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An Evaluation of Elemental Composition of Soils Associated with Ground Nesting Ants in the Family Formicidae in Somerset County, Maine

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UNIVERSITY OF SOUTHERN MAINE

Portland, Maine

An Evaluation of Elemental Composition of Soils Associated with Ground Nesting Ants in the Family Formicidae in Somerset County, Maine

A Thesis

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An Evaluation of Soil Composition Associated with Two Genera of Ground Nesting Ants

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Abstract

Ants are keystone organisms and engineers in many ecosystems, playing an important role in nutrient cycling and rearrangement of organic and inorganic materials through foraging, waste management and, in subterranean species, nest construction and maintenance. In this study, I conducted elemental analysis of soils for calcium, copper, iron, potassium, manganese and zinc in and around the colonies of subterranean nesting ants. The two-step analysis used in this study utilizes the efficiency of X-Ray Fluorescence (XRF) Spectrometry to look for overall trends in elemental composition followed by Flame Atomic Absorbance (AA) to achieve higher resolution of select elements of interest as justified in XRF results. No relationships were found between concentration of elements with depth nor distance. Additional observations made throughout this research revealed significant taxon-specific differences in elemental concentrations.

An Evaluation of Soil Composition Associated with Two Genera of Ground Nesting Ants

Chapter 1

Introduction

Purpose. In the present study, concentrations of copper, iron, potassium, calcium, and manganese of soils in and around ant colonies were evaluated in two subterranean ant genera commonly found in Maine, *Aphaenogaster rudis* and *Myrmica*, belonging to the family Formicidae and subfamily Myrmicinae. Throughout New England, these genera include 6 species of *Aphaenogaster* and 21 species of *Myrmica* (Ellison, Gotelli, Farnsworth & Alpert 2012). In addition, I assessed a multilevel approach to soil analysis that incorporated the convenience of X-Ray Fluorescence Spectroscopy with the accuracy and precision of Atomic Absorbance Spectroscopy.

The current literature on specific relationships between subterranean nesting ants and the physical and chemical characteristics of the soils they nest in is limited, with little overlap between studies evaluating members of the same genera occurring in similar habitats. Thus, further research is necessary to clarify relationships between subterranean nesting ants and characteristics of the soils they inhabit.

Determining the extent to which ants are involved in chemical cycling in soils will provide better insight into their role in ecosystem function and stability. By characterizing differences in the concentrations of copper, iron, potassium, calcium, and manganese in soils as a function of depth and distance from colonies of *Aphaenogaster rudis* and *Myrmica,* I hope to provide a better understanding of the extent to which ground nesting ants are involved with chemical cycling and composition of the biological communities they inhabit and/or factors that may be influencing location of colony formation.

Previous research. It is well established that ants are keystone organisms in many ecosystems by influencing the distribution of organic and inorganic materials (Barroso, Cerdá & Boulay. 2013; Boots, Kieth, Niechoi, Breen, Schmidt & Clipson 2012; Dattilo, Rico-Gray, Rodtigues & Izzo. 2013; DeFauw, Vogt & Boykin 2008; Jílková*.,* Matějíček & Frouz 2011; Richards 2009; Sanders & Frank van Veen 2011; van Gils, Gaigl & Gómez 2010; Veen & Olff 2011). Previous findings characterizing relationships between subterranean nesting ants and surrounding soils has been inconsistent, with some authors reporting a clear influence on physiochemical features of soils (Anderson, Lanoue & Radford 2010; Boots *et al*. 2012; Dostal, Březnová, Kozlíčková, Herben & Kovář 2005; Eldridge & Myers 1998; Frouz *et al*. 2003; Holec & Frouz 2003; Jílková*.,* Pech, Mihaljevič & Frouz 2017; Richards 2009; van Gils *et al.* 2010; Veen & Olff 2011; Véle, Frouz, Holuša & Kalčík 2010) while others have found little or no influence (Dattilo *et al*. 2013; Jacquemin, Drouet, Delsinne, Roisin & Leponce 2012; Jílková *et al.* 2017; Milks Fuxa, Richter & Moser 2007). Soil features that have been associated with ground nesting ants include texture, organic content, carbon to nitrogen ratio, pH and concentration of metal ions relative to surrounding soils.

When correlations between physiochemical properties of soils and proximity to the colony have been found, researchers generally adopt two basic hypotheses to explain this observation: 1.) sites are selected for colony formation based on favorable physical and chemical characteristics; 2.) the activity of the ant colony through bioturbation changes basic structure and/or composition of the soil; or some combination of the two hypotheses (Eldridge & Myers 1998; Jílková *et al.* 2017; Milks *et al.* 2007; Richards 2009; Veen & Olff 2011; Véle *et al.* 2010). It is possible that conflicting findings are the result of a conditional relationship and that the exact outcomes depend on several variables including colony size, climate, location, previously existing soil features, and ecological niche. Additional findings support that ants can execute top-down influence over their communities by acting as keystone predators of other invertebrates which promote or reduce biodiversity (McPhee, Garnas, Drummond & Groden 2012; Sanders & van Veen 2011; Warren, McMillan, King, Chick & Bradford 2015).

Prior to the late 1970s, the influence ants had on the composition of their ecological community was not well documented. Though the impacts of ants in an ecosystem had long been recognized, they were not widely viewed as ecosystem engineers. Early documents by Grabham (1921) recounted observations of a rapid increase in population of an unspecified species of *Lecanium* scale insect as a direct result of care by a "small Argentine ant," where under the ant's tending were able to reach populations large enough to heavily infest and greatly reduce the health of a lemon tree. In 1932, Cole documented the plant cutting activities of the Western Harvester Ant, *Pogonomyrmex occidentalis,* and documented distinct patterns of cleared foliage in the immediate proximity of ant mounds. In 1934, Talbot was the first to report on ant colonies exhibiting ecological succession. Talbot found that several species of ants in the Chicago region followed a distinct succession in colonization of deadwood that correlated with the wood's state of decay. These findings lead Talbot to propose that, due to the large number of ants found in many ecosystems, these insects likely were involved in shaping their ecosystems as a whole.

Culver and Beattie (1978) were the first to publish experimental findings investigating the role of ants in seed distribution, now termed myrmecochory. This study found several species of ants in southern West Virginia, including *Formica subsericea*, *Lasius alienus*, *Leptothorax* spp., *Myrmica punctiventris* and *Tapinoma sessile*, collected seeds from the six of the nine species of *Viola* tested. The most significant finding was the role *Aphaenogaster* spp. had in the distribution of these flowers: unintentional dispersal of the seeds occurred when dropped along the return trip to their nest. While the majority of seeds were successfully returned to the colony, those that were dropped and abandoned before reaching the final destination had been transported to locations with reduced risk of predation by other species as a result of proximity to the ant nests. This allowed for an increased successful germination rate of the translocated seeds relative to those untouched by ants. The other genera of ants tested had a lower harvest rate of seeds from *Viola* and so fewer seeds were distributed. Additional studies have since supported the role of myrmecochory in community structure, further illustrating the fundamental role ants play in shaping their ecosystems (Barroso *et al.* 2013; Hilley & Thiet 2015; Lubertazzi 2012; Richards 2009; Thomson, Auld, Ramp & Kingsford 2016; Warren *et al.* 2015).

Early studies examining the influence of subterranean ant colonies on the physiochemical properties of soil focused on broad changes in texture and mixing, pH, conductivity, nitrogen and phosphorus, and organic carbon content. In 1968, Thomas reported the contributions of ants as being among the four species of invertebrates primarily involved in the decomposition of lobolly pine needles. The effects of subterranean nesting ants on physiochemical properties of soil in and around the colonies was not characterized by a major study until the early 1980s. Then, Culver and Beattie (1983) conducted detailed research on the subjects, by evaluating 15 abandoned ant mounds for plant diversity and concentrations of elements including phosphorus, potassium, zinc, iron, manganese, copper, cadmium, lead, nickel, nitrate, and nitrite. In the same study, Culver and Beattie also analyzed physiochemical properties of soil including organic content, sodium absorption and the pH and conductivity of soils in and around the mounds. In this study, elemental analysis was carried out *via* ammonium bicarbonate extraction diethylenetriaminepentaacetic acid (DPTA). Nitrate and nitrite were determined using colorimetric analysis. Culver and Beattie also noted that areas without mounds had higher plant diversity than areas associated with mounds.

Soils associated with colonies contained lower concentrations of most chemicals analyzed except for phosphorus, potassium, copper, nickel, and nitrate (Culver & Beattie, 1983). Conductivity was higher in ant colonies, while sodium absorption and pH were roughly the same in soils within colonies vs. soils at a distance.

Differences in soil texture associated with proximity to subterranean ant colonies have been found to vary by species. This is believed to be due to characteristics that influence the suitability of soils for nest construction. A study by Milks *et al.* (2007) evaluated nest site characteristics of *Solenopsis invicta* in Louisiana for soil texture, sodium and phosphorous concentrations, and organic matter content and found that numbers of ant nests were higher at locations where soils contained relatively lower levels of silt, clay, and sodium. Milks *et al.* (2007) speculates the possibility that this association is the result of poor drainage at sites with relatively higher concentrations of these characteristics posing a risk of drowning the nest. In addition, Milks *et al.* (2007) noted these soil characteristics were also associated with poor plant growth and so may be less desirable due to decreased food availability relative to other locations nearby. In contrast, van Gils *et al.* (2010) found colonies of *Atta sexdens* in the Colombian Amazon formed more frequently in soils with relatively more clay and sand, but less silt. Similarly, Jacquemin *et al.* (2012) found that *Acropyga fuhrmanni* in Andean forests more frequently nested in soils within sample sites featuring relatively higher clay content, but this did not hold true for other sample sites evaluated, which showed no associations between nest location and soil characteristics. Richards (2009) reported that Australian species of *Aphaenogaster* had distinct preferences for soil characteristics of nesting sites that varied greatly between species, with these contrasts being particularly noticeable when comparing species that occupy different niches or biomes. A two year study by Eldridge and Pickard (1994) on bioturbation at the site of *Aphaenogaster barbigula* colonies found over a quarter millimeter of soil was generated per year. Due to frequent movement of the colony at two moves per year, cumulative effects were limited. Reports of association of *Myrmica* spp. with soil texture was not found in this review, though this may be due to their nature of constructing relatively small nests that would not be as prone to exhibiting the same amount of accumulative changes in their soils that are seen in other genera (Lenoir 2009). Physical characteristics of soils in association with ants is a good indicator that there are differences in chemical characteristics as well.

Subterranean nesting ants have been associated with increased soil fertility near their colonies as a byproduct of foraging, waste production, and nest construction and maintenance (Boots *et al*. 2012; Eldridge & Myers 1998; Frouz, Holec & Kalčík 2003; Richards 2009; Sanders & van Veen 2011). Several studies have found direct associations between these activities of colonies and increased available nitrogen, phosphorus, and carbon in colonized soils. The redistribution and concentration of organic materials at nest sites as well as the burial of decaying organic matter and excavation of deeper soils has been found to encourage the proliferation of bacteria and fungi as well as other decomposers, resulting in the production of nutrient-rich environments favorable to plant life (Boots *et al.* 2012; Frouz *et al*. 2003; Holec & Frouz 2006; Sanders & van Veen 2011). The amount of change observed appears to be species specific. Jílková *et al.* (2017) compared the effects of *Formica sanguinea*, *Lasius niger,* and *Tetramorium cf. caespitum* and found *F. sanguinea* to have the most influence*,* while *Lasius niger,* and *Tetramorium cf. caespitum* had no significant influence on these characteristics. DeFauw *et al.* (2008) found significantly higher carbon to nitrogen ratios in the nests of *Solenopsis* spp. relative to surrounding soils, while Boots *et al.* (2012) found no significant difference in this ratio in the nests of neither *Lasius flavus* nor *Myrmica sabuleti* relative to surrounding soils. One genus that has been widely found to influence organic carbon and nitrogen concentration through bioturbation is *Aphaenogaster*. A study by Eldridge and Myers (1998) found *Aphaenogaster barbigula* increased concentrations of nitrogen, phosphorus and organic carbon at nest entrances. A study by Véle *et al.* (2010) on the influence of *Myrmica ruginodis* on the chemical characteristics of the soils they nest in suggests that the changes associated with ant activity are dependent on the initial condition of their surrounding soil. Véle *et al.* (2010) evaluated the physiochemical properties of soils associated with *M. ruginodis* in a spruce forest in Norway and reported sample sites exhibited an inverse relationship between concentrations of phosphorous and reduced carbon. Véle *et al.* (2010) observed that sample sites where soils contained relatively high levels of phosphorous had lower concentrations of reduced carbon at the location of ant colonies, whereas sample sites with soils containing relatively low levels of phosphorous showed higher concentrations of reduced carbon at the location of ant colonies. These findings support that concentration of phosphorus closely corresponds with that of reduced carbon, a characteristic associated with decaying organic matter. Concentrations of reduced carbon, available nitrogen and available phosphorus are closely tied to concentrations of decomposing organic materials (Boots *et al.* 2012; Eldridge & Myers 1998; Holec & Frouz 2006; Jílková *et al.* 2017; Véle *et al.* 2010). Changes in distribution of organic materials from the activity of ants and the resulting chemical characteristics of soil can result directly from the actions of ants.

Trends in conductivity and pH of soils in and around subterranean ant colonies vary based on numerous factors. The variables that have so far been characterized include species of ant (Boots *et al*. 2012; Jacquemin *et al.* 2012), how long a colony has been at a given location (Boots *et al.* 2012), influence of pH in existing soils on prey or species that host prey such as flowering plants (Dattilo *et al*. 2013), relative pH of surrounding soils (Frouz *et al*. 2003), and organic content of soils (Jílková *et al.* 2011). Jílková *et al.* (2011) reported increased pH in soils in proximity to *Formica sanguinea*, while *Lasius niger,* and *Tetramorium cf. caespitum* had no significant influence on soil pH. The increased pH found in soils from the nests of *F. sanguinea* correlate with higher concentrations of organic material. Findings by Eldridge & Myers (1998) and Véle *et al.* (2010) support those of Jílková *et al.* (2011), with soils from the entrances of *Aphaenogaster barbigula* and *Myrmica ruginodis* colonies featuring a significantly higher pH as well as concentration of organic materials. Reports by Boots *et al.* (2012) suggest that colonies that had been established longer at a given location have more of an influence on pH than younger or recently relocated colonies in *Lasius flavus* and *Formica lemani*. Like the relationship observed in phosphorus levels in the Véle *et al.* (2010) study, Frouz *et al.* (2003) found that pH in soils associated with colonies of *Lasius niger* is influenced by surrounding soils, with increases in pH in soils that had relatively lower pH relative to control plots, and vice versa. Acidity has a direct effect on how readily molecules can ionize and as a result influences other chemical characteristics of affected soils.

Elemental concentrations in soils of subterranean ant colonies, including calcium, magnesium and potassium, have been found to vary by nest site and species. Dostál *et al.* (2005) found that soils occupied by *Lasius flavus* featured decreased concentrations of calcium and magnesium ions and increased concentration of potassium relative to control soils. Findings by Véle *et al.* (2010) suggest that concentration of organic materials and nest construction and maintenance by *Lasius flavus* may be associated with higher concentrations of potassium relative to surrounding soil. Frouz *et al.* (2003) found that another member of this genus, *Lasius niger,* showed increased concentrations of potassium and sodium and pH increased where colonies were present and concentrations of calcium and total carbon varied independently of colonies. As calcium and total carbon concentrations increased in surrounding soils, potassium and sodium decreased at the site of the colony. All three of these metals, calcium, potassium, and magnesium, are essential micronutrients to many forms of life including plants.

Bioaccumulation is another means by which ants are involved in chemical cycling. Research suggests that the bodies of ants accumulate heavy metals including zinc, lead and arsenic (Burgess, Davis & Edwards 2018; Del Toro*,* Floyd, Gardea-Torresdey & Borrok 2010; Grześ 2012). Ants have not been found to have internal mechanisms to regulate accumulation of these metals, though speed and intensity of uptake varies by species (Burgess *et al.* 2018; Del Toro *et al.* 2010; Grześ 2012). Two studies had conflicting results with regards to bioaccumulation of copper, however both examined ants of different genera and so these disparities may be the result of taxonomic differences (Del Toro, *et al.* 2010; Grześ 2012). A study by Del Toro *et al.* (2010) was the first to evaluate bioaccumulation of heavy metals in ants. The results of their research found copper concentrations to decrease in the bodies of *Pogonomyrmex rugosus* and the grass seeds they fed on with increasing distance from a copper smelter. In contrast, a study by Gramigni *et al.* (2013) found no correlation between the copper concentrations in the bodies of *Crematogaster scutellaris* and the soils in which they lived. In a study by Burgess *et al.* (2018), concentrations of lead in the bodies of *Pogonomyrmex barbatus* from soils, bodies, and rinse water from ants did not fit the theoretical bioaccumulation model, suggesting that there is more to be learned as to the mechanisms responsible for bioaccumulation in this species.

Additional factors. Anthropogenic disturbances can directly impact the composition of local biological communities. Chemical and physical properties of soil can be directly or indirectly manipulated by anthropogenic means and the cascading effects rendered by these manipulations influencing the surrounding ecosystem (Dominati, Patterson & Mackay 2010; Gupta, Kumar, Ahmad, Pandey & Chauhan 2017; Huang, Jia, Zhang & Shao 2017; Nadezhda, Rogovaya, Ivashchenko, Vasenev, Sarzhanoy, Ryzhkov & Kudeyarov 2016; Whittinghill, Currie, Zak, Burton & Pregitzer 2012). These manipulations include redistributing existing soils and introducing foreign substrates, traffic levels altering physical characteristics such as the packing of soils, manipulation of soil chemistry through introduced chemicals such as fertilizers, pesticides, herbicides, and ice-melt treatments such as those used on roads. Anthropogenic activity can lead to skewed communities through either intentional or accidental perpetuation of crops as well as the introduction of foreign species.

Though bioaccumulation of toxic materials generally receives the most research and discussion, the chemical properties of soil can change the nutritional characteristics of subterranean nesting invertebrates. A better understanding of this type of relationship could lend to the development of better quality food sources for captive wildlife that naturally would rely on such species as a substantial part of their diet. A 2015 study by Janzow & Judd of the subterranean termite *Reticulitermes flavipes* found through a series of controlled experiments of captive termite colonies that internalized concentrations of four biologically necessary minerals-- calcium, iron, magnesium, and manganese-- was directly linked to the chemical composition of the soils in which they nested.

Relationships have been observed between many different physical and chemical characteristics of soils and the presence of subterranean nesting ants. Associations between ant and soil characteristics appear to be species specific. Despite the number and magnitude of studies conducted evaluating soils in the proximity of subterranean ant colonies, relationships between the two require further investigation to fully understand the role of ants in nutrient cycling in soils.

The study presented here is the first to investigate concentrations of zinc, iron, copper, and manganese in soils as a function of proximity to subterranean ant colonies. In addition, I evaluated concentrations of potassium and calcium, which have previously been studied in a limited number of species. All elements measured in this study are essential to biological functioning of most organisms. Concentrations in each sample were compared to evaluate for associations between concentrations of different elements.

Chapter 2

Materials and Methods

Introduction. Samples were collected in Canaan, Maine, in October 2018 from an open lot with a moderate southwestern slope and bordering a mixed temperate deciduous conifer forest and a manmade pond. The lot had been a recreational vehicle park that appears to have been closed for over a decade, as suggested by abundant overgrowth occurring throughout the site. The suitability of individual sample sites was determined by accessibility and visual indications of lower disturbance, to ensure that ant colonies had been able to establish colonies that were at least one season old. Accessibility was based on the ability to gain appropriate permissions by the land owner and by physical features of the sample site (*i.e.* dry and walkable). Anthropogenic disturbances consisting of alterations to physical features of the substrate including redistribution of native soil and the introduction of foreign soils or chemicals were consistent across the park.

In this study, I compared the concentrations of copper, iron, potassium, calcium, and manganese in soils in and around the hills of subterranean ant colonies. The primary objective for this research was to determine if ground-nesting ants influenced soil elemental composition. I also explored the sequential use of portable X-ray fluorescence (XRF) and atomic absorbance (AA) spectroscopy in a stepwise sequence that allows for both the speed and convenience of portable XRF analysis and the resolution of AA analysis. I hypothesized that the activities of these subterranean nesting genera are correlated with elemental concentrations in proximity to their nests, as observed by Eldridge & Myers (1998), Richards (2009), and Véle *et al.* (2010).

Sample site. The open portion of the lot was primarily populated with an assortment of grasses, wild strawberries (*Fragaria* sp.), black-eyed Susans (*Rudbeckia hirta*), Queen Anne's lace

(*Daucus carota*), red clover (*Trifolium pratense*), goldenrod (*Solidago* sp.), many flowered aster (*Symphyotrichum ericoides*), burdock (*Arctium* sp.), common dandelion (*Taraxacum officinale*), sensitive fern (*Onoclea sensibilis*), sapling white pine (*Pinus strobus*) and quaking aspen (*Populus tremuloides*), with the roadside edge of the field featuring milkweed (*Asclepias* sp.). The adjacent forest consisted primarily of white pine, quaking aspen, staghorn sumac (*Rhus typhina*), and sugar maple (*Acer saccharum*) (Figure 1). The manmade pond was surrounded by black raspberry (*Rubus occidentalis*) and unidentified grasses.

Sample sites were labelled 1 through 8. Sites 1, 2, 6, 7, and 8 were collected from the open portion of the lot, with Sample Site 8 being slightly elevated as if debris had been dumped there at some point in the past and having the most ant activity (Figure 2). Sample Site 3 was omitted from this study as it consisted of a tree containing one or more colonies of wood nesting ants, likely belonging to the genus *Camponotus*, which are not the focus of this research. Sample Site 4, containing a colony of the common ant, *Aphaenogaster rudis,* with tunnel openings beneath a rock, was located at the base of a mature *Acer saccharum.* Sample Site 5 was located on the edge of the pond and also exhibited a relatively high level of ant activity. Ants in the genus *Myrmica* inhabited Sample Sites 1, 2 and 5. *Aphaenogaster* inhabited Sample Sites 4, 6, 7 and 8.

An open area was selected for the sample site to optimize visibility for locating ant hills. Colonies needed to be dispersed enough to minimize overlap between colonies to ensure that I was collecting colony specific data. Although I made every effort to collect colony-specific data, this location had many colonies so possibly overlapping boundaries cannot be fully accounted for.

Sample collection. Samples were collected using a 17.5 cm wide steel shovel to carefully cut and extract 8 cm squares of soil 10 cm or deeper at 0, 1, and 2 m from each ant hill evaluated, following methods adapted from Jacquemin *et al.* (2012), Anderson *et al.* (2010), Dostál *et al.* (2005) and Frouz *et al.* (2003). Samples were carefully placed into clear plastic Ziploc® bags to ensure they maintained their structure, and secured in boxes for transport to the laboratory. The 0 m sample was collected directly from the primary tunnel opening of the colony. The 1 m and 2 m samples were taken along a transect in three directions radiating out from the primary tunnel opening of the colony roughly 50-110º apart forming a "Y" shape with the primary tunnel opening of the colony at the intersection of the three rows (Figure 3).

Elemental soil analysis-- X-ray fluorescence (XRF). Samples were returned to the University of Southern Maine's soil chemistry lab in Gorham and stored in Ziploc® bags at room temperature until used for analysis. Samples were prepared by dividing each vertically at 2 cm increments starting from the ground surface to the sample's depth. Sample preparation consisted of drying fractions in an oven at 37 ºC for 1-7 days with an average drying time of approximately two days. Rocks and sticks were removed before dried samples were ground in a mortar and pestle and sieved using a 1 mm^2 mesh. Samples were then placed in 12 mL MC-1520 Premier Lab Supply XRF cups (Fort St. Lucie, FL), covered on the bottom with Spectrocertified thin-film 6.0 µm gauge Mylar polyester (Chemplex, Palm City, FL) and capped with a fitted plastic cover. The cups were filled nearly to maximum capacity with the dried and sieved samples. For the samples collected at 0 m, triplicates were prepared from each vertical fraction to allow for calculating an average from each distance measured. The prepared samples were evaluated using a Thermo Niton XL3t X-Ray Fluorescence Spectrometer set to soil analysis in benchtop mode. Samples were examined for a 90 second interval and measurements reported in ppm.

Figure 1: Samples were collected from different habitats within 50 m of each other in order to evaluate elemental concentrations as a function of depth and distance from ant hills across varied communities and conditions. **A**: Sample Site 1 is located in an open field that once was a recreational vehicle park. This location was primarily populated with an assortment of grasses, wild strawberries (*Fragaria* sp.), black-eyed Susans (*Rudbeckia hirta*), Queen Anne's lace (*Daucus carota*), red clover (*Trifolium pratense*), goldenrod (*Solidago* sp.), many flowered aster (*Symphyotrichum ericoides*), burdock (*Arctium* sp.), common dandelion (*Taraxacum officinale*), sensitive fern (*Onoclea sensibilis*), sapling white pine (*Pinus strobus*), quaking aspen (*Populus tremuloides*) and milkweed (*Asclepias* sp.). **B**: Sample Site 2, located in the same field as Sample Site 1. **C**: Sample Site 5, located amongst black raspberries (*Rubus occidentalis*) at the edge of a manmade pond. **D**: Sample Site 4, located at the base of a sugar maple (*Acer saccharum*).

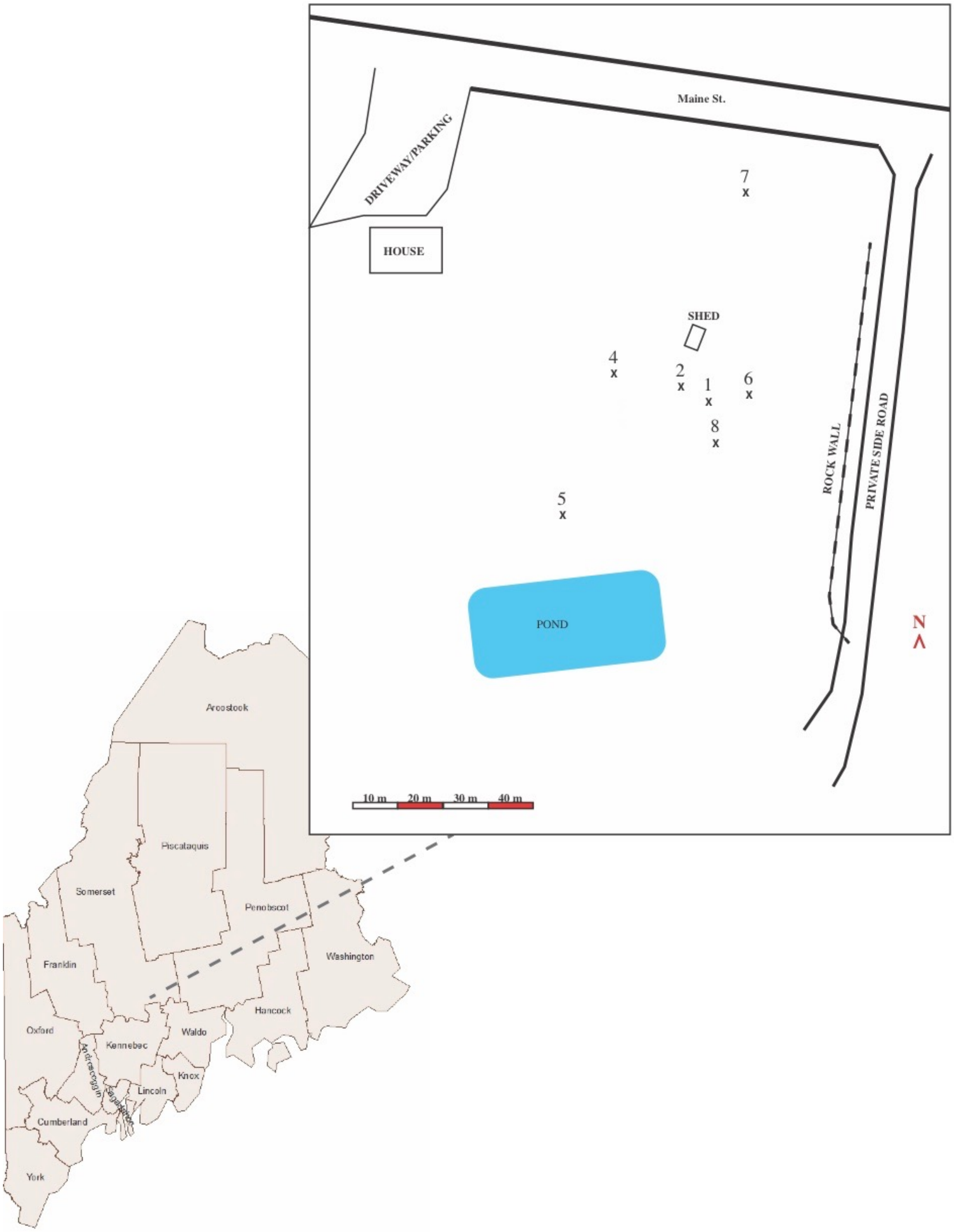

Figure 2: Sample Site Map. Samples were collected from Canaan, Somerset County. Adapted from "Maine County Selection Map" United States Department of Agriculture. Adapted from UNH Carsey School of Public Policy. Demographic Trends in U.S. Counties: Maine County Selection Map. Site inhabited with Aphaenogaster rudis: 4, 6, 7, 8. Sites inhabited with members of Myrmica: 1, 2, 5. Sample Site 3 was omitted from this study.

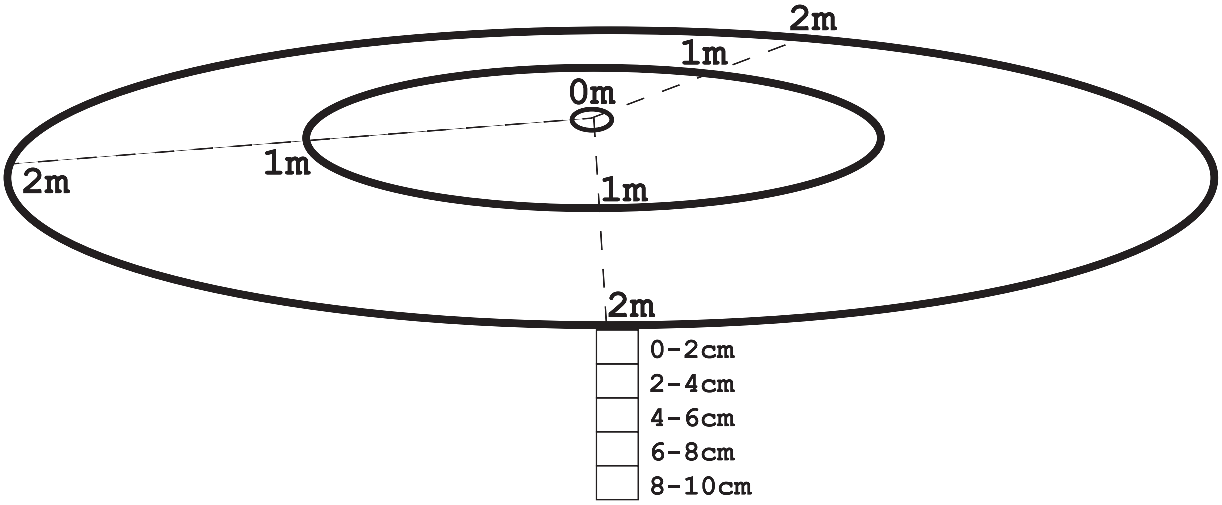

Figure 3: General schematic of the sample layout for each colony. Three samples were collected from each sample site at 0 m (the primary tunnel opening to the colony), 1 m, and 2 m from the ant hills that make up each sample site to a depth of 10 cm. Compass direction was variable for each transect. Samples were later divided vertically into 2 cm increments in The University of Southern Maine's soil chemistry lab in Gorham, Maine.

Elemental soil analysis-- atomic absorbance (AA). In this project, select samples were further analyzed *via* atomic absorbance in order to assess the functionality of this multistep approach to elemental analysis. Atomic absorbance has greater resolution than XRF, but requires a more extensive sample preparation procedure (VanCott, McDonald & Seelos 1999). A multistep approach that involves both analytical methods allows for the speed of XRF to be combined with the precision of AA, where samples are quickly evaluated using XRF before undergoing further analysis with AA for select samples and elements of interest. One sample from each transect 0 m, 1 m, and 2 m making up one of the "Y" shaped arms from each Sample Sites 5 and 8 were used for this assessment. Sample Site 5 was inhabited by members of *Myrmica* and Sample Site 8 inhabited by *Aphaenogaster rudis.*

Samples were taken from XRF cups after undergoing XRF analysis. Samples were prepared using a method adapted from EPA standard method 3050B (1996). All glassware used during AA was soaked in 5% nitric acid overnight before use. Each sample was divided into triplicates each roughly 80 mg in weight. To digest the samples and free the metals they contained, 10 mL of each concentrated nitric acid (Fisher Scientific) and 30% hydrogen peroxide (Fisher Scientific) were added to the soil sample. Each sample was then boiled for 1 hour before centrifuging and diluting to 25 mL using 5% nitric acid. In addition to the soil samples, ants collected from each sample site were also analyzed. For each sample site, 10-15 mg of ants were digested in 3 mL of each concentrated nitric acid and 30% hydrogen peroxide and boiled for an hour before diluting to 10 mL with 5% nitric acid. Both soil samples and ant bodies were analyzed using Thermo Scientific iCE 3000 Series Flame Atomic Absorption Spectrometer. Calibration curves were formed using 1000 ppm stock solutions of calcium, copper, and zinc (Fisher Scientific). Standards were prepared at 0.5 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 10 ppm (Appendix E). The cathode lamps used operated at wavelengths of 422.7 nm for calcium, 324.8 nm for copper, and 213.9 nm for zinc. Concentrations were determined using Beer's Law and Equations 1 and 2 (adapted from JoVE Science Education Database 2019, Appendix E). Samples were ionized using a flame fueled by acetylene and air.

Samples were analyzed for three elements *via* flame atomic absorbance: calcium, copper, and zinc. Calcium was used as a reference between XRF and AA because the standard deviation for this element was the lowest of those analyzed by XRF at 2%. Copper and zinc both had standard deviations greater than 20% for XRF and so required further analysis.

Statistical analysis. Variance among sites for XRF was conducted using a factorial 2 x 1 ANOVA with genus (Table 1) and combined distance & depth (Table 2) as the two factors and elemental concentration as the dependent variable. The following hypotheses were tested to determine if there were significant differences in elemental concentration among individual factors and interactions between factors and individual levels within factors:

Hypothesis 1: Distance & Depth. Elemental concentrations will differ between soil samples for each distance and depth.

Hypothesis 2: Genera. Elemental concentrations will differ between soil samples for each genus inhabiting the sample site.

Hypothesis 3: Genera x Distance & Depth. Elemental concentrations will vary based on combined effects of genera, distance and depth.

AA results were analyzed using a one-way ANOVA with genera and both distance and depth serving as independent variables and elemental concentration serving as the dependent variable. For data obtained *via* AA, post-hoc analysis was done using t-tests and corrected for alpha based on the number of data sets being compared (0.5/N) to compare to the p-value. Data analysis for XRF results were performed using Statistica version 13 software. Data analysis for AA results and comparisons between AA and XRF results were performed using and Microsoft Excel version 15.32. The following hypotheses were tested to determine if there were differences between individual general and combined distance and depth at each level:

Hypothesis 4: Distance & Depth. Elemental concentrations will differ between soil samples for each distance and depth.

Hypothesis 5: Genera. Elemental concentrations will differ between soil samples for each genus inhabiting the sample site.

Two hypotheses were considered for potential relationships between metal content in ant bodies and the soils they nest in:

Hypothesis 6: No relationship. Elemental concentrations in ant bodies and the soils they live in have no relationship.

Hypothesis 7: Elemental concentrations in soil correlate with concentration in ant bodies. Elemental concentrations in both soil samples and ant bodies will correlate.

Statistical analysis was not conducted on ant body samples due to insufficient sample size.

Table 1: Genera consisting of two levels.

Levels	Description
1	*Aphaenogaster*
2	*Myrmica*

Table 2: Genera consisting of 15 levels for combined Distance & Depth.

Levels	Description
1	Entrance 0-2 cm
2	1 m: 0-2 cm
3	2 m: 0-2 cm
4	Entrance 2-4 cm
5	1 m: 2-4 cm
6	2 m: 2-4 cm
7	Entrance 4-6 cm
8	1 m: 4-6 cm
9	2 m: 4-6 cm
10	Entrance 6-8 cm
11	1 m: 6-8 cm
12	2 m: 6-8 cm
13	Entrance 8-10 cm
14	1 m: 8-10 cm
15	2 m: 8-10 cm

Chapter 3

Results

Overview. Through this study, I sought to gain a better understanding on the role of subterranean nesting ants on nutrient cycling through elemental analysis of the soils in and around their colony. The results from both X-ray fluorescence and atomic absorbance indicate no significant relationship of concentration of the elements tested as a function of either depth or distance, with ANOVA giving $P > 0.05$ (Figures 4 through Figure 11, Tables 3, 5 and 6). X-ray fluorescence spectrometry results show concentrations of iron, manganese and zinc were significantly higher in *Aphaenogaster rudis* than in *Myrmica* when averages were taken across all sample sites, distances and depths combined (Table 4). The mean concentration of potassium was lower in *Aphaenogaster rudis* than in *Myrmica* when averages were taken across all sample sites, distances and depths combined (Table 4). Copper was below the detection limit for XRF and thus there was no data to report.

While XRF did not show a statistical difference between genera and concentrations of calcium, with $P = 0.507$ (Table 3), AA revealed significantly elevated concentrations of calcium in soils inhabited by *Myrmica* at Sample Site 5 relative to *Aphaenogaster* at Sample Site 8 (Figure 9, Tables 1 and 3). However, one must bear in mind the small number of samples analyzed and the overall differences in the characteristics of the two sample sites tested. Sample Site 5 was located at the edge of a manmade pond and had far higher clay content than Sample Site 8, so the differences in content found through AA may not be genus specific.

Copper was not able to be detected through XRF, however it was detected by AA, revealing a statistically significant ($P < 0.05$) trend in Sample Site 8 with concentrations decreasing as a function of distance to a depth of 6 cm (Figure 10, Table 5). There were no significant differences found as a function of depth or genus ($P > 0.05$) (Figure 10, Tables 3 and 5). The findings for zinc by AA support those by XRF with sample sites associated with *Aphaenogaster rudis* showing significantly higher concentrations of zinc than those associated with *Myrmica* (Figure 11, Tables 3, 5 and 6).

For all samples sites analyzed by AA, XRF indicated the presence of overall higher concentrations of calcium than was suggested by results obtained through AA (Figure 8 and Figure 9, Table 4, Appendices A and B). Both forms of instrumentation gave very similar results for concentrations of zinc (Figure 6 and Figure 11). It is not unreasonable to suspect that calcium, due to matrix effects, may be more difficult to ionize in the flame than zinc (Skoog, Douglus, Holler, James, Nieman, & Timothy 1998). Two common functional groups that could cause this include sulfates and phosphates (Skoog *et al.* 1998). To avoid this, additional chemicals can be added that bind to sulfates and phosphates to free calcium for ionization.

Ant bodies analyzed by AA exhibited higher concentrations in calcium, copper, and zinc in ants belonging to *Myrmica* collected from Sample Site 5 than samples analyzed from *Aphaenogaster rudis,* collected from Sample Site 8 (Table 9, Appendix D). Ants from Sample Site 5 had roughly five times the concentration of calcium and zinc than ants from Sample Site 8, and three times as much copper. Calcium and copper are found to have higher concentrations in soil samples from Sample Site 5 relative to Sample Site 8, and zinc in higher concentrations in soil samples from Sample Site 8 relative to Sample Site 5. Because ants from Sample Site 5 have consistently higher concentrations in the elements tested relative to ants from Sample Site 8, I speculate that this observation is the result of undescribed species specific characteristics as opposed to a direct relationship with the soils the colonies inhabit.

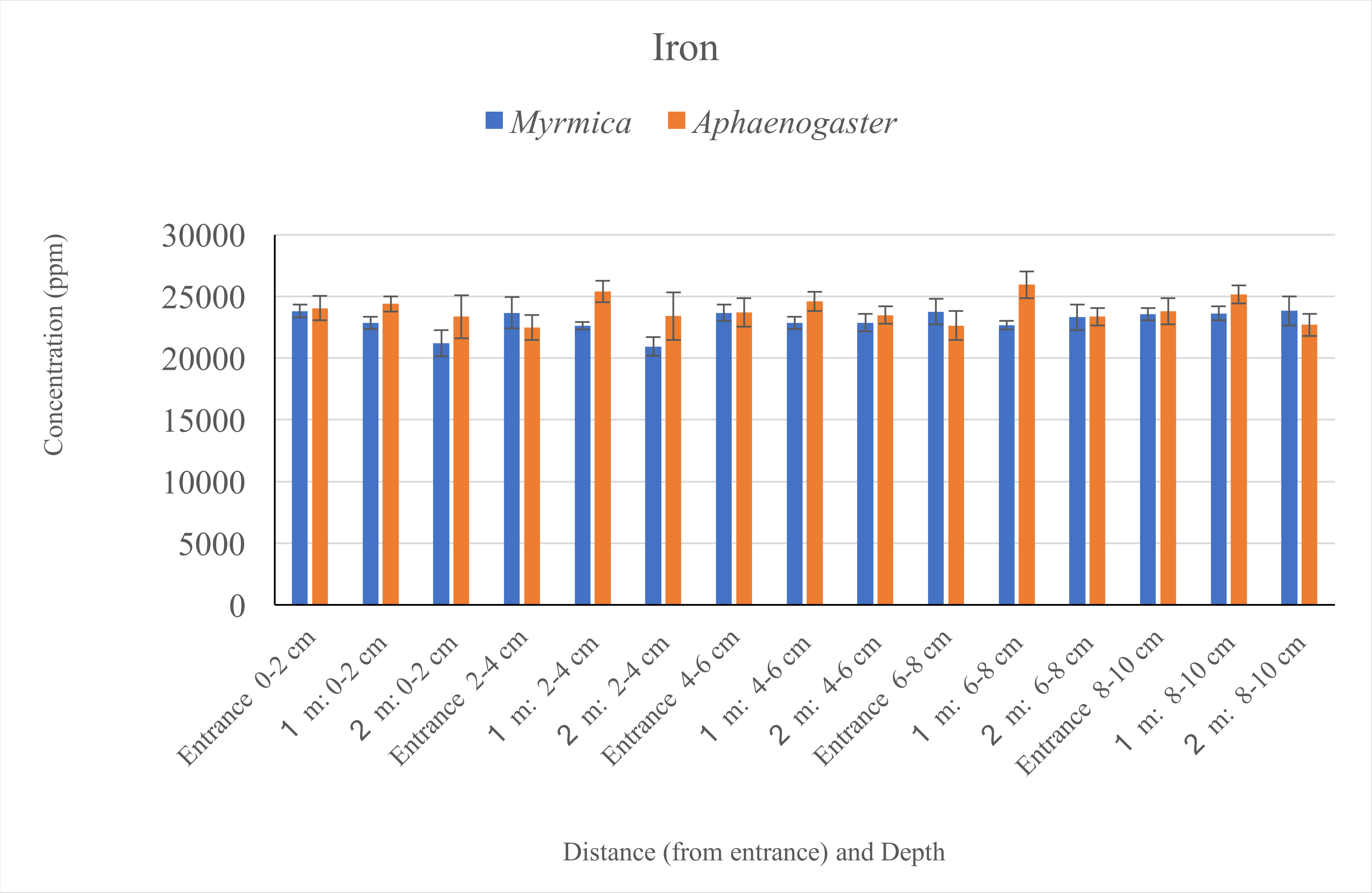

Figure 4: X-Ray Fluorescence results: iron as a function of each distance and depth in *Aphaenogaster rudis* (orange) and *Myrmica* (blue). Error bars represent standard error. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Entrance 0-2 cm N = 18, 1 m 0-2 cm N = 21, 2 m 0-2 cm N = 21, Entrance 2-4 cm N = 18, 1 m 2-4 cm N = 21, 2 m 2-4 cm N = 21, Entrance 4-6 cm N = 17, 1 m 4-6 cm N = 21, 2 m 4-6 cm N =21 , Entrance 6-8 cm N = 17, 1 m 6-8 cm N = 21, 2 m 6-8 cm N = 21, Entrance 8-10 cm N = 15, 1 m 8-10 cm N = 19, 2 m 8-10 cm N = 20.

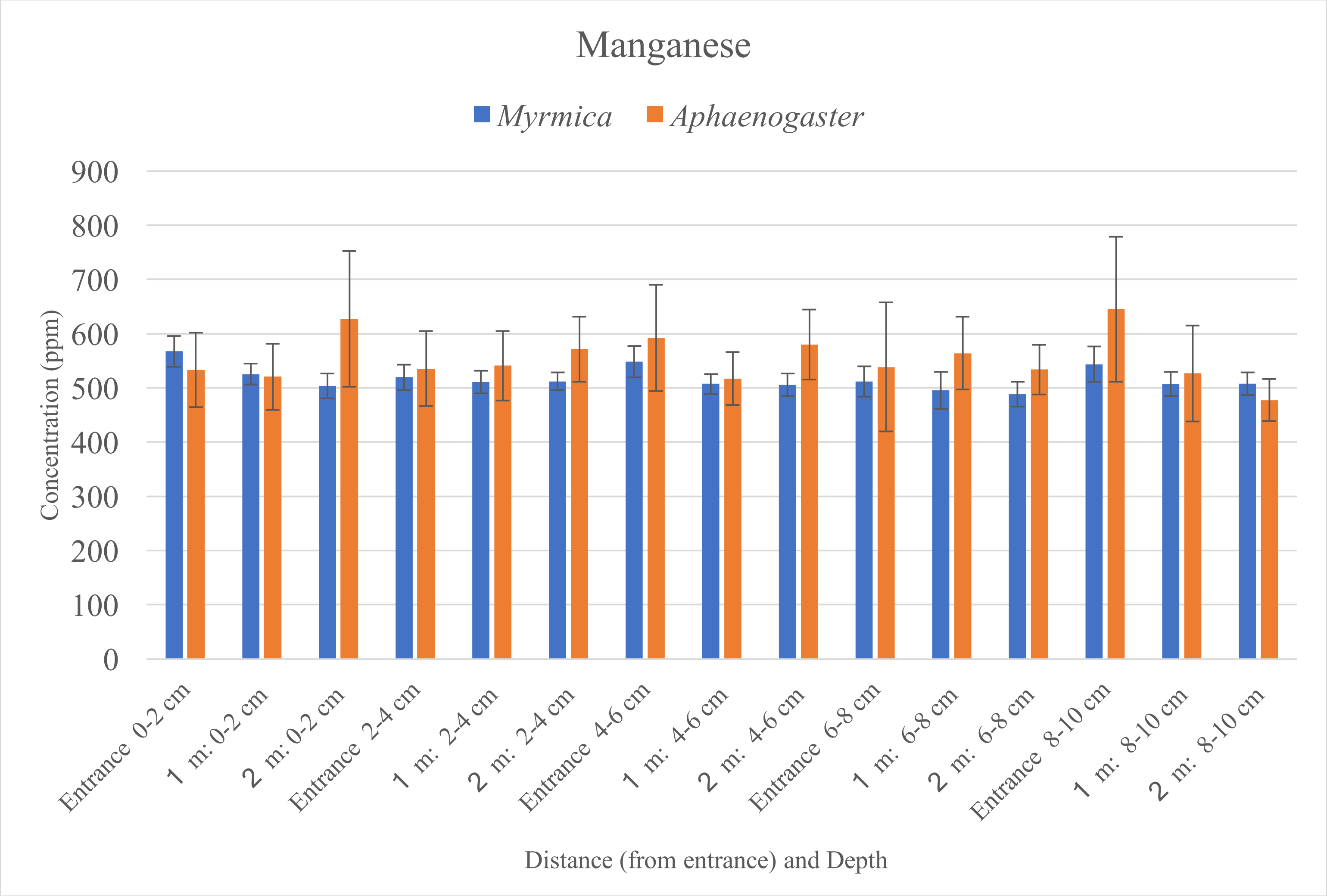

Figure 5: X-Ray Fluorescence results: manganese as a function of each distance and depth in Aphaenogaster rudis (orange) and Myrmica (blue). Error bars represent standard error. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Entrance 0-2 cm N = 18, 1 m 0-2 cm N = 21, 2 m 0-2 cm N = 21, Entrance 2-4 cm N = 18, 1 m 2-4 cm N = 21, 2 m 2-4 cm N = 21, Entrance 4-6 cm N = 17, 1 m 4-6 cm N = 21, 2 m 4-6 cm N =21 , Entrance 6-8 cm N = 17, 1 m 6-8 cm N = 21, 2 m 6-8 cm N = 21, Entrance 8-10 cm N = 15, 1 m 8-10 cm N = 19, 2 m 8-10 cm N = 20.

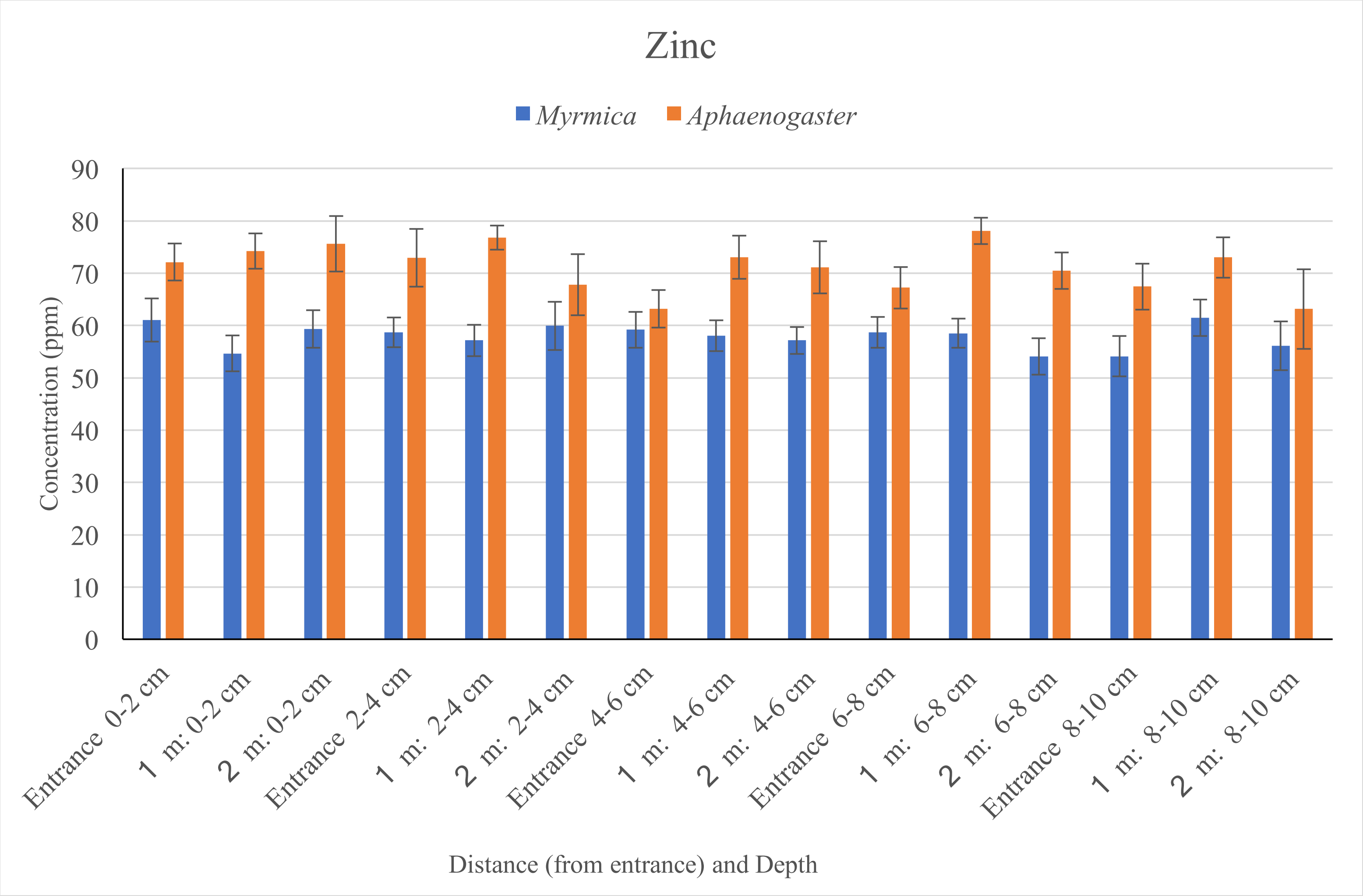

Figure 6: X-Ray Fluorescence results: zinc as a function of each distance and depth in *Aphaenogaster rudis* (orange) and *Myrmica* (blue). Error bars represent standard error. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Entrance 0-2 cm N = 18, 1 m 0-2 cm N = 21, 2 m 0-2 cm N = 21, Entrance 2-4 cm N = 18, 1 m 2-4 cm N = 21, 2 m 2-4 cm N = 21, Entrance 4-6 cm N = 17, 1 m 4-6 cm N = 21, 2 m 4-6 cm N =21 , Entrance 6-8 cm N = 17, 1 m 6-8 cm N = 21, 2 m 6-8 cm N = 21, Entrance 8-10 cm N = 15, 1 m 8-10 cm N = 19, 2 m 8-10 cm N = 20.

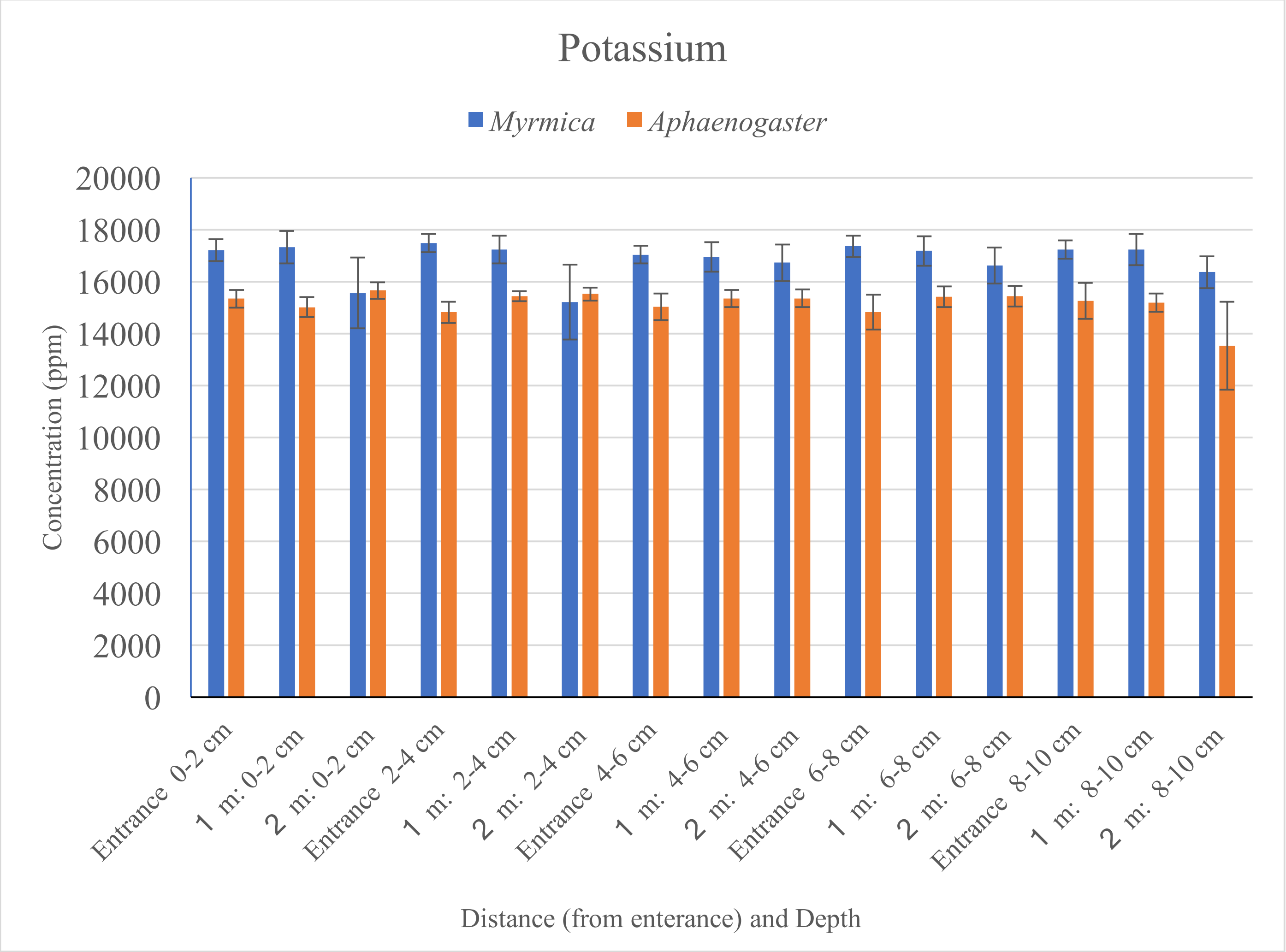

Figure 7: X-Ray Fluorescence results: potassium as a function of each distance and depth in *Aphaenogaster* (orange) and *Myrmica* (blue). Error bars represent standard error. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Entrance 0-2 cm N = 18, 1 m 0-2 cm N = 21, 2 m 0-2 cm N = 21, Entrance 2-4 cm N = 18, 1 m 2-4 cm N = 21, 2 m 2-4 cm N = 21, Entrance 4-6 cm N = 17, 1 m 4-6 cm N = 21, 2 m 4-6 cm N =21 , Entrance 6-8 cm N = 17, 1 m 6-8 cm N = 21, 2 m 6-8 cm N = 21, Entrance 8-10 cm N = 15, 1 m 8-10 cm N = 19, 2 m 8-10 cm N = 20.

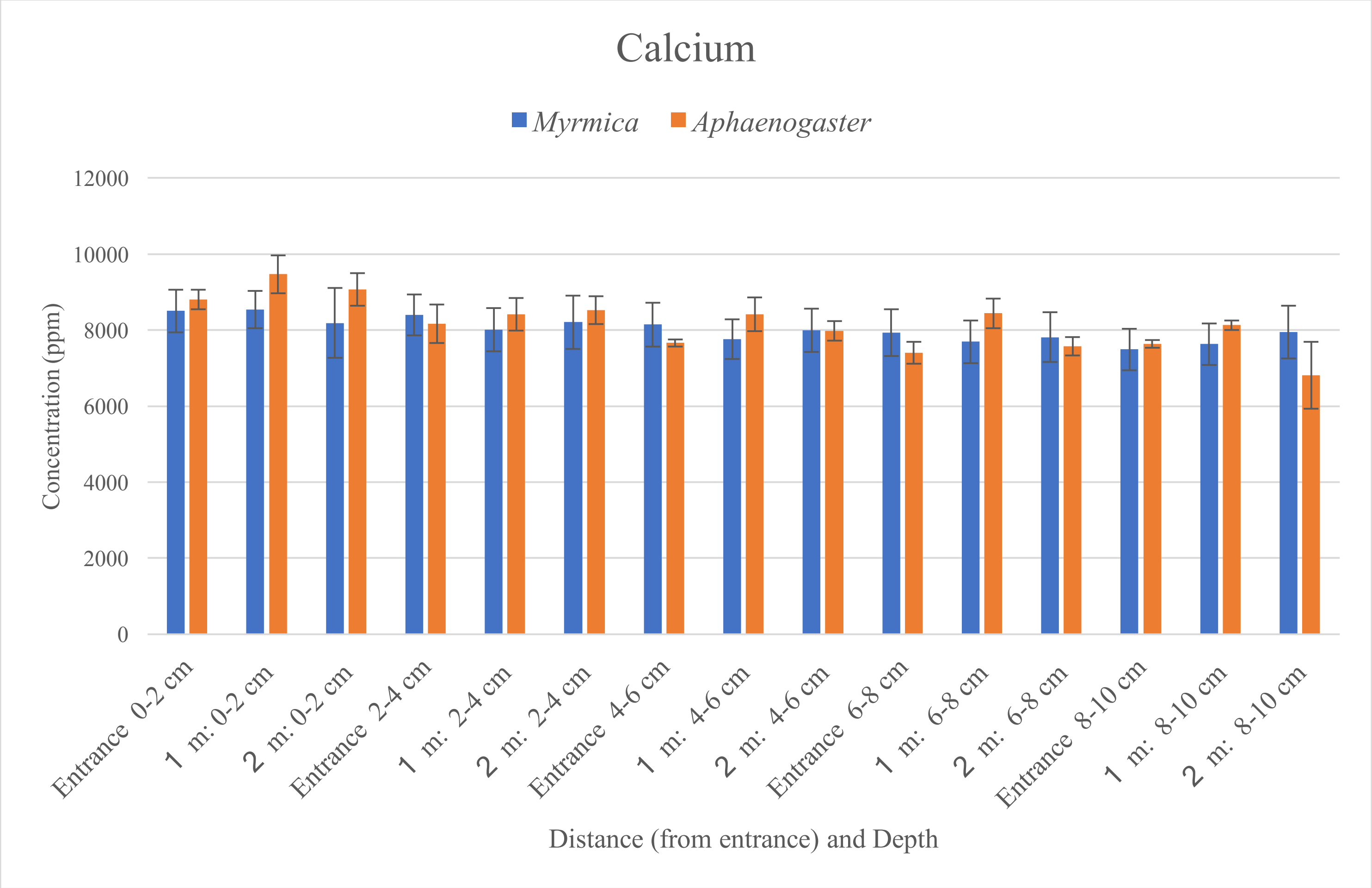

Figure 8: X-Ray Fluorescence results: calcium as a function of each distance and depth in *Aphaenogaster rudis* (orange) and *Myrmica* (blue). Error bars represent standard error. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Entrance 0-2 cm N = 18, 1 m 0-2 cm N = 21, 2 m 0-2 cm N = 21, Entrance 2-4 cm N = 18, 1 m 2-4 cm N = 21, 2 m 2-4 cm N = 21, Entrance 4-6 cm N = 17, 1 m 4-6 cm N = 21, 2 m 4-6 cm N =21 , Entrance 6-8 cm N = 17, 1 m 6-8 cm N = 21, 2 m 6-8 cm N = 21, Entrance 8-10 cm N = 15, 1 m 8-10 cm N = 19, 2 m 8-10 cm N = 20.

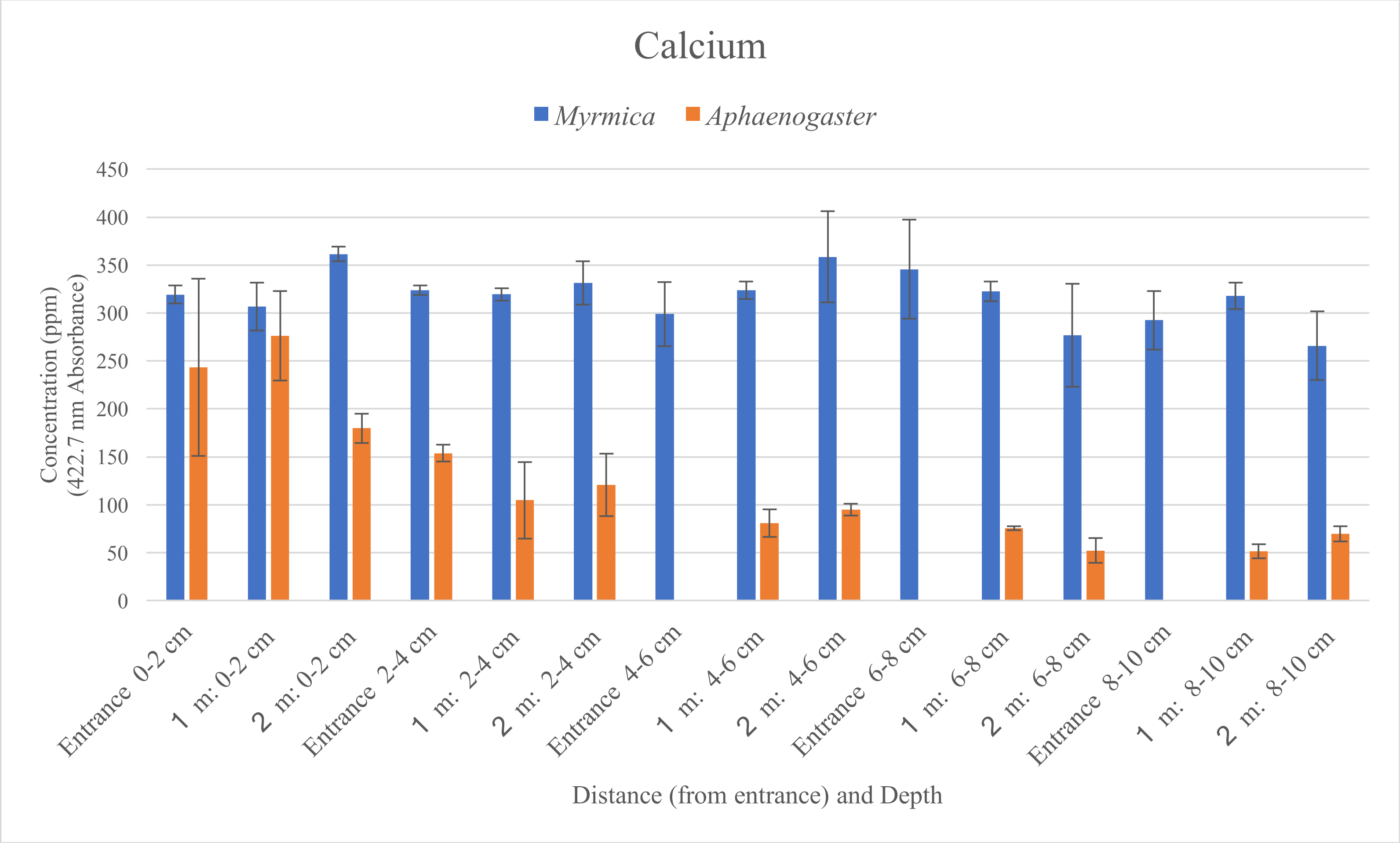

Figure 9: Atomic Absorbance results: calcium as a function of each distance and depth in *Aphaenogaster rudis* (orange, Sample Site 8) and *Myrmica* (blue, Sample Site 5). Error bars represent standard deviation. Samples from the tunnel entrance at Site 8 beyond a depth of 4 cm were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth.

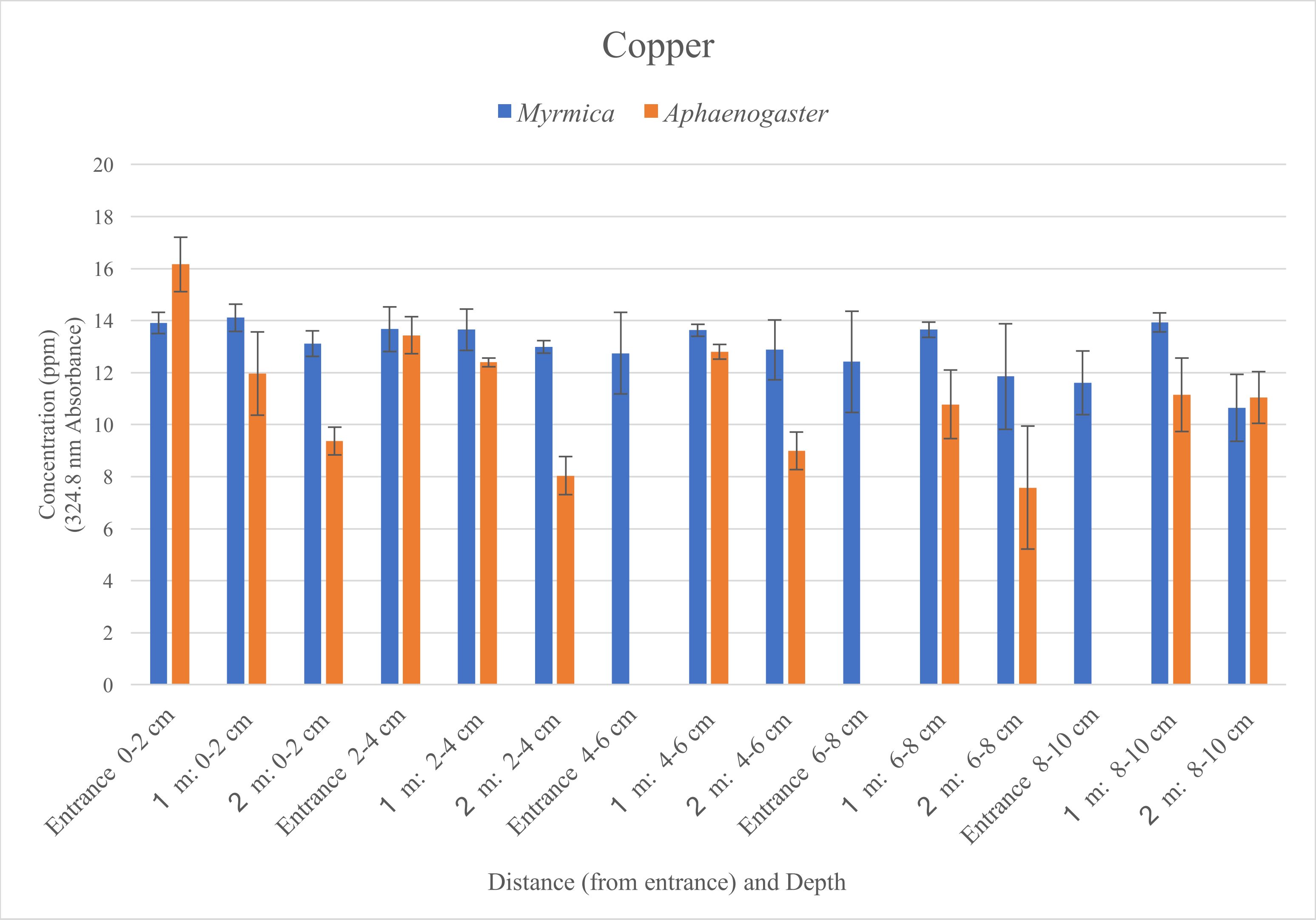

Figure 10: Atomic Absorbance results: copper as a function of each distance and depth in *Aphaenogaster rudis* (orange, Sample Site 8) and *Myrmica* (blue, Sample Site 5). Error bars represent standard deviation. Samples from the tunnel entrance at Site 8 beyond a depth of 4 cm were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth.

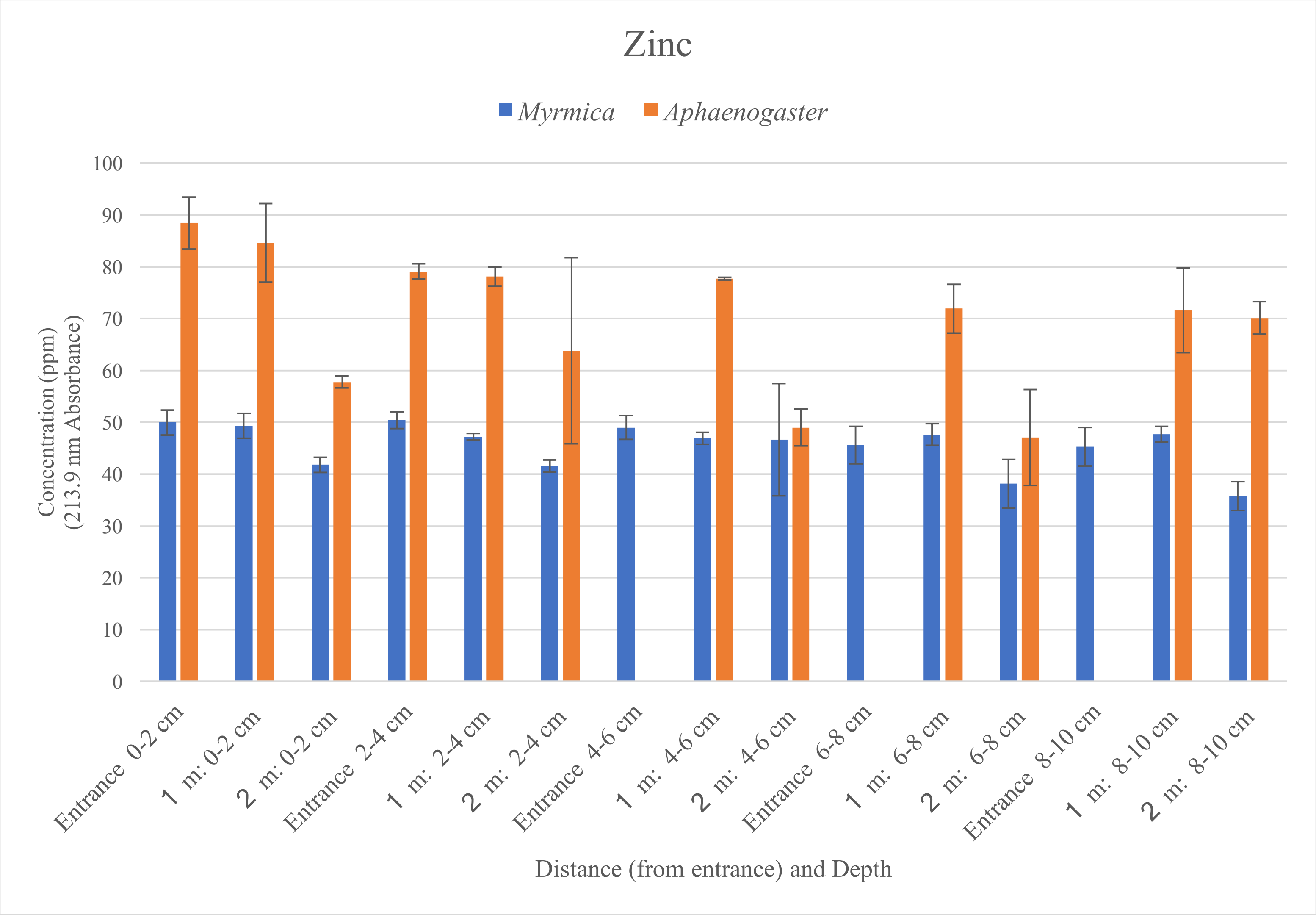

Figure 11: Atomic Absorbance results: zinc as a function of each distance and depth in *Aphaenogaster ruids* (orange, Sample Site 8) and *Myrmica* (blue, Sample Site 5). Error bars represent standard deviation. Samples from the tunnel entrance at Site 8 beyond a depth of 4 cm were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth.

Table 3: XRF ANOVA results. Each Genus and Depth + Distance results were analyzed with one-way ANOVA. Genus x Depth + Distance results were analyzed via two-way factorial ANOVA. Bold text indicates statistically significant results. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth

Element	Intendent Factors	*df*	*F*	*P*
Zinc	**Genus**	**1**	**76.756**	**0**
	Depth+Dist	14	0.842	0.62
	Genus x (Depth+Dist)	14	0.693	0.78
	Error	263		
Iron	**Genus**	**1**	**4.93**	**0.02**
	Depth+Dist	14	0.76	0.71
	Genus x (Depth+Dist)	14	0.92	0.54
	Error	263		
Manganese	**Genus**	**1**	**4.226**	**0.04**
	Depth+Dist	14	0.534	0.91
	Genus x (Depth+Dist)	14	0.419	0.96
	Error	261		
Calcium	Genus	1	0.442	0.50
	Depth+Dist	14	1.111	0.35
	Genus x (Depth+Dist)	14	0.492	0.93
	Error	263		
Potassium	**Genus**	**1**	**39.16**	**0**
	Depth+Dist	14	0.61	0.85
	Genus x (Depth+Dist)	14	0.79	0.67
	Error	264		

Table 4: Mean overall concentrations for individual metals by genera for each XRF and AA results. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. LOD = limit of detection. nd = no data.

Aphaenogaster rudis:	Ca	Cu	Fe	K	Mn	Zn	N
XRF Mean (ppm)	8214.96	LOD	23974.93	15159.03	551.67	71.60	294
XRF Mean Standard Deviation	126.28		213.75	161.05	18.93	1.19	
AA Mean (ppm)			nd	nd	nd	nd	36
AA Mean Standard Deviation			nd	nd	nd	nd	
Myrmica:							
XRF Mean (ppm)	8022.25	LOD	23013.91	16847.00	516.80	57.91	294
XRF Mean Standard Deviation	153.45		277.35	184.83	6.27	0.88	
AA Mean (ppm)			nd	nd	nd	nd	45
AA Mean Standard Deviation			nd	nd	nd	nd	

Table 5: AA one-way ANOVA results for distance. Samples with P < 0.05 are considered statistically significant. Samples with F < 0.05 are considered statistically significant. Samples from the tunnel entrance at Site 8 beyond a depth of 4 cm were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Source of variation is reported within and between groups of the same depth across 0 m, 1 m, and 2 m distances for each sample site.

	Source of Variation	*df*	*F*	*P-value*
Copper: Distance SITE 8				
(0-2) cm	Between Groups	2.00	8.81	0.02
	Within Groups	6.00		
	Total	8.00		
(2-4) cm	Between Groups	2.00	45.37	0.00
	Within Groups	6.00		
	Total	8.00		
(4-6) cm	Between Groups	1.00	48.90	0.00
	Within Groups	4.00		
	Total	5.00		
(6-8) cm	Between Groups	1.00	2.80	0.17
	Within Groups	4.00		
	Total	5.00		
(8-10) cm	Between Groups	1.00	0.01	0.93
	Within Groups	4.00		
	Total	5.00		
Copper: Distance SITE 5				
(0-2) cm	Between Groups	2.00	2.43	0.17
	Within Groups	6.00		
	Total	8.00		
(2-4) cm	Between Groups	2.00	0.64	0.56
	Within Groups	6.00		
	Total	8.00		
(4-6) cm	Between Groups	2.00	0.35	0.72
	Within Groups	6.00		
	Total	8.00		
(6-8) cm	Between Groups	2.00	0.64	0.56
	Within Groups	6.00		
	Total	8.00		
(8-10) cm	Between Groups	2.00	5.18	0.05
	Within Groups	6.00		
	Total	8.00		
Calcium: Distance SITE 8				
(0-2) cm	Between Groups	2.00	1.32	0.33

Table 6, continued.

	Source of Variation	*df*	*F*	*P-value*
	Within Groups	6.00		
	Total	8.00		
(2-4) cm	Between Groups	2.00	1.39	0.32
	Within Groups	6.00		
	Total	8.00		
(4-6) cm	Between Groups	1.00	1.61	0.27
	Within Groups	4.00		
	Total	5.00		
(6-8) cm	Between Groups	1.00	6.07	0.07
	Within Groups	4.00		
	Total	5.00		
(8-10) cm	Between Groups	1.00	5.62	0.08
	Within Groups	4.00		
	Total	5.00		
Calcium: Distance SITE 5				
(0-2) cm	Between Groups	2.00	6.47	0.03
	Within Groups	6.00		
	Total	8.00		
(2-4) cm	Between Groups	2.00	0.39	0.69
	Within Groups	6.00		
	Total	8.00		
(4-6) cm	Between Groups	2.00	1.55	0.29
	Within Groups	6.00		
	Total	8.00		
(6-8) cm	Between Groups	2.00	1.30	0.34
	Within Groups	6.00		
	Total	8.00		
(8-10) cm	Between Groups	2.00	1.69	0.26
	Within Groups	6.00		
	Total	8.00		
Zinc: Distance SITE 8				
(0-2) cm	Between Groups	2.00	19.92	0.00
	Within Groups	6.00		
	Total	8.00		
(2-4) cm	Between Groups	2.00	1.35	0.33
	Within Groups	6.00		
	Total	8.00		
(4-6) cm	Between Groups	1.00	310.76	0.00
	Within Groups	4.00		
	Total	5.00		
(6-8) cm	Between Groups	1.00	11.44	0.03

Table 6, continued.

	Source of Variation	*df*	*F*	*P-value*
	Within Groups	4.00		
	Total	5.00		
(8-10) cm	Between Groups	1.00	0.06	0.82
	Within Groups	4.00		
	Total	5.00		
Zinc: Distance SITE 5				
(0-2) cm	Between Groups	2.00	8.81	0.02
	Within Groups	6.00		
	Total	8.00		
(2-4) cm	Between Groups	2.00	28.12	0.00
	Within Groups	6.00		
	Total	8.00		
(4-6) cm	Between Groups	2.00	0.08	0.93
	Within Groups	6.00		
	Total	8.00		
(6-8) cm	Between Groups	2.00	3.83	0.08
	Within Groups	6.00		
	Total	8.00		
(8-10) cm	Between Groups	2.00	10.08	0.01
	Within Groups	6.00		
	Total	8.00		

Table 6: AA one-way ANOVA results for depth. Samples with $P < 0.05$ are considered statistically significant. Samples with $F < 0.05$ are considered statistically significant. Samples from the tunnel entrance at Site 8 beyond a depth of 4 cm were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Source of variation is reported within and between groups of the same distance across 0-2 cm, 2-4 cm, 4-6 cm, 6-8 cm, and 8-10 cm depths for each sample site.

	Source of Variation	*df*	*F*	*P-value*
Copper: Depth				
SITE 8 0 m	Between Groups	1.00	9.15	0.04
	Within Groups	4.00		
	Total	5.00		
SITE 8 1 m	Between Groups	4.00	1.12	0.40
	Within Groups	10.00		
	Total	14.00		
SITE 8 2 m	Between Groups	4.00	2.29	0.13
	Within Groups	10.00		
	Total	14.00		
SITE 5 0 m	Between Groups	4.00	1.01	0.45
	Within Groups	10.00		
	Total	14.00		
SITE 5 1 M	Between Groups	4.00	0.40	0.81
	Within Groups	10.00		
	Total	14.00		
SITE 5 2 M	Between Groups	4.00	1.50	0.27
	Within Groups	10.00		
	Total	14.00		

	Source of Variation	*df*	*F*	*P-value*
Calcium: Depth				
SITE 8 0 m	Between Groups	1.00	1.87	0.24
	Within Groups	4.00		
	Total	5.00		
SITE 8 1 m	Between Groups	4.00	20.29	0.00
	Within Groups	10.00		
	Total	14.00		
SITE 8 2 m	Between Groups	4.00	15.97	0.00
	Within Groups	10.00		
	Total	14.00		
SITE 5 0 m	Between Groups	4.00	0.92	0.49
	Within Groups	10.00		
	Total	14.00		

Table 6, continued.

	Source of Variation	*df*	*F*	*P-value*
SITE 5 1 M	Between Groups	4.00	0.45	0.77
	Within Groups	10.00		
	Total	14.00		
SITE 5 2 M	Between Groups	4.00	2.91	0.08
	Within Groups	10.00		
	Total	14.00		
Zinc: Depth				
SITE 8 0 m	Between Groups	1.00	6.34	0.07
	Within Groups	4.00		
	Total	5.00		
SITE 8 1 m	Between Groups	4.00	1.92	0.18
	Within Groups	10.00		
	Total	14.00		
SITE 8 2 m	Between Groups	4.00	1.82	0.20
	Within Groups	10.00		
	Total	14.00		
SITE 5 0 m	Between Groups	4.00	1.47	0.28
	Within Groups	10.00		
	Total	14.00		
SITE 5 1 M	Between Groups	4.00	0.60	0.67
	Within Groups	10.00		
	Total	14.00		
SITE 5 2 M	Between Groups	4.00	1.13	0.40
	Within Groups	10.00		
	Total	14.00		

Table 7: AA, mean value of triplicates. Samples from the tunnel entrance at Site 8 beyond a depth of 4 cm were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. The first number in the sample name (leftmost column) denotes the sample site, the second number the distance from the tunnel openings of the colony, and the third and fourth the depth range of the fraction evaluated.

Sample site, distance and depth	mean Ca (ppm)	mean Cu (ppm)	mean Zn (ppm)
8-0 0-2	243.51	16.16	88.47
8-0 2-4	153.81	13.44	79.12
8-1B 0-2	276.36	11.96	84.64
8-1B 2-4	104.53	12.40	78.13
8-1B 4-6	80.74	12.81	77.75
8-1B 6-8	75.38	10.78	71.91
8-1B 8-10	51.26	11.15	71.60
8-2B 0-2	179.70	9.37	57.78
8-2B 2-4	120.67	8.04	63.76
8-2B 4-6	94.82	8.99	48.96
8-2B 6-8	52.23	7.57	47.07
8-2B 8-10	69.54	11.04	70.09
5-0B 0-2	319.33	13.90	49.94
5-0B 2-4	323.56	13.67	50.40
5-0B 4-6	298.90	12.75	48.96
5-0B 6-8	345.65	12.42	45.60
5-0B 8-10	292.42	11.62	45.24
5-1B 0-2	306.60	14.11	49.28
5-1B 2-4	319.47	13.65	47.19
5-1B 4-6	323.79	13.63	46.93
5-1B 6-8	322.81	13.65	47.63
5-1B 8-10	317.94	13.92	47.66
5-2B 0-2	361.64	13.12	41.81
5-2B 2-4	331.58	12.99	41.56
5-2B 4-6	358.56	12.88	46.62
5-2B 6-8	276.82	11.85	38.12
5-2B 8-10	265.92	10.64	35.73

Table 8: AA 1-way ANOVA two-tailed post-hoc t-test results. Post-hoc analysis was conducted on all AA results with P < 0.05.

Calcium, Sample Site 8, Significance of depth at each distance

1 m (0-2 cm vs 2-4 cm)	*Variable 1*	*Variable 2*
t Stat	3.95	
P(T<=t) two-tail	0.02	
t Critical two-tail	5.60	

*0-2 and 2-4 are similar at 1 m;

1 m (0-2 cm vs 4-6 cm)	*Variable 1*	*Variable 2*
t Stat	5.66	
P(T<=t) two-tail	0.03	
t Critical two-tail	14.09	

*0-2 and 4-6 are similar at 1 m;

1 m (0-2 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
t Stat	6.07	
P(T<=t) two-tail	0.03	
t Critical two-tail	14.09	

*0-2 and 6-8 are similar at 1 m

1 m (0-2 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	6.72	
P(T<=t) two-tail	0.02	
t Critical two-tail	14.09	

*0-2 and 8-10 are similar at 1 m;

1 m (2-4 cm vs 4-6 cm)	*Variable 1*	*Variable 2*
t Stat	0.79	
P(T<=t) two-tail	0.49	
t Critical two-tail	7.45	

*2-4 and 4-6 are similar at 1 m;

1 m (2-4 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
t Stat	1.03	
P(T<=t) two-tail	0.41	
t Critical two-tail	14.09	

*2-4 and 6-8 are similar at 1 m;

Calcium, Sample Site 8, Significance of depth at each distance

1 m (2-4 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	1.85	
P(T<=t) two-tail	0.21	
t Critical two-tail	14.09	

*2-4 and 8-10 are similar at 1 m;

1 m (4-6 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
t Stat	0.52	
P(T<=t) two-tail	0.65	
t Critical two-tail	14.09	

*4-6 and 6-8 are similar at 1 m;

1 m (4-6 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	2.57	
P(T<=t) two-tail	0.08	
t Critical two-tail	7.45	

*4-6 and 8-10 are similar at 1 m;

1 m (6-8 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	4.42	
P(T<=t) two-tail	0.05	
t Critical two-tail	14.09	

*6-8 and 8-10 are similar at 1 m;

Table 8, continued.

Calcium, Sample Site 8, Significance of depth at each distance

2 m (0-2 cm vs 2-4 cm)	*Variable 1*	*Variable 2*
t Stat	2.33	
P(T<=t) two-tail	0.10	
t Critical two-tail	7.45	

corrected alpha = alpha (0.05)/# tests performed = 0.05/10 = 0.005

*0-2 and 2-4 are similar at 2 m;

2 m (0-2 cm vs 4-6 cm)	*Variable 1*	*Variable 2*
t Stat	7.21	
P(T<=t) two-tail	0.01	
t Critical two-tail	7.45	

*0-2 and 4-6 are similar at 2 m (although slightly different)

2 m (0-2 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
t Stat	8.90	
P(T<=t) two-tail	0.00	
t Critical two-tail	5.60	

*0-2 and 6-8 are different at 2 m

2 m (0-2 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	8.98	
P(T<=t) two-tail	0.00	
t Critical two-tail	7.45	

*0-2 and 8-10 are different at 2 m;

2 m (2-4 cm vs 4-6 cm)	*Variable 1*	*Variable 2*
t Stat	1.11	
P(T<=t) two-tail	0.38	
t Critical two-tail	14.09	

*2-4 and 4-6 are similar at 2 m;

2 m (2-4 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
t Stat	2.77	
P(T<=t) two-tail	0.07	
t Critical two-tail	7.45	

*2-4 and 6-8 are similar at 2 m;

Calcium, Sample Site 8, Significance of depth at each distance

2 m (2-4 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	2.17	
P(T<=t) two-tail	0.16	
t Critical two-tail	14.09	

*2-4 and 8-10 are similar at 2 m;

2 m (4-6 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
t Stat	4.13	
P(T<=t) two-tail	0.03	
t Critical two-tail	7.45	

*4-6 and 6-8 are similar at 2 m;

2 m (4-6 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	3.53	
P(T<=t) two-tail	0.02	
t Critical two-tail	5.60	

*4-6 and 8-10 are similar at 2 m;

2 m (6-8 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
t Stat	-1.59	
P(T<=t) two-tail	0.21	
t Critical two-tail	7.45	

*6-8 and 8-10 are similar at 2 m;

Table 8, continued.

Calcium, Sample Site 5, Significance of distance at each depth

0-2 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*0-2 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*0-2 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
t Stat	0.68		t Stat	-4.99		t Stat	-2.13	
P(T<=t) two-tail	0.55		P(T<=t) two-tail	0.01		P(T<=t) two-tail	0.28	
t Critical two-tail	4.85		t Critical two-tail	3.96		t Critical two-tail	38.11	
*Sample Site 5 0 m and 1 m is different at 0-2 cm			*Sample Site 5 0 m and 2 m is similar at 0-2 cm * only nest and 2m from nest (5-0B and 5-2B) are significantly different			*Sample Site 5 1 m and 2 m is different at 0-2 cm		

Copper, Sample Site 8, Significance of distance at each depth

0-2 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*0-2 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*0-2 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
t Stat	3.10		t Stat	8.15		t Stat	3.09	
P(T<=t) two-tail	0.05		P(T<=t) two-tail	0.00		P(T<=t) two-tail	0.09	
t Critical two-tail	4.86		t Critical two-tail	4.86		t Critical two-tail	4.30	
			*8-0 and 8-2B are significantly different (8-1B has bigger variance so is similar to both 8-0 and 8-2B)					

Table 8, continued.

Copper, Sample Site 8, Significance of distance at each depth

2-4 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*
t Stat	2.00	
P(T<=t) two-tail	0.18	
t Critical two-tail	7.66	

2-4 cm (0 m vs 2 m)	*Variable 1*	*Variable 2*
t Stat	7.43	
P(T<=t) two-tail	0.00	
t Critical two-tail	3.97	

*8-0 and 8-2B at 2-4 cm, and 8-1B and 8-2B at 2-4 cm are significantly different (8-0 and 8-1B similar)

2-4 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
t Stat	8.14	
P(T<=t) two-tail	0.01	
t Critical two-tail	7.66	

4-6 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*
t Stat	6.99	
P(T<=t) two-tail	0.01	
t Critical two-tail	3.18	

*they are significantly different (same results as ANOVA since only 2 groups)

Table 8, continued.

Zinc, Sample Site 8, Significance of distance at each depth

0-2 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*
t Stat	0.59	
P(T<=t) two-tail	0.59	
t Critical two-tail	4.85	

corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166

8-0 and 8-1B at 0-2 cm are not significantly different however, if 0.05 instead of 0.0166 were used for p value comparison, 8-0 and 8-2B, as well as 8-1B and 8-2B would be significantly different

0-2 cm (0 m vs 2 m)	*Variable 1*	*Variable 2*
t Stat	8.40	
P(T<=t) two-tail	0.01	
t Critical two-tail	7.64	

8-0 and 8-2B at 0-2 cm are significantly different

0-2 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
t Stat	4.95	
P(T<=t) two-tail	0.04	
t Critical two-tail	7.64	

8-1B and 8-2B at 0-2 cm are not significantly different

4-6 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
t Stat	17.63	
P(T<=t) two-tail	0.00	
t Critical two-tail	4.30	

corrected alpha = alpha (0.05)/# tests performed = 0.05/1 = 0.05

*they are significantly different (same results as ANOVA since only 2 groups)

6-8 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
t Stat	3.38	
P(T<=t) two-tail	0.04	
t Critical two-tail	3.18	

corrected alpha = alpha (0.05)/# tests performed = 0.05/1 = 0.05

Table 8, continued.

Zinc, Sample Site 5, Significance of distance at each depth								
t Stat	0.28		t Stat	4.05		t Stat	3.74	
P(T<=t) two-tail	0.80		P(T<=t) two-tail	0.03		P(T<=t) two-tail	0.03	
t Critical two-tail	3.96		t Critical two-tail	4.85		t Critical two-tail	4.85	
corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166 * 5-0B and 5-1B are not significantly different at 0-2 cm *because of larger variances in 5-0 and 5-1B, there appear to be no differences, even with 5-2B at 0-2 cm			* 5-0B and 5-2B are not significantly different at 0-2 cm			* 5-1B and 5-2B are not significantly different at 0-2 cm		
2-4 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*2-4 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*2-4 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
t Stat	2.66		t Stat	6.33		t Stat	6.06	
P(T<=t) two-tail	0.08		P(T<=t) two-tail	0.00		P(T<=t) two-tail	0.01	
t Critical two-tail	4.85		t Critical two-tail	3.96		t Critical two-tail	4.85	
corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166 *5-0 and 5-1B are not significantly different at 2-4 cm			*5-0 and 5-2B are significantly different at 2-4 cm			*5-1B and 5-2B are significantly different at 2-4 cm		

Table 8, continued.

Zinc, Sample Site 5, Significance of distance at each depth

8-10 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*8-10 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*8-10 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
t Stat	-0.86		t Stat	2.90		t Stat	5.37	
P(T<=t) two-tail	0.45		P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.01	
t Critical two-tail	4.85		t Critical two-tail	3.96		t Critical two-tail	4.85	

corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166

*5-0 and 5-1B are not significantly different at 8-10 cm

RESULT: it appears that Zn conc decreases with distance from the nest until a depth of 4-6cm (but jumps up again at 8-10cm?) - post hoc negates some of this due to greater variance
however, if 0.05 instead of 0.0166 for p value comparison, Zn conc decreases at 1 to 2 m at depths of 0-2 cm, 2-4 cm, (not diff 4-6 cm or 6-8 cm), and back again 8-10 cm

*5-0 and 5-2B are not significantly different at 8-10 cm

*5-1B and 5-2B are significantly different at 8-10 cm

Table 9: Elemental concentrations in ant bodies, AA.

Genus	Metal	Concentration (ppm)
Aphaenogaster	Calcium	276.04
Myrmica	Calcium	1190.97
Aphaenogaster	Copper	-5.91
Myrmica	Copper	9.19
Aphaenogaster	Zinc	38.21
Myrmica	Zinc	141.95

Discussion

With a few exceptions, ANOVA results did not show statistically significant differences in elemental concentrations as a function of depth and distance except for AA analysis of copper. Concentrations measured by XRF for each sample were averaged site by distance and depth and yielded P values of 0.507 for calcium, 0.715 for iron, 0.715 for potassium, 0.912 for manganese, and 0.623 for zinc (Table 3). Concentrations measured by AA were based on the mean elemental concentrations of triplicates for each sample and yielded P values of $P > 0.05$ for calcium and zinc and $P < 0.05$ for copper (Table 5). Exceptions include an XRF P value of 0.0002 for calcium Sample Site 8 at two meters, AA P values for zinc of 0.002 and 0.028 at Sample Site 8 at depths of 0-2 cm and 6-8 cm, and AA P values of 0.016, 0.001, and 0.012 for zinc at Sample Site 5 at depths of 0-2 cm, 2-4 cm, and 8-10 (Tables 3 and 5). These exceptions are likely due to previously existing variations in the elemental concentrations at each sample site, as these differences do not coincide with changes in proximity to ant hills.

Though XRF analysis of copper could not be obtained because concentrations within samples were below the manufacturer's recommended lower detection limit, AA was able to detect copper (Table 4, Appendices A and B). Findings by AA indicate a statistically significant decrease in concentrations of copper as a function of distance to a depth of 4 cm at Sample Site 8 (Figure 10, Table 8, Appendices B and D). The ability for AA to detect lower concentrations of an element that were too low for analysis by XRF support the utility of a multilevel approach to soil analysis of using XRF followed by AA where needed.

Of AA results with $P < 0.05$, two-tailed post-hoc T test analysis found that calcium concentrations were significantly different at Sample Site 8 at 2 m between depths of 0-2 cm and 6-8 cm, and Sample Site 5 at 0-2 cm deep between all distances (Table 8). This could be the result of additional factors influencing the chemical characteristics of surface soils that are not measured in this study. Post-hoc T test analysis indicated that copper is significantly different across all distances down to a depth of 6-4 cm at Sample Site 8 (Table 8). In Sample Site 8, a concentration gradient of copper occurs with concentrations decreasing as a function of distance (Figure 10). This could potentially indicate influence by *Aphaenogaster rudis*, though more sample sites would need to be evaluated by AA to support this. Post-hoc T test analysis of AA results found zinc to be significantly different at Sample Site 8 between ant hills (0 m) and 2 m at a depth of 0-2 cm and between 1 and 2 m at a depth of 4-6 cm, and at Sample Site 5 was found to be significantly different at depths of 4-6 cm between ant tunnels (0 m) and 2 m away as well as 1 m and 2 m but not between 0 m and 1 m, and at a depth of 8-10 cm between distances of 1 m and 2 m (Table 8). These findings could potentially suggest association of zinc concentrations with subterranean nesting ants, though more sample sites would need to be evaluated by AA to support this. In Sample Site 8, a concentration gradient of zinc occurs with concentrations decreasing as a function of distance, though large variance occurs at depths at 6-8 cm and greater (Figure 11).

Results obtained through XRF and AA cannot be directly compared due to the small number of samples that were analyzed with AA. However, some of the findings from AA analysis support observations from XRF analysis. For example, zinc had significantly ($P = 0$, Table 3) higher concentrations in colonies inhabited by *Aphaenogaster rudis* than those inhabited by members of *Myrmica* (Figures 6 and 11, Table 8). For calcium, findings by AA contradicted those by XRF, with AA showing sites inhabited by *Aphaenogaster rudis* to have concentrations of calcium that are statistically significantly higher than concentrations of calcium at sites inhabited by members of *Myrmica* (Figures 8 and 9, Table 4, Appendices A and B). XRF detected far higher concentrations of calcium than AA (Figures 8 and 9, Table 4). This is most likely due to matrix influences of sulfates and phosphates resulting in calcium being more difficult to ionize (Skoog *et al.* 1998). Another potential explanation for the unexpectedly low concentrations of calcium found in AA results relative to those found by XRF include having a faulty calibration curve in the AA. XRF results showed significantly higher concentrations of iron and potassium at sites inhabited by *Aphaenogaster rudis* than those inhabited by members of *Myrmica* (Figures 4 and 7, Table 4). A mildly significant difference was found between genera for manganese (P = 0.040, Figure 5, Table 3).

Though AA showed higher concentrations of copper as a function of genus and no difference in concentrations of calcium between genera in Sample Site 5, which was inhabited by members of *Myrmica*, relative to that of Sample Site 8, which was colonized by *Aphaenogaster rudis*, the two sample sites had very different physical characteristics including soil texture and habitat and so differences cannot be directly correlated to genus. Raw AA data appears to show elemental concentrations decreasing with distance from colonies, however large variance prevents these results from being significant (Table 8, Appendix B). To minimize variance in future AA analysis more trials should be conducted to allow for the omission of outliers.

Conclusions. I believe this study to be the first to evaluate the concentrations of iron, copper, zinc, manganese, calcium, and potassium in soils associated with *Aphaenogaster rudis* and members of *Myrmica*, except for one study that evaluated the concentration of potassium in association with a member of *Myrmica* by Véle *et al.* (2010). My findings support the findings of Véle *et al.* (2010), where a correlation between proximity to colonies of *Myrmica ruginodis* was associated with elevated concentrations of potassium. The findings from my study support the species- and genus-specificity of relationships between subterranean nesting ants and chemical characteristics of the soils in and around colonies as documented by Anderson *et al.* (2010), Boots *et al.* (2012), Holec & Frouz (2006), Jacquemin *et al.* (2012), Jílková *et al.* (2017) and Richards (2009). I was unable to directly attribute variations in elemental concentrations in soils to ant activity and thus not able to define any sort of ant-soil interactions that are involved in nutrient cycling. Because no statistically significant trends were observed besides in AA results for copper, it is likely the differences between genera are a result of site selection for colonization rather than changes brought on by ant activity. If changes resulted from the presence of colonies, I would have expected there to be more differences in elemental concentrations with depth or as samples moved away from the primary tunnel opening of the colony. The genus-specific variations seen in elemental concentrations between sample sites has provided an unexpected detail into habitat preferences of these organisms.

Future research. The multistep analytical method for elemental composition of soils outlined in this study appears to be an effective means of streamlining the process through the use of XRF for overview of concentration trends followed by further review with AA for better resolution of samples and elements of interest. To prevent chemical interference when evaluating calcium concentrations *via* AA, the preparation method needs to be modified to include chemical that sequester sulfates and phosphates (Skoog *et al.* 1998).

Analysis of more colonies in different stages of development, over the course of seasons and in a less disturbed habitat would allow for a more detailed understanding of the relationship between elemental compositions of soils and the presence and activities by *Aphaenogaster rudis* and subterranean members of *Myrmica* in central Maine. Observation of soils selected by foundress queens to start their colony would provide a better understanding of abiotic factors that influence location of new forming colonies. Evaluation of additional characteristics such as soil texture, pH, nitrogen and phosphorus would allow for further comparison of these species with those of previously published findings.

Appendix A

X-Ray Fluorescence Data

All elemental concentrations were recorded in ppm. For all samples, sample site, distance, depth, genus and compass direction relative to the primary tunnel opening of the colonies were recorded. Some samples were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth. Myr = *Myrmica* Aph = *Aphaenogaster rudis* LOD = limit of detection

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	1	0-M	0-2	Myr	54.5	14.9	23002.7	432.8	571.2	103.0	7185.9	369.8	17621.2	746.1
SE	1	0-M	2-4	Myr	46.9	14.3	23806.5	437.7	585.0	103.8	7413.1	382.2	17818.5	765.3
SE	1	0-M	4-6	Myr	56.3	15.2	23718.5	434.1	510.6	98.3	7586.3	380.6	17167.6	744.3
SE	1	0-M	6-8	Myr	51.4	13.7	20776.9	391.9	365.1	83.5	6963.8	368.5	18644.3	769.7
SE	1	0-M	8-10	Myr	42.0	13.5	22847.1	423.0	547.6	99.8	6753.3	361.2	17363.9	741.7
SW	1	0-M	0-2	Myr	52.1	14.0	19612.2	381.7	438.6	88.3	7584.0	370.3	17350.2	727.7
SW	1	0-M	2-4	Myr	52.3	14.4	22316.6	419.2	522.7	98.8	6930.3	361.1	17514.8	737.0
SW	1	0-M	4-6	Myr	42.4	12.0	16863.4	331.9	414.9	80.2	6949.3	367.4	17181.3	742.7
SW	1	0-M	6-8	Myr	52.3	14.6	23583.5	436.9	566.6	102.3	6703.8	358.5	18352.2	754.5
SW	1	0-M	8-10	Myr	59.2	15.4	21809.4	421.8	556.3	101.8	6062.5	344.3	18009.9	746.7
W	1	0-M	0-2	Myr	53.3	14.5	21418.3	412.4	546.2	99.0	7195.7	360.8	17334.9	723.3

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
W	1	0-M	2-4	Myr	50.5	13.5	20309.0	384.8	440.8	88.1	7597.5	381.3	17874.1	757.6
W	1	0-M	4-6	Myr	55.7	14.7	22455.6	421.8	467.4	94.8	7254.0	367.2	17643.9	738.9
W	1	0-M	6-8	Myr	57.2	12.4	15183.7	301.6	364.3	72.6	6217.3	367.6	17569.9	778.1
W	1	0-M	8-10	Myr	41.0	13.2	19696.6	387.9	397.0	87.3	6593.0	352.7	17567.5	734.5
SE	1	1-M	0-2	Myr	55.2	14.4	22417.3	418.5	562.7	100.4	7198.2	366.9	17782.1	742.4
SE	1	1-M	2-4	Myr	53.8	14.4	21815.6	411.0	460.0	92.8	6748.0	362.8	17972.5	755.4
SE	1	1-M	4-6	Myr	43.4	12.5	19182.5	366.3	414.0	83.7	6551.5	353.9	16686.8	723.5
SE	1	1-M	6-8	Myr	48.2	14.0	21376.5	412.0	477.0	94.2	6539.7	356.0	17685.0	745.3
SE	1	1-M	8-10	Myr	54.9	14.8	22749.5	427.7	474.1	94.9	6360.7	348.9	17460.6	734.6
SW	1	1-M	0-2	Myr	45.1	13.8	21300.9	409.5	495.4	96.5	7501.4	369.5	16701.2	718.4
SW	1	1-M	2-4	Myr	63.4	13.2	16126.2	317.7	450.7	79.9	6263.8	348.4	16088.4	712.5
SW	1	1-M	4-6	Myr	46.1	14.0	21572.5	413.3	532.2	98.3	6347.8	345.1	16673.9	713.7
SW	1	1-M	6-8	Myr	59.2	14.9	21949.4	416.3	380.9	88.1	6448.8	349.7	17294.3	729.5
SW	1	1-M	8-10	Myr	62.0	14.7	19641.9	381.7	480.5	90.4	6813.2	361.3	16764.4	729.4
W	1	1-M	0-2	Myr	47.8	13.9	22262.2	415.0	464.7	93.5	7271.0	363.5	17298.3	725.1

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
W	1	1-M	2-4	Myr	46.7	13.8	21911.3	411.3	473.6	93.3	7102.6	367.4	18374.6	757.4
W	1	1-M	4-6	Myr	67.8	15.4	22746.0	422.2	569.3	100.9	7210.2	372.9	17826.3	755.1
W	1	1-M	6-8	Myr	53.7	14.7	22659.8	429.4	619.3	105.5	6064.8	337.4	17093.2	717.3
W	1	1-M	8-10	Myr	58.1	14.9	21866.8	417.0	470.2	94.0	6995.4	358.7	17257.8	725.3
SE	1	2-M	0-2	Myr	56.9	14.9	21888.7	417.0	414.6	91.7	7572.1	370.6	16614.5	716.5
SE	1	2-M	2-4	Myr	48.3	14.5	22292.9	424.4	496.2	97.6	7245.7	353.1	15734.6	679.0
SE	1	2-M	4-6	Myr	47.9	13.8	20913.7	402.4	386.2	86.2	7202.3	364.9	18060.4	742.8
SE	1	2-M	6-8	Myr	31.9	12.5	20514.6	399.2	581.7	100.0	6381.0	349.9	17375.4	734.3
SE	1	2-M	8-10	Myr	40.6	12.8	19954.7	380.3	499.6	91.2	6904.2	360.6	16928.3	727.2
SW	1	2-M	0-2	Myr	56.3	14.6	21757.8	416.5	493.9	95.9	8410.4	383.4	15852.8	697.6
SW	1	2-M	2-4	Myr	38.4	12.7	20958.0	402.2	593.3	101.1	7731.9	367.8	15625.4	687.9
SW	1	2-M	4-6	Myr	42.0	13.6	22199.8	417.7	545.8	99.6	7418.6	369.3	17485.9	733.9
SW	1	2-M	6-8	Myr	36.6	13.3	22570.3	424.5	435.2	93.0	6487.2	352.8	17386.6	735.9
SW	1	2-M	8-10	Myr	48.7	14.1	21210.8	409.4	488.1	95.4	6668.9	353.6	16459.7	714.4
W	1	2-M	0-2	Myr	43.8	13.3	20943.9	399.9	642.9	102.7	7650.6	367.7	17302.9	719.3

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
W	1	2-M	2-4	Myr	56.8	14.7	23062.2	426.1	493.0	96.2	7105.6	366.2	17090.2	733.1
W	1	2-M	4-6	Myr	62.3	15.4	24270.3	442.3	487.0	97.8	6547.5	353.1	16642.9	721.4
W	1	2-M	6-8	Myr	58.8	14.7	22302.8	419.3	427.0	91.8	7101.0	366.3	17354.9	738.2
W	1	2-M	8-10	Myr	45.4	13.5	21822.0	404.6	497.7	93.8	6588.4	342.1	16044.1	687.6
E	2	0-M	0-2	Myr	64.5	15.5	24473.4	440.6	543.5	100.2	8428.4	396.0	15157.5	706.6
E	2	0-M	2-4	Myr	65.1	15.5	24690.4	441.5	455.1	95.0	8130.1	393.6	16624.3	739.3
E	2	0-M	4-6	Myr	68.4	15.9	25952.4	455.2	532.6	102.1	7065.9	376.0	16490.9	742.9
E	2	0-M	6-8	Myr	84.4	17.3	26091.0	463.0	555.9	103.9	6481.7	351.8	14609.6	685.3
E	2	0-M	8-10	Myr	65.9	15.9	25780.5	460.6	635.5	108.6	6237.2	358.0	15761.1	728.3
S	2	0-M	0-2	Myr	84.5	16.9	24439.7	438.6	565.8	101.6	8440.4	402.7	15674.2	727.8
S	2	0-M	2-4	Myr	79.5	16.5	23574.3	428.9	577.3	100.8	7160.3	369.7	15710.1	713.4
S	2	0-M	4-6	Myr	63.0	14.9	22816.0	414.9	476.4	93.2	7516.8	373.6	14516.5	685.3
S	2	0-M	6-8	Myr	61.8	15.1	24807.0	441.2	530.7	98.9	6936.3	369.1	15489.5	716.8
S	2	0-M	8-10	Myr	74.0	16.2	24333.6	435.3	549.9	99.5	6849.9	368.9	15161.5	713.4
SW	2	0-M	0-2	Myr	68.8	15.8	24079.3	434.9	580.6	102.6	7532.5	375.0	15220.4	700.5

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SW	2	0-M	2-4	Myr	58.9	15.0	23158.4	424.5	425.4	91.8	7406.2	375.3	15373.8	708.7
SW	2	0-M	4-6	Myr	68.4	15.8	23345.3	430.1	557.7	101.1	6819.2	366.0	14930.5	705.0
SW	2	0-M	6-8	Myr	57.2	15.0	25496.3	451.5	490.1	97.1	7592.5	380.0	15726.5	716.8
SW	2	0-M	8-10	Myr	57.8	14.9	25697.4	449.2	526.4	99.7	7210.6	376.5	15515.0	720.4
E	2	1-M	0-2	Myr	60.7	15.1	23187.5	424.2	602.5	101.7	9834.3	429.8	16477.5	745.7
E	2	1-M	2-4	Myr	79.9	16.2	24215.5	435.2	531.8	98.8	8148.6	393.4	15879.6	724.9
E	2	1-M	4-6	Myr	66.5	15.4	24130.4	433.4	531.1	98.1	8062.2	401.5	15907.7	743.0
E	2	1-M	6-8	Myr	67.5	15.4	24684.7	436.8	505.6	96.1	7837.1	390.6	15759.7	728.4
E	2	1-M	8-10	Myr	77.1	16.5	24993.9	444.6	474.9	95.9	7132.0	381.1	16659.7	753.5
S	2	1-M	0-2	Myr	70.0	16.4	25236.7	456.5	559.0	103.2	7423.5	365.0	15582.1	692.8
S	2	1-M	2-4	Myr	58.2	15.0	23864.0	433.7	466.3	94.9	7415.3	376.4	15485.4	712.2
S	2	1-M	4-6	Myr	72.0	16.0	23931.2	436.6	470.1	95.4	7324.1	377.7	15199.8	712.7
S	2	1-M	6-8	Myr	66.0	15.5	25029.4	444.1	438.5	93.5	6727.7	369.5	16227.4	739.3
S	2	1-M	8-10	Myr	61.0	15.1	25086.9	444.4	459.9	95.0	6677.9	360.8	15246.5	706.3
SW	2	1-M	0-2	Myr	61.5	15.4	22874.7	424.7	544.2	99.5	8973.7	393.9	15217.9	686.5

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SW	2	1-M	2-4	Myr	63.6	15.1	24030.3	435.9	486.3	96.0	7294.0	376.5	16916.3	743.1
SW	2	1-M	4-6	Myr	51.3	12.8	19426.3	359.0	481.7	85.5	6697.7	360.3	15439.1	707.6
SW	2	1-M	6-8	Myr	68.5	16.1	24797.3	446.6	621.1	106.0	6974.2	362.6	15381.1	700.8
SW	2	1-M	8-10	Myr	66.9	16.0	23736.0	432.5	458.8	93.8	6810.6	354.9	14827.6	682.3
E	2	2-M	0-2	Myr	52.7	10.3	3121.6	118.5			917.6	233.7	2594.9	423.7
E	2	2-M	2-4	Myr	101.0	24.9	621.7	97.0					834.3	152.2
E	2	2-M	4-6	Myr	57.9	15.2	21474.1	416.8	470.6	95.4	7001.9	355.9	16815.7	712.1
E	2	2-M	6-8	Myr	49.5	14.7	23738.5	441.5	458.7	95.7	5583.5	315.0	13734.3	634.1
E	2	2-M	8-10	Myr	56.0	14.8	23790.3	431.5	515.2	97.0	6472.1	345.0	15394.6	686.9
S	2	2-M	0-2	Myr	70.0	15.9	25295.0	447.1	478.9	96.3	9881.4	429.1	15367.2	721.8
S	2	2-M	2-4	Myr	57.8	14.9	23642.9	431.3	435.0	92.7	7605.4	376.6	15608.6	708.1
S	2	2-M	4-6	Myr	51.7	14.6	25288.6	445.4	494.4	96.4	8184.0	395.9	14968.6	710.6
S	2	2-M	6-8	Myr	63.6	15.4	25716.6	452.2	422.0	93.3	7632.4	390.6	15169.0	724.4
S	2	2-M	8-10	Myr	76.0	16.4	28876.1	483.1	376.7	92.5	8683.5	421.8	14126.2	721.9
SW	2	2-M	0-2	Myr	60.3	13.9	20909.6	379.5	436.7	84.8	8704.1	405.0	15891.3	727.0

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SW	2	2-M	2-4	Myr	71.2	16.0	24206.5	437.2	485.9	96.1	8571.8	401.3	16027.4	727.5
SW	2	2-M	4-6	Myr	66.5	15.5	24800.4	443.0	485.9	96.3	9350.0	411.9	14830.2	698.5
SW	2	2-M	6-8	Myr	72.0	16.3	24380.9	440.0	494.0	96.8	8208.9	395.2	15388.1	716.8
SW	2	2-M	8-10	Myr	92.4	17.7	27104.3	471.4	609.9	107.3	7969.2	397.6	15523.6	732.0
SE	4	0-M	0-2	Aphr	60.5	15.3	25056.4	448.3	782.0	115.5	9090.1	413.9	16719.4	745.8
SE	4	0-M	2-4	Aphr	64.1	15.6	23105.6	428.5	735.7	111.7	8161.6	384.7	16172.7	714.1
SE	4	0-M	4-6	Aphr	72.9	16.3	25880.9	457.4	871.1	120.7	7943.5	387.4	16237.2	727.4
SE	4	0-M	6-8	Aphr	76.8	16.6	25320.4	448.3	747.9	112.9	8333.0	403.0	16381.4	745.2
SE	4	0-M	8-10	Aphr	71.5	16.1	24649.3	443.1	934.9	123.5	7973.1	392.7	17204.1	753.8
NE	4	0-M	0-2	Aphr	72.4	16.0	22504.3	423.2	707.6	110.0	8549.0	387.6	15851.6	700.6
NE	4	0-M	2-4	Aphr	67.0	16.0	22739.4	426.8	763.1	113.2	8043.3	373.7	15484.1	685.4
NE	4	0-M	4-6	Aphr	55.6	14.8	23653.8	434.9	757.0	113.8	7720.8	377.8	16245.4	717.8
NE	4	0-M	6-8	Aphr	71.1	16.0	21119.4	408.9	894.5	119.6	7681.6	367.6	16477.4	704.1
NE	4	0-M	8-10	Aphr	49.8	14.4	22046.8	417.9	1004.	125.3	7348.1	362.7	16662.6	709.9
SE	4	1-M	0-2	Aphr	62.4	15.8	24735.2	450.2	744.8	113.8	8240.5	394.8	16407.2	734.0

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	4	1-M	2-4	Aphr	87.7	17.5	25435.5	453.9	710.2	111.7	7928.4	394.5	16218.4	741.0
SE	4	1-M	4-6	Aphr	82.7	16.8	25129.7	447.6	590.0	103.5	7744.6	389.6	16060.4	735.6
SE	4	1-M	6-8	Aphr	88.2	17.2	26341.6	460.4	658.1	108.6	7516.1	388.3	16828.8	755.8
SE	4	1-M	8-10	Aphr	64.8	15.4	24579.4	439.6	638.9	105.8	8198.2	396.7	16674.1	743.8
NE	4	1-M	0-2	Aphr	74.6	16.2	24169.1	437.6	709.2	110.6	9346.6	415.0	16458.1	734.7
NE	4	1-M	2-4	Aphr	78.1	17.0	25540.5	460.8	839.3	120.7	7880.3	373.3	15350.4	687.7
NE	4	1-M	4-6	Aphr	58.7	15.4	22545.0	429.5	710.9	111.7	8638.7	393.6	15206.4	696.3
NE	4	1-M	6-8	Aphr	74.4	16.3	25169.1	445.4	928.8	122.2	9279.5	420.1	17360.2	762.5
W	4	1-M	0-2	Aphr	71.0	16.7	24005.2	445.4	819.1	118.9	8867.9	405.2	16119.1	726.4
W	4	1-M	2-4	Aphr	75.7	16.9	26071.6	465.0	802.0	118.6	8141.8	392.1	16007.4	725.3
W	4	1-M	4-6	Aphr	79.8	16.2	24305.1	433.2	735.9	110.5	8925.8	416.7	17070.0	762.6
W	4	1-M	6-8	Aphr	75.1	16.5	26263.2	463.5	831.1	119.9	8422.5	408.8	16577.4	756.6
W	4	1-M	8-10	Aphr	84.0	17.3	28185.3	479.6	995.1	128.6	8723.7	416.4	16220.8	753.2
SE	4	2-M	0-2	Aphr	70.7	16.3	23556.3	437.2	717.2	111.7	8193.9	385.5	16095.8	713.1
SE	4	2-M	2-4	Aphr	73.6	16.5	25796.8	458.5	868.7	120.8	7586.7	380.5	15902.4	721.5

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	4	2-M	4-6	Aphr	62.7	15.6	25557.9	456.1	761.4	115.8	7399.5	369.4	16140.8	711.8
SE	4	2-M	6-8	Aphr	71.4	16.2	25027.6	448.9	622.3	106.4	7840.2	385.9	15349.7	712.0
SE	4	2-M	8-10	Aphr	LOD		LOD		LOD		260.3	74.8	229.7	108.8
NE	4	2-M	0-2	Aphr	74.0	15.9	24933.6	435.6	717.9	108.6	8197.9	409.0	17185.1	775.9
NE	4	2-M	2-4	Aphr	51.8	13.6	19292.4	375.9	519.4	92.9	7775.1	370.7	16152.6	700.8
NE	4	2-M	4-6	Aphr	57.5	15.5	22499.0	426.9	770.6	114.7	7269.2	360.2	15624.5	690.1
NE	4	2-M	6-8	Aphr	55.1	14.6	20055.1	394.1	598.5	100.7	6143.7	334.6	17772.7	718.7
NE	4	2-M	8-10	Aphr	36.3	13.1	19555.6	394.0	542.6	99.1	5691.9	316.2	16774.6	683.4
W	4	2-M	0-2	Aphr	74.8	16.6	24292.1	441.5	1522.5	150.8	10199.7	418.3	15382.1	695.3
W	4	2-M	2-4	Aphr	51.2	14.4	23686.2	434.4	850.4	118.3	10555.7	431.0	16499.6	727.0
W	4	2-M	4-6	Aphr	66.4	15.6	23681.5	435.1	905.3	121.2	9565.2	420.9	17225.1	752.2
W	4	2-M	6-8	Aphr	61.3	15.2	22155.4	420.4	804.8	115.4	8203.0	382.2	16303.1	710.3
W	4	2-M	8-10	Aphr	41.4	13.6	19679.5	397.7	561.9	100.4	7404.6	350.1	15681.5	667.3
E	5	0-M	0-2	Myr	42.5	13.7	21261.3	411.5	521.7	97.8	7061.1	364.9	18581.8	757.1
E	5	0-M	2-4	Myr	46.3	13.5	19512.8	378.8	456.1	89.4	7445.2	373.2	18749.6	761.8

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
E	5	0-M	4-6	Myr	61.5	15.2	21473.8	412.3	467.7	94.6	7151.2	369.5	18504.9	761.5
E	5	0-M	6-8	Myr	55.4	14.8	21691.2	415.1	514.3	97.9	6568.3	352.0	18272.1	745.2
E	5	0-M	8-10	Myr	45.0	13.9	20046.1	399.6	450.6	91.9	6654.2	347.1	17885.7	725.7
SE	5	0-M	0-2	Myr	54.4	14.7	20892.8	410.4	528.2	99.0	6918.2	365.9	20041.9	788.1
SE	5	0-M	2-4	Myr	51.6	14.5	19886.3	394.6	397.9	88.9	7261.5	363.9	19045.4	754.9
SE	5	0-M	4-6	Myr	37.4	12.9	19787.4	391.0	492.8	93.8	6703.0	349.6	17811.6	727.8
SE	5	0-M	6-8	Myr	49.1	14.2	20183.4	398.7	409.3	89.8	6779.6	359.7	19371.1	770.3
SE	5	0-M	8-10	Myr	38.3	13.2	19562.5	392.2	514.9	96.8	6956.1	364.7	18527.7	760.5
SW	5	0-M	0-2	Myr	38.2	13.1	20053.8	390.5	426.3	88.7	6972.8	359.3	18770.5	752.1
SW	5	0-M	2-4	Myr	57.1	14.7	20690.0	406.2	541.4	98.9	7194.9	362.7	19359.7	759.6
SW	5	0-M	4-6	Myr	51.0	14.5	20622.6	403.5	620.7	103.8	6483.3	353.3	18151.4	750.0
SW	5	0-M	6-8	Myr	44.2	13.9	21448.8	415.2	498.9	97.4	6604.0	349.4	18053.6	735.4
SW	5	0-M	8-10	Myr	41.5	13.7	20973.2	410.0	393.3	90.0	6891.0	355.3	18328.0	740.2
E	5	1-M	0-2	Myr	36.3	12.7	19601.1	385.5	441.8	89.5	7514.6	374.9	19575.0	775.7
E	5	1-M	2-4	Myr	39.6	13.3	20270.5	393.6	489.2	92.8	7401.3	370.6	18235.6	749.8

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
E	5	1-M	4-6	Myr	43.6	13.8	21039.7	410.5	464.0	94.1	6814.2	349.2	18668.0	736.1
E	5	1-M	6-8	Myr	49.5	12.1	14995.3	303.4	349.1	72.5	7181.8	377.0	18092.7	767.0
E	5	1-M	8-10	Myr	56.2	14.9	21248.3	409.0	510.8	96.5	6742.6	351.6	18370.7	739.1
SE	5	1-M	0-2	Myr	35.0	13.0	21100.6	408.6	426.0	91.3	7132.0	357.6	19205.3	749.2
SE	5	1-M	2-4	Myr	52.0	14.4	20854.8	404.7	466.9	93.9	6797.7	359.2	19002.7	762.8
SE	5	1-M	4-6	Myr	56.4	15.0	20672.8	407.8	431.7	91.8	6829.7	348.7	17933.0	722.9
SE	5	1-M	6-8	Myr	47.5	13.9	20830.5	401.6	443.2	91.2	6914.8	358.3	18360.1	746.1
SE	5	1-M	8-10	Myr	43.8	13.9	21629.4	416.8	445.8	94.1	7268.7	370.0	19734.6	777.9
SW	5	1-M	0-2	Myr	57.3	14.6	20364.8	395.1	462.0	91.3	7639.5	375.0	19702.6	773.2
SW	5	1-M	2-4	Myr	58.7	15.1	21972.0	419.4	566.0	101.2	7446.1	369.0	19390.3	763.6
SW	5	1-M	4-6	Myr	53.8	14.5	22032.6	416.1	437.6	92.7	7395.7	368.3	19073.4	759.0
SW	5	1-M	6-8	Myr	44.4	12.1	17171.0	335.3	337.0	75.5	6771.2	370.6	19694.6	797.5
SW	5	1-M	8-10	Myr	45.4	14.2	22267.8	424.7	417.9	92.1	6573.5	357.9	20358.3	790.4
E	5	2-M	0-2	Myr	71.6	16.0	22601.5	424.8	383.5	89.3	6575.2	362.3	21263.9	813.3
E	5	2-M	2-4	Myr	59.3	15.1	22809.0	426.8	496.2	97.6	6888.1	373.8	19992.4	805.7

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
E	5	2-M	4-6	Myr	49.9	14.4	22989.6	425.2	530.3	97.9	7024.8	376.1	20083.9	806.0
E	5	2-M	6-8	Myr	49.6	14.6	23513.2	434.3	459.3	95.0	6856.4	369.8	20727.6	809.4
E	5	2-M	8-10	Myr	50.8	14.6	24049.6	439.8	580.9	103.4	6927.0	373.5	19721.9	798.9
SE	5	2-M	0-2	Myr	42.3	13.5	21107.4	405.1	478.9	94.6	7573.7	375.1	18393.8	754.9
SE	5	2-M	2-4	Myr	48.5	14.0	20520.6	404.7	499.9	96.9	7135.6	360.1	18571.6	744.2
SE	5	2-M	4-6	Myr	59.0	15.4	20274.1	407.2	573.3	103.0	7046.3	356.2	18406.9	736.9
SE	5	2-M	6-8	Myr	53.8	14.4	19872.0	396.0	408.8	89.5	7224.1	362.3	17731.9	731.7
SE	5	2-M	8-10	Myr	55.7	14.8	20760.0	405.5	571.4	100.4	6779.5	354.9	18140.0	740.8
SW	5	2-M	0-2	Myr	62.6	15.6	20845.3	409.2	531.5	99.4	7963.5	375.2	17469.7	725.5
SW	5	2-M	2-4	Myr	50.9	14.3	19274.8	386.4	543.0	97.2	7770.5	373.6	18489.2	746.2
SW	5	2-M	4-6	Myr	49.8	14.4	19597.1	393.4	472.0	93.1	7675.2	370.3	18456.9	743.0
SW	5	2-M	6-8	Myr	45.7	13.5	19628.7	394.8	461.1	92.8	7947.4	367.5	17114.1	705.6
SW	5	2-M	8-10	Myr	35.5	12.9	20237.3	399.6	413.7	90.1	7555.2	368.6	17475.9	727.4
SE	6	0-M	0-2	Aphr	75.7	16.0	23502.1	423.6	402.9	88.8	8162.7	397.3	15074.9	715.9
SE	6	0-M	2-4	Aphr	79.1	16.3	23999.3	431.5	485.4	95.0	7392.5	376.5	13909.6	683.0

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	6	0-M	4-6	Aphr	67.8	15.8	23984.4	435.4	386.6	90.1	7438.6	379.4	14667.1	701.4
SE	6	0-M	6-8	Aphr	70.2	15.8	23483.4	428.5	369.3	88.0	7296.9	365.2	14000.1	667.7
SE	6	0-M	8-10	Aphr	72.3	15.9	23493.3	425.8	489.1	94.7	7564.5	382.7	14214.6	694.0
SW	6	0-M	0-2	Aphr	67.5	15.5	23686.2	429.7	462.5	93.4	8311.6	394.2	15008.0	704.2
SW	6	0-M	2-4	Aphr	70.6	13.4	16626.3	316.4	387.6	75.0	7434.7	389.6	13803.4	701.4
SW	6	0-M	4-6	Aphr	54.2	14.5	23038.9	424.6	382.0	88.7	7486.7	376.0	14118.5	682.8
SW	6	0-M	6-8	Aphr	53.5	12.9	19551.9	362.6	293.0	74.1	7068.3	371.3	13392.0	674.6
SW	6	0-M	8-10	Aphr	71.6	16.1	24609.8	444.9	382.3	89.9	7667.3	378.7	14374.0	686.3
NW	6	0-M	0-2	Aphr	70.4	16.0	23461.6	431.0	442.3	92.7	8817.3	401.9	14516.4	691.6
NW	6	0-M	2-4	Aphr	59.9	15.3	22771.4	424.6	421.6	91.9	7376.1	374.1	14126.9	683.3
NW	6	0-M	4-6	Aphr	65.5	15.6	21940.1	414.6	564.9	100.0	7717.3	373.3	13872.0	665.2
NW	6	0-M	6-8	Aphr	64.6	15.3	23721.5	432.7	388.8	89.8	6659.2	355.6	13890.0	671.6
NW	6	0-M	8-10	Aphr	72.1	16.4	24127.9	441.7	416.0	93.0	7660.7	381.1	13827.4	679.9
SE	6	1-M	0-2	Aphr	75.6	16.6	26876.0	471.8	467.4	98.1	9068.4	415.8	13495.4	686.4
SE	6	1-M	2-4	Aphr	73.3	16.2	26825.3	465.9	458.0	96.7	7749.9	394.6	16079.2	744.7

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	6	1-M	4-6	Aphr	90.2	17.7	27200.7	465.9	536.0	100.9	7806.9	398.5	14292.7	715.1
SE	6	1-M	6-8	Aphr	80.8	16.7	27028.4	461.4	350.0	87.8	8401.4	406.3	14528.2	713.0
SE	6	1-M	8-10	Aphr	84.2	16.8	25956.1	448.3	506.0	97.6	8209.0	408.7	14253.6	718.0
SW	6	1-M	0-2	Aphr	55.3	14.2	23173.4	418.9	372.8	86.8	9008.8	415.5	15152.4	721.3
SW	6	1-M	2-4	Aphr	70.5	15.7	23647.6	429.4	456.0	93.8	8020.4	390.1	14682.9	700.5
SW	6	1-M	4-6	Aphr	61.2	15.0	23656.1	429.4	428.8	91.5	7687.2	384.5	14500.2	698.3
SW	6	1-M	6-8	Aphr	61.5	15.4	24978.8	449.5	406.0	92.0	8094.5	397.7	14832.0	714.3
SW	6	1-M	8-10	Aphr	77.4	16.2	23972.5	431.7	418.0	90.5	7718.0	385.7	14635.6	701.9
NW	6	1-M	0-2	Aphr	87.1	17.2	26659.1	461.4	447.0	94.9	7987.7	394.0	13480.4	684.2
NW	6	1-M	2-4	Aphr	84.2	16.9	25644.6	450.2	352.4	88.1	7850.7	393.5	14664.1	712.0
NW	6	1-M	4-6	Aphr	79.8	16.6	24490.6	439.1	394.3	91.1	7476.2	380.4	14313.4	694.9
NW	6	1-M	6-8	Aphr	79.4	16.8	26244.5	457.4	451.7	94.9	7831.6	393.9	13909.4	698.1
NW	6	1-M	8-10	Aphr	72.7	15.7	24560.7	431.0	403.2	89.2	8113.5	401.3	14699.9	717.4
SE	6	2-M	0-2	Aphr	60.9	14.4	24300.4	422.7	447.9	89.9	8756.0	417.0	14660.6	722.4
SE	6	2-M	2-4	Aphr	72.3	15.7	24537.8	431.9	560.2	98.8	8239.8	403.7	14583.9	714.9

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	6	2-M	4-6	Aphr	72.3	15.9	25135.6	439.0	447.4	92.3	8180.9	400.7	13696.0	693.6
SE	6	2-M	6-8	Aphr	82.7	16.9	25837.9	453.5	481.9	98.3	7651.2	389.8	14600.4	711.2
SE	6	2-M	8-10	Aphr	63.9	15.4	26472.6	458.1	507.2	98.8	7855.5	398.3	15407.8	735.2
SW	6	2-M	0-2	Aphr	97.7	15.6	15783.3	312.3	273.6	67.6	7875.1	380.2	14772.4	688.8
SW	6	2-M	2-4	Aphr	71.6	15.7	21445.0	404.5	480.6	92.3	8167.6	379.3	14993.3	682.3
SW	6	2-M	4-6	Aphr	65.8	14.8	19161.2	378.0	408.8	86.4	7868.6	374.9	14812.5	681.1
SW	6	2-M	6-8	Aphr	77.6	14.8	17279.0	341.3	405.2	81.4	7462.4	368.2	14571.7	678.3
SW	6	2-M	8-10	Aphr	59.3	14.6	21711.9	404.0	360.6	84.6	8424.7	395.3	13994.0	682.6
NW	6	2-M	0-2	Aphr	82.6	16.3	24772.4	433.3	437.3	91.2	10052.5	442.8	14987.2	731.9
NW	6	2-M	2-4	Aphr	67.6	15.5	24377.5	437.1	366.4	87.6	9371.8	424.7	14841.7	719.5
NW	6	2-M	4-6	Aphr	73.7	15.5	23249.6	413.1	363.7	84.0	8244.6	407.6	14780.4	725.7
NW	6	2-M	6-8	Aphr	75.8	16.6	26682.8	464.4	401.6	92.9	8215.0	409.6	14143.6	717.2
NW	6	2-M	8-10	Aphr	94.2	17.7	26242.2	458.1	408.9	92.4	8316.2	408.9	14508.0	719.9
SE	7	0-M	0-2	Myr	67.9	16.0	28897.4	482.5	589.9	105.7	11738.3	488.2	16659.4	791.1
SE	7	0-M	2-4	Myr	69.2	16.2	28699.3	482.6	656.8	109.5	11359.7	480.8	17094.4	798.3

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	7	0-M	4-6	Myr	73.7	16.5	30155.3	498.8	754.2	116.9	11804.6	490.1	17281.7	804.5
SE	7	0-M	6-8	Myr	61.8	15.4	27523.1	468.6	686.3	110.6	11470.5	477.6	18018.3	807.5
SE	7	0-M	8-10	Myr	64.9	15.7	29119.4	487.3	736.9	115.1	11053.9	466.8	18121.9	803.1
SW	7	0-M	0-2	Myr	77.3	17.0	29458.3	490.9	734.5	115.0	11101.1	466.6	17287.0	785.7
SW	7	0-M	2-4	Myr	61.7	15.5	29226.6	485.6	601.5	107.0	11786.4	484.5	17471.7	799.5
SW	7	0-M	4-6	Myr	76.1	16.8	28492.2	481.1	597.2	106.4	11031.4	472.0	17324.4	797.7
SW	7	0-M	6-8	Myr	63.0	15.7	29013.5	487.9	622.5	108.9	11263.7	469.8	16954.5	780.2
SW	7	0-M	8-10	Myr	66.1	16.2	29203.4	490.0	669.9	111.7	11158.4	473.8	17335.8	797.2
NW	7	0-M	0-2	Myr	74.6	16.7	28139.6	481.7	760.7	116.5	11910.9	482.5	16862.4	781.1
NW	7	0-M	2-4	Myr	65.4	16.0	28188.2	481.5	573.4	104.8	11182.3	466.4	17231.4	782.2
NW	7	0-M	4-6	Myr	56.6	15.2	28369.5	479.1	687.5	111.0	11379.4	470.6	17457.1	787.8
NW	7	0-M	6-8	Myr	66.7	16.2	29392.6	497.1	537.4	104.4	11564.2	475.9	17330.2	788.9
SE	7	1-M	0-2	Myr	56.6	14.3	24940.5	434.8	609.1	102.0	12639.7	484.5	12550.9	675.6
SE	7	1-M	2-4	Myr	58.3	15.0	27552.6	471.1	644.2	109.1	13634.9	509.0	12901.2	695.4
SE	7	1-M	4-6	Myr	63.5	15.5	26958.2	460.8	585.0	104.5	13056.1	496.8	12388.5	679.7

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	7	1-M	6-8	Myr	61.2	15.5	27946.9	471.0	650.1	107.9	13166.9	500.4	12585.8	686.7
SE	7	1-M	8-10	Myr	82.3	16.9	27797.0	476.2	623.6	108.7	13191.3	497.9	13253.1	697.7
SW	7	1-M	0-2	Myr	58.0	14.8	25162.1	444.4	611.1	104.0	10273.5	452.2	19060.4	818.5
SW	7	1-M	2-4	Myr	48.5	14.2	26864.4	458.8	646.8	106.8	9137.1	432.2	17995.0	800.5
SW	7	1-M	4-6	Myr	65.1	15.5	26390.7	458.6	582.9	103.8	8664.0	417.6	18799.3	803.8
SW	7	1-M	6-8	Myr	63.9	15.5	25976.2	460.1	669.3	110.1	9056.8	425.4	18582.3	801.4
SW	7	1-M	8-10	Myr	75.3	16.8	26246.9	458.5	632.8	106.8	8230.1	411.5	18240.5	798.4
NW	7	1-M	0-2	Myr	72.7	16.3	25950.2	454.0	523.1	98.9	9163.4	429.3	18761.4	807.7
NW	7	1-M	2-4	Myr	63.4	14.2	21983.0	389.1	447.5	86.0	8783.4	422.3	18628.6	805.5
NW	7	1-M	4-6	Myr	67.3	15.8	26300.7	457.8	587.3	103.9	8267.0	416.7	18719.1	815.6
NW	7	1-M	6-8	Myr	72.8	15.6	24660.3	431.6	459.5	92.2	8708.0	417.0	19383.0	811.1
NW	7	1-M	8-10	Myr	54.8	14.6	26268.6	449.9	632.1	104.6	8800.2	425.1	18642.9	810.6
SE	7	2-M	0-2	Myr	51.3	14.2	25351.2	439.6	555.2	98.9	15271.6	524.8	10974.2	637.5
SE	7	2-M	2-4	Myr	69.8	15.2	24211.0	421.7	621.6	100.5	14885.0	523.6	11341.6	652.3
SE	7	2-M	4-6	Myr	61.7	13.0	20140.6	354.0	475.8	82.9	13789.9	522.9	10558.3	654.5

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
SE	7	2-M	6-8	Myr	61.2	15.4	27194.8	467.7	679.9	110.9	14566.6	508.4	11407.4	640.6
SE	7	2-M	8-10	Myr	60.6	15.1	27521.2	470.0	594.7	105.4	15191.3	526.0	11435.4	651.8
SW	7	2-M	0-2	Myr	87.5	17.0	25437.1	444.3	575.3	102.3	9718.0	443.3	17114.8	784.6
SW	7	2-M	2-4	Myr	54.4	14.2	23795.0	423.0	489.0	94.3	8802.8	421.5	16535.9	765.5
SW	7	2-M	4-6	Myr	63.9	15.5	27545.2	472.9	676.8	110.5	7922.0	406.0	17093.1	778.8
SW	7	2-M	6-8	Myr	67.0	15.9	26184.2	456.4	547.4	101.6	8115.6	404.0	17456.4	774.4
SW	7	2-M	8-10	Myr	44.7	14.1	25301.0	445.8	484.0	95.8	8653.8	414.7	17541.4	776.9
NW	7	2-M	0-2	Myr	56.7	14.9	25049.6	442.8	545.1	99.5	8005.3	402.1	17902.1	782.6
NW	7	2-M	2-4	Myr	63.1	14.9	25923.7	448.5	476.6	95.4	6577.6	367.3	16607.7	748.0
NW	7	2-M	4-6	Myr	73.2	16.0	24936.0	441.9	467.3	96.4	6870.7	381.2	17284.4	775.4
NW	7	2-M	6-8	Myr	60.0	14.7	23995.5	423.0	486.2	93.7	7640.3	394.2	18583.5	792.8
NW	7	2-M	8-10	Myr	67.1	15.8	25242.3	444.3	459.0	94.5	7034.2	382.7	17588.6	776.9
	8	0-M	0-2	Aphr	86.4	16.9	26077.5	443.9	400.2	89.3	9920.2	440.1	14851.3	728.6
	8	0-M	2-4	Aphr	97.1	17.3	25640.9	436.1	418.9	89.8	10592.7	461.6	15430.9	755.8
NE	8	1-M	0-2	Aphr	77.5	15.3	23038.5	402.2	402.4	85.0	12019.6	488.5	14766.0	745.1

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
NE	8	1-M	2-4	Aphr	72.8	15.6	25991.0	445.2	512.8	96.4	11738.8	476.8	15158.2	742.6
NE	8	1-M	4-6	Aphr	83.4	16.4	26508.6	451.8	469.3	94.3	11661.2	477.3	15078.3	743.9
NE	8	1-M	6-8	Aphr	84.0	16.6	27121.6	456.9	544.1	99.5	11108.8	466.9	14881.3	738.4
NE	8	1-M	0-2	Aphr	85.9	16.8	24037.5	427.2	372.9	85.9	11930.6	469.0	14448.6	711.1
SE	8	1-M	2-4	Aphr	82.6	16.7	25212.2	442.6	367.3	88.3	8652.3	415.9	15222.9	735.1
SE	8	1-M	4-6	Aphr	58.1	14.1	23848.6	416.6	281.4	78.0	8208.3	409.8	15186.8	738.8
SE	8	1-M	6-8	Aphr	79.7	16.8	26567.7	458.2	462.0	95.6	8146.2	402.5	14544.2	715.0
SE	8	1-M	8-10	Aphr	55.8	13.9	24770.6	424.0	277.3	78.0	8200.1	407.6	14499.9	721.4
W	8	1-M	0-2	Aphr	78.8	16.1	22826.8	416.0	348.2	83.9	8756.0	400.8	14838.7	697.9
W	8	1-M	2-4	Aphr	66.6	15.6	24225.8	436.0	371.8	87.6	7799.0	394.0	15540.2	731.9
W	8	1-M	4-6	Aphr	63.9	15.3	23820.6	430.2	506.1	96.7	7600.8	386.5	16408.3	741.1
W	8	1-M	6-8	Aphr	80.2	16.8	23795.6	436.2	444.4	94.2	7191.1	374.2	15282.7	712.7
W	8	1-M	8-10	Aphr	72.4	15.9	24092.9	438.2	447.4	94.5	7777.8	387.7	15282.7	716.0
NE	8	2-M	0-2	Aphr	101.6	17.9	26581.2	453.8	727.5	110.0	8715.8	423.2	15175.0	744.7
NE	8	2-M	2-4	Aphr	99.6	18.1	25972.7	454.4	623.0	105.8	7756.7	391.2	14698.1	711.6

Appendix A, continued.

Dir	Site	Dist. (m)	Depth (cm)	Genus	Zn	Zn Err	Fe	Fe Err	Mn	Mn Err	Ca	Ca Err	K	K Err
NE	8	2-M	4-6	Aphr	106.4	18.3	25997.8	448.1	641.3	105.4	7623.7	401.1	15188.7	744.0
NE	8	2-M	6-8	Aphr	84.6	17.1	26647.9	460.5	620.3	105.9	7316.9	379.4	14100.1	694.3
NE	8	2-M	8-10	Aphr	93.6	16.9	25485.6	436.5	666.9	105.1	6719.0	362.5	13437.3	672.7
SE	8	2-M	0-2	Aphr	61.7	15.3	24675.3	436.7	361.4	87.7	11640.3	465.8	15610.8	737.3
SE	8	2-M	2-4	Aphr	80.7	16.4	24424.2	435.0	430.5	91.2	9736.4	423.4	15878.4	726.3
SE	8	2-M	4-6	Aphr	77.6	16.6	24432.0	440.7	488.0	96.7	8577.2	406.2	14838.7	713.2
SE	8	2-M	6-8	Aphr	59.1	14.9	23744.7	428.1	438.0	91.7	8377.7	395.0	16048.1	722.8
SE	8	2-M	8-10	Aphr	66.2	15.4	24374.7	434.8	395.1	89.9	9238.4	415.5	15954.6	729.5
W	8	2-M	0-2	Aphr	56.3	14.7	21313.1	404.8	438.3	90.4	8023.4	390.2	17026.3	744.1
W	8	2-M	2-4	Aphr	41.9	13.4	21128.7	399.5	442.7	89.4	7506.1	382.3	16164.3	732.5
W	8	2-M	4-6	Aphr	57.9	14.6	21652.7	409.3	434.1	91.5	7101.4	366.0	15924.9	712.4
W	8	2-M	6-8	Aphr	66.8	15.7	22876.5	422.9	431.5	91.3	6940.0	368.8	16044.4	726.0
W	8	2-M	8-10	Aphr	50.5	13.0	18036.9	345.5	378.1	78.8	7399.1	384.5	15796.0	733.5

Appendix B

Atomic Absorbance Data for Soil Samples

All elemental concentrations were recorded in ppm. All samples analyzed via atomic absorbance were split into triplicates to allow for a mean value of concentration to be obtained from each sample (Table 7). Samples from the tunnel entrance at Site 8 beyond a depth of 4 cm were not analyzed due to their structural integrity having been compromised and so making it impossible to collect fractions by depth.

Sample	Cu Absorbance	y=0.127x+0.0033 R^2=0.9997	wt of soil sample(mg)	conc(ppm) *	average conc	std dev	%RSD
8-0 0-2							
I	0.07	0.5252	775	16.94			
II	0.063	0.4701	801	14.67	16.16	1.05	6.52
III	0.065	0.4858	720	16.87			
8-0 2-4 I	0.057	0.4228	801	13.20			
II	0.062	0.4622	802	14.41	13.44	0.72	5.32
III	0.055	0.4071	801	12.71			
8-1B 0-2							
I	0.044	0.3205	801	10.00			
II	0.06	0.4465	802	13.92	11.96	1.60	13.36
III	0.053	0.3913	817	11.97			
8-1B 2-4							
I	0.055	0.4071	806	12.63			
II	0.055	0.4071	825	12.34	12.40	0.17	1.35
III	0.054	0.3992	816	12.23			
8-1B 4-6							
I	0.056	0.4150	816	12.71			
II	0.055	0.4071	813	12.52	12.81	0.28	2.22
III	0.058	0.4307	816	13.20			
8-1B 6-8							
I	0.046	0.3362	811	10.36			
II	0.042	0.3047	810	9.41	10.78	1.32	12.27

Appendix B, continued.

Sample	Cu Absorbance	y=0.127x+0.0033 R^2=0.9997	wt of soil sample(mg)	conc(ppm) *	average conc	std dev	%RSD
III	0.055	0.4071	810	12.56			
8-1B 8-10 I	0.053	0.3913	811	12.06			
II	0.053	0.3913	800	12.23	11.15	1.41	12.63
III	0.041	0.2969	810	9.16			
8-2B 0-2 I	0.042	0.3047	809	9.42			
II	0.044	0.3205	802	9.99	9.37	0.53	5.64
III	0.039	0.2811	808	8.70			
8-2B 2-4 I	0.036	0.2575	816	7.89			
II	0.033	0.2339	810	7.22	8.04	0.74	9.19
III	0.04	0.2890	802	9.01			
8-2B 4-6 I	0.037	0.2654	814	8.15			
II	0.044	0.3205	809	9.90	8.99	0.72	7.98
III	0.04	0.2890	810	8.92			
8-2B 6-8 I	0.021	0.1394	824	4.23			
II	0.042	0.3047	825	9.23	7.57	2.36	31.23
III	0.042	0.3047	823	9.26			
8-2B 8-10 I	0.032	0.2260	490	11.53			
II	0.035	0.2496	522	11.95	11.05	1.00	9.06
III	0.031	0.2181	565	9.65			

Appendix B, continued.

Sample	Cu Absorbance	y=0.127x+0.0033 R^2=0.9997	wt of soil sample(mg)	conc(ppm) *	average conc	std dev	%RSD
			Average Cu Concentration PPM (distance)				
			0m	14.80			
			1m	11.82			
			2m	9.00			
			Average Cu Concentration PPM (depth)				
			0-2cm	12.50			
			2-4cm	11.29			
			4-6cm	10.90			
			6-8cm	9.18			
			8-10cm	11.10			
5-0B 0-2							
I	0.061	0.4543	832	13.65			
II	0.06	0.4465	822	13.58	13.90	0.41	2.95
III	0.064	0.4780	825	14.48			
5-0B 2-4							
I	0.06	0.4465	810	13.78			
II	0.063	0.4701	802	14.65	13.67	0.85	6.22
III	0.055	0.4071	809	12.58			
5-0B 4-6							
I	0.06	0.4465	818	13.64			
II	0.062	0.4622	822	14.06	12.75	1.57	12.31
III	0.047	0.3441	816	10.54			
5-0B 6-8							
I	0.043	0.3126	809	9.66			
II	0.061	0.4543	823	13.80	12.42	1.95	15.69
III	0.061	0.4543	824	13.78			

Appendix B, continued.

Sample	Cu Absorbance	y=0.127x+0.0033 R^2=0.9997	wt of soil sample(mg)	conc(ppm) *	average conc	std dev	%RSD
5-0B 8-10 I	0.045	0.3283	812	10.11			
II	0.051	0.3756	808	11.62	11.62	1.23	10.57
III	0.058	0.4307	821	13.12			
5-1B 0-2 I	0.061	0.4543	811	14.01			
II	0.059	0.4386	810	13.54	14.11	0.52	3.66
III	0.064	0.4780	808	14.79			
5-1B 2-4 I	0.062	0.4622	835	13.84			
II	0.063	0.4701	810	14.51	13.65	0.79	5.78
III	0.056	0.4150	823	12.61			
5-1B 4-6 I	0.059	0.4386	820	13.37			
II	0.061	0.4543	816	13.92	13.63	0.23	1.65
III	0.062	0.4622	850	13.59			
5-1B 6-8 I	0.06	0.4465	821	13.59			
II	0.062	0.4622	823	14.04	13.65	0.30	2.18
III	0.06	0.4465	838	13.32			
5-1B 8-10 I	0.063	0.4701	818	14.37			
II	0.061	0.4543	815	13.94	13.92	0.37	2.63
III	0.059	0.4386	814	13.47			
5-2B 0-2 I	0.058	0.4307	831	12.96			
II	0.057	0.4228	838	12.61	13.12	0.49	3.74
III	0.061	0.4543	824	13.78			
5-2B 2-4 I	0.058	0.4307	835	12.90			

Appendix B, continued.

Sample	Cu Absorbance	y=0.127x+0.0033 R^2=0.9997	wt of soil sample(mg)	conc(ppm) *	average conc	std dev	%RSD
II	0.059	0.4386	823	13.32	12.99	0.24	1.87
III	0.057	0.4228	829	12.75			
5-2B 4-6							
I	0.058	0.4307	815	13.21			
II	0.05	0.3677	811	11.34	12.88	1.15	8.93
III	0.062	0.4622	820	14.09			
5-2B 6-8							
I	0.055	0.4071	822	12.38			
II	0.063	0.4701	838	14.02	11.85	2.03	17.10
III	0.042	0.3047	833	9.15			
5-2B 8-10 I	0.041	0.2969	802	9.25			
II	0.046	0.3362	814	10.33	10.64	1.28	12.06
III	0.056	0.4150	840	12.35			

Average Cu Concentration PPM (distance)

0m	12.87
1m	13.79
2m	12.30

Average Cu Concentration PPM (depth)

0-2cm	13.71
2-4cm	13.44
4-6cm	13.09
6-8cm	12.64
8-10cm	12.06

Appendix B, continued.

Ca Absorbance	y=0.048x+0.0107 R^2=0.9986	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.536	10.94	775	353.02			
0.206	4.07	801	126.99	243.51	92.41	37.95
0.357	7.21	720	250.51			
0.262	5.24	801	163.40			
0.23	4.57	802	142.42	153.81	8.66	5.63
0.25	4.99	801	155.60			
0.42	8.53	801	266.14			
0.357	7.21	802	224.89	276.37	46.76	16.92
0.541	11.05	817	338.06			
0.222	4.40	806	136.54			
0.215	4.26	825	128.98	104.53	40.05	38.31
0.086	1.57	816	48.06			
0.126	2.40	816	73.59			
0.168	3.28	813	100.77	80.74	14.36	17.78
0.117	2.21	816	67.85			
0.124	2.36	811	72.76			
0.129	2.46	810	76.04	75.38	1.93	2.56
0.131	2.51	810	77.35			
0.079	1.42	811	43.86			
0.106	1.99	800	61.51	51.26	7.48	14.59
0.086	1.57	810	48.42			
0.316	6.36	809	196.55			
0.256	5.11	802	159.30	179.70	15.41	8.58
0.295	5.92	808	183.26			
0.132	2.53	816	77.42			
0.252	5.03	810	155.16	120.67	32.33	26.80
0.21	4.15	802	129.43			
0.152	2.94	814	90.41			
0.151	2.92	809	90.32	94.82	6.29	6.64
0.172	3.36	810	103.72			

Appendix B, continued.

Ca Absorbance	y=0.048x+0.0107 R^2=0.9986	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.064	1.11	824	33.69			
0.11	2.07	825	62.69	52.23	13.15	25.17
0.106	1.99	823	60.31			
0.066	1.15	490	58.78			
0.083	1.51	522	72.14	69.54	7.94	11.42
0.095	1.76	565	77.71			

Average Ca Concentration PPM (distance)	
0m	198.66
1m	117.66
2m	103.39

Average Ca Concentration PPM (depth)	
0-2cm	233.19
2-4cm	126.33
4-6cm	87.78
6-8cm	63.81
8-10cm	60.40

0.525	10.71	832	321.95			
0.53	10.82	822	329.04	319.33	9.18	2.88
0.497	10.13	825	307.01			
0.505	10.30	810	317.84			
0.519	10.59	802	330.10	323.56	5.04	1.56
0.512	10.44	809	322.74			
0.504	10.28	818	314.09			
0.532	10.86	822	330.30	298.90	33.60	11.24
0.406	8.24	816	252.31			
0.661	13.55	809	418.66			
0.506	10.32	823	313.45	345.65	51.74	14.97

Appendix B, continued.

Ca Absorbance	y=0.048x+0.0107 R^2=0.9986	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.493	10.05	824	304.85			
0.401	8.13	812	250.35			
0.483	9.84	808	304.44	292.42	30.64	10.48
0.519	10.59	821	322.46			
0.522	10.65	811	328.36			
0.433	8.80	810	271.54	306.61	25.03	8.16
0.507	10.34	808	319.91			
0.517	10.55	835	315.81			
0.499	10.17	810	313.98	319.47	6.52	2.04
0.53	10.82	823	328.64			
0.501	10.21	820	311.42			
0.525	10.71	816	328.27	323.79	8.86	2.74
0.552	11.28	850	331.68			
0.534	10.90	821	331.98			
0.529	10.80	823	328.00	322.81	10.28	3.18
0.507	10.34	838	308.46			
0.536	10.94	818	334.47			
0.51	10.40	815	319.08	317.94	13.98	4.40
0.48	9.78	814	300.28			
0.572	11.69	831	351.80			
0.594	12.15	838	362.53	361.64	7.70	2.13
0.597	12.21	824	370.59			
0.494	10.07	835	301.46			
0.544	11.11	823	337.50	331.58	22.57	6.81
0.577	11.80	829	355.79			
0.563	11.51	815	352.95			
0.483	9.84	811	303.32	358.56	47.56	13.26
0.671	13.76	820	419.40			
0.491	10.01	822	304.33			
0.533	10.88	838	324.62	276.82	53.89	19.47

An Evaluation of Soil Composition Associated with Two Genera of Ground Nesting Ants

Appendix B, continued.

Ca Absorbance	y=0.048x+0.0107 R^2=0.9986	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.333	6.71	833	201.52			
0.346	6.99	802	217.75			
0.444	9.03	814	277.24	265.92	35.62	13.39
0.499	10.17	840	302.77			

Average Ca Concentration PPM (distance)	
0m	315.97
1m	318.12
2m	318.90

Average Ca Concentration PPM (depth)	
0-2cm	329.19
2-4cm	324.87
4-6cm	327.08
6-8cm	315.10
8-10cm	292.09

Zn Absorbance	y=0.2617x+0.024 R^=0.994	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.74	2.74	775	88.26			
0.715	2.64	801	82.41	88.47	5.033	5.69
0.738	2.73	720	94.73			
0.68	2.51	801	78.24			
0.706	2.61	802	81.24	79.12	1.505	1.90
0.677	2.50	801	77.88			
0.821	3.05	801	95.05			
0.71	2.62	802	81.71	84.65	7.589	8.97
0.684	2.52	817	77.17			

Appendix B, continued.

Zn Absorbance	y=0.2617x+0.024 R^=0.994	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.702	2.59	806	80.36			
0.698	2.58	825	78.04	78.13	1.789	2.29
0.673	2.48	816	75.98			
0.689	2.54	816	77.85			
0.688	2.54	813	78.02	77.75	0.270	0.35
0.685	2.53	816	77.38			
0.616	2.26	811	69.73			
0.597	2.19	810	67.58	71.91	4.690	6.52
0.689	2.54	810	78.43			
0.67	2.47	811	76.09			
0.682	2.51	800	78.57	71.60	8.164	11.40
0.534	1.95	810	60.15			
0.522	1.90	809	58.81			
0.514	1.87	802	58.37	57.78	1.158	2.00
0.499	1.82	808	56.16			
0.783	2.90	816	88.86			
0.431	1.56	810	48.00	63.76	17.936	28.13
0.481	1.75	802	54.43			
0.442	1.60	814	49.06			
0.51	1.86	809	57.39	53.96	3.558	6.59
0.494	1.80	810	55.43			
0.317	1.12	824	33.97			
0.484	1.76	825	53.26	47.07	9.268	19.69
0.489	1.78	823	53.97			
0.401	1.44	490	73.50			
0.411	1.48	522	70.82	70.09	3.129	4.46
0.414	1.49	565	65.94			

Average Zn Concentration PPM
(distance)

Appendix B, continued.

Zn Absorbance	y=0.2617x+0.024 R^=0.994	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
		0m	83.79			
		1m	76.81			
		2m	58.53			
		Average Zn Concentration PPM (depth)				
		0-2cm	76.96			
		2-4cm	73.67			
		4-6cm	65.86			
		6-8cm	59.49			
		8-10cm	70.85			
0.443	1.60	832	48.11			
0.44	1.59	822	48.35	49.95	2.431	4.87
0.485	1.76	825	53.38			
0.441	1.59	810	49.18			
0.466	1.69	802	52.65	50.40	1.595	3.16
0.442	1.60	809	49.36			
0.446	1.61	818	49.28			
0.468	1.70	822	51.60	48.96	2.294	4.68
0.417	1.50	816	46.01			
0.367	1.31	809	40.50			
0.439	1.59	823	48.17	45.60	3.601	7.90
0.439	1.59	824	48.11			

Appendix B, continued.

Zn Absorbance	y=0.2617x+0.024 R^=0.994	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.364	1.30	812	40.00			
0.432	1.56	808	48.24	45.24	3.716	8.22
0.432	1.56	821	47.47			
0.463	1.68	811	51.71			
0.414	1.49	810	46.00	49.28	2.409	4.89
0.448	1.62	808	50.13			
0.429	1.55	835	46.33			
0.428	1.54	810	47.65	47.19	0.606	1.28
0.434	1.57	823	47.59			
0.417	1.50	820	45.78			
0.438	1.58	816	48.47	46.93	1.131	2.41
0.438	1.58	850	46.53			
0.435	1.57	821	47.82			
0.455	1.65	823	50.03	47.63	2.046	4.30
0.419	1.51	838	45.03			
0.449	1.62	818	49.63			
0.428	1.54	815	47.35	47.66	1.498	3.14
0.416	1.50	814	46.00			
0.375	1.34	831	40.35			
0.386	1.38	838	41.27	41.81	1.470	3.51
0.402	1.44	824	43.82			
0.374	1.34	835	40.04			
0.384	1.38	823	41.79	41.57	1.164	2.80
0.396	1.42	829	42.87			
0.382	1.37	815	41.96			
0.332	1.18	811	36.28	46.62	10.860	23.29
0.553	2.02	820	61.63			
0.359	1.28	822	38.93			
0.405	1.46	838	43.43	38.12	4.704	12.34

Appendix B, continued.

Zn Absorbance	y=0.2617x+0.024 R^=0.994	wt of soil sample (mg)	conc(ppm)*	average conc	std dev	%RSD
0.303	1.07	833	32.00			
0.3	1.05	802	32.88			
0.321	1.13	814	34.86	35.73	2.760	7.72
0.371	1.33	840	39.46			

Average Zn Concentration PPM (distance)	
0m	48.03
1m	47.74
2m	40.77

Average Zn Concentration PPM (depth)	
0-2cm	47.01
2-4cm	46.38
4-6cm	47.50
6-8cm	43.78
8-10cm	42.88

Appendix C

Atomic Absorbance Data for Ant Body Samples

Sample	Cu Absorbance	y=0.127x+0.0033 R^2=0.9997	wt of soil sample(mg)	conc(ppm)*
Ant 5	0.004	0.0055	15	9.19
Ant 8	0.003	-0.0024	10	-5.91
	Ca Absorbance			
Ant 5	0.045	0.71	15	1190.97
Ant 8	0.016	0.11	10	276.04
	Zn Absorbance			
Ant 5	0.051	0.10	15	171.95
Ant 8	0.028	0.02	10	38.21

Appendix D
AA Post-Hoc Two-Tailed T Test Results

Post-hoc t tests were conducted on all AA samples that were statistically significant, with a P value < 0.05.

Calcium, Sample Site 8, Significance of depth at each distance

1 m (0-2 cm vs 2-4 cm)	*Variable 1*	*Variable 2*
Mean	276.37	104.53
Variance	3280.30	2405.49
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	4.00	
t Stat	3.95	
P(T<=t) two-tail	0.02	
t Critical two-tail	5.60	

*0-2 and 2-4 are similar at 1 m;

1 m (0-2 cm vs 4-6 cm)	*Variable 1*	*Variable 2*
Mean	276.37	80.74
Variance	3280.30	309.26
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	5.66	
P(T<=t) two-tail	0.03	
t Critical two-tail	14.09	

*0-2 and 4-6 are similar at 1 m;

1 m (0-2 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
Mean	276.37	75.38
Variance	3280.30	5.59
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
Df	2.00	
t Stat	6.07	
P(T<=t) two-tail	0.03	
t Critical two-tail	14.09	

*0-2 and 6-8 are similar at 1 m

1 m (0-2 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
Mean	276.37	51.26
Variance	3280.30	83.89
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	6.72	
P(T<=t) two-tail	0.02	
t Critical two-tail	14.09	

*0-2 and 8-10 are similar at 1 m;

1 m (2-4 cm vs 4-6 cm)	*Variable 1*	*Variable 2*
Mean	104.53	80.74
Variance	2405.49	309.26
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	3.00	
t Stat	0.79	
P(T<=t) two-tail	0.49	
t Critical two-tail	7.45	

*2-4 and 4-6 are similar at 1 m;

1 m (2-4 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
Mean	104.53	75.38
Variance	2405.49	5.59
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	1.03	
P(T<=t) two-tail	0.41	
t Critical two-tail	14.09	

*2-4 and 6-8 are similar at 1 m;

Appendix D, continued.

Calcium, Sample Site 8, Significance of depth at each distance

1 m (2-4 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
Mean	104.53	51.26
Variance	2405.49	83.89
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	1.85	
P(T<=t) two-tail	0.21	
t Critical two-tail	14.09	

*2-4 and 8-10 are similar at 1 m;

1 m (4-6 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
Mean	80.74	75.38
Variance	309.26	5.59
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	0.52	
P(T<=t) two-tail	0.65	
t Critical two-tail	14.09	

*4-6 and 6-8 are similar at 1 m;

1 m (4-6 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
Mean	80.74	51.26
Variance	309.26	83.89
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	3.00	
t Stat	2.57	
P(T<=t) two-tail	0.08	
t Critical two-tail	7.45	

*4-6 and 8-10 are similar at 1 m;

1 m (6-8 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
Mean	75.38	51.26
Variance	5.59	83.89
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	4.42	
P(T<=t) two-tail	0.05	
t Critical two-tail	14.09	

*6-8 and 8-10 are similar at 1 m;

Appendix D, continued.

Calcium, Sample Site 8, Significance of depth at each distance								
2 m (0-2 cm vs 2-4 cm)	*Variable 1*	*Variable 2*	*2 m (0-2 cm vs 4-6 cm)*	*Variable 1*	*Variable 2*	*2 m (0-2 cm vs 6-8 cm)*	*Variable 1*	*Variable 2*
Mean	179.70	120.67	Mean	179.70	94.82	Mean	179.70	52.23
Variance	356.36	1568.19	Variance	356.36	59.40	Variance	356.36	259.21
Observations	3.00	3.00	Observations	3.00	3.00	Observations	3.00	3.00
Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00	
df	3.00		df	3.00		df	4.00	
t Stat	2.33		t Stat	7.21		t Stat	8.90	
P(T<=t) two-tail	0.10		P(T<=t) two-tail	0.01		P(T<=t) two-tail	0.00	
t Critical two-tail	7.45		t Critical two-tail	7.45		t Critical two-tail	5.60	
corrected alpha = alpha (0.05)/# tests performed = 0.05/10 = 0.005 *0-2 and 2-4 are similar at 2 m;			*0-2 and 4-6 are similar at 2 m (although slightly different)			*0-2 and 6-8 are different at 2 m		
2 m (0-2 cm vs 8-10 cm)	*Variable 1*	*Variable 2*	*2 m (2-4 cm vs 4-6 cm)*	*Variable 1*	*Variable 2*	*2 m (2-4 cm vs 6-8 cm)*	*Variable 1*	*Variable 2*
Mean	179.70	69.54	Mean	120.67	94.82	Mean	120.67	52.23
Variance	356.36	94.64	Variance	1568.19	59.40	Variance	1568.19	259.21
Observations	3.00	3.00	Observations	3.00	3.00	Observations	3.00	3.00
Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00	
df	3.00		df	2.00		df	3.00	
t Stat	8.98		t Stat	1.11		t Stat	2.77	
P(T<=t) two-tail	0.00		P(T<=t) two-tail	0.38		P(T<=t) two-tail	0.07	
t Critical two-tail	7.45		t Critical two-tail	14.09		t Critical two-tail	7.45	
*0-2 and 8-10 are different at 2 m;			*2-4 and 4-6 are similar at 2 m;			*2-4 and 6-8 are similar at 2 m;		

Appendix D, continued.

Calcium, Sample Site 8, Significance of depth at each distance

2 m (2-4 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
Mean	120.67	69.54
Variance	1568.19	94.64
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	2.17	
P(T<=t) two-tail	0.16	
t Critical two-tail	14.09	

*2-4 and 8-10 are similar at 2 m;

2 m (4-6 cm vs 6-8 cm)	*Variable 1*	*Variable 2*
Mean	94.82	52.23
Variance	59.40	259.21
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	3.00	
t Stat	4.13	
P(T<=t) two-tail	0.03	
t Critical two-tail	7.45	

*4-6 and 6-8 are similar at 2 m;

2 m (4-6 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
Mean	94.82	69.54
Variance	59.40	94.64
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	4.00	
t Stat	3.53	
P(T<=t) two-tail	0.02	
t Critical two-tail	5.60	

*4-6 and 8-10 are similar at 2 m;

2 m (6-8 cm vs 8-10 cm)	*Variable 1*	*Variable 2*
Mean	52.23	69.54
Variance	259.21	94.64
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	3.00	
t Stat	-1.59	
P(T<=t) two-tail	0.21	
t Critical two-tail	7.45	

*6-8 and 8-10 are similar at 2 m;

Appendix D, continued.

Calcium, Sample Site 5, Significance of distance at each depth								
0-2 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*0-2 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*0-2 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
Mean	319.33	306.61	Mean	319.33	361.64	Mean	299.95	361.64
Variance	126.48	940.00	Variance	126.48	88.87	Variance	1614.37	88.87
Observations	3.00	3.00	Observations	3.00	3.00	Observations	2.00	3.00
Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00	
df	3.00		df	4.00		df	1.00	
t Stat	0.68		t Stat	-4.99		t Stat	-2.13	
P(T<=t) two-tail	0.55		P(T<=t) two-tail	0.01		P(T<=t) two-tail	0.28	
t Critical two-tail	4.85		t Critical two-tail	3.96		t Critical two-tail	38.11	
*Sample Site 5 0 m and 1 m is different at 0-2 cm			*Sample Site 5 0 m and 2 m is similar at 0-2 cm * only nest and 2m from nest (5-0B and 5-2B) are significantly different			*Sample Site 5 1 m and 2 m is different at 0-2 cm		

Copper, Sample Site 8, Significance of distance at each depth								
0-2 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*0-2 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*0-2 cm (1 m vs 2 m)*	*10.00*	*9.42*
Mean	16.16	11.96	Mean	16.16	9.37	Mean	12.95	9.34
Variance	1.66	3.83	Variance	1.66	0.42	Variance	1.89	0.84
Observations	3.00	3.00	Observations	3.00	3.00	Observations	2.00	2.00
Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00	
df	3.00		df	3.00		df	2.00	
t Stat	3.10		t Stat	8.15		t Stat	3.09	
P(T<=t) two-tail	0.05		P(T<=t) two-tail	0.00		P(T<=t) two-tail	0.09	
t Critical two-tail	4.86		t Critical two-tail	4.86		t Critical two-tail	4.30	
			*8-0 and 8-2B are significantly different (8-1B has bigger variance so is similar to both 8-0 and 8-2B)					

Appendix D, continued.

Copper, Sample Site 8, Significance of distance at each depth

2-4 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*
Mean	13.44	12.40
Variance	0.77	0.04
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	2.00	
P(T<=t) two-tail	0.18	
t Critical two-tail	7.66	

2-4 cm (0 m vs 2 m)	*Variable 1*	*Variable 2*
Mean	13.44	8.04
Variance	0.77	0.82
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	4.00	
t Stat	7.43	
P(T<=t) two-tail	0.00	
t Critical two-tail	3.97	

*8-0 and 8-2B at 2-4 cm, and 8-1B and 8-2B at 2-4 cm are significantly different (8-0 and 8-1B similar)

2-4 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
Mean	12.40	8.04
Variance	0.04	0.82
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	8.14	
P(T<=t) two-tail	0.01	
t Critical two-tail	7.66	

4-6 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*
Mean	12.81	8.99
Variance	0.12	0.77
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	3.00	
t Stat	6.99	
P(T<=t) two-tail	0.01	
t Critical two-tail	3.18	

*they are significantly different (same results as ANOVA since only 2 groups)

Appendix D, continued.

Zinc, Sample Site 8, Significance of distance at each depth

0-2 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*
Mean	88.47	84.65
Variance	38.00	86.38
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	3.00	
t Stat	0.59	
P(T<=t) two-tail	0.59	
t Critical two-tail	4.85	

corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166

8-0 and 8-1B at 0-2 cm are not significantly different. However, if 0.05 instead of 0.0166 were used for p value comparison, 8-0 and 8-2B, as well as 8-1B and 8-2B would be significantly different

0-2 cm (0 m vs 2 m)	*Variable 1*	*Variable 2*
Mean	88.47	57.78
Variance	38.00	2.01
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	8.40	
P(T<=t) two-tail	0.01	
t Critical two-tail	7.64	

8-0 and 8-2B at 0-2 cm are significantly different

0-2 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
Mean	84.65	57.78
Variance	86.38	2.01
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	4.95	
P(T<=t) two-tail	0.04	
t Critical two-tail	7.64	

8-1B and 8-2B at 0-2 cm are not significantly different

4-6 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
Mean	77.75	48.96
Variance	0.11	7.89
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	17.63	
P(T<=t) two-tail	0.00	
t Critical two-tail	4.30	

corrected alpha = alpha (0.05)/# tests performed = 0.05/1 = 0.05

6-8 cm (1 m vs 2 m)	*Variable 1*	*Variable 2*
Mean	71.91	47.07
Variance	33.00	128.85
Observations	3.00	3.00
Hypothesized Mean Difference	0.00	
df	3.00	
t Stat	3.38	
P(T<=t) two-tail	0.04	
t Critical two-tail	3.18	

corrected alpha = alpha (0.05)/# tests performed = 0.05/1 = 0.05

Appendix D, continued.

*they are significantly different (same results as ANOVA since only 2 groups)

Zinc, Sample Site 5, Significance of distance at each depth

0-2 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*0-2 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*0-2 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
Mean	49.95	49.28	Mean	49.95	41.81	Mean	49.28	41.81
Variance	8.87	8.71	Variance	8.87	3.24	Variance	8.71	3.24
Observations	3.00	3.00	Observations	3.00	3.00	Observations	3.00	3.00
df	4.00		df	3.00		df	3.00	
t Stat	0.28		t Stat	4.05		t Stat	3.74	
P(T<=t) two-tail	0.80		P(T<=t) two-tail	0.03		P(T<=t) two-tail	0.03	
t Critical two-tail	3.96		t Critical two-tail	4.85		t Critical two-tail	4.85	

corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166

* 5-0B and 5-1B are not significantly different at 0-2 cm
*because of larger variances in 5-0 and 5-1B, there appear to be no differences, even with 5-2B at 0-2 cm

* 5-0B and 5-2B are not significantly different at 0-2 cm

* 5-1B and 5-2B are not significantly different at 0-2 cm

2-4 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*2-4 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*2-4 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
Mean	50.40	47.19	Mean	50.40	41.57	Mean	47.19	41.57
Variance	3.81	0.55	Variance	3.81	2.03	Variance	0.55	2.03
Observations	3.00	3.00	Observations	3.00	3.00	Observations	3.00	3.00
Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00	
df	3.00		df	4.00		df	3.00	
t Stat	2.66		t Stat	6.33		t Stat	6.06	
P(T<=t) two-tail	0.08		P(T<=t) two-tail	0.00		P(T<=t) two-tail	0.01	
t Critical two-tail	4.85		t Critical two-tail	3.96		t Critical two-tail	4.85	

corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166

*5-0 and 5-1B are not significantly different at 2-4 cm

*5-0 and 5-2B are significantly different at 2-4 cm

*5-1B and 5-2B are significantly different at 2-4 cm

Appendix D, continued.

Zinc, Sample Site 5, Significance of distance at each depth

8-10 cm (0 m vs 1 m)	*Variable 1*	*Variable 2*	*8-10 cm (0 m vs 2 m)*	*Variable 1*	*Variable 2*	*8-10 cm (1 m vs 2 m)*	*Variable 1*	*Variable 2*
Mean	45.24	47.66	Mean	45.24	35.73	Mean	47.66	35.73
Variance	20.72	3.36	Variance	20.72	11.42	Variance	3.36	11.42
Observations	3.00	3.00	Observations	3.00	3.00	Observations	3.00	3.00
Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00		Hypothesized Mean Difference	0.00	
df	3.00		df	4.00		df	3.00	
t Stat	-0.86		t Stat	2.90		t Stat	5.37	
P(T<=t) two-tail	0.45		P(T<=t) two-tail	0.04		P(T<=t) two-tail	0.01	
t Critical two-tail	4.85		t Critical two-tail	3.96		t Critical two-tail	4.85	

corrected alpha = alpha (0.05)/# tests performed = 0.05/3 = 0.0166

*5-0 and 5-1B are not significantly different at 8-10 cm

RESULT: it appears that Zn conc decreases with distance from the nest until a depth of 4-6cm (but jumps up again at 8-10cm?) - post hoc negates some of this due to greater variance
however, if 0.05 instead of 0.0166 for p value comparison, Zn conc decreases at 1 to 2 m at depths of 0-2 cm, 2-4 cm, (not diff 4-6 cm or 6-8 cm), and back again 8-10 cm

*5-0 and 5-2B are not significantly different at 8-10 cm

*5-1B and 5-2B are significantly different at 8-10 cm

Appendix E

Atomic Absorbance Equations and Calibration Curves

Calibration curves were formed using 0.5, 1, 2, 3, 4, and 10 ppm solutions of calcium, copper, and zinc (Fisher Scientific). The calibration curve ($y = mx + b$) was generated based on the known elemental concentration of the standard solutions used and the corresponding resulting absorbance. The calibration curves generated have good correlation ($R^2 > 0.99$).

The cathode lamps used operated at wavelengths of 422.7 nm for calcium, 324.8 nm for copper, and 213.9 nm for zinc. Concentrations were determined using Beer's Law and Equations 1 and 2 (adapted from JoVE Science Education Database 2019).

Beer's Law allows for the absorbance measured to be converted to a concentration of the metal in the sample. Equation 1 determines the concentration of metal in the soil sample in milligrams. Equation 2 converts the results from Equation 1 to parts per million.

Beer's Law: $A = \varepsilon\, b\, C$

Equation 1:

Digestion solution volume (mL) X weight of metal (mg)/L solution X 1 L/1000 mL

= weight of metal in solution sample (mg)

Equation 2:

Weight of metal in solution sample (mg)/weight of soil (g) X 1000 g/1 kg

= ppm concentration of metal in soil

Appendix E, continued.

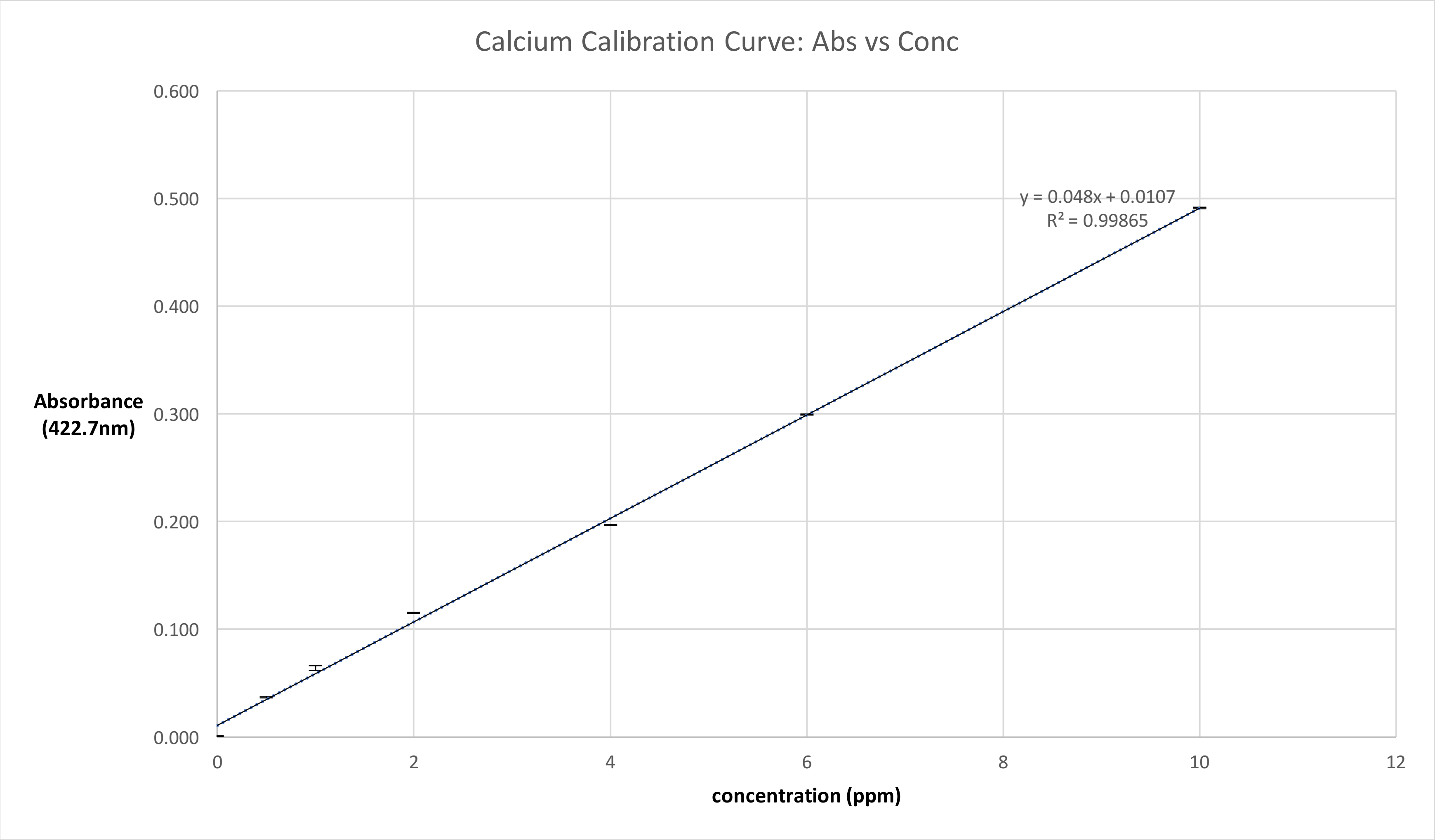

Figure 12: Calibration curve and line equation for atomic absorbance of calcium.

Appendix E, continued.

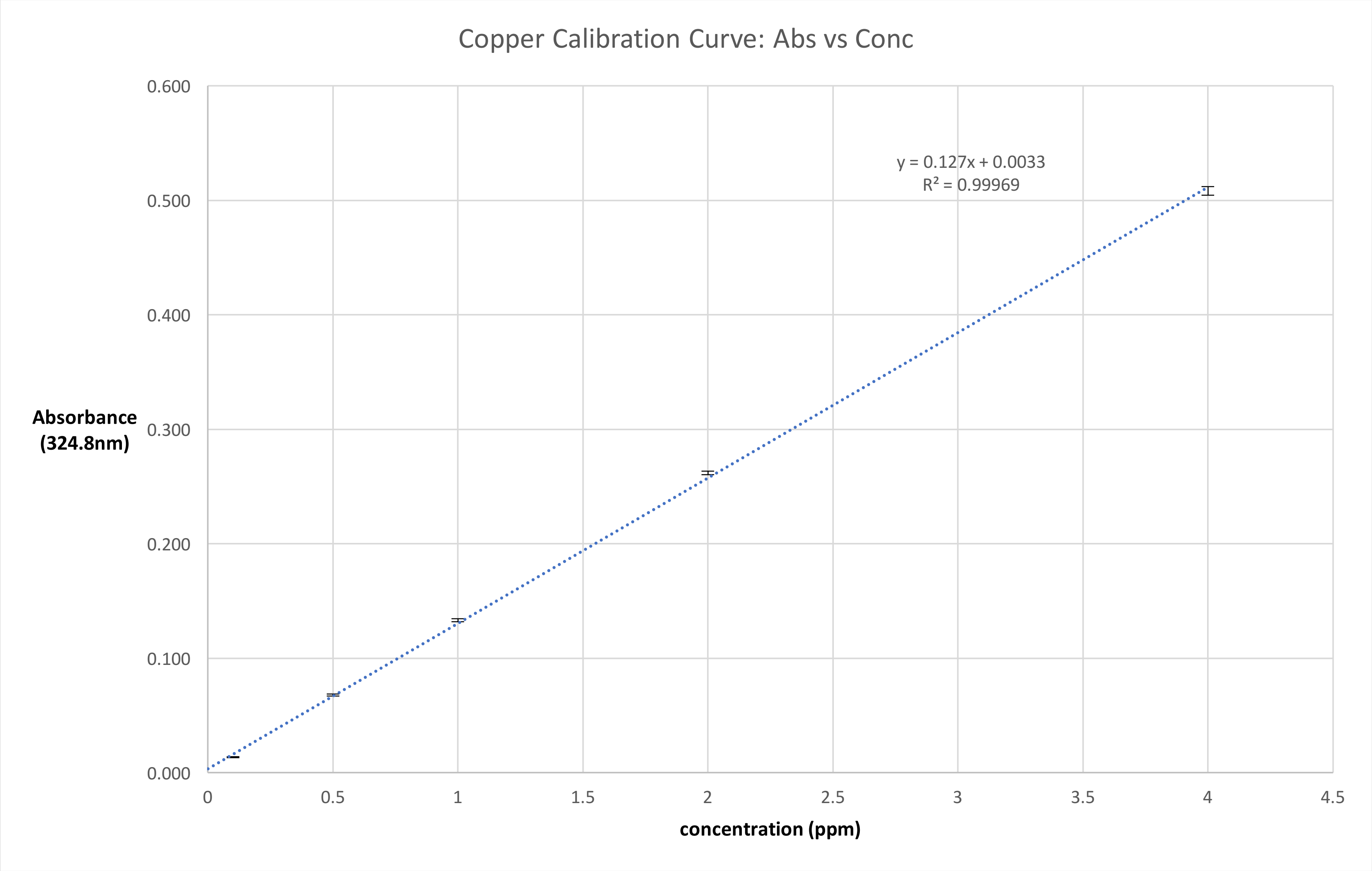

Figure 13: Calibration curve and line equation for atomic absorbance of copper.

Appendix E, continued.

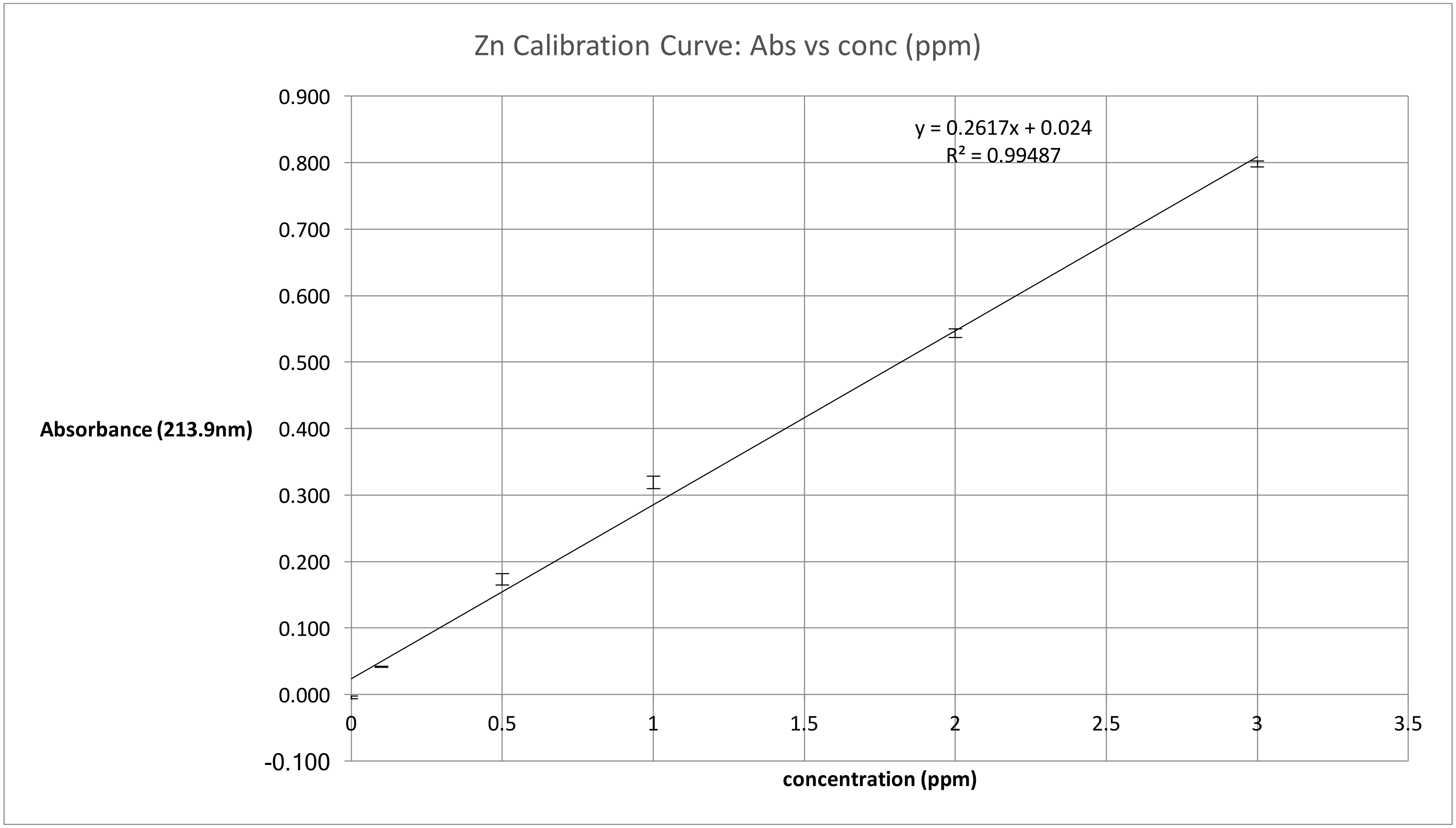

Figure 14: Calibration curve and line equation for atomic absorbance of zin

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