QUANTIFICATION AND COMPARISON OF CALCIUM IN JUNIPER ASH AND SOIL USED IN TRADITIONAL NAVAJO FOODS

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ABSTRACT

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In order to achieve healthy bone formation and structure, the human body needs an adequate source of calcium in their diet. A calcium deficiency can cause multiple bone-related disorders such as osteoporosis and rickets in children. Although most Native Americans, due to being lactose intolerant, are unable to consume dairy products, previous research has demonstrated that elderly Native American women had fewer hip fractures than elderly Caucasian women. This suggested that they are receiving an adequate non-dairy based source of calcium in their diet on a daily basis.

A potential source of calcium is traditional Navajo foods, specifically blue corn-based dishes that contain juniper ash which comes from branches from a juniper tree that are burned until only an ash is left. Previous work done by Christensen quantified calcium in juniper ash collected on the Navajo reservation and suggested juniper ash as an adequate source of calcium comparable to milk. The focus of the present research was to expand on this previous research by (1) quantifying calcium in different segments of the juniper tree, (2) comparing two different ashing techniques (laboratory and traditional Navajo ashing process), and (3) establishing a relationship between calcium concentration of the juniper tree and the soil beneath the tree.

During sample collection, 20 sampling sites were selected for analysis, along with five juniper samples collected from a flea market as well as a blue corn meal sample; a total of 27

samples were collected. Quantification of calcium was done via flame atomic absorption spectroscopy utilizing external calcium calibration standards. Results suggest the following: (1) there is a greater average concentration of calcium in the juniper branch (309 mg/g) compared to the juniper leaves (279 mg/g). (2) The type of ashing process will determine the amount of calcium in juniper samples. Samples ashed via muffle furnace had a greater average concentration of calcium (289 mg/g) compared to traditional Navajo process (242 mg/g). (3) Compared to the calcium concentration in a juniper tree, the soil collected beneath had an extremely low amount of calcium (11.8 mg/g).

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Chapter 1: Introduction

1.1 The Navajo People and their Traditional Foods

As shown throughout their history, the Navajo people have stood the test of resiliency, suffering and more. Not only did they survive every conflict thrown to them, they came out a stronger people. They adapted to the changes that surround them. Their culture, traditions, and food molded as the people did. They absorbed other native and non-native cultures (Iverson 1983).

Navajos, among other Athabaskan tribes from the north, migrated to the southwestern area of what is now the United States hundreds of years ago. After this migration, Navajos were primarily hunter/gatherers, foraging for wild plants and wild game in their surrounding area. This continued until their neighbors, the Pueblo, had a significant influence on cultivating the land to produce the fruits and vegetables needed. Farming became their primary source of food, with their main staples being corn, beans, and squash. After adopting these farming techniques, the Navajo people became less nomadic and leaned toward settling in one particular area. Once arrived, the Spanish culture had an influence on the Navajos as well. The Spanish conquistadors brought with them horses, sheep, goats and cattle. Now having obtained livestock, Navajos transitioned from hunter/gatherers to farmers/herders. Livestock, specifically sheep, was utilized for their wool and meat (on rare occasions, milk) (Iverson 1983). The number of livestock had a direct correlation to social status and wealth.

In 1864, after conflict with the Anglo-Americans in the surrounding area, they would be rounded up into camps by the government to be removed from their homeland, this was known as

The Long Walk. They were sent to Fort Sumner, where the soil was barren, crops that were grown failed at that location. They were not in a position to farm or herd livestock. They relied on the food and water provided by the government, who was unable to successfully provide for all the people in the camps. Traditional foods are defined as foods that utilize native plants and animals that are indigenous to the area where a native tribe resides. They were also unable to provide any type of traditional foods to the people because of their relocation (Gurney et al. 2015). The food they relied on was not culturally appropriate to the Navajo people. Unable to protect themselves and the government not providing protection, they would be attacked and raided by other tribes (Iverson and Porter 1990). Similar situations for other Native American tribes were occurring as well. Over the course of time, these teachings developed a culture that the Navajo have to this day. This culture, along with its foods has survived through trials, tribulations and the birth of the modern world that the Navajo now live.

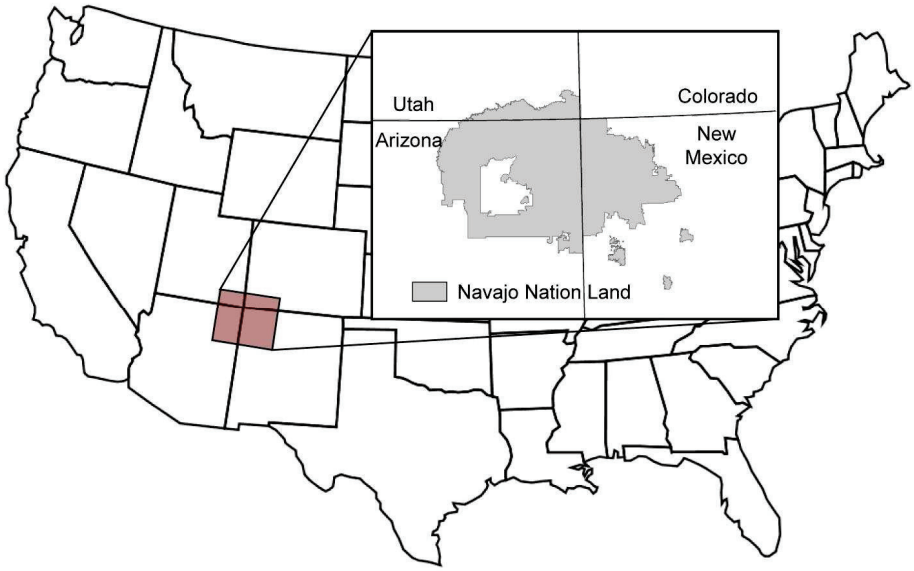

Figure 1.1: Map of the Navajo Nation in respects to the United States (earthzine.org)

Today, the Navajo tribe is primarily located on the Navajo Nation Reservation that encompasses three states as shown in Figure 1.1, with a total population of 174,000. Figure 1.2 showed 153,323 who identify as Navajo only, and Figure 1.3 showed 156,823 who identify as Navajo with combination of other races living on the Navajo Nation (Nation) and 332,129 claiming tribal enrollment with the Navajo tribe across the United States (2010 Census 2010). It is the largest Native American reservation in the U.S. and area is roughly the same size as West Virginia. The Navajo reservation can be described as a wide open area with many mountains, grasslands, and canyons, but most areas spanning between are dry desert lands. Many towns are isolated from one another; many family homes are scattered among the reservation connected by dirt roads, many not having access to a nearby grocery store. Due to the unfortunate events that directly affected the Navajos, like most Native American tribes, their people are among the highest in obesity, diabetes, alcoholism and much more (IHS 2016). A list of these health issues of Native American people compared to the United States as a whole can be seen in Table 1. Due to the isolation of most towns on the Navajo reservation and the convenience of certain foods, the people have an increased likelihood to consume foods with little to no nutritional content.

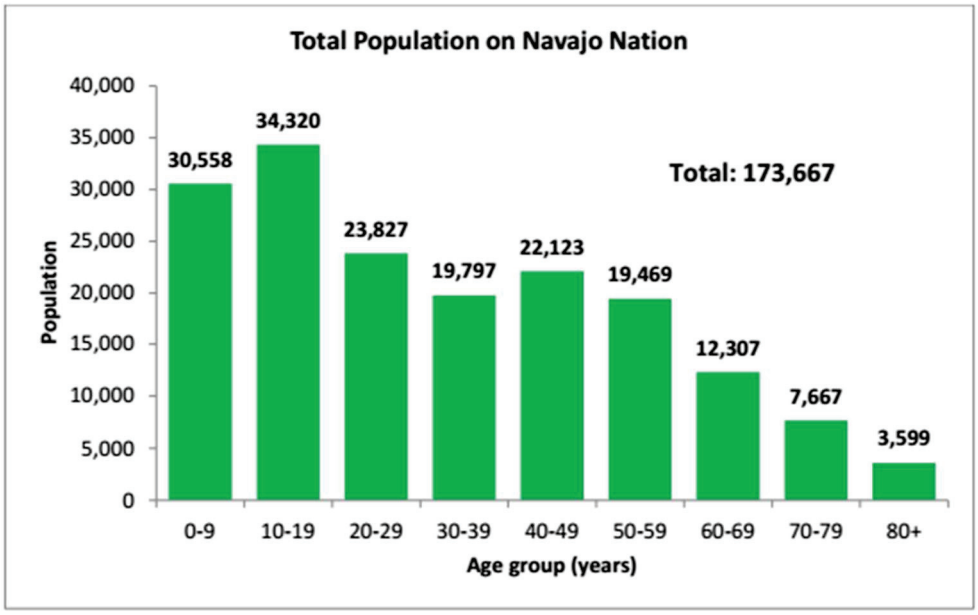

Figure 1.2: Graph of the Total Population of the Navajo Nation (Taken from Navajo Population Profile 2010 US Census)

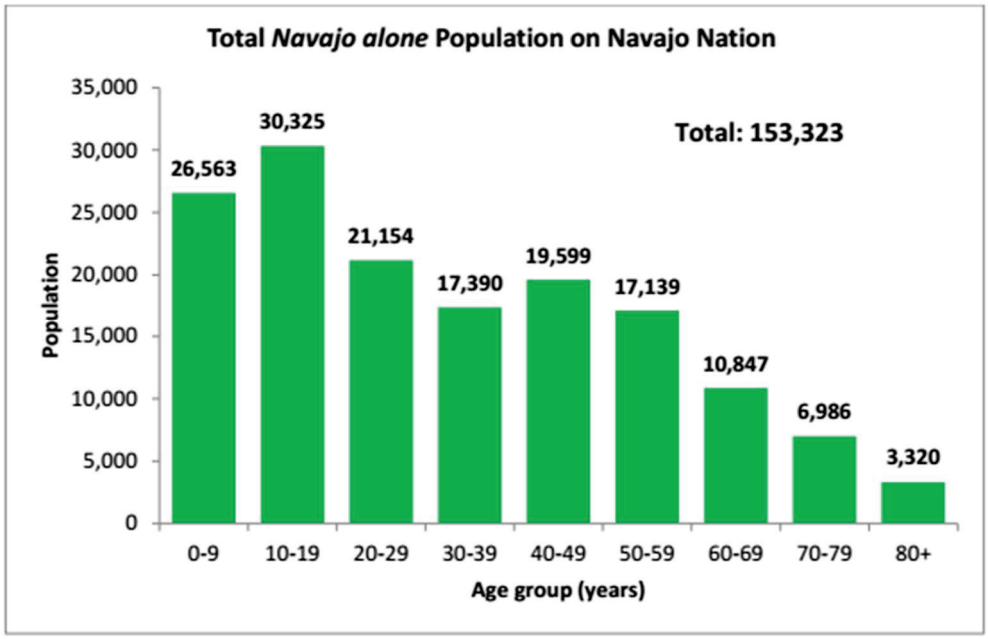

Figure 1.3: Graph of the Total Navajo alone Population on the Navajo Nation (Taken from Navajo Population Profile 2010 US Census)

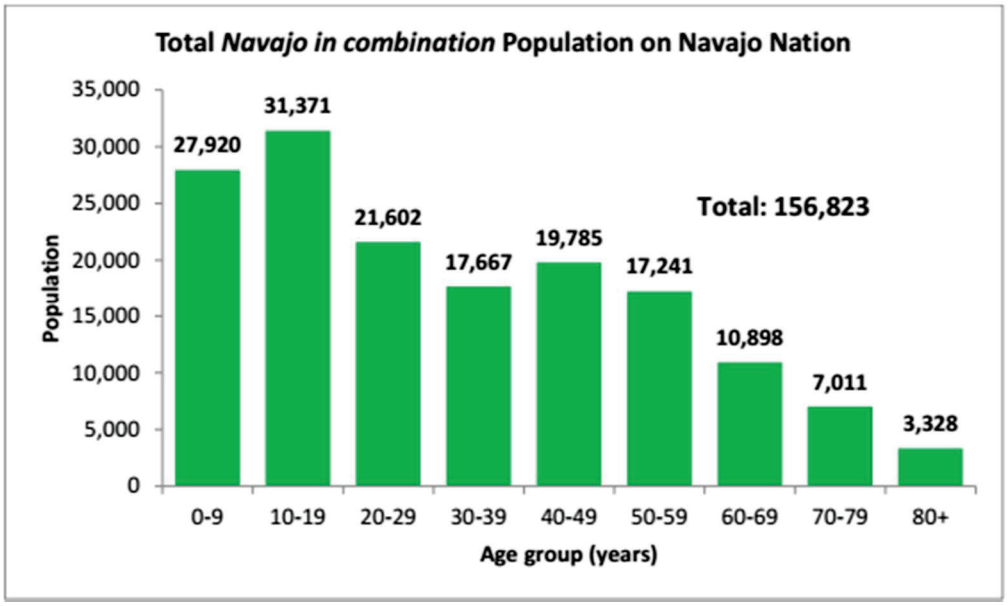

Figure 1.4: Graph of the Total Navajo in Combination Population on the Navajo Nation (Taken from Navajo Population Profile 2010 US Census)

	AI/AN Rate 2007-2009	U.S. All Races Rate – 2008	Ratio: AI/AN to U.S. All Races
ALL CAUSES*	943.0	774.9	1.2
Diseases of the heart	182.4	192.1	0.9
Malignant neoplasm	170.8	176.4	1.0
Unintentional injuries	94.5	39.2	2.4
Chronic lower respiratory diseases	43.2	44.7	1.0
Diabetes mellitus	61.0	22.0	2.8
Chronic liver disease and cirrhosis	43.1	9.2	4.7
Cerebrovascular diseases	39.1	42.1	0.9
Influenza and pneumonia	24.1	17.8	1.4
Nephritis, nephrotic syndrome	22.1	15.1	1.5
Intentional self-harm (suicide)	18.5	11.6	1.6
Septicemia	16.5	11.3	1.5
Alzheimer's disease	14.6	24.4	0.6
Assault (homicide)	11.0	5.9	1.9
Hypertensive heart and/or kidney disease	12.8	13.9	0.9
Parkinson's disease	5.1	6.6	0.8

* Unintentional injuries include motor vehicle crashes.

NOTE: Rates are adjusted to compensate for misreporting of American Indian and Alaska Native race on state death certificates. American Indian and Alaska Native age-adjusted death rate columns present data for the 3-year period specified. U.S. All Races columns present data for a one-year period. ICD-10 codes were introduced in 1999; therefore, comparability ratios were applied to deaths for years prior to 1999. Rates are based on American Indian and Alaska Native alone; 2000 census with bridged-race categories.

Table 1.1: 2008 IHS Disparities Fact Sheet. Comparing health issues of Native Americans to the United States population (IHS 2016)

There are three major limitations on the dietary choices of the Navajo people: cost, availability, and shelf life (Ballew et al. 1997). On the Navajo Nation, more than one-third (32%) of all households have a household income of less than $15,000. This percentage is twice the number of households compared to that of the State of Arizona (17%). These poverty rates still hold consistent in all three states compared to people of the Navajo Nation (Arizona, Utah, New Mexico) (Arizona Rural Policy Institute 2010). These statistics show that households on the reservations will find it more difficult to afford foods. On the same note, most grocery stores on the Navajo Nation do not provide a wide variety of meats, fruits, and vegetables due to the grocery

stores being located in rural areas or near Navajo communities (Ford and Harris 1988). Households that live in extremely rural areas have no electricity and must transport their own water from water sources (Energy Consumption and Renewable Energy Development Potential on Indian Lands 2000). As a result of the lack of electricity, these households are unable to store certain foods that require refrigeration to prolong their shelf life. Foods that do not require refrigeration and increased shelf life become more favorable than healthier dietary choices.

To support the claims of the limitations on dietary choices, a study was done on Dine' Food Sovereignty in 2014. In this study, 230 Navajo people from five communities participated in a survey to patterns food assistance programs, access to food, traditional foods, and diets. When responding to the question, *Are there certain foods that you need or would like to eat that are difficult to get, or not available in your community?*, more than half (60%) said that there are foods they would like but are not available to them. Many have stated that they would like access to fresh meats, produce, natural and organic foods. When asked, *Where do you do your grocery shopping?*, half (51%) of the participants said they travel to stores in border towns off the reservation. Contributing factors are cheaper prices, wide variety, and fresh quality. Of these participants, more than half (58%) will travel to these border towns three or more times per month with a little less than half (46%) spending more than $300 each trip. (Dine Food Sovereignty, 2014)

The US Department of Agriculture (USDA), through the Food Distribution Program on Indian Reservations (FDPIR), still supplies over 250 tribes with commodity foods. These foods include canned goods (meats, beans, vegetables, soups, and fruits), bottled juices, cereals, rice, dried pasta, flour, processed cheese products, powdered egg mix, powdered milk, butter spread, and vegetable oil (FDPIR 2016). All of these foods are meant to have a long shelf life; thus they have large numbers of preservatives. Supplemental Nutrition Assistance Program (SNAP),

formerly the Food Stamps Program, is another USDA program that provides low/no income American Indians with assistance to purchase foods. Unlike FDPIR, where pre-packaged foods are delivered to a household, SNAP allows households assistance to purchase allowed foods at the grocery store, farmer's markets, and other SNAP-approved centers (Vantrease and Vantrease 2013).

The FDPIR aims to assist low-income families with USDA foods living in tribal communities on American Indian reservations as a supplemental food source. As of 2015, an average of 88,600 individuals participated monthly in the FDPIR program. An USDA study of participating households in 1989 and 2013 show a decrease in household earnings (USDA 2016). Though the foods packages are meant to be a supplemental food source, it is the primary source of food for 38 percent of households. This was an overall study of American Indian/Alaskan Native (AI/AN) tribes, not solely the Navajo tribe. According to a demographic analysis done on the Navajo reservation, tribal members are twice more likely to have SNAP assistance than residents of Arizona (Arizona Rural Policy Institute 2010).

1.2 Nixtamalization and Similar Processes

Navajo people had adopted developing the land for agricultural purposes from neighboring tribes. Corn being the main staple, Navajos developed many corn-based meals such as blue corn mush, hominy corn stew, dumplings and others. Most North American indigenous tribes use a type of ash (plant, wood etc.) as a mixture of cornmeal. In other studies, when indigenous tribes are asked about the use this technique, they would say it is a tradition passed down from generation to generation. Not much explanation as to the reason why this is done, other than deeper flavor and

color (Christensen et al. 1998). The process of adding ash to food may be compared to a process called nixtamalization that was first discovered in Mesoamerica, a process still used to this day. Like some North American tribes, Mesoamerican tribes' main food staple was corn. In order to prepare and improve the quality of corn, they would use the process of nixtamalization, where the corn was cooked in an aqueous alkaline solution and allowed to soak from 8 to 16 hours. It is then washed by hand to remove the outer layer of the corn (which is difficult to remove without this process). The inner layer is then stone ground to obtain the dough end product, *masa*. It can then be used to make tortillas, tamales, tortilla chips, and much more (Dickerson 2003).

This traditional nixtamalization utilized calcium oxide (CaOH), also called *lime*, and prepare a mixture with water to produce calcium hydroxide ($Ca(OH)_2$), also called *slaked lime*. This aqueous solution has a high alkalinity due to the slaked lime, which assisted in softening the hard outer shell of the corn kernels, also called the pericarp, absorption of calcium into the kernels, and water uptake during the steeping process. The grinding process de-constructs the structure of the grain, distributing the starch granules, lipids, and fragmented parts of the cell structure. Grinding is an important process in order to retain the puffing consistency during kneading to prepare *masa* (Wacher 2003). There are significant improvements to the nutritional value of the alkaline-cooked corn. This process frees up previously bounded and unavailable niacin, an increase in calcium content. However, this traditional process is considered "polluting", due to the amount of pericarp-containing waste collected at the end of the nixtamalization process. There is a new process that substitutes lime with calcium salts (such as calcium carbonate, etc.), and does not produce the same waste as the traditional process (Bello-Pérez et al. 2015). This new process shows promise for future use.

These processes are very similar to the one used by Native American tribes with ash when cooking/preparing corn-based dishes. There are variations of these cooking methods based on different tribes and also may vary between communities of the same tribe. The Navajo people, for the purpose of research, will be the focus. There have been two methods observed so far, a dry mixture of both juniper ash and roasted blue corn meal, then adding water. The other method is boiling water with juniper ash, straining the mixture into roasted blue corn meal.

These ash-mixture processes can also be observed in East and Central African countries, who utilize bean-debris ash and magadi soda in cooking their cultural dishes containing cereal or grain legumes. Cooking with the ash and magadi soda improves the flavor and decreases the cooking time significantly (Mamiro 2011). There has been no study found as to whether cooking with juniper ash shortens the cooking time of dishes.

1.3 Blue Corn Meal and Juniper Ash

On July 2015, I had the opportunity to interview Lillie Pete, a Navajo traditional consultant who has extensive knowledge on cultural and traditional ways of the Navajo lifestyle. She gives instructional lessons on how to cook traditional Navajo foods and the history behind each food. The interview focused on the traditional dishes consisting of roasted blue corn meal and the ash from a juniper tree (Refer to Appendix B for transcript of the interview). Ash was mixed with boiled water and the ash/water mixture was strained and used to mix with blue cornmeal and prepared for different dishes such as blue corn mush, bread, tamales, dumplings, and pancakes. Blue corn mush is the most popular to eat (Alford and Nance 1976). Before contact with non-

indigenous people, these dishes were commonly consumed on a daily basis, which was part of the Navajo lifestyle.

Lillie Pete stated that the Navajo people have used blue corn meal with ash "From the beginning, it has always been used...". This suggests that after the end of the nomadic lifestyle and beginning of the stationary one (focus on agriculture of corn), they created the dishes that has developed into the foods they would eat for centuries.

Blue cornmeal dishes (with juniper ash) are considered a critical food for the Navajo culture, but in the present day, few people know how to make these dishes. This could be due to the older generation of Navajos refusing to teach the younger generation, or the younger generation refusing to learn. No matter which it is, knowledge of how to prepare these dishes is diminishing. This is the reason Lillie Pete goes town-to-town and school-to-school, to teach the younger generation of Navajos of their culture and traditional foods.

In one article, after observations of the Navajo lifestyle, after a pregnant woman gives birth, she would only eat cornmeal mush and drink juniper tea to get her strength back. Juniper tea is a blend of juniper twigs and hot water, given to women after childbirth, to clean out her blood (Bailey 1940).

Not only were these dishes used for day-to-day meals, they also served a ceremonial purpose for the Navajo people. Medicine men are people who perform a ceremonial healing process using chants and bundles of herbs (Wyman 1983). Today, these medicine men are monetarily compensated, but before this, they were compensated with food. Blue corn meal dishes were considered a valuable food. Cornmeal is a part of a medicine man's tool kit in order to perform ceremonies along with other indigenous plants. Blue cornmeal and other types of

cornmeal had significant importance during types of ceremonies such as healing, rain, and funerals (Carlson 1985).

1.4 Dietary Calcium Intake of Navajo People (Diet of Navajo People)

Studies on the diet on Native American tribes have shown their many health issues and possible links associated. Many conclusions suggested that the sudden change in lifestyle is the main responsible factor (Kunitz 1983) . Prior to colonization, all Native American tribes relied on foods indigenous to their region to prepare their traditional dishes. Foods ranged from wild game to edible plants, all which required physical exertion in order to gather or hunt as stated by Darby (Adams et al. 1956). This created a healthy balance of physical activities with consumption of nutritious foods. With colonization, brought tribes removed from their land and forced to adopt a new lifestyle which consisted of consuming government-issued foods that were foreign to the tribes of people, junk food high in fats, and a significant decrease in physical activity. An assessment of the nutritional quality of foods provided by the FDPIR and other food assistance programs such as Supplemental Nutrition Assistance Program (SNAP), showed they had they had a higher nutritional value compared to other food-assistance programs available to Native American reservations. The assessment showed potential areas where the Food Distribution Program on Indian Reservations (FDPIR) could improve on nutritional value but overall it is up to Native American communities of people to be consistent with the consumption of healthy choices of foods (Byker Shanks et al. 2016).

Today, like most Native American tribes, Navajos have a high prevalence of obesity, diabetes, and many other health concerns (Kunitz 1983). These same issues can be seen in

neighboring tribes, such as the Hopi (Brown and Brenton 1993). In 1990, a study done by Sugarman supported the increased health concerns showed an increase in weight, height, and obesity in Navajo children from 1989 compared to children examined from 1955 to 1979 (Sugarman et al. 1990). This supports the hypothesis that a radical change in dietary intake can cause drastic effects to a group of people. A later study in 1992 focused on obesity in Navajo children also showed a positive correlation between body mass index and blood pressure. It also showed that Navajo children were only consuming less than two-thirds their recommended daily allowance for select nutrients such as calcium (Gilbert et al. 1992). A questionnaire done by Navajo women in 1985 showed that there is a significant decrease in the consumption of pre-Columbian traditional Navajo foods (Wolfe et al. 1985) since the introduction of government-aided foods. Another study showed Navajo women who volunteered to give one-day dietary reports found that they were not receiving adequate nutritional intake from meals they were consuming on a daily basis, which included fast food, contemporary Navajo foods, and foods received from food-assistance programs. These women were asked questions about a number of traditional foods they consume, which concluded to be infrequent compared to other foods listed (Wolfe and Sanjur 1988). Blue corn mush and blue corn dumpling (both mixed with ash) were among the most frequently consumed foods, which was on a daily and weekly basis. Other foods such as hominy corn and Navajo cake were mostly consumed on a monthly or yearly basis. Most pre-Columbian traditional foods have been replaced with a modified version of traditional foods. Today, tortilla, frybread, coffee, potatoes, eggs, and sugar are the most common foods found in the Navajo diet. Foods that are high in fat and that are generally unhealthy to consume on a daily basis increase the likelihood of developing chronic diseases such as diabetes, along with low vegetable/fruit intake (Serdula et al. 1996) (Trude et al. 2015). Frybread and commodity foods are

now closely associated with the Navajo lifestyle, and more broadly, Native American culture and traditional foods (Vantrease and Vantrease 2013).

Based on the current diet of the Navajo, there is no denying that there is a lack of adequate nutritional intake. Dietary calcium intake has been below the recommended levels in the United States and is especially low among Native Americans living in rural areas compared to other races (Bell et al. 2002). These low levels of dietary calcium intake can be attributed to low consumption of dairy products, which is a substantial source of dietary calcium. The consumption of dairy products in the Navajo diet was extremely rare, with goat milk being the sole dairy product used. Many Native American people, along with other minority groups, are more susceptible to lactose intolerance or lactose maldigestion (McBean and Miller 1998) due to their inability to fully digest lactose. Lactose intolerance/maldigestion is another variable for the low intakes of calcium in the Native American diet. Native American tribes such as the Navajo must find a good source of dietary calcium, along with other essential nutrients by other means in order to avoid health disparities such as osteoporosis (Jackson and Savaiano 2001).

1.5 Calcium in the Body

Calcium intake is typically associated with the consumption of milk to build and fortify the formation and structure bones and teeth. It is one of the most abundant elements in the human body and 99% of the body's calcium is found in bones (vertebrate skeleton), 0.6% in teeth, both as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) and 0.6% in soft tissues, 0.03% in plasma and 0.06% in extravascular fluids (Nordin and B.E.Christopher 1997). Calcium in bone gives it its structure, rigidity, strength, and flexibility, allowing the body the ability to move (Theobald 2005). The

bones and teeth also act as a reserve source of calcium for the body to meet its metabolic needs in case of calcium deficiency. Throughout our lifetime, bone is constantly replenishing itself by resorption and deposition of calcium into newly forming bone. This calcium balance (resorption and deposition) in the human body will be negatively affected with advancing age (Committee to Review Dietary Reference...). The rest of the calcium (>1%) has an essential role in the human body dealing with the circulatory system, extracellular fluid, muscle, and other tissues in its ionized form (Ca^{+2}) (Weaver et al. 2006; Raue et al. 2006). Non-ionized calcium found in the extracellular/intracellular fluids is bound to proteins and anions. These major ionic complexes in serum are calcium phosphate, calcium carbonate, and calcium oxalate. Calcium is reserved and stored in the bone tissue, this essential element mediates vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signaling and hormonal secretion (Robertson and Marshall 1981). The body's calcium inventory consists of these components: skeleton, extracellular fluids, and intracellular fluids.

Calcium metabolism is defined as all movement of calcium throughout the body. This includes dietary calcium intake, serum ionized calcium, digestive juices calcium, bone calcium and the receptors that regulate them throughout different organs and systems. The movement of dietary calcium can be defined by the mechanisms for intake, absorption, and excretion of calcium from food. The metabolism can be broken down into three separate mechanisms: intestinal absorption, renal reabsorption, and bone turnover (Allgrove and Jeremy 2003).

Calcium homeostasis throughout the body is directly/indirectly regulated by the parathyroid hormones (PTH). Some calcium systems are more tightly regulated than others (e.g. serum ionized calcium). There are two other hormones involved in calcium homeostasis,

calcitonin, and calcitriol (Peacock 2010). Because serum calcium is tightly regulated, parathyroid glands can detect even the smallest of changes in the serum calcium levels. The glands will secrete PTH when the changes are detected, usually within seconds. Small changes in the serum ionized calcium will be mediated by the PTH system. During intake of dietary calcium, plasma ionized calcium levels will play a factor in the rate of calcium absorption (Heaney et al. 1975). However, if there is a lack of dietary calcium for long periods of time, it will increase the likelihood of developing hypocalcemia (Centeno et al. 2009) (Centeno et al. 2009) (Abrams et al. 2005) (Boonen et al. 2006).

Approximately 90% of calcium absorption takes place in the small intestines. Less than 10% of calcium absorption takes place in the colon. The remaining (less than 1%) absorption takes place in the stomach. This mechanism has two components that are situational: active (transcellular) and diffusion. Active transport occurs when there is a low/medium intake of calcium. Diffusion is utilized at higher intakes of calcium (Nordin 1976). During the acid digestion process, the dietary calcium is absorbed in the soluble form Ca^{2+} or bound to a soluble organic molecule that is able to cross the intestinal wall (Vavrusova et al. 2014). After calcium absorption, the rest of the unabsorbed dietary calcium is excreted in the fecal matter (Need et al. 1991). Other factors such as salts, proteins, and potassium can have an effect on the retention of calcium in the body (Weaver et al. 2006).

Fractional calcium absorption is defined as the amount of calcium absorbed with regard to the amount of calcium taken into body (Bronner 1987). Most foods availability is measured by fractional absorption percentage. For example, in Table 2, cheddar cheese has 303 mg of calcium

per serving, but after measuring, only 97.2 mg/serving was absorbed into the body. This gives cheddar cheese a 32.1% fractional absorption percentage (Fishbein and Lawrence 2004) .

Bioavailability is defined as the portion of a measured amount of a substance (in this case, dietary calcium) that is absorbed and retained in the body and used. There are many factors that influence the bioavailability of dietary calcium that gets absorbed and utilized in the body. The dietary calcium that enters the body can form complexes with ions (such as phosphate ions) that render it insoluble, unable to be absorbed or used in the absorption mechanism (Nordin and B.E.Christopher 1997). The active form of vitamin D, calcitriol, promotes calcium absorption in the intestines. Low levels of vitamin D correlate to lowered calcium absorption from the diet. Serum calcitriol concentrations are shown to have a positive correlation with calcium absorption (Fishbein and Lawrence 2004).

The food and nutrients consumed can be considered a dietary variable, as they can promote or inhibit calcium absorption. In order to promote calcium intake, some foods which generally are not a viable source of calcium are fortified with a variety of calcium salts. The foods and nutrients are taken in can complex with calcium to form different calcium compounds (Skibsted 2016). The solubility of these calcium compounds in the stomach and intestines is very important to absorption and its mechanism because the gastric acids react with the complexes causing ionization of calcium. Additionally, excessive consumption of alcohol (Sampson 1997), and tannin (Theobald 2005) have a negative effect upon calcium uptake.

The culture and geographical location of the people will have an effect on which type of calcium-rich food sources are readily available to them (Bronner 1987; Charoenkiatkul et al.

2008). Countries such as the United States, Canada, and the United Kingdom will have dairy products (milk, cheese, butter, etc.) as the main source of calcium-rich foods, as these foods are affordable, available (Van Dokkum et al. 1996), and convenient. Other countries (such as Thailand), or even smaller communities within large countries such as Native American reservations within the United States, rely on non-dairy products for their daily intake of calcium. These communities rely upon diets consisting of plant foods and some cereal products that are indigenous to their surrounding area. (Theobald 2005) Depending on the regulation of water in the area, it can be treated as a source of calcium as well (Hallfrisch et al. 2000).

Dairy products are primarily associated with calcium intake and building strong bones. Dairy products such as milk have higher bioavailability than certain foods, such as vegetables (Willows 2015). These dairy products are an excellent source of calcium, as shown in Table 2, has a high fractional absorption (32.1%). There are factors in the milk that is suggested to promote absorption. These factors are lactose, proteins and calcium/vitamin D fortification.

There is an extensive list of plants/vegetables that have a high quantity of calcium, such as spinach. Some examples of plant foods are shown in Table 1.2. But with plant foods as a source of calcium, it becomes more complicated. Some plants contain oxalic, phytic and uronic acid which are known to inhibit calcium absorption (Graf 1983). Phytic acid bind strongly with metals such as calcium (also zinc and magnesium) to create insoluble complexes that cannot be absorbed (Vavrusova et al. 2014). Some plants will have a high quantity of calcium but a low bioavailability associated with calcium absorption (Heaney et al. 1988). Plants such as spinach, rhubarb, walnuts, celery, okra, and beans can be seen as an adequate source of calcium; however, they contain high levels of oxalic acid which when combined with calcium, form a less soluble calcium oxalate. For

example, spinach contains a high concentration of calcium, but due to the present of oxalic acid, only about 5% of the calcium is absorbed from the spinach (Amalraj and Pius 2015). Locations like India, there is a low intake of dairy products, receive 30-50% of their calcium intake from plant foods (Sanwalka et al. 2010). But if all-plant food diet is carefully planned, these foods can be an excellent source of calcium (Charoenkiatkul et al. 2008). If the diet is not carefully planned, there is the potential for malnutrition and deficiency in calcium.

Cereal products such as wheat, millet, oats, rice, barley can be considered an adequate source of calcium that has the potential to compete with dairy products as a primary source of calcium (Amalraj and Pius 2015; Weaver et al. 1991). Examples of cereal products with their measured dietary calcium intake are located on Table 1.3. Compared to milk (Table 1.2) to whole wheat flour (Table 1.3), whole wheat flour has a measured calcium content that is comparable, however, keep in mind that the fractional absorption of calcium for whole wheat flour is not listed. Some foods like unleavened bread, nuts, and grain, which contain a high concentration of phytic acid, exhibit very low fractional calcium absorption in the body.

Food	Serving size (g)	Calcium content per serving (mg)	Fractional absorption (%)	Estimated absorbable calcium (mg)	Number of servings needed to equal 240 g milk (total amount*)
Milk	240	300	32.1	96.3	1.0
Cheddar cheese	42	303	32.1	97.2	1.0 (42 g)
Yoghurt	240	300	32.1	96.3	1.0 (240 g)
Chinese mustard greens	85	212	40.2	85.3	1.1 (93.5 g)
Tofu fortified with calcium	126	258	31.0	80.0	1.2 (151 g)
Spinach	85	115	5.1	5.9	16.3 (1386 g)
Kale	85	61	49.3	30.1	3.2 (272 g)
Chinese spinach	85	347	8.36	29	3.3 (281 g)
Broccoli	71	35	61.3	21.5	4.5 (320 g)
Pinto beans	86	44.7	26.7	11.9	8.1 (697 g)
Rhubarb	120	174	8.54	10.1	9.5 (1140 g)
Bok choy	85	79	53.8	42.5	9.7 (825 g)
Sweet potatoes	164	44	22.2	9.8	9.8 (1607 g)

*Total amount in g needed to provide the same amount of calcium as a 240 g serving of milk.
Source: Weaver *et al.* (1999), based on US calcium concentrations. Reproduced with kind permission of the author.

Table 1.2: Foods as a source of dietary calcium (Theobald 2005)

	Calcium (mg/100 g)		Calcium (mg/100 g)
Wheat flour, brown	130	Brown bread	186
Wheat flour, white, plain	140	White bread	177
Wheat flour, white, self-raising	350	Wholemeal bread	106
Wheat flour, wholemeal	38	Pitta bread, white	138
Rice, brown, boiled	4	Rye bread	80
Pasta, fresh, cooked	37	Cornflakes	5
Digestive biscuits, plain	92	Weetabix	35
Gingernut biscuits	130	Frosties	453
Doughnuts, jam	72	Sultana bran	50
Sponge cake	69	Porridge, made with water	7

Table 1.3: Calcium content of cereal products (Theobald 2005)

Intake of dietary calcium is used for the skeletal growth, bone remodeling, and non-skeletal roles vary throughout the lifecycle of a human. Stages such as infancy and in older adulthood require more dietary calcium intake for the purpose of initial bone growth in infants and to make

up for calcium loss in older adults. All calcium dietary reference intakes for different life stages can be seen in Table 1.4.

As shown in Table 1.4, the fetus will need about 200 to 250 mg of calcium per day. As shown in Table 1.4, infants receive roughly 200 mg/day of calcium via breastfeeding and formula feeding. (Abrams et al. 1997). During the childhood period, and going into adolescence, calcium absorption is at its maximum peak. However, Table 1.4 shows that the Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for both male and female are identical (Matkovic et al. 1994). This increased calcium intake is extremely important because about 40% of total skeletal growth is during a small 3 to 5-year range. This is where bone disorders such as rickets will begin to develop if the calcium intake needs are not met.

There is a significant decrease in the EAR and RDA from the 14-18 year to 19-30 year old age range. During this young adult period, if calcium intake requirements are being met, bone formation and resorption will be equal (Bonjour et al. 1994). EAR and RDA for dietary calcium remain relatively the same for male and females until they begin to reach their early 70's. Both older male and females (over 65 years old) will begin to experience the effects of age-related bone loss. At this stage, the bones resorption will outweigh the formation of new bone (Hannan et al. 1992). Postmenopausal women will experience an increased loss of bone because of the decrease in estrogen levels. Resorption of bone will increase as the formation of new bone will decrease. Low estrogen levels will have a negative effect on calcium absorption (Riggs et al. 1998).

TABLE S-1 Calcium Dietary Reference Intakes by Life Stage (amount/day)

Life Stage Group	AI	EAR	RDA	UL
Infants				
0 to 6 mo	200 mg	—	—	1,000 mg
6 to 12 mo	260 mg	—	—	1,500 mg
Children				
1–3 y	—	500 mg	700 mg	2,500 mg
4–8 y	—	800 mg	1,000 mg	2,500 mg
Males				
9–13 y	—	1,100 mg	1,300 mg	3,000 mg
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
51–70 y	—	800 mg	1,000 mg	2,000 mg
> 70 y	—	1,000 mg	1,200 mg	2,000 mg
Females				
9–13 y	—	1,100 mg	1,300 mg	3,000 mg
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
51–70 y	—	1,000 mg	1,200 mg	2,000 mg
> 70 y	—	1,000 mg	1,200 mg	2,000 mg
Pregnancy				
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg
Lactation				
14–18 y	—	1,100 mg	1,300 mg	3,000 mg
19–30 y	—	800 mg	1,000 mg	2,500 mg
31–50 y	—	800 mg	1,000 mg	2,500 mg

NOTE: AI = Adequate Intake; EAR = Estimated Average Requirement; RDA = Recommended Dietary Allowance; UL = Tolerable Upper Intake Level.

Table 1.4: Calcium Dietary References Intakes by Life Stages (Theobald 2005)

1.6 Calcium interactions with Juniper and Soil

Soils can be categorized by three different grain sizes, clay, silt, and sand. Each of three categories types has different effects in how nutrients are stored and move based on soil texture, permeability, size. Many soils will have a mixture of all three components as shown in Figure 1.5 below. Sand is the largest of the soil types with a particle size of 0.05mm - 2.0mm. A comparison of different sized particle is shown in Figure 1.6. Due to its large particle size, there is a large amount of space in between particles, causing faster rates of water drainage. A slightly finer particle, silt, has a particle size of 0.002mm - 0.05mm. Silt having a smaller particle size, has a higher retention of water in between silt particles. The finest particle, clay, has a particle size of <0.002mm and cannot be seen with the naked eye (Maher 2005). Clay has a higher water retention

rate as there is very little space in between the particles for water to move around freely. Apart from the soil type, organic matter (humus) in the top soil consists of decomposed animal and plant matter, humus frequently contains high nutrient contents that can be useful for nutrient absorption from plant roots. Humus having a small particle size gives it a high water retention rate. Particle size comparison can be seen in Figure 6. Chemical reactions of nutrients take place on the surface of soil particles; because of this, the soil particle size distribution is important for efficient soil chemical reaction. Soil particle types like clay and humus can only be seen through an electron microscope because of the small size, and have a negative surface charge. Clay and humus small particle size means they have a large surface-to-volume ratio, giving the surface of the particle the most negative charge sites. Sand particles, on the other hand, are larger in size, resulting in a lower surface-to-volume ratio and lower number of negative charge sites than clay and humus (Raven 2005).

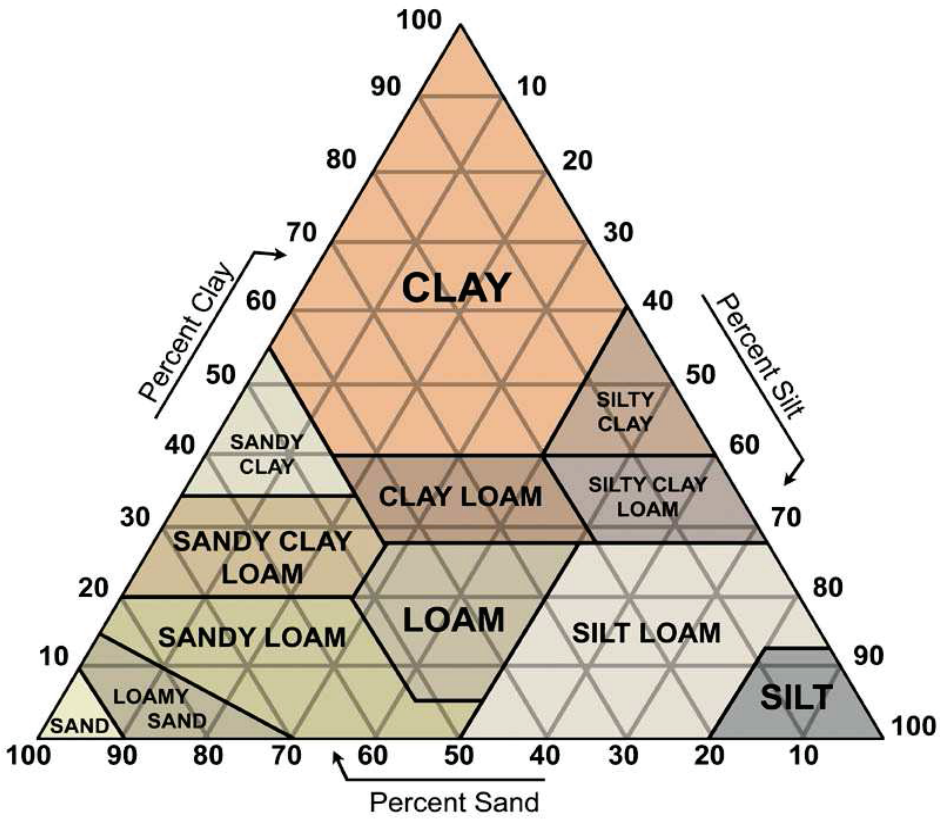

Figure 1.5: Soil Type Triangle (Soil Sensor, 2017)

In order to have significant plant growth, the plant must have a substantial source of nutrients uptake from the soil. Without these nutrients, plants with ceasing to growth and/or develop disorders. There are over a dozen nutrients that are essential for healthy plant growth. They can be broken down into two categories as shown in Table 1.5: Macronutrients and micronutrients. Macronutrients are nutrients that a plant needs in large quantities, such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur. Micronutrients are nutrients required in trace amounts, such as iron, chlorine, zinc, molybdenum, boron, manganese, copper, sodium, and cobalt. Carbon, oxygen, and hydrogen are nutrients that are supplied through the air and water.

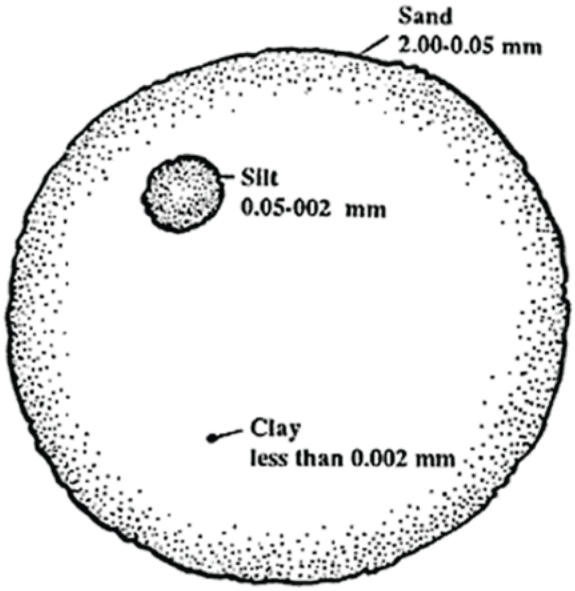

Figure 1.6: Particle size of soil types (Soil Minerals)

The macronutrient that is the subject of this study is calcium. Identified as a macronutrient, calcium is an essential part of the growth, stability, and structure for all types of plants. One of calcium's major roles in plants is to provide structure to the plant cell walls and membranes. Other roles are counter-cation for inorganic and organic anions.

Macronutrients	Micronutrients
Nitrogen	Boron
Phosphorus	Chlorine
Potassium	Manganese
Calcium	Iron
Sulfur	Zinc
Magnesium	Molybdenum
-	Nickel
-	Cobalt

Table 1.5: Macronutrients and micronutrients respectively

Many of the macro/micronutrients listed have a positive charge associated, identifying them as cations. There are also nutrients that are present in negatively charged species forms; these are identified as anions. These positively charged nutrient ions are electrostatically attracted to the negatively charged soil particles, which will adhere to the surface of these particles, allowing the cation nutrient to be stored on the soil particle surface until absorption from a plant root. The number of negatively charged sites on the surfaces of the soil are important for the adherence of cations. Different types of soils will have a different number of negatively charged sites on the particle surface. Clay-rich soils have a high concentration of negative charge. However soils like pure sand will carry little to no negative charge to its particles (Agronomy Fact Sheet Series). An increase in the total quantity of negative charge, increases the number of cations that can potentially adhere to the surface. Because these cations adhere to the surface of the particle, they are not readily available for absorption from the plant roots. This is known as cation exchange capacity (CEC), with units of measurement as meq/100g (milliequivalents per gram of soil). CEC is the soil's total capacity to hold exchangeable cations. Different types of soils have a different capacity, where the percentage of clay in the soil has significant impact on the CEC. Along with clay, organic matter within the soil also has a high CEC. Sand, which has a low CEC, relies on the percentage of clay and organic matter in order to have a moderate CEC to hold nutrients (Chem Cation Exchange PDF). In order to free these essential cations, the electrostatically attached Ca^{2+} ions are exchanged for hydronium ions. To aid in this process, a plant root will release carbon dioxide in combination with water in the soil, will exchange the essential cation with a hydronium ion, as illustrated in Figure 1.7. This process of cation exchange frees the cation, making it readily available for absorption from the plant root (Maher 2005). Research done on the relationship

between CEC and calcium uptake showed a positive and significant correlation that with an increase in the root CEC, there is an increase in calcium uptake (Asher and Ozanne 1961).

$$[soil]Ca^{2+} + CO_2 + H_2O \rightarrow [soil]H^+ + HCO_3 + Ca^{2+}$$

Figure 1.7: Cation exchange of calcium from soil to root

In order for the nutrients from the soil to reach the roots of a plant, the nutrients need a means of traveling to root. There are three mechanisms for nutrient uptake from root: Mass flow, diffusion, and root interception. Not all nutrients needed for plant growth can reach the root by only one mechanism, this is caused by the chemistry of the element or one type of mechanism only brings a small amount of one nutrient. *Mass flow* brings dissolved nutrients within the soil water towards the plant roots. This mechanism is used to bring in large amounts of nutrients such as calcium. Nutrients that are identified to have little to no mobility in the soil with any assistance (potassium and phosphorus) will gain mobility by *diffusion*. Diffusion allows for nutrients to move from a highly concentrated area in the soil to an area where there is a lower concentration. Last, as a plant grows, the roots beneath will grow into new areas in the soil in order to absorb more nutrients not found in the original area, this is known as *root interception* (Raven 2005).

Nutrients such as calcium are exchanged; they are absorbed through the plant root and are transported into the shoot through the xylem (White 2003). They xylem, being the primary path for calcium transport, will eventually make its way to the ends of branches, specifically the leaves. These nutrients are transported with the water that is being pulled up by evaporation caused by air passing by leaves of the tree.

Deficiency of calcium in plants is a rare occurrence because most soils have more than enough calcium for sufficient growth. An investigation done on the soil composition of soils on the Navajo reservation showed that a majority of soils consist mostly of sand. Sand having a low CEC, will not adequately hold cation nutrients unless there is a substantial amount of clay or organic matter in the soil. As studied in 1985, these facts were supported through studies of the soil composition of juniper trees in Utah (White 2003; Bunderson et al. 1985). The high calcium content of the soil is absorbed into the juniper tree through the root system, giving the juniper a high calcium content as well; as reported recently (Nedjimi et al. 2015).

1.7 Calcium Analysis in Foods and Soils

The determination of calcium in plants and foods typically commences with a sample decomposition and dissolution process. There are two possible ways for this: dry ashing and wet ashing (or wet oxidation) (EPA 600/R-95/077). Wet ashing is a process that uses a combination of high temperature acid digestion under pressure (Akinyele and Shokunbi 2015). Nitric, sulfuric, hydrochloric, and perchloric acids can be used in the wet ashing process (Zheljazkov and Warman 2002), but the use of sulfuric acid can produce insoluble calcium sulfate which can decrease the concentration of calcium measured in an elemental analysis. Dry ashing is the recommended method for the quantification of calcium in foods (Srivastava et al. 2008). A study showed the comparison with five different digestion procedures for recovery nutrients from plants tissues such as corn and barley. A comparison between nitric acid, aqua regia, and dry ashing with a Standard Reference Material (SRM) for calcium, dry ashing had the highest recovery of the SRM (90% recovery) (Zheljazkov and McNeil 2008). Food samples are placed in a porcelain crucible and

heated in a muffle furnace in a temperature-ramp procedure. This process removes all organic material in the food sample, leaving behind only inorganic material such as calcium. Once ashed, a common acid such as nitric acid is introduced to dissolve the ashed sample (Srivastava et al. 2008; Azcue and Mudroch 1994). Hydrogen peroxide is then added to acid digestion in order to increase the efficacy of acid to ashed sample. The digested sample is then brought to a known volume.

An example of this method, researchers observed the chemical changes in corn before and after the nixtamalization process. They measured the nutritional changes in thiamine, riboflavin and elements such as calcium. Changes in calcium were determined by analytical methods from the Association of Official Agricultural Chemists' (AOAC). Their recommended method includes a dry ashing process, followed by the use of a common acid for the digestion process. Another journal article measured the mineral content of different seeds belonging to the *Brachystegia eurycoma harms* and *Pipper guineense schum and thonn*. The sample preparation method of choice was also the method proposed by AOAC (Bolanle 2014). A similar method is seen with the EPA 600/R-95/077 Method focusing on the analysis of soils and foliage in the environment.

Preliminary work already done by other researchers that has shown a high calcium content in juniper trees (an average of 285 mg/g of juniper ash). Further, work done on the determination of elemental composition of wood ash showed high calcium content when comparing temperature levels of ashing process (217 mg/g of ash at 600^{o}C) (Etiégni and Campbell 1991). This has been used as a template for type of sample preparation method and elemental instrument of choice that has been used for my research in measuring calcium content in juniper trees. There are multiple analytical techniques that can be used, e.g. atomic absorption spectroscopy and inductively

coupled plasma atomic emission spectroscopy. These two techniques are excellent choices due to high sensitivity with minimal interference (depending on element of choice) (Kalra 1997).

There are multiple methods for the elemental analysis of soils, but the method of choice is determined by the type of element wanted to analyze. In the case of analyzing calcium in soils, there are two methods in consideration: Total Acid Digestion (EPA 3050) and Partial Acid Digestion (EPA 3051). A total acid digestion utilizes acids such as hydrofluoric, nitric, hydrochloric acid with the optional assistance of a microwave. A partial digestion will utilize acids such as nitric and hydrochloric in a microwave. In the case of determining the mineral composition of the entire soil particle (Bunderson et al. 1985), a total digestion would be the best option. Determining the concentrations of exchangeable cations such as calcium will only measure surface-bond calcium of the soil particles, and will not measure calcium bound within the silica structure of the soil particle. This would involve a partial acid digestion that would extract desirable minerals from the surface of the particle (EPA 3051a). Once digestion is complete, the sample may be filtered and transferred to container and brought the desired volume for further preparation for elemental analysis.

Previous work that was referenced above, utilized an ICP-AES as analytical instrument of choice. Due to availability of analytical instruments on hand, Flame-AAS was utilized as the instrument of choice for the research reported here. As discussed in great detail in EPA method 7000B, Flame-AAS can be used for a wide variety of environmental samples such as ground water, aqueous samples, soils, etc. Plant samples prepared become aqueous samples using these methods. There are some limitations to Flame-AAS, such as chemical interferences when analyzing certain elements such as calcium, magnesium, and barium. This is caused when the high temperature flame atomizes an element such as calcium and is immediately bound to compound such as

phosphate, silicon, aluminum, and sulfate. The newly bound compound such as calcium-phosphate is stable and will not dissociate because the flame is not hot enough, resulting in a loss of calcium within the sample (EPA 7000B). This chemical interference can be overcome by the addition of lanthanum nitrate/chloride as a complexing agent in large concentrations (1000 mg per liter). Phosphate will more readily complex to the large amount of lanthanum nitrate/chloride rather than calcium. Another type of interference can be caused by the ionization of elements. If the flame has a sufficiently high temperature, it will have the potential to even remove an electron from neutral atoms, giving them a positive charge. This high potential for ionization can cause signal interference, distorting the true signal of element in question. Ionization interference can be controlled by the addition of an ionization suppressant agent in large amounts (1000 milligrams per liter) to standard and sample solutions.

1.8 Research Goal

Since many Navajo people are lactose intolerant (McBean and Miller 1998), they have a low intake of dairy products, which is a primary source of calcium in the majority of the population in the US. There is minimal dairy intake included in the daily diet of the Navajo people in the past. Because of this, there must be an alternative source of dietary calcium being consumed. This research was inspired by a previous study that investigated lower rates of hip fracture rates among older Navajo people compared to the majority population of older women (Ericksen 1976). Their research examined the mineral content of water sources on the Navajo reservation to determine if the environment was a factor that contributed to the low hip fractures (Hallfrisch et al. 2000). They also suggested work done by Christensen played a factored, whose research first proposed juniper

ash as a potential source of calcium for Navajo people (Christensen et al. 1998). This is important due to the fact that juniper ash is commonly mixed with traditional Navajo foods containing roasted blue corn meal. In recent years, consumption of traditional Navajo foods has declined (Dine Food Sovereignty, 2014).

The goal of this research is to investigate the calcium content in juniper trees to determine if it is an adequate source of dietary calcium for the communities of Navajo people who consumed traditional Navajo foods containing ashed juniper. The approach is to quantify calcium in juniper trees on the Navajo reservations. Calcium quantification will be done by investigating different segments of juniper tree (branch, berries, leaves, species, location) to pin-point the area of the juniper tree that contains the most calcium for dietary intake. Additionally, calcium concentrations will be measured in samples that contain all three components of the juniper tree prepared in a laboratory setting. The study will measure calcium concentration for juniper sample prepared in the traditional Navajo setting and compare to results of juniper samples prepared in a laboratory setting. Last, the calcium concentration for soil samples taken from respective juniper trees will be determined in order to establish the relationship between calcium in the juniper tree and soil. The species and location of juniper samples collected have been identified and will be used to investigate the relationship between species and location.

Chapter 2: Materials and Methods

2.1 Method Introduction

The focus of these experiments was to quantify calcium in juniper, blue corn meal, and soil in order to understand the relationship of calcium in juniper trees used in the preparation of traditional Navajo dishes. Quantification was performed using Flame Atomic Absorption Spectroscopy (FAAS) for elemental analysis of calcium. Different ashing processes were used during the sample preparation stage. First, a general quantification of segments of a whole juniper tree branch separated by branch, leaves, and berries was measured individually. Second, two whole juniper branches in close proximity on the same tree were collected and prepared by different ashing methods. A laboratory ashing process in a muffle furnace (Kalra 1997) and a traditional Navajo ashing process involving manual ignition by fire were utilized. The samples' calcium content were compared using the two ashing processes. Last, where the two juniper branch samples were collected, a soil sample was also collected at the base of juniper tree.

2.2 Sample Collection (Field Work)

Juniper samples were collected in two sampling trips from different sites on the Navajo reservation in Arizona and Utah (August 2014 and March 2016). Sample ID, location description, Global Positioning System (GPS) coordinates and elevation for sample locations are provided in Table 2.1. In Aug 2014, juniper samples were collected along the route from Kayenta, AZ to Flagstaff, AZ (Highway 160 and 89). At each sample site, GPS coordinates and elevation were

recorded; large branches were extracted from trees via pruning shears. This method was used in order to determine the calcium content of different segments of the juniper tree (leaves, branch, and berries). Samples were sealed in plastic Zip-Loc bags and labeled; a total of seven samples were collected. On this same sampling trip, five bags of juniper/ash and one bag of roasted blue corn meal were purchased at the local flea market in Kayenta, AZ. Two bags contained pre-ashed juniper, while the other three consisted of different types of non-ashed juniper; the samples were identified as Canadian Red Flat, Oregon Incense, and Navajo Cedar. Overall, there were a total of 13 samples collected for elemental analysis of calcium. In March 2016, juniper samples were collected at previously sampled sites as well as at new sampling sites. At each sample site, GPS coordinates and elevations were recorded; two smaller branches stemming from a larger branch were extracted from tree, via pruning shears. This new method was utilized in order to compare different ashing techniques with branches of similar mineral composition. Both samples (at each sampling site) were sealed in a plastic Zip-Loc bag and labeled. An overall total of 13 samples were collected.

Soil samples were only collected during the second sampling trip (March 2016) at different sites on the Navajo reservation in Arizona and Utah. The sample ID, location description, Global Positioning System (GPS) coordinates and elevation for sample locations are given in Table 2.1. At each sampling site, GPS coordinates and elevations were recorded, and a trowel was used to grab a soil sample at the surface at the base of the tree and stored in a WhirlPak bag. At the time of the collection of juniper branches during the March 2016 sampling trip, a single soil sample was collected at the base of the juniper tree. The objective was to establish a relationship between the calcium content of the juniper branch with the calcium content of the soil, specifically calcium as a nutrient that could be absorbed from the soil. There were a total of 13 soil samples.

During both sampling trips, once the juniper branch was collected and placed in the plastic Zip-Loc bag, moisture from branch condensed in bag. One juniper branch sample in particular from the 2014 sampling trip was left in the bag (with moisture build-up) for an extended period of time and began to mold. Mold was removed via pruning shears. The sample may have been compromised and was noted. Other samples from 2014 showed no signs of mold.

Two different GPS devices were used for both 2014/2016 sampling trips. The GPS device used in the 2014 sampling trip displayed coordinates in the standard "degrees, minutes, seconds" format, whereas the GPS device used in the 2016 sampling trip displayed coordinates in the "decimal" format. This same issue of different formats also occurred with the units for elevation of sampling sites. The 2014 GPS device used units of meters (m), while the 2016 GPS device used units of feet (ft). In order to keep consistency with units, the "degree, minutes, second" format used in 2014 was converted to the "decimal" format used in 2016. The 2014 units of meters were converted to units of feet for all sampling sites.

ID	Location	GPS Coordinates	Elevation (feet)
S-1	Hwy 160 W of Kayenta, AZ	N 36.465986, W -110.679239	5255
S-2	Mile Post 349	N 36.382279, W -110.885671	NR
S-3	Cameron Visitor Center	N 35.854709, W -111.425837	4169
S-4	Schultz Pass	N 35.372500, W -111.577222	6837
S-5	Monument Valley	N 37.13045, W -110.09621	5688
S-6	Near ASONDW Windmill	N 35.22631, W -11029741	NR
S-7	Near 17-M246 Well	N 35.64732, W -10983089	6452
S-8	Beclabito, AZ	N 36.84036, W -109.02006	5513
S-9	Near Red Mesa, AZ	N 36.90373, W -109.29533	NR
S-10	Monument Valley, UT	N 37.06581, W -110.06641	5426
S-11	Near Kayenta, AZ	N 36.6991, W -110.29636	5893
S-12	Near Navajo National M.	N 36.70772, W -11058820	6826
S-13	Windmill 2T-504	N 36.82559, W -110.61204	6932
S-14	Windmill 2T-252	N 37.11974, W -110.61925	NR

S-15	Navajo Mtn South	N 36.9714, W -110.83300	6247
S-16	Navajo Mtn SW	N 36.98064, W -110.88126	6000
S-17	Cameron Trading Post	N 35.85469, W -111.42576	4377
S-18	Schultz Pass	N 35.37226, W -111.57756	7181
S-19	Near Red Mesa, AZ	N 36.90859, W -109.30157	5590
S-20	Near Red Mesa, AZ	N 36.90090, W -10930467	5666
Navajo Cedar	Flea Market	NR	NR
Can. Red Flat	Flea Market	NR	NR
Org. Inc. Flat	Flea Market	NR	NR
Cedar Ash	Flea Market	NR	NR
Juniper Ash	Flea Market	NR	NR

Table 2.1: GPS Coordinates and Elevation of Sampling Sites. NR Not Recorded

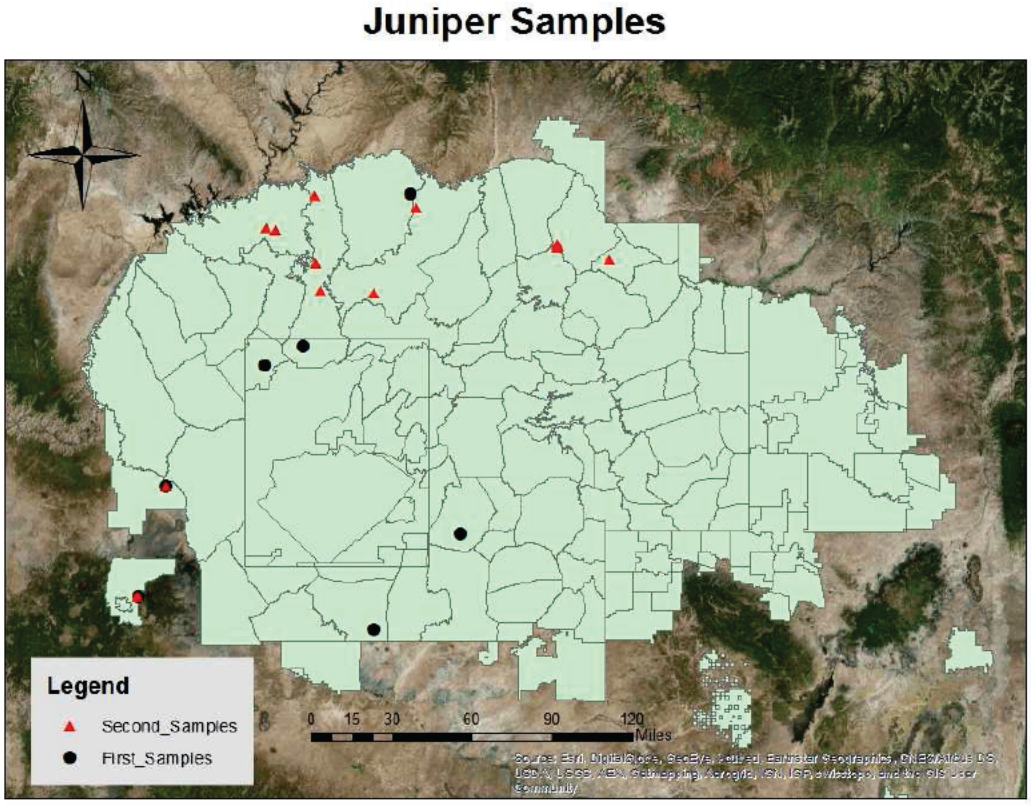

Figure 2.1: Map of Navajo reservation with sampling sites plotted

During the first sampling trip (Aug 2014), only seven sites were sampled. The rest of the samples were collected at the local flea market in Kayenta, AZ. At each of these sites, two or three samples were collected. All of these samples were collected in Arizona and Utah; all but one sample was gathered on the Navajo Reservation. During the second sampling trip (March 2016), 13 sites were sampled as seen in Figure 2.2. New and previous sites were sampled. Of these sites, there was one site in New Mexico.

2.3 Juniper Species Characterization

Sample ID	Species	Sample ID	Species
S-1[a]	*J. monosperma*	**S-8**	*J. osteosperma*
S-2	n/a	**S-9**	*J. osteosperma*
S-3[b]	*J. monosperma*	**S-10**	*J osteosperma*
S-4[c]	*J. deppeana*	**S-11[a]**	*J monosperma*
S-5	n/a	**S-12**	*J. osteosperma*
S-6	n/a	**S-13**	*J. osteosperma*
S-7	n/a	**S-14**	*J. osteosperma*
Navajo Cedar	n/a	**S-15**	*J. osteosperma*
Can. Red Flat	n/a	**S-16**	*J. osteosperma*
Org. Inc. Flat	n/a	**S-17[b]**	*J. monosperma*
Cedar Ash	n/a	**S-18[c]**	*J. deppeana*
Juniper Ash	n/a	**S-19**	*J. osteosperma*
-	-	**S-20**	*J. osteosperma*

Table 2.2: Species of juniper samples. Samples demarcated with letters (a, b, and c respectively) reflect trees that had been previously sampled

The juniper samples collected in the March 2016 field work were taken to Northern Arizona University's Biology Deaver Herbarium for species identification. During this sampling trip, three sample sites were previous collection sites from the first trip; each site is mapped in

Table 2.2. Juniper samples taken from the August 2014 sampling trip were immediately prepared for elemental analysis and were not taken to the Herbarium for species identification. Once juniper samples (from the second trip) were dried, the whole branches were bagged individually and labeled. The Deaver Herbarium (https://nau.edu/merriam-powell/biodiversity-center/deaver-herbarium/) has an enormous collection of dried specimens ranging from ferns to flowering plants that were predominantly collected in Arizona.

2.4 Juniper and Blue Corn Meal Preparation

Preparation of juniper samples varied depending on which groups of samples (2014/2016) were being investigated. Juniper samples obtained during the 2014 sampling trip were used to determine the calcium content of various segments of the juniper tree samples (leaves, branch, and berries). Juniper samples obtained during the 2016 sampling trip were used to compare the calcium content of different ashing process: laboratory dry ashing and traditional Navajo ashing. The blue corn meal used a standard dry ashing in order to obtain total calcium content of samples. See Appendix C for more detailed on sample preparation methods used.

2.4.1 Dry Ashing:

Sample preparation of juniper samples from the 2014 sampling trip began with removing them from the Zip-Loc bags and placing them on paper plates; they were allowed to dry for one to two weeks. Once dried, samples were separated by hand into three categories: leaves, branches, and berries. Because of the season during sampling, most of the juniper trees' berries had fallen off, resulting in only two samples (S-1 and S-7) having berries present. While wearing gloves, separated segments were broken down into smaller pieces and finely ground via a coffee grinder.

This process is done in order to create a homogenous, ground mixture of sample for analysis. Ground samples were transferred to a new paper plate, labeled, and left to dry for one to two weeks. Once dried, ceramic crucibles were completely filled with ground sample and put in muffle furnace (Rows of 3 by 3). The amount of ground sample added to crucible varied because a known mass of the ashed product would be used for further sample preparation. Using a time-temperature muffle furnace (Protocol provided in Appendix), samples were ashed using a 12 hour ashing process (200ºC for 2.00 hours, 400ºC for 4.00 hours, 600ºC for 6.00 hours). The ashed sample was transferred to a storage container until ready for acid digestion.

Similarly, sample preparation of juniper samples from the 2016 sampling trip began with removing the sample from the Zip-Loc bags and placing on paper plates to dry for one to two weeks. There were two sets of the samples collected from the same juniper tree. The first sample set was prepared using Method 1 (described below). The second set was prepared using Method 2 (described below). Both methods followed identical acid digestion procedure.

Method 1 was used to obtain a total ash of the entire juniper branch as a whole. Once the sample was dried, both leaves and branch were broken down into smaller pieces (there was very few berries for 2016 samples). In order for leaves and branches to be ground consistently, the juniper leaves were ground separately from the branches. At the end of the grinding process, both ground leaves and branches were combined to form a homogenous mixture. Six ceramic crucibles were completely filled with the ground sample and ashed using identical ashing protocol via muffle furnace. Ashed samples were transferred to storage container until ready for acid digestion.

Method 2 was used to obtain a total ash of an entire juniper branch as a whole via the traditional Navajo ashing process. In my interview with Lillie Pete, a Navajo tradition consultant, she explained the traditional Navajo ashing process for juniper (See Appendix for transcribed

interview). This process involved building a fire using wood as the fuel, taking an entire branch and placing it above the flame, igniting the branch. Once the branch is on fire, it is placed on a sheet of aluminum foil, in order to burn in an undisturbed fashion. The ash fell onto the foil and once completely burned, the ash was collected in the foil and left to cool. There were complications to this process, such as difficulties to efficiently ignite juniper samples (S-15, 16, 17, 18) that was prematurely broken into smaller pieces. These samples had to have consistent heat applied in order to burn. It was stated that gray ash is more desirable than black ash. Each sample was ashed until the majority of sample became a white ash. For the samples mentioned above, there may be more black ash than the rest of the juniper samples. The ash was transferred into storage container until it was ready for acid digestion.

2.4.2 Acid Digestion:

Three subsamples (0.025 grams each) for each ashed sample was prepared in order to have triplicates of each collected sample. The subsamples were weighed out and the exact mass was recorded (actual mass recorded to the nearest 0.0001 grams), then it was transferred into a 50 mL centrifuge tube, 5 mL of Trace Metal Grade nitric acid (~70%) was pipetted into the centrifuge tube along with 5 mL of Trace Metal Grade hydrogen peroxide (~30%); a total volume of 10 mL for each sample achieved before acid digestion. Caps on the centrifuge tubes were loosened and placed in an oven at 80^{o}C for 24 hours. Evaporation of nitric acid and hydrogen peroxide occurred for most samples. This was possibly due to oven temperature being higher than 80^{o}C or samples being left in oven longer than the 24 hour period. After the 24 hour period, samples were taken out and cooled for a period of time. If evaporation occurred, acidified samples were transferred to a 10 mL volumetric flask and brought to volume with deionized water. Samples were transferred to a newly labeled 50 mL centrifuge tube. This was done in order to ensure each sample's volume

(10 mL) is consistent and to simplify calculations of concentrations after elemental analysis. For each sample, 0.5 mL (500 uL) were pipetted into 50 mL volumetric flask and brought to volume with Nanopure water. Next, the diluted sample was transferred to 50 mL centrifuge tube and labeled. This process provided a 100-fold dilution of the extracted sample.

Utilizing FAAS has the disadvantages of chemical and ionization interference as discussed in greater detail in Section 1.7. In order to account for chemical interference, 1 mL of a 1000 mg/L complexing agent lanthanum nitrate (Matrix Modifier SPEX CertiPrep) was added to each acidified sample. In order to account for ionization interference, 1 mL of a 1000 mg/L ionization suppressant agent cesium chloride (EMD OmniPure) was added to each acidified samples. Both agents were added before samples were brought to the final volume of 50 mL. Once this was completed, the samples were ready for FAAS analysis.

2.5 Soil Preparation and Acid Digestion

Soil samples obtained were used to establish a relationship between the calcium content of the juniper tree samples and corresponding soil sample. Each soil samples was transferred to a plate and left to dried for one to two weeks. Once dried, soil samples were sieved using a 1mm sieve; the sieve was cleaned between soil samples. Large organic/inorganic material (twigs, leaves, rocks) was collect along with soil samples, during the sieving process, these materials were discarded from the sample. A mixer mill and WC_2 grinding jar was used to crush the sieved soil samples to a relatively uniform particle size. Samples were placed in ball mill for two to two and a half minutes. Once complete, samples were ready for acid digestion.

The soil acid digestion process used was different than that of the juniper sample digestion. This process is EPA method 3051, which utilizes microwave-assisted (CEM Mars Xpress) acid digestion for sediments, sludges, soils, and oils (EPA 3051). For each sample, 0.200 grams was weighed out for transfer to digestion vessel. Weighed soil samples were transferred, weighed by difference using the actual mass and was recorded to the nearest 0.0001 gram via balance. 10 mL of concentrated nitric acid (~70%) was added into each vessel. Any reaction between the soil and nitric acid that occurred was given time to dissipate before capping the vessel. Each vessel was carefully placed in the microwave carousel and the microwave program was inputted, starting the microwave digestion process. Sample vessels were irradiated for 10 minutes. At the end of the microwave process, the sample vessels were allowed to cool for a minimum of 5 minutes before taking them out of the microwave carousel. Once cooled, digested samples were transferred to clean 50 mL centrifuge tubes. Because EPA Method 3051 is not meant to be a total soil digestion, at the end of the microwave process there was soil particulates present in each sample. Soil particulates were allowed to settle in centrifuge tubes overnight.

During digestion, all samples were enclosed in a vessel; thus, there should be no evaporation that occurred. For each sample, 0.5 mL (500 uL) was pipetted into 50 mL a volumetric flask and brought to volume with deionized water. Diluted sample was transferred to 50 mL centrifuge tube and labeled. This process is utilized to give samples a 100-fold dilution. This was the same dilution done for the digested juniper samples.

Utilizing FAAS has the disadvantages of chemical and ionization interference as discussed in greater detail in Section 1.7. In order to account for chemical interference, 1 mL a concentrated complexing agent lanthanum nitrate (1000 mg/L) was added to each acidified sample. In order to account for ionization interference, 1 mL of a concentrated ionization suppressant agent cesium

chloride (1000 mg/L) was added to each acidified samples. Both agents were added before samples were brought to the final volume of 50 mL. Once this is done, they were ready for FAAS analysis.

2.6 FAAS Analysis of Juniper and Analysis of Soil

All elemental analysis was performed with a FAAS (AAnalyst 200 Spectrometer). A mixture of air and acetylene gas was used as the carrier gas for the sample. A multi-elemental (Ca/Mg) C-hollow cathode lamp was used in the instrument, at a wavelength of 422.67 nm and a slit of 2.7/0.6 mm. Refer to Appendix for detailed FAAS Method and Specifications.

The acetylene flame was lit and allowed to run for 20 minutes prior to analysis. A solution of Nanopure water was aspirated through the instrument when the acetylene flame was lit. Data collection of each sample consisted of three analyses (triplicate) which were averaged. Before each sample run, 5 external standards were prepared. External calibration standards were chosen based on the expected linear dynamic range for Ca. To initially test the concentration of calcium in a sample, an undiluted sample was introduced into the FAAS. The absorbance measured for the undiluted sample was out of the linear dynamic range for calcium and out of the external calibration concentration range. In order to account for the small linear range, each sample was diluted by 100-fold.External calibration standards (0, 1, 2, 5, 10 mg/L) were used to create a calibration curve for determination of calcium content of different sample types. External calibration standards were prepared using Peak Performance Certified Reference Material P/N S4400-10M91 50,000 mg/L Single-Element Calcium Standard (CPI International). During analysis, counts were plotted in a scatter plot along the external calibration curve to determine concentration of each sample prior to analysis. External calibration curves were created in Microsoft Excel.

2.7 Statistical Analysis

Once elemental analysis was complete, the experimental data were calculated and processed using Microsoft Excel. With each set of experimental data calculated, there will always be some type of random error that occurs. By use of some statistical tools provided, there is a high degree of certainty to accept or reject experimental data points as being correct or incorrect. Statistical techniques used were standard deviation (s), percent relative standard deviation (%RSD), student t-test, Grubb's test and Analysis of Variance (ANOVA).

2.7.1 Standard Deviation:

After elemental analysis, a Gaussian distribution can be created by the mean and standard deviation of the experimental data. The average ($\bar{x}$) of the experimental data is the center of the Gaussian distribution, while the standard deviation is related to the width of the Gaussian distribution. The equation for calculating the average is given in Equation 2.1. The average and standard deviation of the experimental data will determine the precision and reproducibility of the experiment done. The smaller the standard deviation, the higher the precision of the data collected. Although precision is important, it does not ensure accuracy of the data collected. Another statistical tool must be used to ensure the data collected is close to the "true" value. Equation for standard deviation can be seen below in Equation 2.2.

$$\bar{x} = \frac{\sum_i x_i}{n} = \frac{1}{n}(x_1 + x_2 + \cdots + x_n)$$

Equation 2.1: Statistical equation used to calculate the average value of a set of data

$$s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n - 1}}$$

Equation 2.2: Statistical equation used to calculate the standard deviation of an average value of data

2.7.2 Percent Relative Standard Deviation:

As standard deviation is used to compare uncertainties with a set of data points from the same population, percent relative standard deviation is used to compare the uncertainties of sets of data points from different populations. This is useful because elemental analysis were done on different types and numbers of samples (juniper, soil, standard reference material and blue corn meal). Equation for percent relative standard deviation can be seen in Equation 2.3 below.

$$\%rsd = \left(\frac{s}{\bar{x}}\right) \times 100$$

Equation 2.3: Statistical equation used to calculate the percent relative standard deviation for a set of data

2.7.3 Student t-test:

The t-test is a statistical tool used to compare two sets of data and identify if they are statistically different or the same with a confidence of 50% to 99.9% depending on the confidence level. This equation is used rather than the traditional student t-test because the standard deviations for each compared data set is different from one another. The equation used to calculate degrees of freedom is different as well for the same reasoning. Once $t_{calculated}$ is calculated, it can then be compared to t_{table}. If the calculated $t_{calculated}$ is greater than t_{table}, the two sets of data are considered statistically different from one another. Microsoft Excel was utilized to compare individual

samples in the Results and Discussion chapters below. Statistical equation used to calculate student's t-test and determine the degrees of freedom can be seen below in Equation 2.4 and 2.5 respectively. The null hypothesis stated for the student's t-test is stated in Equation 2.6 below.

$$t_{calculated} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{s_1^2/n_1 + s_2^2/n_2}}$$

Equation 2.4: Student t-test equation used to identify if two sets of samples (with different standard deviations) are statistically different from one another

$$Degrees\ of\ Freedom = \left\{ \frac{(s_1^2/n_1 + s_2^2/n_2)^2}{\frac{(s_1^2/n_1)^2}{n_1 + 1} + \frac{(s_2^2/n_2)^2}{n_2 + 1}} \right\} - 2$$

Equation 2.5: Equation used to calculate degrees of freedom for a sample set with different standard deviations

$$t_{calculated} > t_{table}; two\ data\ sets\ are\ different$$

$$t_{calculated} < t_{table}; two\ data\ sets\ are\ the\ same$$

Equation 2.6: Definitions of the result of the student's t-test when comparing t values

2.7.4 Grubb's Test:

A Grubb's test is a statistical test used to evaluate whether suspect points can be considered outliers. Due to random error, there will be data sets with irregular patterns and some data points within a set will assume to be outliers (Grubbs 1950). This test will determine whether or not the data point can be confidently discarded and the sample will continue to be truly represented. $G_{(critical)}$ is selected based on number of measurements done and the level of confidence preferred.

Once $G_{(calculated)}$ is found, it is compared to $G_{(critical)}$ in order to accept or discard potential outlier. The equation used to calculate $G_{calculated}$ is seen below in Equation 2.7. The null hypothesis is stated below in Equation 1.8 below as well.

$$G = \frac{\max_{i=1,\dots,N} |x_i - \bar{x}|}{s}$$

Equation 1.7: Equation used to detect outliers within a set of data.

$$G_{calculated} > G_{critical}; Data\ point\ is\ discarded$$

$$G_{calculated} < G_{critical}; Data\ point\ is\ accepted$$

Equation 1.8: Definition of the results of the Grubb's test on data set in order to accept or discard potential outliers

2.7.5 Analysis of Variance:

Commonly known as ANOVA, is a statistical technique used to test if two or more populations of data are the same or different. This is normally used in order to identify if two or more experimental sample sets with some variation in sample techniques are statistically different. However, it is normally used to compare three or more data sets, if two sets are done it can be seen as an equivalent to the t-test, which compares individual data sets with another. The objective of ANOVA is to either accept or reject the null hypothesis (Equation 2.10); all data sets are statistically equal to one another. If the null hypothesis is rejected, this means one of the data sets is statistically different to the other sets. ANOVA does not specify which data set is different; a Post-Hoc test must be done after ANOVA in order to identify which data set is different (Anscombe 1948).

Using a series of calculations, the $F_{calculated}$ can be calculated by the ratio of Mean Square Treatment (MSTR) and Mean Square Error (MSE) as seen in Equation 2.9. $F_{critical}$ can be found by F values table, using the degrees of freedom to find the critical value for a specific data set. As with the t-test and Q-test, the calculated value is compared to table value. If the $F_{calculated}$ is greater than $F_{critical}$, the null hypothesis is rejected; one of the data sets is statistically different from the rest. If $F_{calculated}$ is less than the $F_{critical}$, the null hypothesis is accepted; all data sets are statistically equal to one another. In this experiment, Microsoft Excel was utilized to calculate the $F_{calculated}$ and the $F_{critical}$.

$$F_{calculated} = \frac{MSTR}{MSE}$$

Equation 2.9: Equation used to calculate F(calculated) in order to compared to F(Critical)

$$F_{calculated} < F_{critical};\ Null\ Hypothesis\ Accepted;\ \mu_1 = \mu_2 = \mu_3$$

$$F_{calculated} > F_{critical};\ Null\ Hypothesis\ Rejected;\ \mu_1 \neq \mu_2 \neq \mu_3$$

Equation 2.10: Definition of null hypothesis for ANOVA

Chapter 3: Results and Discussion (Juniper)

3.1 Results and Discussion Introduction

The overall goal of this research was to quantify the amount of calcium in juniper ash that is used in the traditional Navajo dish, blue corn mush. In this chapter, the goal was to gather information on the amount of calcium found in different segments of the juniper tree used in blue corn mush. The samples analyzed were collected during the 2014 sampling trip. To accurately measure each set of samples, a standard reference material was utilized to ensure accuracy. A set of blue corn meal samples was also prepared using the same method mentioned in Chapter 2. The quantification of calcium utilized FAAS for the elemental analysis. The signal collected from the analytical instrument is directly proportional to concentration of the analyte of interest as per Beer's Law (Harris, 2016). Because of this relationship between signal and concentration, external calibration calcium standards were first analyzed for the purpose of creating a calibration curve with a range of 0 mg/L to 10 mg/L.

3.2 FAAS Analysis of Juniper Segments

Initially, one individual sample was prepared for each sample site, run in triplicate. After elemental analysis, standard deviations and % RSDs were small indicating good reproducibility. However, the low standard deviations observed may have been because only one sample was prepared for each sample site. To obtain an accurate representation of the sample for each sample site, two more samples for each site was prepared and analyzed making each sample prepared and

analyzed in triplicate. The sample analysis took place April 2014, while the second analysis of samples took place August 2014. These sample analyses were 5 months apart and still showed similar results to previous sample of the same collection. Once all samples were analyzed, Microsoft Excel was used to process data. The calcium concentration for each sample was calculated utilizing the raw absorbance signal and the calibration line equation. Results were determined in units of mg/L. The original calcium concentrations of samples were obtained by multiplying sample concentration by original volume, dilution factor, and dividing by mass of juniper ash. The final concentration is represented in units of milligrams of calcium per gram of juniper ash (mg/g). Below are the collective tables of external calibration standards respectively with their absorbance values, and bar graphs for juniper samples from the 2014 sample collection.

Ca Concentration (mg/L)	Abs
0.0	0
1.0	0.103
2.0	0.202
5.0	0.483
10.0	0.913

Table 3.1: Calcium external calibration standards and absorbance values used for the first run of the 2014 sample collection

Ca Concentration (mg/g)	Abs
0	0
1	0.105
2	0.204
5	0.474
10	0.893

Table 1 : Calcium external calibration standards and absorbance values used for the second run of the 2014 sample collection

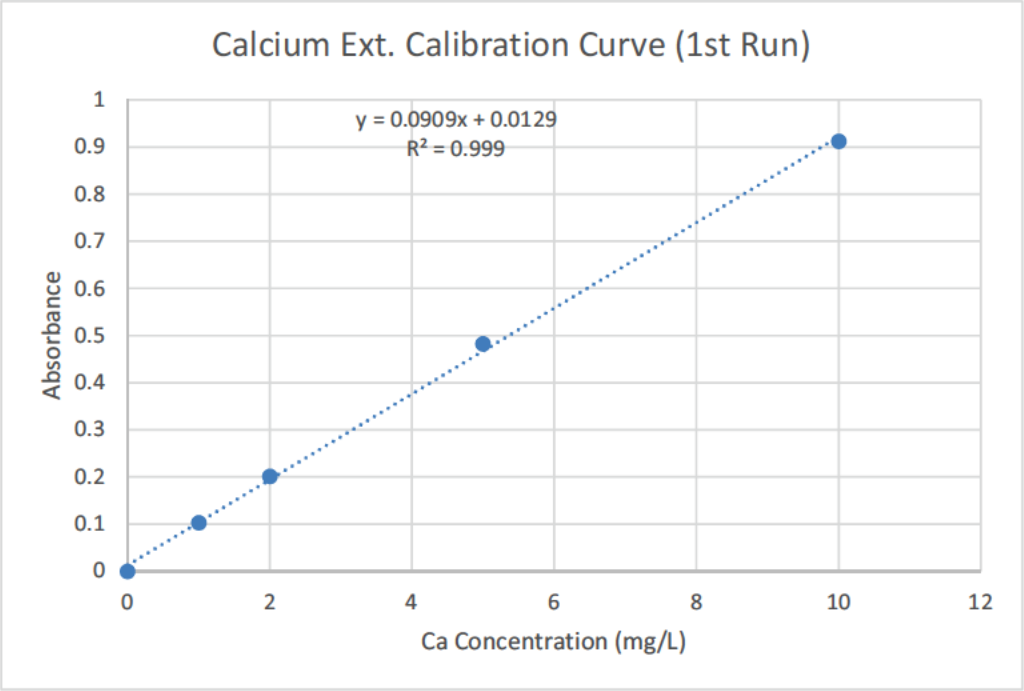

Figure 3.1: External calibration curve of the first sample run from 2014 sample collection. Solid line represents the line between each point. Dotted line represents the linear trendline. Calibration range: 0, 1, 2, 5, 10 mg/L

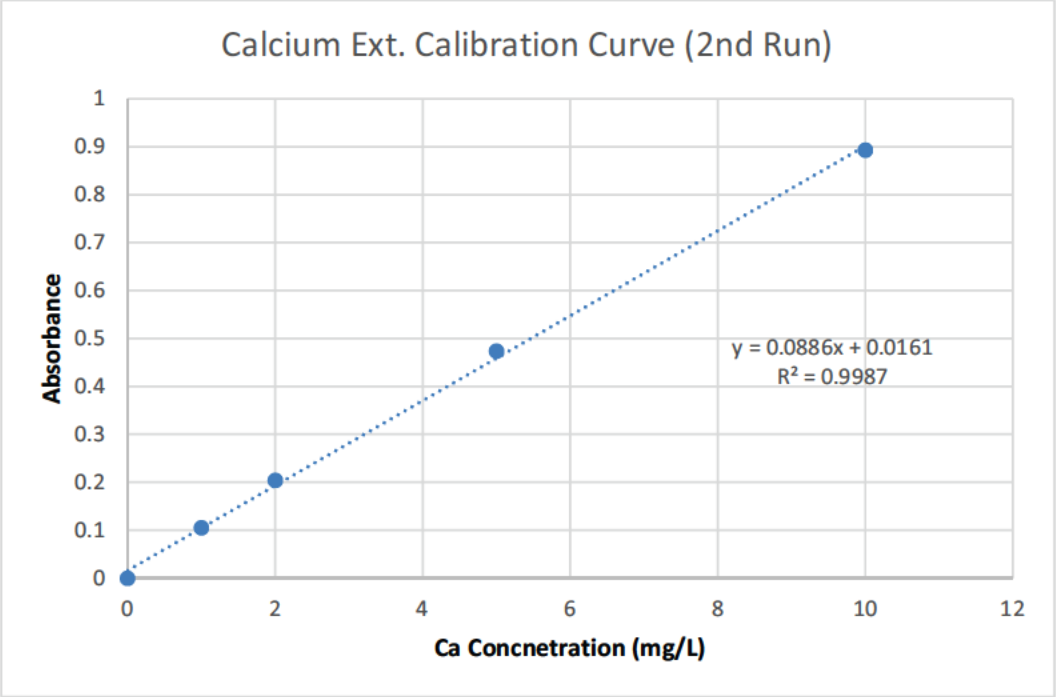

Figure 3.2: External calibration curve of the second sample run from 2014 sample collection. Solid line represents the line between each point. Dotted line represents the linear trendline.

ID	Type	mg/g	s	%RSD
S1-J	Juniper	225	22.7	10.1%
S1-B	Branch	308	22.9	7.42%
S1-Berry	Berry	148	8.61	5.82%
S2-J	Juniper	239	2.25	0.94%
S2-B	Branch	272	11.4	4.19%
S3-J	Juniper	164	3.15	1.92%
S3-B	Branch	332	13.1	3.96%
S4-J	Juniper	236	11.5	4.88%
S4-B	Branch	321	8.04	2.50%
S5-J	Juniper	318	7.65	2.40%
S5-B	Branch	343	17.7	5.16%
S6-J	Juniper	235	7.28	3.10%
S6-B	Branch	261	17.2	6.58%
S7-J	Juniper	174	14.1	8.13%
S7-B	Branch	325	24.7	7.58%
S7-W	Deformed	166	7.72	4.65%
FM1-OI	Juniper	308	4.21	1.37%
FM2-CRF	Juniper	248	3.75	1.51%

FM3-CA	Juniper	281	5.69	2.03%
FM4-JA	Whole	285	11.6	4.09%
FM5-NC	Whole	275	3.67	1.34%
SRM-1	Spinach	92.3	0.25	0.27%
BC-1	Blue Corn	2.19	0.73	33.3%

Table 3.3: A list compiling samples collected, along with standard reference material and blue corn. This table consists of type of sample, calcium concentration, standard deviation, and percent relative standard deviation

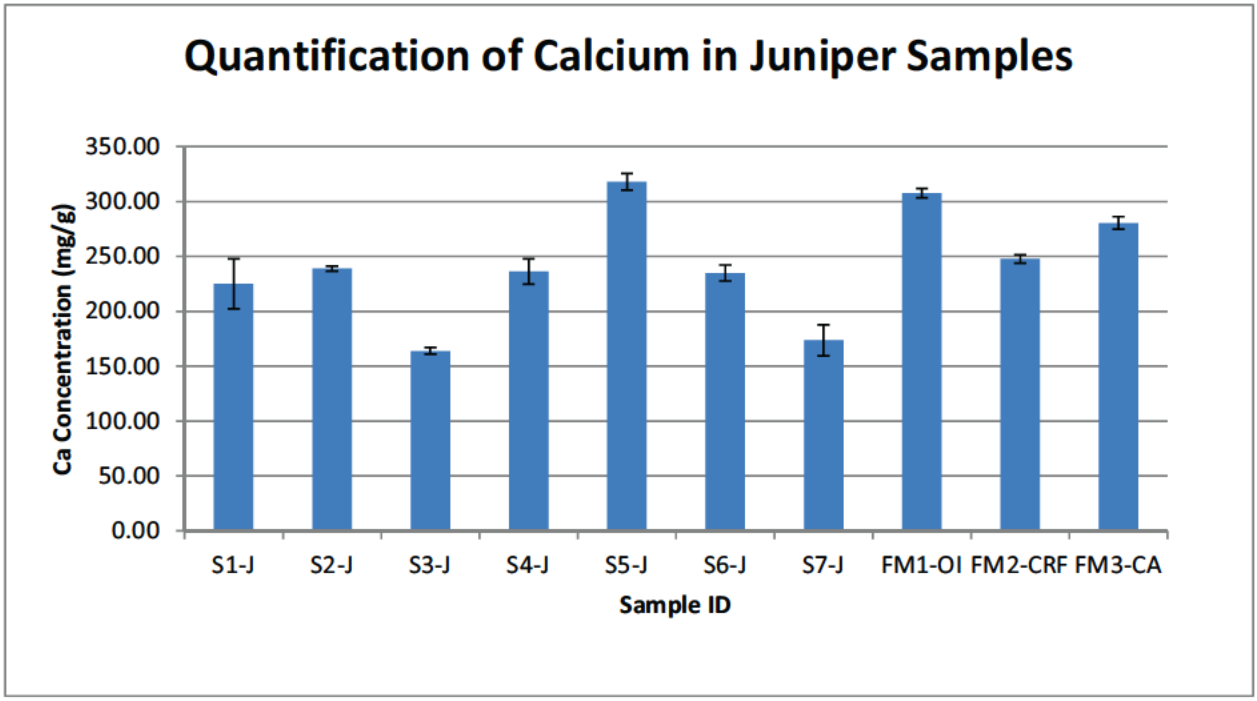

Figure 3.3: Bar graph of calcium concentration in ashed juniper samples (mg/g)

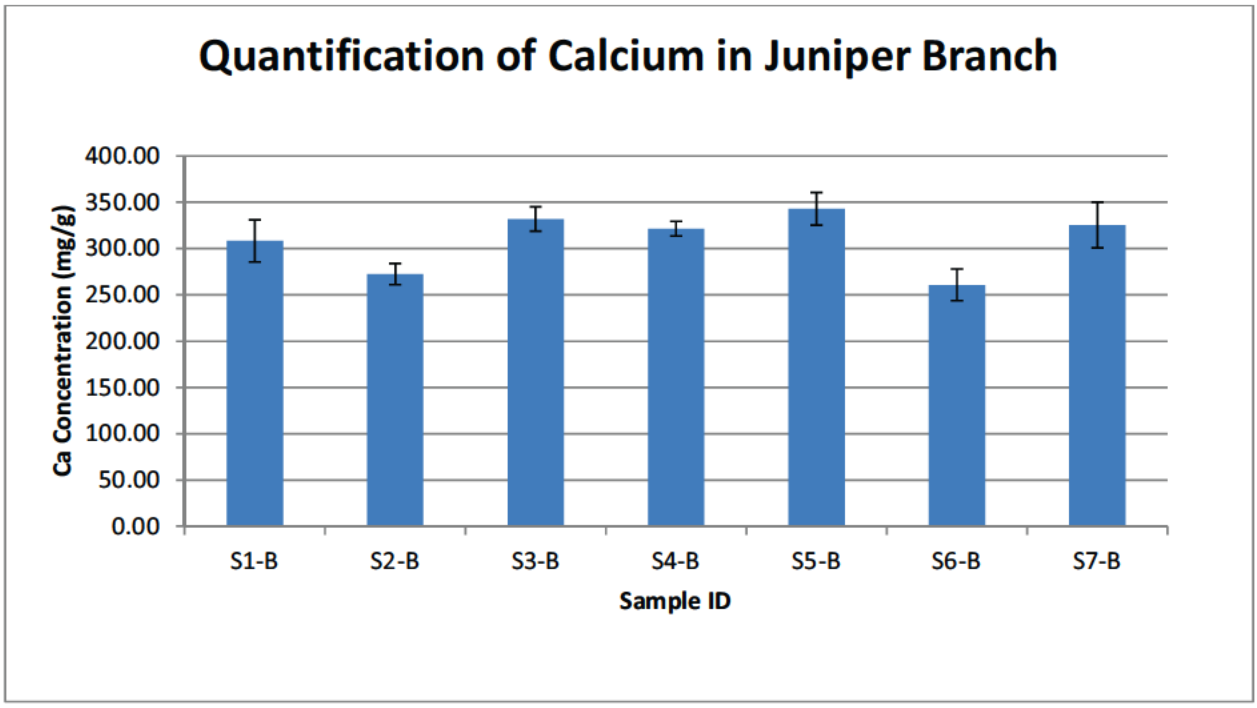

Figure 3.4: Bar graph of calcium concentration in ashed juniper branch samples(mg/g)

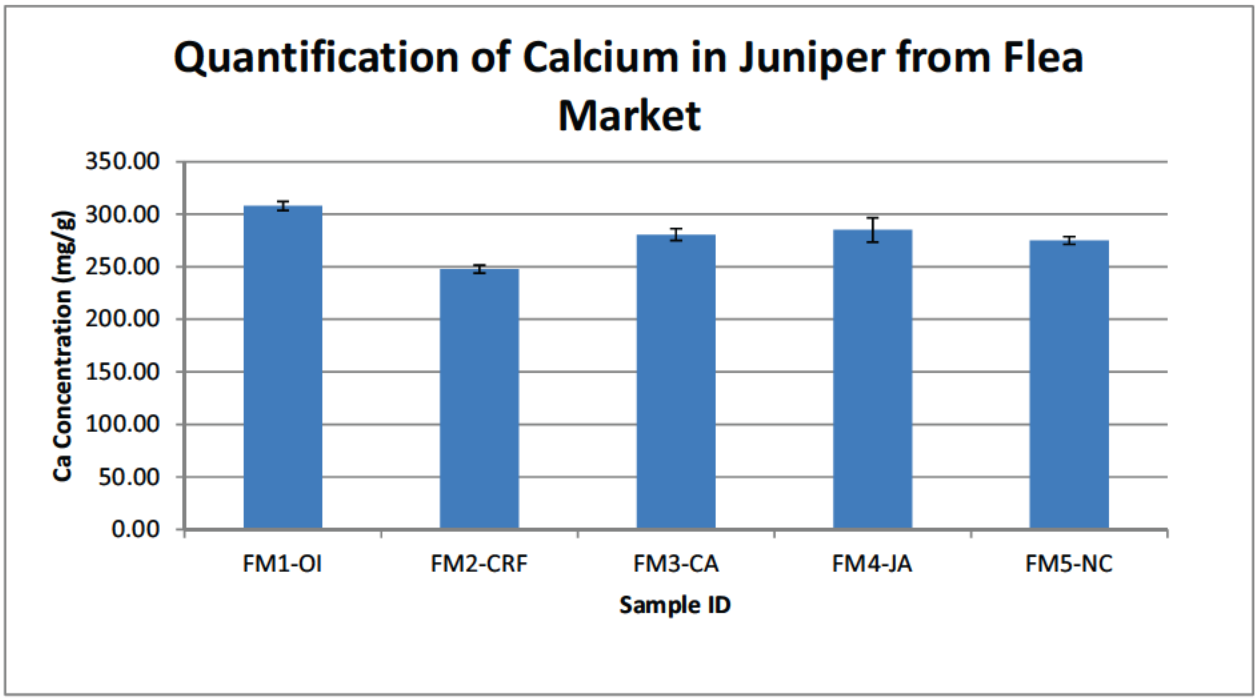

Figure 3.5: Bar graph of calcium concentration in ashed juniper samples (mg/g) from flea

market collected in Kayenta, AZ

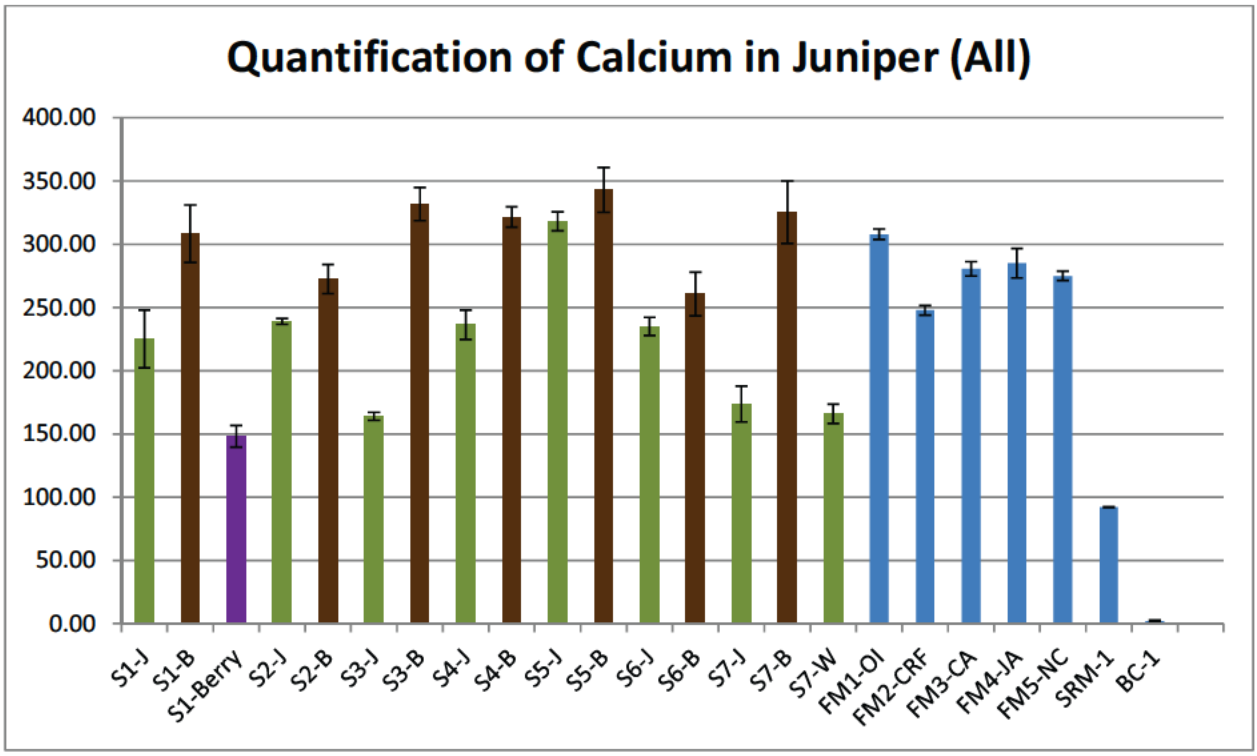

Figure 3.6: Bar graph of concentrations of calcium in collective ashed juniper samples (mg/g) collected from 2014 sampling collection. Green Juniper Leaves; Brown Branch; Purple Berries

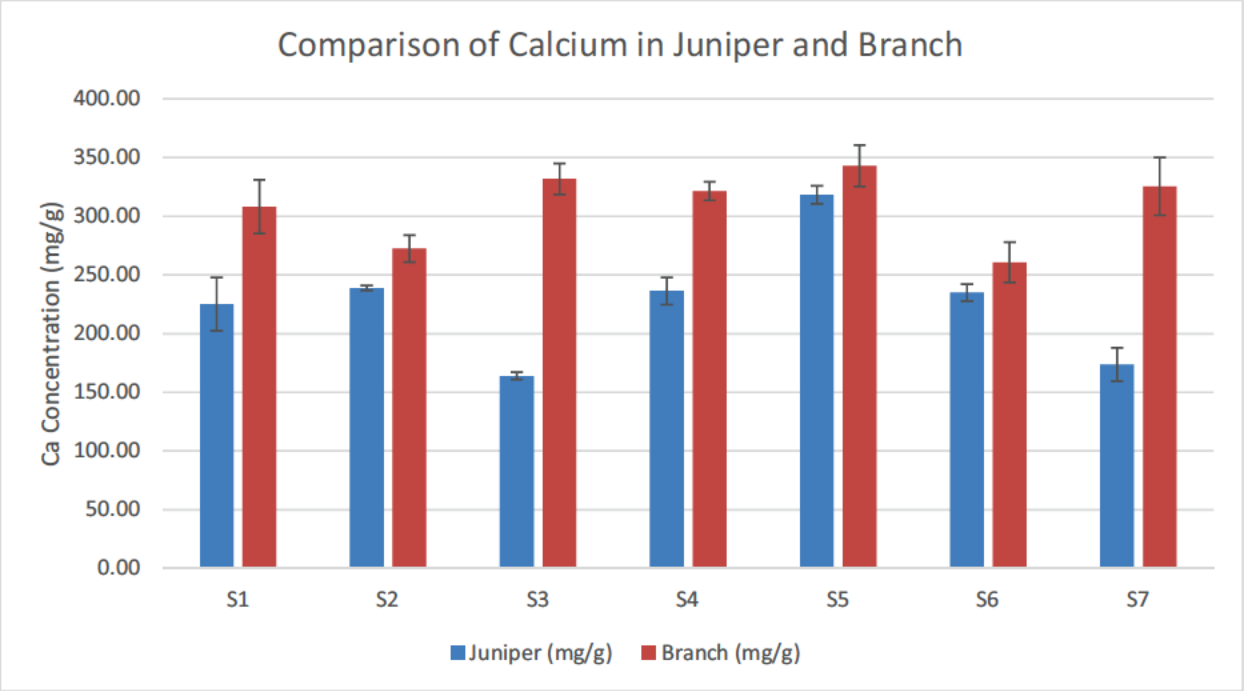

Figure 3.7: Bar graph for comparing the calcium concentration between ashed juniper leaves and branch (mg/g)

ID	Ca (Juniper /Branch) Ratio
S1	0.73
S2	0.88
S3	0.49
S4	0.73
S5	0.93
S6	0.90
S7	0.53

Table 3.4: Calcium ratio created to compare the calcium concentration between ashed juniper and branch (mg/g) from the same sample site

Table 3.3 shows the overall calcium concentration (mg/g), standard deviation (s), and percent relative standard deviation (% RSD). This also includes juniper samples and blue corn collected from flea market in Kayenta, AZ and a Standard Reference Material 1570a (Trace

Elements in Spinach Leaves, 60 grams). The bar graphs for each sample were organized based on type of sample in order to clearly compare and contrast between similar samples. Figure 3.7 is a bar graph of all the samples and their calcium concentration with their standard deviations from Table 3.3.

Figure 3.3 shows all samples that consisted only of juniper and juniper collected from flea market. The juniper collected from the flea market only contained juniper and no branch. In this graph, the calcium concentration ranged from 164 ± 3.15 to 318 ± 7.65 mg of calcium per gram of juniper ash, with a mean of 242 mg of calcium per gram of ash. The two samples with the lowest calcium concentration were S3-J (164 ± 3.15 mg/g) and S7-J (174 ± 14.12 mg/g). The two samples with the highest calcium concentration were S5-J (318 ± 7.65 mg/g) and FM1-OIF (308 ± 4.21 mg/g). The highest %RSD in this group of samples was 10.1%.

Figure 3.5 included all the samples that consisted only of the juniper branch. For these samples, the calcium concentration ranged from 261 ± 17.17 to 343 ± 17.68 mg/g of juniper branch ash. The mean calcium concentration was 309 mg of calcium per gram of juniper branch ash. The sample with the lowest calcium concentration was S6-B (261 ± 17.17 mg/g) and the sample with the highest concentration was S5-B (343 ± 17.68 mg/g).

Figure 3.6 included all the samples that consisted only of juniper collected from the flea market in Kayenta, AZ. Though these samples were collected from one location, they were all gathered elsewhere from merchants. Specifically, FM-CRF is a juniper sample identified as Canadian Red Flat Cedar, and FM-OIF is a juniper sample identified as Oregon Incense Flat Cedar. There was not enough information on these samples in order to identify the species of juniper. The rest of the flea market juniper samples (FM-NC,-CA,-JA) were ashed juniper samples from the Navajo reservation. These samples had a uniform light gray colored ash. There was not enough

information on these samples to identify the species of juniper, but samples being gathered on the Navajo reservation, there is high probability that it is *Juniperus Osteosperma/Monosperma.* Exact locations of all flea market juniper samples were not stated by merchants due to their personal reasons. The calcium concentration for these samples ranged from 248 ± 3.75 to 308 ± 4.21 mg of calcium per gram of juniper ash. The mean calcium concentration was 279 mg of calcium per gram of flea market juniper ash. The sample with the lowest calcium concentration was FM2-CRF (248 ± 3.75 mg/g) and the highest calcium concentration was FM1-OI (308 ± 4.21 mg/g). An interesting point in examining these calcium concentrations, FM3-JA and FM4-CA are juniper samples that were pre-ashed when purchases, while the rest of the flea market samples were not ashed when purchased. The pre-ashed and sampled ashed in the lab have similar calcium concentrations. This suggest that the pre-ashed flea market samples were ashed with an extremely hot flame and was able to achieve a completely uniformed burn that was comparable to samples ashed in a laboratory muffle furnace.

All but four samples have been mentioned in the figures above (S1-Berry, S7-W, SRM-1, and BC-1). As mentioned before in the Methods and Materials chapter, most juniper samples in the 2014 sample collection did not have many juniper berries during collection. As a result, only the first sample (S1) had enough juniper berries for an analysis. The calcium concentration of this sample was 148 ± 8.61 mg of calcium per gram of juniper berry. During collection, S7's juniper tree had many branches with deformed juniper that looked different from the normal juniper. A sample of a deformed branch was collected from this juniper tree and labeled S7-W. The calcium concentration of this sample was 166 ± 7.72 mg of calcium per gram of juniper. Each analysis had a Standard Reference Material analyzed as well. The SRM had a calcium concentration of 92.3 ± 0.25 mg of calcium per gram of SRM. Last, a sample of blue corn meal was analyzed in order for

the calcium concentration to be compared to juniper ash. The blue corn meal sample had a calcium concentration of 2.19 ± 0.73 mg of calcium per gram of blue corn meal.

Though the standard reference material (SRM) was analyzed as well, the concentration of the SRM did not match the certified value. The mean concentration was 92.3 ± 0.25 mg of calcium per gram of SRM. While the certified value was 1.526% or 15.26 mg of calcium per gram of SRM. The reason for the high calcium concentration in the SRM used in the analysis was that the SRM was ashed along with the other samples. On the other hand, the certified value of calcium for the SRM was significantly lowered. The reason for this was that the analysis of SRM was not ashed, but freeze-dried. During the ashing process, the high temperature used in the furnace removed the water and organic material from the sample, all that remains is the inorganic material such as calcium. When the samples are weighed out for acid digestion, there was significantly more inorganic material within each sample; there was more calcium within the sample. Resulting in more calcium per gram of ashed sample analyzed. On the other hand, the freeze-dried SRM contained inorganic and organic material within the sample. This resulted in less inorganic material (such as calcium) per gram of non-ashed sample analyzed. Even though the certified value was not used to compare to the actual value calculated, this analysis was able to validate the reproducibility of the analytical instrument used because the same ashed SRM was analyzed during two different sample analyses with the same FAAS instrument and using different external calibration standards.

The last bar graph and table (Figure 3.7 and Table 3.4) focused on the comparison of calcium in the ashed juniper and branch. In each pair of samples (juniper and branch for each sample), the calcium concentration was higher in the branch than the juniper. Samples such as S2, S5, and S6 were the samples that had calcium concentrations in close proximity to one another.

Samples S3 and S7 were the samples that has Ca concentrations that were the furthest from one another. No matter the samples, the juniper branch was always higher in calcium concentration to the juniper. Similar results for comparing juniper versus branch was seen in other journal articles.

In Figure 3.7, S-2, S-5, and S-6 were both samples in which the juniper and branch were close in calcium concentration. Among those, S-5 and S-6 juniper/branch samples' standard deviations fell within range of one another. In order to conclude whether or not these samples are statistically different from one another, statistical tests were done. These results are discussed in the Statistical Analysis section of this chapter (Section 3.3).

3.3 Statistical Analysis of Data

All calculations including statistical calculations were done on Microsoft Excel in order to perform mass calculations all at once. Along with the calcium concentrations (mg/g), standard deviation (s), and percent relative standard deviation (%RSD) was calculated with each sample. In the previous section, it was stated that two samples (S-5 and S-6) had juniper and branch calcium concentrations that were similar and also fell within range of each other's standard deviation. The purpose of the comparison between both juniper and branch was to conclude that their calcium concentrations for significantly different from one another.

A student's t-test was utilized for both samples in order to conclude if they are statistically different from one another. The purpose of this test is to accept or reject the null hypothesis; the two samples' averages are not different. The variances for both samples are not known, in that case, the type of t-test used was 'Two Samples Assuming Unequal Variance' using a $t_{critical}$ value from a p-value of 0.05 (or 95% confidence). Each sample had three separate analyses done (in triplicate) for juniper and branch. Degrees of freedom (df) used in calculation was based on

variance and standard deviation of samples of interest. Normally, degrees of freedom would be based on the number of observations, only if there is similarity in variance. The t-test as shown in Table 3.5, have similarity in variance, thus the degrees of freedom are equal to the number of observations. The same results can be seen on Table 3.6. The values of interest are **t_{stat}, P(T< t) two-tail** and **$t_{critical}$ two-tail.** The t_{stat} calculated was compared to $t_{crtical}$ in order to determine the hypothesis. As stated in Equation 6, if t_{stat} is greater than $t_{critical}$ then the null hypothesis is rejected; the two data sets are different. If t_{stat} is less than $t_{critical}$ then the null hypothesis is accepted; the two data sets are the same. Another way to determine the null hypothesis is to compare the calculated p-value (P(T< t) two-tail) to the p-vaule used for the t-test (p-value 0.05). If the calculated two-tail p-value is less than the p-value used, then the null hypothesis is rejected.

t-Test: Two-Sample Assuming Unequal Variances		
S-5	*Juniper*	*Branch*
Mean	318	342
Variance	58.5	312
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-2.22	
P(T<=t) one-tail	0.0561	
t Critical one-tail	2.35	
P(T<=t) two-tail	0.112	
t Critical two-tail	3.18	

Table 3.5: A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is accepted.

Table 3.5 shows the calculated values for juniper and branch samples of S-5. The calculated t_{stat} value (-2.22) is less than the $t_{critical}$ value (3.18). Also, the two-tail p-value (0.112) is greater

than the p-value used in the t-test (0.05). Both results conclude that the null hypothesis is accepted; both juniper and branch samples are statistically the same to one another.

t-Test: Two-Sample Assuming Unequal Variances		
S-6	*Juniper*	*Branch*
Mean	234	260
Variance	52.9	294
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-2.39	
P(T<=t) one-tail	0.0482	
t Critical one-tail	2.35	
P(T<=t) two-tail	0.0965	
t Critical two-tail	3.18	

Table 3.6: A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is accepted.

Table 3.6 showed the calculated values for juniper and branch samples of S-6. The calculated t_{stat} value (-2.39) is less than the $t_{critical}$ value (3.18). Also, the two-tail p-value (0.096) is greater than the p-value used in the t-test (0.05). Both results concluded that the null hypothesis is accepted; both juniper and branch samples are statistically the same to one another. Both S-5 and S-6's juniper/branch samples are statistically the same to one another.

Chapter 4: Results and Discussion (Ashing/Soil)

4.1 Introduction

The overall goal of this research was to quantify the amount of calcium found in juniper used in the traditional Navajo food, blue corn mush. In the previous chapter, the average concentration of calcium in different segments of the juniper was established. In this chapter, multiple goals will be addressed pertaining to gathering information on calcium concentration is juniper and soil samples. Specifically, the first goal was to gather information on the amount of calcium found in a sample of juniper that consisted of a homogenous mixture of all segments of the tree (juniper, branch, and berry). The next goal was to compare the calcium concentration of whole juniper samples using two different ashing techniques: a laboratory ashing process utilizing a muffle furnace and a traditional Navajo ashing process utilizing an open flame to ignite juniper. The last goal of this chapter was to quantify the calcium concentration in the soil collected at the base of the juniper tree and compare the calcium concentrations with its respective juniper samples (both ashing techniques) and establish relationship between calcium concentrations from the soil to the juniper tree. To ensure the accuracy of the measured samples, a blank solution and a Standard Reference Material (SRM) was utilized during the elemental analysis process. The quantification of calcium utilized FAAS for the elemental analysis. Based on Beer's law (Harris 2010), the absorbance signal collected from the analytical instrument is directly proportional to the concentration of the analyte of interest. This relationship between absorbance signal and

concentration allowed for external calibration calcium standards to be analyzed prior to sample analysis in order to establish an external calibration curve with a range of 0 mg/L to 10 mg/L.

4.2 FAAS Analysis of Whole Juniper Samples

Concen	Abs
0	0
1	0.093
2	0.178
5	0.419
10	0.795

Table 4.1: External Calcium Calibration Standards and absorbance values used for 2016 Sample Analysis

ID	Mean Conc. (mg/g)	Standard Dev.	%RSD
LA-S-8	311	7.23	2.32%
LA-S-9	253	7.44	2.94%
LA-S-10	323	7.86	2.43%
LA-S-11	284	6.76	2.38%
LA-S-12	253	10.9	4.31%
LA-S-13	304	6.24	2.05%
LA-S-14	299	12.9	4.32%
LA-S-15	333	12.1	3.63%
LA-S-16	323	8.17	2.53%
LA-S-17	238	8.50	3.57%
LA-S-18	290	7.14	2.46%
LA-S-19	258	9.40	3.65%
LA-S-20	295	14.5	4.90%
LA-S-21	281	7.19	2.56%
SRM	16.8	2.60	15.5%
Blank	0.0222	0.01	61.0%
TA-S-8	319	3.15	0.99%
TA-S-9	212	10.6	4.98%
TA-S-10	228	78.4	34.4%
TA-S-11	234	13.8	5.89%

TA-S-12	254	47.8	18.8%
TA-S-13	194	52.7	27.2%
TA-S-14	250	76.2	30.5%
TA-S-15	238	18.2	7.65%
TA-S-16	272	18.8	6.91%
TA-S-17	190	7.46	3.93%
TA-S-18	249	14.0	5.63%
TA-S-19	265	6.62	2.50%
TA-S-20	241	66.7	27.7%

Table 4.2: A list compiling samples collected, along with standard reference material and blank. This table consists of type of sample, calcium concentration (mg/g), standard deviation, and percent relative standard deviation. ***LA Laboratory Ashing TA Traditional Ashing***

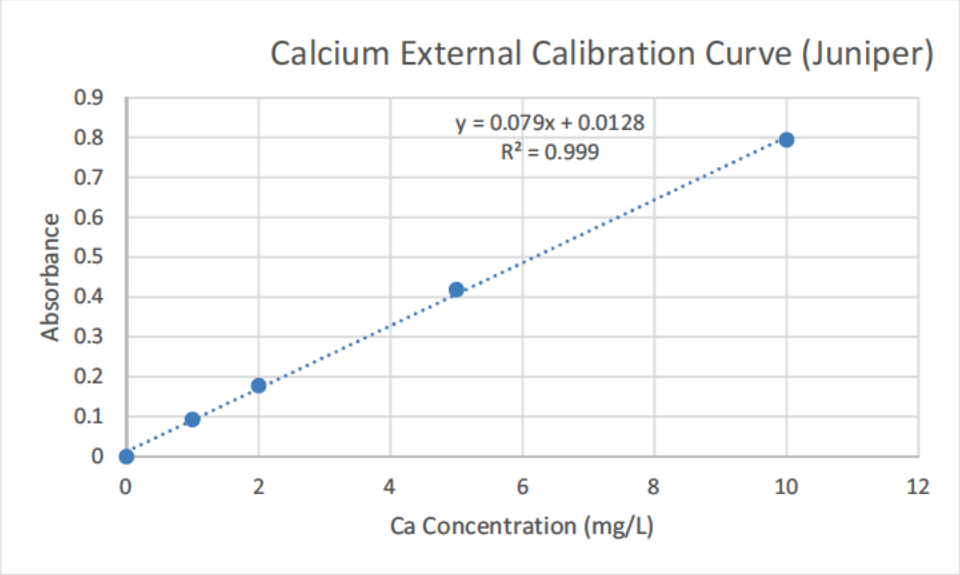

Figure 4.1: External Calibration Curve for Juniper Analysis. Solid line represents the line between each point. Dotted line represents the linear trendline. Calcium standards (0, 1, 2, 5, 10 mg/L)

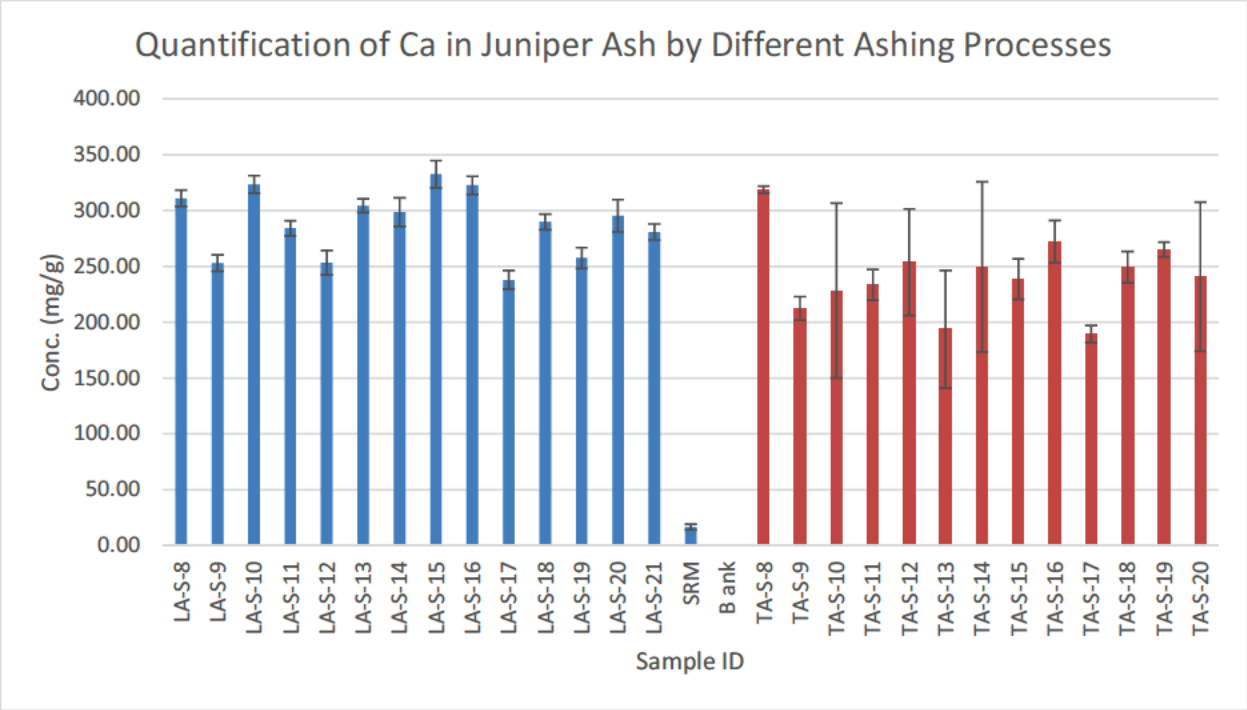

Figure 4.2: Bar graph of concentrations of calcium in collective ashed juniper samples (mg/g) collected from 2016 sampling collection. ***LA Laboratory Ashing TA Traditional Ashing***

The external calcium calibration standards (0, 1, 2, 5, 10 mg/L) and their corresponding absorbance values can be seen above in Table 4.1. These values were plotted in a scatter plot graph (Figure 16) with the calcium concentrations as the x-axis and the absorbance values as the y-axis. Linear trendline was established with the data points plotted. The R^2 value reported for the linear trendline was 0.9990, which is considered good. The external calibration equation established in Figure 4.1 was used to find the concentration of calcium in units of mg per liter of analyte (mg/L). The original calcium concentration (mg/g) was calculated by multiplying the analyte concentration with the original volume (10 mL), the dilution factor (100-fold dilution), then dividing by the mass (grams) of the ashed sample analyzed.

Table 4.2 is a compiled list of all the samples calcium concentration (mg/g), standard deviation (s), and percent relative standard deviation (%RSD). Table 4.1 included two collected juniper samples from the same sampling site, a single juniper sample collected from a flea market, a Standard Reference Material 1570a (Trace Elements in Spinach Leaves, 60 grams), and a blank standard; a total of 29 samples altogether. Figure 4.2 is a bar graph that consists of the calcium concentrations of all the samples included in Table 4.2. There were 13 sampling sites with two juniper samples collected at each (S-8 through S-20); a total of 26 juniper samples. For each pair of juniper samples, two different ashing techniques were used; laboratory ashing (LA) and a traditional Navajo ashing (TA). The single juniper sample (S-21) was taken from a flea market with a stated origin of Mt. Hesperus. There was no additional information given for sample and only contained juniper leaves and no branch.

As shown in Table 4.2 and Figure 4.2, the SRM that was analyzed had a mean calcium concentration of 16.8 ± 2.6 mg/g. The SRM used in the 2014 sample analysis had a mean calcium concentration of 92.27 ± 0.25 mg/g. Though the SRMs had significantly different calcium concentrations in both analyses, they were prepared using the same Standard Reference Material 1570a. It was concluded that the reason for such drastically different calcium concentrations was due to different sample preparation. The SRM used in the 2014 sample analysis was dry ashed utilizing the muffle furnace. While on the other hand, the SRM used in the 2016 sample analysis was not dry ashed. Comparing the two calcium concentrations, the SRM used in the 2016 sample analysis fell within range of the SRM certified value for calcium concentration (15.26 mg/g). As mentioned in the previous section, the certified SRM was not dry ashed before analysis but was only freeze-dried. Thus, the analytical approach used in this research is validated in comparing

the preparation of the freeze-dried (i.e. as is) SRM results (16.8 mg/g) to the certified results (15.26 mg/g)

Excluding the SRM and blank, the calcium concentration ranged from 190 ± 7.46 mg/g (TA-S-17) to 333 ± 12.1 mg/g (LA-S-15) and with an average of 265 ± 39.9 mg/g. The calcium concentration of this sample collection compared to samples collected in 2014 fall into relatively the same range. A comparison of both ashing techniques is discussed in greater depth the next section of this chapter.

4.3 Comparing and Contrasting Ashing Process

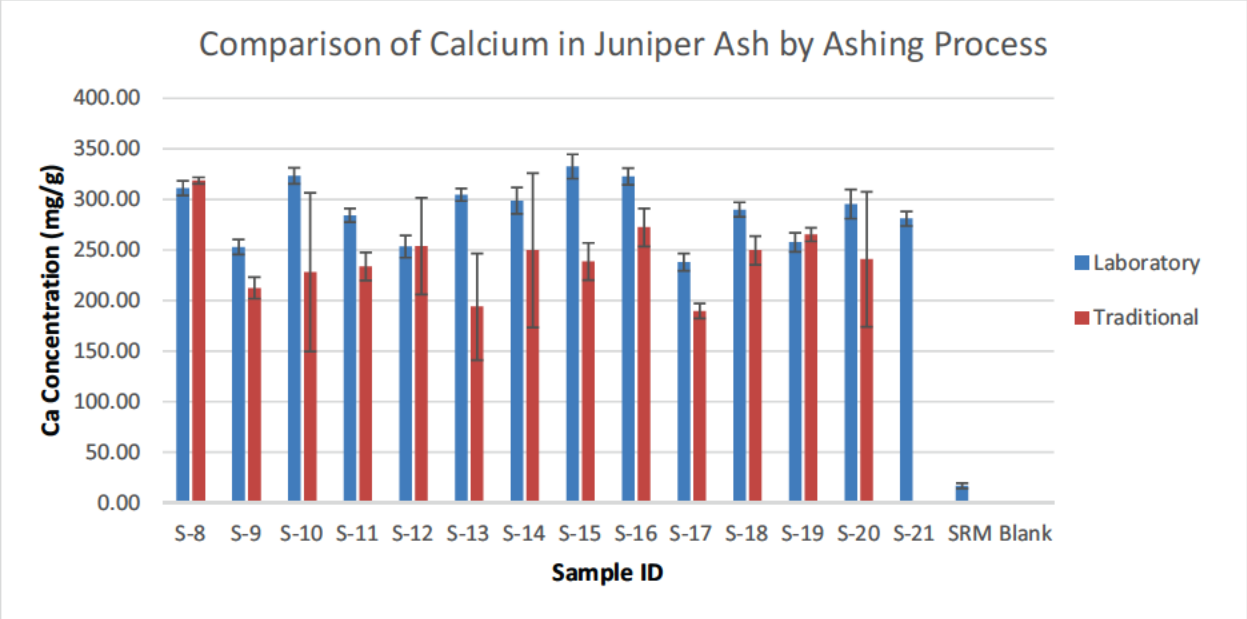

Figure 4.3: Bar graph for comparing calcium concentrations of juniper ashed with different ashing techniques (mg/g)

ID	Ca (TA/LA) Ratio
S-8	1.02
S-9	0.84
S-10	0.71
S-11	0.82
S-12	1.00
S-13	0.64
S-14	0.84
S-15	0.72
S-16	0.84
S-17	0.80
S-18	0.86
S-19	1.03
S-20	0.82

Table 4.3: Ca ratio created to compare the calcium concentration between juniper from the same sample site samples using different ashing techniques

The bar graph shown above (Figure 4.3) was created to show the comparison of the calcium concentration between two different ashing techniques; laboratory ashing and a traditional Navajo ashing. In relation to Figure 4.3, a table (Table 4.3) was also created to show the ratio between the different ashing techniques (TA/LA). Comparing both populations with one another, majority of the laboratory ashed juniper samples have a higher calcium concentration than the traditionally ashed juniper samples. The LA samples have a mean calcium concentration of 289 ± 30.6 mg/g and the TA samples have a mean calcium concentration of 242 ± 28.2 mg/g. This is most likely due to the different ashing processes used and will be discussed further in the next chapter. Given their standard deviations, these two populations have overlapping values that could conclude they are statistically within range to one another. Additional statistical tests were done in order to conclude results; these results were discussed in the Statistical Analysis section of this chapter.

By examining both Figure 4.3 and Table 4.3, there is a clear trend of high standard deviations for the TA samples compared to the LA samples. The highest standard deviation for the LA samples was 14.5 mg/g, while the highest for TA samples was 78.4 mg/g. A reason for TA samples' high standard deviation was due to the sample preparation process, specifically the ashing process. The muffle furnace used to ash the LA samples performed a clear and concise ashing that was programmed to ramp in temperature in a timely matter. While the TA samples were ashed over an open flame, where there is limited control over operating conditions. Because of this limited control, the juniper would not ash uniformly, where some parts of the juniper would not fully ash. Based on the traditional ashing process, higher standard deviations for the TA samples was to be expected.

Samples S-8, S-12, and S-19 are of interest because the TA samples were either greater than or equal in calcium concentrations to LA samples. TA/LA ratios in Table 15 with values close to 1 are TA and LA samples that are close in calcium concentration. Samples such as S-14 and S-20 were examined and tested as well due to LA/TA samples' standard deviations being within range of one another to be considered different. Additional statistical tests were done in order to conclude results; these results were discussed in the Statistical Analysis section of this chapter along with previously mentioned tests.

4.4 Relationship between Juniper and Soil

Ca Concentration (mg/L)	Abs
0	0.000
1	0.093
2	0.180
5	0.422
10	0.806

Table 4.4: External Calcium Calibration Standards and absorbance valued used for 2014 sample analysis

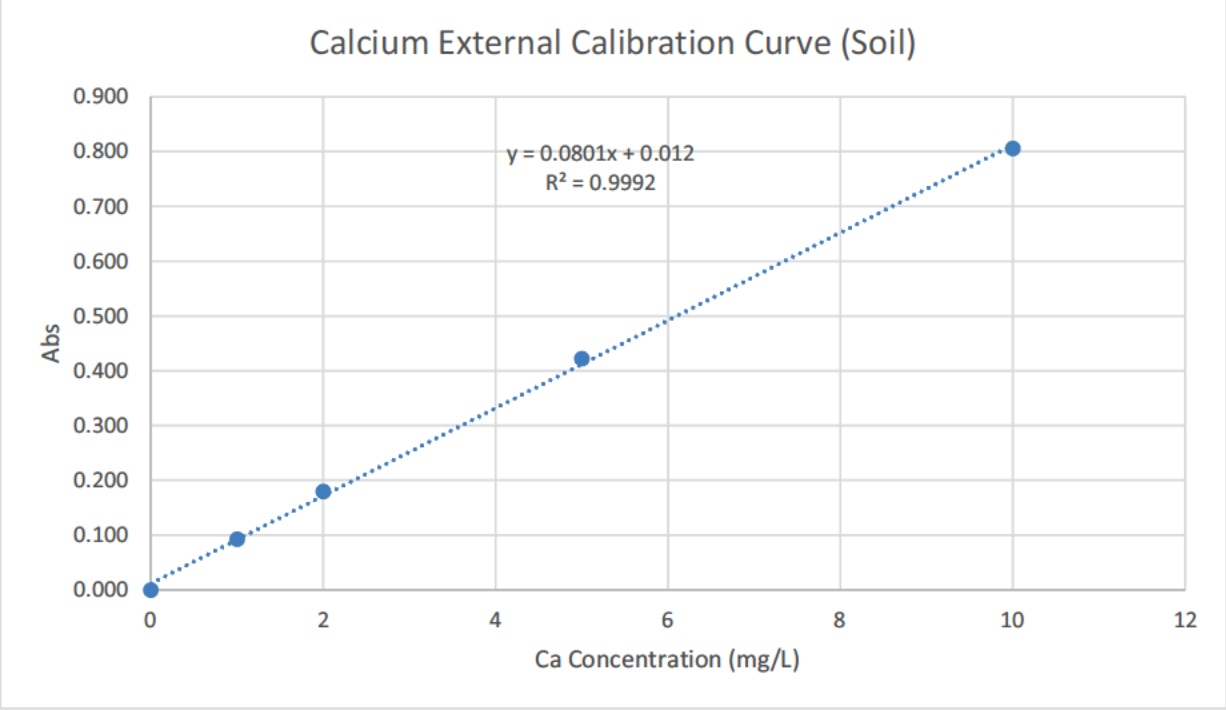

Figure 4.4: External Calibration Curve for Soil Analysis. Solid line represents the line between each point. Dotted line represents the linear trendline. Calcium Standards (0, 1, 2, 5, 10 mg/L)

ID	Ca Conc. (mg/g)	s	%RSD
R1-S-8	26.3	0.776	2.95%
R1-S-9	19.3	0.602	3.12%
R1-S-10	21.2	1.22	5.73%
R1-S-11	14.8	1.13	7.64%
R1-S-12	0.820	0.237	28.8%
R1-S-13	6.15	0.284	4.61%
R1-S-14	10.6	1.29	12.2%
R1-SRM	5.93	0.378	6.37%
R1-BLANK	0.00	0.00	0.00%
R2-S-15	0.806	0.0625	7.75%
R2-S-16	0.382	0.00117	0.31%
R2-S-17	33.4	0.444	1.33%
R2-S-18	1.33	0.0629	4.71%
R2-S-19	14.3	0.225	1.58%
R2-S-20	6.42	0.196	3.06%
R2-SRM	6.65	0.164	2.47%
R2-BLANK	0.00	0.00	0.00%

Table 4.5: A table consisting of the calcium concentration (mg/g) in soil at the base of juniper trees where their respective juniper samples were collected. The sample marked in red needed a Grubb's test in order to identify and discard an outlier in R2-S-18. ***R1 1st Run R2 2nd Run***

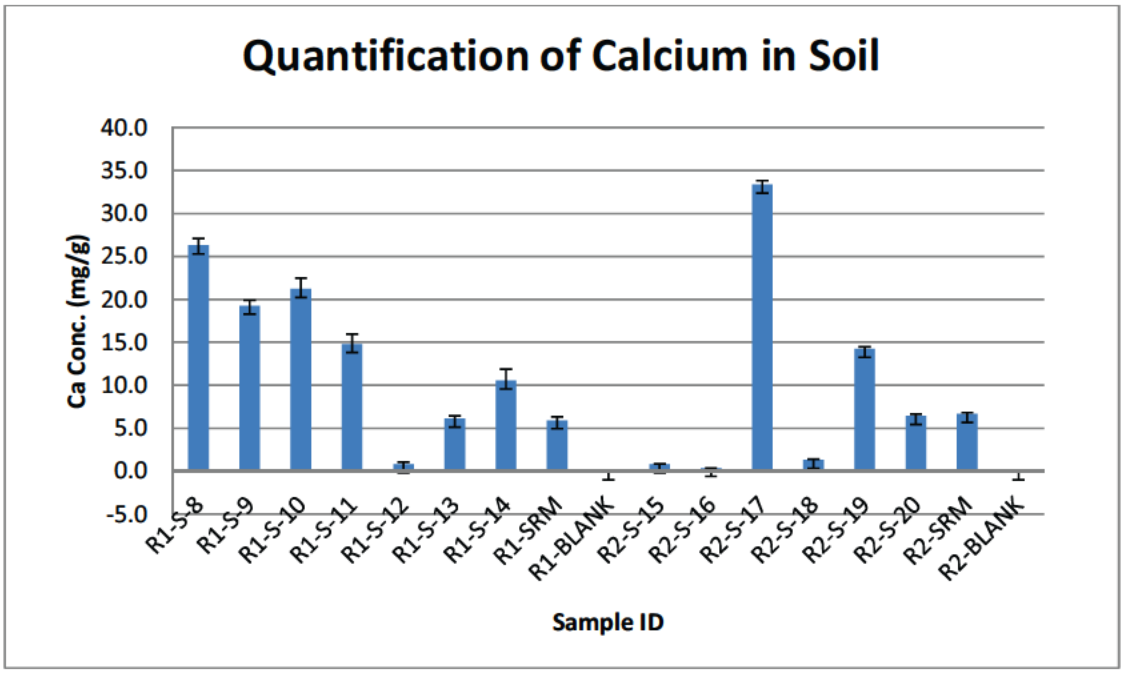

Figure 4.5: Bar graph of the calcium concentrations of soil samples (mg/g) from same sample sites as juniper sample in Table 14 during 2016 sample collection

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Type	ID	mg/g	Certified Value (mg/g)	s	%RSD
Soil	R1-SRM	5.93	19.1	0.38	6.37%
Soil	R2-SRM	6.65	19.1	0.16	2.47%

Table 4.6: A table that consists of the SRMs analyzed with their calcium concentrations (mg/g), standard deviations (s), and percent relative standard deviation (%RSD) in comparison to the certified value of the SRM used (mg/g)

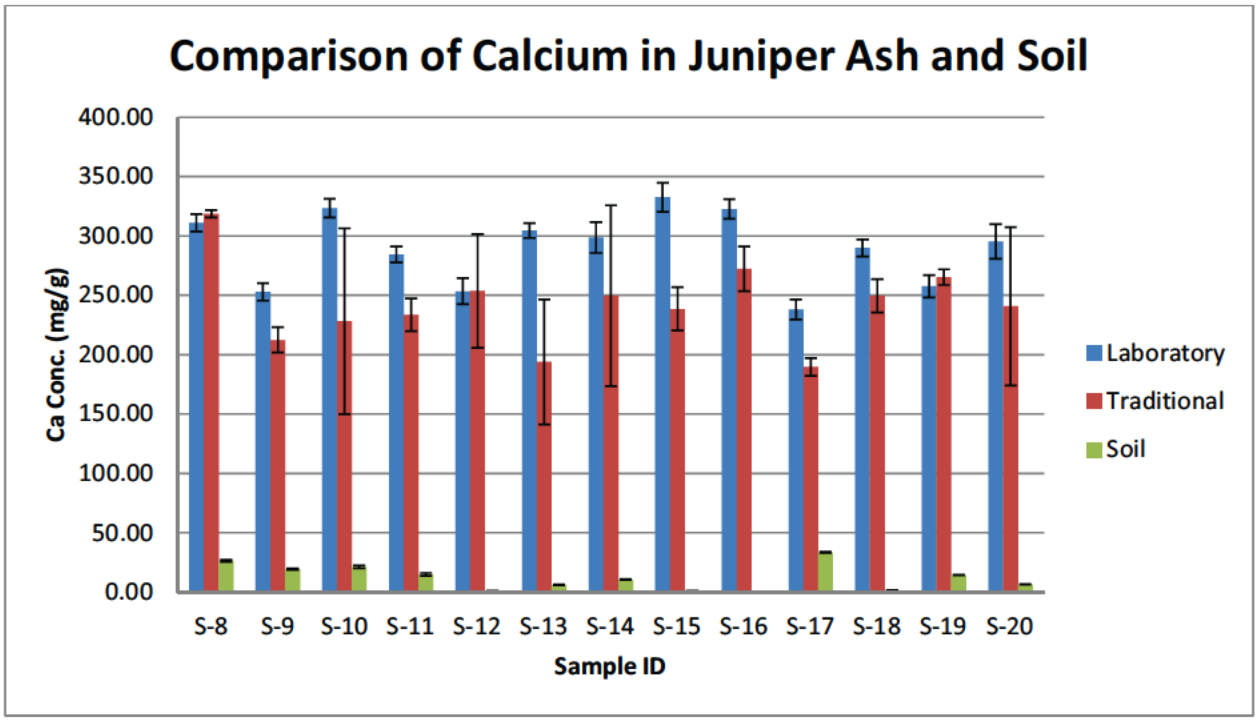

Figure 4.6: Bar graph for comparing the calcium concentrations (mg/g) to the concentrations of ashed juniper samples collected during the same 2016 sample collection

Five external calcium calibration standards (0, 1, 2, 5, 10 mg/L) and their corresponding absorbance values are listed in Table 4.4. These values were plotted in a scatter plot graph as seen above in Figure 4.4, with the external calibration calcium concentration (mg/L) in the x-axis and the absorbance values in the y-axis. A linear trendline was established along the data points in Figure 4.4. Through the linear trendline, the calibration equation and the R^2 value were calculated and can also be seen in Figure 4.4. The R^2 value for this calibration curve was 0.99918, which is considered good. The original calcium concentration (mg/g) was calculated by multiplying the analyte concentration (mg/L) with the original volume (10 mL), the dilution factor (500-fold dilution), then dividing by the mass (grams) of the ashed sample analyzed. Calculating the original concentration was different from similar calculations because during sample preparation, the

sample was subjected to a five-fold dilution, then later a 100-fold dilution; a 500-fold dilution overall.

A list of all the soil samples analyzed was compiled into Table 4.5. In this table, the calcium concentration (mg/g), standard deviation(s), and percent standard deviation (%RSD) of 13 soil samples, two SRMs, and two blank solutions were included. During the 2016 sample collection, along with the two juniper samples taken, there was one soil sample collected at the base of each juniper tree site; a total of 13 samples. During sample preparation, the microwave acid digester's carousel was limited to 40 slots for samples, and 13 soil samples needed to be run in triplicates; a total of 39 soil samples. Microwave-assisted digestion of samples was divided into two because for each digestion, there needed to be one blank and one SRM both prepared in triplicates; a total of six samples. The first sample preparation consisted of the first seven soil samples (S-8 through S-14), one SRM and blank prepared in triplicate; a total of 27 samples. The second sample preparation consisted of the rest of the six soil samples (S-15 through S-20), one SRM and blank prepared in triplicate; a total of 24 samples. The first set of samples digested were labeled with R1 (Run 1), the second set of samples digested were labeled with R2 (Run 2), which can be seen in Table 4.5.

The calcium concentrations of the soil samples were plotted into a bar graph (Figure 4.5) that can be seen above. Excluding the SRMs and blanks, the calcium concentration of the soil samples ranged from 0.382 ± 0.00117 mg/g to 33.4 ± 0.444 mg/g. The mean calcium concentration for the soil samples was 11.8 ± 10.7 mg/g. Both the highest (R2-S-17) and lowest (R2-S-16) calcium concentrations were in samples from the second sample preparation sample set (R2). Most samples except R1-S-12 and R1-S-14, had %RSDs under 10%, which is considered good.

SRM samples were used in the sample analysis in order to ensure the accuracy of the entire measurement process. As stated, there were two SRM samples analyzed along with the soil samples. R1-SRM had a calcium concentration of 5.93 ± 0.378 mg/g and R2-SRM had a calcium concentration of 6.65 ± 0.164 mg/g. The mean calcium concentration of both SRM samples was 6.29 ± 0.504 mg/g. SRM calcium concentrations were compared to the certified value of SRM which was 19.1 mg/g. These results can be seen in Table 4.6. The SRMs analyzed was significantly lower than the certified value, giving a 32.9% recovery. These results can be considered reasonable as the certified value for the SRM was prepared for analysis using a total acid digest protocol (i.e. using hydrofluoric acid for total dissolution), while the sample preparation done for this sample analysis utilized a microwave digester and EPA method 3051, a partial acid digestion. EPA Method 3051 was not meant to fully digest soil samples, as it only uses nitric acid and does not dissolve calcium incorporated into aluminosilicates. As stated in the Methods and Materials Chapter, this sample analysis aimed to measure the amount of calcium on the surface of the soil particles because calcium on the surface are considered exchangeable cations that have the ability to absorb into the roots of the juniper tree.

Figure 4.6 is a bar graph with the compiled calcium concentration data from the LA/TA samples and the soil samples from the same sampling site. Calcium concentrations in soil compared to LA/TA samples is significantly lower. Soil in samples such as S-12, S-15, S-16, and S-18 show minute amounts of calcium compared to the high concentrations found in the juniper tree samples. Previous work was done with similar methods showed lower calcium concentrations in soil samples compared to juniper samples (Bunderson 1980). Based on their results, it was expected that calcium in soil samples would be lower in comparison to juniper samples.

Soil/Juniper ratios for calcium were also done in their work, but because of different sample preparation methods, their ratios would be inconsistent with results discussed.

S-17 were interesting samples as they had the lower calcium concentration in the juniper samples and a higher calcium concentration in the soil samples (compared to other soil samples). Some explanation for this reasoning could be due to a high amount of a specie of calcium that is not bioavailable to the juniper tree. Another reason, even though there was high amounts of calcium in the soil, there was not enough water within the soil in order to assist in the transport of the exchangeable cation nutrients such as calcium. Most of the juniper trees involved in the sample collection were surrounded in sandy soils. This type of soil has a high permeability and is unable to adequately retain water.

4.5 Statistical Analysis of Data

All calculations including statistical calculations were done on Microsoft Excel in order to perform calculations all at once. Along with the calcium concentrations (mg/g), standard deviation (s), and percent relative standard deviation (%RSD) was calculated with each sample. The equations used to find these values can be found in Section 2.7. In the previous sections, it was stated that five samples (S-8, S-12, S-14, S-19, and S-20) had LA/TA calcium concentrations that were similar and also fell within range of each other's standard deviation. The purpose of the comparison between both juniper and branch was to conclude that their calcium concentrations for significantly different from one another.

A student's t-test was utilized for both samples in order to conclude if they are statistically different from one another. The purpose of this test is to accept or reject the null hypothesis; the two samples are statistically the same. The variances for both samples are not known, in that case,

the type of t-test used was 'Two Samples Assuming Unequal Variance' using a $t_{critical}$ value from a p-value of 0.05 (or 95% confidence). Each sample had three separate analyses done (in triplicate) for juniper and branch. Degrees of freedom (df) used in calculation was based on variance and standard deviation of samples of interest. The values of interest are **t_{stat}, P(T< t) two-tail** and **$t_{critical}$ two-tail.** The t_{stat} calculated was compared to $t_{crtical}$ in order to determine the hypothesis. As stated in Equation 6, if t_{stat} is greater than $t_{critical}$ then the null hypothesis is rejected; the two data sets are different. If t_{stat} is less than $t_{critical}$ then the null hypothesis is accepted; the two data sets are the same. Another way to determine the null hypothesis is to compare the calculated p-value (P(T< t) two-tail) to the p-value used for the t-test (p-value 0.05). If the calculated two-tail p-value is less than the p-value used, then the null hypothesis is rejected.

t-Test: Two-Sample Assuming Unequal Variances		
S-8	*LA*	*TA*
Mean	311	318
Variance	52.2	9.90
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-1.66	
P(T<=t) one-tail	0.0970	
t Critical one-tail	2.35	
P(T<=t) two-tail	0.194	
t Critical two-tail	3.18	

Table 4.7: A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is accepted.

Table 4.7 shows the calculated values for LA/TA juniper samples for S-8. The calculated t_{stat} value (-1.66) is less than the $t_{critical}$ value (3.18). Also, the two-tail p-value (0.194 is greater than

the p-value used in the t-test (0.05). Both results conclude that the null hypothesis is accepted; both LA/TA juniper samples are statistically the same to one another.

t-Test: Two-Sample Assuming Unequal Variances		
S-12	*LA*	*TA*
Mean	253	253
Variance	119	2286
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.0132	
P(T<=t) one-tail	0.495	
t Critical one-tail	2.91	
P(T<=t) two-tail	0.990	
t Critical two-tail	4.30	

Table 4.8: A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is accepted.

Table 4.8 shows the calculated values for LA/TA juniper samples for S-12. The calculated t_{stat} value (-0.013) is less than the $t_{critical}$ value (4.30). Also, the two-tail p-value (0.99) is greater than the p-value used in the t-test (0.05). Both results conclude that the null hypothesis is accepted; LA/TA juniper samples are statistically the same to one another.

t-Test: Two-Sample Assuming Unequal Variances		
S-14	*LA*	*TA*
Mean	298	249
Variance	166	5803
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	1.097	

P(T<=t) one-tail	0.193	
t Critical one-tail	2.91	
P(T<=t) two-tail	0.386	
t Critical two-tail	4.30	

Table 4.9: A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is accepted.

Table 4.9 shows the calculated values for LA/TA juniper samples of S-14. The calculated t_{stat} value (1.09) is less than the $t_{critical}$ value (4.30). Also, the two-tail p-value (0.386) is greater than the p-value used in the t-test (0.05). Both results conclude that the null hypothesis is accepted; both LA/TA juniper samples are statistically the same to one another.

t-Test: Two-Sample Assuming Unequal Variances		
S-19	*LA*	*TA*
Mean	257	265
Variance	88.4	43.8
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.14	
P(T<=t) one-tail	0.157	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.314	
t Critical two-tail	2.77	

Table 4.10 A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is accepted.

Table 4.10 shows the calculated values for LA/TA juniper samples of S-19. The calculated t_{stat} value (-1.14) is less than the $t_{critical}$ value (2.77). Also, the two-tail p-value (0.314) is greater than the p-value used in the t-test (0.05). Both results conclude that the null hypothesis is accepted; both LA/TA juniper samples are statistically the same to one another.

t-Test: Two-Sample Assuming Unequal Variances		
S-20	*LA*	*TA*
Mean	295	240
Variance	209	4449
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	1.38	
P(T<=t) one-tail	0.150	
t Critical one-tail	2.91	
P(T<=t) two-tail	0.301	
t Critical two-tail	4.30	

Table 4.11:A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is accepted.

Table 4.11 shows the calculated values for LA/TA juniper samples of S-20. The calculated t_{stat} value (1.38) is less than the $t_{critical}$ value (4.30). Also, the two-tail p-value (0.300) is greater than the p-value used in the t-test (0.05). Both results conclude that the null hypothesis is accepted; both LA/TA juniper samples are statistically the same to one another. This concluded that all the LA/TA juniper samples tested using the Student's t-test accepted the null hypothesis; the two data sets are statistically the same to one another.

In Chapter 5, two juniper samples were prepared using different ashing techniques. There were two ways to determine if these sample populations could be considered statistically different; Analysis of Variance (ANOVA) and a Student's t-test. Microsoft Excel's 'Data Analysis' toolpak was utilized in determining if two sample populations were considered statistically similar to one another. A One-Way ANOVA was utilized to test if the two different ashing populations (LA/TA S-8 through LA/TA S-20) could be considered statistically different from one another. Using the

'Data Analysis' in Microsoft Excel, the $F_{observed}$ was calculated and compared to the $F_{critical}$. As shown in Equation 2.10, the purpose of this test is to determine whether to accept or reject the null hypothesis; the two populations are statistically different from one another.

ANOVA: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Laboratory	13	3763	289	937		
Traditional	13	3145	241	1149		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14700	1	14700	14.1	0.000980	4.25
Within Groups	25045	24	1043			
Total	39745	25				

Table 4.12: A table consisting of variables used to calculate $F_{observed}$ and $F_{critical}$ in order to determine whether to accept or reject the null hypothesis. This is test, the null hypothesis is rejected; the two population groups are statistically different.

For Table 4.12, the important values to consider are $F_{observed}$ and $F_{critical}$. They will determine the results of the test. In this case, the $F_{observed}$ value (14.08) is greater than the $F_{critical}$ value (4.25). This concludes that the calcium concentrations for the LA technique are statistically different from the TA technique; both ashing techniques give different calcium concentrations from one another. A Student's t-test was also used to verify the ANOVA test. Table 4.13 shows the calculated values for LA/TA juniper samples. The calculated t_{stat} value (3.75) was greater than the $t_{critical}$ value (2.06). Also, the two-tail p-value (0.0009) was less than the p-value used in the t-

test (0.05). Both results conclude that the null hypothesis is rejected; both LA/TA juniper samples are statistically the different from one another.

t-Test: Two-Sample Assuming Unequal Variances		
2016 Sample Analysis	*Laboratory*	*Traditional*
Mean	289	241
Variance	937	1149
Observations	13	13
Hypothesized Mean Difference	0	
df	24	
t Stat	3.75	
P(T<=t) one-tail	0.000490	
t Critical one-tail	1.71	
P(T<=t) two-tail	0.000980	
t Critical two-tail	2.06	

Table 4.13: A table consisting of the variables used in order to determine whether to accept or reject the null hypothesis. Based on $t_{critical}$ and t_{stat}, the null hypothesis is rejected; Both sample groups are statistically different.

ID	mg/g
R2-S-18	34.2
R2-S-18	1.29
R2-S-18	1.38
Mean	12.3
s	19.0

Table 4.14: Original data set for R2-S-18; run in triplicate. The first sample run is higher in concentration than the other two.

A Grubb's Test is a statistical test used to determine if a potential outlier in a data set should be accepted or discarded. This is determined by calculating for the $G_{calculated}$ and comparing the

$G_{critical}$ provided based on p-value (0.05) and number of observations. Equation 2.7 and 2.8 are utilized to determine the $G_{calculated}$.

Grubbs Calculated	Grubbs Critical
1.155	1.153

Table 4.15: The values calculated for the Grubb's Test. $G_{calculated}$ is greater than $G_{critical}$. This determined that the potential outlier can be discarded.

ID	mg/g
R2-S-18	1.29
R2-S-18	1.38
Mean	1.33
s	0.0628

Table 4.16: Updated data set for R2-S-18; run in duplicate. The first sample was identified as an outlier and discarded from data set.

This is case, soil sample R2-S-18 was analyzed in triplicates and one of the three absorbance values from the analyses was significantly higher than the rest as shown in Table 4.14. Because of this potential outlier, the standard deviation and %RSD of the data set are also higher than any other samples in the population. A Grubb's test was used to calculate the $G_{calculated}$ for R2-S-18 and compared to its $G_{critical,}$ values shown in Table 4.15. The $G_{calculated}$ value (1.155) was greater than the $G_{critical}$ value (1.153). Based on Equation 2.8, this comparison determined that the one of the replicated values (34.2 mg/g) was an outlier and was allowed to be discarded from the data set. Table 4.16 is the data used in the Results and Discussion for R2-S-18, also with a new calculated standard deviation and %RSD in Table 4.5.

Chapter 5: Conclusion

The goal of this research was to quantify the amount calcium in juniper ash that is used in the traditional Navajo dish, blue corn mush. Specifically, the goal was to quantify the amount of calcium in different segments of the juniper tree and compare the calcium concentration in juniper ashed using different ashing techniques. Along with this goal was to quantify the amount of calcium in the soil at the base of the juniper trees in order to determine the relationship between juniper tree and soil. The following section in this Conclusion chapter will elaborate on all research goals.

5.1 Calcium Concentration in Juniper

In order to gain a better understanding on which segment of the juniper tree has the highest and lowest calcium concentrations, different segments (leaves, branch, and berry) of the juniper tree were analyzed via FAAS. Results from this analysis concluded that majority of the juniper branch samples contained the highest amount of calcium (309 ± 30.9 mg/g), the second highest being the juniper leaves (242 ± 50.3 mg/g) and the lowest being the juniper berries (148 ± 8.61 mg/g). However, the findings regarding juniper berries were limited, as a small set of juniper berry samples were analyzed. Two other studies focusing on the mineral composition of juniper trees showed contradictory results low in calcium concentration, roughly 14.8 and 15.9 mg/g (Brotherson and Osayande, 1980) (Bunderson et al., 1985) Similar results were seen in another journal article doing a similar study, where they were receiving average calcium concentrations of 285 ± 14 mg/g in whole juniper samples (Bunderson et al., 1985; Christensen et al., 1998). These

results are vastly different and this could be due to the type of sample preparation methods used. The studies that resulted in lower calcium concentrations did not utilize a dry ashing process before acid digestion. On the other hand, the study that resulted in a higher calcium concentration did utilize a dry ashing process before acid digestion. The high source of calcium in the juniper branch could be the result of the calcium transport within the juniper tree, as the tree is constantly uptaking nutrients such as calcium from the soil (White, 2003). Though not having the highest calcium concentration, the juniper leaves also have a significant amount that could also be contributed to the same transport as the branch.

Looking through the lens of nutritional value, juniper ash from either branch or leaves can be considered an excellent source of calcium in a daily diet. Milk is considered one of the best sources of calcium in a daily, which contains about 300 mg of calcium per serving (240 g). Although this is a considered a high concentration of calcium, this does not consider the fractional absorption (32.1%) of calcium when consumed, which would only absorb 93.6 mg per serving. On the other hand, one tablespoon of ash is roughly equivalent to one gram of ash. In the form of calcium oxide, the ash is highly soluble in stomach fluids, resulting in a high bioavailability for absorption within the body when consumed.

5.2 Calcium Concentration: Lab Ashing/ Traditional Ashing and Soil

Although the laboratory ashing process for the whole juniper samples resulted in a higher calcium concentration (289 ± 30.6 mg/g), a muffle furnace was utilized for this process, which is a machine that is not readily available to use unless in a laboratory setting. The majority of juniper ash sold at flea markets or even prepared at home were prepared in a traditional Navajo way, over an open flame. The traditional Navajo ashing process clearly had a lower calcium concentration

(242 ± 28.2 mg/g). However the lower calcium concentration in the TA samples could be due to ashing in an inefficient way or not having a hot enough flame. This is suggested from the data presented in Figure 4.6, where flea market samples (both ashed and non-ashed were purchased at flea market) were analyzed and calcium concentration results were similar. The flea market samples that were pre-ashed were possibly ashed using a more efficient ashing process than the one used in this research; utilizing a hotter flame.

Though the traditional ashing process had a lower calcium concentration, compared to other sources of calcium, this is still considered a high source of calcium in the Navajo diet. Examining a Navajo Foods recipe book created by Lillie Y. Pete, the Navajo Traditional Consultant, all the dishes that contain blue corn meal also contain juniper ash. For example, the Blue Corn Mush dish taken from the recipe book contains 2 cups of roasted blue corn and 2 tablespoons of juniper ash. This Navajo dish alone has two tablespoons of juniper ash. In respect to calcium concentration of traditionally ashed juniper, that is roughly 484 mg of calcium per recipe. The juniper ash added to blue corn dishes is important because as shown in the Results and Discussion section, there is little to no calcium found in blue corn meal.

Quantifying the amount of calcium in soil did not necessarily pertain to determining juniper ash as an excellent source of calcium, but utilized in order to understand the calcium concentration uptake relationship between the soil and juniper tree. Compared to the juniper ash, the soil beneath the juniper trees had a significantly lower calcium concentration (12.8 ± 10.2 mg/g). As stated in Results and Discussion, the low calcium concentration in the soil was contributed to the type of acid digestion completed on soil. The partial acid digestion's purpose what only to strip away the surface of soil particles, where exchangeable cations such as calcium adhere. The results suggest that although the soil contained low amount of exchangeable Ca, there is a large accumulation of

calcium once absorbed into the roots and transported to the rest of the juniper tree. This low calcium concentrations found in the soils can also be due to the characteristics of the soil itself. As discussed in a previous section (Section 1.6), sandy soils are unable to hold water (low permeability) and nutrients within itself due to the size of the soil particles (Agrawal 1991). These characteristics possibly contributed to the low levels of calcium in the soil samples.

5.3 Dissemination to Communities of the Navajo Reservation

There have been multiple suggestions on ways to share this information with the Navajo communities that would benefit from. One suggestion, creating a clear and concise poster with information on juniper ash and posting at local chapter houses across the Navajo reservation. Another suggestion and ultimately the next major step after finishing this research is to publish findings in a journal article that focuses on Food Chemistry and/or Environmental concerns. Finally, it is planned to take this information and present it at the 2017 Navajo Research Conference sponsored by the Navajo Nation Human Research Review Board. The Navajo Research Conference aims to support research that promotes the interest and visions of the Navajo people on the Navajo reservation.

5.4 Future Work

Throughout sample preparation and research, sample protocols have been edited in order to obtain accurate calcium concentration results. Now that there is better understanding on calcium in juniper ash, research can be expanded upon. Focusing research on an in-depth quantification of calcium based on specie of juniper, elevation, type of soil, sample extraction from bottom compared to top of juniper tree, and sampling juniper tree root.

The calcium concentration difference between the LA and TA samples was most likely due to the inefficient ashing of the TA samples. This could be further explored by re-ashing the TA samples to determine if a more efficient ashing process would account for the loss of calcium within the non-ashed portions of the TA samples.

Another component for future work would be implementing a focus on uranium contamination. During the 2016 sample collection, Sample 9 (S-9) was collected on a ridge near an abandon uranium mine. There are thousands of abandoned uranium mines on the Navajo Reservation; wastes from these mines can be solubilized in the ambient environment. In these surrounding areas, juniper trees and other vegetation grow and could possibly take up uranium along with the exchangeable cations normally taken up. Investigating the possible contamination of juniper trees near uranium mines could provide vital information for the Navajo community on locations to avoid in harvesting juniper biomass intended for the blue corn preparation.

Appendix A

Additional Information

Instrument Parameters:

Flame Atomic Absorption Spectroscopy (FAAS)
Instrument Information: Perkin Elmer Atomic Absorption Spectrometer AAnalyst 200

Method	Spectrometer
Element	Ca
Wavelength (nm)	422.67
Slit (mm)	2.7/0.6

Table A.1: Instrumental Parameters used for analysis in juniper/soil samples

Signal	
Type	AA BG
Measurement	Time Average

Table A.2: Signal type used for calcium analysis in juniper/soil samples

Lamp	
Element	Ca, Mg
Wavelength (nm)	422.67
Current (mA)	15
Lamp Type	C HCL

Table A.3: Lamp type used for calcium analysis in juniper/soil samples

Appendix B

Interview with Lillie Pete

Interview with Lillie Pete, Navajo Traditional Consultant (7/28/15)
Cell: (928) 349-2657
Email: lypete@yahoo.com

[Me]: How far back have Navajo people used juniper ash for foods?

[Lille]: From the beginning. It's always been used. It's always used with the blue corn meal. Not with the other corn meal. As far back as I can remember, my grandma used to do a lot of cornmeal cooking. That's when we didn't have many stores around. We always had cornmeal food to eat. We had sheep but it always went hand and hand with the meat. Never meat alone, it was with the cornmeal. All the time. Morning, noon, evening. We had plenty of that growing up. Just like in ceremonial use, like the one I did a while ago, the blue corn roll I made, I get asked to make that roll to use as a payment for medicine man for doing the rain ceremony. Dudley Yazzie from White Cone, AZ said that medicine man used to get paid with. Thought of as a valuable food. That's all he would get. Now they get paid over $1000 to do ceremony. It was effective during the ceremony. D. Yazzie only wants the cornmeal roll. He asks me to make that with other traditional foods for an offering during the ceremony.

[L]: They say there is a certain time of year in the fall when the [juniper] tree- in the spring it would regrow, and toward the fall, it would have little red ends on it. That's the best time to make the ash. When it has a little red tip on it. Stronger taste. Sometimes there's ash that's not strong and you keep adding more and it doesn't seem to be coming out right. When my sister and I made the recipe we used ash that was not strong. I got ash from an old man and the one he brings me, it works well and I can use less, because it's stronger.

[Me]: Would you say that is knowledge that everyone knows about? [Speaking about the season to pick juniper]

[L]: No, some people don't know. I just found out recently. It is good knowledge to pass around.

[Me]: Do you think in the past, everyone knew about this practice?

[L]: Probably. I feel like the Navajos did an injustice to all of us because they knew so much, but they wanted to get paid to tell you what they knew. They held back. Being a former teacher, I feel like we need to pass on that knowledge. The knowledge of our culture has gone down so much. We are on the brink of losing everything. So we need to go out and teach what we know. So [the knowledge] can thrive, it can go on. Just like the puberty ceremony, for a long time, you hardly heard of it. Girls were ashamed to do it. I do give a talk where I tell the young girls, that haven't done it that this is one of the biggest blessing you will have in your life.

That's the biggest kick in these programs, like the suicide prevention program, they are trying to pass on on knowledge so that kids will have pride in themselves and know that they have a foundation. That their people knew a lot. And they used to talk down to us a lot, back in the day, but now you can't do that. It's an injustice to the kids. But there was a time, when they talk about boarding school, they got their mouths washed out for talking in Navajo. A lot of that has really ruined the teaching, but now we're trying to rebuild it.

[Me]: Is that why go from town to town doing this [program]?

[L]: Yes, and I also go from school to school. That's what I do. When I go to the school, I usually cut out the recipe and give it to the students to do. They have to work among themselves and it's always a lot of fun.

I was just telling the people before, when we were eating more cornmeal in the past, there was more healthy people. And now the trading posts and the store, they have really ruined us. Because we're looking for fast foods and when you go into the stores, all you see in junk food. We need to teach our kids to back away from that. Let's go back to eating more traditional foods that was good for us. More grain, that's what we need. That's one of the things we're doing too.

[Me]: So you said you can only use the ash with blue corn meal, what kind of dishes can you make with blue corn meal?

[L]: Well, with my recipes, we have round blue bread. Blue corn tamale, that's wrapped like a tamale. Blue marble, where it is rolled like a ball, pressed down and boiled in water. It's like a dumpling. There's another one called the Drop Bread, you mix blue cornmeal with cold water and slowly drop into boiling water. Take it off the stove and mix it until thick. Then you take a serving spoon and scoop and drop into tray and eat it like bread.

[We look over the recipe that we're handed out during program]

[L]: There's the blue corn meal mush. Kids love this one because you can mix it with some sugar and milk and eat it.

[Me]: So these foods are used during ceremonies?

[L]: Yes, foods like the Blue Corn Meal Pancakes are more for kids. But all of these are used in a Ya Be Chei ceremony. They call it "Feeding the Ya be chei's". They lay out the mats. There are about 18-24 mats. You have to have the wall spinach, the wall banana, and another plant that is like oregano, it's a wild seasoning. There's a cornmeal one. I haven't done it but I will soon. You build a fire and move the ashes away. And you pour the mix into the ash and cover it again. It bakes in there and when you pull it out, it will be a bread. Brush the ash off and pour water on it. You bring it in all in small trays, one of the men who are helping the Ya Be Cheis will pinch a piece from each food in his hands and get water and mix it together to make a mush. Goes

among the mats and brush it on the mouthpiece. This when the traditional foods come in handy. Same goes for the the Rain Ceremony, they do the same thing, they get all the traditional foods. They will also get the foods baked in the ground. It goes by season, the first thing that comes out is the Mariposa Lily, its bud is almost like a little round onion but it's sweet. You pick them out and you dry it mix it with cornmeal. Then there is the wild onion. Then there's the wild carrot, then the wild spinach. Then all the other foods. Early spring is when all these come in. Then you have the wild corn, when it gets ripe, you dry it and grind it. You make it into [Navajo Term] Ath-k'ad, for the puberty ceremony cake. That's the very last one. You line all these up and bless it with water. Food has a big part in the ceremonies.

[Me]: In present day, how often do you think people eat blue corn meal? I want to take this information and show people that [blue cornmeal and juniper ash] have a high source of calcium. I believe that Navajos were healthy when we ate more traditional foods. Do you think people eat enough blue cornmeal or corn-based foods?

[L]: No, because people don't know how to do it. Somewhere, our generation really dropped the ball. Because we didn't teach our kids, and now there's this big gap where everyone hardly knows anything anymore. If we can reteach it, and get back to where we were, we could have a successful impact.

[Interrupted]

[Me]: So for my research, I'm taking samples from different juniper trees on the reservation. I'm separating the branch, the leaves, and the cedar and seeing which has the most calcium.

[L]: You better let me know which part does. People also say that the best trees are the ones with the fewest berries. Those are the good ones.

[Me]: Do you burn the branches with berries on there?

[L]: Yes, sometimes. It's not good to have a lot of [berries] in there. They say it gives it a bitter taste. I put a tub down and a grill on it. And build a fire off the the side, i take a branch and let it catch fire really well and bring it on the grill, so the ash gets in the tub. Don't disturb it. Let the gray ash be collected. You don't want any of the black ash. Then repeat. That's the way my mom told me. I take the tub and let it cool. I sift the ash. Then it can be used.

Appendix C

Juniper/Soil Sample Preparation Protocol

Quantification of Calcium in Juniper and Soil Samples on Navajo Reservation – Sample Preparation

During the Spring Break 2016 Sampling Trip, eleven juniper trees were sampled throughout the Navajo Reservation. At each location of sampling, two juniper samples were extracted from the tree (cedar, branch and berries), along with one soil sample at the base of the tree, three samples in total. Resulting in 33 samples overall.

Main Purpose: Comparing calcium concentration of juniper samples that have been prepared by two methods. Also, relating concentration of calcium in soil to juniper sample from same location.

- Method 1: Standard plant ashing protocol via furnace. Using a temperature-time ramp program of 200ºC→400ºC →600ºC over the course of 12 hrs.
- Method 2: Traditional ashing process of the Navajo people. Igniting the juniper as a whole; letting juniper burn untouched.

*Both methods follow identical plant digestion protocol.

Minor Purpose: Quantifying U^{238} contamination in juniper and soil samples. Specifically in locations near abandoned uranium mines.

Pre-Digestion Juniper Sample Preparation:

There are two plates of juniper for each sample. One plate is prepared by Method 1 and the other plate by Method 2.

Method 1 Prep:

1. Juniper is left to dry for +1 week on paper plate.
2. **Wearing gloves**, juniper sample is broken down by hand into pieces that will fit into coffee grinder.
3. The entirety of the juniper (branch, cedar and berries) on the plate will be finely ground via coffee grinder together. (This is meant to create a homogenous sample of juniper for analysis.)
4. **Thoroughly wash and dry coffee grinder between samples.**
    a. **Do not use mixer mill for this protocol.**
5. Ground samples transferred to new paper plate. Label accordingly.
6. Ground samples are left to dry for +1 week.
7. Transferred to whirlpak. Label accordingly.
8. Fill crucible with ground sample.
9. Samples are ashed via furnace for 12 hrs. See “Box Furnace” Protocol for details.

- **To avoid contamination, must be the same sample during ashing process. Do not ash juniper from different samples together.**

10. Transfer ashed sample to "Pee Cup". Label accordingly.
11. See "Acid Digestion Juniper Sample Protocol".

Method 2 Prep:

1. Juniper is left to dry for +1 week on paper plate.
2. **Wearing gloves**, juniper sample (intact as whole) is transferred to sheet of aluminum foil.
3. Using an open flame (preferably wood fire), ignite the whole juniper branch. Place juniper on aluminum foil.
4. Allow for the juniper sample to uniformly burn to ash. Refrain from touching during this process.
5. Collect ash and transfer to "Pee Cup". Label accordingly.
   - **To avoid contamination, change foil between sample ashing.**
6. See "Acid Digestion Juniper Sample Protocol".

Acid-Digestion Juniper Sample Protocol:

1. 0.025 grams are weighed out and transferred to 50 mL centrifuge tubes. Label accordingly.
2. 5 mL of Trace Metal Grade Nitric Acid (~70%) is pipetted into centrifuge tube.
3. 5 mL of Trace Metal Grade Hydrogen Peroxide (~30%) is pipetted into centrifuge tube.
4. Loosely cap each centrifuge tube.
5. Samples are heated in oven (located in Rm 308) at 80^{o}C for 24 hrs.
   - **Evaporation may occur due to temperature being higher than 80^{o}C or left in oven longer than 24 hrs.**
6. Samples are cooled for a period of time.
7. If evaporation occurred, transfer sample to a 10 mL volumetric flask and bring to volume with Nanopure water. Transfer to new 50 mL centrifuge tube. Label accordingly.
   - **This is done for simpler calculation of concentration after analysis.**
8. For each sample, pipet 0.5 mL (500 uL) in a newly labeled 50 mL centrifuge tube.
   - **This will give you a 100x dilution of sample.**
9. Pipet 1.0 mL of 1000 mg/L Lanthanum Nitrate.
10. Pipet 1.0 mL of 1000 mg/L Cesium Chloride.
11. Bring centrifuge to volume with Nanopure water.
12. Analysis via Flame Atomic Absorption Spectroscopy (FAAS).

Pre-Digestion Soil Sample Preparation Protocol:

1. Collect samples from various locations.
2. Let samples dry for +1 week on paper plates. Label accordingly.
3. Sift through dirt using 1mm sifter.
4. Powder samples using a mixer mill for 2-2.5 mins.
5. Transfer to new whirlpak bag. Label accordingly.
6. See "EPA Method 3051 for acid digestion of soil".

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