DETERMINATION OF CALCIUM, MAGNESIUM, AND ALUMINUM IN RED SPRUCE (*Picea rubens)* FOLIAGE AND SURROUNDING SOIL FROM THE GREAT SMOKY MOUNTAINS NATIONAL PARK, BLUE RIDGE PARKWAY, AND MOUNT MITCHELL STATE PARK USING INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY

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ABSTRACT

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Red spruce (*Picea rubens)* trees are medium size conifers found in the Appalachian Mountains at high elevations (above 4500 ft.). Since the 1970's, several reports indicate a decline of spruce-fir forests in the Southern Appalachian Mountains caused by acid deposition. Acid deposition leaches essential nutrients out of the soil, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations, and increases the availability of toxic metals to plants, such as aluminum cations (Al^{3+}). Investigation of acid deposition effects on red spruce forests was achieved by analyzing calcium, magnesium, and aluminum in foliage and soils of these forests.

Samples were collected from various locations on the Blue Ridge Parkway (NC), within the Great Smoky Mountains National Park (NC/TN) and Mt. Mitchell State Park (NC). Foliar and soil samples were collected from 30 red spruce trees (each consisted of 10 matures, 10 saplings, and 10 seedlings,) at each sample site. The concentrations of calcium, magnesium, and aluminum in the foliage and surrounding soils of red spruce

trees were determined by using an acid digestion and cation exchange method, respectively. Foliar and soil samples were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Statistical (Student's *t* – test, analysis of variance, and linear regression analysis) and geospatial analysis were performed on the results.

There was some correlation in nutrient or toxic metal concentrations found in the foliage or surrounding soils of red spruce trees with respect to elevation of red spruce forests located in the Southern Appalachian Mountains. In spite of the proximity of coal burning power plants located in eastern Tennessee, the majority of western samples sites did not exhibit lower nutrient and higher toxic metal concentrations when compared to eastern sample sites. Inconclusive evidence suggested that soil pH did not influence the nutrient or toxic metal concentrations found in the foliage or surrounding soils of red spruce forests. When foliar nutrient or toxic metal concentrations from red spruce trees were investigated as a function of soil metal concentrations, the majority of the results did not follow the hypothesis that the concentration of nutrients or toxic metals found in the surrounding soils of red spruce trees would correlate with the quantity found within the red spruce tree's foliage.

The majority of the results indicated that foliar or soil metal concentrations in mature red spruce, red spruce saplings, and red spruce seedlings were not significantly different. Soil calcium/aluminum molar ratios taken from red spruce trees located in the Southern Appalachian Mountains suggested that almost all sample sites are at high risk of adverse forests health effects. A comparison of previous studies of foliar calcium/aluminum ratios taken from red spruce saplings located at Clingman's Dome, NC/TN suggested a possible improvement, since in the 1980's, in red spruce forest health. A comparison with previous studies, which spanned 40 years, at Richland Balsam, NC of foliar calcium and magnesium concentrations taken from saplings red spruce trees, suggested a possible improvement in red spruce health at that site since 1994.

1. INTRODUCTION

1.1 Red Spruce Background

Red spruce and Fraser fir trees form a unique ecosystem in the Southern Appalachian Mountains.[1-6] Red spruce trees are medium size conifers that can grow over 100 feet tall, have needle like foliage, and grow on the steep slopes at high elevation (above 4500 ft.) in Southern Appalachian Mountains, where they receive moderate amounts of precipitation annually. Spruce-fir forests in the Southern Appalachian Mountains have declined since the 1960's.[2-3] Since the 1970's, spruce-fir forests in Northern Appalachian Mountains and Europe have experienced similar decline. Deusen et al.[7] suggested the decline of red spruce could be related to climate changes or natural causes, such as hurricanes, inset attacks, disease, etc. However, several authors have developed a hypothesis correlating the decline of red spruce to acid deposition.[1-3,7-9]

1.2 Acid Deposition

The burning of fossil fuels, such as coal, releases oxides of sulfur (SO_x) and nitrogen (NO_x) that react with the atmosphere to form nitric acid and sulfuric acid, which may eventually fall to earth as acid deposition.[10] Acid deposition is a complex mixture that includes H^+, SO_4^{2-}, NH_4^+, and NO_3^- ions and can be found in the wet or dry form.[11] Most coal-burning power plants minimize local pollution by erecting tall exhaust stacks, but this approach creates a problem downwind, away from the plant.[10] Several coal-burning power plants are located in the Southeast United States (North Carolina and

Tennessee). For example, when an eastern Tennessee coal-burning power plant releases emissions, then acid deposition has been shown to deposit in the Southern Appalachian Mountains (North Carolina) due to weather patterns (i.e., the wind generally blows from west (Tennessee) to east (North Carolina))[12]. In Figure 1.1, the locations of coal-burning power plants in eastern Tennessee are represented by red stars.[13] Since the industrial revolution (late 18th century), acid deposition has been increasing dramatically.[8, 10]

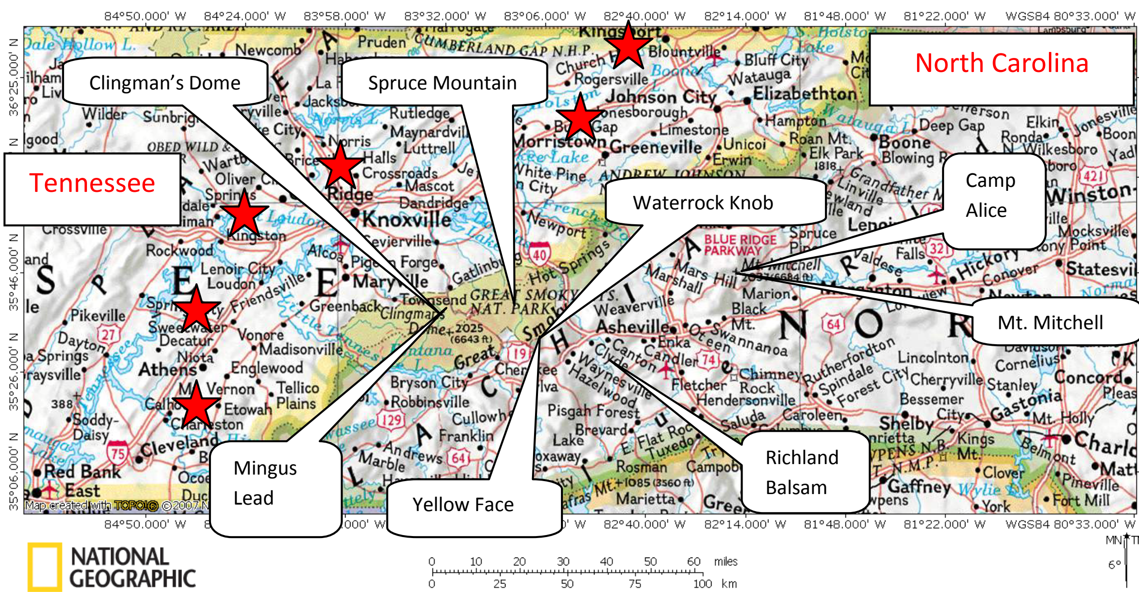

Figure 1.1: Map of Locations of Coal-burning Power Plants in Eastern Tennessee and Sample Sites.

After a rainfall, soil particles are converted into a "soil solution" consisting of mineral ions, which are then available for uptake through the root system of plants. [10] During this process of a "normal" rainfall (i.e., no acid in the rain), negatively charged ions, such as nitrate (NO_3^-) and sulfate (SO_4^{2-}), are quickly leached to the groundwater, whereas positively charged ions, including nutrients such as calcium and magnesium, remain tightly bonded to the soil particles.[10] However, after an "acidic" rainfall, the acid in the rain dissolves the calcium and magnesium minerals and then nutrients are leached from the soil into the groundwater. Acid deposition may increase the availability of aluminum (Al^{3+}) in the soil, which may elevate the concentration of aluminum in plants to toxic levels.[1, 10, 14] In particular, red spruce trees are affected by acid deposition in several ways including: increase in aluminum concentrations,[1, 8, 10, 14] reduced cold temperature tolerance,[3, 8] susceptibility to freezing injury,[8,11] and reduced winter hardiness.[3]

1.3 Effects of Acid Deposition on Red Spruce Forests

Red spruce trees are classified as shallow rooted plants with root depths found in the topsoil layer, which is no more than 60 cm from the surface.[15] In eastern North America, soils of low elevation red spruce forests, consist of a thick organic layer, but in high elevation forests, the topsoil layer are located above rock.[16] Rock composition underlying the soils of Southern Appalachian Mountains consist of Thunderhead Sandstone.[17] Due to the geological rock formation that occurred during the Pleistocene period (i.e. 1.8 million years ago), red spruce forests now grow in high amounts of “weatherable” minerals and on unstable slopes.[17] Soils found in red spruce forests in the Southern Appalachian Mountains are considered to be extremely acidic, with an average range for the topsoil layer to be 3.0 to 4.5 pH.[15-16] These soils are considered to be acidic because of two components: (1) the dissociation of hydrogen ions from organic matter, and (2) the presence of aluminum cations, which may make the trees sensitive to aluminum mobilization as the concentration of strong acid anions increases in soil solution.[18]

Changes in soil chemistry of red spruce trees could result in alterations to the physiological and biochemical processes that could endanger the plant; therefore, detections of the changes before visible symptoms of nutrients deficiencies are important.[19] In 1990, D.W. Johnson et al. concluded that nitrogen and sulfur depositions (i.e., acid rain) have two possible affects on nutrient cycling of base cations (i.e., calcium and magnesium): (1) a decrease in soil pH, and (2) an increase in aluminum concentrations in the soil solution.[20] At several sites in the Southern Appalachian Mountains, the majority of aluminum present, with a reported value of 80-90%, within the mineral soil solution is in the inorganic form.[16]

The biogeochemistry of aluminum foliar or soil concentrations taken from red spruce forests could be important to investigate, because the bioavailable form of aluminum is considered poisonous to plants (i.e. phytotoxic).[21] Interference with cation uptake and damage to plant cells could result from an increase in aluminum concentrations, resulting in a decrease in cellular inorganic cation concentrations such as Ca^{2+}, Mg^{2+}, Mn^{2+}, and K^{+}.[19] Some possible symptoms from an increase in aluminum concentrations found in red spruce trees are needle biomass, decrease in seedling height, inhibition in DNA synthesis, effects on root growth, and cell division complications.[19] Numerous theories related to the mechanisms responsible for these visible symptoms that have been observed.

Studies have revealed several possible mechanisms responsible for the decline of red spruce, caused by acid deposition, in the Southern Appalachian Mountains. Increased aluminum (Al^{3+}) cation mobility may reduce soil storage of calcium cation (Ca^{2+}) and the uptake of other essential nutrients.[11] Magnesium ion (Mg^{2+}) is a component of chlorophyll; therefore, magnesium deficiency may lead to chlorosis which results in yellow-colored leaves.[10] Calcium deficiency in red spruce may reduce photosynthesis and lead to secondary stress, such as reduced resistance to freezing conditions or diseases.[11] Low concentrations of calcium in red spruce may lead to destabilization of the plasma membrane-cell wall in mesophyll and alter the carbon metabolism.[11] Currently, the actual mechanisms responsible for the decline in red spruce, due to calcium and magnesium depletion in soil of spruce-fir forests, are uncertain.[11]

1.4 Previous Studies of Red Spruce Decline in the Southern Appalachian Mountains

Evidences that acid deposition is causing red spruce forest decline in the Southern Appalachian Mountains varies in the literature. Some studies have reported inconclusive results.[3, 6] Other studies can support the acid deposition hypothesis by analyzing foliage and soil from several sites in the Southern Appalachian Mountains.[1-2] McLaughlin et al.[28] designed laboratory experiments to simulate acid deposition on red spruce seedlings in order to investigate any alterations in red spruce physiology and their results support field observations from other studies.[22]

Shepard et al.[1] compared results to a previous study of red spruce saplings foliar magnesium and calcium at Richland Balsam, North Carolina. Bintz et al. determined low concentrations of calcium and magnesium, and high concentrations of aluminum in some red spruce foliar and soil samples.[2] A possible increase in nutrient levels was observed by Bintz et al. study in 2005[2] compared to results from previous studies in 1969[23] and 1994[1]. In the 1990's, under Clean Air Act Amendments, the federal government imposed regulations to reduce the sulfur dioxide emissions from coal-burning power plants.[10-11] The increase in nutrient concentrations of red spruce trees reported by Bintz et al.[2] in 2005 could be explained by the Clean Air Act Amendments, which may have reduced acid depsoition in the Southern Appalachian Mountians.

Some studies revealed that higher elevation sites, above the 5500 ft. cloud base, could be affected more by acid deposition.[24] These higher elevations sites are subject to an increased rate of acid deposition due to acidic fog and/or clouds. Hence, comparison of higher elevation sites with lower elevation sites may allow the characterization of the severity of acid deposition effects on red spruce forest health.

Fraser fir trees have suffered severe infestation by the Balsam Wooly Adelgid (BWA) in recent years and this could create problems for investigating spruce-fir forests health effects caused by acid deposition. Studies have shown that red spruce are sensitive to acid deposition and are not affected by the BWA, which could make red spruce trees a more reliable gauge of acid deposition effects on the forests than Fraser fir trees. These previous studies suggest that the effects of acid deposition on the spruce-fir forests by analyzing calcium, magnesium, and aluminum in red spruce foliage and surrounding soils.

1.5 Goals and Hypotheses

In this project the concentration of calcium, magnesium, and aluminum were analyzed in red spruce foliage and surrounding soils to determine if acidic deposition increased toxic metal (Aluminum) levels and reduced nutrient availability (calcium and magnesium), which may affect red spruce forests health. There are four major goals of this project. First, determine if there was any correlation between elevation and acidic deposition on red spruce forests. The second goal was to determine if the coal-burning power plants located in eastern Tennessee has any affect on western sites located in the Great Smoky Mountain National Park due to acid deposition. The third goal was to estimate the health of red spruce forests located in the Southern Appalachian Mountains by examining soil calcium/aluminum molar ratios. Our last goal was to compare our results of this project with similar previous studies.

Bintz et al.[2] reported inclusive results when determining the effects of acid deposition on red spruce forests' geography (i.e., sample sites were located in close proximity of each other). Sample sites in this study were chosen relatively farther apart, as suggested by Bintz, shown in Figure 1.3. Western samples sites include Clingman's Dome, Mingus Lead, and Spruce Mountain located within the Great Smoky Mountains National Park (NC); Central located samples in our study include Richland Balsam, Waterrock Knob, and Yellow Face on the Blue Ridge Parkway (NC); and eastern sample sites include Mount Mitchell and Camp Alice located in Mt. Mitchell State Park (NC).

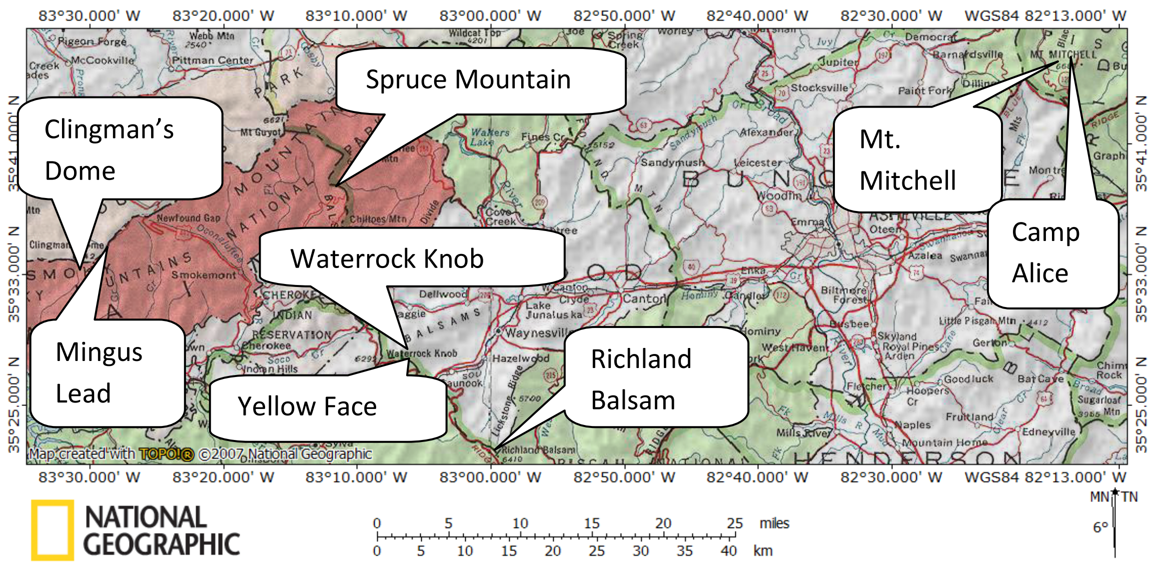

Figure 1.2: Map of Location of All Selected Sample Sites in This Study.

We hypothesized that higher elevation sites would exhibit lower nutrient (calcium and magnesium) levels and higher levels of toxic metal (aluminum). Also, we proposed that western sample sites were expected to exhibit lower nutrient (calcium and magnesium) levels and higher toxic metal (aluminum) levels caused by enhanced acid deposition due to proximity of the coal-burning power plants located in eastern Tennessee. Acid deposition on red spruce forests may increase the concentrations of H^+ in the soil which would thereby increase the mobility of aluminum; for that reason, we speculated that as soil pH from our sample sites decreases, then the concentration of aluminum found in red spruce foliage and surrounding soils would increase. Due the Clean Air Act of 1990, we proposed that a comparison with previous studies will show an improvement in red spruce forests health at Clingman's Dome, NC/TN and Richland Balsam, NC.

2. EXPERIMENTAL

2.1 Sample Site Selection

Red spruce foliar and soil samples were collected at eight sites, located on the Blue Ridge Parkway (North Carolina), within the Great Smoky Mountains National Park (North Carolina /Tennessee) and Mt. Mitchell State Park (North Carolina), listed in Table 2.1 and represented in Figure 2.1. The criteria for selecting sample sites included: spruce-fir forest, broad distribution of trees, within 10 km of the trailhead, elevations above 1370 m (4500 ft.). Each sample site location was recorded with a Garmin Global Positioning System (GPS) 76CSx receiver unit, data shown in Table 2.1.

Table 2.1: All Sample Sites Coordinates and Elevations (Blue Ridge Parkway (BRP), Great Smoky Mountains National Park (GSMNP), and Mt. Mitchell State Park (MMSP)).

SITE	Measured Latitude (North)	Measured Longitude (West)	Measured Elevation (ft.)
Clingman's Dome	35° 33' 0.742"	83° 29' 6.788"	6610 ± 40
Mingus Lead	35° 36' 0.723"	83° 26' 0.760"	5600 ± 20
Mount Mitchell	35° 45' 0.823"	82° 15' 0.755"	6600 ± 40
Camp Alice	35° 45' 0.399"	82° 15' 20.617"	5760 ± 20
Richland Balsam	35° 21' 10.626"	82° 59' 0.321"	6200 ± 130
Spruce Mountain	35° 36' 0.752"	83° 10' 0.520"	5630 ± 20
Waterrock Knob	35° 27' 0.810"	83° 8' 0.340"	6130 ± 150
Yellow Face	35° 27' 0.305"	83° 8' 0.665"	5780 ± 40

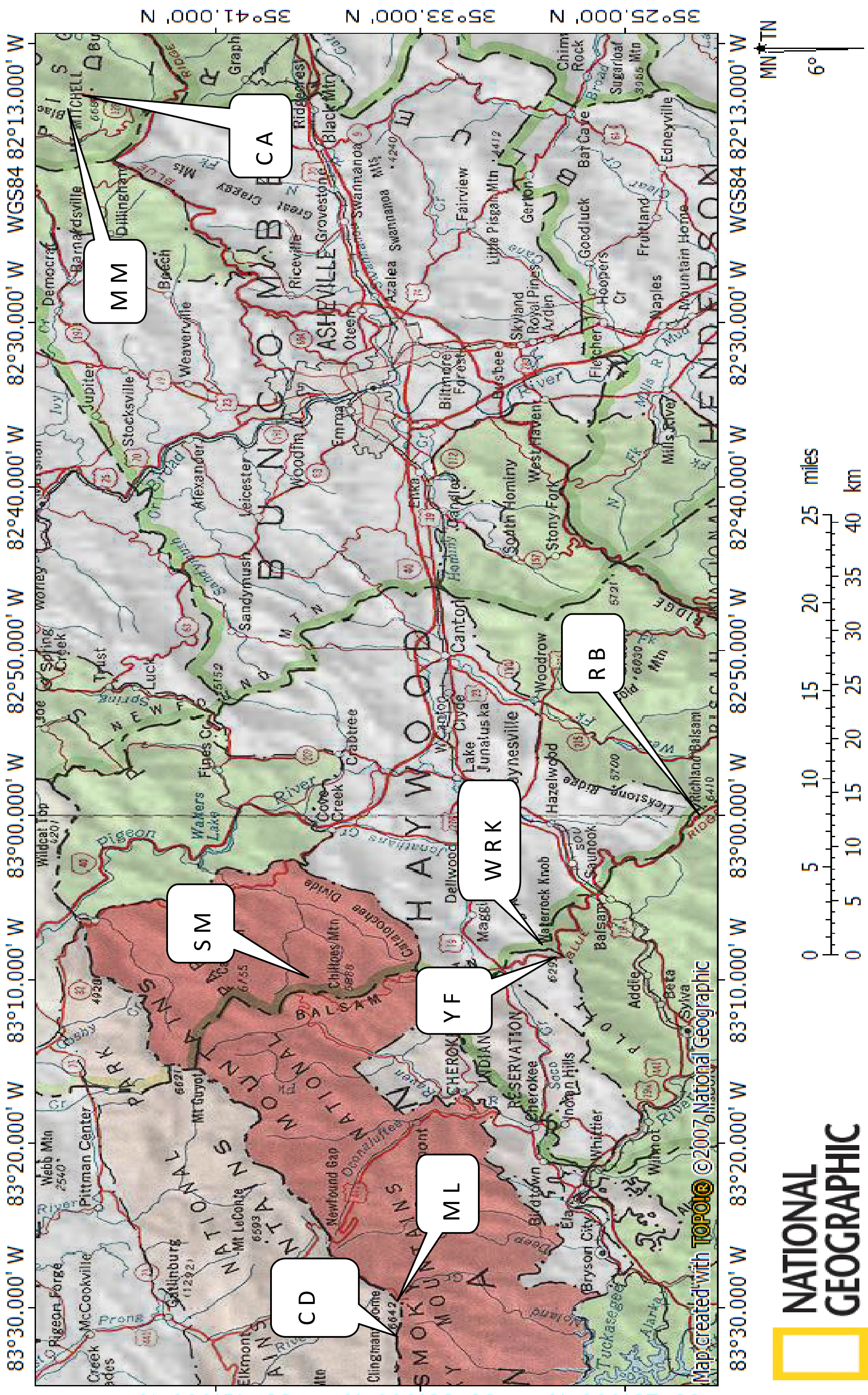

Figure 2.1: Map of All Sample Sites

Foliar and soil samples were collected from 30 red spruce trees at each sample site. Sample Site ID, soil and foliar population, date of samplings and location of each sample sites are shown in Table 2.2. The 30 red spruce trees (at each sample site) were then divided into three categories by height: 10 seedlings (less than 7 ft.), 10 saplings (7 ft. to 13 ft.), and 10 mature (above 13 ft.).

Table 2.2: Sample Site ID, Elevations, Soil and Foliar Sample Population.

SITE	SITE ID	Soil Pop.	Foliar Pop.	Location	Date of Sampling
Clingman's Dome	CD	30	30	GSMNP	16 July 2009
Mingus Lead	ML	30	30	GSMNP	26 July 2009
Mount Mitchell	MM	30	30	MMSP	11 August 2009
Camp Alice	CA	30	30	MMSP	12 August 2009
Richland Balsam	RB	30	30	BRP	18 June 2009
Spruce Mountain	SM	30	30	GSMNP	30 July 2009
Waterrock Knob	WRK	30	30	BRP	19 May 2009
Yellow Face	YF	30	30	BRP	04 August 2009

The criteria used for comparing a low and high elevation sample sites include: samples sites are located within 5 miles of each other in any direction and/or the total difference in elevation is greater than 1000 ft. between the two sites, shown in Figure 2.2. High elevation samples are defined as being above 6100 ft. and low elevation sample sites are below 6100 ft. Therefore, Mount Mitchell and Camp Alice, sample sites were compared because they meet all of the criteria as well as Clingman's Dome and Mingus

Lead. Waterrock Knob and Yellow Face were examined together but did not meet the elevation requirement.

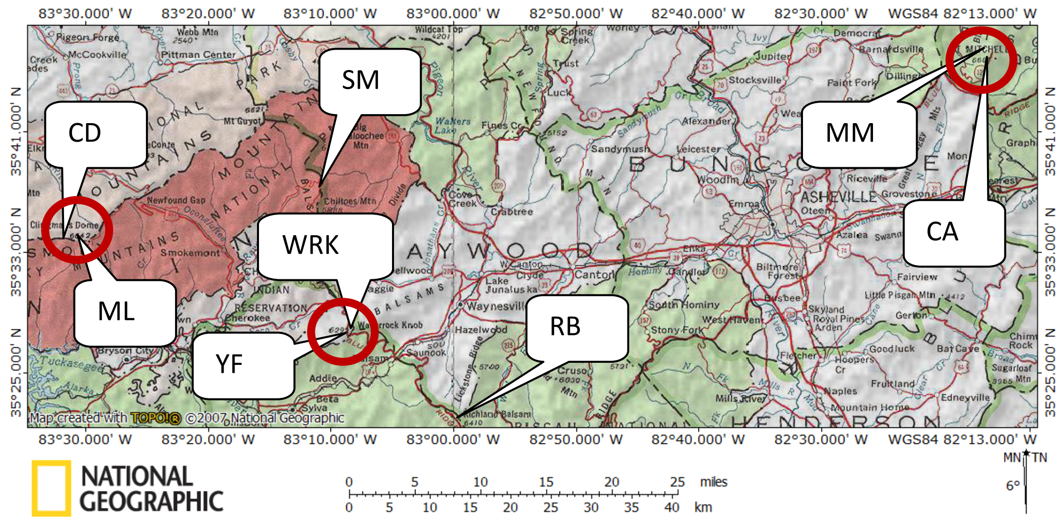

Figure 2.2: Map of All Sample Sites for Elevational Studies, When Comparing Individual Sample Sites.

The significance of geography on nutrients and toxic metal concentrations due to the coal-burning power plants located in eastern Tennessee was examined by comparing western located sample sites with central sites, Central located sites with eastern, and western located sample sites with eastern sites, shown in Figures 2.3 and 2.4. Western samples sites include Clingman’s Dome, Mingus Lead, and Spruce Mountain located within the Great Smoky Mountains National Park (North Carolina /Tennessee); Central located samples are in our study (i.e., these sites are located approximately in the central of the sampling area) include Richland Balsam, Waterrock Knob, and Yellow Face on the Blue Ridge Parkway (North Carolina); and eastern sample sites include Mount Mitchell and Camp Alice located in the Mt. Mitchell State Park (North Carolina).

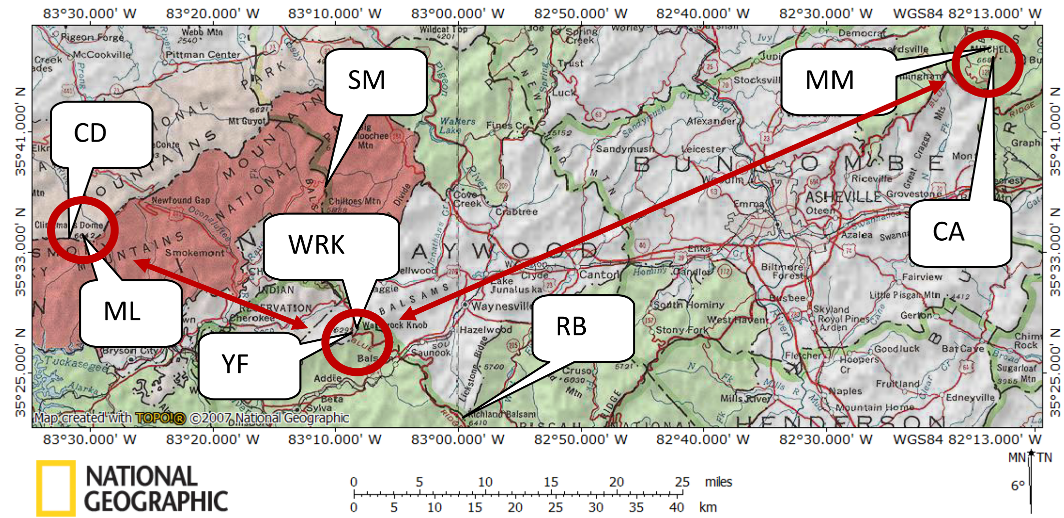

Figure 2.3: Map of All Sample Sites for Longitude Studies of Western, Central, and Eastern Sample sites.

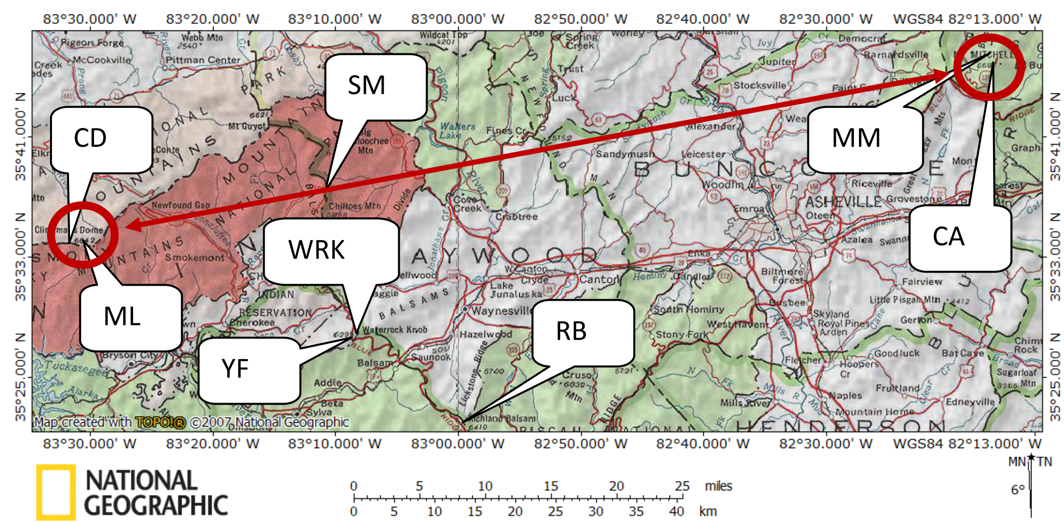

Figure 2.4: Map of Western Sample Sites Compared to Eastern sites.

2.2 Collection and Preparation of Foliar Samples

Approximately 100 grams of foliage was collected from each red spruce tree by using stainless steel pruning shears. Foliage was cut at various locations, up to 2 m from the ground, around each tree. The location of each tree was recorded using the Garmin GPS 76CSx receiver unit (Garmin Ltd., Olathe, KS). Samples were labeled and then placed in polyethylene bags for transport and storage.

After the current year's foliage growth was separated, each foliar sample was dried in a Precision Economy Oven (Thermo Fisher Scientific Inc., Waltham, MA) at 110°C for a period of 24 hours. The red spruce needles were removed from the limbs, leaving between 5-10 grams of dried foliage, which was then placed into pre-labeled polyethylene bags for storage. Composite samples were prepared by removing 1.0000 ± 0.0020 gram of dried foliage from each sample composed of a particular sample class at each site and placed into a polyethylene bag (e.g., a saplings' sample class was composed of 10 red spruce specimens, so 1.0000 ± 0.0020 gram was removed from each specimen). The polyethylene bag composed of the 10.0000 grams of foliage was mixed until homogenous. The needles were removed and pulverized for approximately 30 minutes using a Spex mixer/mill 8000 (SPEX SamplePrep, LLC, Metuchen, New Jersey).

2.3 Foliar Digestion Procedure

Foliar samples were acid digested using a modification of the procedure from Shepard et al.[1]

Aliquots of 0.2000 ± 0.01 gram of composited foliage samples were introduced into Fisherband (Fisher Scientific, Pittsburgh, PA) 16 x 150 mm borosilicate glass test tubes in replicates of five using a stainless steel spatula. A Finnpipette (Fisher Scientific LLC, Pittsburgh, PA) was used to place 1.0 mL of concentrated nitric acid (FisherChemical, A200-c212) into each test tube. The solution was vortexed and then allowed to stand for 1 hour in a test tube rack. These solutions were then placed into a laboratory-constructed aluminum heating block and heated to reflux for 3 hours. Once 140ºC was reached on the heating block, 1.0 mL of concentrated nitric acid was carefully added to each sample. During the reflux, the samples were vortexed every 10-15 minutes to ensure complete digestion. Care was taken in the first 30 minutes of the initial heating process to prevent the "foam/froth" of the sample from leaving the test tube; therefore, the samples were vortexed every 1-2 minutes when the temperature was between 60 and 110 ºC.

The solutions were covered with Parafilm (Pechiney Plastic Packaging, Menasha, WI) and allowed to cool overnight to room temperature and 0.5 mL of cold (35ºC) 30% hydrogen peroxide (FisherChemical, BP2633-500) was added to each sample. These solutions were vortexed and allowed to stand for 30 minutes before placing them back on the heating block. The solutions were refluxed at approximately 150ºC for an additional 2 hours. During the reflux, the samples were vortexed every 10-15 minutes to ensure complete digestion. In order to remove undigested particles, the solutions were gravity filtered by using Fisherband Filter Paper P8 (Fisher Scientific LLC, Pittsburgh, PA) before being transferred into plastic 100 mL Nalgene volumetric flasks (Thermo Fisher Scientific Inc., Waltham, MA) and diluted with NANOpure water from a Barnstead NANOpure water purification (Thermo Fisher Scientific Inc., Waltham, MA).

All glassware was washed with a 1% Alconox solution for 24 hours, rinsed with NANOpure water, and then placed into a 20% nitric acid solution for 24 hours and rinsed with NANOpure water.

2.4 Collection and Preparation of Soil Samples

Approximately 100 grams of soil were collected within 10 feet from the base of each red spruce tree. A stainless steel hand trowel was used to displace the leaf litter to obtain a 10 cm^2 by 15 cm deep “topsoil” sample. The location of the specimen was recorded using a Garmin GPS 76CSx receiver unit. Soil samples were labeled and placed in polyethylene bags for transport and storage.

The samples were dried in a Precision Economy Oven at 110°C for a period of 24 hours. Debris in the dried soil was removed by using stainless steel USA Standard Testing Sieves No. 10, 2mm and No. 18, 1mm made by Fisher Scientific (Fisher Scientific LLC, Pittsburgh, PA). Composite samples were prepared by removing 1.0000 ± 0.0020 gram of dried soil from each sample composed of a particular sample class at each site and placed into a polyethylene bag. Each polyethylene bag was composed of 10.0000 grams of soil, which was mixed until homogeneous. The samples were removed from the bag and placed into a steel canister with two steel shots. The canister was placed into a Spex mixer/mill 8000 and pulverized for approximately 5 to 15 minutes.

2.5 Soil Exchangeable Cations Extraction Procedure

Soil exchangeable cations were extracted by using a modification of the procedure from Carter.[25] Aliquots of 0.5000 ± 0.01 grams of dried soil were placed into

Falcon Blue Max™ (Becton Dickinson and Company, Franklin Lakes, NJ) 50 mL polypropylene conical tubes in replicates of five. A graduated cylinder was used to place 30.0 mL of 0.100 M barium chloride (Fisher Chemical, B34-500) solution into each conical tube. These solutions were placed on a Lab-Line Orbit Shaker (Lab-Line Instruments, Inc., Melrose Park, IL) and shaken at 100 rpm for 2 hours. In order to remove large unextracted particles, the solutions were gravity filtered before being transferred into plastic 100 mL Nalgene volumetric flasks and diluted with NANOpure water.

All glassware was washed with a 1% Alconox solution for 24 hours, rinsed with NANOpure water, and then placed into a 20% nitric acid solution for 24 hours and rinsed with NANOpure water.

2.6 Soil pH Analysis

Soil pH from each of the composite samples was measured by using the procedure from Carter.[25] Aliquots of 1.0000 ± 0.002 grams of dried soil were placed into Falcon Blue Max™ 50 mL polypropylene conical tubes. A graduated cylinder was used to place 20.0 mL of NANOpure water into each conical tube. These solutions were placed on a Lab-Line Orbit Shaker and shaken at 100 rpm for 30 minutes. These 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

Aluminum, calcium, and magnesium standards were prepared using a SpexCertiPrep (SPEX CertiPrep, LLC, Metuchen, New Jersey) 1,000 ppm Custom Assurance Standard in 2% nitric acid. 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. Only recovery checks were used for quality control for the soil samples because SRM from NIST are unavailable to provide concentration of exchangeable cations in soil. A selected example of SRM 1575a and recovery checks are represented in Table 2.3. All samples analyzed are within the certified concentrations values for each element of the NIST SRM 1575a, except for aluminum. The pine needles in NIST SRM 1575a were digested using a procedure involving the use hydrofluoric acid and concentrated nitric acid which completely digested the aluminum. Since our goal was to measure the concentration of the aluminum only available to the plants, we were not interested in the aluminum bound in the silica found in the foliage. The good agreement of the recovery checks indicates the accuracy of our procedure.

Table 2.3: Quality Control for Aluminum, Calcium, and Magnesium Concentrations from NIST 1575a Standard Reference Material and Recovery Checks

Element	SRM 1575a Average Conc.	SRM 1575a NSIT Accepted Values	Recovery Checks	RSD Recovery Checks
Al	540 ± 8 µg/g	580 ± 30 µg/g	99 ± 6 %	6 %
Ca	0.25 ± 0.002 %	0.25 ± 0.02 %	102 ± 1 %	1 %
Mg	0.094 ± 0.001 %	0.106±0.017 %	96 ± 5 %	5 %

2.8 Sample Analysis

Foliar and soil samples were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Perkin-Elmer Optima 4100 DV) for aluminum, calcium, and magnesium. Detection limits were determined to between 1-26 ppb for aluminum, calcium, and magnesium, shown in Table 2.4.

Table 2.4: Calculated Detection Limits for ICP-OES.

Element	Calculated Detection Limits (ng/mL)
Magnesium 285 nm	0.005
Magnesium 279 nm	0.001
Magnesium 280 nm	0.008
Aluminum 308 nm	0.026
Aluminum 394 nm	0.002
Aluminum 237 nm	0.006
Calcium 315 nm	0.002
Calcium 317 nm	0.001
Calcium 393 nm	0.001

2.9 Analytes, Wavelengths, and ICP-OES Conditions

The conditions of the ICP-OES, selected analytes, and wavelengths are represented in Table 2.5 and were given in Bintz et al.[2] Wavelengths were chosen based on the lowest detection limits and least amount of spectral interferences for the element being analyzed.

Table 2.5: Selected Analytes, Wavelengths and Instrumental Conditions for ICP-OES

Analyte	Wavelength (nm)
Aluminum	398.215
Aluminum	394.401
Aluminum	315.313
Calcium	315.887
Calcium	317.933
Calcium	393.366
Magnesium	285.213
Magnesium	279.077
Magnesium	280.271
Radio Frequency (watts)	1300
Pump Rate (mL/min)	1.25
Aux. Gas Flow (L/min)	0.2
Nebuilzer Gas Flow (L/min)	0.8
Plasma View	Axial

2.10 Statistical Analysis

Statistical analysis, a Student's *t*-test, linear regression analysis, and analysis of variance, were performed on the results by using R version 2.10.1 program (R Foundation for Statistical Computing; Vienna, Austria) to determine if a statistical difference existed between red spruce populations and classes. Geographic Information System (GIS) mapping software was used to perform geospatial analysis. A summary of the statistical analysis include: Student's *t*-test was employed to compare foliar or soil concentrations between two sites, linear regression was used to determine any correlation, when comparing all sites together, in foliar or soil concentration with elevation, pH, or longitude, and analysis of variance was employed to compare foliar or soil concentrations between multiple sites. An alpha value of 0.05 was used as the decision criterion for all statistical tests. If the p-value was greater than the alpha value (0.05), then the concentrations in either soil or foliage between the sample sites was determined to be the same. If the p-value was less than the alpha value (0.05), then a statistical difference existed between samples sites' concentrations.

3. RESULTS AND DISCUSSION

Detailed maps of sample sites locations and approximate selected sampling areas are shown in Appendix A. Average and standard deviations of elevations, longitude, and latitude values for individual trees are in Appendix B. This chapter is divided into 7 main sections involving statistical analysis of the following parameters: (1) the correlation between elevation of sample sites with nutrient (calcium and magnesium) and toxic metal (aluminum) concentrations, (2) a comparison of nutrient and toxic metal levels based on distance from coal-fired power plants located in eastern Tennessee, (3) the correlation between foliar and soil metal concentrations with soil pH, (4) life stage comparison of foliar and soil metal concentrations in mature red spruce, red spruce saplings, and red spruce seedlings, (5) the correlation between foliar metal concentrations with soil metal concentrations, (6) investigation of red spruce forest health using soil calcium/aluminum molar ratios, and (7) comparison of the results obtained in this work with data collected in previous studies. Results from theses statistical analyses are shown in Appendix C.

3.1 Foliage Data

Average foliar elemental concentrations, elevations, and soil pH of mature red spruce, red spruce saplings, and red spruce seedlings are represented in Table 3.1, Table 3.2, and Table 3.3, respectively. Foliar elemental concentrations represent the concentration of metals found within each class of red spruce foliage at each site.

Table 3.1: Average Foliar Elemental Concentrations, Elevations, and Soil pH of Mature Red Spruce from All Sites Located in the Southern Appalachian Mountains

SITE	Foliar Al conc.(μg/g)	Foliar Ca conc.(μg/g)	Foliar Mg conc.(μg/g)	Mature Elevation (m)	Mature Soil pH
Clingman's Dome	81 ± 3	2870 ± 20	566 ± 4	2010 ± 10	3.9 ± 0.1
Mingus Lead	93 ± 4	3520 ± 70	543 ± 10	1700 ± 9	3.6 ± 0.1
Mount Mitchell	91 ± 10	2720 ± 30	676 ± 3	2010 ± 10	3.7 ± 0.1
Camp Alice	104 ± 6	3930 ± 40	631 ± 5	1760 ± 6	5.1 ± 0.1
Richland Balsam	71 ± 8	2330 ± 20	321 ± 10	1850 ± 30	3.7 ± 0.1
Spruce Mountain	106 ± 10	3030 ± 20	562 ± 4	1720 ± 6	3.8 ± 0.1
Waterrock Knob	118 ± 5	2470 ± 30	469 ± 4	1910 ± 6	3.9 ± 0.1
Yellow Face	82 ± 3	2380 ± 9	437 ± 6	1760 ± 10	3.7 ± 0.1

Table 3.2: Average Foliar Elemental Concentrations, Elevations, and Soil pH of Red Spruce Saplings from All Sites Located in the Southern Appalachian Mountains

SITE	Foliar Al conc.(µg/g)	Foliar Ca conc.(µg/g)	Foliar Mg conc.(µg/g)	Saplings Elevation (m)	Saplings Soil pH
Clingman's Dome	65 ± 9	3310 ± 40	625 ± 5	2010 ± 20	4.0 ± 0.1
Mingus Lead	91 ± 7	3140 ± 40	611 ± 7	1710 ± 6	3.6 ± 0.1
Mount Mitchell	73 ± 4	2520 ± 50	730 ± 4	2010 ±10	3.7 ± 0.1
Camp Alice	163 ± 5	3130 ±70	613 ± 10	1750 ± 3	5.4 ± 0.1
Richland Balsam	70 ± 4	2320 ± 50	570 ± 4	1910 ± 40	3.9 ± 0.1
Spruce Mountain	90 ± 8	3230 ± 10	450 ± 2	1710 ± 6	3.8 ± 0.1
Waterrock Knob	82 ± 6	2070 ± 20	558 ± 6	1850 ± 40	3.8 ± 0.1
Yellow Face	75 ± 4	2890 ± 50	490 ± 6	1760 ± 10	3.8 ± 0.1

Table 3.3: Average Foliar Elemental Concentrations, Elevations, and Soil pH of Red Spruce Seedlings from All Sites Located in the Southern Appalachian Mountains

SITE	Foliar Al conc.(μg/g)	Foliar Ca conc.(μg/g)	Foliar Mg conc.(μg/g)	Seedlings Elevation (m)	Seedlings Soil pH
Clingman's Dome	79 ± 6	3090 ± 70	760 ± 6	2010 ± 9	3.8 ± 0.1
Mingus Lead	91 ± 5	4030 ± 50	701 ± 10	1710 ± 9	3.9 ± 0.1
Mount Mitchell	74 ± 5	2710 ± 30	814 ± 6	2010 ± 9	3.8 ± 0.1
Camp Alice	155 ± 3	4410 ± 10	617 ± 4	1760 ± 6	5.4 ± 0.1
Richland Balsam	71 ± 4	2410 ± 7	593 ± 8	1890 ± 40	3.9 ± 0.1
Spruce Mountain	74 ± 5	2780 ± 17	510 ± 6	1710 ± 6	3.9 ± 0.1
Waterrock Knob	62 ± 3	2040 ± 50	472 ± 6	1840 ± 50	3.6 ± 0.1
Yellow Face	55 ± 4	3490 ± 50	716 ± 8	1770 ± 6	3.7 ± 0.1

3.2 Soil Data

Average soil exchangeable concentrations, elevations, and soil pH of mature red spruce, red spruce saplings, and red spruce seedlings are represented in Table 3.4, Table 3.5, and Table 3.6, respectively. These concentrations represent the concentration of exchangeable cations (i.e., aluminum Al^{3+}, calcium Ca^{2+} and magnesium Mg^{2+}) found from the surrounding soils of red spruce trees at each site.

Table 3.4: Average Soil Exchangeable Concentrations, Elevations, and Soil pH of Mature Red Spruce from All Sites Located in the Southern Appalachian Mountains

SITE	Soil Al conc. (µg/g)	Soil Ca conc. (µg/g)	Soil Mg conc. (µg/g)	Mature Elevation (m)	Mature Soil pH
Clingman's Dome	1030 ± 8	100 ± 9	79 ± 2	2010 ± 10	3.9 ± 0.1
Mingus Lead	761 ± 30	174 ± 10	87 ± 6	1700 ± 9	3.6 ± 0.1
Mount Mitchell	1620 ± 30	190 ± 7	133 ± 2	2010 ± 10	3.7 ± 0.1
Camp Alice	355 ± 20	168 ± 9	152 ± 3	1760 ± 6	5.1 ± 0.1
Richland Balsam	772 ± 8	174 ± 2	82 ± 1	1850 ± 30	3.7 ± 0.1
Spruce Mountain	876 ± 20	107 ± 4	79 ± 1	1720 ± 6	3.8 ± 0.1
Waterrock Knob	551 ± 20	827 ± 20	133 ± 4	1910 ± 6	3.9 ± 0.1
Yellow Face	1270 ± 20	126 ± 4	108 ± 1	1760 ± 10	3.7 ± 0.1

Table 3.5: Average Soil Exchangeable Concentrations, Elevations, and Soil pH of Red Spruce Saplings from All Sites Located in the Southern Appalachian Mountains

SITE	Soil Al conc. (µg/g)	Soil Ca conc. (µg/g)	Soil Mg conc. (µg/g)	Saplings Elevation (m)	Saplings Soil pH
Clingman's Dome	1050 ± 40	375 ± 10	114 ± 4	2010 ± 20	4.0 ± 0.1
Mingus Lead	680 ± 6	195 ± 3	77 ± 1	1710 ± 6	3.6 ± 0.1
Mount Mitchell	1420 ± 40	171 ± 6	139 ± 3	2010 ± 10	3.7 ± 0.1
Camp Alice	217 ± 20	86 ± 4	164 ± 3	1750 ± 3	5.4 ± 0.1
Richland Balsam	785 ± 20	125 ± 4	93 ± 3	1910 ± 40	3.9 ± 0.1
Spruce Mountain	905 ± 10	167 ± 10	61 ± 1	1710 ± 6	3.8 ± 0.1
Waterrock Knob	1090 ± 20	236 ± 6	112 ± 1	1850 ± 40	3.8 ± 0.1
Yellow Face	1220 ± 20	76 ± 6	89 ± 2	1760 ± 10	3.8 ± 0.1

Table 3.6: Average Soil Exchangeable Concentrations, Elevations, and Soil pH of Red Spruce Seedlings from All Sites Located in the Southern Appalachian Mountains

SITE	Soil Al conc. (µg/g)	Soil Ca conc. (µg/g)	Soil Mg conc. (µg/g)	Seedlings Elevation (m)	Seedlings Soil pH
Clingman's Dome	1010 ± 9	95 ± 4	87 ± 1	2010 ± 9	3.8 ± 0.1
Mingus Lead	763 ± 3	155 ± 8	69 ± 1	1710 ± 9	3.9 ± 0.1
Mount Mitchell	1310 ± 10	105 ± 7	124 ± 1	2010 ± 9	3.8 ± 0.1
Camp Alice	258 ± 10	106 ± 5	177 ± 6	1760 ± 6	5.4 ± 0.1
Richland Balsam	746 ± 20	128 ± 3	81 ± 2	1890 ± 40	3.9 ± 0.1
Spruce Mountain	1080 ± 10	70 ± 1	54 ± 1	1710 ± 6	3.9 ± 0.1
Waterrock Knob	929 ± 20	338 ± 6	141 ± 4	1840 ± 50	3.6 ± 0.1
Yellow Face	1150 ± 8	122 ± 5	90 ± 1	1770 ± 6	3.7 ± 0.1

3.3 Elevational Studies

3.3.1 Elevational Studies of All Sites Compared Together

Nutrient concentrations (calcium and magnesium) were hypothesized to exhibit a negative correlation and the toxic metal concentration (aluminum) was hypothesized to exhibit a positive correlation with elevation due to enhanced acid deposition at higher elevations. Linear regression analysis was employed to study the effects of elevation on foliar or soil metal concentrations when comparing all sites together. The statistical analyses of foliar concentration *vs.* elevation are presented in Table C.1 and the results from soil concentration *vs.* elevation are shown in Table C.2. Figure 3.1 shows a representative example of a graph of foliar aluminum concentration in mature red spruce trees *vs.* elevation. A summary of results from the linear regression analyses, listed in Table 3.7, shows that there was no correlation between either soil or foliar metal concentrations with elevation.

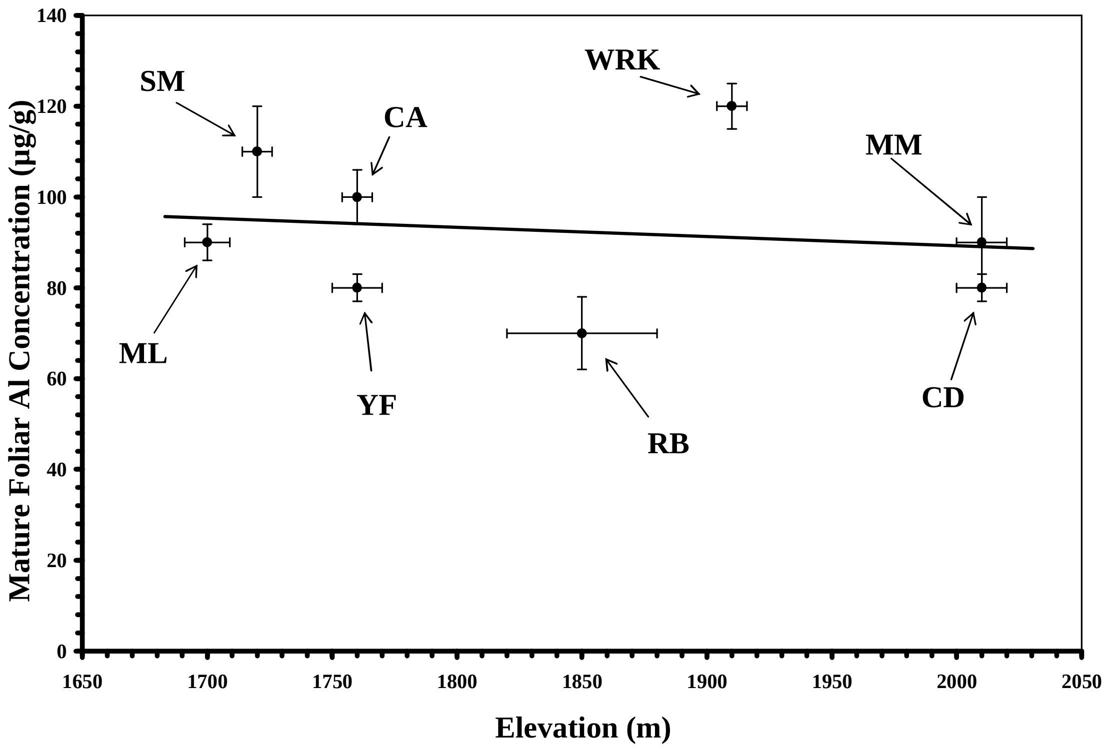

Figure 3.1: Foliar Aluminum Concentration Taken from Mature Red Spruce Trees *vs.* Elevation. Figure represents an example of an independent relationship of foliar aluminum concentration with respect to elevation. Slope = -0.0201 ± 0.0538 (S.E.), intercept = 129.43 ± 99.25 (S.E.), DF = 6, R-squared = 0. 0227, p-value = 0.722.

Table 3.7: Summary of Statistical Analysis of Elevational Studies, When Comparing All Sites Together.

Statistical Analysis (all concentrations in µg/g)	**Aluminum**	**Calcium**	**Magnesium**
Linear Regression of Foliar Conc. *vs.* Elevation (all sites)	Independent (did not follow hypothesis)	Independent (did not follow hypothesis)	Independent (did not follow hypothesis)
Linear Regression of Soil Conc. *vs.* Elevation (all sites)	Independent (did not follow hypothesis)	Independent (did not follow hypothesis)	Independent (did not follow hypothesis)

Follow Hypothesis: Higher elevation sites were expected to exhibit **lower nutrient** (Ca & Mg) concentrations and **higher toxic metal** (Al) concentrations due to acid deposition. **Independent**: Higher elevation sites have **higher or same nutrient** (Ca & Mg) concentrations and **lower or same toxic metal** (Al) concentrations.

3.3.2 Higher Elevation Sites Compared to Lower Adjacent Elevation Sites

Instead of comparing all the sample sites together, selective individual high elevation sites were compared to adjacent low elevation sites. The significance of investigating these sample sites was to determine if acid deposition had any effect on metal foliar concentrations with elevation at a particular location. In order to compare the effects of elevation among these sample sites' foliar concentrations, a Student's *t*-test analysis was employed. The *t*-test analyses of foliar concentrations between Mt. Mitchell and Camp Alice are shown in Table C.3, the comparison between Clingman's Dome and Mingus Lead are shown in Table C.4 and the comparison between Waterrock Knob and Yellow Face are shown in Table C.5.

A summary of the Student's *t*-tests are shown in Table 3.8 and foliar calcium concentrations seemed to most closely follow the hypothesis. Seven out of nine Student's *t*-tests for foliar calcium concentrations followed the hypothesis. The majority of the Student's *t*-tests did not follow the hypothesis for either foliar aluminum or magnesium concentrations. In addition, foliar aluminum concentrations in red spruce saplings trees at Waterrock Knob and Yellow Face were statistically the same.

Table 3.8: Summary of Statistical Analysis of Elevational Studies Comparing Individual High and Adjacent Low Sample Sites.

Statistical Analysis (all concentrations in µg/g)	**Aluminum**	**Calcium**	**Magnesium**
A. *t*-test between adjacent high elevation site, Mount Mitchell, and low elevation site, Camp Alice	All Life Stages Reject Hypothesis	All Life Stages Follow Hypothesis	All Life Stages Reject Hypothesis
B. *t*-test between adjacent high elevation site Clingman's Dome, and low elevation site, Mingus Lead	All Life Stages Reject Hypothesis	Only Mature and Seedlings Follow Hypothesis	All Life Stages Reject Hypothesis
C. *t*-test between adjacent high elevation site, Waterrock Knob, and low elevation site, Yellow Face	Only Mature and Seedlings Follow Hypothesis; Saplings are Statistically the Same	Only Saplings and Seedlings Follow Hypothesis	Only Seedlings Follow Hypothesis

Follow Hypothesis: Higher elevation sites were expected to exhibit **lower nutrient** (Ca & Mg) concentrations and **higher toxic metal** (Al) concentrations due to acid deposition.
Reject Hypothesis: Higher elevation sites have **higher nutrient** (Ca & Mg) concentrations and **lower toxic metal** (Al) concentrations.
Statistically the Same: Higher elevation sites have the **same nutrient** (Ca & Mg) concentrations and the **same toxic metal** (Al) concentrations.

3.3.3 Elevational Studies of Selective High Sites Compared Together

Foliar metal concentrations at all high elevation sites were hypothesized to contain statistically identical concentrations levels of same nutrients (calcium and magnesium) and toxic metal (aluminum) levels. Analysis of variance (ANOVA) was employed to study the effects on foliar metal concentrations among all high elevation sites, at elevations above 6100 ft., and the results are shown in Table C.6. Results from the statistical analyses showed that there were differences in foliar concentrations among these sample sites.

3.3.4 Elevational Studies of Selective Low Sites Compared Together

Metal concentrations taken from the foliage of red spruce trees for all low elevation sites, at elevations below 6100 ft., were expected to exhibit the same nutrient (calcium and magnesium) and toxic metal (aluminum) levels. In order to investigate the effects of foliar metal concentrations among all low elevation samples sites, analysis of variance (ANOVA) was utilized and the results are shown in Table C.7. Results of the statistical analyses showed that there were differences in foliar concentrations among these sample sites.

3.4 Longitude Studies

Refer to maps in section 2.1 (pages 19-23) for the locations of sample sites used in the longitude studies.

3.4.1 Longitude Studies of All Sites Compared Together

The significance of the longitude studies were to determine if more westerly sample sites exhibited lower nutrients (calcium and magnesium) levels and higher toxic metal (aluminum) levels compared to more easterly sites caused by enhanced acid deposition based on distance from coal-burning power plants located in eastern Tennessee. Linear regression analysis was employed to study these effects of longitude on foliar or soil metal concentrations in red spruce trees. The statistical analyses of foliar concentrations *vs.* longitude are shown in Table C.8 and the results from soil metal concentrations *vs.* longitude are shown in Table C.9. Figure 3.2 is a representative example of the correlation of exchangeable soil magnesium concentrations in mature red spruce trees *vs.* longitude. A summary of the results from the linear regression analyses of foliar and soil metal concentrations *vs.* longitude are shown in Table 3.9. Only magnesium concentrations taken from surrounding soils of mature red spruce and red spruce seedlings trees exhibited the predicted correlation with longitude.

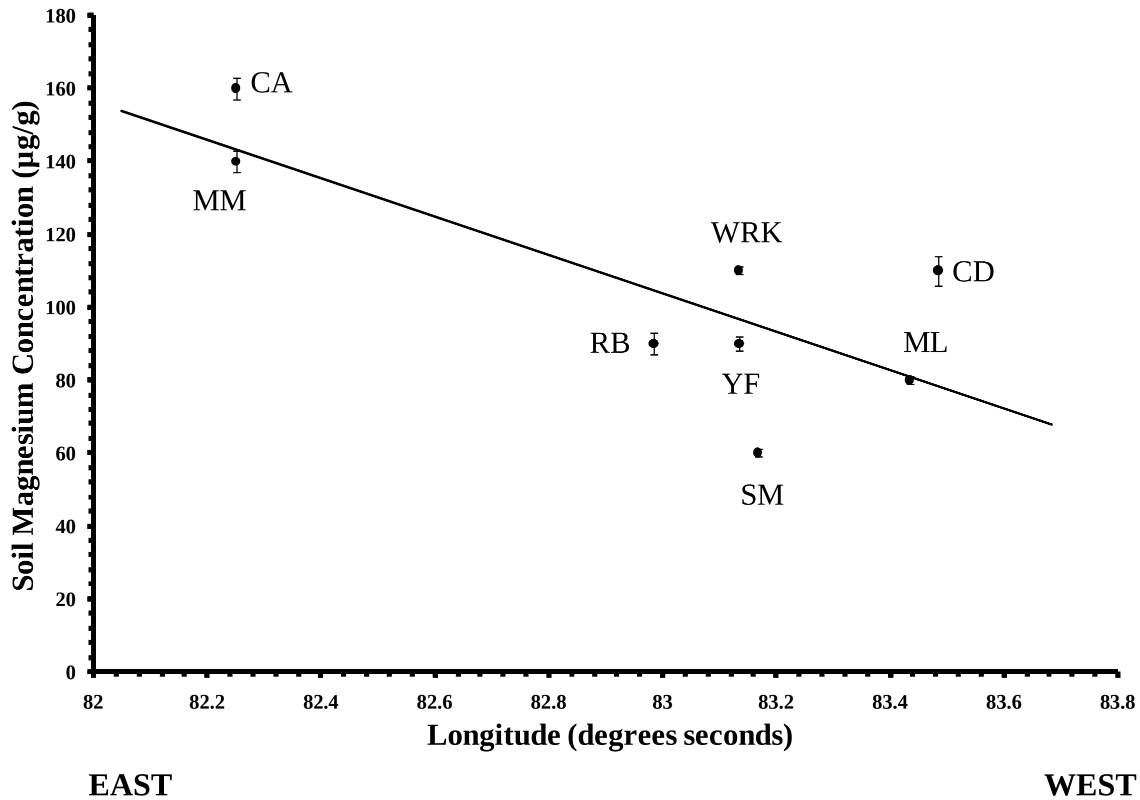

Figure 3.2: Soil Magnesium Concentration Taken From Mature Red Spruce Trees *vs.* Longitude. Slope = -51.6 ± 17.5(S.E.), intercept = 4469.34 ± 1453.16(S.E.), DF = 6, R-squared = 0.6005, p-value = 0.0239.

Table 3.9: Summary of Statistical Analysis of Longitudinal Studies, When Comparing All Sites.

Statistical Analysis (all concentrations in µg/g)	**Aluminum**	**Calcium**	**Magnesium**
Linear Regression of Foliar Conc. *vs.* Longitude (all sites)	Independent (did not follow hypothesis)	Independent (did not follow hypothesis)	Independent (did not follow hypothesis)
Linear Regression of Soil Conc. *vs.* Longitude (all sites)	Independent (did not follow hypothesis)	Independent (did not follow hypothesis)	Only Mature and Saplings Support Hypothesis

Hypothesis: Western sample sites were expected to exhibit **lower nutrient** (Ca & Mg) concentrations and **higher toxic metal** (Al) concentrations due to acid deposition.
Independent: Western elevation sites have **higher or same nutrient** (Ca & Mg) concentrations and **lower or same toxic metal** (Al) concentrations.

3.4.2 Western Sites Compared to Central Sites

Rather than comparing all sites together, western sample sites were compared to central (both high and low elevation sites) sample sites to examine the effects of longitude on foliar metal concentrations by using Student's *t*-test analysis. Central sites were expected to exhibit higher nutrient levels and lower toxic metal levels due to reduced acid deposition. Results of *t*-test analyses comparing foliar metal concentrations at high elevation sample sites' Clingman’s Dome (western) and Waterrock Knob (central) are shown in Table C.10 and the results from the Mingus Lead (western) and Yellow Face (central) low elevation sample sites comparison are shown in Table C.11. A summary of *t*-test results from the comparisons of western sample sites with central sample sites can be found in Table 3.10 and the results showed that only foliar aluminum concentration found in red spruce saplings and seedlings trees supported our hypothesis.

Table 3.10: Summary of Statistical Analysis of Longitudinal Studies, When Comparing Metal concentrations of Western Sample Sites with Central Sample Sites.

Statistical Analysis (all concentrations in µg/g)	**Aluminum**	**Calcium**	**Magnesium**
t-test between western high elevation site, Clingman's Dome, and central high elevation site, Waterrock Knob	All Life Stages Reject Hypothesis	All Life Stages Reject Hypothesis	All Life Stages Reject Hypothesis
t-test between western low elevation site, Mingus Lead, and central low elevation site, Yellow Face	Only Saplings and Seedlings Follow Hypothesis	All Life Stages Reject Hypothesis	All Life Stages Reject Hypothesis

Follow Hypothesis: Western sites were expected to exhibit **lower nutrient** (Ca & Mg) concentrations and **higher toxic metal** (Al) concentrations due to acid deposition.
Reject Hypothesis: Western sites have **higher nutrient** (Ca & Mg) concentrations and **lower toxic metal** (Al) concentrations.
Statistically the Same: Western elevation sites have the **same nutrient** (Ca & Mg) concentrations and the **same toxic metal** (Al) concentrations.

3.4.3 Central Sites Compared to Eastern Sites

Foliar metal concentrations taken from central sample sites were compared to those taken from (both high and low elevation) eastern sample sites by means of Student's *t*-test. Eastern sample sites were expected to exhibit higher nutrient levels and lower toxic metal levels due to enhanced acid deposition. For the high elevation comparison, results of *t*-test analyses (comparing foliar metal concentrations) for central sample site Waterrock Knob and eastern sample site Mt. Mitchell are shown in Table C.12. For low elevation sample site comparison, results from *t*-test analyses comparing foliar metal concentrations between Yellow Face (central) and Camp Alice (eastern) are shown Table C.13. The results of these *t*-test analyses are summarized in Table 3.11. For foliar aluminum concentrations, only mature red spruce and red spruce saplings followed the hypothesis. All Student's *t*-tests for foliar calcium concentrations in red spruce trees followed the hypothesis. Only red spruce seedlings for foliar magnesium concentrations did not follow the hypothesis (i.e., five out of six Student's *t*-tests did follow the hypothesis).

Table 3.11: Summary of Statistical Analysis of Longitude Studies, When Comparing Metal Concentrations of Central Sample Sites with Eastern Sample Sites.

Statistical Analysis (all concentrations in μg/g)	**Aluminum**	**Calcium**	**Magnesium**
t-test between Central elevation site, Waterrock Knob, and eastern high elevation site, Mount Mitchell	Only Mature and Saplings Follow Hypothesis	All Life Stages Follow Hypothesis	All Life Stages Follow Hypothesis
t-test between Central low elevation site, Yellow Face, and eastern low elevation site, Camp Alice	All Life Stages Reject hypothesis	All Life Stages Follow Hypothesis	Only Mature and Saplings Follow Hypothesis

Follow Hypothesis: Western sites were expected to exhibit **lower nutrient** (Ca & Mg) concentrations and **higher toxic metal** (Al) concentrations due to acid deposition.
Reject Hypothesis: Western sites have **higher nutrient** (Ca & Mg) concentrations and **lower toxic metal** (Al) concentrations.
Statistically the Same: Western elevation sites have the **same nutrient** (Ca & Mg) concentrations and the **same toxic metal** (Al) concentrations.

3.4.4 Eastern Sites Compared to Western Sites

Comparing eastern sample sites (both high and low elevations) with western samples sites were important comparisons because the distance between eastern and western sample sites is the greatest among all the sample sites in this study. Student's *t*-test analysis was used to investigate the effects of acid deposition on geography. The results of the foliar metal concentrations comparison between Mt. Mitchell (high elevation, eastern) and Clingman's Dome (high elevation, western) are shown Table C.14. For low elevation sample sites, results of the foliar metal concentrations comparison between Camp Alice (eastern) and Mingus Lead (western) are shown in Table C.15.

Summarized results from these statistical analyses can be found in Table 3.12. For foliar aluminum concentrations, all trees were statistically the same or did not follow the hypothesis. Two out of the six Student's *t*-tests for foliar calcium concentrations in red spruce trees followed the hypothesis. Four out of the six Student's *t*-tests for foliar magnesium concentrations in red spruce trees followed the hypothesis. In addition, foliar magnesium concentrations in red spruce saplings tress were statistically the same at Mingus Lead and Camp Alice sample sites.

Table 3.12: Summary of Statistical Analysis of Longitude Studies, When Comparing Metal Concentrations Eastern Sample Sites with Western.

Statistical Analysis (all concentrations in µg/g)	**Aluminum**	**Calcium**	**Magnesium**
t-test between western high elevation site, Clingman's Dome, and eastern high elevation site, Mount Mitchell	All Life Stages Statistically the Same	All Life Stages Reject hypothesis	All Life Stages Follow Hypothesis
t-test between western low elevation site, Mingus Lead, and eastern low elevation site, Camp Alice	All Life Stages Reject hypothesis	Only Mature and Seedlings Follow Hypothesis; Saplings are Statistically the Same	Only Mature Follow Hypothesis; Saplings are Statistically the Same

Follow Hypothesis: Western sites were expected to exhibit **lower nutrient** (Ca & Mg) concentrations and **higher toxic metal** (Al) concentrations due to acid deposition.
Reject Hypothesis: Western sites have **higher nutrient** (Ca & Mg) concentrations and **lower toxic metal** (Al) concentrations.
Statistically the Same: Western elevation sites have the **same nutrient** (Ca & Mg) concentrations and the **same toxic metal** (Al) concentrations.

3.4.5 Longitude Studies of Selected High Sites Compared Together

In this statistical analysis all of the selective high elevations sites (at elevations above 6100 ft.) used in the previous longitude studies were compared. Nutrient concentrations were hypothesized to increase and toxic metal levels were hypothesized to decrease as longitude decreased (i.e., traveling west to east) due to acidic deposition. Analysis of variance was employed to compare the effects of longitude on foliar metal concentrations among theses sample sites and the results are shown in Table C.16.

Results from these analyses showed that a statistical difference in foliar metal concentrations exists among all sample sites. However, foliar metal concentrations in all red spruce trees did not follow the hypothesis. For instance, the results from the Student's *t*-test of foliar aluminum concentrations in mature red spruce trees indicated that the foliar concentrations increased from the western sample sites to central sample sites and then the foliar concentrations decreased from the central sample sites to eastern sample sites. In another example, the results from the Student's *t*-test of foliar aluminum concentrations in red spruce seedlings trees indicated that the foliar concentrations were statistically the same for western and eastern sample sites.

3.4.6 Longitude Studies of Selected Low Sites Compared Together

All low elevation sample sites (at elevations below 6100 ft.) used in the previous longitude studies were compared by using analysis of variance. Toxic metal concentrations were expected to decrease and nutrient concentrations were expected to increase as longitude decreased (i.e., traveling west to east) due to reduced acid deposition. The results from the analysis of variance are shown in Table C.17, and indicated that the majority of the foliar metal concentrations among all low elevation sample sites were statistically different. However, foliar metal concentrations in all red spruce trees did not follow the hypothesis. For instance, the results from the Student's *t*-test of foliar magnesium concentrations in mature red spruce trees indicated that the foliar concentrations decreased from the western sample sites to central sites and then the foliar concentrations increased from the central sample sites to eastern sample sites. In another example, the result from the Student's *t*-test of foliar magnesium concentrations in red spruce saplings trees indicated that the foliar concentrations were statistically the same for western and eastern sample sites.

3.5 pH Studies

Acid deposition (i.e., nitric acid and sulfuric acid) on soils found in spruce-fir forests will increase the amount of hydrogen ions (H^+) in the soil which will subsequently influence soil pH. Linear regression analysis was used for studying the effects of soil pH on foliar or soil metal concentrations in red spruce trees. The statistical analyses of foliar metal concentrations *vs.* soil pH are shown in Table C.18 and the results from soil metal concentrations *vs.* soil pH are shown in Table C.19. Results from elevation of sample sites *vs.* soil pH are shown in Table C.20.

Only a positive correlation between foliar aluminum concentrations in red spruce saplings and seedlings *vs.* soil pH was observed from the results of the linear regression analyses (i.e., as the soil becomes more basic, the foliar aluminum concentration increased). Conversely, the results from the linear regression analyses indicated that only soil aluminum concentrations in red spruce saplings and seedlings showed a weak negative correlation with soil pH (i.e., as the soil becomes more acidic, the soil aluminum concentration increases). This was significant because acid deposition may increase the concentration of H^+ ions in the soil thereby, increasing the mobility of the toxic metal (aluminum) found in the soil. However, soil pH taken from red spruce trees at the Camp Alice sample site seemed to be reproducibly different from all the other sample sites. Soil properties were different at Camp Alice (i.e., the soil appeared lighter in color and sandy when compared to other sample sites); consequently, this may have affected the soil pH at Camp Alice.

3.6 Life Stage Studies

To determine if any statistical differences exist in foliar or soil metal concentrations among mature red spruce, red spruce saplings, and red spruce seedlings, analysis of variance was implemented. The life stages of the red spruce trees were hypothesized to exhibit the statistically same foliar or soil metal concentrations. In this analysis, a statistical model was constructed that removed the variation in each sample sties (i.e., elevation, geography, etc.); therefore, the model only analyzed the foliar or soil metal concentrations in each of the life stage of the red spruce trees. These results from the analyses of variance are shown in Table C.21. All foliar or soil metal concentrations in red spruce trees were statistically the same except for magnesium concentrations found in the foliage of mature red spruce, red spruce saplings, and red spruce seedlings trees. The majority (five out of six statistical tests) of these results suggested that the age of the red spruce tree does not influence the foliar or soil metal concentration.

3.7 Foliar Metal Concentration vs. Soil Metal Concentration Studies

As the metal concentrations found in the surrounding soils of red spruce trees increased, then the concentration of metals found in the foliage of red spruce trees were hypothesized to increase. Linear regression analysis was used to investigate the dependence of foliar metal concentrations on the soil metal concentrations and the results are shown in Table C.22. The results from the linear regression analyses showed that only foliar aluminum concentrations *vs.* soil aluminum concentrations in red spruce saplings and seedlings trees had a correlation. These correlation were negative (i.e., as the aluminum soil concentration increased, the foliar aluminum concentration decreased). This was contrary to what was hypothesized.

3.8 Red Spruce Forest Health using Soil Molar Calcium/Aluminum Ratios

Red spruce forests located in the Southern Appalachians Mountains were investigated for health effects by using a model developed by Cronan and Grigel,[26] which used soil molar calcium/aluminum ratios taken from red spruce trees at all sample sites. Cronan and Grigel[26] estimated "that there is a 50:50 risk of impacts on tree growth or nutrition when soil solution Ca/Al ratio is as low as 1.0, a 75% risk when the soil solution ratio is as low as 0.5, and nearly a 100% risk when the soil solution Ca/Al molar ratio is as low as 0.2." Mature red spruce, red spruce saplings, and red spruce seedlings soil calcium/aluminum molar ratios, and risk assessments are given in Table 3.13, Table 3.14, and Table 3.15, respectively.

According to the model developed by Cronan and Grigel,[26] all of our sample sites exhibited nearly 100% risk of adverse forests health effects due to acid deposition except for Camp Alice and Waterrock Knob. Soil samples collected at Camp Alice had an estimated 90% risk for mature red spruce trees, and an 85% risk for saplings and seedlings red spruce trees for adverse forests health effects. Waterrock Knob exhibited an estimate 50% risk for mature red spruce trees, and 90% risk for seedlings red spruce trees of adverse forests health effects.

Table 3.13: Mature Red Spruce Soil Molar Calcium/Aluminum Ratios at All Sample Sites

SITE	Mature Soil Molar Ca/Al Ratio	Adverse Impacts
Clingman's Dome	0.066 ± 0.006	Nearly 100% risk
Mingus Lead	0.151 ± 0.012	Nearly 100% risk
Mount Mitchell	0.079 ± 0.003	Nearly 100% risk
Camp Alice	0.319 ± 0.018	90% risk
Richland Balsam	0.149 ± 0.002	Nearly 100% risk
Spruce Mountain	0.084 ± 0.003	Nearly 100% risk
Waterrock Knob	1.019 ± 0.020	50% risk
Yellow Face	0.069 ± 0.002	Nearly 100% risk

Table 3.14: Red Spruce Saplings Soil Molar Calcium/Aluminum Ratios at All Sample Sites

SITE	Saplings Soil Molar Ca/Al Ratio	Adverse Impacts
Clingman's Dome	0.362 ± 0.017	90% risk
Mingus Lead	0.294 ± 0.005	Nearly 100% risk
Mount Mitchell	0.120 ± 0.005	Nearly 100% risk
Camp Alice	0.409 ± 0.041	85% risk
Richland Balsam	0.164 ± 0.007	Nearly 100% risk
Spruce Mountain	0.189 ± 0.011	Nearly 100% risk
Waterrock Knob	0.220 ± 0.007	Nearly 100% risk
Yellow Face	0.066 ± 0.005	Nearly 100% risk

Table 3.15: Red Spruce Seedlings Soil Molar Calcium/Aluminum Ratios at All Sample Sites

SITE	Seedlings Soil Molar Ca/Al Ratio	Adverse Impacts
Clingman's Dome	0.099 ± 0.004	Nearly 100% risk
Mingus Lead	0.211 ± 0.011	Nearly 100% risk
Mount Mitchell	0.084 ± 0.005	Nearly 100% risk
Camp Alice	0.423 ± 0.25	85% risk
Richland Balsam	0.173 ± 0.006	Nearly 100% risk
Spruce Mountain	0.065 ± 0.001	Nearly 100% risk
Waterrock Knob	0.366 ± 0.010	90% risk
Yellow Face	0.104 ± 0.004	Nearly 100% risk

3.9 Comparison of Results with Previous Studies

3.9.1 Comparison of Foliar Calcium/Aluminum Ratios

Bintz et al. proposed a model in which red spruce saplings were sampled at Clingman's Dome, North Carolina/Tennessee to compare foliar calcium/aluminum ratios from 1988 and 2005.[2] A comparison with this model involving foliar calcium/aluminum ratios taken at Clingman's Dome, North Carolina/Tennessee between McLaughlin et al.[28], Bintz et al.[2], and this study was performed to determine the risk of adverse forest health effects on red spruce trees and the results are shown in Table 3.16. Foliar calcium/aluminum ratios were examined because exchangeable soil molar calcium/aluminum ratios were not available from either McLaughlin or Bintz. No statistical analysis was performed on the data because McLaughlin did not report standard deviation. The foliar calcium/aluminum ratios suggested a possible improvement in the health of the red spruce forest at Clingman's Dome, NC/TN in 2009 compared to 1988 and 2005.

Table 3.16: Comparison of Foliar Calcium/Aluminum Ratios Between McLaughlin (1988), Bintz (2005), and Rosenberg (2009) at Clingman's Dome, NC/TN

Molar Foliar Saplings Ca:Al Ratio	McLaughlin (1988)	Bintz (2005)	Rosenberg (2009)
Clingman's Dome	20 ± NR*	30 ± 15	47 ± 6

NR* = No Standard Deviation Reported.

3.9.2 Comparison at Richland Balsam, North Carolina

A comparison of calcium and magnesium concentrations found in red spruce saplings taken at Richland Balsam, North Carolina by Weaver et al.[23], Shepard et al.[1], Bintz et al.[2] and this study are shown in Table 3.17. Aluminum concentrations found in red spruce saplings were not reported by Weaver or Shepard; therefore, no statistical analysis was performed on foliar aluminum concentrations. The importance in comparing this project results with previous studies was to investigate any trends in the foliar nutrients (calcium and magnesium) concentrations over time at Richland Balsam, NC.

Analysis of variance was used to determine if any statistical differences existed in the concentrations of magnesium and calcium in the foliage of red spruce saplings among researchers, and the results showed that a significant difference exist among foliar calcium and magnesium concentrations. Student's *t*-test analysis was utilized to determine if any statistical differences existed in the concentrations of foliar magnesium and calcium of red spruce saplings trees between Bintz (2005) and this study (2009). This *t*-test analysis was preformed because samples were taken within four years from each other and the results showed no statistical differences existed between nutrient concentrations, shown in Table 3.17.

Calcium and magnesium concentrations found in the foliage of red spruce saplings at Richland Balsam, NC decreased from 1969 to 1994. Since 1994, the nutrient levels have increased to an intermediate level when compared to 1969. These trends could be explained by the Clean Air Act of 1990, in which legislation was imposed to reduce the emissions of greenhouse gases. A graph of foliar calcium and magnesium concentrations found in red spruce saplings at Richland Balsam as a function of time, is shown in Figure 3.3 and Figure 3.4, respectively.

Table 3.17: Comparison of Red Spruce Saplings Foliar Calcium and Magnesium Concentration between Weaver (1969), Shepard (1994), Bintz (2005), and Rosenberg (2009) at Richland Balsam, NC

Researcher	Foliar Ca Conc. (µg/g)	Foliar Mg Conc. (µg/g)
Weaver (1969) n = 14 Ca, n = 12 Mg	4164 ± 388^{a}	788 ± 62^{a}
Shepard (1994) n = 10	1932 ± 225^{b}	330 ± 22^{b}
Bintz (2005) n = 10	2690 ± 300^{c}	584 ± 36^{c}
Rosenberg (2009) n =10	2320 ± 16^{c}	570 ± 1^{c}
Statistical Analysis- ANOVA for all Researchers (α = 0.05)	p-value = 2.13×10^{-18}	p-value = 8.76×10^{-24}
Decision	Different	Different
Statistical Analysis- *t*-test between Bintz and Rosenberg (α = 0.05)	p-value = 0.407	p-value = 0.827

*Statistical differences are indicated by superscripts with different letters.

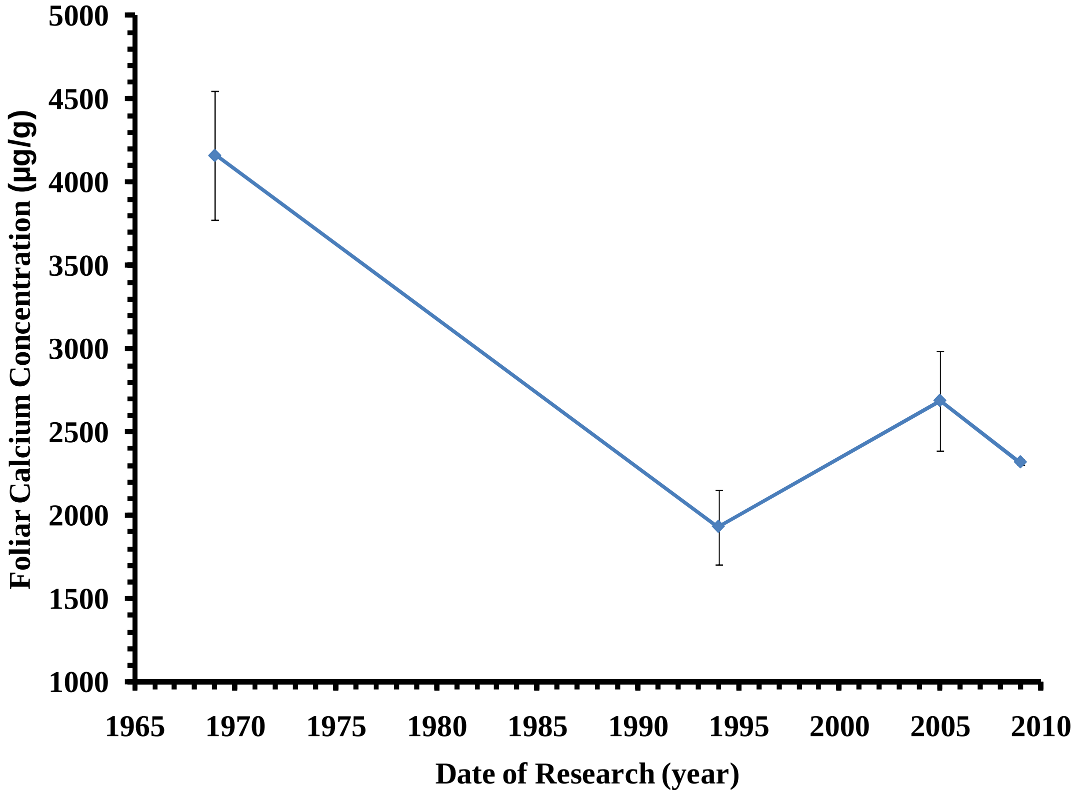

Figure 3.3: Red Spruce Saplings Foliar Calcium Concentrations as a Function of Time at Richland Balsam, North Carolina.

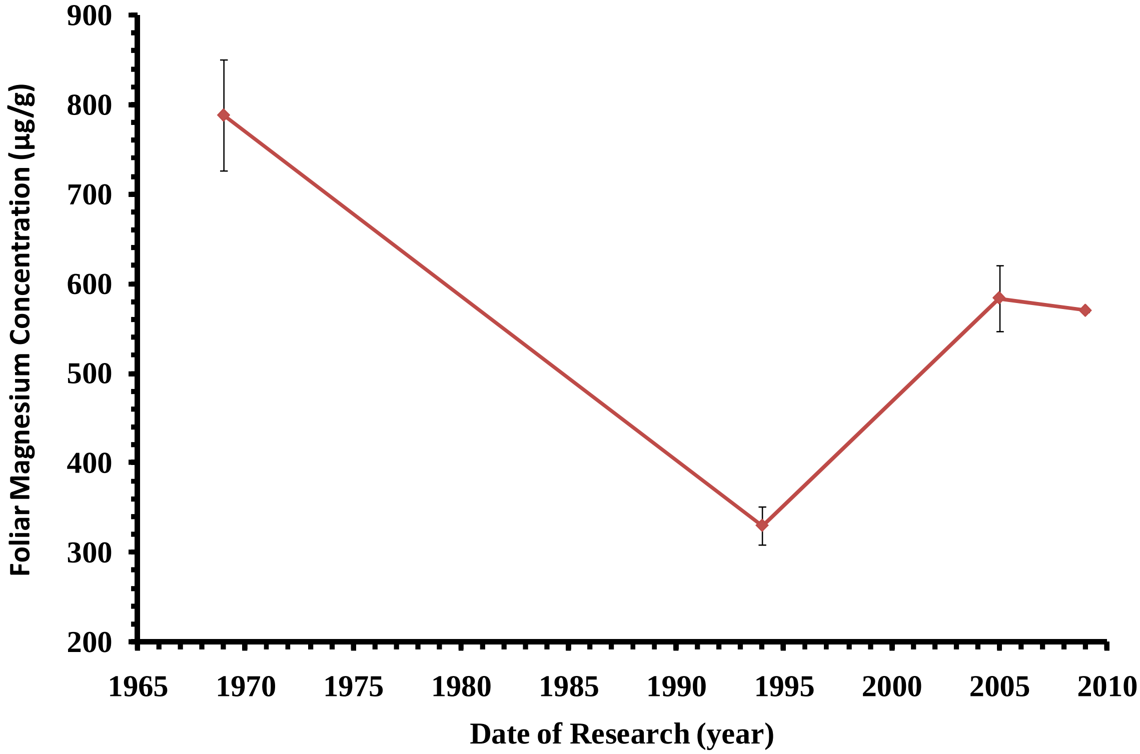

Figure 3.4: Red Spruce Saplings Foliar Magnesium Concentrations as a Function of Time at Richland Balsam, North Carolina.

4. Conclusion

Nutrient (calcium and magnesium) and toxic metal (aluminum) concentrations in foliage and surrounding soils from red spruce trees located in the Southern Appalachian Mountains were measured. In addition, the pH of the soil was measured. Statistical (Student's *t* – test, analysis of variance, and linear regression analysis) analyses were used to compare the metal concentrations in various populations. These results were used to assess the effects of acid deposition upon red spruce forests.

High elevation sample sites are subjected to higher amounts of acid deposition due to acidic fog and/or clouds. For elevational studies, results from the linear regression analyses showed no correlation in soil or foliar metal concentrations with elevation. The majority of the Student's t-test analyses comparing individual selected high elevation sample sites with adjacent low elevation sample sites did not follow the hypothesis that higher elevation sample sites exhibited lower nutrients (calcium and magnesium) levels and higher toxic metal (aluminum) levels due to enhanced acid deposition.

Due to the presence of coal-burning power plants located in eastern Tennessee, western sample sites were examined for enhanced acid deposition effects when compared to eastern sample sites. Nutrient concentrations (calcium and magnesium) were hypothesized to exhibit a positive correlation with longitude (i.e., traveling west to east) and toxic metal concentrations were expected to exhibit a negative correlation with longitude due to enhanced acid deposition; however, results from the linear regression analyses did not support this hypothesis. Selective individual western sample sites were compared to eastern sample sites and the data yielded inconsistent results to suggest that western sample sites exhibited lower nutrients (calcium and magnesium) levels and higher toxic metal (aluminum) levels due to acid deposition.

There was insufficient evidence to suggest soil pH influences the nutrients (calcium and magnesium) or toxic metal (aluminum) concentrations found in foliage or surrounding soils of red spruce forests. No overall conclusion can be made that indicated foliar or soil metal concentrations in mature red spruce, red spruce saplings, and red spruce seedlings trees were significantly different. There was inconsistent evidence to suggest that the nutrient or toxic metal concentrations found in the surrounding soils of red spruce trees influenced the metal concentrations found in the foliage of red spruce trees.

In order to monitor the health of red spruce forests located in the Southern Appalachian Mountains, a model developed by Cronan and Grigel,[26] involving soil molar calcium/aluminum ratios and comparisons of the results obtained in this work with data collected in previous studies were used. Soil calcium/aluminum molar ratios taken from mature red spruce, red spruce saplings, and red spruce seedlings trees suggested that almost all sample sites are at a high risk of adverse forests health effects. Foliar calcium/aluminum ratios taken from red spruce saplings trees at Clingman's Dome, North Carolina/Tennessee showed a possible improvement in the forest health when compared to previous studies since 1980's. A comparison, that spanned over 40 years, with previous studies at Richland Balsam, North Carolina of foliar calcium and magnesium concentrations taken from red spruce saplings trees suggested a possible improvement in red spruce forests health since 1994, but foliar concentrations are not at the same level as in 1969.

In future studies, it is proposed that only red spruce saplings would be collected at each sample site because: (1) previous studies only used red spruce saplings, (2) if future researchers only sampled red spruce saplings, then the amount of samples collected at each site will be reduced. In addition, this will allow for more samples sites to be investigated in the Southern Appalachian Mountains, which could provide more information/data on the effects of acid deposition on red spruce forests.

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Appendix A: Maps and Approximate Sampling Area for All Sites

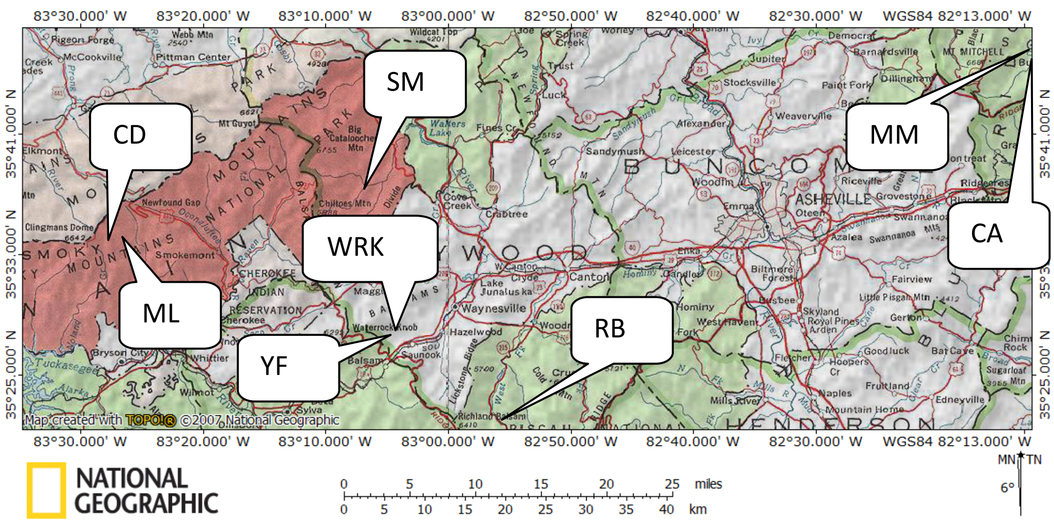

Figure A.1: Map of All Sample Sites

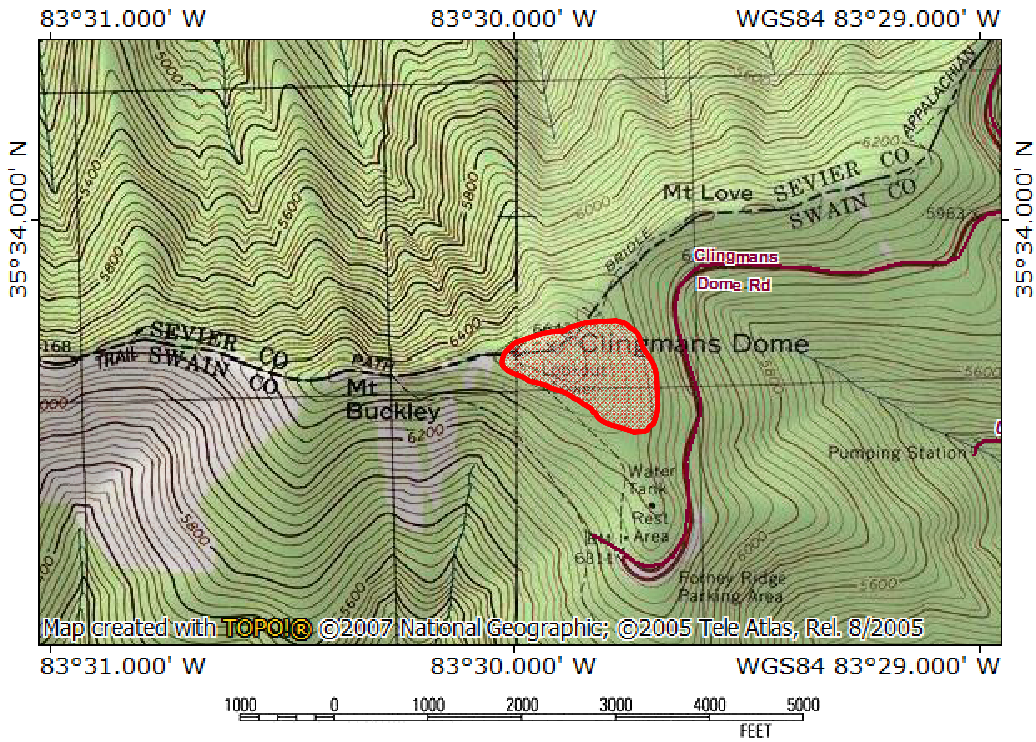

Figure A.2: Map of Sampling Area at Clingman’s Dome

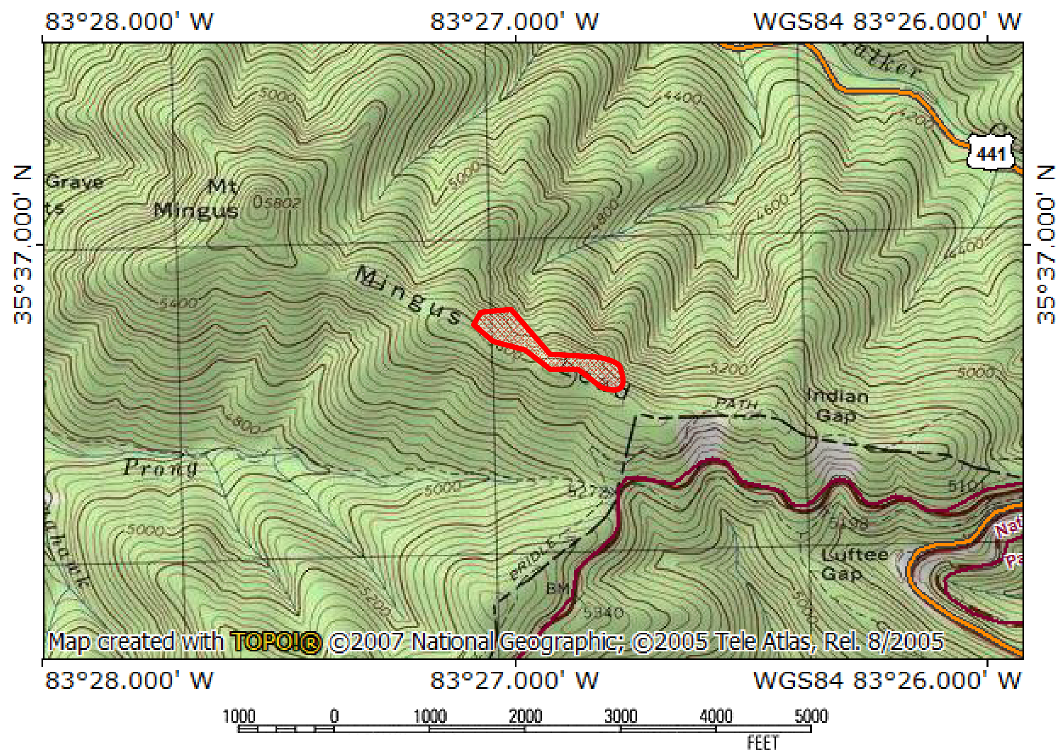

Figure A.3: Map of Sampling Area at Mingus Lead

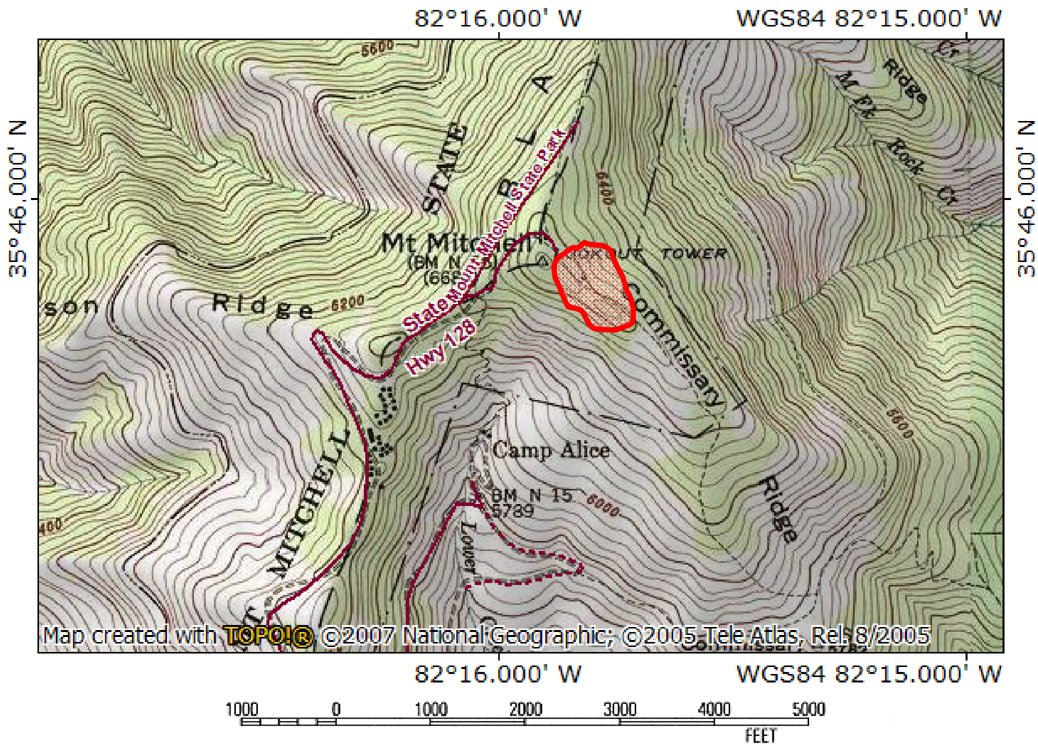

Figure A.4: Map of Sampling Area at Mount Mitchell

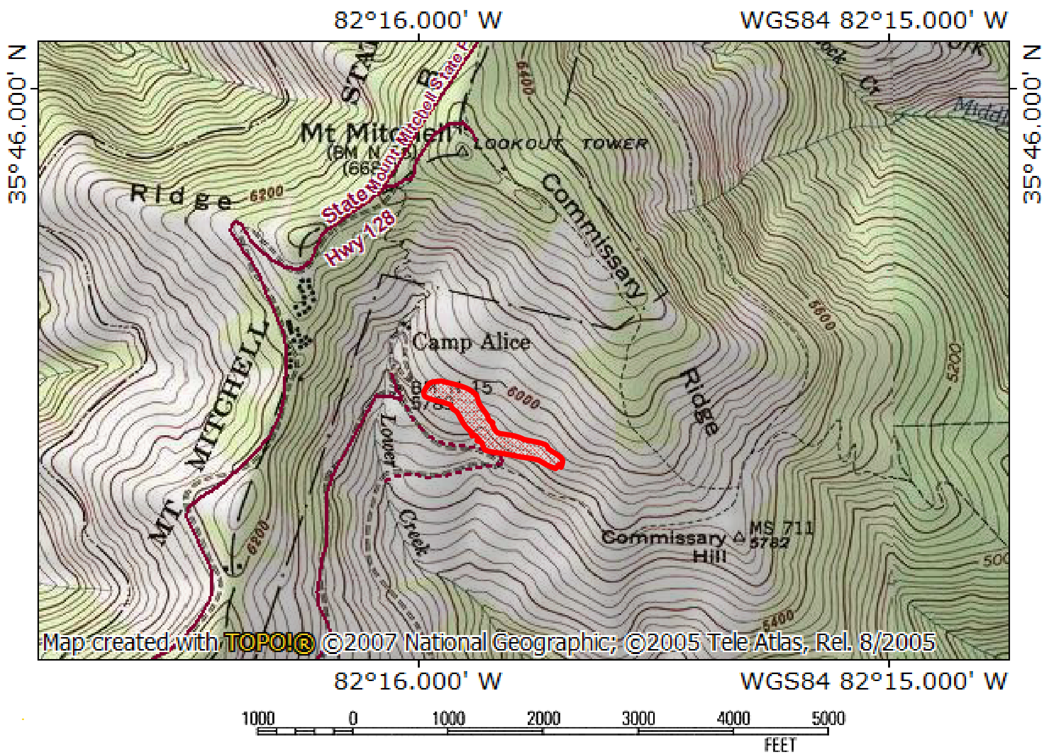

Figure A.5: Map of Sampling Area at Camp Alice

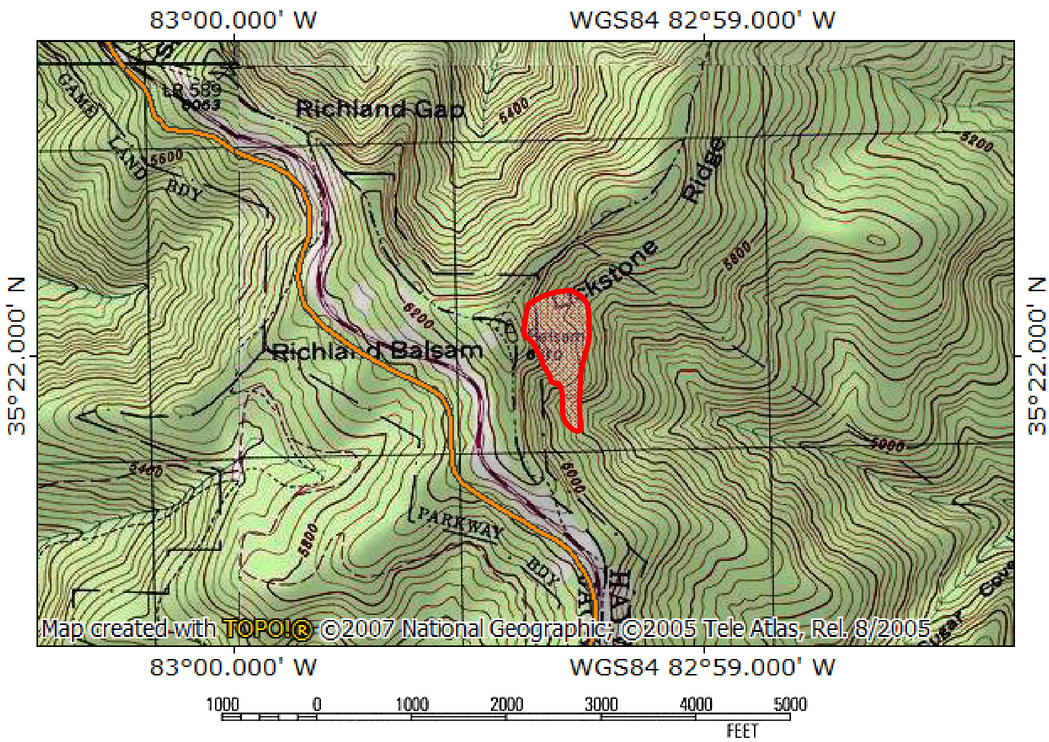

Figure A.6: Map of Sampling Area at Richland Balsam

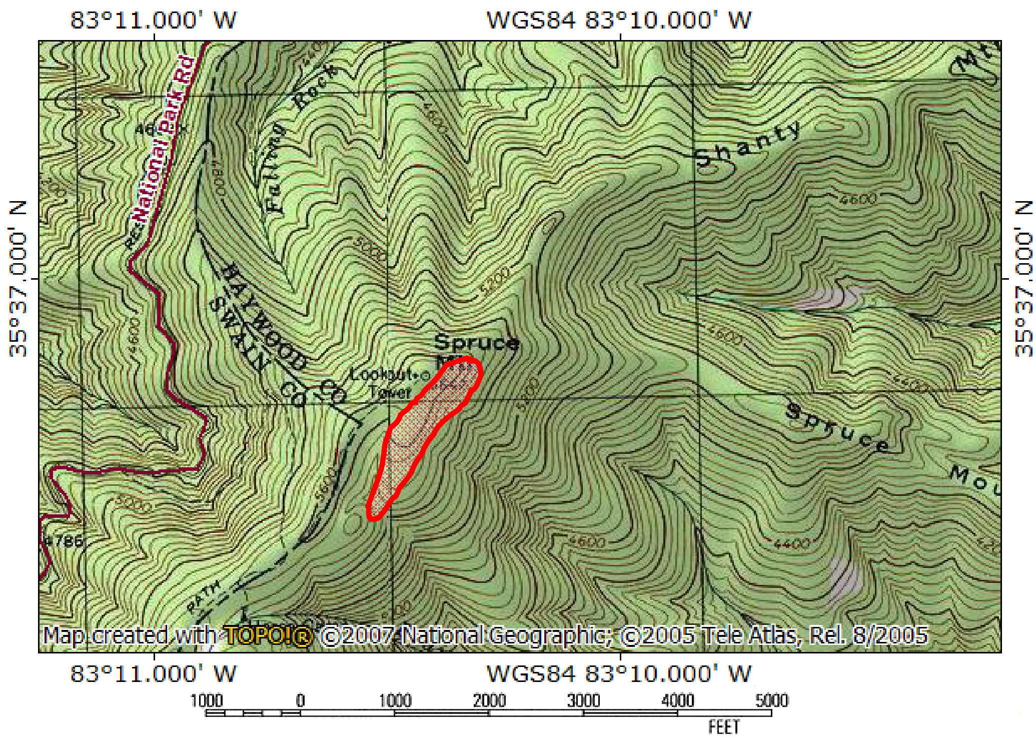

Figure A.7: Map of Sampling Area at Spruce Mountain

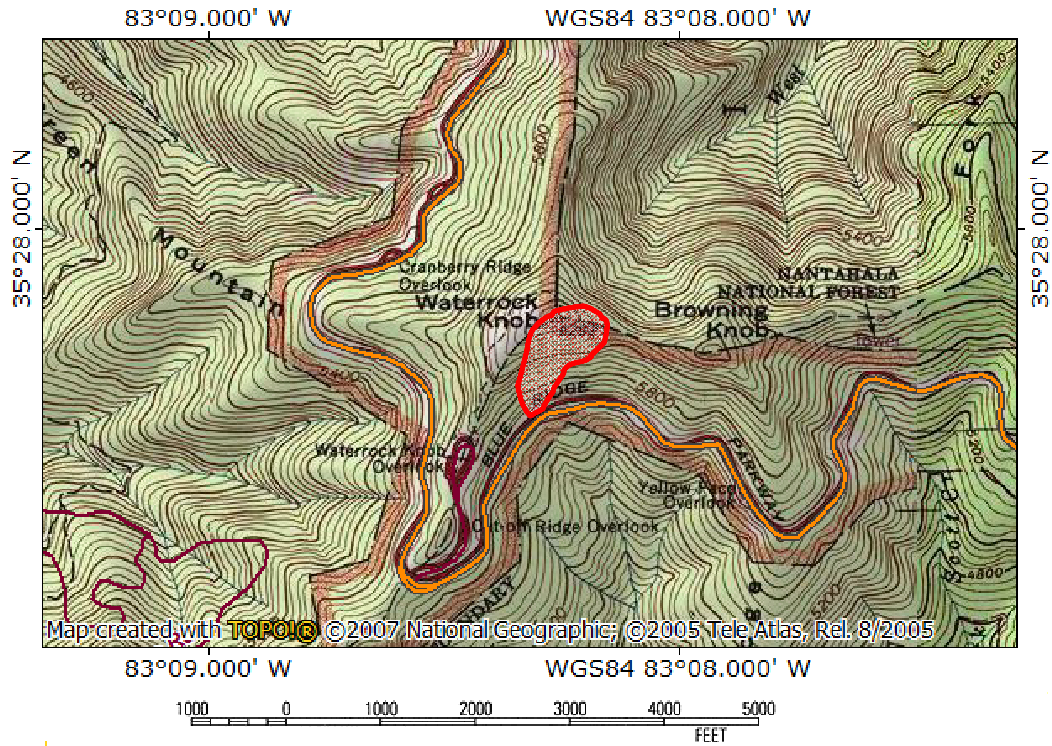

Figure A.8: Map of Sampling Area at Waterrock Knob

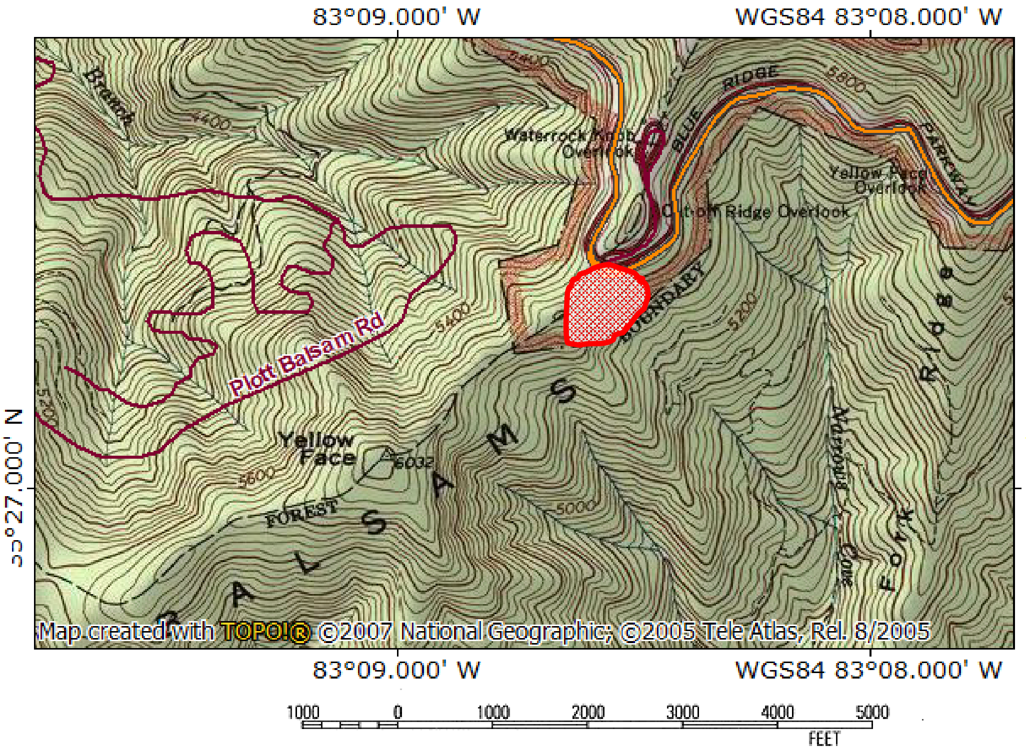

Figure A.9: Map of Sampling Area at Yellow Face

Appendix B: Data Tables

Table B.1: Coordinates and Elevations Taken at Clingman's Dome for Mature Red Spruce

CD Mature Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.55021	83.48358	6658
2	35.55020	83.48357	6627
3	35.55020	83.48357	6622
4	35.55023	83.48357	6592
5	35.55023	83.48357	6601
6	35.55018	83.48356	6530
7	35.55019	83.48358	6601
8	35.55020	83.48358	6602
9	35.55020	83.48358	6612
10	35.55020	83.48358	6625
Average	35.55020	83.48360	6600
SD			30

Table B.2: Coordinates and Elevations Taken at Clingman's Dome for Red Spruce Saplings

CD Sapling Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.55025	83.48358	6649
2	35.55025	83.48358	6666
3	35.55020	83.48357	6636
4	35.55020	83.48357	6639
5	35.55020	83.48357	6639
6	35.55023	83.48357	6593
7	35.55018	83.48356	6512
8	35.55018	83.48356	6534
9	35.55019	83.48358	6572
10	35.55019	83.48358	6579
Average	35.55021	83.48360	6600
SD			50

Table B.3: Coordinates and Elevations Taken at Clingman's Dome for Red Spruce Seedlings

CD Seedlings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.55023	83.48357	6628
2	35.5502	83.48357	6628
3	35.5502	83.48357	6632
4	35.5502	83.48357	6595
5	35.5502	83.48358	6617
6	35.5502	83.48358	6620
7	35.5502	83.48358	6639
8	35.5502	83.50001	6590
9	35.5502	83.50003	6566
10	35.5502	83.50006	6552
Average	35.55021	83.48850	6600
SD			30

Table B.4: Coordinates and Elevations Taken at Mingus Lead for Mature Red Spruce

ML Mature Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.60021	83.43356	5596
2	35.60020	83.43355	5595
3	35.60020	83.43355	5597
4	35.60020	83.43355	5618
5	35.60020	83.43354	5608
6	35.60020	83.43354	5598
7	35.60020	83.43354	5600
8	35.60020	83.43354	5584
9	35.60020	83.43353	5561
10	35.60019	83.43353	5671
Average	35.60020	83.43350	5600
SD			30

Table B.5: Coordinates and Elevations Taken at Mingus Lead for Red Spruce Saplings

ML Sapling Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.60021	83.43356	5589
2	35.60021	83.43356	5606
3	35.60021	83.43356	5606
4	35.60020	83.43356	5589
5	35.60020	83.43354	5603
6	35.60020	83.43354	5599
7	35.60020	83.43354	5604
8	35.60020	83.43354	5602
9	35.60019	83.43353	5580
10	35.60019	83.43352	5558
Average	35.60020	83.43350	5600
SD			20

Table B.6: Coordinates and Elevations Taken at Mingus Lead for Red Spruce Seedlings

ML Seedlings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.60021	83.43357	5599
2	35.6002	83.43356	5604
3	35.6002	83.43356	5613
4	35.6002	83.43356	5602
5	35.6002	83.43356	5595
6	35.6002	83.43355	5598
7	35.6002	83.43354	5599
8	35.6002	83.43354	5599
9	35.6002	83.43352	5543
10	35.6002	83.43352	5543
Average	35.60020	83.43350	5600
SD			30

Table B.7: Coordinates and Elevations Taken at Mount Mitchell for Mature Red Spruce

MM Mature Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.75024	82.25023	6631
2	35.75023	82.25023	6642
3	35.75023	82.25022	6610
4	35.75023	82.25022	6608
5	35.75022	82.25021	6612
6	35.75022	82.25021	6606
7	35.75022	82.25019	6590
8	35.75022	82.25019	6579
9	35.75023	82.25019	6508
10	35.75023	82.25019	6559
Average	35.75023	82.25020	6600
SD			40

Table B.8: Coordinates and Elevations Taken at Mount Mitchell for Red Spruce Saplings

MM Sapling Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.75024	82.25024	6646
2	35.75024	82.25024	6639
3	35.75023	82.25023	6648
4	35.75022	82.25019	6582
5	35.75022	82.25019	6590
6	35.75022	82.25019	6588
7	35.75023	82.25020	6579
8	35.75023	82.25020	6534
9	35.75026	82.25023	6596
10	35.75024	82.25021	6544
Average	35.75023	82.25020	6600
SD			40

Table B.9: Coordinates and Elevations Taken at Mount Mitchell for Red Spruce Seedlings

MM Seedlings Sample #	MM (°N)	MM (°W)	MM (ft.)
1	35.75024	82.25024	6647
2	35.7502	82.25024	6646
3	35.7502	82.25024	6639
4	35.7502	82.25023	6643
5	35.7502	82.25019	6592
6	35.7502	82.25019	6586
7	35.7502	82.25019	6581
8	35.7502	82.25019	6587
9	35.7502	82.25019	6590
10	35.7502	82.25019	6570
Average	35.75023	82.25020	6600
SD			30

Table B.10: Coordinates and Elevations Taken at Camp Alice for Mature Red Spruce

CA Mature Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.75011	82.25025	5751
2	35.75010	82.25025	5748
3	35.75011	82.25026	5739
4	35.75011	82.25028	5760
5	35.75011	82.25028	5767
6	35.75012	82.26667	5784
7	35.75012	82.26667	5787
8	35.75012	82.26667	5785
9	35.75012	82.26667	5781
10	35.75013	82.26669	5804
Average	35.75012	82.25850	5770
SD			20

Table B.11: Coordinates and Elevations Taken at Camp Alice for Red Spruce Saplings

CA Saplings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.75010	82.25024	5721
2	35.75010	82.25024	5724
3	35.75010	82.25023	5731
4	35.75010	82.25024	5727
5	35.75010	82.25025	5740
6	35.75010	82.25026	5751
7	35.75010	82.25025	5749
8	35.75010	82.25025	5751
9	35.75010	82.25026	5751
10	35.75011	82.25026	5739
Average	35.75010	82.25020	5740
SD			10

Table B.12: Coordinates and Elevations Taken at Camp Alice for Red Spruce Seedlings

CA Seedlings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.75010	82.25025	5748
2	35.7501	82.25026	5748
3	35.7501	82.25026	5747
4	35.7501	82.25026	5738
5	35.7501	82.25026	5739
6	35.7501	82.26667	5768
7	35.7501	82.26667	5774
8	35.7501	82.26667	5776
9	35.7501	82.26667	5785
10	35.7501	82.26667	5782
Average	35.75011	82.25850	5760
SD			20

Table B.13: Coordinates and Elevations Taken at Spruce Mountain for Mature Red Spruce

SM Mature Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.60023	83.16678	5671
2	35.60023	83.16678	5666
3	35.60023	83.16678	5666
4	35.60023	83.16679	5665
5	35.60022	83.16680	5630
6	35.60021	83.16680	5630
7	35.60022	83.16681	5631
8	35.60021	83.16681	5634
9	35.60021	83.16682	5643
10	35.60021	83.16682	5628
Average	35.60022	83.16680	5650
SD			20

Table B.14: Coordinates and Elevations Taken at Spruce Mountain for Red Spruce Saplings

SM Saplings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.60023	83.16678	5658
2	35.60022	83.16680	5634
3	35.60021	83.16681	5629
4	35.60021	83.16682	5630
5	35.60020	83.16682	5621
6	35.60020	83.16682	5619
7	35.60020	83.16682	5615
8	35.60019	83.16683	5607
9	35.60019	83.16683	5607
10	35.60016	83.16684	5591
Average	35.60020	83.16680	5620
SD			20

Table B.15: Coordinates and Elevations Taken at Spruce Mountain for Red Spruce Seedlings

SM Seedlings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.60023	83.16679	5633
2	35.6002	83.16679	5659
3	35.6002	83.16681	5627
4	35.6002	83.16681	5632
5	35.6002	83.16682	5631
6	35.6002	83.16682	5628
7	35.6002	83.16682	5625
8	35.6002	83.16682	5625
9	35.6002	83.16684	5593
10	35.6002	83.16684	5588
Average	35.60021	83.16680	5620
SD			20

Table B.16: Coordinates and Elevations Taken at Richland Balsam for Mature Red Spruce

RB Mature Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.35022	82.98343	6184
2	35.35022	82.98343	6214
3	35.35022	82.98343	6214
4	35.35022	82.98344	6252
5	35.35022	82.98344	6270
6	35.36667	82.98344	6397
7	35.35018	82.98340	6071
8	35.35018	82.98339	6073
9	35.35018	82.98339	6073
10	35.35018	82.98339	6068
Average	35.35185	82.98340	6060
SD			110

Table B.17: Coordinates and Elevations Taken at Richland Balsam for Red Spruce Saplings

RB Sapling Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.35021	82.98342	6094
2	35.35022	82.98343	6184
3	35.35023	82.98344	6275
4	35.35023	82.98344	6279
5	35.35022	82.98344	6298
6	35.36668	82.98345	6407
7	35.36668	82.98345	6408
8	35.36667	82.98345	6410
9	35.35018	82.98339	6055
10	35.35018	82.98339	6063
Average	35.35515	82.98340	6250
SD			140

Table B.18: Coordinates and Elevations Taken at Richland Balsam for Red Spruce Seedlings

RB Seedlings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.35022	82.98343	6208
2	35.3502	82.98343	6214
3	35.3502	82.98344	6284
4	35.3502	82.98345	6310
5	35.3502	82.98344	6311
6	35.3667	82.98345	6404
7	35.3502	82.98339	6063
8	35.3502	82.98339	6054
9	35.3502	82.98339	6058
10	35.3502	82.98339	6059
Average	35.35186	82.98340	6200
SD			130

Table B.19: Coordinates and Elevations Taken at Waterrock Knob for Mature Red Spruce

WRK Mature Sample #	**Latitude (°N)**	**Longitude (°W)**	**Elevation (ft.)**
1	35.45023	83.13341	6287
2	35.45023	83.13341	6287
3	35.45023	83.13341	6293
4	35.45023	83.13341	6270
5	35.45023	83.13341	6266
6	35.45023	83.13341	6268
7	35.45023	83.13341	6262
8	35.45023	83.13341	6271
9	35.45024	83.13341	6220
10	35.45024	83.13341	6235
Average	35.45024	83.13340	6260
SD			20

Table B.20: Coordinates and Elevations Taken at Waterrock Knob for Red Spruce Saplings

WRK Saplings Sample #	**Latitude (°N)**	**Longitude (°W)**	**Elevation (ft.)**
1	35.45024	83.13342	6206
2	35.45024	83.13342	6204
3	35.45024	83.13342	6198
4	35.45022	83.13345	6029
5	35.45023	83.13345	6017
6	35.45025	83.13343	6102
7	35.45025	83.13342	6113
8	35.45024	83.13343	6140
9	35.45018	83.13346	5906
10	35.45018	83.13346	5904
Average	35.45023	83.13340	6080
SD			120

Table B.21: Coordinates and Elevations Taken at Waterrock Knob for Red Spruce Seedlings

WRK Seedlings Sample #	Latitude (N)	Longitude (°W)	Elevation (ft.)
1	35.45024	83.13341	6235
2	35.4502	83.13341	6244
3	35.4502	83.13341	6244
4	35.4502	83.13341	6243
5	35.4502	83.13345	6004
6	35.4502	83.13346	5908
7	35.4502	83.13346	5900
8	35.4502	83.13346	5905
9	35.4502	83.13346	5904
10	35.4502	83.13347	5897
Average	35.45021	83.1334	6050
SD			170

Table B.22: Coordinates and Elevations Taken at Yellow Face for Mature Red Spruce

YF Mature Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.45006	83.13356	5741
2	35.45006	83.13356	5738
3	35.45007	83.13354	5722
4	35.45007	83.13354	5752
5	35.45007	83.13357	5825
6	35.45009	83.13351	5830
7	35.45009	83.13351	5821
8	35.45009	83.13351	5803
9	35.45009	83.13351	5797
10	35.45009	83.13350	5787
Average	35.45008	83.13350	5780
SD			40

Table B.23: Coordinates and Elevations Taken at Yellow Face for Red Spruce Saplings

YF Saplings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.45006	83.13356	5744
2	35.45007	83.13355	5718
3	35.45008	83.13351	5831
4	35.45009	83.13351	5786
5	35.45009	83.13351	5787
6	35.45009	83.13350	5782
7	35.45010	83.13350	5786
8	35.45010	83.13350	5731
9	35.45010	83.13350	5731
10	35.45010	83.13350	5735
Average	35.45009	83.13350	5760
SD			40

Table B.24: Coordinates and Elevations Taken at Yellow Face for Red Spruce Seedlings

YF Seedlings Sample #	Latitude (°N)	Longitude (°W)	Elevation (ft.)
1	35.45008	83.13352	5818
2	35.4501	83.13351	5830
3	35.4501	83.13351	5829
4	35.4501	83.13351	5822
5	35.4501	83.13351	5793
6	35.4501	83.13351	5794
7	35.4501	83.13351	5793
8	35.4501	83.13351	5794
9	35.4501	83.13351	5783
10	35.4501	83.13350	5781
Average	35.45009	83.13350	5800
SD			20

Table B.25: Soil pH of Mature Red Spruce Taken from All Sample Sites

Mature Sample #	CD (pH)	ML (pH)	MM (pH)	CA (pH)	RB (pH)	SM (pH)	WRK (pH)	YF (pH)
1	4.0	3.6	3.7	5.1	3.7	3.9	3.9	3.7
2	3.9	3.6	3.7	5.1	3.8	3.8	3.9	3.7
3	3.9	3.6	3.8	5.1	3.7	3.9	3.9	3.7
Average	3.9	3.6	3.7	5.1	3.7	3.8	3.9	3.7
SD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table B.26: Soil pH of Red Spruce Saplings Taken from All Sample Sites

Saplings Sample #	CD (pH)	ML (pH)	MM (pH)	CA (pH)	RB (pH)	SM (pH)	WRK (pH)	YF (pH)
1	4.0	3.7	3.7	5.5	3.9	3.8	3.8	3.8
2	4.0	3.6	3.7	5.4	3.9	3.8	3.8	3.8
3	4.0	3.7	3.8	5.4	3.9	3.9	3.8	3.8
Average	4.0	3.6	3.7	5.4	3.9	3.8	3.8	3.8
SD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table B.27: Soil pH of Red Spruce Seedlings Taken from All Sample Sites

Seedlings Sample #	CD (pH)	ML (pH)	MM (pH)	CA (pH)	RB (pH)	SM (pH)	WRK (pH)	YF (pH)
1	3.8	3.9	3.8	5.5	3.9	3.9	3.6	3.7
2	3.8	3.8	3.8	5.4	3.9	3.9	3.6	3.7
3	3.8	3.9	3.8	5.4	3.9	3.9	3.6	3.7
Average	3.8	3.9	3.8	5.4	3.9	3.9	3.6	3.7
SD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table B.28: Mature Red Spruce Foliar Aluminum, Calcium, and Magnesium Concentrations for All Sample Sites

Site	**CD**	**ML**	**MM**	**CA**	**RB**	**SM**	**WRK**	**YF**
Mature Foliar Al Conc. (µg/g)	83	93	91	104	71	106	118	82
SD	3	4	10	6	8	10	5	3
RSD	4	5	10	5	10	10	4	4
SE	1	1	3	2	3	3	2	1
Mature Foliar Ca Conc. (µg/g)	2870	3520	2720	3930	2330	3030	2470	2380
SD	20	70	30	40	20	20	30	9
RSD	1	2	1	1	1	1	1	1
SE	6	20	9	13	6	6	9	3
Mature Foliar Mg Conc. (µg/g)	566	543	676	631	321	562	469	437
SD	4	10	3	5	10	4	4	6
RSD	1	2	1	1	3	1	1	1
SE	1	3	1	2	3	1	1	2

Table B.29: Red Spruce Sapling Foliar Aluminum, Calcium, and Magnesium Concentrations for All Sample Sites

Site	**CD**	**ML**	**MM**	**CA**	**RB**	**SM**	**WRK**	**YF**
Saplings Foliar Al Conc. (µg/g)	65	91	73	163	70	90	82	75
SD	9	7	4	5	4	8	6	4
RSD	10	8	5	3	5	9	7	6
SE	3	2	1	2	1	3	2	1
Saplings Foliar Ca Conc. (µg/g)	3310	3140	2520	3130	2320	3230	2070	2890
SD	40	40	50	70	50	10	20	50
RSD	1	1	2	2	2	1	1	2
SE	10	10	20	20	20	3	6	20
Saplings Foliar Mg Conc. (µg/g)	625	611	730	613	570	450	588	490
SD	5	7	4	10	4	2	6	6
RSD	1	1	1	2	1	1	1	1
SE	2	2	1	3	1	1	2	2

Table B.30: Red Spruce Seedling Foliar Aluminum, Calcium, and Magnesium Concentrations for All Sample Sites

Site	CD	ML	MM	CA	RB	SM	WRK	YF
Seedlings Foliar Al Conc. (µg/g)	79	91	74	155	71	74	62	55
SD	6	5	5	3	4	5	3	4
RSD	8	5	6	2	6	7	5	7
SE	20	20	9	3	2	5	20	20
Seedlings Foliar Ca Conc. (µg/g)	3090	4030	2710	4410	2410	2780	2040	3490
SD	70	50	30	10	7	17	50	50
RSD	2	1	1	1	1	1	3	1
SE	20	20	9	3	2	5	20	20
Seedlings Foliar Mg Conc. (µg/g)	760	701	814	617	593	510	472	716
SD	6	10	6	4	8	6	6	8
RSD	1	1	1	1	1	1	1	1
SE	2	3	2	1	3	2	2	3

Table B.31: Surrounding Soil Aluminum, Calcium, and Magnesium Concentrations for Mature Red Spruce for All Sample Sites

Site	CD	ML	MM	CA	RB	SM	WRK	YF
Mature Soil Al Conc. (μg/g)	1030	761	1620	360	770	880	550	1270
SD	8	33	32	16	8	15	15	20
RSD	1	4	2	4	1	2	3	2
SE	3	10	10	5	3	5	5	6
Mature Soil Ca Conc. (μg/g)	100	174	190	168	174	107	827	126
SD	9	13	7	9	2	4	16	4
RSD	9	7	4	5	1	3	2	3
SE	3	4	2	3	1	1	5	1
Mature Soil Mg Conc. (μg/g)	79	87	133	152	82	79	133	108
SD	2	6	2	3	1	1	4	1
RSD	3	7	1	2	1	1	3	1
SE	1	2	1	1	1	1	1	1

Table B.32: Surrounding Soil Aluminum, Calcium, and Magnesium Concentrations for Red Spruce Saplings for All Sample Sites

Site	CD	ML	MM	CA	RB	SM	WRK	YF
Saplings Soil Al Conc. (µg/g)	1050	680	1420	220	790	900	1090	1220
SD	40	6	40	20	20	10	20	20
RSD	4	1	3	8	3	1	2	2
SE	3	1	2	1	1	3	2	2
Saplings Soil Ca Conc. (µg/g)	375	195	171	86	125	167	236	76
SD	10	3	6	4	4	10	6	6
RSD	3	1	3	4	3	6	2	8
SE	3	1	2	1	1	3	2	2
Saplings Soil Mg Conc. (µg/g)	114	77	139	164	93	61	112	89
SD	4	1	3	3	3	1	1	2
RSD	4	1	2	2	3	1	1	2
SE	1	1	1	1	1	1	1	1

Table B.331: Surrounding Soil Aluminum, Calcium, and Magnesium Concentrations for Red Spruce Seedlings for All Sample Sites

Site	CD	ML	MM	CA	RB	SM	WRK	YF
Seedlings Soil Al Conc. (μg/g)	1010	760	1310	260	750	1080	930	1150
SD	9	3	10	10	20	10	20	8
RSD	1	1	1	6	2	1	2	1
SE	3	1	3	3	6	3	6	3
Seedlings Soil Ca Conc. (μg/g)	95	155	105	106	128	70	338	122
SD	4	8	7	5	3	1	6	5
RSD	4	5	7	5	2	2	2	4
SE	1	3	2	2	1	1	2	2
Seedlings Soil Mg Conc. (μg/g)	87	69	124	177	81	54	141	90
SD	1	1	1	6	2	1	4	1
RSD	1	2	1	3	2	1	3	1
SE	1	1	1	2	1	1	1	1

Appendix C: Statistical Analysis Tables

Table C.1: Linear Regression Analysis of Total Metal Foliar Concentration *vs.* Elevation when comparing all sites together.

Linear Regression Analysis (for all sites) (foliar Concentration in µg/g))	**p-value (α = 0.05)**	**Decision***
Mature Al Foliar Conc. *vs.* Elevation	0.7220	No correlation
Mature Ca Foliar Conc. *vs.* Elevation	0.2877	No correlation
Mature Mg Foliar Conc. *vs.* Elevation	0.6306	No correlation
Saplings Al Foliar Conc. *vs.* Elevation	0.1839	No correlation
Saplings Ca Foliar Conc. *vs.* Elevation	0.4131	No correlation
Saplings Mg Foliar Conc. *vs.* Elevation	0.0725	No correlation
Seedlings Al Foliar Conc. *vs.* Elevation	0.5446	No correlation
Seedlings Ca Foliar Conc. *vs.* Elevation	0.2619	No correlation
Seedlings Mg Foliar Conc. *vs.* Elevation	0.1872	No correlation

*Nutrient concentrations (Ca & Mg) were expected to exhibit a negative correlation with elevation and toxic metals concentration were expected exhibit a positive correlation due to acid deposition.

Table C.2: Linear Regression Analysis of Exchangeable Metal Soil Concentration vs. Elevation when comparing all sites together.

Linear Regression Analysis (for all sites) (soil Concentration in µg/g)	**p-value (α = 0.05)**	**Decision***
Mature Al Soil Conc. *vs.* Elevation	0.2759	No correlation
Mature Ca Soil Conc. *vs.* Elevation	0.5906	No correlation
Mature Mg Soil Conc. *vs.* Elevation	0.8009	No correlation
Saplings Al Soil Conc. *vs.* Elevation	0.1758	No correlation
Saplings Ca Soil Conc. *vs.* Elevation	0.1851	No correlation
Saplings Mg Soil Conc. *vs.* Elevation	0.3410	No correlation
Seedlings Al Soil Conc. *vs.* Elevation	0.3319	No correlation
Seedlings Ca Soil Conc. *vs.* Elevation	0.9466	No correlation
Seedlings Mg Soil Conc. *vs.* Elevation	0.7210	No correlation

*Nutrient concentrations (Ca & Mg) were expected to exhibit a negative correlation with elevation and toxic metals concentration were expected exhibit a positive correlation due to acid deposition.

Table C.3: Student's *t*-test Foliar Metal Concentrations at High Elevation Site Mt. Mitchell (MM) and Adjacent Low Elevation Site Camp Alice (CA)

Statistical Analysis: *t*-test between MM and CA (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	0.0405	Different	No, MM < CA
Mature Ca Foliar Conc.	1.267×10^{-11}	Different	Yes, MM < CA
Mature Mg Foliar Conc.	9.320×10^{-08}	Different	No, MM > CA
Saplings Al Foliar Conc.	6.215×10^{-10}	Different	No, MM < CA
Saplings Ca Foliar Conc.	3.651×10^{-07}	Different	Yes, MM < CA
Saplings Mg Foliar Conc.	8.109×10^{-08}	Different	No, MM > CA
Seedlings Al Foliar Conc.	9.080×10^{-10}	Different	No, MM < CA
Seedlings Ca Foliar Conc.	1.120×10^{-14}	Different	Yes, MM < CA
Seedlings Mg Foliar Conc.	3.901×10^{-12}	Different	No, MM > CA

*Higher elevation sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.4: Student's *t*-test Foliar Metal Concentrations at High Elevation Site Clingman's Dome (CD) and Adjacent Low Elevation Site Mingus Lead (ML).

Statistical Analysis: *t*-test between CD and ML (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	0.0007	Different	No, CD < ML
Mature Ca Foliar Conc.	5.975×10^{-08}	Different	Yes, CD < ML
Mature Mg Foliar Conc.	0.0025	Different	No, CD > ML
Saplings Al Foliar Conc.	0.0011	Different	No, CD < ML
Saplings Ca Foliar Conc.	0.0002	Different	No, CD > ML
Saplings Mg Foliar Conc.	0.0105	Different	No, CD > ML
Seedlings Al Foliar Conc.	0.0123	Different	No, CD < ML
Seedlings Ca Foliar Conc.	5.054×10^{-09}	Different	Yes, CD < ML
Seedlings Mg Foliar Conc.	3.146×10^{-06}	Different	No, CD > ML

* Higher elevation sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.5: Student's *t*-test Foliar Metal Concentrations at High Elevation Site Waterrock Knob (WRK) and Adjacent Low Elevation Site Yellow Face (YF).

Statistical Analysis: *t*-test between WRK and YF (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	7.857×10^{-07}	Different	Yes, WRK > YF
Mature Ca Foliar Conc.	0.0001	Different	No, WRK >YF
Mature Mg Foliar Conc.	1.274×10^{-05}	Different	No, WRK > YF
Saplings Al Foliar Conc.	0.0509	No Difference	No
Saplings Ca Foliar Conc.	8.581×10^{-10}	Different	Yes, WRK < YF
Saplings Mg Foliar Conc.	1.159×10^{-07}	Different	No, WRK > YF
Seedlings Al Foliar Conc.	0.0156	Different	No, WRK < YF
Seedlings Ca Foliar Conc.	6.497×10^{-11}	Different	Yes, WRK < YF
Seedlings Mg Foliar Conc.	1.431×10^{-11}	Different	Yes, WRK < YF

*Higher elevation sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.6: Analysis of Variance (ANOVA) Foliar Metal Concentrations at High Elevation Sites Clingman's Dome, Mt. Mitchell, Richland Balsam, and Waterrock Knob (CD, MM, RB & WRK).

Statistical Analysis: ANOVA of CD, MM, RB and WRK) (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	1.75×10^{-07}	Different	No
Mature Ca Foliar Conc.	3.22×10^{-15}	Different	No
Mature Mg Foliar Conc.	$< 2.2 \times 10^{-16}$	Different	No
Saplings Al Foliar Conc.	5.30×10^{-4}	Different	No
Saplings Ca Foliar Conc.	$< 2.2 \times 10^{-16}$	Different	No
Saplings Mg Foliar Conc.	$< 2.2 \times 10^{-16}$	Different	No
Seedlings Al Foliar Conc.	2.17×10^{-04}	Different	No
Seedlings Ca Foliar Conc.	4.72×10^{-16}	Different	No
Seedlings Mg Foliar Conc.	$< 2.2 \times 10^{-16}$	Different	No

*All higher elevation sites were expected to exhibit the same nutrient (Ca & Mg) and toxic metals (Al) concentrations. No indicates same concentrations were observed for both sample sites.

Table C.7: Analysis of Variance (ANOVA) Foliar Metal Concentrations at Low Elevation Sites Mingus Lead, Camp Alice, Spruce Mountain, and Yellow Face (ML, CA, SM, and YF).

Statistical Analysis: ANOVA of ML, CA, SM, and YF) (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	1.41×10^{-04}	Different	No
Mature Ca Foliar Conc.	$<2.2 \times 10^{-16}$	Different	No
Mature Mg Foliar Conc.	$<2.2 \times 10^{-16}$	Different	No
Saplings Al Foliar Conc.	4.01×10^{-13}	Different	No
Saplings Ca Foliar Conc.	9.53×10^{-08}	Different	No
Saplings Mg Foliar Conc.	5.71×10^{-16}	Different	No
Seedlings Al Foliar Conc.	7.17×10^{-16}	Different	No
Seedlings Ca Foliar Conc.	$<2.2 \times 10^{-16}$	Different	No
Seedlings Mg Foliar Conc.	$<2.2 \times 10^{-16}$	Different	No

*All lower elevation sites were expected to exhibit the same nutrient (Ca & Mg) and toxic metals (Al) concentrations. No indicates same concentrations were observed for both sample sites.

Table C.8: Linear Regression Analysis of Foliar Metal Concentration *vs.* Longitude when comparing all sites together.

Linear Regression Analysis (for all sites) (foliar concentration in µg/g)	**p-value (α = 0.05)**	**Decision***
Mature Al Foliar Conc. *vs.* Longitude	0.8155	No correlation
Mature Ca Foliar Conc. *vs.* Longitude	0.5565	No correlation
Mature Mg Foliar Conc. *vs.* Longitude	0.2513	No correlation
Saplings Al Foliar Conc. *vs.* Longitude	0.2128	No correlation
Saplings Ca Foliar Conc. *vs.* Longitude	0.5875	No correlation
Saplings Mg Foliar Conc. *vs.* Longitude	0.2496	No correlation
Seedlings Al Foliar Conc. *vs.* Longitude	0.2167	No correlation
Seedlings Ca Foliar Conc. *vs.* Longitude	0.7211	No correlation
Seedlings Mg Foliar Conc. *vs.* Longitude	0.6972	No correlation

*Nutrient concentrations (Ca & Mg) were expected to exhibit a positive correlation with longitude (traveling west to east) and toxic metals concentration were expected to exhibit a negative correlation (traveling west to east) due to acid deposition.

Table C.9: Linear Regression Analysis of Soil Metal Concentration *vs.* Longitude when comparing all sites together.

Linear Regression Analysis (for all sites) (soil concentration in µg/g)	**p-value (α = 0.05)**	**Decision**
Mature Al Soil Conc. *vs.* Longitude	0.8128	No correlation
Mature Ca Soil Conc. *vs.* Longitude	0.9219	No correlation
Mature Mg Soil Conc. *vs.* Longitude	0.0293	Positive correlation
Saplings Al Soil Conc. *vs.* Longitude	0.7445	No correlation
Saplings Ca Soil Conc. *vs.* Longitude	0.1658	No correlation
Saplings Mg Soil Conc. *vs.* Longitude	0.0239	Positive correlation
Seedlings Al Soil Conc. *vs.* Longitude	0.6105	No correlation
Seedlings Ca Soil Conc. *vs.* Longitude	0.6931	No correlation
Seedlings Mg Soil Conc. *vs.* Longitude	0.0625	No correlation

*Nutrient concentrations (Ca & Mg) were expected to exhibit a positive correlation with longitude (traveling west to east) and toxic metals concentration were expected to exhibit a negative correlation (traveling west to east) due to acid deposition.

Table C.10: Student's *t*-test of Foliar Metal Concentrations at Western High Elevation Site Clingman's Dome (CD) and Central High Elevation Site Waterrock Knob (WRK).

Statistical Analysis: *t*-test between CD and WRK (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	7.491×10^{-07}	Different	No, CD < WRK
Mature Ca Foliar Conc.	8.712×10^{-09}	Different	No, CD > WRK
Mature Mg Foliar Conc.	4.640×10^{-10}	Different	No, CD > WRK
Saplings Al Foliar Conc.	0.0094	Different	No, CD < WRK
Saplings Ca Foliar Conc.	5.582×10^{-12}	Different	No, CD > WRK
Saplings Mg Foliar Conc.	7.907×10^{-08}	Different	No, CD > WRK
Seedlings Al Foliar Conc.	0.0005	Different	Yes, CD > WRK
Seedlings Ca Foliar Conc.	3.111×10^{-09}	Different	No, CD > WRK
Seedlings Mg Foliar Conc.	1.188×10^{-12}	Different	No, CD > WRK

*Western sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.11: Student's *t*-test of Foliar Metal Concentrations at Western Low Elevation Site Mingus Lead (ML) and Central Low Elevation Site Yellow Face (YF).

Statistical Analysis: *t*-test between ML and YF (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	0.0011	Different	Yes, ML > YF
Mature Ca Foliar Conc.	5.667×10^{-10}	Different	No, ML > YF
Mature Mg Foliar Conc.	5.203×10^{-08}	Different	No, ML > YF
Saplings Al Foliar Conc.	0.0021	Different	Yes, ML > YF
Saplings Ca Foliar Conc.	3.954×10^{-05}	Different	No, ML > YF
Saplings Mg Foliar Conc.	3.142×10^{-09}	Different	No, ML > YF
Seedlings Al Foliar Conc.	1.434×10^{-06}	Different	Yes, ML > YF
Seedlings Ca Foliar Conc.	9.964×10^{-08}	Different	No, ML > YF
Seedlings Mg Foliar Conc.	0.0268	Different	Yes, ML < YF

* Western sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.12: Student's *t*-test of Foliar Metal Concentrations at Central High Elevation Site Waterrock Knob (WRK) and Eastern High Elevation Site Mt. Mitchell (MM).

Statistical Analysis: *t*-test between WRK and MM (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	0.0009	Different	Yes, WRK > MM
Mature Ca Foliar Conc.	1.265×10^{-06}	Different	Yes, WRK < MM
Mature Mg Foliar Conc.	2.612×10^{-13}	Different	Yes, WRK < MM
Saplings Al Foliar Conc.	0.0204	Different	Yes, WRK > MM
Saplings Ca Foliar Conc.	1.381×10^{-07}	Different	Yes, WRK < MM
Saplings Mg Foliar Conc.	2.199×10^{-11}	Different	Yes, WRK < MM
Seedlings Al Foliar Conc.	0.0007	Different	No, WRK < MM
Seedlings Ca Foliar Conc.	6.839×10^{-09}	Different	Yes, WRK < MM
Seedlings Mg Foliar Conc.	2.773×10^{-13}	Different	Yes, WRK < MM

*Western sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.13: Student's *t*-test of Foliar Metal Concentrations at Central Low Elevation Site Yellow Face (YF) and Eastern Low Elevation Site Camp Alice (CA).

Statistical Analysis: *t*-test between YF and CA (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	5.461×10^{-05}	Different	No, YF < CA
Mature Ca Foliar Conc.	2.262×10^{-13}	Different	Yes, YF < CA
Mature Mg Foliar Conc.	1.244×10^{-11}	Different	Yes, YF < CA
Saplings Al Foliar Conc.	1.006×10^{-09}	Different	No, YF < CA
Saplings Ca Foliar Conc.	0.0003	Different	Yes, YF < CA
Saplings Mg Foliar Conc.	7.679×10^{-08}	Different	Yes, YF < CA
Seedlings Al Foliar Conc.	8.231×10^{-11}	Different	No, YF < CA
Seedlings Ca Foliar Conc.	1.121×10^{-10}	Different	Yes, YF < CA
Seedlings Mg Foliar Conc.	5.653×10^{-09}	Different	No, YF > CA

* Western sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.14: Student's *t*-test of Foliar Metal Concentrations at Eastern High Elevation Site Mt. Mitchell (MM) and Western High Elevation Site Clingman's Dome (CD).

Statistical Analysis: *t*-test between MM and CD (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	0.0745	No Difference	No
Mature Ca Foliar Conc.	3.592×10^{-05}	Different	No, CD > MM
Mature Mg Foliar Conc.	4.893×10^{-11}	Different	Yes, CD < MM
Saplings Al Foliar Conc.	0.1311	No Difference	No
Saplings Ca Foliar Conc.	4.574×10^{-09}	Different	No, CD > MM
Saplings Mg Foliar Conc.	6.399×10^{-10}	Different	Yes, CD < MM
Seedlings Al Foliar Conc.	0.2119	No Difference	No
Seedlings Ca Foliar Conc.	2.038×10^{-06}	Different	No, CD > MM
Seedlings Mg Foliar Conc.	4.336×10^{-07}	Different	Yes, CD < MM

*Western sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.15: Student's of t-test Foliar Metal Concentrations at Eastern Low Elevation Site Camp Alice (CA) and Western Low Elevation Site Mingus Lead (ML).

Statistical Analysis: *t*-test between CA and ML (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	0.0115	Different	No, CA > ML
Mature Ca Foliar Conc.	3.840×10^{-06}	Different	Yes, CA > ML
Mature Mg Foliar Conc.	1.574×10^{-07}	Different	Yes, CA > ML
Saplings Al Foliar Conc.	5.657×10^{-08}	Different	No, CA > ML
Saplings Ca Foliar Conc.	0.8365	No Difference	No
Saplings Mg Foliar Conc.	0.7856	No Difference	No
Seedlings Al Foliar Conc.	8.073×10^{-09}	Different	No, CA > ML
Seedlings Ca Foliar Conc.	1.239×10^{-07}	Different	Yes, CA > ML
Seedlings Mg Foliar Conc.	1.156×10^{-07}	Different	No, CA < ML

* Western sites were expected to exhibit lower nutrient (Ca & Mg) levels and higher toxic metals (Al) levels due to acid deposition.

Table C.16: Analysis of Variance (ANOVA) of Foliar Metal Concentrations at High Elevation Sites Mt. Mitchell, Clingman's Dome, and Waterrock Knob (MM, CD, and WRK) of Longitude Studies.

Statistical Analysis: ANOVA of MM, CD, and WRK) (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	6.914×10^{-06}	Different	No, CD < WRK > MM
Mature Ca Foliar Conc.	1.055×10^{-09}	Different	No, CD > MM > WRK
Mature Mg Foliar Conc.	8.148×10^{-16}	Different	No, MM > CD > WRK
Saplings Al Foliar Conc.	6.966×10^{-03}	Different	No, CD = MM > WRK
Saplings Ca Foliar Conc.	1.638×10^{-14}	Different	No, MM > CD > WRK
Saplings Mg Foliar Conc.	7.719×10^{-15}	Different	No, MM > CD > WRK
Seedlings Al Foliar Conc.	2.562×10^{-04}	Different	No, CD =MM > WRK
Seedlings Ca Foliar Conc.	1.739×10^{-12}	Different	No, MM > CD > WRK
Seedlings Mg Foliar Conc.	$< 2.2 \times 10^{-16}$	Different	No, MM > CD > WRK

* All higher elevation sites were expected to exhibit the lower nutrient (Ca & Mg) and higher toxic metal (Al) concentrations as the sample site's longitude increased (i.e., traveling east to west).

Table C.17: Analysis of Variance (ANOVA) of Foliar Metal Concentrations at Low Elevation Sites Camp Alice, Mingus Lead, and Yellow Face (CA, ML, and YF) of Longitude Studies.

Statistical Analysis: ANOVA of CA, ML, and YF) (foliar concentration in µg/g)	**p-value (α =0.05)**	**Decision (conc. Different or Same)**	**Expected?***
Mature Al Foliar Conc.	2.580×10^{-05}	Different	No, CA > ML > YF
Mature Ca Foliar Conc.	5.997×10^{-15}	Different	No, CA > ML > YF
Mature Mg Foliar Conc.	1.522×10^{-13}	Different	No, CA > ML > YF
Saplings Al Foliar Conc.	1.473×10^{-11}	Different	No, CA > ML > YF
Saplings Ca Foliar Conc.	2.292×10^{-05}	Different	No, CA = ML > YF
Saplings Mg Foliar Conc.	1.035×10^{-10}	Different	No, CA = ML > YF
Seedlings Al Foliar Conc.	2.519×10^{-13}	Different	No, CA > ML > YF
Seedlings Ca Foliar Conc.	4.254×10^{-13}	Different	No, CA > ML > YF
Seedlings Mg Foliar Conc.	2.144×10^{-10}	Different	No, YF > ML > CA

*All lower elevation sites were expected to exhibit the lower nutrient (Ca & Mg) and higher toxic metal (Al) concentrations as the sample site's longitude increased (i.e., traveling east to west).

Table C.18: Linear Regression Analysis of Metal Foliar Concentration *vs.* soil pH when comparing all sites together.

Linear Regression Analysis (for all sites) (foliar concentration in µg/g)	**p-value (α = 0.05)**	**Decision***
Mature Al Foliar Conc. *vs.* pH	0.5132	No correlation
Mature Ca Foliar Conc. *vs.* pH	0.0722	No correlation
Mature Mg Foliar Conc. *vs.* pH	0.3622	No correlation
Saplings Al Foliar Conc. *vs.* pH	0.0019	Positive Correlation
Saplings Ca Foliar Conc. *vs.* pH	0.5248	No correlation
Saplings Mg Foliar Conc. *vs.* pH	0.8189	No correlation
Seedlings Al Foliar Conc. *vs.* pH	3.971×10^{-5}	Positive Correlation
Seedlings Ca Foliar Conc. *vs.* pH	0.0616	No correlation
Seedlings Mg Foliar Conc. *vs.* pH	0.8743	No correlation

*Nutrient concentrations (Ca & Mg) were expected exhibit a negative correlation and toxic metals concentration should were expected to a positive correlation with pH due to acid deposition.

Table C.19: Linear Regression Analysis of Soil Metal Concentration *vs.* soil pH when comparing all sites together.

Linear Regression Analysis (for all sites) (Soil concentration in µg/g)	**p-value (α = 0.05)**	**Decision***
Mature Al Soil Conc. *vs.* pH	0.1277	No correlation
Mature Ca Soil Conc. *vs.* pH	0.9938	No correlation
Mature Mg Soil Conc. *vs.* pH	0.0922	No correlation
Saplings Al Soil Conc. *vs.* pH	0.0340	Weak Negative Correlation
Saplings Ca Soil Conc. *vs.* pH	0.4734	No correlation
Saplings Mg Soil Conc. *vs.* pH	0.0655	No correlation
Seedlings Al Soil Conc. *vs.* pH	0.0111	Weak Negative Correlation
Seedlings Ca Soil Conc. *vs.* pH	0.4950	No correlation
Seedlings Mg Soil Conc. *vs.* pH	0.0927	No correlation

*Nutrient concentrations (Ca & Mg) were expected exhibit a negative correlation and toxic metals concentration were expected to exhibit a positive correlation with pH due to acid deposition.

Table C.20: Linear Regression Analysis of pH vs. Elevation when comparing all sites together.

Linear Regression Analysis (all sites)	**p-value (α = 0.05)**	**Decision***
pH of Mature Red Spruce Soil *vs.* Elevation	0.7309	No correlation
pH of Red Spruce Saplings Soil *vs.* Elevation	0.6690	No correlation
pH of Red Spruce Seedlings Soil *vs.* Elevation	0.5032	No correlation

*Soil pH was hypothesized to exhibit a negative correlation with elevation due to acid deposition.

Table C.21: Comparison of Life Stage of Red Spruce Metal Foliar and Soil Concentration when comparing all sites together.

Analysis of Variance (all sites)	**p-value (α = 0.05)**	**Decision (conc. Different or Same)**	**Expected ?***
Comparison of Life Stage for Foliar Al	0.5924	No Difference	No
Comparison of Life Stage for Foliar Ca	0.2759	No Difference	No
Comparison of Life Stage for Foliar Mg	0.0212	Different	Yes
Comparison of Life Stage for Soil Al	0.9644	No Difference	No
Comparison of Life Stage for Soil Ca	0.3915	No Difference	No
Comparison of Life Stage for Soil Mg	0.8531	No Difference	No

* Mature, saplings, and seedlings were hypothesized to exhibit different nutrient (Ca & Mg) and toxic metals (Al) concentrations.

Table C.22: Linear Regression Analysis of Soil Metal Concentration *vs.* Foliar Metal Concentration when comparing all sites together.

Linear Regression Analysis (for all sites) (soil and foliar concentration in µg/g)	**p-value (α = 0.05)**	**Decision***
Mature Al Soil Conc. *vs.* Mature Al Foliar Conc.	0.3237	No correlation
Mature Ca Soil Conc. *vs.* Mature Ca Foliar Conc.	0.4955	No correlation
Mature Mg Soil Conc. *vs.* Mature Mg Foliar Conc.	0.2777	No correlation
Saplings Al Soil Conc. *vs.* Saplings Al Foliar Conc.	0.0119	Negative Correlation (does not follow expected trend)
Saplings Ca Soil Conc. *vs.* Saplings Ca Foliar Conc.	0.7154	No correlation
Saplings Mg Soil Conc. *vs.* Saplings Mg Foliar Conc.	0.0562	No correlation
Seedlings Al Soil Conc. *vs.* Seedlings Al Foliar Conc.	0.0080	Negative Correlation (does not follow expected trend)
Seedlings Ca Soil Conc. *vs.* Seedlings Ca Foliar Conc.	0.2998	No correlation
Seedlings Mg Soil Conc. *vs.* Seedlings Mg Foliar Conc.	0.9215	No correlation

*Foliar nutrient concentrations (Ca & Mg) and toxic metals concentration were hypothesized to exhibit a positive correlation with respect to soil concentrations.