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DETERMINING THE PRESENCE OF REMAINS USING THE CHEMICAL COMPOSITION OF BURNED CADAVER ASH

By

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Bachelor of Arts, University of Montana, Missoula, MT, 2012

Thesis

presented in partial fulfillment of the requirements
for the degree of

Master of Arts
in Anthropology, Forensic Anthropology Option

The University of Montana
Missoula, MT

May 2017

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Abstract

Richardson, Megan, M.A., Spring 2017 Anthropology

Determining the Presence of Remains Using the Chemical Composition of Burned Cadaver Ash

Chairperson: Dr. Randall R. Skelton

When humans are reduced to ash by cremation or mass disasters, determining the presence of remains can be very difficult for investigators (Eckert et al., 1988; Dirkmaat, 2002; Schmidt and Symes, 2008; Ubelaker, 2009). This study attempted to identify elements and minerals that were indicative of burned remains in a forensic context. There were two distinct parts of this study, one in the field, and one in the laboratory. Ash was collected from the three outdoor fire pits, two containing pig cadavers, and analyzed for elemental content by CNS and ICP, and for mineral content by XRD. The statistical differences between the cadaver fires and the control fires were calculated by 2-tailed t-tests. The elements phosphorus, sodium, and zinc, and the minerals periclase and hydroxyapatite were both significantly different at the 95% confidence interval and higher in the cadaver fires. In the laboratory, slices of pig limbs were combusted at a range of temperatures in a muffle furnace. The resulting ash was analyzed for carbon, nitrogen, sulfur, and phosphorus. Carbon and nitrogen levels both decreased with increased temperature, with near complete volatilization at 600°C. Sulfur content followed a less consistent pattern, eventually increasing at higher temperatures. Phosphorus increased as the temperature increased and other elements were volatilized. The muffle furnace results emphasize phosphorus as a means of identifying remains. As other elements are volatilized, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), containing phosphorus, also was persistent in the cadaver ash and absent in the control ash.

Acknowledgements

I would like to acknowledge my appreciation of my advisor and chairperson, Dr. Randall R. Skelton, as well as my committee members Dr. Meradeth Snow and Mr. Stephen Baker. I will always be grateful for your support and guidance. I would like to acknowledge and thank the Missoula Fire Sciences Laboratory for partially funding this research. Finally, I would like to thank my family and friends, especially Rachel Fife, Jenny Cavallari, and my husband, Mr. Jeremy Richardson.

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Chapter 1: Introduction

Several activities can lead to human remains being reduced to ash: individuals trying to cover up crimes, airplane crashes, explosions, and bombs (Ubelaker, 2009). Fire scenes can be some of the most difficult scenes to investigate (Eckert et al., 1988; Glassman and Crow, 1996; Schmidt and Symes, 2008). It is challenging to distinguish fragmented, burned bone from other burned items such as glass, wood, and leather (Dirkmaat, 2002; Ubelaker, 2009). It has long been suspected that in severe fire scenarios some human remains are not identified or recovered because they have been entirely consumed due to high temperature incineration.

There are many examples of burned remains in forensic contexts. Sledzik et al. (2009) describe the recovery of human remains from the 9/11 World Trade Center disaster, the nearly simultaneous attack on the Pentagon, and the related airliner crash in Pennsylvania. Many human remains associated with these events were fragmentary and burned. No remains have ever been found for over 1,000 victims of the World Trade Center attack (Hartocollis, 2007). A lawsuit filed by families of 9/11 victims sought to require New York City to sift through ash and debris taken to a landfill from the site of the World Trade Center to look further for human remains (Hartocollis, 2007). Eckert et al. (1988) describe a forensic case where a woman was incinerated in her home. No physical remains were found despite sifting through the ashes.

Sifting through ashes and physical examination does not provide certainty of the absence of human remains. Chemical analysis of the ash can provide a more complete assessment of the site. An important factor is what materials burned at the site and the resulting chemical content remaining in the ash. It can be hard to identify human ash mixed in with everything else that burned. What is needed are chemical species that are in large amounts in mammalian bodies, such as humans, and not in the other objects burned at the site.

In forensic taphonomy, pigs (*Sus scrofa domesticus*) are often used as a proxy for a human body due to their size, diet, and lack of dense body hair (Schoenly et al., 2006). There has been controversy over this in recent years, though mostly relating to differences in decomposition (Keough et al., 2016). Beckett et al. (2011) analyzed combusted femora of 12 species, including humans, and determined hydroxyapatite responds differently to heating depending on species. Their study showed combusted human hydroxyapatite was closest to pigs and dogs. Brooks et al. (2006) was unable to detect a difference between the cremains of humans and dogs. There may be variations in how pigs and humans burn, however, the results should be similar. Using pigs in this research was also easier and cheaper than procuring human cadavers.

For this study, two pig carcasses were burned to ash in outdoor fires along with a control fire to investigate the ability to identify remains by the chemical composition of the ash. This simulates a crime scene or cremation site. The other main burned component was wood, whose ash can be well characterized chemically by a third control fire, where the same amount and type of wood was burned without the pig. My hypothesis was that the chemical composition of the ash would be significantly higher in phosphorus, nitrogen, and sulfur for the pig carcass fires vs. the control fire, as these are important chemical components of pig and human tissue, and these elements were higher in my preliminary study (Richardson, 2013). I also measured the concentration of other elements in the ash of both the pig and control fires to look for indicator chemical species from the pig carcass ash such as calcium, magnesium, and trace metals.

In the second part of this study pig limbs, cut into slices, were combusted in a muffle furnace, at a range of possible fire temperatures. In such a controlled lab environment, the effect of combustion temperature on ash chemical composition could be examined. The hypothesis was that volatilization of nitrogen and sulfur will increase at higher temperatures, and therefore their relative amounts would decrease in the ash. With the loss of nitrogen and sulfur, as well as carbon, at higher burn temperatures, residual phosphorus and other element concentrations should increase.

Chapter 2: Literature Review

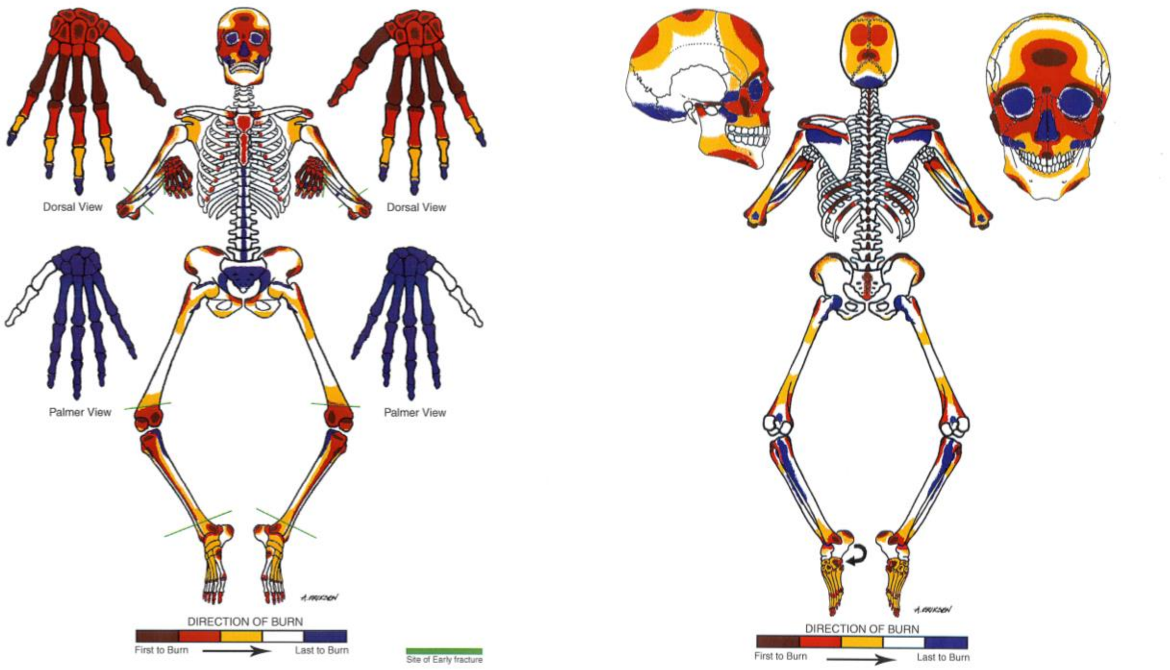
To study the chemical composition of remains left after a human body is combusted, one must look at what the human body and other natural fuels such as wood are composed of, as well as how bodies burn, both physically and chemically. Previous research that can provide insight and a starting point for research into this question includes a number of separate topics.

Consumption and Reduction to Ash of Human Remains by Fire

According to Bass (1984), the average building fire cannot completely consume a body since fragments are left which can still be identified by osteologists. Average house fires burn at temperatures between 750-850^{o}C (Piga et al., 2009). A typical house fire lacks the concentration of fuels to produce higher temperatures. However, as previously mentioned, bodies can be reduced to ash, but it requires a high temperature event or an intentional act such as cremation (Eckert et al., 1988; Ubelaker, 2009). Simple funeral pyres can exceed 1000°C (Brown and Brown, 2011). Bohnert et al. (1998) observed and documented the commercial cremation of fifteen human individuals in a modern cremation oven. After cremation, but prior to pulverizing, calcined bone fragments from all the bodies could still be accurately identified. Fire can alter remains and make the circumstances of the fire scenario difficult to interpret (Eckert et al., 1988). Hands and feet may be exposed to more heat, therefore burning more completely than other areas, making them appear to have been missing prior to burning. Skin can split resulting in incorrect observations of sharp force trauma. Skin splitting can expose subcutaneous fat, and when the fat is absorbed into a substrate, such as furniture or flooring, the fat can act as a fuel producing an in-situ cooking fire (DeHaan, 2012). DeHaan (2012) found subcutaneous fat to be the most significant fuel in a human body which can accelerate and increase combustion.

Normal Burn Pattern of Cadavers

When a body is subjected to fire, there is a known overall pattern of burning that occurs, from what body parts burn first to what parts are the last to burn (Symes et al., 2008). Figures 1 and 2 show this pattern. The uniform burn pattern only applies to whole, fresh bodies (Symes et al., 2008; Symes et al., 2014). Muscles, when heated, contract. The larger muscles will overpower the smaller muscles, resulting in the pugilistic position, or curling up, of burned extremities. Restraining, dismembering, and defleshing all change how a body responds to heat and fire, and can aid in determining how a body was treated postmortem. Decomposing can cause the breakdown of muscle tissues, which prevents the pugilistic form.



Figures 1a and 1b. Normal pattern of human burning on a fresh cadaver from Symes et al., 2008.

Glassman and Crow (1996) used this pattern to develop a standardization of description for burned bodies for on-scene professionals. The Crow-Glassman Scale uses five levels based on visual inspection of burn severity:

- Level one: bodies are recognizable with hair singed and skin blistered.
- Level two: bodies are charred, with the possible loss of hands, feet, genitals, and/or ears.
- Level three: bodies are not recognizable, the head is articulated, but large areas missing from the legs or arms.
- Level four: bodies do not have a head, however parts of the legs or arms may be attached to the body.
- Level five: individuals have fragmentary and incomplete remains with minimal or no soft tissue.

Basic Stages of Burning

Bones are protected from fire by the soft tissues as well as by other bones at the joints (Symes et al., 2014). When the soft tissue is destroyed or removed, bone goes through chemical, size, shape, and color changes. These relate to the four stages of changes in bone subjected to heat as described by Thompson (2004). These stages are dehydration, decomposition, inversion, and fusion. There is overlapping of these stages at certain temperatures, and these stages are connected to one another. Chemically, bones carbonize and, if bones continue to be exposed to fire, calcify (Symes et al., 2014). Carbonization is the process of releasing elements other than carbon from organic compounds through heat. The elements either are volatilized or form other bonds and carbon is left. During calcination, the carbon bonds with oxygen to form carbon monoxide or carbon dioxide, leaving only the hydroxyapatite making up the shape of the bone.

Color Changes of Bone Due to Heating

Carbonization and calcination causes color changes of the residual bone. Bones go through a transition from the natural color of bone to black when carbonized and to white when completely calcined with several different shades between the stages (Shipman, 1984; Mays, 1988; Symes et al., 2014). Some studies have focused on determining the temperature of the fire from the color of the bones, but there is not an agreement (Shipman et al., 1984; Mays, 1988). Shipman, et al. (1984) found five stages of color change, using a muffle furnace to burn cleaned mandibles and vertebrae of goats and sheep. The muffle furnace was set at temperatures between 185^{o}C and 940^{o}C. They found the colors overlapped and were not sufficient to determine the temperature of the heating. Mays (1998) heated animal bones in a muffle furnace to temperatures between 185^{o}C and 1200^{o}C, following the methods outlined in Shipman et al. (1984). He found six stages of color change, including no further change after 645^{o}C. There were inconsistencies between his observations and the observations by Shipman et al. (1984). This suggests that using color to infer temperature is subjective and not as accurate as investigators would require.

Bone Shrinkage

Bones shrink when subjected to heat and fire. The amount of shrinkage that occurs is correlated to bone density, as well as the temperature and duration of the heat (Van Vark, 1970; Eckert et al., 1988). Van Vark (1970) found bones shrink between one and twenty-five percent, depending on bone density and combustion temperature. Shrinkage was most significant between 700^{o}C and 900^{o}C. Shipman et al. (1984) found a statistically significant correlation between combustion temperature and shrinkage of bone. They observed bone shrinkage as early as 185^{o}C, the lowest combustion temperature studied, and found the maximum shrinkage was fifteen percent.

Age-Related Changes in Bone that Affect Burning

Waterhouse (2013) researched the age differences in fragmentation due to burning of bone. Using *S. scrofa* limbs from piglets and 8-10 month old pigs in outdoor fires, Waterhouse found that piglet limbs had complete bones or epiphyses, as opposed to fragmented bones of the older pigs.

Survivability of mtDNA After Burning

The ability to obtain reproducible mtDNA from burned remains was examined by Harbeck et al. (2011). Cattle tibiae were cleaned of fat, cut into sections, and subjected to heating in a muffle furnace. When heated for an hour and a half, mtDNA was found to be identifiable until 700^{o}C. When heated for four hours, mtDNA was only obtained until 600^{o}C. No mtDNA was identified after 700^{o}C.

Survivability of Stable Isotopes after Burning

The effect of heat on stable isotopes was also investigated by Harbeck et al. (2011) using the same cattle tibiae. They found that values of light elemental isotopes δ15N, δ13C, and δ18O all dramatically decrease at temperatures above 200^{o}C. Munro et al. (2007) studied the effect of heat on the δ18O isotope using cleaned deer leg bones, and found decreasing values starting at 300^{o}C.The heavier isotopes decrease at increased temperatures causing the decrease in δ ratios. The δ87Sr did not change, even at the maximum temperature examined, 1000^{o}C (Harbeck et al., 2011).

Changes from Burning in the Crystalline Structure of Bone

Shipman et al. (1984) used a scanning electron microscope to analyze the changes in microscopic morphology of dentin, enamel, and bone tissues of goats and sheep after burning. The study found five very similar stages of variation in morphology related to combustion temperature in each type of tissue. Magnifications below 1000X were insufficient to observe changes. Castillo et al. (2013) observed the microscopic changes of the bone matrix of the ilium resulting from combustion temperatures between 100°C and 1100°C. At each increase of 100°C, differences were observed, but only four distinct stages were found. Collagen deformation occurred at 100-300°C, crystal formations appeared glass-like at 400-600°C. At 700-800°C crystals were not homogenous and appeared rounded and cubical, and surfaces were granular over 900°C.

While hydroxyapatite crystals get larger with an increase in combustion temperature, the chemical content species remain intact (Shipman et al., 1984). Piga et al. (2008; 2009) and Hiller et al. (2003) studied the effect of temperature and heat duration on the growth of hydroxyapatite crystals. Piga et al. (2008) heated human femoral fragments from Spain in a muffle furnace with temperatures from 200°C to 1000°C for zero, eighteen, and sixty minutes. X-ray diffraction was utilized to analyze the hydroxyapatite crystal growth. The study found the correlation between hydroxyapatite crystal size and temperature. Piga et al. (2009) used X-ray diffraction to analyze modern femoral fragments and teeth heated 200°C to 1000°C for zero, eighteen, thirty-six, and sixty minutes in an air (not oxygenated) muffle furnace. The study found a sigmoid function relationship between heating temperature and duration of heating for both bones and teeth. They found that while the teeth have a larger untreated hydroxyapatite crystal size than femora, the hydroxyapatite crystal size is larger in bones than teeth when exposed to heat greater than 900°C. Hiller et al. (2003) used X-ray scattering to analyze the increase in hydroxyapatite crystals thickness when exposed to heat. Fresh, de-fleshed, cortical portions of bones from sheep were heated to 500°C, 700°C, and 900°C in a muffle furnace for fifteen and forty-five minutes. Minimal changes were observed for the samples heated at 500°C for fifteen minutes. The thickness of the hydroxyapatite crystals increased with temperature. Bergslien et al. (2008) were able to use the hydroxyapatite peaks, with X-ray diffraction (XRD), to identify cremains from common fillers, such as wood ash, charcoal, and plaster of Paris.

Average Elemental Content in Human Bodies

The human body is 96.2% by weight oxygen, carbon, hydrogen, and nitrogen (Shyamala, 2009). Calcium makes up 1.5%; phosphorus 1%; potassium 0.4%; sodium 0.2%; magnesium 0.1%; and sulfur 0.04%. There are several elements that are found in the human body in concentrations less than 0.01% including chromium, cobalt, copper, iron, manganese, vanadium, and zinc.

Causes of Elemental Differences between Individuals

Many elements can substitute for parts of hydroxyapatite (typically $Ca_{10}(PO_4)_6(OH)_2$) (Bergslien et al., 2008). The OH- can be replace by F-; the Ca+ can be replaced by Na+, K+, Fe2+, Zn2+, Sr2+, Mg2+, Cd2+, Ba2+, Mn2+ and Pb2+; and the P5+ can be replaced by As5+, V5+, Si4+, S6+, and Sb5+. These substitutions depend on the local water, soil, and dust, the proximity to and abundance of emissions from automobiles, power plants, and factories, as well as any foreign materials in the body such as bullets (Brooks et al., 2006; Bergslien et al., 2008).

Elemental Volatilization Due to Heating

Different elements volatilize at different temperatures (Knoepp et al., 2005). Nitrogen starts to volatilize at 200°C, phosphorus at 774°C, magnesium at 1107°C, and calcium at 1484°C.

The hotter the fire, and the longer the fire burns, will change the relative amounts of elements present in the ash.

Elemental Analysis Studies

Few studies have been conducted on the elemental components of burned remains. Brooks et al. (2006) used statistical analysis of Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) data to find a discriminate function to determine the authenticity of cremains. Twenty-one elements were analyzed from samples including known commercial human cremations, questionable human cremations, dog cremains, wood ash, concrete, and mixtures of different ratios of cremains with concrete. Seven elements were found to be significant: antimony, boron, lithium, manganese, strontium, thallium, and vanadium. Brooks et al. were able to classify the sample as cremains if it contained 90% or more of cremains. If a sample contained less than 50% cremains, it was classified with concrete, and if it was between 50% and 90% remains, the sample was not classifiable. X-ray fluorescence (XRF) was used by Bergslien et al. (2008) to analyze the elements in cremated bone and enamel, as well as commonly used fillers for cremains.

Grupe and Hummel (1991) burned defleshed cortical bone from pigs in an oxygenated muffle furnace. They were able to estimate original, unburnt, amounts of calcium, phosphorus, strontium, barium, and lead through regression analysis.

The body of research mentioned in this review has assisted in the design and interpretation of the results of this multi-faceted project.

Chapter 3: Materials and Methods

Field Materials and Methods

Outdoor Fires

Three experimental fires were performed: two with fresh pig cadavers and one control fire with wood only. The study was conducted on private property in Arkansaw, WI, in July, 2013. The pigs were purchased from a local pig farm, and freshly killed just prior to burning. They were burned using local wood species (oak, birch, poplar, and bass). Excelsior (finely shredded wood) and Coleman fuel were added ignition aids. All of the wood, excelsior, and pigs used were weighed using a spring scale in the field. The Coleman fuel was measured using a volumetric flask. Seven large pieces of firewood formed a stacked lattice structure (see figure 3). Smaller logs and the excelsior were placed in the center of the firewood lattice. The pig carcass was placed at the top of wood arrangement just prior to ignition. The measured Coleman fuel was poured over the entire fuel structure, and ignited. Smoke emissions samples in canisters were taken during all phases of each fire.

The fires were built five meters apart. After ignition, firewood was added to maintain a fairly constant rate of combustion until the pig carcasses were consumed and reduced to ash.

The temperature and humidity on burn day was obtained from the National Weather Service (weather.gov). Photographs of the fires were taken using a Sony Cyber-shot DSC-HX20V camera as well as an iPhone. The temperatures of the pig carcass fires were measured using thermocouples and a data logger. While the plan was to use the thermocouples for all fires, after the first fire, the ends were brittle and crumbled. The damaged ends of the thermocouples were cut off, with the undamaged wires tightly twisted together for the second fire. After the second fire, the usable section of wires was too short for continued use with the control fire. The Crow-Glassman scale (CGS) was utilized for standard description scoring of burn damage (Glassman and Crow, 1996).

Figure 2. The lattice structure of the fires, with thermocouple wires visible.

The first pig carcass fire SS1 (*Sus scrofa domesticus*1) took place on July 29. The carcass weight was 40kg. Thermocouples were placed around the pig, with three on the ground, secured with fencing wire; three touching the carcass; and two in the air above the carcass. SS1 was ignited at 11:18, (00:00). The carcass was scored a CGS1 (Crow-Glassman Scale 1) at 00:05, with hair singed and blistering on the abdomen. By 00:14, the carcass exhibited pugilistic form, with extremities flexed (figure 3). At 00:36, with an internal temperature of 82.2°C, there was a complete tibia fracture, and both a foot and an ear were missing, scoring a CGS2. The back of the skull was missing and the front metacarpals were exposed at 01:03. At 12:55, the carcass was unrecognizable and no longer had limbs, which would be scored at a CGS4, with an internal probe temp of 200°C. At 02:37, the pig carcass was scored as a CGS5, since there was only a 15cm^2 section of meat from the shoulder area, and the rest of the remains consisted of bone fragments. The bone fragments were bluish white at 03:54, with mostly epiphyses visible. The data logger for temperature was stopped at 05:18. The coals were allowed to extinguish on their own.

Figure 3. Pig carcass from SS1 at 11:35, exhibiting pugilistic form.

The second pig carcass fire (SS2) carcass weighed 52kg and was killed at 17:48 on the same day as SS1. The repaired thermocouples were used, with thermocouples one, two, and three on the ground; five and six in the air on wire, and seven in the fire. The fire was ignited at 18:04 (00:00). Hair singed and abdomen blistered on ignition, scoring a CGS1. By 00:03, the legs started flexing. The internal temperature was 40^{o}C at 00:06. By 00:41, blood was bubbling from the snout, the intestines had ruptured, and the right foot was missing, scoring a CGS2. The internal probe temperature was 49^{o}C at that time. SS2 was not placed as centrally on the fire lattice and was exposed to uneven fire, causing the rear end of the pig to combust more quickly than the front of the pig. At 01:01, the pig fell out of the fire, and was placed more centrally over the fire site. At 01:25, the pig was pushed further into the fire, and the thermocouple wires were no longer under the pig. An ember temperature of 805^{o}C was recorded by probe at 03:22. By 04:29, all flesh had been burned, leaving small fragments of bone, scoring a CGS5. The data logger was stopped at 05:53, and the fire was allowed to extinguish. Both SS1 and SS2 were still radiating heat the following morning.

The control fire (CF) was ignited at 11:52, (00:00) on July 30. It had rained the night before, making the wood damp and the fire smokier than the 2 pig carcass fires. This was very much a typical outside fire. Due to damaged thermocouples, temperatures were measured with only the probe. The ember temperature was 702^{o}C at 01:06, and 782^{o}C at 01:54. Like the carcass fires, the control fire was allowed to extinguish on its own.

Table 1. Summary of Fire Conditions Data

FIRE	DATE	AMBIENT TEMP	HUMIDITY	WIND	WOOD	SUBJECT SIZE	IGNITION TIME	TIME BURNED
SS1	7/29/13	22°C	76%	1 mph	336 kg	44 kg	11:18	5.3 hours
SS2	7/29/13	25°C	54%	6 mph	285 kg	52 kg	18:04	5.9 hours
CF	7/30/13	19°C	79%	0 mph	114 kg		11:52	2.2 hours

Ash Collection at Burn Sites

The burn sites were sampled two days after the fires had burned to ash, in order to allow the ash to cool. A mixture of char and calcined (white) bone fragments and epiphyses were visible at SS1 and SS2. It would be clear after only two days and no further intentional disturbance of the fire sites that bones had been burned in a fire. A grid was created over each fire pit using nylon twine and plastic garden stakes, as shown in Figure 4. The rows of the grid were labeled with numbers and the columns were labeled with letters. Ash was collected from each grid square using a plastic spoon, which was cleaned between sampling of each fire. A total of 12-15 ash samples were collected at each burn site. Each ash sample was labeled with the corresponding alphanumeric code and placed into a separate paper bag to inhibit mold. The paper bag would not contaminate the ash samples.

Figure 4. Ash bed from SS1 at 16:06, showing bone fragments, epiphyses, char, and thermocouples.

Figure 5. Ash bed from SS1, with grid and collection labels.

Laboratory Processing of Ash Samples

At the Missoula Fire Sciences laboratory each ash sample was sieved with a 1.2mm screen to remove larger bone or wood pieces. Larger bone fragments weighing 0.41kg and 0.73kg were removed from SS1 and SS2 respectfully. Samples were then pulverized using a mortar and pestle to create more homogenous and reproducible samples, and stored in a glass vial labeled with the corresponding alphanumeric code. The sieve, as well as the mortar and pestle, were vacuumed and dusted with Kimwipes to remove residual ash between samples.

Method for Muffle Furnace Combustion of Pig Tissue

In the second part of the study, to test the effect of temperature on the loss of element content of the ash through volatilization, samples were combusted at temperatures from 400^{o} to 1000^{o}C in 100^{o} increments. One freshly-slaughtered pig was butchered at H & H meats and the limbs were cut into sections. The limb sections were stored in a freezer until processing and testing. The limb sections were sliced, while still frozen, into pieces weighing approximately 40 grams using a PVC saw. Each section contained skin, fat, muscle, and bone to simulate, as closely as possible, a human body part, since the bone would still be partially protected from combustion by soft tissues. The sample was placed into a 250mL pre- weighed porcelain crucible and allowed to thaw overnight. The thawed sample in the crucible was weighed and placed into a muffle furnace (Blue Electric Company Lab-Heat muffle furnace fitted with an Omega CN142 digital controller) at the Missoula Fire Sciences Laboratory. The muffle furnace was near room temperature at the start of each run and ramped up to the set temperature from 400^{o}C to 1000^{o}C. Each sample was combusted for 3 hours at the designated temperature and then immediately removed to allow for quicker cooling. As soon as the crucible was cool enough to handle, it was weighed again (to minimize absorbing ambient moisture) to determine post-combustion (ash) weight. Before re-use after a sample run, crucibles were cleaned by combustion of the residue in the muffle furnace, at 1000^{o}C. After a complete set of samples were burned, duplicates were run at each temperature.

Figure 6. Sample T-4 prior to burning in muffle furnace.

Figure 7. Sample T-4 after burning in muffle furnace at 700°C.

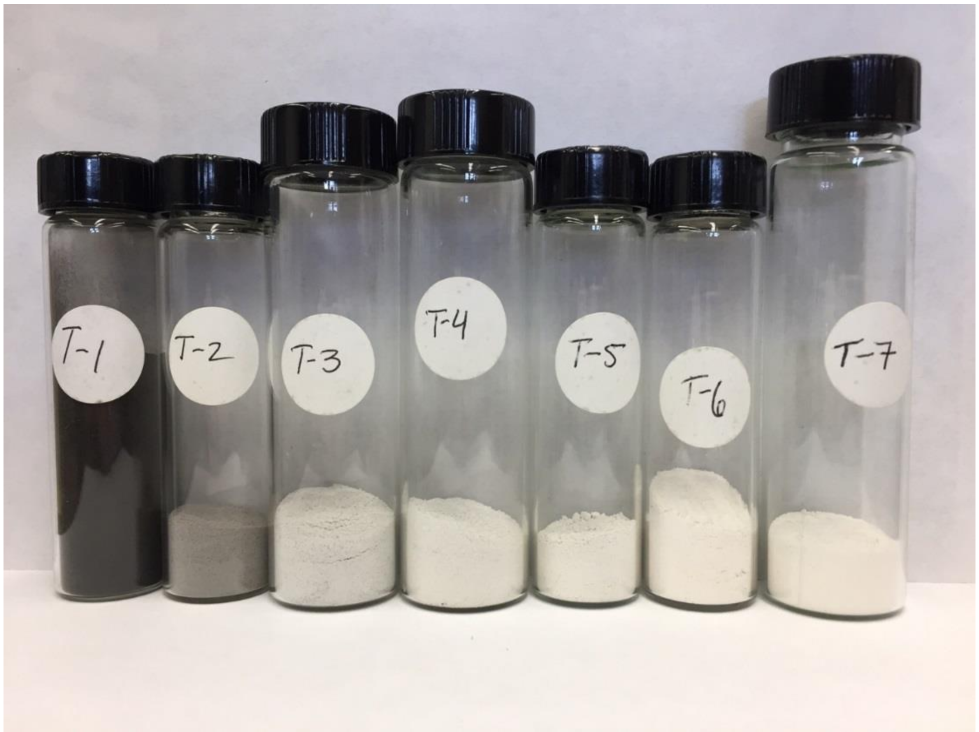


Figure 8. Samples T-1 – T-7, pulverized in labeled glass vials.

A database was created using an alphanumeric system to document the sample origin and description, temperature of combustion, pre- and post-weights. After combustion the samples were photographed and the thoroughness of combustion was recorded. Each sample was removed from the crucible, pulverized using a mortar and pestle, sieved, and stored in a glass vial with the corresponding alphanumerical code, for later chemical analysis.

All data from outdoor fires was entered into spreadsheets using Microsoft Excel 2013. Graphs were created when appropriate. Data was then imported into SPSS for statistical analysis. Several T-tests were conducted. Each method of analysis was tested comparing the first and second pig fires, as well as fire with a pig and fire without a pig. Results are shown for elements and minerals at both the 90% and 95% confidence intervals.

Chapter 4: Chemistry Methods

The analysis methods used for this study were selected on the basis of being standard methods for the types of chemical analysis required for this project. Carbon, nitrogen and sulfur are key elements of tissue composition and therefore significant for this study. A CNS analyzer was available at the Missoula Fire Lab for use to analyze unburned and ash samples.

Inductively coupled plasma optical emission spectroscopy (ICP-OES) was selected as an efficient and affordable method for analysis of 19 separate elements that may occur in tissue.

X-ray diffraction (XRD) analyzed the mineral content of the same eight samples from each field fire. Knowing that hydroxyapatite remains after heating bone, it was important to look at this and other mineral content for significant differences between cadaver and control fires.

Phosphorus is another key element to measure, as a component of tissue and bone as well as present in soil. The molybdenum blue phosphorus colorimetric test was utilized for the lab muffle furnace samples, which did not undergo ICP analysis.

CNS Content of Field and Muffle Furnace Ash

The total carbon, nitrogen, and sulfur was analyzed with a LECO CNS analyzer, at the Missoula Fire Sciences Laboratory. The CN analyzer combusts samples and standards at 950^{o}C in a pure oxygen environment. Carbon is oxidized to carbon dioxide and measured in an IR cell. Nitrogen is converted to nitrogen dioxide, then reduced to nitrogen gas and measured by a thermo-conductivity detector (TCD). LECO EDTA and soil standards were used for the carbon nitrogen calibration. Before weighing the samples were put in a drying oven at 58 degrees Celsius for a minimum of an hour to remove any moisture in order to achieve an accurate mass measurement. During the analysis gloves were worn to prevent contamination and safety glasses and a lab coat were worn for protection. For the sample analysis, a 0.10g sample of pulverized ash was weighed into a tin foil cup on a balance. Only 0.07g of sample was used for the muffle furnace samples due to the small amount of ash remaining. The sample was compressed into a pill shape by hand and placed into the sampling carousel per standard operating procedure for the LECO. Each sample was run in duplicate. Each analysis took about four minutes and subsequent samples are dropped automatically into the analyzer from the carousel. The instrument parameters were monitored and set with LECO software, and a database of CN results was produced by the program. The results were subsequently transferred to an EXCEL spreadsheet for analysis.

Sulfur was analyzed in a separate module that combusts the sample at 1450°C, converting sulfur to sulfur dioxide which is measured by IR spectroscopy. Another LECO software program controls the instrument, and collects data. A LECO soil standard of known sulfur content was used for calibration of the instrument. A sample of 0.1 gram of pulverized ash was weighed into LECO ceramic boats. Per LECO standard method (LECO, 2010) 0.1 gram of LECO Com-Cat was placed on top of the sample in the ceramic boat and they were loaded manually one at a time into the sulfur furnace using a LECO loading rod. After approximately 4 minutes, when the sulfur analysis was complete, the ceramic boat was removed and the next sample was loaded into the furnace. Duplicates were prepared and run for each sample.

Molybdenum Blue Phosphorus Analysis of Muffle Furnace Ash

Phosphorus from the muffle furnace burns was analyzed by Stephen Baker at the Missoula Fire Sciences Laboratory using the colorimetric molybdenum blue method (Grimshaw et al. 1989). Phosphorus calibration standards were prepared at 2ppm, 5ppm, 10ppm, and 20ppm. The ash samples were digested using the technique described below for ICP analysis. Fresh ammonium molybdate-sulfuric acid reagent and stannous chloride reagent were prepared prior to analysis. One blank, the standards, and the digested ash samples were pipetted into 50mL volumetric flasks. Deionized water, and 2mL each ammonium molybdate and stannous chloride were added. The sample and standard flasks were brought to volume and allowed to set for 30 minutes. The absorbance of each liquid was measured at 700nm using a Milton Roy Spectronic 301 spectrophotometer. The spectrophotometer was zeroed before each sample measurement using the prepared blank. A calibration curve from the standards was used to calculate the sample P concentrations.

ICP Analysis

Ash samples from the field study were analyzed for arsenic, barium, calcium, cadmium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, potassium, phosphorus, sodium, sulfur, vanadium, and zinc by simultaneous inductively coupled plasma spectroscopy (ICP) at the University of Idaho. Subsamples of ash were shipped in labeled glass vials. Simultaneous ICP is a method where a liquid sample is nebulized into an argon plasma torch, a spectrum of element specific emission lines are produced, and the emitted light is measured in an array of charge-coupled devices (CCD's) positioned for the characteristic wavelength of each element analyzed. The amount of emitted light collected at each CCD is ratioed to calibration standard emissions to determine the element concentration. Eight samples were selected from each fire, the center grid squares, as well as randomly selected perimeter grid squares, in order to achieve representative results. To prepare the ash for ICP analysis a 0.05 gram sample was dissolved in 5ml of ultra- pure HCl in a cleaned crucible and heated on a hotplate to boiling. After cooling the liquid was transferred to 50ml volumetric flask and brought to volume with high purity distilled water. A blank sample was also produced using the same technique. The liquid samples were transferred to clean Nalgene bottles for ICP analysis.

XRD Analysis of Field Burn Ash

XRD analysis is based on the interaction of monochromatic X-rays and a crystalline sample. The X-rays are generated by a cathode ray tube, processed, and directed toward the sample. The characteristic X-ray diffraction pattern generated in a typical XRD analysis provides a unique “fingerprint” of the crystals present in the sample. When interpreted by comparison with standard reference patterns and measurements, this fingerprint allows identification of the crystalline form in the sample (Bergslien, 2008). The ash samples from the three field fires were analyzed using X-ray diffraction (XRD) for crystalline structures. In preparation for the XRD, the samples were sieved with a 0.246mm screen, with the remaining material being pulverized again and sieved. The X’Pert Pro PANalytical XRD was located in the geosciences laboratory at the University of Montana. The XRD used a nickel filter with a copper X-ray tube. The tension was 45kV and the current was 40mA. The equipment was set to three absolute 5.5 minute scans from 2-70^{o}. A calibration blank was run before each set of samples. A magazine of fifteen back-loaded samples was loaded at a time for analysis. Integration of peaks was performed using X’Pert HighScore Plus. A subset of compounds was created using known minerals in wood (Ragland et al., 1991) and compounds I found to facilitate more efficient integrating. Minerals were converted from pattern to phase, and then a Rietveld refinement was run three times. The percentages from each Rietveld refinement were entered into a Microsoft Excel spreadsheet. Averages were calculated for each sample and then for each fire.

Chapter 5: Results

The following graphs and tables show the results of the two components of this study – the field cadaver fires and the laboratory muffle furnace experiment. The field cadaver fire data includes thermocouple data that measure the combustion temperatures, which can then be compared to the laboratory muffle furnace burn temperatures. The results for CNS, ICP, and phosphorus indicate the significant elements for identification of cadaver residue. The XRD results show the similarities and differences between crystalline structures in the field fire samples as related to bone and other human remains..

Results for Field Fires

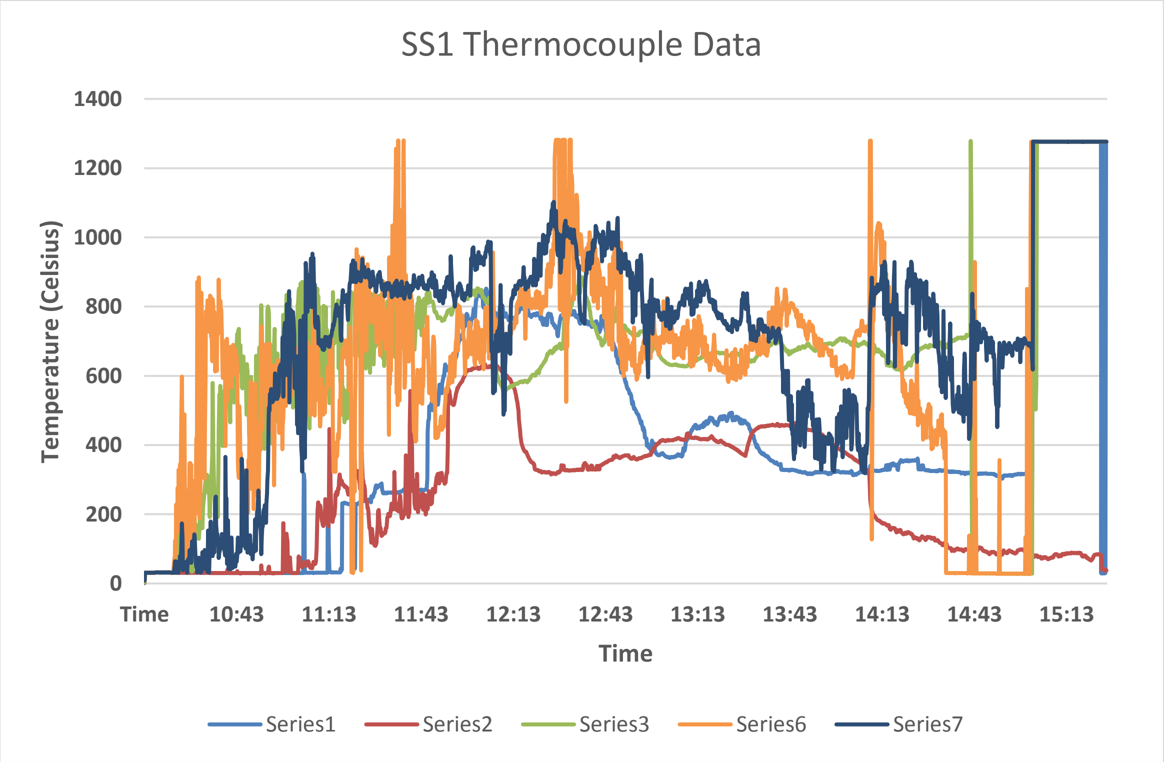


Figure 9. Graph of SS1 Temperatures (in ^{o}C) Measured by Thermocouples.

Figure 9 shows the temperatures obtained from five thermocouples used in SS1. The points where the temperature is over 1200^{o}C, mostly shown in Series6, is likely when the thermocouples where not in contact with the fuels and were reading flame temperature to the maximum allowed by the thermocouples. Thermocouples labeled Series4, Series5, and Series8 were removed from the graph data due to being open, meaning the weld was broken, and they were not measuring temperature. SS1 was mainly between 600^{o}C and 900^{o}C, with a few peaks approaching 1100^{o}C.

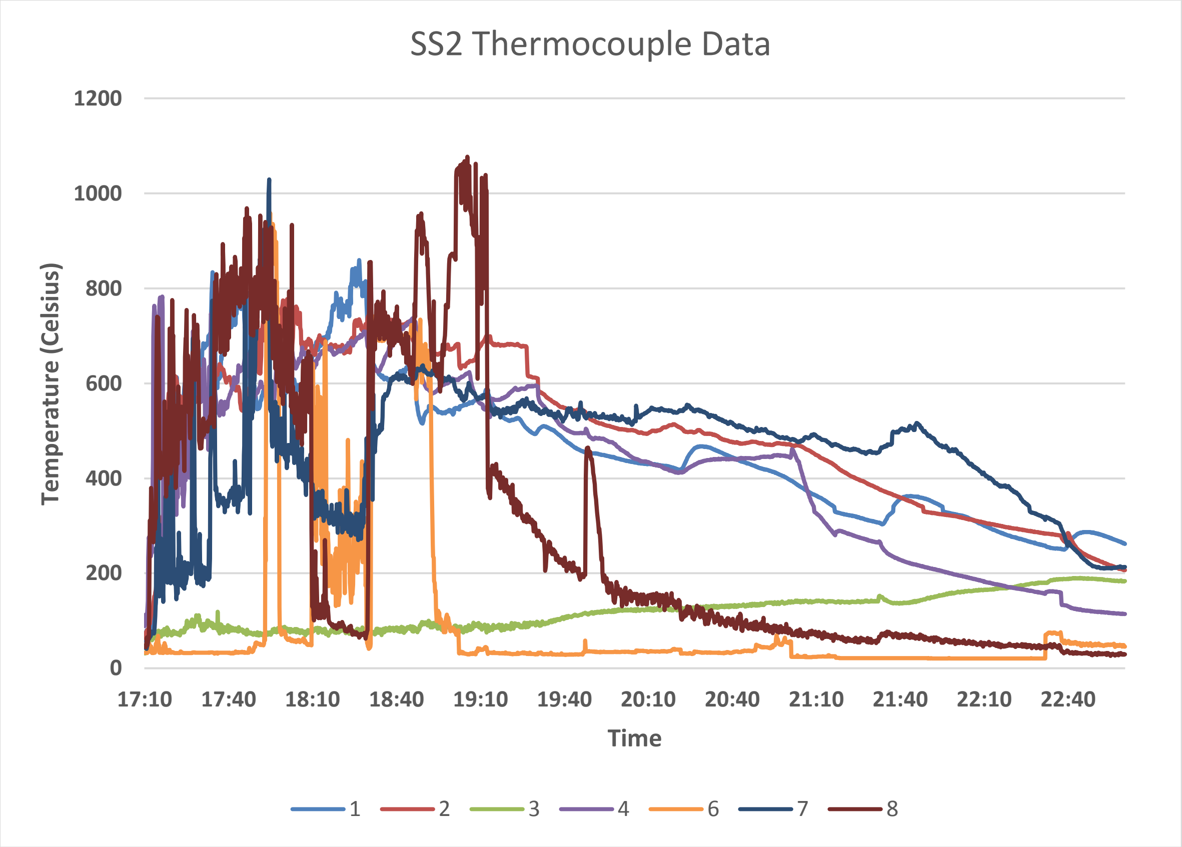


Figure 10. Graph of SS2 Temperatures (in ^{o}C) Measured by Thermocouples.

The temperatures measured from SS2 are found in figure 10. The start of the fire shows a similar temperature pattern to SS1. When the pig was moved at 19:26, and the thermocouple wires were no longer under the pig, is shown well on this graph, as the temperatures drop significantly around that time. The Thermocouple labeled Series5 was removed from the graph data for being open and not measuring.

CNS

The CNS results for the pig cadaver fire ash are presented in Table 1. The average nitrogen content for all three fires was similar, 0.18 to 0.22 percent. Carbon content of SS1 was 5.29 percent, with SS2 and control very similar at 6.82 and 6.78 percent. Sulfur which is an element that one might predict to be higher for the cadaver fires vs. control, was lower for SS1 than the control and higher for SS2.

Fire	**n**	**Nitrogen**	**std.dev.**	**Carbon**	**std.dev.**	**Sulfur**	**std.dev.**
SS1	12	0.20	0.03	5.29	0.68	0.29	0.09
SS2	15	0.22	0.08	6.82	1.67	0.42	0.20
CF	12	0.18	0.01	6.87	0.66	0.36	0.08
p value		0.085		0.120		0.909	
Level of Significance		90%		None		None	

Table 2. Percent Nitrogen, Carbon, and Sulfur content of ash from pig cadaver fires and control. The results are means from the grid samples collected from each ash bed. The p value is for the average of the 2 cadaver fires vs. the control fire ash content.

ICP Results for Field Fires

The ICP results are reported in two tables: macro elements with concentrations generally above 5000 ppm, and micro- elements, which consists mostly of trace metals. The macro elements calcium (Ca), potassium (K), and magnesium (Mg) were presented in high concentrations for all three ash beds, with calcium in the range of 18.5 to 20.5 percent, or 185000 to 205000 ppm (Table 3). Calcium and potassium were almost identical in fire 2 to the control. The two elements which were noticeably different in concentration between the cadaver fire ash and the control were phosphorus (P) and sodium (Na). Both P and Na were much lower in the control vs. the two treatment fires. There were thirteen micro elements analyzed for, of these nine had measurable concentrations above the limit of detection of the ICP for all samples and are reported in Table 4. Arsenic (As), molybdenum (Mo) and Cadmium (Cd) were below the limit of detection for all samples with the exception of one sample; A-1 from fire SS1 with a cadmium concentration of 1.1 ppm. Lead (Pb) was only found in five samples from the pig cadaver fire ash and the concentration varied greatly, from 28-1200 ppm. The nine remaining micro element concentrations are shown in Table 4. For most of the elements, the elemental concentration is higher in the control fire ash than the 2 pig cadaver fires. Iron (Fe) concentrations were noticeably high in all ash samples, possibly indicating a high metal background at the site, possibly from the soil or the wood. Zinc (Zn) was the only trace element with a lower concentration in the control vs. the pig cadaver fire ash. Table 3 and table 4 show the p value and level of significance from 2-tailed t-tests comparing the combined pig fire samples with the control fire for the macro and micro elements respectfully. Table 5 shows the p value and level of significance comparing the pig fires to each other with a 2-tailed t-test. The entire set of ICP results can be found in Appendices B and C.

Table 3. Mean macro-element concentrations (in ppm) of 2 pig cadaver fires ash and control fire ash from ICP analysis.

Fire	Ca	K	Mg	Na	P	S
SS1	205000	66250	18250	8700	23700	2825
SS2	185000	79875	19250	11637	29125	4937
CF	186250	79250	21125	324	11487	4300
p value	0.458	0.460	0.098	0.001	0.002	0.554
Level of Significance	None	None	90%	95%	95%	None

Table 4. Mean micro-element concentrations (in ppm) of 2 pig cadaver fires ash and control fire ash from ICP analysis.

Fire	Ba	Co	Cr	Cu	Fe	Mn	Ni	V	Zn
SS1	757	2.03	6.92	68.8	4750	1147	14.1	10.2	223
SS2	549	1.49	4.92	75.5	2712	746	10.5	6.0	418
CF	680	2.83	9.08	88.8	5850	1147	14.6	14.4	186
p value	0.656	0.001	0.000	0.058	0.012	0.128	0.173	0.001	0.024
Level of Significance	None	95%	95%	90%	95%	None	None	95%	95%

Table 5. T-Test (2-tailed) results comparing SS1 to SS2, including p value and level of significance.

Element	Analysis Method	p Value	Level of Significance
Nitrogen	CNS	0.380	None
Carbon	CNS	0.004	95%
Sulfur	CNS	0.044	95%
Calcium	ICP	0.109	None
Potassium	ICP	0.072	90%
Magnesium	ICP	0.448	None
Sodium	ICP	0.301	None
Nickel	ICP	0.056	90%
Phosphorus	ICP	0.376	None
Sulfur	ICP	0.026	95%
Barium	ICP	0.000	95%
Cobalt	ICP	0.052	90%
Vanadium	ICP	0.033	95%
Chromium	ICP	0.034	95%
Copper	ICP	0.405	None
Iron	ICP	0.180	None
Manganese	ICP	0.000	95%
Zinc	ICP	0.004	95%

XRD Results for Field Fires

The complete XRD results are in appendices D. The average XRD results (in %) for each outside fire are shown in table 6. The p value and level of significance is from a two-tailed t-test comparing the combined pig fires vs the control fire.

Table 6. Mean mineral concentrations (in percent) of 2 pig cadaver fires ash and control fire ash from XRD analysis.

Fire	Calcite	Quartz	Lime	Periclase	Hydroxyapatite
SS1	54.8	11.1	4.9	10.8	18.4
SS2	44.9	6.6	3.1	27.1	17.8
CF	69.5	24.2	5.7	0.6	0.0
p value	0.012	0.002	0.076	0.005	0.000
Level of Significance	95%	95%	90%	95%	95%

The diffractogram for sample SS1 E-3 (figure 11) is an example of the output from the XRD for samples from the fires containing a pig. The orange lines visible at the top of the diffractogram correspond to the peaks found. The “V” above the first line indicates that peak is not from one of the five minerals identified. The peak list (figure 12) shows the locations of the peaks found, first row, with the locations of the peaks from the minerals found.

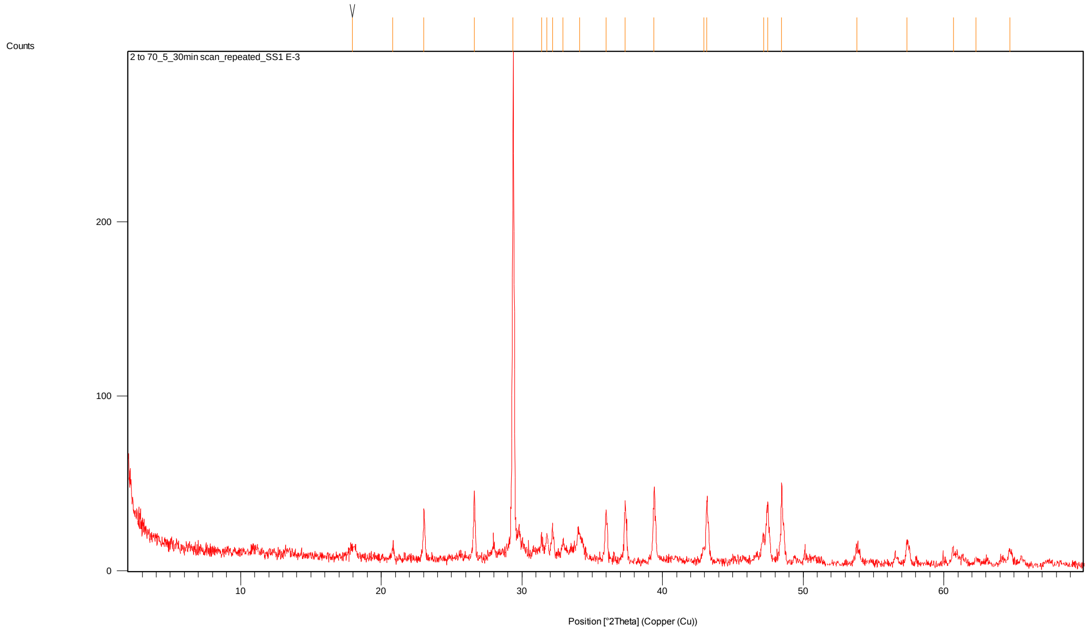


Figure 11. XRD Diffractogram for SS1 E-3.

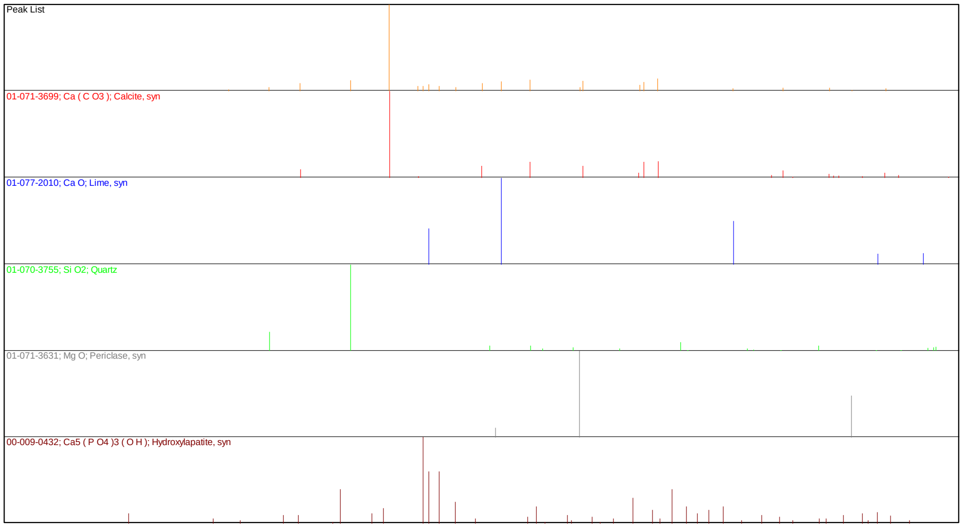


Figure 12. XRD Peak List for SS1 E-3.

Results for Laboratory Muffle Furnace Experiment

Two sets of samples were combusted in the muffle furnace. One complete set, from 400^{o}C to 1000^{o}C was completed before starting over at 400^{o}C with the second set of samples. Those sets are referred to as "series1" and "series2" in Figures 13-17. The individual sample names and temperatures of the muffle furnace can be found in Table 7, as well as in the CNS data in Appendix E.

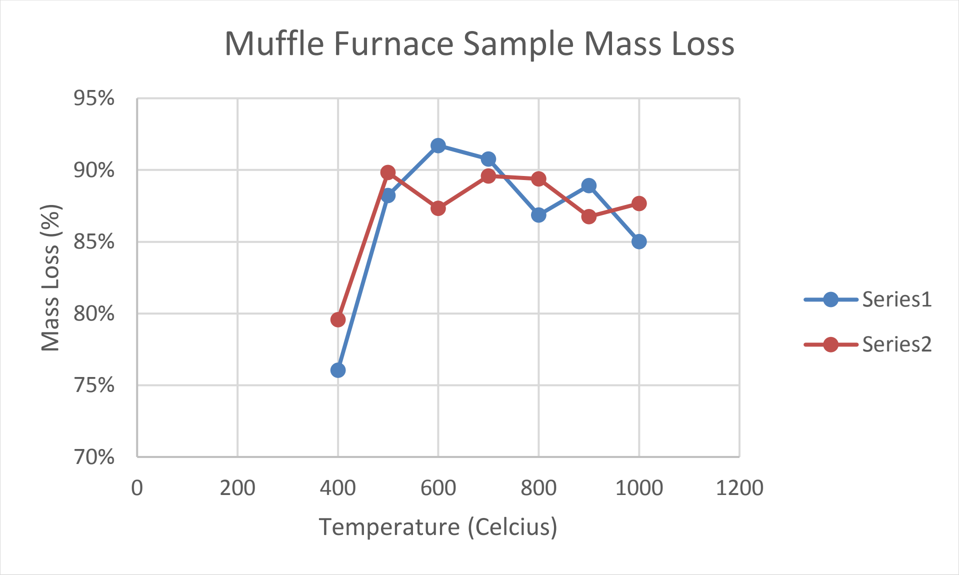


Figure 13. Mass loss (in %) of muffle furnace samples based on temperature (in Celsius).

The mass lost (Figure 13) does not follow as much of a linear pattern based on temperature as in some other studies, including Grupe and Hummel (1991). This is most likely because the remaining substance after muffling is mostly the mineral content of the bones (hydroxyapatite). Many other researchers used only cleaned bone. The samples in this study were from the same S. scrofa and included all surrounding tissues, making them variable in their bone content.

CNS

The results of the analyses of ash samples are displayed in Figures 14-16. Carbon and nitrogen are essentially burned off when 600°C is reached (Figures 14 & 15). Almost half of each C and N are volatilized at 400°C. Sulfur percent followed a more interesting pattern, increasing from 400° to 500°C, then dropping sharply at 700° to 800°C, before increasing slightly at 800° to 1000°C (Figure 16). This is probably related to other elements, like carbon burning out of the sample, thus increasing the relative amount of S, but unlike carbon and nitrogen, sulfur is not completely volatized at higher temperatures.

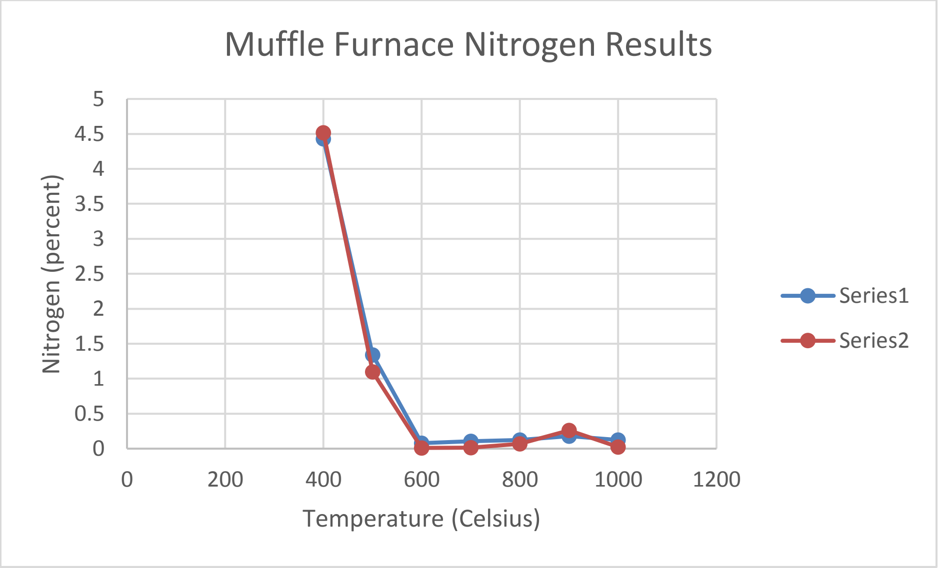


Figure 14. Nitrogen Percentage in Relation to Muffle Furnace Temperature.

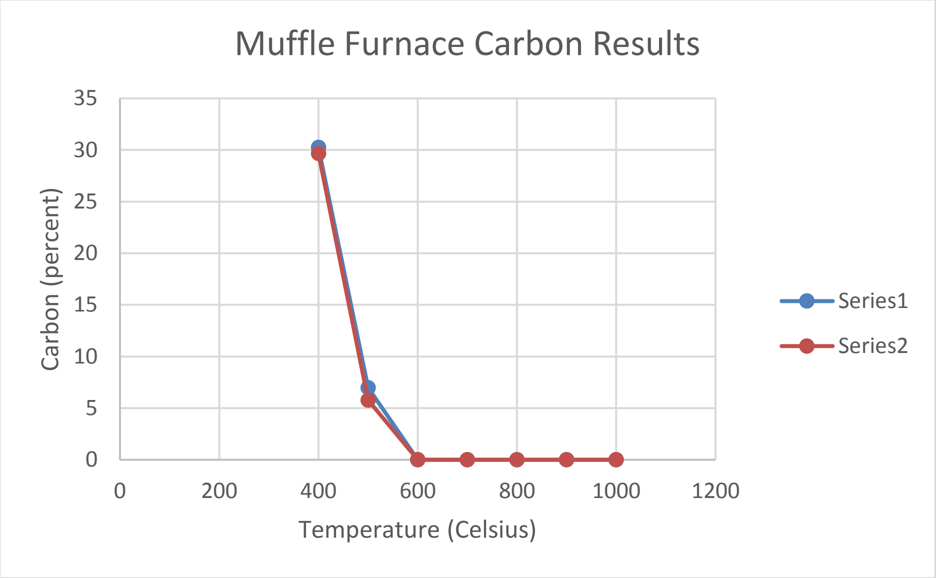


Figure 15. Carbon Percentage in Relation to Muffle Furnace Temperature

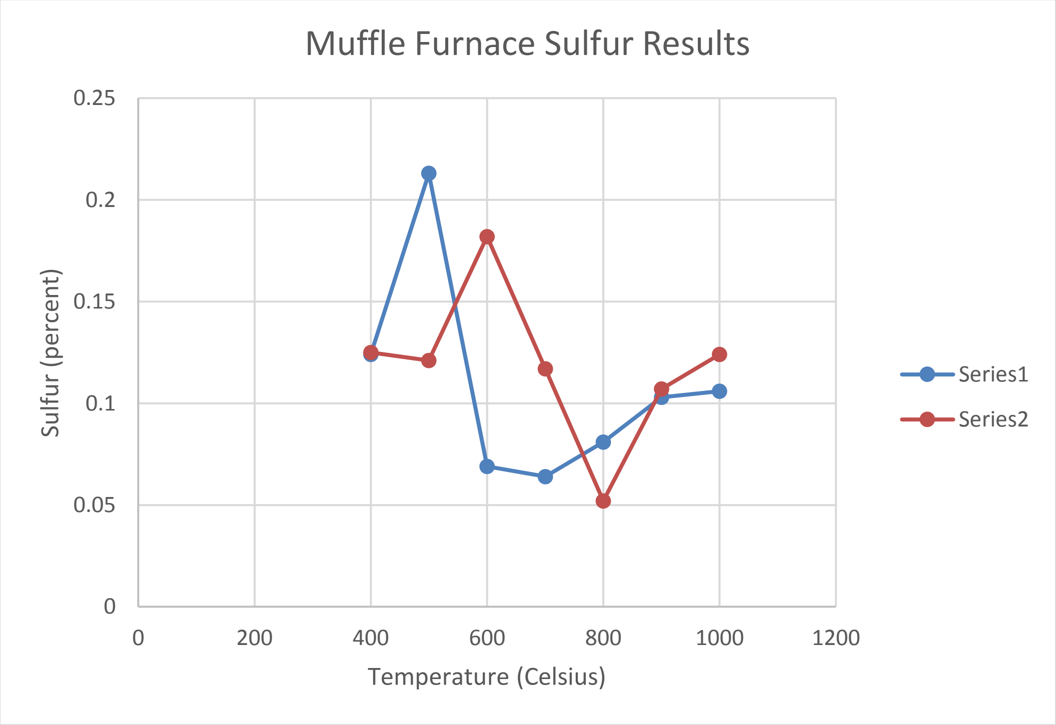


Figure 16. Sulfur Percentage in Relation to Muffle Furnace Temperature.

Phosphorus Results for Laboratory Burns

Phosphorus concentration increased with increasing temperatures (Figure17). This is likely due to higher P levels in cadaver tissue vs. wood, and the lack of volatilization of P at higher temperatures compared to other major elements like carbon and nitrogen, resulting in increasing relative P levels. At 1000^{o}C the concentration of P was high, at 18 to 20 percent. There was a big difference in the phosphorus concentration at 400^{o}C between the 2 sets of samples, probably due to the variability of the limb samples.

Table 7. Phosphorus Concentration (in ppm) in Relation to Muffle Furnace Temperature (^{o}C)

Sample	Temperature	P (%)
R-1	400	2.8
R-3	500	8.2
R-4	600	14
R-5	700	14.7
R-6	800	14.3
R-7	900	13.2
R-8	1000	14.5
T-1	400	10
T-2	500	12.6
T-3	600	13.8
T-4	700	14
T-5	800	14.7
T-6	900	15.3
T-7	1000	18.8

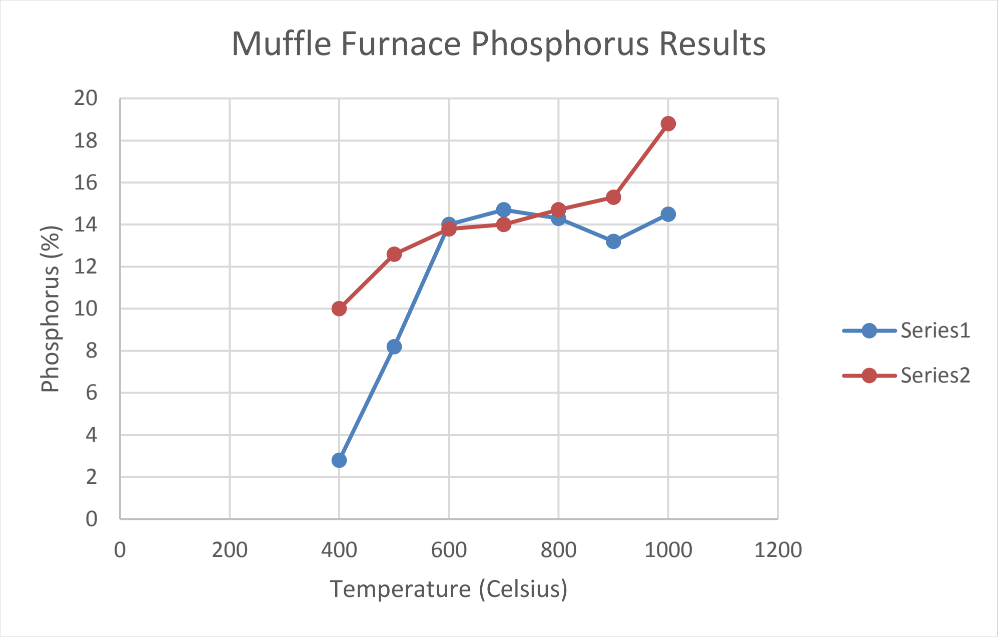


Figure 17. Phosphorus in Relation to Muffle Furnace Temperature (in %).

Chapter 6: Discussion

Chemical Content of Ash

Table 5 shows the statistical differences between the two pig fires using a 2-tailed t-test. Many elements: carbon, sulfur, barium, vanadium, chromium, manganese, and zinc, are all significantly different at the 95% confidence interval, meaning there is a 95% probability that the difference is not due to chance. Potassium, nickel, and cobalt, are statistically different at the 90% confidence interval. These differences are possibly caused by variations in the wood content or differences the amount of wood burned between the 2 fires. A 40kg pig cadaver was consumed using 336kg of firewood in Fire SS1, while Fire SS2 burned a 52kg pig with 285kg of firewood. The SS2 cadaver had 130% of the weight of the SS1 cadaver, while using only 85% of the firewood of SS1. The differences in the ratio of cadaver size to wood consumed resulted in different concentrations of elements prominent in the ash content of the cadavers.

Nitrogen, sodium, phosphorus, cobalt, chromium, iron, vanadium, zinc, calcite, quartz, lime, and periclase in the SS2 ash all have a greater difference from the control (CF) than was true for SS1. Variation in the pig cadavers may have caused additional variation between samples.

The means of the elemental concentrations and t-test significance results for the 3 fires (treatment vs. control) are in tables 2-4. The results of the 2 treatment (cadaver) Fires SS1 and SS2 were grouped together for the t-tests. There were no significant differences found for carbon, sulfur, calcium, potassium, nickel, barium, or manganese between the treatment (cadaver) and control (wood only) fires. Significant differences were found at the 90% confidence interval for nitrogen, magnesium, and copper. There were significant differences at the 95% confidence interval identified for sodium, phosphorus, cobalt, vanadium, chromium, iron, and zinc.

The majority of previous work conducted on the elemental content of burned remains has been to allow investigators to determine the legitimacy of commercial cremains. While that is not what this study aimed to do, some comparisons can be made. Brooks et al., (2006), while comparing cremains to common fillers found that using their discriminant function wood ash was categorized as concrete, but was not statistically the same. Six elements analyzed by Brooks et al. were also analyzed in this study: cobalt, chromium, copper, manganese, nickel, and vanadium. They found cobalt, copper, and vanadium to be statistically different with a p value of 0.001, with cobalt and copper higher in cremains and vanadium higher in concrete. This study did find a statistical difference for vanadium, which was a trace amount so not an important finding. This most likely is due to the differences in materials used.

All minerals in the ash that were analyzed by XRD were found to be statistically different between the cadaver and control fires, as shown in Table 6. But only hydroxyapatite and periclase were at higher concentrations in the treatment than the control ash. These two elements were at near zero levels in the control, and greater than 10 ppm in the treatments, resulting in p values of 0.000, and 0.005 respectively. Of these two, hydroxyapatite is a known component of bone. This makes it a key marker compound in detecting animal or human remains chemically in ash.

While there were many statistically different elements and crystalline structures, most of the differences are not of value because of the direction of the difference, higher in the control than the treatment. Magnesium, cobalt, chromium, copper, iron, and magnesium were all statistically significant, with values higher in the control fire ash. When analyzing for remains, an investigator would not look for lower concentrations of elements or minerals as evidence of remains.

These results indicate that five elements and minerals that were analyzed were higher in the treatment fires than the control and statistically significant: zinc, sodium, phosphorus, periclase, and hydroxyapatite. Each will be explained below:

Zinc, although lower overall than in a preliminary study I performed, was again significantly higher in the pig cadaver ash, especially SS2. Zinc is an important trace metal in the body and is found mainly in bone and muscle (Tapiero and Tew, 2003). Zn^{2+} can replace the Ca^{2+} in hydroxyapatite (Bergslien, 2008). This indicates that zinc may be of use in identifying remains in wood ash.

Sodium (Na) was significantly higher in the cadaver ash than the control, with mean concentrations of 8700 and 11637ppm, vs 324ppm in the control, and a p value of 0.001. Sodium can also replace the calcium cation in hydroxyapatite. It accounts for about 0.2% of bodies by weight (Shyamala, 2009). Wood contains a trace amount, from below reporting limits to about 63ppm (Pettersen, 1984). This indicates that sodium is a valuable indicator of human or animal remains in ash.

Magnesium was significantly higher in the control fire, at the 90% confidence interval. Magnesium oxide, or periclase, from XRD analysis was significantly higher, at the 95% confidence interval, in the pig cadaver fire ash. This results in the overall mean concentration of magnesium being higher in the control fire, but the magnesium in the ash from the pig fires is in the form of magnesium oxide crystal, so magnesium is not important forensically.

Phosphorus makes up 1% by weight of bodies (Shyamala, 2009). It is one of the main components of bone mineral. Phosphorus is found in very low concentrations, from below reporting limits to 0.04% in wood (Pettersen, 1984). This makes phosphorus a clear indicator of the presence of remains.

Hydroxyapatite in particular, is probably the most important finding, since it was present in only the samples from fires containing a pig. As Shipman et al. (1984) showed, with heating, hydroxyapatite crystal size increases, but the substance remains hydroxyapatite. No matter what temperature a body was combusted at, hydroxyapatite should still be recognizable by XRD.

The Effect of Temperature on Ash Content

The nitrogen and carbon percentages from the muffle furnace burns follow similar patterns to each other (Figures 14 and 15). From the starting experimental temperature of 400^{o}C, there is a sharp drop in both nitrogen and carbon until 500^{o}C, indicating volatilization at these temperatures. There is a less drastic drop in the mass percentage from 500^{o}C until 600^{o}C, as less and less of these elements remain. After 600^{o}C, there is no detectable nitrogen or carbon left in the sample, it has all burned off. Above 600^{o}C, the LECO results became negative. When the blank standards are adjusted for the weight of the analyzed samples, the samples are at or above the blank standard percentages. This demonstrates that carbon and nitrogen are not good indicators of the presence of remains, as both are burned off at relatively low temperatures. Both elements, as shown in Figures 14 and 15, volatilize almost completely by 600^{o}C. Nitrogen was different at a 90% confidence interval for the pig fires, but the means of the fires were only 0.04% different.

The carbon concentrations from all of the field fires are in a range between the muffle furnace results at 500^{o} and 600^{o}C. The color of the ash is also in between the colors of the muffle furnace samples. The temperatures reported from the thermocouples (Figures 9 and 10) indicate a hotter fire, however, an outdoor fire is not as efficient as a burn performed in a muffle furnace. Calcined bone fragments were sieved out of the ash, and had they been included, would have diluted the carbon and nitrogen concentrations.

Sulfur percentages in muffle furnace samples did not follow a clear pattern, (Figure 16). The sulfur was very similar among the different muffle furnace samples, regardless of temperature (0.05-0.21%). Both sets of samples, while not as consistent as the nitrogen and carbon results, show a similar pattern to each other. The relative amount of sulfur initially increases, then decreases, then increases again. This reflects the volatilization of other elements, however it is not clear enough to use as an indicator of burning temperatures. There may have been differences caused by the samples themselves, as sulfur is found more in muscle and hair, and samples varied in their contents.

The relative phosphorus increased as the temperature of the muffle furnace increased, following a positive correlation (Table 7). The curves created from the two sets of phosphorus data, while both increasing with temperature, do not match each other, as shown in figure 17. The first sample set follows what I would expect to see, a dramatic increase in phosphorus from 400^{o} to 600^{o}C, which corresponds to the sharp decrease in the carbon and nitrogen. The second sample set has a more subtle increase in phosphorus, with a higher initial and final ppm. This could be caused by the standards being made slightly different on consecutive days. The muffle furnace thermocouple also melted between the sample sets and had to be replaced, however, given the carbon and nitrogen results, the new thermocouple did not likely affect the temperature of the muffle furnace. The muffle furnace burns have a much higher concentration of phosphorus than the treatment fires due to consisting solely of animal tissues.

Chapter 7: Conclusion

The purpose of this study was to see whether chemistry of the residue and ash can be useful in determining if a human or animal cadaver has been burned in an outdoor fire. Three elements: P, Na, and Zn; as well as two minerals: periclase (MgO) and hydroxyapatite

($Ca_{10}(PO_4)_6(OH)_2$) were at significantly increased amount in ash from fires containing pig carcasses. I had hypothesized that the phosphorus, nitrogen, and sulfur would be statistically higher in the experimental fires than in the control fire. My results show I can only accept part of that hypothesis. The phosphorus was indeed statistically higher, the nitrogen was not enough to be conclusive, and sulfur was not significantly different.

Furthermore, this study shows the differences in the ability to make the distinction between a fire with a cadaver and without, between three different methods: XRD, ICP, and CNS. Hydroxyapatite being the remaining material when a cadaver is reduced to ash, causes the phosphorus, and especially the hydroxyapatite compound itself to be very telling substances. I agree with Bergslien et al. (2008) that XRD is a better method than elemental analysis in many situations for the determination of the presence of remains, given the resources. The x-ray diffraction (XRD) analysis was very clear in the detection of hydroxyapatite. If hydroxyapatite was present, the sample analyzed was from a fire that contained a cadaver.

In the laboratory study, the last of my hypotheses was tested. The nitrogen did volatilize more as the temperature increased, and the phosphorus remained and had a relative increase. The sulfur, however, did not decrease as the temperature increased. The sulfur concentration was very low and did not follow a clear pattern. I can therefore only accept part of my hypothesis. I reject any part of my hypothesis as it pertains to sulfur content.

Appendix A. Field CNS Data

Sample	Nitrogen %	Carbon %	Sulfur %
SS1 D-1	0.24	6.09	0.33
SS1 D-2	0.22	5.80	0.23
SS1 D-3	0.19	5.58	0.49
SS1 E-1	0.20	3.97	0.29
SS1 E-2	0.23	5.01	0.26
SS1 E-3	0.19	4.54	0.40
SS1 F-1	0.21	5.77	0.35
SS1 F-2	0.24	6.09	0.25
SS1 F-3	0.18	4.62	0.30
SS1 G-1	0.20	4.82	0.22
SS1 G-2	0.18	5.61	0.19
SS1 G-3	0.16	5.54	0.21
SS2 A-1	0.25	5.85	0.88
SS2 A-2	0.45	6.76	0.91
SS2 A-3	0.22	6.44	0.41
SS2 A-4	0.16	5.84	0.31
SS2 A-5	0.21	6.05	0.35
SS2 B-1	0.22	6.22	0.37
SS2 B-2	0.33	8.47	0.37
SS2 B-3	0.27	11.62	0.30
SS2 B-4	0.23	9.30	0.39
SS2 B-5	0.16	5.90	0.28
SS2 C-1	0.16	6.04	0.38
SS2 C-2	0.19	4.80	0.41
SS2 C-3	0.17	6.41	0.42
SS2 C-4	0.17	6.62	0.33
SS2 C-5	0.17	5.54	0.17
CF H-1	0.18	7.43	0.48
CF H-2	0.18	7.18	0.30
CF H-3	0.20	7.59	0.28
CF H-4	0.16	7.07	0.27
CF I-1	0.19	7.00	0.51
CF I-2	0.18	7.06	0.38
CF I-3	0.18	6.29	0.38
CF I-4	0.17	6.77	0.45
CF J-1	0.19	6.63	0.27
CF J-2	0.18	7.69	0.39
CF J-3	0.17	6.10	0.30
CF J-4	0.22	5.50	0.31

Appendix B. Field ICP Macro Element Results

Sample	Fire	Calcium	Potassium	Magnesium	Sodium	Phosphorus	Sulfur
D-1	SS1	230000	67000	16000	2100	7600	3100
E-1	SS1	160000	51000	17000	6000	14000	2800
E-2	SS1	190000	67000	19000	10000	23000	2400
E-3	SS1	220000	85000	20000	15000	38000	3900
F-1	SS1	220000	66000	18000	6900	24000	3600
F-2	SS1	230000	66000	17000	14000	35000	2400
F-3	SS1	180000	77000	22000	14000	37000	2800
G-2	SS1	210000	51000	17000	1600	11000	1600
A-1	SS2	180000	100000	25000	7400	24000	9300
A-2	SS2	140000	100000	19000	19000	47000	7600
A-4	SS2	180000	77000	22000	3700	16000	3100
B-2	SS2	200000	71000	17000	15000	30000	4300
B-3	SS2	200000	77000	16000	16000	37000	4000
B-4	SS2	200000	61000	17000	13000	27000	3100
C-2	SS2	180000	61000	18000	5000	13000	3500
C-3	SS2	200000	92000	20000	14000	39000	4600
H-2	CF	220000	57000	19000	270	13000	3200
H-3	CF	220000	60000	20000	270	11000	3100
I-1	CF	180000	120000	26000	<200	17000	7200
I-2	CF	190000	86000	23000	360	12000	4200
I-3	CF	160000	93000	22000	400	10000	4000
I-4	CF	190000	78000	21000	320	10000	5500
J-1	CF	190000	98000	23000	<200	12000	3500
J-4	CF	140000	42000	15000	<200	6900	3700

Appendix C. Field Micro Element Results

Sample	Arsenic	Barium	Cadmium	Cobalt	Chromium	Copper	Iron
D-1		780		0.94	3.1	53	1900
E-1		600		3.1	10	43	7200
E-2		730		2.1	7.5	70	5000
E-3		790		1.6	6.3	99	3300
F-1		790		2.1	7	67	4200
F-2		840		1.7	5.8	69	4000
F-3		710		2.5	8.7	93	7300
G-2		820		2.2	7	56	5100
A-1		530	1.1	1.9	5.9	82	3600
A-2		380		1.1	4.8	98	2000
A-4		620		1.4	2.8	68	1400
B-2		540		1.4	4.5	66	2200
B-3		610		1.4	5.4	78	3600
B-4		610		1.4	4.2	65	2300
C-2		540		1.6	5.3	73	3200
C-3		560		1.7	6.5	74	3400
H-2		800		2.7	8.8	88	5100
H-3		710		2.7	8.2	79	5100
I-1		700		2.5	10	120	4400
I-2		740		2.8	8.3	89	5600
I-3		730		2.8	9.5	94	6300
I-4		820		3.2	9.2	100	6300
J-1		520		1.9	6.6	89	4400
J-4		420		4.1	12	51	9600

Appendix C. Field ICP Micro Element Results

Sample	Manganese	Molybdenum	Nickel	Lead	Vanadium	Zinc
D-1	990		11	28	3.7	130
E-1	990		12	160	18	230
E-2	1100		14	1200	11	240
E-3	1300		16		7.4	270
F-1	1200		12	34	10	230
F-2	1200		12		7.2	280
F-3	1200		24		12	310
G-2	1200		12		12	95
A-1	660		14		8.8	580
A-2	570		8.4		3.5	590
A-4	500		8.5		3.6	210
B-2	700		9.3		5	400
B-3	1000		11		6.1	490
B-4	920		10	60	4.8	330
C-2	730		12		8	260
C-3	890		11		8.3	490
H-2	1500		11		14	160
H-3	1400		12		13	200
I-1	960		18		10	330
I-2	1200		17		14	200
I-3	1200		19		17	140
I-4	1400		18		16	180
J-1	640		10		10	150
J-4	880		12		21	130

Appendix D. Field XRD Mean Percentage Results

Fire	**Sample**	**Calcite**	**Quartz**	**Lime**	**Periclase**	**Hydroxyapatite**
SS1	D-1	65.2	1.2	4.0	5.4	24.2
SS1	E-1	39.9	27.0	2.7	6.8	23.6
SS1	E-2	40.3	10.7	3.0	29.0	17.0
SS1	E-3	47.2	3.4	5.0	10.5	33.9
SS1	F-1	69.0	15.5	5.7	2.5	7.4
SS1	F-2	72.2	10.2	7.1	0.0	10.6
SS1	F-3	30.8	6.9	3.5	32.6	26.1
SS1	G-2	73.9	14.2	7.8	0.0	4.1
SS2	A-1	26.1	12.2	0.9	39.9	20.8
SS2	A-2	20.8	3.1	1.4	46.2	28.5
SS2	A-4	76.9	1.9	5.5	5.2	10.4
SS2	B-2	37.2	5.2	1.8	38.8	16.9
SS2	B-3	45.1	2.7	3.1	34.2	14.9
SS2	B-4	73.4	11.9	4.1	2.6	8.1
SS2	C-2	34.3	10.6	3.6	29.5	22.0
SS2	C-3	45.2	5.4	4.4	20.6	20.7
CF	H-2	75.7	17.0	7.3	0.0	0.0
CF	H-3	76.0	17.1	7.0	0.0	0.0
CF	I-1	83.5	13.8	2.7	0.0	0.0
CF	I-2	71.7	18.0	8.0	2.3	0.0
CF	I-3	59.6	30.4	7.5	2.5	0.0
CF	I-4	65.4	29.3	5.3	0.0	0.0
CF	J-1	70.1	26.0	3.9	0.0	0.0
CF	J-4	54.3	42.1	3.6	0.0	0.0

Appendix E. Muffle Furnace CNS Results

Series 1

Sample	Temperature	N%	C%	S%
R-1	400	4.44	29.66	0.12
R-3	500	1.02	5.78	0.21
R-4	600	-0.07	-0.91	0.07
R-5	700	-0.06	-1.37	0.06
R-6	800	0.00	-1.43	0.08
R-7	900	0.18	-1.38	0.10
R-8	1000	-0.05	-1.52	0.11

Series 2

Sample	Temperature	N%	C%	S%
T-1	400	4.35	30.24	0.125
T-2	500	1.26	6.98	0.121
T-3	600	0.48	-1.15	0.182
T-4	700	0.50	-1.39	0.117
T-5	800	0.52	-1.45	0.052
T-6	900	0.57	-1.46	0.107
T-7	1000	0.52	-1.51	0.124

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