'	83° 26.764'	1705 m
Spruce Mountain	SM	35° 36.732'	83° 10.540'	1714 m
Yellow Face	YF	35° 27.305'	83° 08.656'	1765 m
Mount Mitchell	MM	35° 45.842'	82° 15.773'	2011 m
Camp Alice	CA	35° 45.424'	82° 15.989'	1760 m

Figure 2.1: Map Showing All Sample Sites Used in This Study.



Foliage and surrounding soil samples were obtained from 30 trees at each sample site. Of these 30 trees, 10 were seedlings (height less than 2 m), 10 were saplings (2-4 m), and 10 were mature trees (greater than 4 m). Table 2.2 includes each site and its location, along with the date sampled and the mountain range in which the site is located. See Appendix B for latitude, longitude, and elevation of every sample acquired.

Table 2.2: Sample Sites and Their Respective Mountain Ranges, the Location, and the Dates Sampled

Site	**Range**	**Location**	**Date Sampled**
Water Rock Knob	Balsam (Central)	BRP	May 19, 2009
Richland Balsam	Balsam (Central)	BRP	June 18, 2009
Clingmans Dome	Great Smoky (West)	GSMNP	July 16, 2009
Mingus Lead	Great Smoky (West)	GSMNP	July 26, 2009
Spruce Mountain	Balsam (Central)	GSMNP	July 30, 2009
Yellow Face	Balsam (Central)	BRP	August 4, 2009
Mount Mitchell	Black (East)	MMSP	August 11, 2009
Camp Alice	Black (East)	MMSP	August 12, 2009

In order to compare two sites of high and low elevation, the sites had to differ in elevation by 300 m, and had to be within 10 km in proximity. The sites compared were Mt. Mitchell and Camp Alice, and Clingmans Dome and Mingus Lead. The Water Rock Knob and Yellow Face sites were not compared due to an inadequate differential in elevation. These proximity of these sites can be viewed in Figure 2.1.

Another comparison was based on longitude of the sites, to examine nutrient/toxic metal levels possibly affected by the coal burning power plants found in Tennessee. Sites were compiled into three categories: western, central, and eastern. The Great Smoky Mountains contained the western-most sites (Clingmans Dome, Mingus Lead), the Balsam Mountain range (Spruce Mountain, Yellow Face, Water Rock Knob, Richland Balsam) was considered central, and the Black Mountains (Mt. Mitchell, Camp Alice) were the eastern sites. The three longitudinal groupings can be seen in Figure 2.1.

2.2 Collection and Preparation of Foliar Samples

Approximately 100 g of foliage was collected from each sample using pruning shears, and stored in labeled polyethylene bags for transport. Foliage was collected from all sides of the tree, ranging 1 to 2 m from ground level. The location of the sample was recorded in the GPS. The current year's growth was removed, and the foliage was dried in a Precision Economy Oven (Thermo Fisher Scientific Inc., Waltham, MA) at 110^{o}C for 24 hrs. Once dried, the needles were separated from the limbs, and placed in labeled polyethylene bags for storage. Composite samples were prepared by measuring 1.0000 ± .0200 g of needles from each of 10 samples in each age class (seedling, sapling, mature). Each composite group was stored in polyethylene bags, and shaken well until homogeneous. The composite was packed into a cartridge and loaded into a SPEX 8000 mixer/mill (SPEX SamplePrep, LLC, Metuchen, NJ) and pulverized 5 minutes.

2.3 Foliar Digestion Procedure

To prevent sample contamination, all glassware was cleaned by a 1% Alconox solution (24 hrs), and a 20% nitric acid bath (24 hrs). Upon removal from the acid bath, glassware was rinsed with NANOpure water, and glassware was ready for use. The samples were digested using a modified method of the procedure from Shepard *et. al.* (1994). Approximately (0.2000 ± .01 g) of foliage was introduced into Fisher Brand (Fisher Scientific, Pittsburgh, PA) 16 x 150 mm borosilicate test tubes using a stainless steel spatula, in replicates of 5 for each age class of sample from each site. A Finnpipette (Fisher Scientific LLC, Pittsburgh, PA) was used to transfer 1.0 mL concentrated nitric acid (Fisher Chemical, A200-c212) to each test tube. Each test tube was vortexed immediately and allowed to stand at room temperature for 30 minutes.

The test tubes were introduced into a laboratory constructed heating block, and the temperature of the block was raised to 140^{o}C, at which point an additional 1.0 mL concentrated nitric acid was added to the mixture. The test tubes were refluxed for 3 hrs. Vortexing occurred once every 10-15 minutes during reflux, to ensure thorough digestion. It should be noted that between 60-110^{o}C, samples tended to foam/froth. To avoid losing sample, the tubes were vortexed constantly during this temperature range.

The samples were allowed to return to room temperature, and 0.5 mL of 0^{o}C 30% hydrogen peroxide (Fisher Chemical, BP2633-500) was added to the tubes. The solutions were vortexed and reheated to 140^{o}C and allowed to reflux for an additional 90 minutes, vortexing once every 10-15 minutes.

Blank solutions (reagents only, no sample in tube) were subject to both acid and peroxide, but not heated to avoid splattering. Blanks were mixed in an ice bath and remained in the bath while the samples were refluxing. Once samples reached room temperature, they were gravity filtered with Fisher Brand Q8 Quantitative Filter Paper

(Fisher Scientific LLC, Pittsburgh, PA) to remove any undigested particles. Solutions were filtered into 100 mL glass volumetric flasks, and diluted to volume with deionized water.

2.4 Collection and Preparation of Soil Samples

Within 2 m of the base of a sampled tree, approximately 500 g of soil was acquired from a depth between 10 and 20 cm using a gardening spade. Leaf litter was not part of the sampled soil. The sample was placed in an appropriately labeled polyethylene bag. The location of the sample was recorded into the GPS as mentioned in section 2.2. The soil samples were dried in a Precision Economy Oven at 110^{o}C for 24 hr. Debris was removed from the sample using stainless steel USA Standard Testing Sieves No. 10, 2 mm and No. 18, 1 mm by Fisher Scienctific (Fisher Scientific LLC, Pittsburgh, PA). Composite samples were prepared by measuring out 1.0000 ± 0.02 g of each sample in each age class (seedling, sapling, mature), and combining them in a polyethylene bag. The composite was shaken until homogenous, and loaded into a cartridge for pulverizing. Pulverization was achieved using a SPEX 8000 mixer/mill with two steel shots for 5 minutes. The pulverized sample was stored in a labeled polyethylene bag.

2.5 Soil Exchangeable Cation Extraction Procedure

All glassware involved in soil extraction was soaked in a 1% Alconox solution for 24 hrs followed by soaking in a 20% nitric acid bath for an additional 24 hrs for cleaning. Soil exchangeable cations were extracted using a modification described by Carter (1993). Approximately 0.5000 ± .02 g of soil was added to Falcon Blue Max™ (Becton Dickinson and Company, Franklin Lakes, NJ) 50 mL polypropylene conical tubes in replicates of 5. Using a 100 mL graduated cylinder, 30.0 mL of 0.100 M barium chloride (Fisher Chemical, B34-500) solution was added to the soil. These were placed onto a Lab-Line Orbit Shaker (Lab-Line Instruments, Inc., Melrose Park, IL), and shaken at 100 rpms for 2 hrs. To remove unwanted soil particles, the samples were gravity filtered into glass 100 mL volumetric flasks and were diluted using deionized water.

2.6 Soil pH Analysis

Soil pH of each composite sample was measured using the method described by Carter. 1.000 ± 0.0100 g of dried, sieved soil was added to Falcon Blue Max™ 50 mL polypropylene conical tubes. A graduated cylinder was used to measure 20.0 mL of NANOpure water into the tubes. These samples were placed on the Lab-Line Orbit Shaker and shaken at 100 rpms for 30 minutes. The solutions were allowed to stand for approximately 1 hour and then an electrode from a Mettler Toledo SevenGo pH meter SG2 (Mettler-Toledo International, Inc., Switzerland) was immersed into the clear supernatant. The pH was recorded and triplicate pH readings were obtained for each sample.

2.7 Standards Preparation and Quality Control

Instrumental standards for calcium, magnesium, and aluminum were prepared using a SpexCertiPrep (SPEX CertiPrep, LLC, Metuchen, New Jersey) 1,000 ppm Custom Assurance Standard in 2% nitric acid. This solution was diluted in glassware from the 1% Alconox and 20% nitric acid cleaning baths. Quality control for foliage samples was determined by using a National Institute of Standards and Technology (NIST) (U.S. Department of Commerce, Washington, DC) Standard Reference Material

(SRM) 1575a (Pine Needles) and recovery checks. The SRM was subject to identical methodology as foliar samples. For soil, only recovery checks were utilized due to the lack of SRM for the exchangeable metal extraction method used. The acceptable recovery range for ICP-OES used was 80-120% recovery, and all samples fell within this range.

2.8 Sample Instrumental Analysis

Concentrations of calcium, magnesium, and aluminum in sample solutions were determined using Inductively-coupled Plasma Optical Emission Spectroscopy (ICP-OES)(Perkin Elmer Optima 4100 DV). The elements analyzed, along with the wavelength selected for analysis, and detection limits for each are given in Table 2.3. Instrumental conditions are listed in Table 2.4.

Table 2.3: Elements Analyzed With Their Wavelengths and Detection Limits

Element	**Wavelength**	**Detection Limit (ppm)**
Aluminum	308.215	0.032
Calcium	315.887	0.006
Magnesium	285.213	0.002

Table 2.4: Instrumental Conditions for ICP-OES During Sample Analysis

Radio Frequency (watts)	1350
Pump Rate (mL/min)	1.25
Auxillary Gas Flow (L/min)	0.2
Nebulizer Gas Flow (L/min)	0.80
Plasma Gas Flow (L/min)	15
Plasma View	Axial

2.9 Statistical Analysis

Student’s *t*-test, linear regression, and analysis of variance (ANOVA), were performed on the appropriate datasets by using “R” computer software (R Foundation for Statistical Computing, Vienna, Austria) to determine if there was statistical difference between Fraser fir populations and classes. The Student’s t-test was used to determine if a statistical difference exists in soil or foliar samples between two sites. Linear regression was used to determine whether a linear relation existed between all sites when comparing metal concentrations with elevation, pH, or longitude. ANOVA was used to compare soil and foliar concentrations of metals between multiple sites. For all, an alpha value of .05 was used. For Student's t-testing and ANOVA, if the p-value determined was greater than the alpha value, then the concentrations of compared samples was considered the same. If the p-value was less than the alpha value, then the sites were considered statistically different.

3. RESULTS AND DISCUSSION

This chapter contains data and results of the experimentation. Data was obtained and given in Tables 3.1-3.6, grouped by element. Upon acquisition of data, statistical analysis was performed on data to test a number of hypotheses. Linear regression analysis was used to find possible correlation when comparing all sites together. The slope, p-value, and R^2 value have much importance in confirming or dismissing a hypothesis. All values may not be given in this section, but all details from linear regression analysis are in the Appendix. Analysis of variance (ANOVA) was another statistical tool used on the data, when comparing multiple sites together. Not all ANOVA values were utilized in hypothesis confirmation, but the p-values are all included in the Appendix. Student's t-testing was also used in comparisons of two sites or life stage classes. This section summarizes the results from the Student's t-testing in tables, but p-values are all given in the Appendix. The chapter is divided into 6 statistical sections, grouped from the different statistical tests used on the data: (1) Effects of elevation on concentrations of foliar and exchangeable soil metals, (2) the correlation of concentration of foliar and exchangeable soil nutrients and toxic metal with distance from coal burning power plants of Tennessee, (3) the effect of pH on concentrations of foliar and exchangeable soil metals, (4) the dependence of concentration of foliar and exchangeable soil metal on life stage of Fraser fir, (5) correlation between exchangeable metals in soil and concentrations of nutrients and toxic metal in foliage, and (6) comparison of current data to previously collected data to characterize changes in Fraser fir over the course of several years.

3.1 Foliage Data

Average concentrations (and standard deviations) of aluminum, calcium, and magnesium in seedlings, saplings, and mature trees are listed in Tables 3.1, 3.2, and 3.3. Values given are averages and standard deviations for each life stage grouping of Fraser fir sampled.

Table 3.1: Average Concentrations of Aluminum in Foliage of Seedling, Sapling, and Mature Samples From Each Site, and Average Elevation of Samples

SITE	Seedling Concentration (µg/g)	Sapling Concentration (µg/g)	Mature Concentration (µg/g)	Elevation (m)
WR	139 ± 4	177 ± 5	220 ± 7	1907 ± 5
RB	202 ± 3	164 ± 3	186 ± 9	1908 ± 47
CD	215 ± 4	178 ± 13	167 ± 8	2016 ± 12
ML	161 ± 1	149 ± 5	139 ± 1	1705 ± 5
SM	188 ± 3	220 ± 2	254 ± 3	1714 ± 7
YF	254 ± 3	207 ± 3	225 ± 2	1765 ± 11
MM	173 ± 4	161 ± 5	127 ± 3	2011 ± 12
CA	369 ± 4	311 ± 3	281 ± 8	1760 ± 9

Table 3.2: Average Concentrations of Calcium in Foliage of Seedling, Sapling, and Mature Samples From Each Site, and Average Elevation of Samples

SITE	Seedling Concentration (μg/g)	Sapling Concentration (μg/g)	Mature Concentration (μg/g)	Elevation (m)
WR	2260 ± 10	2970 ± 40	3620 ± 70	1907 ± 5
RB	3350 ± 30	3870 ± 70	4270 ± 500	1908 ± 47
CD	3790 ± 40	4110 ± 210	4110 ± 80	2016 ± 12
ML	3600 ± 20	3970 ± 70	5270 ± 100	1705 ± 5
SM	3560 ± 60	4090 ± 10	3700 ± 40	1714 ± 7
YF	3530 ± 150	3020 ± 30	5230 ± 10	1765 ± 11
MM	3600 ± 40	3650 ± 30	5090 ± 20	2011 ± 12
CA	4870 ± 70	5420 ± 50	6570 ± 100	1760 ± 9

Table 3.3: Average Concentrations of Magnesium in Foliage of Seedling, Sapling, and Mature Samples From Each Site, and Average Elevation of Samples

SITE	Seedling Concentration (μg/g)	Sapling Concentration (μg/g)	Mature Concentration (μg/g)	Elevation (m)
WR	713 ± 10	729 ± 3	694 ± 20	1907 ± 5
RB	895 ± 6	838 ± 20	819 ± 80	1908 ± 47
CD	735 ± 4	827 ± 50	861 ± 20	2016 ± 12
ML	1130 ± 100	934 ± 20	856 ± 20	1705 ± 5
SM	926 ± 8	879 ± 4	847 ± 9	1714 ± 7
YF	1230 ± 10	983 ± 10	1060 ± 3	1765 ± 11
MM	1170 ± 7	981 ± 10	859 ± 7	2011 ± 12
CA	733 ± 4	736 ± 7	809 ± 12	1760 ± 9

3.2 Soil Data

Average concentrations (and standard deviations) of exchangeable metals aluminum, calcium, and magnesium in soil surrounding sampled Fraser fir trees are listed in Tables 3.4, 3.5, and 3.6, respectively. Each table contains the 3 life stage classes of samples from each site, along with soil pH and average elevation.

Table 3.4: Average Exchangeable Aluminum Concentration in Soil For Each Life Stage Class, Average pH of Soil, and Average Elevation of Each Site

SITE	Seedling Concentration (µg/g)	Sapling Concentration (µg/g)	Mature Concentration (µg/g)	Soil pH	Elevation (m)
WR	880 ± 17	973 ± 8	973 ± 16	3.5 ± 0.1	1907 ± 5
RB	832 ± 16	779 ± 17	857 ± 43	3.7 ± 0.1	1908 ± 47
CD	774 ± 76	715 ± 13	791 ± 55	3.7 ± 0.1	2016 ± 12
ML	775 ± 18	947 ± 20	750 ± 40	3.5 ± 0.1	1705 ± 5
SM	947 ± 6	896 ± 70	1100 ± 6	3.6 ± 0.1	1714 ± 7
YF	1080 ± 90	1070 ± 10	1290 ± 10	3.7 ± 0.1	1765 ± 11
MM	1020 ± 60	1090 ± 40	1030 ± 70	3.7 ± 0.1	2011 ± 12
CA	505 ± 10	278 ± 12	543 ± 28	4.4 ± 0.1	1760 ± 9

Table 3.5: Average Exchangeable Calcium Concentration in Soil For Each Life Stage Class, Average pH of Soil, and Average Elevation of Each Site

SITE	Seedling Concentration (µg/g)	Sapling Concentration (µg/g)	Mature Concentration (µg/g)	Soil pH	Elevation (m)
WR	459 ± 7	751 ± 11	175 ± 15	3.5 ± 0.1	1907 ± 5
RB	63 ± 10	90 ± 1	79 ± 13	3.7 ± 0.1	1908 ± 47
CD	57 ± 2	230 ± 11	194 ± 8	3.7 ± 0.1	2016 ± 12
ML	255 ± 5	261 ± 5	363 ± 8	3.5 ± 0.1	1705 ± 5
SM	233 ± 2	254 ± 9	168 ± 11	3.6 ± 0.1	1714 ± 7
YF	147 ± 12	170 ± 3	165 ± 7	3.7 ± 0.1	1765 ± 11
MM	127 ± 6	297 ± 9	229 ± 15	3.7 ± 0.1	2011 ± 12
CA	69 ± 1	91 ± 3	76 ± 3	4.4 ± 0.1	1760 ± 9

Table 3.6: Average Exchangeable Magnesium Concentration in Soil For Each Life Stage Class, Average pH of Soil, and Average Elevation of Each Site

SITE	Seedling Concentration (µg/g)	Sapling Concentration (µg/g)	Mature Concentration (µg/g)	Soil pH	Elevation (m)
WR	172 ± 1	188 ± 3	83 ± 2	3.5 ± 0.1	1907 ± 5
RB	61 ± 5	62 ± 1	56 ± 8	3.7 ± 0.1	1908 ± 47
CD	49 ± 5	75 ± 3	67 ± 3	3.7 ± 0.1	2016 ± 12
ML	101 ± 1	114 ± 1	105 ± 1	3.5 ± 0.1	1705 ± 5
SM	70 ± 1	65 ± 1	66 ± 1	3.6 ± 0.1	1714 ± 7
YF	91 ± 5	90 ± 1	89 ± 1	3.7 ± 0.1	1765 ± 11
MM	84 ± 6	98 ± 4	119 ± 6	3.7 ± 0.1	2011 ± 12
CA	74 ± 2	99 ± 3	84 ± 3	4.4 ± 0.1	1760 ± 9

3.3 Elevation Studies

It was hypothesized that the effects of acid deposition would be amplified at higher elevations due to increased wet and dry deposition, and increased cloud immersion. As a result, higher concentrations of aluminum and lower concentrations of calcium and magnesium were expected at higher sites when compared to lower sites.

3.3.1 All Sites Compared to Observe Elevation Correlation

To determine if a trend existed in elevation and hypothesized metal concentrations, linear regression analysis was used. Average aluminum, calcium, and magnesium concentrations in foliage and soil were used in the model along with average elevations from the site. Each life stage class was tested independent of other classes at the same site. The slope was hypothesized to be positive for aluminum; negative for calcium and magnesium ($\alpha = .05$). Slope/Intercept, R^2, and p-values for each test are listed in the Appendix: foliage is in Table C.1, and soil is in Table C.2. No correlation existed with all sites' metal concentrations against elevation. This might be due to influence of longitude, soil pH, or a number of other variables. By comparing sites in a similar region some variables could be eliminated. This is described in Section 3.3.2.

3.3.2 Adjacent High and Low Elevation Sites Compared

Two sets of adjacent sites differing in elevation by about 300 m were selected to compare toxic metal and nutrient concentrations as a function of elevation. The sites compared were Clingmans Dome and Mingus Lead in the GSMNP, and Mount Mitchell and Camp Alice in MMSP. Student's t-testing was used in making single element concentration comparisons with elevation for each life stage class. If the p-value calculated was less than the alpha value of 0.05, the concentrations compared were considered statistically different. Table 3.7 summarizes the results from the GSMNP comparison, and Table 3.8 summarizes the MMSP comparison. All other statistics from the Student's t-tests for foliage and surrounding soil are located in Tables C.3 and C.4, respectively. The hypothesis of higher toxic metal concentration in both soil and foliage at higher elevation and lower nutrient concentration in both soil and foliage at higher elevation was proven true exactly 50% of the 36 tests. Figure 3.1 is a representative graph including the comparison of foliar aluminum concentrations at two adjacent sites differing in elevation by about 900 m in the GSMNP.

Table 3.7: Results of Student's t-test - Elevation Comparison of Clingmans Dome and Mingus Lead in the GSMNP

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	Followed hypothesis	Did not follow hypothesis	Followed hypothesis
Sapling Foliage	Followed hypothesis	Did not follow hypothesis	Followed hypothesis
Mature Foliage	Followed hypothesis	Followed hypothesis	Did not follow hypothesis
Seedling Soil	Did not follow hypothesis	Followed hypothesis	Followed Hypothesis
Sapling Soil	Followed hypothesis	Followed hypothesis	Followed hypothesis
Mature Soil	Did not follow hypothesis	Followed hypothesis	Followed hypothesis

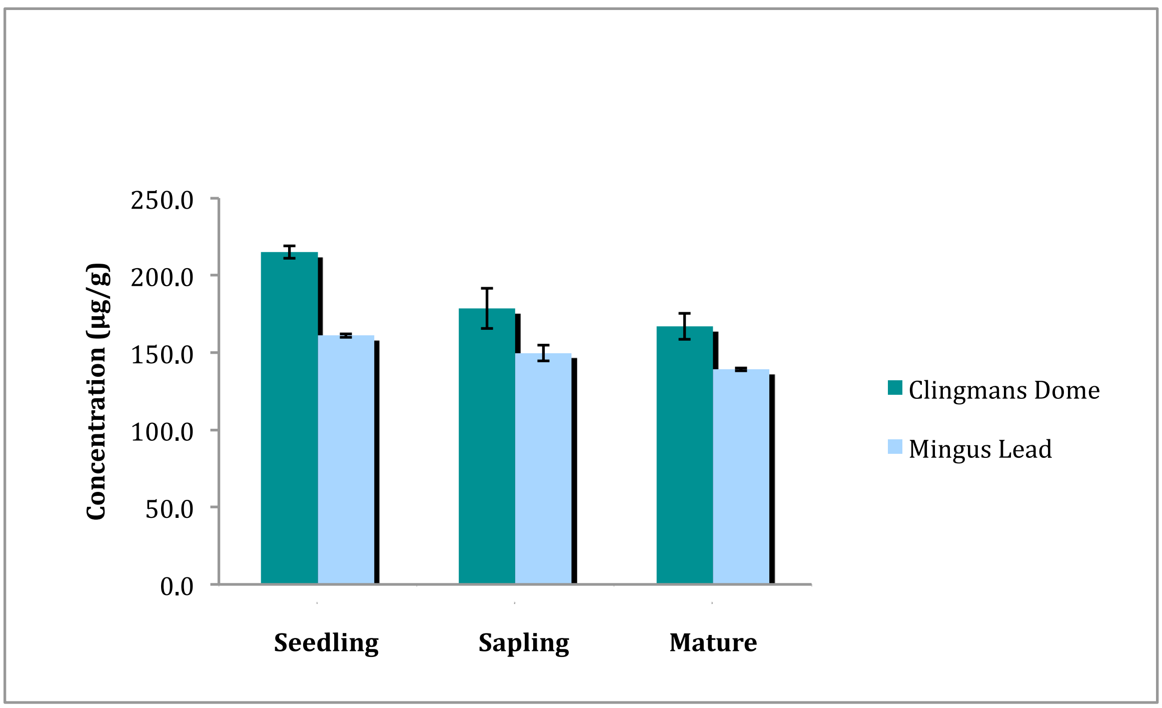
Clingmans Dome was expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to Mingus Lead.

Table 3.8: Summary of Student's t-test - Elevation Comparison of Mt. Mitchell and Camp Alice in MMSP

	Aluminum	Calcium	Magnesium
Seedling Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Sapling Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Mature Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Seedling Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis
Sapling Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis
Mature Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis

Mt. Mitchell was expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to Camp Alice.

Figure 3.1: Comparison of Foliar Aluminum Concentration in Seedlings, Saplings, and Mature Trees At Two Adjacent Sites (Clingmans Dome and Mingus Lead) Differing in Elevation by 900 m



Clingmans Dome was expected to have higher levels aluminum in each life stage class compared to Mingus Lead.

3.3.3 Collective Comparison of High Elevation Sites

All sites above 1900 m (above 6,000 ft) were collectively compared using analysis of variance. Due to similar conditions at these high elevations, the hypothesis was that all concentrations at high elevation sites were statistically the same. All replicates of samples per life stage class were used in the model, as it was operated for an individual element. If the p-value was above the alpha value (0.05), the sites were considered statistically the same. For both foliar and exchangeable soil metal concentrations, all sites and classes were statistically different. Results from the statistical analysis of foliage and soil are in the Appendix in Tables C.5 and C.6, respectively. The hypothesis of statistically similar metal concentrations for both foliage and soil at high elevation sites was not observed in any comparison.

3.3.4 Collective Comparison of Low Elevation Sites

All sites below 1900 m (below 6,000 ft) were evaluated with analysis of variance to determine if the metal concentrations were statistically the same. Similar to the high elevation comparison, the foliar and exchangeable soil metal concentrations were hypothesized to be identical. The statistical test was performed with all replicates of samples from each life stage class, for each element. The concentrations were labeled statistically the same if the p-value was found to be greater than the alpha value of 0.05. For both foliar and exchangeable soil metal concentrations, all sites and classes were found to be different. Results from the statistical analysis can be found in the Appendix in Tables C.7 and C.8, respectively. The hypothesis of statistically similar metal concentrations for both foliage and soil at low elevation sites was not observed in any comparison.

3.4 Longitudinal Studies

Due to down-wind coal burning power plants in Tennessee, it was questioned if longitude was a factor in concentrations of nutrients and toxic metals in the Fraser fir forest. Weather patterns could deliver pollutants and acids from these coal burning plants for deposition in the high elevation mountains, and it was hypothesized that the western most sites (closest to Tennessee) would be affected more by acid deposition than the central or eastern sample sites. Statistical analysis was performed to test the hypothesis.

3.4.1 All Sites Compared to Determine Longitude Correlation

All sites were compared by linear regression analysis, to determine if distance from coal burning power plants had an effect on foliar and exchangeable soil metal concentrations. The model utilized average element concentrations from each life stage class to plot against longitude. Aluminum was hypothesized to have a positive slope; calcium and magnesium were hypothesized to have negative slopes, all with a p-value less than the alpha value (0.05) for correlation to be confirmed. No correlation was found for any element in any class. Statistical results for foliage and soil are in the Appendix in Tables C.9 and C.10, respectively.

3.4.2 Western Sites Compared to Eastern Sites

The largest difference in metal concentrations in foliage and soil was expected between the western sites (Clingmans Dome and Mingus Lead, GSMNP) and the eastern sites (Mt. Mitchell and Camp Alice, MMSP). The high elevation western site was compared to the high elevation eastern site, and the low western site was compared to the low eastern site using Student's t-testing. Higher concentrations of aluminum and lower concentrations of calcium and magnesium were expected at Clingmans Dome and Mingus Lead. The tests were performed individually for each element and used all replicates of each life stage class at each site. If the p-value was less than the alpha value (0.05), a statistical difference was concluded. When comparing western sites to eastern sites, the hypothesis was proven true in exactly 50% of the 36 total tests. A summary of the results from the high elevation comparison is in Table 3.9, and a summary of results from the low elevation comparison is in Table 3.10. Representative results are plotted in Figure 3.2. Graphed are foliar aluminum concentrations for all life stages of samples from the high elevation western site (Clingmans Dome) compared to the high elevation eastern site (Mt. Mitchell). All statistical results from high and low elevation sites are in the Appendix, in Tables C.11 and C.12, respectively.

Table 3.9: Summary of Student's t-test Comparison of Western (Clingmans Dome) and Eastern (Mt. Mitchell) High Elevation Sites

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	Followed hypothesis	Did not follow hypothesis	Followed hypothesis
Sapling Foliage	Followed hypothesis	Did not follow hypothesis	Followed hypothesis
Mature Foliage	Followed hypothesis	Followed hypothesis	Did not follow hypothesis
Seedling Soil	Did not follow hypothesis	Followed hypothesis	Followed hypothesis
Sapling Soil	Did not follow hypothesis	Followed hypothesis	Followed hypothesis
Mature Soil	Did not follow hypothesis	Followed hypothesis	Followed hypothesis

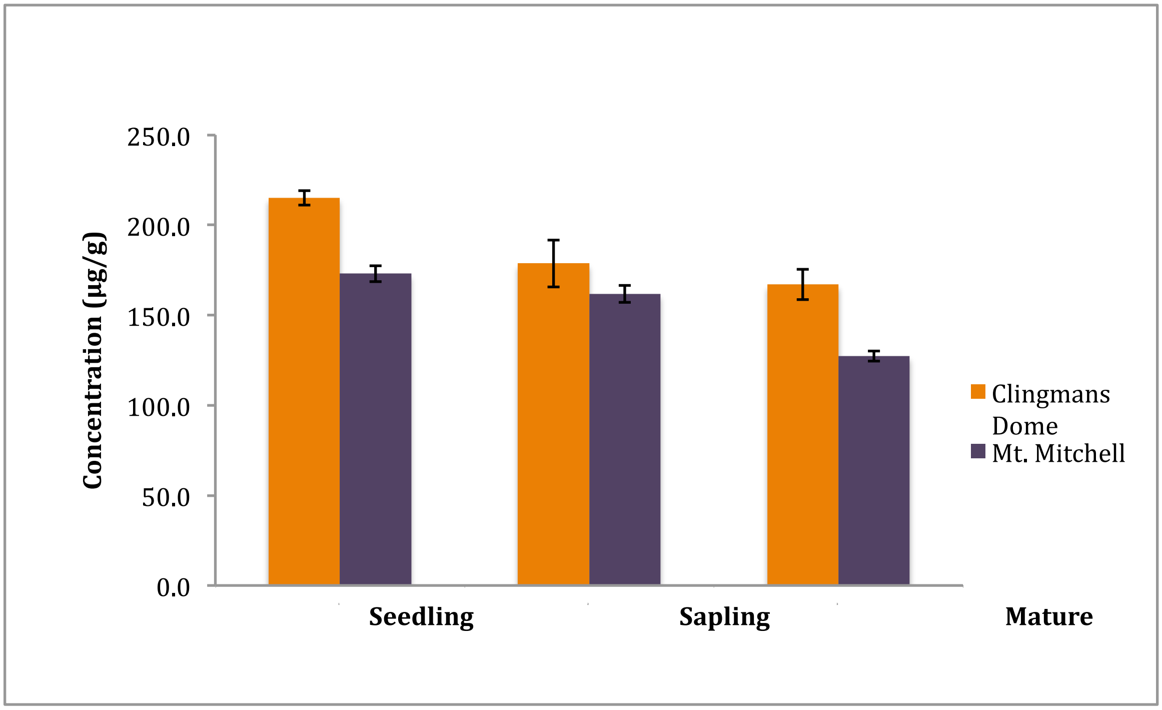
Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to eastern sites. A p-value less than 0.05 indicates difference among samples.

Table 3.10: Summary of Student's t-test Comparison of Western (Mingus Lead) and Eastern (Camp Alice) Low Elevation Sites

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Sapling Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Mature Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Seedling Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis
Sapling Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis
Mature Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis

Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to eastern sites. A p-value less than 0.05 indicates difference among samples.

Table 3.2: Foliar Aluminum Concentrations From Samples at a Western Site (Clingmans Dome) Compared to an Eastern Site (Mt. Mitchell)



Clingmans Dome was expected to have higher concentrations of foliar aluminum compared to Mt. Mitchell, due to closer proximity of the site to coal burning power plants of Tennessee.

3.4.3 Western Sites Compared to Central Sites

Foliar and exchangeable soil element concentrations were compared for further longitude effect analysis. The high elevation western site (Clingmans Dome) was compared to a high elevation central site (Water Rock Knob), and the low elevation western site (Mingus Lead) was compared to a low elevation central site (Yellow Face). Water Rock Knob and Yellow Face were chosen because of their close proximity to one another. Student's t-testing was utilized to compare the metal concentrations individually for all replicates in each life stage class of tree. If the p-value was calculated to be less than the alpha value (0.05), the sites were concluded as statistically different. When comparing western sites to central sites, the hypothesis was proven true in exactly

25% of the 36 total tests. Table 3.11 contains a statistical summary for the high elevation comparison, and the low elevation comparison summary is located in Table 3.12. Statistical results from the Student's t-test are located in the Appendix in Tables C.13 and C.14.

Table 3.11: Summary of Student's t-test Comparison of Western (Clingmans Dome) and Central (Water Rock Knob) High Elevation Sites

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis
Sapling Foliage	Did not follow hypothesis	Did not follow hypothesis	Did not follow hypothesis
Mature Foliage	Did not follow hypothesis	Did not follow hypothesis	Did not follow hypothesis
Seedling Soil	Did not follow hypothesis	Followed hypothesis	Followed hypothesis
Sapling Soil	Did not follow hypothesis	Followed hypothesis	Followed hypothesis
Mature Soil	Did not follow hypothesis	Did not follow hypothesis	Followed hypothesis

Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

Table 3.12: Summary of Student's t-test Comparison of Western (Mingus Lead) and Central (Yellow Face) Low Elevation Sites

	Aluminum	Calcium	Magnesium
Seedling Foliage	Did not follow hypothesis	Did not follow hypothesis	Followed hypothesis
Sapling Foliage	Did not follow hypothesis	Did not follow hypothesis	Followed hypothesis
Mature Foliage	Did not follow hypothesis	Did not follow hypothesis	Followed hypothesis
Seedling Soil	Did not follow hypothesis	Did not follow hypothesis	Did not follow hypothesis
Sapling Soil	Did not follow hypothesis	Did not follow hypothesis	Did not follow hypothesis
Mature Soil	Did not follow hypothesis	Did not follow hypothesis	Did not follow hypothesis

Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

3.4.4 Eastern Sites Compared to Central Sites

Foliar and exchangeable soil element concentrations were compared for further longitude effect analysis. The high elevation eastern site (Mt. Mitchell) was compared to a high elevation central site (Water Rock Knob), and the low elevation eastern site (Camp Alice) was compared to a low elevation central site (Yellow Face). Water Rock Knob and Yellow Face were chosen because of their close proximity to one another. Student's t-testing was utilized to compare the metal concentrations individually for all replicates in each life stage class of tree. If the p-value was calculated to be less than the alpha value (0.05), the sites were concluded as statistically different. When comparing eastern sites to central sites, the hypothesis was proven true in less than 50% of the 36 total tests. Table 3.13 contains a statistical summary for the high elevation comparison, and the low elevation comparison summary is located in Table 3.14. All statistical results are located in the Appendix, in Tables C.15 and C.16.

Table 3.13: Summary of Student's t-test Comparison of Eastern (Mt. Mitchell) and Central (Water Rock Knob) High Elevation Sites

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	Did not follow hypothesis	Followed hypothesis	Followed hypothesis
Sapling Foliage	Followed hypothesis	Followed hypothesis	Followed hypothesis
Mature Foliage	Followed hypothesis	Followed hypothesis	Followed hypothesis
Seedling Soil	Did not follow hypothesis	Did not follow hypothesis	Did not follow hypothesis
Sapling Soil	Did not follow hypothesis	Did not follow hypothesis	Did not follow hypothesis
Mature Soil	Did not follow hypothesis	Followed hypothesis	Followed hypothesis

Eastern sites were expected to have lower concentrations of aluminum and higher concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

Table 3.14: Summary of Student's t-test Comparison of Eastern (Camp Alice) and Central (Yellow Face) Low Elevation Sites

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Sapling Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Mature Foliage	Did not follow hypothesis	Followed hypothesis	Did not follow hypothesis
Seedling Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis
Sapling Soil	Followed hypothesis	Did not follow hypothesis	Did not follow hypothesis
Mature Soil	Followed hypothesis	Did not follow hypothesis	Followed hypothesis

Eastern sites were expected to have lower concentrations of aluminum and higher concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

3.5 Effect of pH on Concentrations of Foliar Metals and Exchangeable Soil Metals

The pH of soil was expected to have an effect on the exchangeable metal concentrations in soil surrounding Fraser fir trees; thus affecting toxic metal and nutrient concentrations in foliage. A decrease in soil pH was hypothesized to result in an increase in foliar and exchangeable soil metal concentrations of aluminum, and decreases in levels of calcium and magnesium. Linear regression analysis was performed to observe these effects, but results were inconclusive. Foliar and exchangeable soil statistical results are in the Appendix, in Tables C.17 and C.18, respectively.

3.6 Life Stage Analysis

The life stage of a tree (be it a seedling, sapling, or mature) was hypothesized to have no effect on foliar metal concentrations or exchangeable soil metal concentrations. Statistical analysis was performed for each element using every replicate, testing throughout all life stages of samples (seedling, sapling, and mature combined).

3.6.1 Life Stage Analysis Involving Foliar Metal Concentrations

Analysis of variance was used to determine any statistical difference in concentrations of metals in foliage between different life stage classes at each site. If the p-value was greater than the alpha value (0.05), the concentrations were statistically the same. Analysis of variance results are shown in Tables C.19, C.20, and C.21 of the Appendix. Table 3.15 is a summary of analysis of variance. Magnesium concentrations at Richland Balsam were the only concentrations that were found to be statistically similar in all foliage data.

Table 3.15: Results of Analysis of Variance When Testing Across All Life Stages of Samples (seedling, sapling, mature) At All Sample Sites

Statistical Test	**Conclusion**
All Sites, Aluminum	No Sites Followed Hypothesis
All Sites, Calcium	No Sites Followed Hypothesis
All Sites, Magnesium	Richland Balsam Followed Hypothesis, No Other Sites Followed Hypothesis

All life stages of samples at each site were tested to determine if differences in foliar metal concentrations existed. All life stages were expected to be the same.

3.6.2 Life Stage Analysis Involving Exchangeable Soil Metal Concentrations

Analysis of variance was used to determine if statistical difference in concentration of exchangeable soil metals existed between different life stages of tree at each sample site. A p-value greater than the alpha value (0.05) indicates statistical similarity. A summary of results from this test is found in Table 3.16, and p-values from ANOVA for each site can be found in the Appendix in Tables C.22, C.23, and C.24.

Table 3.16: Summary of Results From Analysis of Variance When Comparing Exchangeable Concentrations in Soil Over All Life Stages Classes of Samples (seedling, sapling, mature) At All Sample Sites

Site	Conclusions
WR	No Elements Followed Hypothesis
RB	Magnesium Followed Hypothesis No Other Elements Followed Hypothesis
CD	Aluminum Followed Hypothesis No Other Elements Followed Hypothesis
ML	No Elements Followed Hypothesis
SM	No Elements Followed Hypothesis
YF	Aluminum and Magnesium Followed Hypothesis Calcium Did Not Follow Hypothesis
MM	Aluminum Followed Hypothesis No Other Element Followed Hypothesis
CA	No Elements Followed Hypothesis

All life stages of samples at each site were tested to determine if differences in exchangeable soil metal concentrations existed. All life stages were expected to be the same.

Exchangeable aluminum concentrations were found to be the same among all life stages at Clingmans Dome, Yellow Face, and Mt. Mitchell. Concentrations of exchangeable magnesium were the same among all life stages at Richland Balsam and Yellow Face. All other sites, along with all calcium concentrations from all sites differed among different life stages.

3.7 Foliar Metal Concentration vs. Soil Exchangeable Metal Concentration

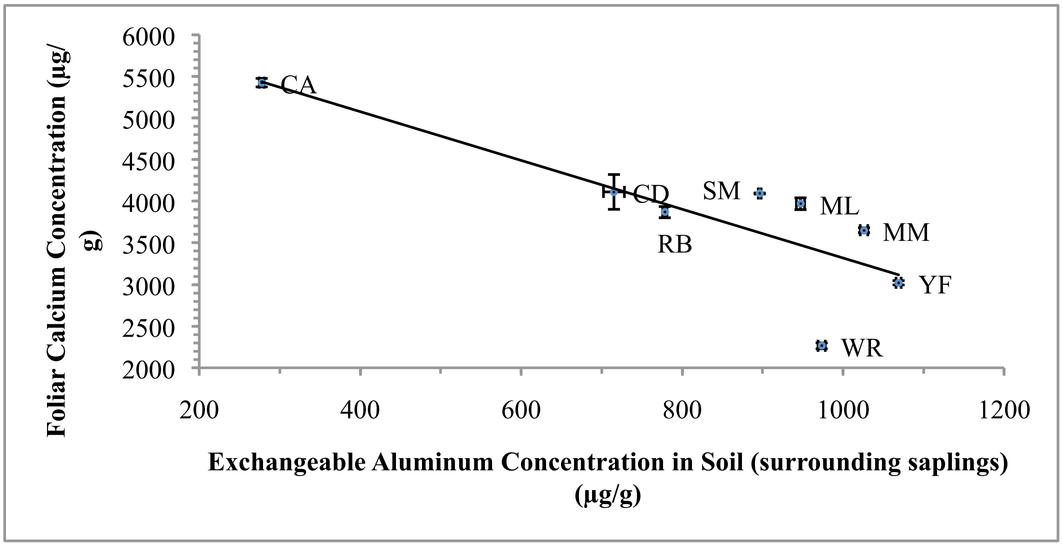
3.7.1 Relationship of Exchangeable Soil Element and Foliar Element

It was hypothesized that as the concentration of a given exchangeable element in soil increased, the concentration of said element in foliage should increase as well. Linear regression analysis was used to challenge the hypothesis. Average concentrations of exchangeable soil metal and foliar metal from each life stage class at each site were used in the model. For all elements, a positive slope was expected with a p-value less than the alpha value (0.05). These statistical results are in the Appendix in Table C.25. No correlation occurred in any life stage class for any element.

3.7.2 Effect of Exchangeable Aluminum in Soil on Foliar Calcium Concentration

Linear regression analysis was used to examine the effect exchangeable aluminum had on concentration of calcium in foliage. Exchangeable aluminum is able to interfere with calcium transport through the roots of the Fraser fir; so increased levels of exchangeable aluminum in soil should result in decreased foliar levels of calcium in foliage. A negative slope was expected in the linear regression analysis, and a p-value less than the alpha value (0.05) was used to indicate correlation. Figure 3.1 is a plot showing the correct correlation, which existed with sapling samples.

Figure 3.3: Plot of Linear Trend Between Foliar Calcium Concentration and Soil Exchangeable Aluminum Concentration. Slope: -2.929 ± 0.843 (S.E.), Intercept: 6248 ± 732.3 (S.E.), R^2 value: 0.668, p-value: 0.013



As concentration of exchangeable aluminum in soil increases, concentration of calcium in foliage was expected to decrease; therefore, a negative slope was expected.

For seedling and mature classes of Fraser fir, a non-linear negative trend was observed in regression analysis of this hypothesis. A negative correlation existed in saplings, confirming this hypothesis. Results of linear regression analysis are located in the Appendix, in Table C.26.

3.8 Comparison of Results to Previous Foliage Data

It was important to compare results from this study to data collected in previous studies to examine the effect of time and air cleansing laws on the effects of acid deposition. Comparisons involving soil could not be completed. Previous soil data was for total concentrations of elements in soil, but this study involved exchangeable elements only. However, previous data of Fraser fir foliar metal concentrations were available from three previous studies, conducted in 1969, 1994, and 1996. The locations of these sites were considered when choosing sample sites, so that comparisons could be made. The 1969 and 1994 studies involved determination of nutrients calcium and magnesium at Richland Balsam, by Weaver and Shepard, respectively (1972, 1994). Lee determined calcium, magnesium, and aluminum at Clingmans Dome (1996).

To compare concentrations of foliar elements between this study and those prior, analysis of variance was used to determine if the concentrations of elements in each study were different or statistically the same. It was hypothesized that the concentrations of foliar elements had changed since previous work. Due to the amendments of the Clean Air Act in 1990, acid deposition should have decreased, and the effects of deposition on foliar metal concentrations should have begun to subside since then. Concentrations of aluminum in foliage should have decreased since the last study, and the concentrations of calcium and magnesium should have increased.

3.8.1 Comparison to Previous Data Acquired From Richland Balsam

The studies conducted at Richland Balsam involved foliar concentrations of tree nutrients calcium and magnesium in saplings. Table 3.17 includes averages, standard deviations, and results of analysis of variance.

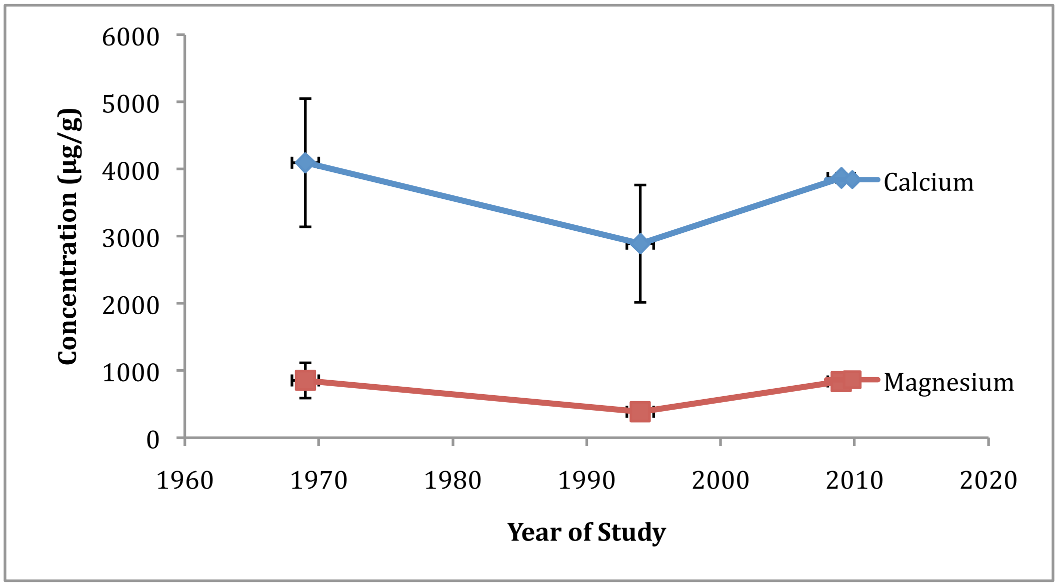
Table 3.17: Nutrient Concentrations Found During Previous Studies, and Analysis of Variance Results When Comparing Data From This Study to Results of the Previous Studies at Richland Balsam (α = 0.05)

Element	Weaver (1969)	Shepard (1994)	Wilson (2009)	p-value	Conclusion
Calcium (µg/g)	4095 ± 955	2890 ± 873	3870 ± 67	1.04×10^{-24}	Different and increased, followed hypothesis
Magnesium (µg/g)	853 ± 262	387 ± 81	838 ± 17	1.08×10^{-22}	Different and increased, followed hypothesis

The results from this study and previous studies were expected to be different (p-value less than 0.05). Calcium and magnesium concentrations were expected to increase from prior studies.

Results from analysis of variance determined difference in concentrations of foliar calcium and magnesium in saplings between the three studies. Figure 3.2 shows concentrations of calcium and magnesium determined at Richland Balsam from the three studies (1969, 1994, 2009). It appears between the 1969 and 1994 studies a loss in foliar calcium and magnesium occurred. This could have resulted from direct leaching from foliage by acid precipitation or soil aluminum inhibition of nutrient uptake. Since 1994, calcium and magnesium concentrations at Richland Balsam have increased some, probably due to the Clean Air Act Amendments of 1990.

Figure 3.4: Three Separate Studies of Foliar Calcium and Magnesium Concentrations Found At Richland Balsam In 1969, 1994, and 2009



Calcium and magnesium concentrations were hypothesized to increase since the 1994 study, due to the amendments to The Clean Air Act of 1990.

3.8.2 Comparison to Previous Data Acquired From Clingmans Dome

Lee collected foliar metal concentrations of eight saplings at Clingmans Dome in 1996. Data from this study is included in Table 3.18, along with data from this study, and results of analysis of variance.

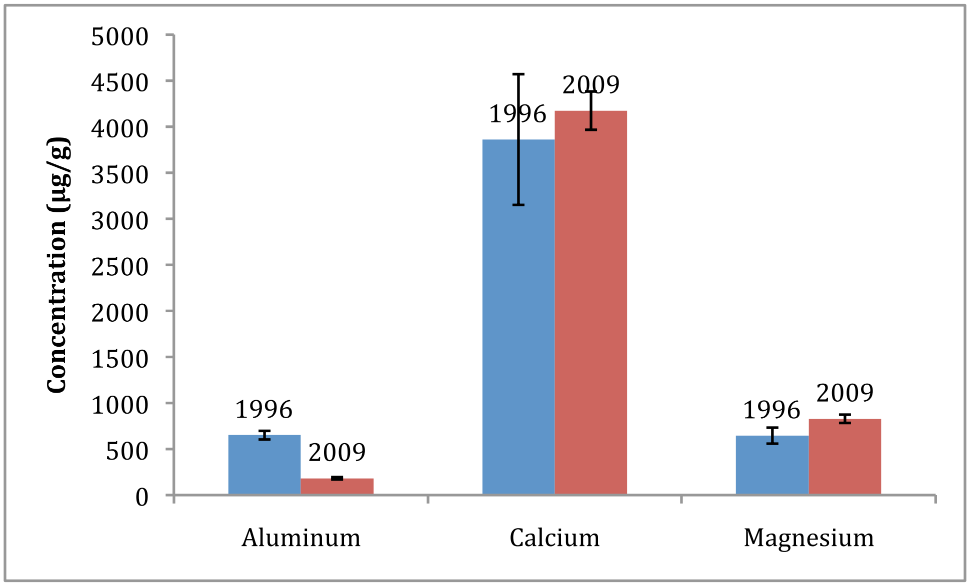
Table 3.18: Analysis of Variance Results When Comparing Data to Results of the Previous Study at Clingmans Dome (α = 0.05)

Element	**Lee (1996)**	**Wilson (2009)**	**p-value**	**Conclusion**
Aluminum	650 ± 47	182 ± 13	4.51×10^{-18}	Different and decreased, follows hypothesis
Calcium	3860 ± 710	4174 ± 208	4.91×10^{-14}	Different and increased, followed hypothesis
Magnesium	645 ± 87	828 ± 45	3.52×10^{-16}	Different and increased, followed hypothesis

The results from this study and the previous study were expected to be different (p-value less than 0.05). Aluminum concentration was expected to decrease, whereas calcium and magnesium concentrations were expected to increase from prior study.

Student's t-testing concluded that the concentrations of all aluminum and magnesium have changed since the 1996 study, in the way suggested by the hypothesis. No change was statistically found for calcium concentrations. As expected in the hypothesis, magnesium concentration increased and toxic metal concentration has decreased since the previous study. Figure 3.3 is a graph showing the changes in all three elements since the 1996 study.

Figure 3.5: Change in Nutrient and Toxic Metal Concentration in Fraser Fir Foliage at Clingmans Dome Since 1996



Aluminum concentration was hypothesized to decrease, whereas calcium and magnesium concentrations were expected to increase since the 1996 study.

4. CONCLUSION

High elevation Fraser fir forests are subjected to increased amounts of rain and snowfall, increased cloud emersion, and higher wind speeds, all of which should result in more intense effects of acid deposition than lower elevation forests. Lower levels of nutrients (calcium and magnesium) and higher levels of toxic metal (aluminum) were expected at the higher elevation sites. Results from linear regression analysis showed no correlation between metal concentrations and elevation when comparing all sites. When comparing two adjacent sites together using a Student's t-test to analyze effect of elevation on metal concentrations, the hypothesis was proven true in exactly 50% of the 36 tests.

The coal burning power plants located downwind from the sample sites in Tennessee were expected to effect the metal concentrations in foliage and soil. Sites located further west were expected to have lower concentrations of nutrients (calcium and magnesium) and higher concentrations of toxic metal (aluminum). No correlation was observed following a linear regression analysis comparing all sites. When comparing western sites to eastern sites, the hypothesis was proven true in exactly 50% of the 36 total tests. When comparing western sites to central sites, the hypothesis was proven true in exactly 25% of the 36 total tests, and proven true less than 50% of the total 36 tests when comparing eastern sites to central sites.

The effect of soil pH on concentrations of foliar and soil metal concentrations was also tested. As pH decreased, it was hypothesized to observe increases in both foliar and exchangeable soil aluminum concentrations and a decrease in both foliar and exchangeable soil nutrient (calcium and magnesium) concentrations. Linear regression analysis indicated inadequate correlation in all life stage classes.

It was hypothesized that all life stage classes of samples should have statistically similar foliar metal concentrations. Analysis of variance results indicated the site Richland Balsam to have statistically similar magnesium concentration, but no other element or site followed the hypothesis. Based on Student's t-test follow-up results, the BWA could be the reason behind differences at Water Rock Knob, Mingus Lead, Spruce Mountain, and Mt. Mitchell. For exchangeable metal similarities between life stage classes, no similarities existed for calcium concentrations. However, aluminum concentrations at Clingmans Dome, Yellow Face, and Mt. Mitchell were the same, and magnesium concentrations at Richland Balsam and Yellow Face were found to be the same.

Linear regression analysis was used to determine if a positive correlation existed between exchangeable metal concentration in soil and foliar metal concentration. No correlation was found for any site. Due to the action of exchangeable toxic metal aluminum in soil, it was hypothesized that foliar calcium would increase as soil aluminum decreased. The negative slope was observed in all life stage classes, but sapling was the only class where the slope was statistically linear.

Results from this study were compared to previously conducted studies to characterize the effects of acid deposition over time. The Clean Air Act amendments of 1990 were expected to have an effect on concentrations of determined elements, with lower concentrations of toxic metal aluminum and higher concentrations of nutrients calcium and magnesium. Comparison of sapling foliar nutrient and toxic metal concentrations to the 1996 work at Clingmans Dome followed the hypothesis for aluminum and magnesium, but not for calcium. Differences in foliar nutrient concentrations of saplings were determined when comparing to two previous studies at Richland Balsam (1969 and 1994). Foliar nutrient concentration in saplings has increased since the 1994 study at Richland Balsam.

REFERENCES

1. Aber, J. D.; Goodale, C. L.; Ollinger, S. V.; Smith, M. L.; Magill, A. H.; Martin, M. E.; Hallett, R. A.; Stoddard, J. L. Is Nitrogen Deposition Altering the Nitrogen Status of Northeastern Forests? *Biosceince.* **2003**, 53, 375-389.

2. Acid Rain and Transported Air Pollutants: Implications for Public Policy (Washington, D. C.: U. S. Congress, Office of Technology Assessment, OTA-O-204, June 1984).

3. Carter, M. R. 1993. Soil Sampling and Methods of Analysis. Boca Raton, FL: Lewis Publishers. 141-142 and 167-169.

4. Center For Global Development. http://carma.org/dig (accessed May 18, 2010).

5. Cronan, C. S.; Schofield, C. L. Aluminum Leaching Response to Acid Precipitation - Effects on High Elevation Watersheds in the Northeast. *Science.* **1979**, 204, 304-306.

6. Dehayes, D. H.; Schaberg, P. G.; Hawley, G. J.; Strimbeck, G. R. Acid Rain Impacts on Calcium Nutrition and Forest Health. *Bioscience.* **1999**, 49, 789-800.

7. Eagar, C. In *The Southern Appalachian Spruce-Fir Ecosystem: Its Biology and Threats*; White, P. S., Ed; U. S. Department of the Interior, National Park Service, Research/Resources Management Report SER-71: Gatlinburg, TN, 1984.

8. Johnson, A. H. Acid Deposition and Forest Decline. *Environmental Science and Technology.* **1983**, 17, A294-A305.

9. Lee, C. E. Determination of Aluminum, Calcium, and Magnesium in Fraser Fir (*Abies fraseri*) Foliage and Surrounding Soil by Atomic Absorption Spectrometry. M. S. Thesis, Western Carolina University, Cullowhee, NC, December 1996.

10. Lovett, G. M. In *The Southern Appalachian Spruce-Fir Ecosystem: Its Biology and Threats*; White, P. S., Ed; U. S. Department of the Interior, National Park Service, Research/Resources Management Report SER-71: Gatlinburg, TN, 1984.

11. McNulty, S. G.; Aber, J.D.; Newman, S. D. Nitrogen Saturation in a High Elevation New England Spruce-fir Stand. *Forest Ecology and Management*. **1996**, 84, 109-121.

12. Park, C. C.; *Acid Rain Rhetoric and Reality*; Methuen: London and New York, 1987; pp. 38-41, 92-96.

13. Schaberg, P.G.; Dehayes, D. H.; Hawley, G. J. Anthropogenic Calcium Depletion: A Unique Threat to Forest Ecosystem Health? *Ecosystem Health.* **2001,** 7, 214-228.

14. Shepard, M. R.; Lee, C. E.; Woosley, R. S.; and Butcher, D. J.. 1995. Determination of Calcium and Magnesium by Flame Atomic Absorption Spectroscopy in Fraser Fir (Abies fraseri) and Red Spruce (Picea rubens) Foliage from Richland Balsam Mountain, North Carolina. Microchemical Journal. 52: 118-126.

15. Shortle, W. C.; Smith, K.T. Aluminum Induced Calcium Deficiency Syndrome in Declining Red Spruce. *Science*. **1988,** 240, 1017-1018.

16. Sutton, B. A. Decline of the Spruce-Fir Forests in the Southern Appalachians: A Study of the Monoterpenes Present in the Oleoresin of Fraser Fir (*Abies fraseri*). *Microchem J.* **1997**, *56*, 332-342.

17. United States Environmental Protection Agency. Overview: The Clean Air Act Amendments of 1990. http://www.epa.gov/air/caa/overview.txt (accessed May 18, 2010).

18. Weaver, G. T. Dry Matter and Nutrient Dynamics in Red-Spruce - Fraser Fir and Yellow Birch Ecosystems in the Balsam Mountains, Western North Carolina. Ph.D. Dissertation, The University of Tennessee, TN, 1972.

19. White, P. S. In *The Southern Appalachian Spruce-Fir Ecosystem: Its Biology and Threats*; White, P. S., Ed; U. S. Department of the Interior, National Park Service, Research/Resources Management Report SER-71: Gatlinburg, TN, 1984.

APPENDIX A: MAPS OF SAMPLE AREAS

Figure A.1: Map of Sampling Area at Water Rock Knob

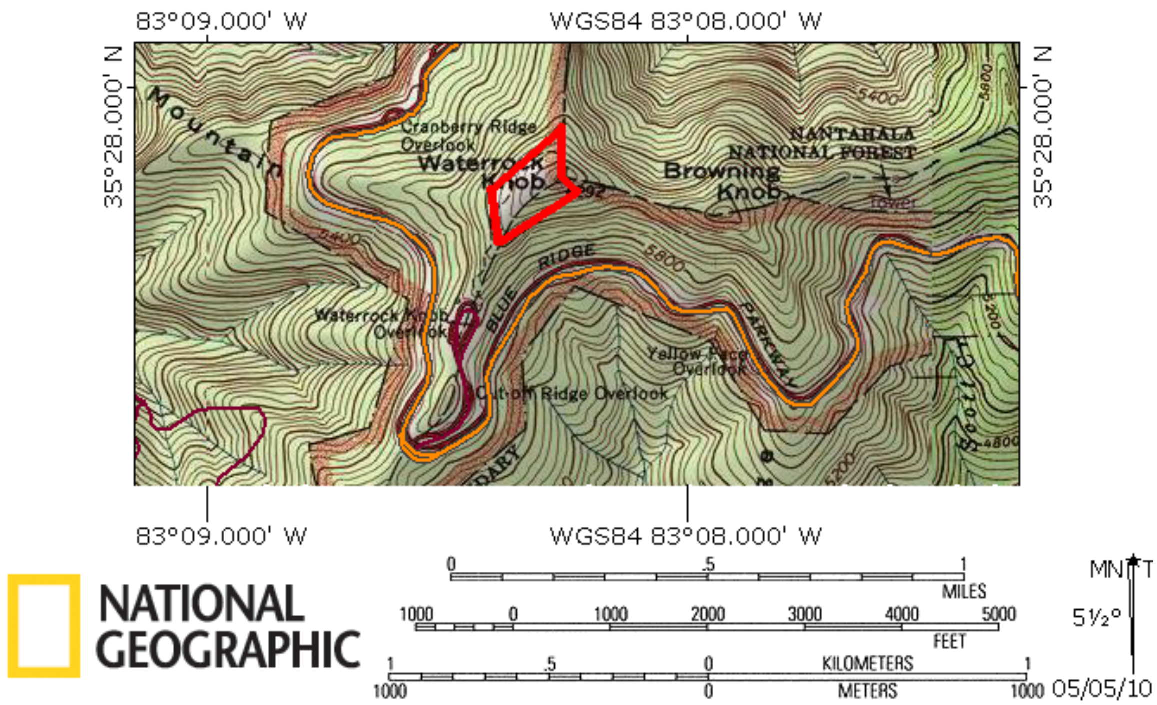


Figure A.2: Map of Sampling Area at Richland Balsam

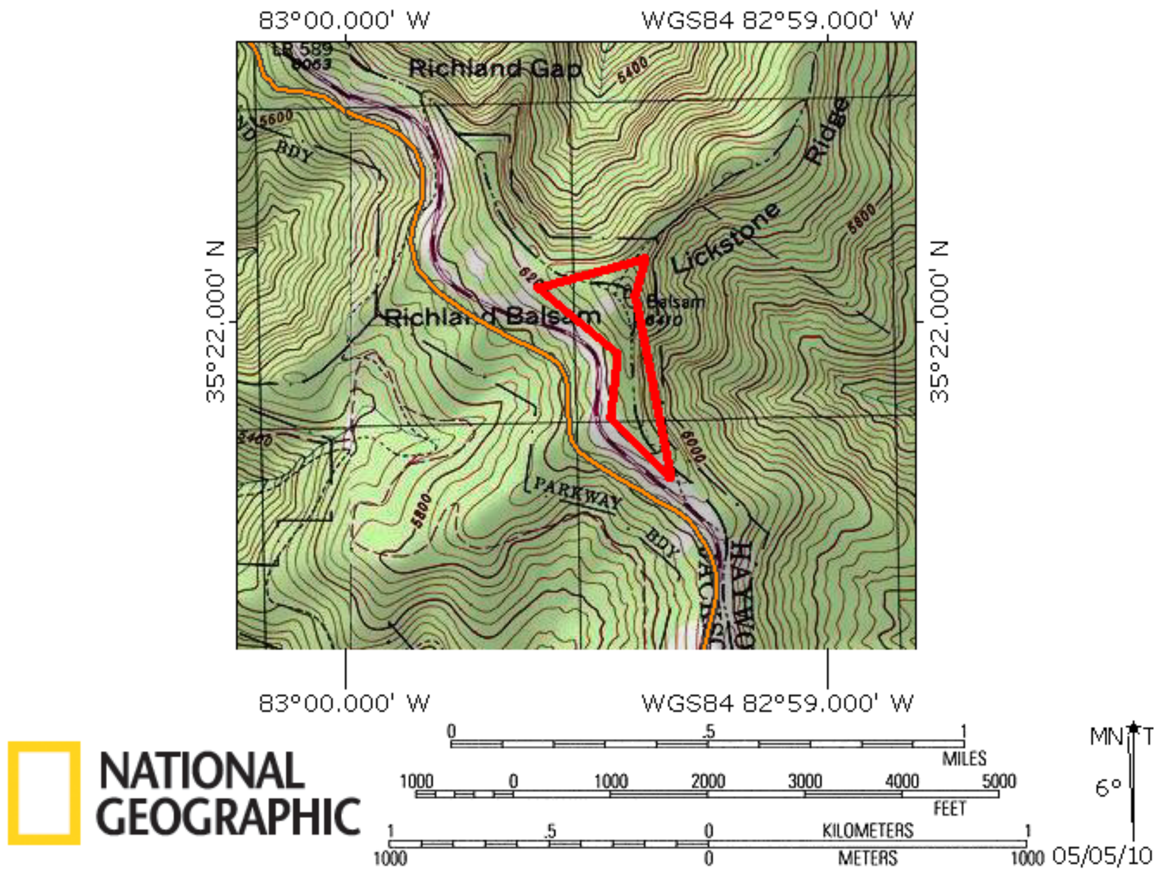


Figure A.3: Map of Sampling Area at Clingmans Dome

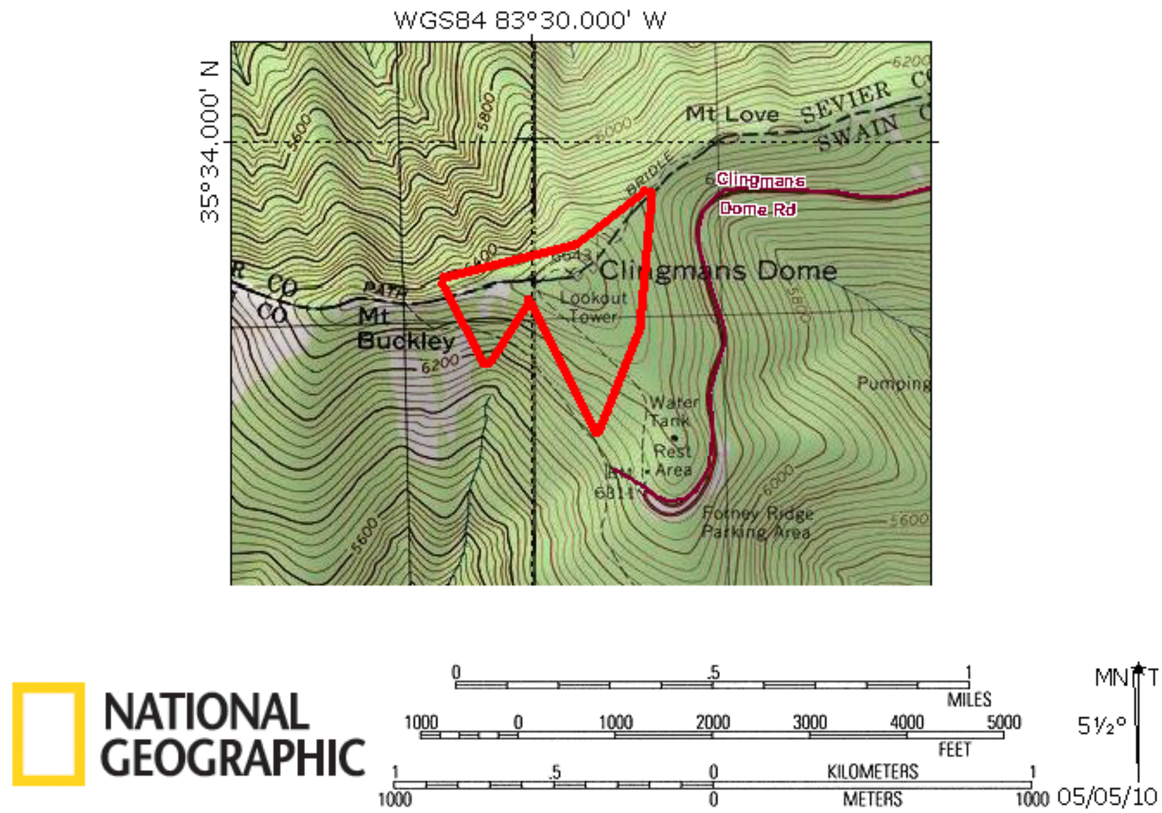


Figure A.4: Map of Sampling Area at Mingus Lead

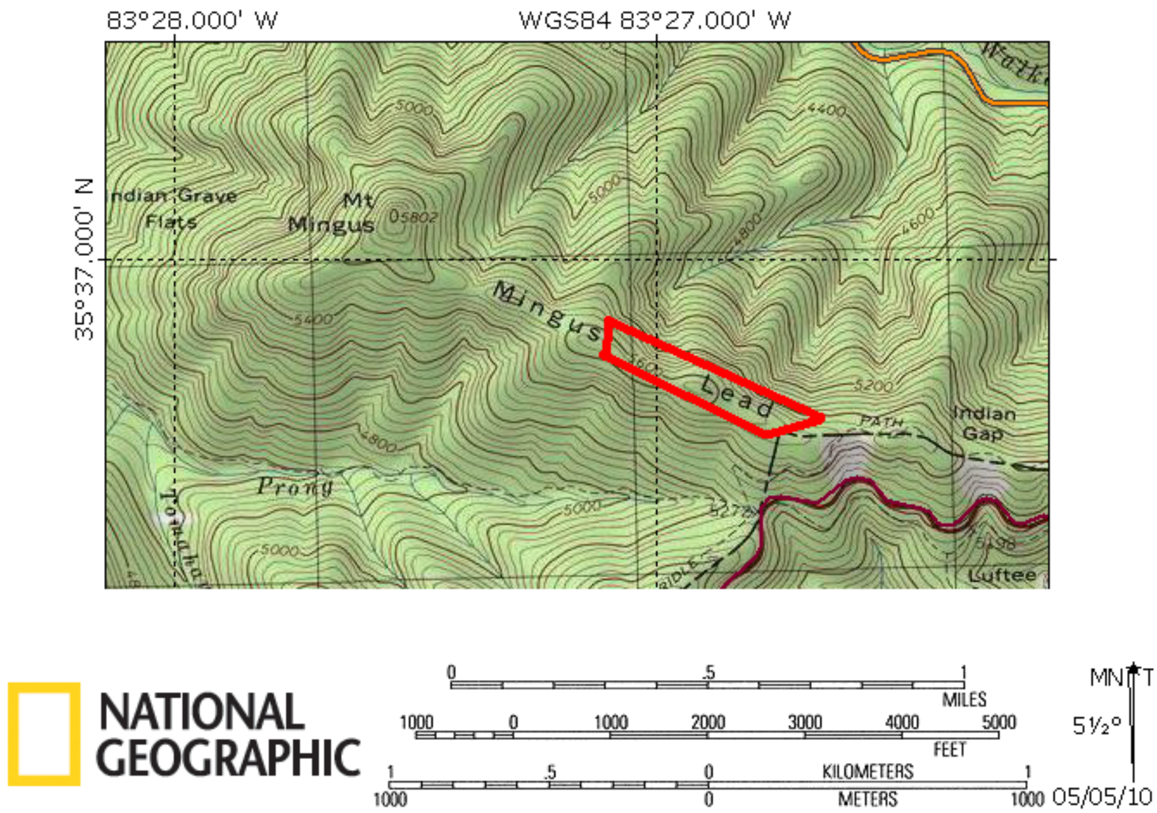


Figure A.5: Map of Sampling Area at Spruce Mountain

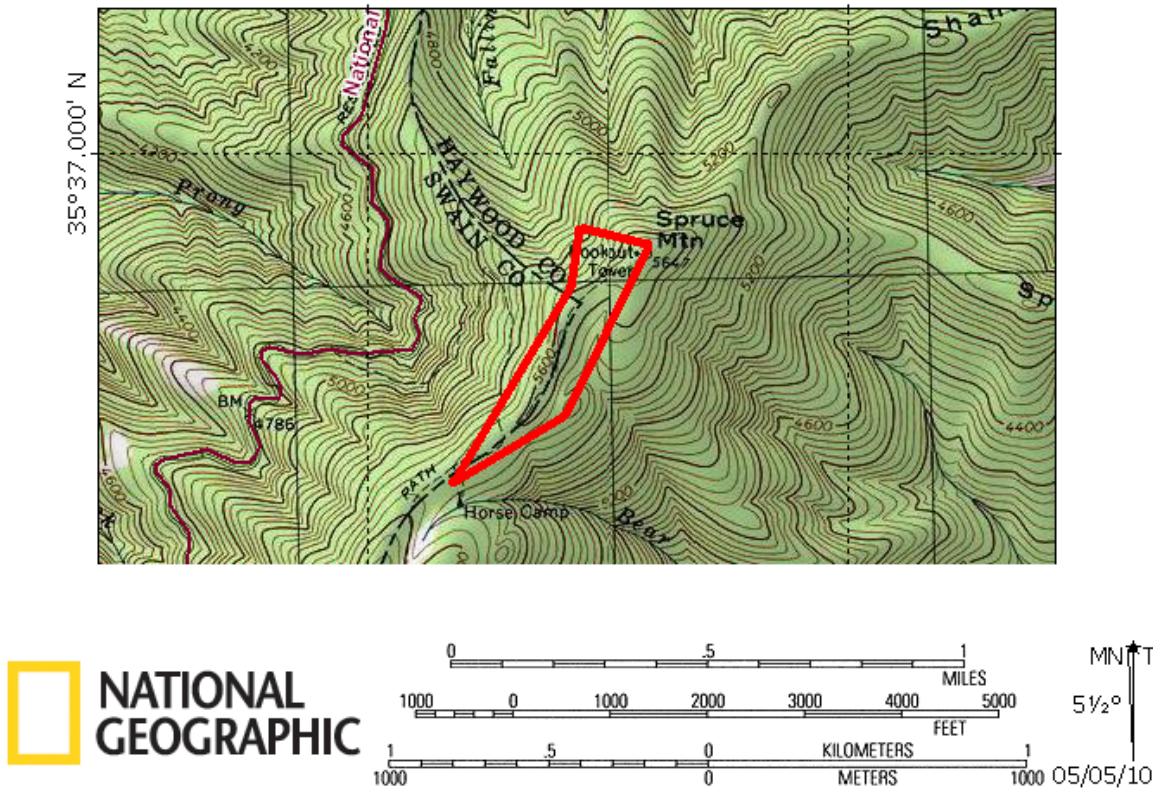


Figure A.6: Map of Sampling Area at Yellow Face

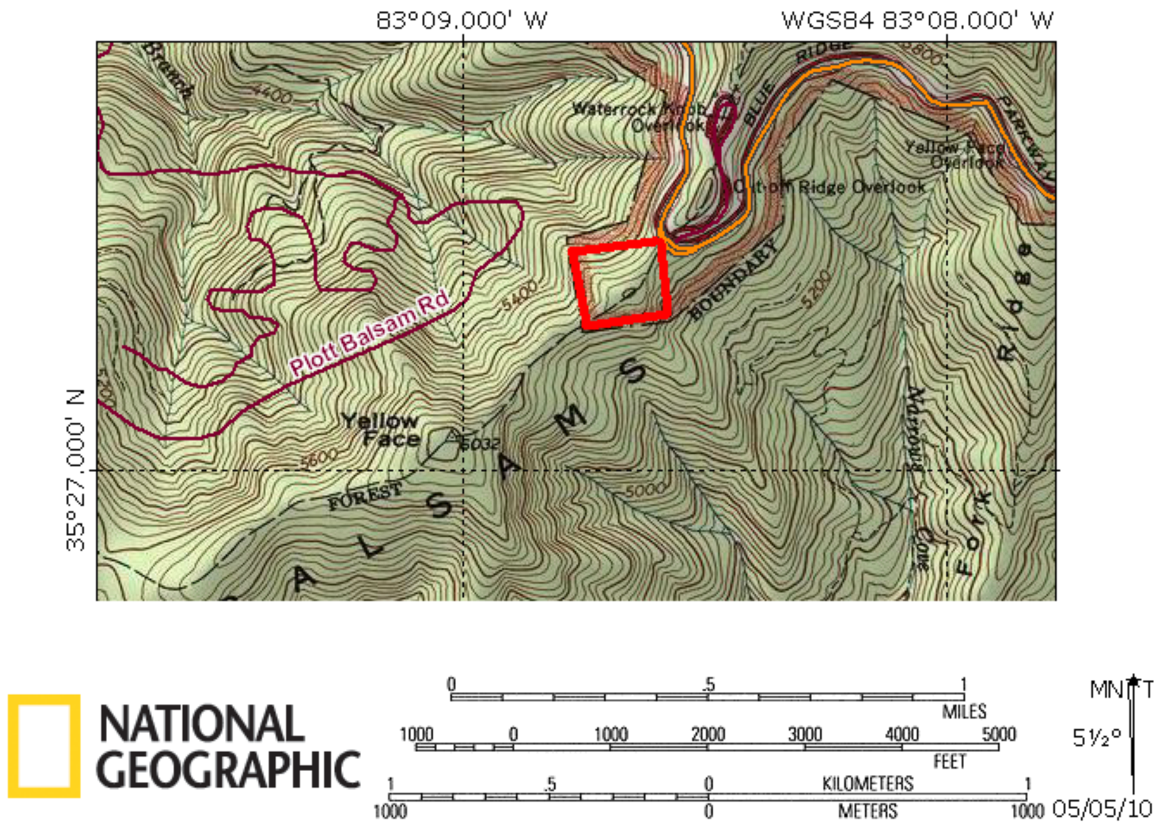


Figure A.7: Map of Sampling Area at Mt. Mitchell

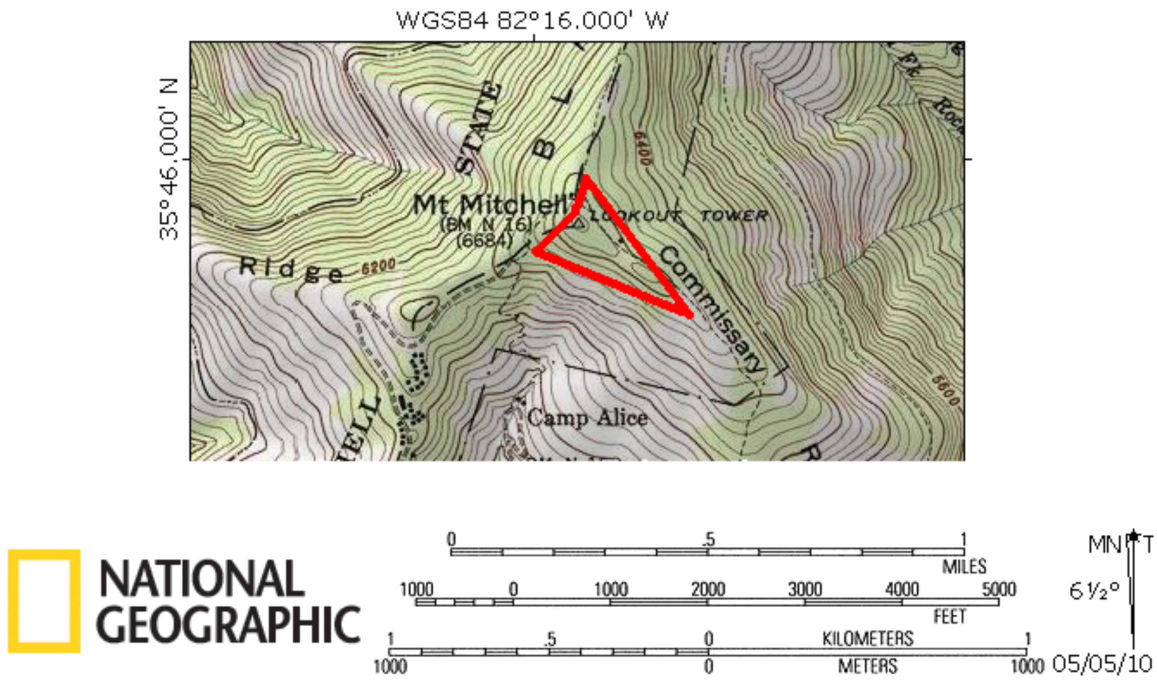
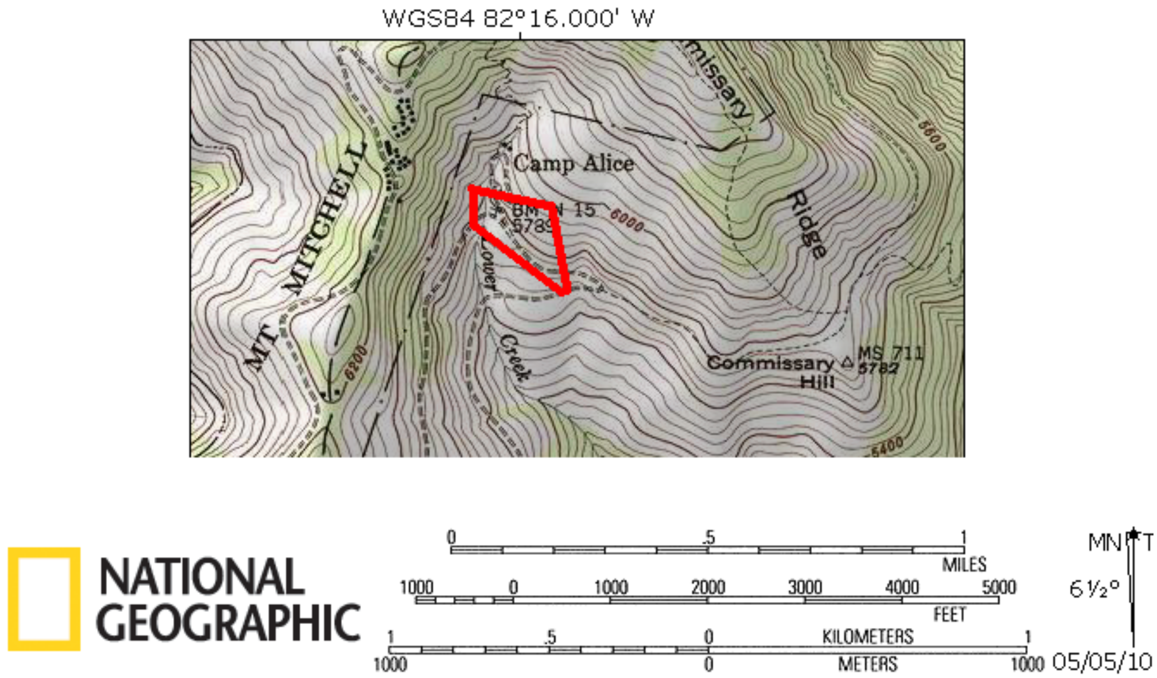


Figure A.8: Map of Sampling Area at Camp Alice



APPENDIX B: INDIVIDUAL SAMPLE INFORMATION

Table B.1: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Water Rock Knob

Sample ID	Latitude (^oN)	Longitude (^oW)	Elevation (ft)
E1	35^o 27.855	83^o 08.265	6266
E2	35^o 27.854	83^o 08.278	6252
E3	35^o 27.848	83^o 08.280	6262
E4	35^o 27.844	83^o 08.277	6260
E5	35^o 27.825	83^o 08.297	6256
E6	35^o 27.816	83^o 08.308	6237
E7	35^o 27.834	83^o 08.291	6258
E8	35^o 27.849	83^o 08.236	6265
E9	35^o 27.859	83^o 08.231	6246
E10	35^o 27.867	83^o 08.235	6226
Average	35^o 27.846	83^o 08.269	6252
Standard Deviation			13

Table B.2: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Water Rock Knob

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
A1	35^o 27.855	83^o 08.248	6270
A2	35^o 27.852	83^o 08.278	6257
A3	35^o 27.850	83^o 08.279	6266
A4	35^o 27.846	83^o 08.297	6243
A5	35^o 27.823	83^o 08.300	6245
A6	35^o 27.831	83^o 08.291	6254
A7	35^o 27.835	83^o 08.272	6276
A8	35^o 27.845	83^o 08.245	6283
A9	35^o 27.855	83^o 08.235	6265
A10	35^o 27.861	83^o 08.222	6225
Average	35^o 27.845	83^o 08.267	6270
Standard Deviation			17

Table B.3: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Water Rock Knob

Sample ID	**Latitude (^{o}N)**	**Longitude (^{o}W)**	**Elevation (ft)**
M1	35^o 27.849	83^o 08.266	6278
M2	35^o 27.854	83^o 08.271	6263
M3	35^o 27.851	83^o 08.280	6255
M4	35^o 27.846	83^o 08.283	6261
M5	35^o 27.833	83^o 08.287	6245
M6	35^o 27.819	83^o 08.307	6238
M7	35^o 27.839	83^o 08.285	6273
M8	35^o 27.850	83^o 08.246	6248
M9	35^o 27.857	83^o 08.229	6241
M10	35^o 27.876	83^o 08.253	6229
Average	35^o 27.847	83^o 08.271	6253
Standard Deviation			16

Table B.4: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Richland Balsam

Sample ID	**Latitude (^{o}N)**	**Longitude (^{o}W)**	**Elevation (ft)**
E1	35^o 22.018	82^o 59.397	6414
E2	35^o 22.022	82^o 59.402	6407
E3	35^o 22.029	82^o 59.415	6402
E4	35^o 22.056	82^o 59.410	6396
E5	35^o 22.040	82^o 59.454	6370
E6	35^o 22.655	82^o 59.199	6046
E7	35^o 22.724	82^o 59.294	6118
E8	35^o 22.773	82^o 59.331	6188
E9	35^o 22.735	82^o 59.344	6080
E10	35^o 22.800	82^o 59.483	6031
Average	35^o 22.385	82^o 59.373	6245
Standard Deviation			167

Table B.5: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Richland Balsam

Sample ID	**Latitude (^{o}N)**	**Longitude (^{o}W)**	**Elevation (ft)**
A1	35^o 22.017	82^o 59.400	6415
A2	35^o 22.020	82^o 59.409	6399
A3	35^o 22.026	82^o 59.419	6400
A4	35^o 22.061	82^o 59.409	6387
A5	35^o 22.045	82^o 59.437	6385
A6	35^o 22.040	82^o 59.453	6373
A7	35^o 22.657	82^o 59.212	6061
A8	35^o 22.789	82^o 59.363	6214
A9	35^o 22.711	82^o 59.304	6077
A10	35^o 22.768	82^o 59.409	6068
Average	35^o 22.313	82^o 59.382	6278
Standard Deviation			155

Table B.6: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Richland Balsam

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
M1	35^o 22.021	82^o 59.398	6418
M2	35^o 22.019	82^o 59.409	6407
M3	35^o 22.044	82^o 59.411	6404
M4	35^o 22.048	82^o 59.435	6391
M5	35^o 22.042	82^o 59.448	6382
M6	35^o 22.696	82^o 59.263	6082
M7	35^o 22.732	82^o 59.295	6122
M8	35^o 22.747	82^o 59.314	6145
M9	35^o 22.720	82^o 59.310	6079
M10	35^o 22.750	82^o 59.369	6087
Average	35^o 22.382	82^o 59.365	6252
Standard Deviation			158

Table B.7: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Clingmans Dome

Sample ID	**Latitude (^{o}N)**	**Longitude (^{o}W)**	**Elevation (ft)**
E1	33^o 33.741	83^o 29.863	6643
E2	33^o 33.733	83^o 29.845	6631
E3	33^o 33.792	83^o 29.885	6642
E4	33^o 33.773	83^o 29.865	6635
E5	33^o 33.790	83^o 29.860	6623
E6	33^o 33.641	83^o 29.817	6526
E7	33^o 33.695	83^o 29.890	6583
E8	33^o 33.712	83^o 29.879	6621
E9	33^o 33.788	83^o 29.960	6629
E10	33^o 33.760	83^o 30.056	6581
Average	33^o 33.743	83^o 29.892	6611
Standard Deviation			37

Table B.8: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Clingmans Dome

Sample ID	**Latitude (^{o}N)**	**Longitude (^{o}W)**	**Elevation (ft)**
A1	33^{o} 33.772	83^{o} 29.894	6667
A2	33^{o} 33.732	83^{o} 29.840	6628
A3	33^{o} 33.778	83^{o} 29.871	6641
A4	33^{o} 33.778	83^{o} 29.861	6629
A5	33^{o} 33.797	83^{o} 29.862	6617
A6	33^{o} 33.644	83^{o} 29.821	6528
A7	33^{o} 33.693	83^{o} 29.886	6603
A8	33^{o} 33.715	83^{o} 29.874	6628
A9	33^{o} 33.772	83^{o} 29.993	6605
A10	33^{o} 33.762	83^{o} 30.043	6596
Average	33^{o} 33.744	83^{o} 29.895	6614
Standard Deviation			37

Table B.9: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Clingmans Dome

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
M1	33^o 33.751	83^o 29.873	6650
M2	33^o 33.804	83^o 29.900	6646
M3	33^o 33.797	83^o 29.892	6654
M4	33^o 33.785	83^o 29.860	6622
M5	33^o 33.805	83^o 29.864	6613
M6	33^o 33.638	83^o 29.829	6512
M7	33^o 33.693	83^o 29.896	6580
M8	33^o 33.706	83^o 29.879	6610
M9	33^o 33.727	83^o 29.875	6635
M10	33^o 33.743	83^o 29.1123	6566
Average	33^o 33.745	83^o 29.899	6608
Standard Deviation			45

Table B.10: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Mingus Lead

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
E1	35^o 36.755	83^o 26.828	5602
E2	35^o 36.750	83^o 26.811	5610
E3	35^o 36.744	83^o 26.803	5604
E4	35^o 36.736	83^o 26.797	5596
E5	35^o 36.733	83^o 26.786	5602
E6	35^o 36.718	83^o 26.758	5602
E7	35^o 36.719	83^o 26.752	5614
E8	35^o 36.703	83^o 26.733	5595
E9	35^o 36.695	83^o 26.677	5560
E10	35^o 36.697	83^o 26.656	5537
Average	35^o 36.725	83^o 26.760	5592
Standard Deviation			24

Table B.11: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Mingus Lead

Sample ID	Latitude (^oN)	Longitude (^oW)	Elevation (ft)
A1	35^o 36.757	83^o 26.838	5585
A2	35^o 36.756	83^o 26.824	5610
A3	35^o 36.735	83^o 26.803	5595
A4	35^o 36.703	83^o 26.781	5594
A5	35^o 36.725	83^o 26.766	5600
A6	35^o 36.723	83^o 26.760	5601
A7	35^o 36.717	83^o 26.745	5614
A8	35^o 36.706	83^o 26.739	5603
A9	35^o 36.703	83^o 26.697	5579
A10	35^o 36.698	83^o 26.671	5556
Average	35^o 36.722	83^o 26.762	5594
Standard Deviation			17

Table B.12: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Mingus Lead

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
M1	35^o 36.760	83^o 26.844	5591
M2	35^o 36.763	83^o 26.820	5613
M3	35^o 36.739	83^o 26.792	5600
M4	35^o 36.733	83^o 26.790	5582
M5	35^o 36.733	83^o 26.783	5592
M6	35^o 36.725	83^o 26.763	5602
M7	35^o 36.709	83^o 26.738	5602
M8	35^o 36.699	83^o 26.728	5594
M9	35^o 36.703	83^o 26.718	5587
M10	35^o 36.700	83^o 26.709	5587
Average	35^o 36.726	83^o 26.769	5595
Standard Deviation			9

Table B.13: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Spruce Mountain

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
E1	35^o 36.835	83^o 10.410	5665
E2	35^o 36.846	83^o 10.423	5651
E3	35^o 36.807	83^o 10.459	5650
E4	35^o 36.766	83^o 10.536	5642
E5	35^o 36.756	83^o 10.540	5628
E6	35^o 36.745	83^o 10.544	5629
E7	35^o 36.710	83^o 10.570	5616
E8	35^o 36.604	83^o 10.619	5591
E9	35^o 36.580	83^o 10.648	5596
E10	35^o 36.532	83^o 10.698	5567
Average	35^o 36.718	83^o 10.545	5624
Standard Deviation			31

Table B.14: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Spruce Mountain

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
A1	35^o 36.840	83^o 10.415	5656
A2	35^o 36.832	83^o 10.425	5634
A3	35^o 36.797	83^o 10.497	5623
A4	35^o 36.767	83^o 10.529	5634
A5	35^o 36.763	83^o 10.538	5633
A6	35^o 36.738	83^o 10.547	5624
A7	35^o 36.736	83^o 10.551	5624
A8	35^o 36.684	83^o 10.589	5602
A9	35^o 36.671	83^o 10.591	5606
A10	35^o 36.552	83^o 10.660	5577
Average	35^o 36.738	83^o 10.534	5621
Standard Deviation			22

Table B.15: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Spruce Mountain

Sample ID	**Latitude (^{o}N)**	**Longitude (^{o}W)**	**Elevation (ft)**
M1	35^{o} 36.827	83^{o} 10.465	5635
M2	35^{o} 36.794	83^{o} 10.491	5640
M3	35^{o} 36.775	83^{o} 10.519	5625
M4	35^{o} 36.774	83^{o} 10.518	5630
M5	35^{o} 36.751	83^{o} 10.541	5625
M6	35^{o} 36.740	83^{o} 10.551	5625
M7	35^{o} 36.730	83^{o} 10.553	5624
M8	35^{o} 36.726	83^{o} 10.566	5616
M9	35^{o} 36.680	83^{o} 10.588	5603
M10	35^{o} 36.616	83^{o} 10.611	5584
Average	35^{o} 36.738	83^{o} 10.534	5621
Standard Deviation			16

Table B.16: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Yellow Face

Sample ID	Latitude (^oN)	Longitude (^oW)	Elevation (ft)
E1	35^o 27.226	83^o 08.803	5740
E2	35^o 27.255	83^o 08.744	5751
E3	35^o 27.309	83^o 08.662	5832
E4	35^o 27.314	83^o 08.642	5821
E5	35^o 27.322	83^o 08.640	5816
E6	35^o 27.309	83^o 08.627	5810
E7	35^o 27.312	83^o 08.624	5806
E8	35^o 27.315	83^o 08.616	5797
E9	35^o 27.322	83^o 08.610	5795
E10	35^o 27.362	83^o 08.598	5748
Average	35^o 27.305	83^o 08.657	5792
Standard Deviation			33

Table B.17: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Yellow Face

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
A1	35^o 27.255	83^o 08.757	5728
A2	35^o 27.257	83^o 08.754	5739
A3	35^o 27.309	83^o 08.655	5827
A4	35^o 27.307	83^o 08.645	5828
A5	35^o 27.314	83^o 08.635	5826
A6	35^o 27.312	83^o 08.634	5808
A7	35^o 27.304	83^o 08.621	5801
A8	35^o 27.317	83^o 08.616	5801
A9	35^o 27.348	83^o 08.600	5762
A10	35^o 27.370	83^o 08.590	5743
Average	35^o 27.309	83^o 08.651	5786
Standard Deviation			39

Table B.18: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Yellow Face

Sample ID	**Latitude (^{o}N)**	**Longitude (^{o}W)**	**Elevation (ft)**
M1	35^{o} 27.215	83^{o} 08.818	5737
M2	35^{o} 27.229	83^{o} 08.804	5737
M3	35^{o} 27.307	83^{o} 08.678	5819
M4	35^{o} 27.306	83^{o} 08.643	5827
M5	35^{o} 27.315	83^{o} 08.638	5838
M6	35^{o} 27.303	83^{o} 08.617	5794
M7	35^{o} 27.315	83^{o} 08.620	5801
M8	35^{o} 27.316	83^{o} 08.608	5780
M9	35^{o} 27.329	83^{o} 08.607	5787
M10	35^{o} 27.372	83^{o} 08.587	5748
Average	35^{o} 27.309	83^{o} 08.651	5787
Standard Deviation			37

Table B.19: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Mt. Mitchell

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
E1	35^o 45.851	82^o 15.820	6639
E2	35^o 45.837	82^o 15.812	6614
E3	35^o 45.811	82^o 15.740	6655
E4	35^o 45.773	82^o 15.699	6602
E5	35^o 45.784	82^o 15.689	6600
E6	35^o 45.829	82^o 15.706	6580
E7	35^o 45.861	82^o 15.749	6543
E8	35^o 45.897	82^o 15.813	6537
E9	35^o 45.935	82^o 15.847	6575
E10	35^o 45.851	82^o 15.820	6616
Average	35^o 45.844	82^o 15.774	6596
Standard Deviation			38

Table B.20: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Mt. Mitchell

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
A1	35^o 45.851	82^o 15.855	6634
A2	35^o 45.870	82^o 15.850	6636
A3	35^o 45.839	82^o 15.815	6656
A4	35^o 45.827	82^o 15.765	6612
A5	35^o 45.774	82^o 15.703	6597
A6	35^o 45.777	82^o 15.694	6586
A7	35^o 45.821	82^o 15.702	6539
A8	35^o 45.841	82^o 15.732	6549
A9	35^o 45.897	82^o 15.827	6584
A10	35^o 45.876	82^o 15.762	6530
Average	35^o 45.837	82^o 15.771	6592
Standard Deviation			43

Table B.21: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Mt. Mitchell

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
M1	35^o 45.864	82^o 15.861	6644
M2	35^o 45.871	82^o 15.845	6640
M3	35^o 45.843	82^o 15.811	6646
M4	35^o 45.834	82^o 15.779	6619
M5	35^o 45.807	82^o 15.734	6600
M6	35^o 45.778	82^o 15.695	6585
M7	35^o 45.807	82^o 15.686	6548
M8	35^o 45.835	82^o 15.708	6554
M9	35^o 45.930	82^o 15.845	6596
M10	35^o 45.890	82^o 15.792	6563
Average	35^o 45.846	82^o 15.776	6600
Standard Deviation			37

Table B.22: Coordinates and Elevations of Fraser Fir Seedling Samples Acquired at Camp Alice

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
E1	35^o 45.369	82^o 15.902	5740
E2	35^o 45.377	82^o 15.910	5749
E3	35^o 45.397	82^o 15.963	5773
E4	35^o 45.402	82^o 15.977	5774
E5	35^o 45.418	82^o 15.978	5779
E6	35^o 45.408	82^o 16.001	5746
E7	35^o 45.440	82^o 16.019	5781
E8	35^o 45.466	82^o 16.038	5796
E9	35^o 45.488	82^o 16.074	5813
E10	35^o 45.498	82^o 16.074	5823
Average	35^o 45.426	82^o 15.994	5777
Standard Deviation			28

Table B.23: Coordinates and Elevations of Fraser Fir Sapling Samples Acquired at Camp Alice

Sample ID	**Latitude (^oN)**	**Longitude (^oW)**	**Elevation (ft)**
A1	35^o 45.371	82^o 15.867	5728
A2	35^o 45.377	82^o 15.921	5740
A3	35^o 45.373	82^o 15.938	5751
A4	35^o 45.387	82^o 15.955	5762
A5	35^o 45.403	82^o 15.973	5774
A6	35^o 45.404	82^o 16.000	5758
A7	35^o 45.436	82^o 16.015	5778
A8	35^o 45.445	82^o 16.020	5789
A9	35^o 45.471	82^o 16.051	5795
A10	35^o 45.479	82^o 16.069	5794
Average	35^o 45.415	82^o 15.981	5767
Standard Deviation			23

Table B.24: Coordinates and Elevations of Fraser Fir Mature Samples Acquired at Camp Alice

Sample ID	**Latitude (°N)**	**Longitude (°W)**	**Elevation (ft)**
M1	35° 45.361	82° 15.846	5696
M2	35° 45.374	82° 15.867	5729
M3	35° 45.388	82° 15.956	5752
M4	35° 45.406	82° 15.970	5785
M5	35° 45.411	82° 15.981	5778
M6	35° 45.426	82° 16.008	5772
M7	35° 45.445	82° 16.026	5791
M8	35° 45.482	82° 16.057	5800
M9	35° 45.479	82° 16.051	5800
M10	35° 45.487	82° 16.063	5798
Average	35° 45.426	82° 15.983	5770
Standard Deviation			35

APPENDIX C: STATISTICAL ANALYSIS RESULTS

Table C.1: Linear Regression Analysis of Metal Concentrations in Foliage *vs.* Elevation When Comparing All Sites Together (α = 0.05). From Section 3.3.1.

Linear Regression Analysis For All Sites	**Slope/Intercept**	**R^2 value**	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	-0.015 / 490	0.069880	0.527	No Correlation
Aluminum Concentration in Saplings	-0.168 / 507	0.174100	0.304	No Correlation
Aluminum Concentration in Matures	-0.219 / 605	0.263400	0.193	No Correlation
Calcium Concentration in Seedlings	-1.373 / 6110	0.061190	0.555	No Correlation
Calcium Concentration in Saplings	-1.749 / 7030	0.060840	0.556	No Correlation
Calcium Concentration in Matures	-2.446 / 9250	0.098470	0.449	No Correlation
Magnesium Concentration in Seedlings	-0.385 / 1650	0.053860	0.580	No Correlation
Magnesium Concentration in Saplings	-0.074 / 997	0.008477	0.828	No Correlation
Magnesium Concentration in Matures	-0.1923 / 1210	0.059310	0.561	No Correlation

Aluminum was expected to have a positive slope; calcium and magnesium were expected to have negative slopes. A p-value less than 0.05 indicates linear correlation.

Table C.2: Linear Regression Analysis of Exchangeable Metal Concentrations in Soil *vs.* Elevation When Comparing All Sites Together (α = 0.05). From Section 3.3.1.

Linear Regression Analysis For All Sites	**Slope Intercept**	**R^2 value**	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	0.21 455	0.235	0.717	No Correlation
Aluminum Concentration in Saplings	0.19 492	8.82×10^{-3}	0.825	No Correlation
Aluminum Concentration in Matures	-0.02 958	1.48×10^{-5}	0.977	No Correlation
Calcium Concentration in Seedlings	-0.22 588	0.043	0.623	No Correlation
Calcium Concentration in Saplings	0.274 -247	0.028	0.691	No Correlation
Calcium Concentration in Matures	-0.084 336	0.014	0.780	No Correlation
Magnesium Concentration in Seedlings	-0.020 124	4.39×10^{-3}	0.876	No Correlation
Magnesium Concentration in Saplings	0.044 19.8	0.019	0.743	No Correlation
Magnesium Concentration in Matures	8.388×10^{-4} 82.1	2.600×10^{-5}	0.990	No Correlation

Aluminum was expected to have a positive slope; calcium and magnesium were expected to have negative slopes. A p-value less than 0.05 indicates linear correlation.

Table C.3: Results of Student's t-test - Elevation Comparison of Clingmans Dome and Mingus Lead in the GSMNP ($\alpha = 0.05$) - Includes p-value and Conclusion. From Section 3.3.2.

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	2.109×10^{-9} Different Followed hypothesis	1.8522×10^{-5} Different Did not follow hypothesis	2.324×10^{-5} Different Followed hypothesis
Sapling Foliage	1.727×10^{-3} Different Followed hypothesis	0.186 Statistically the same Did not follow hypothesis	1.164×10^{-3} Different Followed hypothesis
Mature Foliage	7.705×10^{-5} Different Followed hypothesis	3.186×10^{-8} Different Followed hypothesis	0.651 Statistically the same Did not follow hypothesis
Seedling Soil	0.972 Statistically the same Did not follow hypothesis	3.240×10^{-13} Different Followed hypothesis	5.919×10^{-9} Different Followed Hypothesis
Sapling Soil	1.902×10^{-8} Different Followed hypothesis	4.745×10^{-4} Different Followed hypothesis	2.550×10^{-9} Different Followed hypothesis
Mature Soil	0.215 Statistically the same Did not follow hypothesis	6.553×10^{-10} Different Followed hypothesis	9.155×10^{-9} Different Followed hypothesis

Clingmans Dome was expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to Mingus Lead. A p-value less than 0.05 indicates statistical difference between samples.

Table C.4: Summary of Student's t-test - Elevation Comparison of Mt. Mitchell and Camp Alice in MMSP ($\alpha = 0.05$) - Includes p-value and Conclusion. From Section 3.3.2.

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	1.536×10^{-12} Different Did not follow hypothesis	3.015×10^{-10} Different Followed hypothesis	2.914×10^{-14} Different Did not follow hypothesis
Sapling Foliage	8.863×10^{-12} Different Did not follow hypothesis	2.755×10^{-12} Different Followed hypothesis	3.933×10^{-11} Different Did not follow hypothesis
Mature Foliage	1.179×10^{-9} Different Did not follow hypothesis	4.686×10^{-9} Different Followed hypothesis	1.176×10^{-4} Different Did not follow hypothesis
Seedling Soil	5.221×10^{-8} Different Followed hypothesis	3.965×10^{-8} Different Did not follow hypothesis	4.723×10^{-3} Different Did not follow hypothesis
Sapling Soil	8.404×10^{-11} Different Followed hypothesis	4.446×10^{-11} Different Did not follow hypothesis	0.641 Statistically the Same Did not follow hypothesis
Mature Soil	4.805×10^{-7} Different Followed hypothesis	1.592×10^{-8} Different Did not follow hypothesis	2.308×10^{-6} Different Did not follow hypothesis

Mt. Mitchell was expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to Camp Alice. A p-value less than 0.05 indicates statistical difference between samples.

Table C.5: Analysis of Variance Comparing Foliar Metal Concentrations at All High Elevation Sites ($\alpha = 0.05$). From Section 3.3.3.

Analysis of Variance	p-value	Conclusion
Aluminum Concentration in Seedlings	9.94×10^{-15}	Does not follow hypothesis
Aluminum Concentration in Saplings	2.94×10^{-3}	Does not follow hypothesis
Aluminum Concentration in Matures	7.00×10^{-12}	Does not follow hypothesis
Calcium Concentration in Seedlings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Calcium Concentration in Saplings	1.80×10^{-10}	Does not follow hypothesis
Calcium Concentration in Matures	2.12×10^{-6}	Does not follow hypothesis
Magnesium Concentration in Seedlings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Magnesium Concentration in Saplings	3.59×10^{-10}	Does not follow hypothesis
Magnesium Concentration in Matures	1.60×10^{-5}	Does not follow hypothesis

High elevation sites were expected to have statistically similar concentrations of foliar metals. A p-value greater than 0.05 indicates statistical similarity.

Table C.6: Analysis of Variance Comparing Exchangeable Soil Metal Concentrations at All High Elevation Sites ($\alpha = 0.05$). From Section 3.3.3.

Analysis of Variance	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	5.65×10^{-6}	Does not follow hypothesis
Aluminum Concentration in Saplings	4.24×10^{-14}	Does not follow hypothesis
Aluminum Concentration in Matures	4.39×10^{-6}	Does not follow hypothesis
Calcium Concentration in Seedlings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Calcium Concentration in Saplings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Calcium Concentration in Matures	4.12×10^{-11}	Does not follow hypothesis
Magnesium Concentration in Seedlings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Magnesium Concentration in Saplings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Magnesium Concentration in Matures	$< 2.20 \times 10^{-16}$	Does not follow hypothesis

High elevation sites were expected to have statistically similar concentrations of exchangeable soil metals. A p-value greater than 0.05 indicates statistical similarity.

Table C.7: Analysis of Variance Comparing Foliar Metal Concentrations at All Low Elevation Sites ($\alpha = 0.05$). From Section 3.3.4.

Analysis of Variance	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Aluminum Concentration in Saplings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Aluminum Concentration in Matures	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Calcium Concentration in Seedlings	4.13×10^{-14}	Does not follow hypothesis
Calcium Concentration in Saplings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Calcium Concentration in Matures	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Magnesium Concentration in Seedlings	1.85×10^{-10}	Does not follow hypothesis
Magnesium Concentration in Saplings	4.91×10^{-16}	Does not follow hypothesis
Magnesium Concentration in Matures	1.10×10^{-14}	Does not follow hypothesis

Low elevation sites were expected to have statistically similar concentrations of foliar metals. A p-value greater than 0.05 indicates statistical similarity.

Table C.8: Analysis of Variance Comparing Exchangeable Soil Metal Concentrations at All Low Elevation Sites (α = 0.05). From Section 3.3.4.

Analysis of Variance	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	4.94×10^{-12}	Does not follow hypothesis
Aluminum Concentration in Saplings	1.22×10^{-15}	Does not follow hypothesis
Aluminum Concentration in Matures	1.18×10^{-12}	Does not follow hypothesis
Calcium Concentration in Seedlings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Calcium Concentration in Saplings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Calcium Concentration in Matures	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Magnesium Concentration in Seedlings	3.69×10^{-12}	Does not follow hypothesis
Magnesium Concentration in Saplings	$< 2.20 \times 10^{-16}$	Does not follow hypothesis
Magnesium Concentration in Matures	1.22×10^{-15}	Does not follow hypothesis

Low elevation sites were expected to have statistically similar concentrations of exchangeable soil metals. A p-value greater than 0.05 indicates statistical similarity.

Table C.9: Linear Regression Analysis of Metal Concentrations in Foliage *vs.* Longitude When Comparing All Sites Together (α = 0.05). From Section 3.4.1.

Linear Regression Analysis For All Sites	**Slope Intercept**	**R^2 value**	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	-32.0 2870	0.070	0.527	No Correlation
Aluminum Concentration in Saplings	-24.8 2250	0.088	0.495	No Correlation
Aluminum Concentration in Matures	0.737 139	6.46×10^{-6}	0.985	No Correlation
Calcium Concentration in Seedlings	-443 40300	0.140	0.362	No Correlation
Calcium Concentration in Saplings	-766 67300	0.249	0.208	No Correlation
Calcium Concentration in Matures	-592 53800	0.125	0.390	No Correlation
Magnesium Concentration in Seedlings	89.4 -6470	0.063	0.547	No Correlation
Magnesium Concentration in Saplings	47.6 -3090	0.076	0.509	No Correlation
Magnesium Concentration in Matures	85.9 -6280	0.258	0.199	No Correlation

Aluminum was expected to have a positive slope; calcium and magnesium were expected to have negative slopes. A p-value less than 0.05 indicates linear correlation.

Table C.10: Linear Regression Analysis of Exchangeable Metal Concentrations in Soil *vs.* Longitude When Comparing All Sites Together (α = 0.05). From Section 3.4.1.

Linear Regression Analysis For All Sites	**Slope Intercept**	**R^2 value**	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	129 -9860	0.187	0.284	No Correlation
Aluminum Concentration in Saplings	220 -17400	0.263	0.193	No Correlation
Aluminum Concentration in Matures	207 -16300	0.282	0.176	No Correlation
Calcium Concentration in Seedlings	67.2 -5400	0.086	0.482	No Correlation
Calcium Concentration in Saplings	76.7 -6100	0.047	0.605	No Correlation
Calcium Concentration in Matures	50.8 -4040	0.112	0.418	No Correlation
Magnesium Concentration in Seedlings	10.0 -743	0.025	0.710	No Correlation
Magnesium Concentration in Saplings	-3.07 356	0.002	0.916	Correct Correlation
Magnesium Concentration in Matures	-5.75 560	0.027	0.6997	No Correlation

Aluminum was expected to have a positive slope; calcium and magnesium were expected to have negative slopes. A p-value less than 0.05 indicates linear correlation.

Table C.11: Student's t-test Comparison of Western (Clingmans Dome) and Eastern (Mt. Mitchell) High Elevation Sites (α = 0.05) - Includes p-value and Conclusion. From Section 3.4.2.

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	2.447×10^{-7} Different Followed hypothesis	7.942×10^{-5} Different Did not follow hypothesis	3.394×10^{-14} Different Followed hypothesis
Sapling Foliage	0.026 Different Followed hypothesis	1.219×10^{-3} Different Did not follow hypothesis	7.412×10^{-5} Different Followed hypothesis
Mature Foliage	9.037×10^{-6} Different Followed hypothesis	5.834×10^{-9} Different Followed hypothesis	0.803 Statistically the Same Did not follow hypothesis
Seedling Soil	4.489×10^{-4} Different Did not follow hypothesis	1.163×10^{-8} Different Followed hypothesis	3.094×10^{-6} Different Followed hypothesis
Sapling Soil	4.080×10^{-8} Different Did not follow hypothesis	7.285×10^{-6} Different Followed hypothesis	3.474×10^{-6} Different Followed hypothesis
Mature Soil	3.363×10^{-4} Different Did not follow hypothesis	1.924×10^{-3} Different Followed hypothesis	1.276×10^{-7} Different Followed hypothesis

Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to eastern sites. A p-value less than 0.05 indicates difference among samples.

Table C.12: Student's t-test Comparison of Western (Mingus Lead) and Eastern (Camp Alice) Low Elevation Sites ($\alpha = 0.05$) - Includes p-value and Conclusion. From Section 3.4.2.

	Aluminum	Calcium	Magnesium
Seedling Foliage	2.447×10^{-7} Different Followed hypothesis	7.942×10^{-5} Different Did not follow hypothesis	3.394×10^{-14} Different Followed hypothesis
Sapling Foliage	0.026 Different Followed hypothesis	1.219×10^{-3} Different Did not follow hypothesis	7.412×10^{-5} Different Followed hypothesis
Mature Foliage	9.037×10^{-6} Different Followed hypothesis	5.834×10^{-9} Different Followed hypothesis	0.803 Statistically the Same Did not follow hypothesis
Seedling Soil	4.489×10^{-4} Different Did not follow hypothesis	1.163×10^{-8} Different Followed hypothesis	3.094×10^{-6} Different Followed hypothesis
Sapling Soil	4.080×10^{-8} Different Did not follow hypothesis	7.285×10^{-6} Different Followed hypothesis	3.474×10^{-6} Different Followed hypothesis
Mature Soil	3.363×10^{-4} Different Did not follow hypothesis	1.924×10^{-3} Different Followed hypothesis	1.276×10^{-7} Different Followed hypothesis

Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to eastern sites. A p-value less than 0.05 indicates difference among samples.

Table C.13: Student's t-test Comparison of Western (Clingmans Dome) and Central (Water Rock Knob) High Elevation Sites ($\alpha = 0.05$) - Includes p-value and Conclusion. From Section 3.4.3.

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	2.528×10^{-9} Different Followed hypothesis	8.754×10^{-13} Different Did not follow hypothesis	1.113×10^{-3} Different Did not follow hypothesis
Sapling Foliage	0.907 Statistically the Same Did not follow hypothesis	2.018×10^{-6} Different Did not follow hypothesis	1.346×10^{-3} Different Did not follow hypothesis
Mature Foliage	4.262×10^{-6} Different Did not follow hypothesis	8.543×10^{-6} Different Did not follow hypothesis	1.179×10^{-7} Different Did not follow hypothesis
Seedling Soil	0.017 Different Did not follow hypothesis	4.456×10^{-14} Different Followed hypothesis	6.867×10^{-12} Different Followed hypothesis
Sapling Soil	2.360×10^{-10} Different Did not follow hypothesis	1.126×10^{-12} Different Followed hypothesis	2.863×10^{-12} Different Followed hypothesis
Mature Soil	1.032×10^{-4} Different Did not follow hypothesis	0.036 Different Did not follow hypothesis	2.303×10^{-5} Different Followed hypothesis

Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

Table C.14: Student's t-test Comparison of Western (Mingus Lead) and Central (Yellow Face) Low Elevation Sites (α = 0.05) - Includes p-value and Conclusion. From Section 3.4.3.

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	1.456×10^{-12} Different Did not follow hypothesis	0.319 Statistically the Same Did not follow hypothesis	0.043 Different Followed hypothesis
Sapling Foliage	2.494×10^{-8} Different Did not follow hypothesis	2.695×10^{-9} Different Did not follow hypothesis	5.304×10^{-4} Different Followed hypothesis
Mature Foliage	8.915×10^{-14} Different Did not follow hypothesis	0.395 Statistically the Same Did not follow hypothesis	9.424×10^{-9} Different Followed hypothesis
Seedling Soil	6.069×10^{-5} Different Did not follow hypothesis	4.682×10^{-8} Different Did not follow hypothesis	1.091×10^{-3} Different Did not follow hypothesis
Sapling Soil	2.070×10^{-6} Different Did not follow hypothesis	2.981×10^{-10} Different Did not follow hypothesis	1.325×10^{-9} Different Did not follow hypothesis
Mature Soil	1.484×10^{-9} Different Did not follow hypothesis	1.024×10^{-10} Different Did not follow hypothesis	5.914×10^{-10} Different Did not follow hypothesis

Western sites were expected to have higher concentrations of aluminum and lower concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

Table C.15: Student's t-test Comparison of Eastern (Mt. Mitchell) and Central (Water Rock Knob) High Elevation Sites ($\alpha = 0.05$) - Includes p-value and Conclusion. From Section 3.4.4.

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	1.944×10^{-6} Different Did not follow hypothesis	9.197×10^{-13} Different Followed hypothesis	3.979×10^{-13} Different Followed hypothesis
Sapling Foliage	5.451×10^{-4} Different Followed hypothesis	7.390×10^{-10} Different Followed hypothesis	8.532×10^{-12} Different Followed hypothesis
Mature Foliage	3.259×10^{-9} Different Followed hypothesis	9.704×10^{-11} Different Followed hypothesis	2.247×10^{-8} Different Followed hypothesis
Seedling Soil	8.812×10^{-4} Different Did not follow hypothesis	1.207×10^{-12} Different Did not follow hypothesis	6.163×10^{-10} Different Did not follow hypothesis
Sapling Soil	1.889×10^{-4} Different Did not follow hypothesis	1.630×10^{-12} Different Did not follow hypothesis	6.601×10^{-11} Different Did not follow hypothesis
Mature Soil	0.132 Statistically the Same Did not follow hypothesis	4.901×10^{-4} Different Followed hypothesis	1.300×10^{-6} Different Followed hypothesis

Eastern sites were expected to have lower concentrations of aluminum and higher concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

Table C.16: Student's t-test Comparison of Eastern (Camp Alice) and Central (Yellow Face) Low Elevation Sites (α = 0.05) - Includes p-value and Conclusion. From Section 3.4.4.

	Aluminum	**Calcium**	**Magnesium**
Seedling Foliage	2.328×10^{-11} Different Did not follow hypothesis	7.748×10^{-8} Different Followed hypothesis	9.339×10^{-14} Different Did not follow hypothesis
Sapling Foliage	2.861×10^{-11} Different Did not follow hypothesis	1.877×10^{-13} Different Followed hypothesis	5.351×10^{-11} Different Did not follow hypothesis
Mature Foliage	7.604×10^{-7} Different Did not follow hypothesis	7.292×10^{-9} Different Followed hypothesis	4.915×10^{-10} Different Did not follow hypothesis
Seedling Soil	4.425×10^{-7} Different Followed hypothesis	3.821×10^{-7} Different Did not follow hypothesis	7.013×10^{-5} Different Did not follow hypothesis
Sapling Soil	5.944×10^{-14} Different Followed hypothesis	6.237×10^{-11} Different Did not follow hypothesis	3.604×10^{-4} Different Did not follow hypothesis
Mature Soil	7.927×10^{-12} Different Followed hypothesis	3.765×10^{-9} Different Did not follow hypothesis	4.727×10^{-3} Different Followed hypothesis

Eastern sites were expected to have lower concentrations of aluminum and higher concentrations of calcium and magnesium compared to central sites. A p-value less than 0.05 indicates difference among samples.

Table C.17: Linear Regression Analysis of Metal Concentrations in Foliage *vs.* Soil pH When Comparing All Sites Together (α = 0.05). From Section 3.5.1.

Linear Regression Analysis For All Sites	**Slope Intercept**	**R^2 value**	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	277 -812	0.883	5.208×10^{-4}	No Correlation
Aluminum Concentration in Saplings	124 -266	0.739	6.201×10^{-3}	No Correlation
Aluminum Concentration in Matures	149 -353	0.421	0.082	No Correlation
Calcium Concentration in Seedlings	2640 -6180	0.835	1.507×10^{-3}	Correct Correlation
Calcium Concentration in Saplings	1880 -3220	0.550	0.035	Correct Correlation
Calcium Concentration in Matures	2570 -4840	0.378	0.105	No Correlation
Magnesium Concentration in Seedlings	-250 1870	0.084	0.486	No Correlation
Magnesium Concentration in Saplings	-140 1390	0.240	0.218	No Correlation
Magnesium Concentration in Matures	-23.5 938	3.080×10^{-3}	0.896	No Correlation

Aluminum was expected to have a negative slope; calcium and magnesium were expected to have positive slopes. A p-value less than 0.05 indicates linear correlation.

Table C.18: Linear Regression Analysis of Exchangeable Soil Metal Concentrations *vs.* Soil pH When Comparing All Sites Together ($\alpha = 0.05$). From Section 3.5.2.

Linear Regression Analysis For All Sites	**Slope Intercept**	**R^2 value**	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	-506 2720	0.485	0.055	No Correlation
Aluminum Concentration in Saplings	-642 3230	0.825	1.803×10^{-3}	Correct Correlation
Aluminum Concentration in Matures	-481 2700	0.243	0.215	No Correlation
Calcium Concentration in Seedlings	-395 1640	0.499	0.050	No Correlation
Calcium Concentration in Saplings	-221 1080	0.143	0.355	No Correlation
Calcium Concentration in Matures	-278 1220	0.536	0.039	No Correlation
Magnesium Concentration in Seedlings	-85.3 403	0.304	0.157	No Correlation
Magnesium Concentration in Saplings	-9.28 136	6.682×10^{-3}	0.847	No Correlation
Magnesium Concentration in Matures	-19.1 155	0.047	0.607	No Correlation

Aluminum was expected to have a negative slope; calcium and magnesium were expected to have positive slopes. A p-value less than 0.05 indicates linear correlation.

Table C.19: Analysis of Variance Results Comparing Foliar Aluminum Concentrations of All Life Stage Classes at Each Site (α = 0.05). From Section 3.6.1

Site	p-value	Conclusion
WR	7.940×10^{-11}	Did Not Follow Hypothesis
RB	6.761×10^{-7}	Did Not Follow Hypothesis
CD	7.595×10^{-6}	Did Not Follow Hypothesis
ML	4.315×10^{-7}	Did Not Follow Hypothesis
SM	2.210×10^{-13}	Did Not Follow Hypothesis
YF	8.157×10^{-12}	Did Not Follow Hypothesis
MM	1.824×10^{-9}	Did Not Follow Hypothesis
CA	1.184×10^{-10}	Did Not Follow Hypothesis

All life stages at each site were expected to be the same (p-value greater than 0.05).

Table C.20: Analysis of Variance Results Comparing Foliar Calcium Concentrations of All Life Stage Classes at Each Site (α = 0.05). From Section 3.6.1

Site	p-value	Conclusion
WR	3.853×10^{-14}	Did Not Follow Hypothesis
RB	1.725×10^{-3}	Did Not Follow Hypothesis
CD	2.803×10^{-3}	Did Not Follow Hypothesis
ML	1.744×10^{-13}	Did Not Follow Hypothesis
SM	1.388×10^{-10}	Did Not Follow Hypothesis
YF	9.275×10^{-14}	Did Not Follow Hypothesis
MM	$< 2.2 \times 10^{-16}$	Did Not Follow Hypothesis
CA	$3.651 \times 10\text{-}^{12}$	Did Not Follow Hypothesis

All life stages at each site were expected to be the same (p-value greater than 0.05).

Table C.21: Analysis of Variance Results Comparing Foliar Magnesium Concentrations of All Life Stage Classes at Each Site (α = 0.05). From Section 3.6.1

Site	p-value	Conclusion
WR	7.132×10^{-4}	Did Not Follow Hypothesis
RB	0.053	Followed Hypothesis
CD	3.352×10^{-5}	Did Not Follow Hypothesis
ML	3.403×10^{-5}	Did Not Follow Hypothesis
SM	4.301×10^{-9}	Did Not Follow Hypothesis
YF	1.844×10^{-14}	Did Not Follow Hypothesis
MM	1.149×10^{-15}	Did Not Follow Hypothesis
CA	2.119×10^{-8}	Did Not Follow Hypothesis

All life stages at each site were expected to be the same (p-value greater than 0.05).

C.22: Analysis of Variance Results Comparing Soil Exchangeable Aluminum Concentrations of All Life Stage Classes at Each Site (α = 0.05). From Section 3.6.2.

Site	p-value	Conclusion
WR	1.72×10^{-7}	Did Not Follow Hypothesis
RB	2.54×10^{-3}	Did Not Follow Hypothesis
CD	0.116	Followed Hypothesis
ML	1.51×10^{-7}	Did Not Follow Hypothesis
SM	1.18×10^{-5}	Did Not Follow Hypothesis
YF	0.427	Followed Hypothesis
MM	0.131	Followed Hypothesis
CA	5.17×10^{-11}	Did Not Follow Hypothesis

All life stages at each site were expected to be the same (p-value greater than 0.05).

C.23: Analysis of Variance Results Comparing Soil Exchangeable Calcium Concentrations of All Life Stage Classes at Each Site ($\alpha = 0.05$). From Section 3.6.2.

Site	p-value	Conclusion
WR	$< 2.20 \times 10^{-16}$	Did Not Follow Hypothesis
RB	2.75×10^{-3}	Did Not Follow Hypothesis
CD	7.22×10^{-13}	Did Not Follow Hypothesis
ML	1.92×10^{-12}	Did Not Follow Hypothesis
SM	2.20×10^{-9}	Did Not Follow Hypothesis
YF	1.42×10^{-3}	Did Not Follow Hypothesis
MM	4.38×10^{-11}	Did Not Follow Hypothesis
CA	1.01×10^{-8}	Did Not Follow Hypothesis

All life stages at each site were expected to be the same (p-value greater than 0.05).

C.24: Analysis of Variance Results Comparing Soil Exchangeable Magnesium Concentrations of All Life Stage Classes at Each Site ($\alpha = 0.05$). From Section 3.6.2.

Site	p-value	Conclusion
WR	$< 2.20 \times 10^{-16}$	Did Not Follow Hypothesis
RB	0.194	Followed Hypothesis
CD	2.75×10^{-7}	Did Not Follow Hypothesis
ML	1.06×10^{-9}	Did Not Follow Hypothesis
SM	3.20×10^{-8}	Did Not Follow Hypothesis
YF	0.752	Followed Hypothesis
MM	6.96×10^{-7}	Did Not Follow Hypothesis
CA	3.78×10^{-8}	Did Not Follow Hypothesis

All life stages at each site were expected to be the same (p-value greater than 0.05).

Table C.25: Linear Regression Analysis of Foliar Metal Concentrations *vs.* Exchangeable Soil Metal Concentrations When Comparing All Sites Together (α = 0.05). From Section 3.7.1.

Linear Regression Analysis For All Sites	**Slope Intercept**	**R^2 value**	**p-value**	**Conclusion**
Aluminum Concentration in Seedlings	-0.235 413	0.335	0.133	No Correlation
Aluminum Concentration in Saplings	-0.155 326	0.576	0.029	No Correlation
Aluminum Concentration in Matures	-6.441×10^{-3} 206	7.515×10^{-4}	0.949	No Correlation
Calcium Concentration in Seedlings	-3.88 4260	0.565	0.031	No Correlation
Calcium Concentration in Saplings	-3.16 4620	0.529	0.041	No Correlation
Calcium Concentration in Matures	-0.472 4820	1.839×10^{-3}	0.920	No Correlation
Magnesium Concentration in Seedlings	-0.425 979	5.793×10^{-3}	0.858	No Correlation
Magnesium Concentration in Saplings	-0.837 946	0.111	0.420	No Correlation
Magnesium Concentration in Matures	0.789 784	0.037	0.698	No Correlation

Aluminum, calcium, and magnesium were expected to have positive slopes; a p-value less than 0.05 indicates linear correlation.

Table C.26: Linear Regression Analysis of Foliar Calcium Concentrations *vs.* Soil Exchangeable Aluminum Concentrations When Comparing All Sites Together (α = 0.05). From Section 3.7.2.

Linear Regression Analysis For All Sites	**Slope Intercept**	**R^2**	**p-value**	**Conclusion**
Seedlings	-2.290 5520	0.332	0.135	No Correlation
Saplings	-2.930 6250	0.668	0.013	Correct Correlation
Mature	-1.840 6420	0.184	0.289	No Correlation

All slopes were expected to be negative. A p-value less than 0.05 indicates linear correlation.