

## **Analysis of the Effect of Alkaline Hydrolysis Cremation on Minerals and Trace Metals in Bone**

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### **Abstract**

This research seeks to determine the changes that occur in bone during the Alkaline Hydrolysis Cremation (AHC) process. AHC is a form of cremation, which is considered by many, to be an environmentally friendly alternative to traditional fire cremation. AHC is typically performed in a strongly basic solution under increased pressure and temperature to accelerate the natural decomposition a body typically undergoes. This process results in human or animal remains being completely decomposed with only bone ash remaining, which can be returned to the family. The AHC process was performed using pig femur bones in a pressure cooker with a potassium hydroxide solution to mimic the commercial process. Bone structure was analyzed qualitatively by visual inspection of the bones and quantitatively by monitoring the concentrations of calcium, magnesium, and iron ions during the AHC process. Metal ion analysis was performed using atomic absorption spectroscopy. The concentration of calcium fell within the range of 30.3 ppm to 125 ppm, the iron concentration ranged from 17 ppm to 68 ppm, and the magnesium concentration ranged from 7.65 ppm to 24.3 ppm.

**Keywords:** Alkaline Hydrolysis Cremation, Atomic Absorption Spectroscopy, Bone Decomposition

### **1. Introduction**

For as long as humans have been alive, they have also been dying. Some of the earliest cases of ritual burial of the dead can be found at sites occupied by *Homo neanderthalensis*, or Neanderthals, over 50,000 years ago.<sup>1</sup> This form of symbolic burial has never been seen at other sites of known prehistoric occupation and shows it to be a human-specific trait. The practice of burial as a funerary process did not end with the Neanderthal extinction. Burial is the most popular form of funerary practice in the United States and as of 2005, over 61.4% of deceased individuals were buried.<sup>2</sup> Typical burials involve the deceased individual being placed in a coffin or other vessel and being placed in the ground and marked with a headstone or marker. While burial remains the most common form of funerary practice in the United States, preferences are changing. Over the years, new techniques have been developed and used as an alternative funerary process for those opposed to burial.

The second most popular funerary practice in the United States is traditional fire cremation. This technique uses a combustion reaction with natural gas and the body as the fuel sources, to break down the deceased so that the residual remains can be returned to the family. After the reaction goes to completion, the bones are further crushed into bone ash or small, broken-down particles of bone, to be returned to the family.<sup>3</sup> Over the past years, fire cremation has steadily become a more favorable funerary practice in the United States, with a projected 58% of individuals being cremated in 2020, up from 32.3% in 2005.<sup>2</sup> The reasoning behind the shift from traditional burials to cremation can be due to many factors, including religious and personal reasons. The lower cost for cremation over burial, (a difference of over \$1,000), can also be the deciding factor for many families.<sup>4</sup>

More recently, a new movement for environmentally friendly funerary practices has led individuals to improve on the traditional burial and cremation practices already widely used in the United States and around the world. Traditional fire cremations release a large amount of carbon dioxide gas into the atmosphere and any plastics intentionally or inadvertently left in the casket before cremation can be vaporized to produce toxic fumes that are released into the environment. Medical implants, such as hip and knee replacements or pacemakers, and prosthetics must be removed from the deceased before cremation as they can contain batteries which can lead to explosive ruptures when exposed to the extreme temperatures created during the cremation process.<sup>5</sup> Removal of such implants can cause families to incur additional costs.

The alkaline hydrolysis cremation (AHC) process was developed as a “greener” cremation alternative. This newer technique has been in use since the 1990’s by many research universities to dispose of donated corpses and deceased animals used in disease research. This form of cremation uses an alkaline hydrolysis reaction of aqueous solutions of potassium hydroxide or sodium hydroxide to break down the body into its basic, soluble, organic components and bone ash. At room temperature and pressure, this reaction can take up to twelve hours to fully decompose a human body; when the temperature and pressure are raised, the reaction rate increases to the point where a whole human body will decompose in just three hours.<sup>6</sup> A typical AHC retort is similar to a traditional cremation retort except for heating coils in place of the flame ignition source and an area for the alkali solution to be introduced to the chamber.



Figure 1: Dried, crushed bone resulting from this experiment after the AHC process.

The remaining bone, which can be seen in Figure 1 above, is brittle and soft which allows the bone to be further crushed, if necessary, to be returned to the family. The end result is an organic solution with a basic pH that can be neutralized for use as fertilizer in gardens or other allowed areas. While the entire process requires heating, the carbon dioxide emissions created during the reaction are minimal when compared to fire cremation, and pose little threat to the environment. Medical implants and prosthetics do not have to be removed since they are not harmed during the reaction and can be removed from the bones afterwards, reducing costs for their removal. This technique can also destroy infectious disease precursors, which are difficult to destroy using an incinerator, making AHC the ideal technique for disposal of infectious waste and animal carcasses used for research purposes.<sup>7</sup>

Even though the immediate reaction between the body and the alkaline solution is the breakdown of the flesh and organic tissue, when the flesh is gone, the base reacts with the skeleton, which is much harder to dissolve. Bone is composed of a number of organic and inorganic components, contributing to the strength and stability of bone which causes it to be difficult to break down. The largest organic component present in every human and animal bone is collagen, a large protein molecule that gives bone its flexibility.<sup>8</sup> At birth, bone is composed entirely of collagen fibers, which intertwine to form the bone structure. As the individual and bone ages, the collagen fibers begin to be replaced with a hard, dense, inorganic mineral, called hydroxyapatite.

Hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , is a member of the apatite mineral family whose main component is calcium phosphate.<sup>9</sup> In early bone development, pockets of hydroxyapatite are stored within the collagen fibers as they are created. As the bone ages, the hydroxyapatite mineral covers the collagen fibers and creates the dense bone structure that is most commonly seen.<sup>8</sup> This mineral is the main component used in bone remodeling and it can be broken down when mineral needs to be removed from the bone surface.<sup>9</sup>

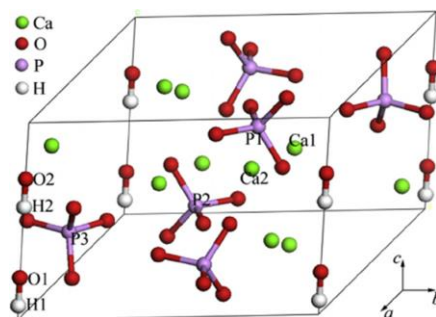
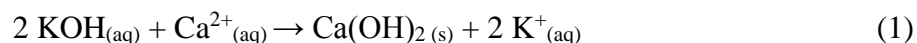


Figure 2: Crystal structure of calcium phosphate mineral with a hexagonal unit cell.<sup>10</sup>

The crystal structure of hydroxyapatite, seen in Figure 2, is only 50 nm in size with lattice parameters of  $a=9.436 \text{ \AA}$  and  $c=6.882 \text{ \AA}$ . Groups of phosphates and alcohols bound to their respective hydrogens are shown.<sup>11</sup> However, all the groups, including the calcium ions, are not bound to each other, allowing ion exchange and changing the function of the hydroxyapatite in specific areas. Some of the exchanges that are possible are magnesium, zinc, strontium, and iron for calcium, carbonate groups for the phosphates, and also fluorine for the alcohol groups.<sup>9</sup>

The metal exchanges in the crystal structure have a large impact on the mineral and bone structure. Magnesium and iron have been found to influence the strength of bone. Iron is essential for collagen synthesis, keeping the bone slightly flexible, so it does not break when force is applied. It can also prevent bone resorption, helping to increase the strength of bone by preventing bone mass loss and boosting hydroxyapatite synthesis.<sup>9</sup> Magnesium, on the other hand, controls osteoblast and osteoclast activity through signaling. The magnesium ions can reside on the surface of the hydroxyapatite or within the apatite structure, which increases the strength of the bone.<sup>12</sup>

The research presented here focuses on the alkaline hydrolysis reaction with bone. While the use of an acidic solution as the reactant with bone is typically considered for disposal, it removes the hydroxyapatite mineral from bone and leaves the collagen behind. This results in a flexible and bendable bone being left over, which is difficult to further break down.<sup>8</sup> The use of a basic solution with bone employs a reaction shown in equation (1) below.



In this reaction, the potassium hydroxide will dissociate into aqueous  $\text{K}^+$  and  $\text{OH}^-$ . The  $\text{OH}^-$  will react and bind with the  $\text{Ca}^{2+}$  within the hydroxyapatite structure in a 2:1 ratio of hydroxide ions to calcium ions. The resulting calcium hydroxide will form a solid and precipitate out of the basic solution, effectively removing the calcium from the crystal lattice structure and leaving a vacancy in its place. The potassium ions can also bond with the  $\text{PO}_4^{3-}$  groups, in a 3:1 ratio, creating soluble  $\text{K}_3\text{PO}_4$ . Solubilizing the phosphate could further destabilize the hydroxyapatite structure. Theoretically, this calcium ion vacancy should result in the degradation of the bone structure and lead to the bone ash that remains after the AHC process is complete.

The objectives of this research were, first, to successfully cremate a pig femur bone, then to identify the changes that occur to the bone structure during the AHC process by monitoring the change in calcium, iron, and magnesium ions being removed from the bone throughout the AHC process.

## 2.1. Procedure

A Presto 01370 8-Qt Stainless Steel pressure cooker was obtained and fitted with a dual temperature/pressure gauge to monitor temperature and pressure changes during the experiment. A Durabrand Single Burner 1100 watts Variable Temperature Control heating element (WS 100) was used for the heating the pressure cooker. Pig bones were used in place of human bones for this experiment since pigs are often used as a suitable model for humans in research. Four femur bones were obtained from Cheplic Meat Processing and analyzed for trauma due to the butchering process. Each bone was weighed prior to cremation and placed in the middle of the pressure cooker. A 2 liter solution of 0.89 M potassium hydroxide (KOH) was added to the pressure cooker and filled with an additional 1.5 liters of deionized water to fill the pressure cooker halfway and prevent the cooker from running dry. The pressure cooker was heated

for a total of three hours and depressurized every thirty minutes to remove samples for later analysis using atomic absorption spectroscopy.

## 2.2. Bone Trauma

Each of the four pig femurs was received with noticeable signs of trauma caused by the butchering process. Any trauma to the bone before cremation began could have impacted the length of time and extent of cremation. Any impact to the cartilage surrounding the bone should have no impact on cremation, while deeper trauma impacting the trabecular or cortical bone will introduce an additional entrance point for the KOH solution and speed up the cremation process. All femurs were sided, weighed, and measured for femur length, head diameter, and diameter at mid-shaft and all trauma was recorded for future analysis. All femurs appeared to have fused epiphyses, indicating adult pigs.

## 2.3. Atomic Absorption Spectroscopy

All samples were analyzed using a Shimadzu AA-7000 atomic absorption spectrometer with an ASC-7000 Shimadzu auto sampler. To analyze the AHC samples by atomic absorption (AA) spectroscopy, the samples required acidification prior to dilution. A 5 mL portion of the collected sample was mixed with 5 mL of 0.8 M HNO<sub>3</sub> in a 10 mL volumetric flask. The samples then had to be filtered using gravity filtration due to a brown foam forming with a peroxide odor on the surface of the liquid sample. From the 10 mL sample, 1.00 mL was removed and diluted with deionized water in a 25 mL volumetric flask. The 25 mL samples were used for AA analysis.

## 3. Results

A total of three cremations were performed and each will be discussed below.

### 3.1. Femur 1

This femur was the smallest bone of the four and was used as an initial trial to test the experimental set-up and procedure before the rest of the bones were tested. Using the smallest femur also eliminated femur size and weight as a variable to influence extent of cremation. As described above in the procedure, the pressure cooker was only filled with 2 liters of 0.89 M KOH solution and left undisturbed for three hours to determine what affect depressurizing the pressure cooker would have on the extent of cremation. After two and a half hours had elapsed, the steady stream of pressure coming from the release valve was interrupted and a yellow substance began to spew out the valve. The heat was immediately turned off and the cooker depressurized. The result of the first trial can be seen in Figure 3 below.

After the lid was removed, the pressure cooker revealed a dark red solution along with the bone. Even though the cremation did not proceed for the full three hours, the resulting bone still showed signs of breakdown.



Figure 3: (A) remaining KOH solution and broken femur. (B) main femur shaft with proximal and distal ends missing and slight red discoloration. (C) partially crushed condyles.

### 3.2. Femur 2

In response to the results of femur 1, an additional 1.5 liters of deionized water was added to fill the pressure cooker halfway to prevent the pot from running dry. A total of 30 mL of the KOH/H<sub>2</sub>O sample were taken every thirty minutes for atomic absorption analysis and the water was replenished to the half-way mark with approximately 100 to 300 mL of deionized water in the pressure cooker after sample removal. Depressurization began five minutes before each sample was taken. At the end of three hours, the solution was filtered to catch any bone particles and to retrieve the larger bone parts which can be seen below in Figure 4.

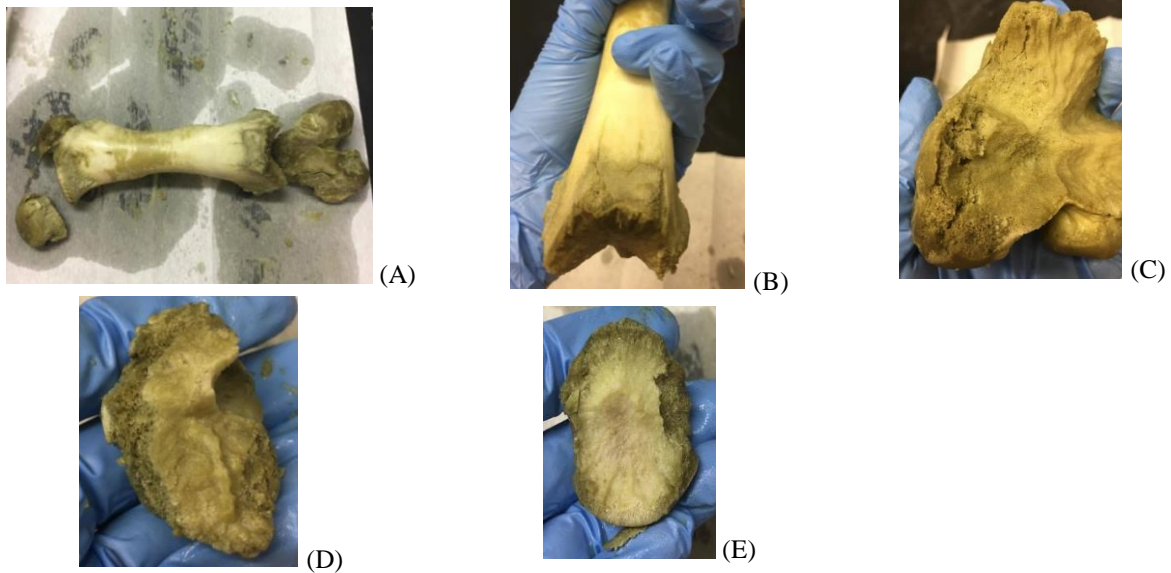


Figure 4: (A) relative placement of each separated bone to the femoral shaft and discoloration. (B) cracking and discoloration of distal femur. (C) cracking of epiphyseal surface of the medial condyle with discoloration. (D) greater trochanter with exposed spongy bone and discoloration. (E) femoral head showing discoloration halting at epiphyseal surface.

Atomic absorption (AA) spectroscopy was used to analyze a total of six sample solutions for calcium concentration in the pressure cooker solution. The standard curve created for sample analysis ranged from 0.5 to 5 ppm with an extremely high linearity of 0.9979. Table 1 below shows the absorbance of each sample and their corresponding concentrations back-calculated to the original pressure cooker environment.

Table 1: Summation of sample absorbance and concentrations to original solution.

<b>Femur 2 calcium concentration</b>			
<b>Collection Time (min)</b>	<b>Abs. in sample</b>	<b>Concentration in sample (ppm)</b>	<b>Concentration in original solution (ppm)</b>
<b>30</b>	0.0486	0.640	560.
<b>60</b>	0.0575	0.751	651
<b>90</b>	0.0597	0.778	669
<b>120</b>	0.0575	0.751	640.
<b>150</b>	0.0516	0.677	731
<b>180</b>	0.0546	0.715	805

### 3.3. Femur 3

In an effort to fully cremate the bone, the cremation time was increased to four hours. Three additional samples were extracted at 0 minutes, 3 hrs and 30 minutes, and 4 hours. Only 20 mL of sample were removed and 100 to 300 mL



of deionized water was once again added to the halfway mark. The time zero sample was removed when the bone was placed in the KOH/H<sub>2</sub>O solution and prior to heating and pressurization. Depressurization began five minutes before each sample was taken. During the third hour, a banging noise was heard within the pressure cooker due to the bone bouncing about in the solution after additional water was added. The solution was filtered before the bones were removed. The bone results can be seen in Figure 5 below.

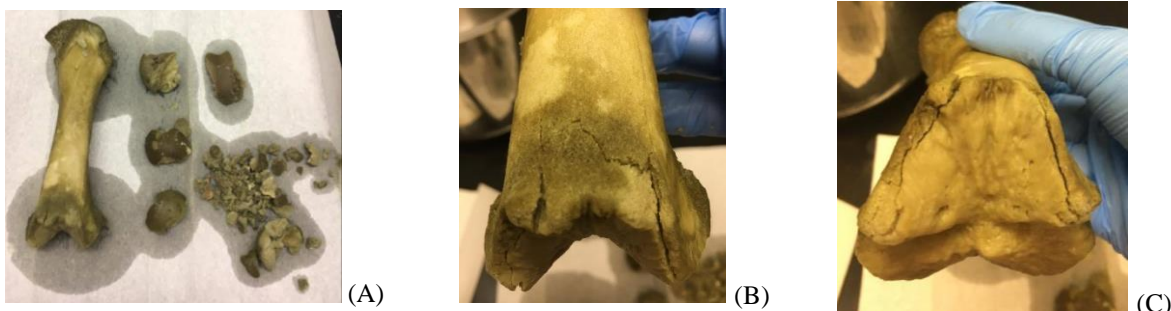


Figure 5: (A) remains of femur 4 with discoloration seen on the majority of the bones. (B) extreme cracking and discoloration of frontal femur. (C) minimal discoloration and extensive cracking on sides of epiphyseal surface.

Femur 3 was left to dry completely to determine its brittleness and ease at which it could be crushed. The bone was left to air dry for five days and was examined for any changes to the color and structural integrity of the bone. Images of the bone can be seen in Figure 6 below.

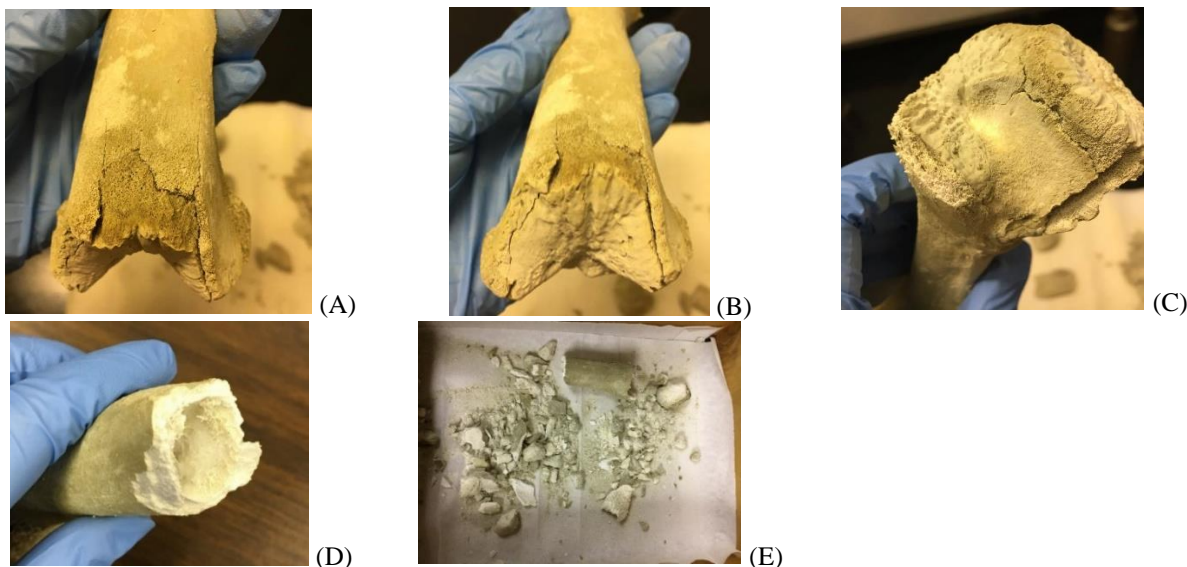


Figure 6: (A) extended cracking of frontal femur. (B) bone peeling on the sides of the epiphyseal surface. (C) crack and gouge on the femoral neck. (D) empty marrow chamber. (E) remains of fully crushed femur 4.

Analysis of concentrations of calcium, iron, and magnesium was conducted by AA. Three standard curves were created to calculate the concentrations of the metals based on their specific absorbance. The curves and their corresponding equations can be seen in Figure 7 below. Calcium was measured in the range of 0 to 1 ppm, iron was ranged from 0 to 0.5 ppm and magnesium ranged from 0 to 0.1 ppm with a high linearity of 1, 0.9964 and 0.9933 respectively. One replicate of each standard was run to create the standard curve. Table 2 displays the absorbance and calculated concentration of each metal.

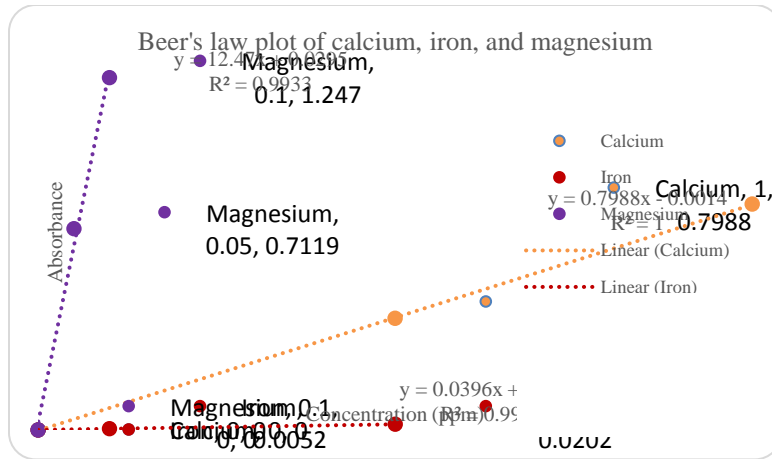


Figure 7: Combined standard curves for calcium, iron, and magnesium with corresponding linear regression equations used to calculate the concentration of each metal sample.

Table 2: Absorbance and corresponding calculated calcium, iron, and magnesium concentrations in the sample and original pressure cooker solution.

Collection Time (min)	Femur 4 calcium concentration		Femur 3 iron concentration		Femur 3 magnesium concentration	
	Abs. in sample	Concentration in original solution (ppm)	Abs. in sample	Concentration in original solution (ppm)	Abs. in sample	Concentration in original solution (ppm)
0	0.0568	63.8	0.0020	31	0.179	10.5
30	0.0264	30.3	0.0014	18	0.180	10.5
60	0.0369	41.5	0.0014	17	0.379	24.3
90	0.0336	38.8	0.0018	27	0.297	19.0
120	0.0359	44.8	0.0023	41	0.238	16.0
150	0.0330	42.6	0.0028	55	0.188	12.6
180	0.0507	68.5	0.0028	58	0.104	6.27
210	0.0313	45.9	0.0030	68	0.115	7.65
240	0.0837	125	0.0019	39	0.182	14.3

## 4. Discussion

### 4.1. Femur 1

As seen in Figure 3 (A), it appeared that all of the water had been boiled out of the solution. The resulting liquid would have consisted of KOH, collagen, and other organic substances which began to turn to a gelatinous substance that began to foam and exit through the release valve. The femur can be seen to have detached into three different parts, as seen in image (A). The detachment points coincide with the epiphyseal or growth plate areas of the femur. Since the femur appeared to have fused epiphyses, the condyles and head should not have detached as easily as they would have become one solid bone with the femoral shaft. This suggests that the pig was much younger than first thought, between two to three years at death.

In image (B and C) the red discoloration is due to the bones sitting in the concentrated solution. The breakdown of the condyles in image (C) is evidence of cremation. The bone was fragile to pick up and the removal of the bone from the pot resulted in the damage. The edges of the proximal and distal femur in image (B) had a softer feel when

compared to the femoral shaft which was still as firm as it was before the cremation. The softer feel to the bone is further evidence of cremation because those are the points where the hydroxyapatite crystal structure is weakest due to calcium removal. In these areas there is a large amount of trabecular bone at the epiphyses, increasing the surface area available for the KOH solution to react with. Therefore, the areas of the epiphyses will breakdown much faster than the rest of the bone.

#### 4.2. Femur 2

The cremation of femur 2 was performed for the full three hours. Since additional water was added to the pressure cooker, it did not run dry and the bone again separated into four different sections. One difference between femur 1 and 2 is a green discoloration seen on the distal portion of the femur 2 shaft, as well as the condyles, greater trochanter, and outline of the femoral head. The placement of the green color coincides with the epiphyseal sites of the bone and is also where a large amount of blood vessels flow to provide blood to the growth plate. Therefore, the green discoloration is due to the breakdown of the haemin in the blood at the ends of the bone. Haemin breakdown is also known to transform into biliverdin and bilirubin which is green in color.<sup>14</sup>

The bones also showed more extensive cracking and breakdown than femur 1. In Figure 4 (B) cracks are present on the anterior surface of the distal portion of the femur. Those cracks only extend as far as the green discoloration but were not present on femur 1. In image (C), a large chunk of the condyle is crumbling and was able to be pulled off of the bone itself. In images (D and E), no cracking is present but the edges of the bone are ragged and spongy looking. This is due to the removal of the layer of dense bone, exposing the spongy bone below. These two bones show evidence of the KOH solution eating its way through the outer bone surface to begin impacting the softer, inner bone. This was not seen in femur 1. Since the reaction conditions were not equal for femur 1 and 2, there is no way to conclusively tell if the cracks and breakdown appeared due to the extra thirty minutes the bone was submerged in the KOH/H<sub>2</sub>O solution. The combination of the green discoloration and the appearance of the breakdown of the bone can be used as indicators of the extent of cremation. The more discoloration of the bone as well as ragged, broken edges, the farther cremated the bone is.

The AA data, in Table 1, revealed a steady increase in calcium concentration throughout the 3 hours of cremation. This is consistent with the breakdown of the bone discussed previously. The slight decrease in calcium concentration between 90 and 120 minutes could be due to a decrease in the amount of calcium being removed from the bone while the sample collection removed the calcium from the solution. Another reason for this decrease can be the formation of a Ca(OH)<sub>2</sub> precipitate. The acidification process was used to dissociate the calcium and hydroxide, releasing the calcium back into solution for analysis. If the acidification did not release all of the calcium from the precipitate, a lower absorbance, and therefore lower concentration of calcium, will be detected by the AA. This can be resolved by using a higher concentration of HNO<sub>3</sub> for acidification and will increase the amount of Ca(OH)<sub>2</sub> that is dissociated.

#### 4.3. Femur 3

In an attempt to fully cremate femur 3, the cremation time was increased to four hours. This resulted in the bone breakdown seen in Figure 5. The bone was broken into significantly more pieces during this cremation. The femur shaft remained intact but the condyles were completely destroyed, the remnants can be seen as the pile of bone in image (A). This was most likely due to the bone being thrown around in the solution and hitting other bones, as well as the sides of the pressure cooker, causing the already brittle bone to break apart much more easily. Similar evidence of bone breakdown was present on femur 3, as with femur 2, except it was more extensive in femur 3. In image (A) the discoloration covers all of the bones, with a small patch of the femur remaining a white color. Since the bone was in the KOH/H<sub>2</sub>O solution an additional hour, the blood within the bone was able to breakdown further in femur 4 than in femur 2.

In image (B) the discoloration is more concentrated in the lower half of the femur and the cracking appears almost identical to the cracks in femur 2, which extends to the edge of the discoloration. The cracking extends to the epiphyseal surface, which can be seen in image (C). The bone appears to be peeling away from the main shaft, extending laterally down the surface. All of the bones were extremely fragile and difficult to remove from the pressure cooker without causing additional damage. Unique to this bone was the condition of the femoral shaft. In femur 2, the shaft was very strong after three hours and appeared to not have been greatly impacted by the KOH/H<sub>2</sub>O solution. In femur 3, indents could be made in the shaft with a fingernail after removal from the solution. This is evidence of the solution breaching the top layers of the trabecular bone and beginning to break down the hydroxyapatite structure.



Femur 3 was left to dry for five days to observe the bone structure both wet and dry. Those images can be seen in Figure 6. All images shows a color change occurred during the drying process with the entire bone taking on a grey/tan coloring as it dried. This could be due to any remaining KOH/H<sub>2</sub>O solution drying on the outside of the bone while the inside remained pure white like in image (D). Images (A and B) show the distal end of the femur where the condyles were attached. The cracking that was previously present in the wet bone has become more prominent and bone appears to have broken off in some areas. Spongy bone can also be seen in the lower middle portion of the bone of image (A and B). This means the dense bone has broken down enough to leave the more pores bone underneath exposed or the basic solution ate away at the dense bone on top, leaving behind pockmarks on the surface to give the appearance of spongy bone.

The proximal end of the femur seen in image (C) shows cracking not previously present in the wet bone. The crack extends parallel to the femoral head on the posterior surface of the bone. Both ends of the bone were extremely fragile, and when squeezed, shattered with a little application of pressure, the results of which can be seen in image (E). The only part of the bone that remained intact was the central shaft seen in image (D). The central marrow channel was completely empty, indicating the marrow liquefied and leached out of the bone sometime during the cremation process and leaving behind the bone white coloring.

Full analysis of calcium, iron, and magnesium ion concentrations was performed for femur 4, resulting in Table 2. A graph of concentration vs. time can be seen in Figure 8 below, comparing the three metal ion concentrations throughout the cremation process.

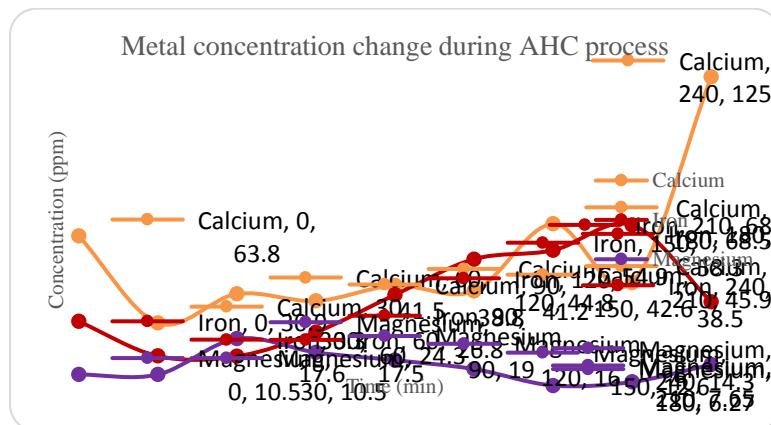


Figure 8: Graph comparing the concentrations of the three metals analyzed by atomic absorption spectroscopy.

The first trend that can be seen in the graph is the high initial concentration at 0 minutes. This can be due to the sample having been collected when the bone was placed in the KOH/H<sub>2</sub>O solution and began to decompose the little amount of soft tissue that remained on the bone. After 30 minutes, the calcium and iron levels began to climb steadily until calcium decreased at 210 minutes and iron decreased at 240 minutes. This shows both calcium and iron were steadily removed from the bone structure over the course of the cremation, which could have been one component of the bone breakdown.

A comparison of the calcium concentrations between femur 2 and 3 shows that the calcium was removed throughout the whole cremation processes but the final concentrations were strikingly different. Femur 2 had a significantly higher calcium concentration over the entire cremation process and had a continuous increase over the full 3 hours. This can be due to the small difference in linear regression equations for each of the calcium samples. Even though the concentrations of calcium do not match for both femur bones, the trend still remains that calcium is removed throughout the cremation process.

An interesting aspect of the iron concentration is a dramatic increase in iron between 90 and 120 minutes. This is most likely the point where the bone marrow was liquefied and removed from the marrow channel within the bone. Since bone marrow is rich in blood, the breakdown of the hemoglobin would have released a large amount of iron into the solution, increasing its concentration. After 120 minutes, the iron levels began to rise slowly again.

The third metal investigated, magnesium, had a very different trend in concentration. Instead of an increasing concentration line, the line slowly decreases over time after a peak at 60 minutes. This could be due to the magnesium not residing within the bone structure at all, but in the small amount of flesh present or even in the cartilage that decomposed rather quickly when exposed to the KOH/H<sub>2</sub>O solution. This is the opposite of what was expected since

the literature suggested magnesium played a key part in the stability of the bone crystal structure. Another reason for the low concentration could be the sensitivity of the instrument coupled with the sample being too diluted. The amount of magnesium in the diluted sample may have been within the sensitivity range of the instrument, causing it to register minimal changes in concentration over time.

Future work on this topic includes using scanning electron microscopy to obtain a visual representation of the inside of the bone structure before and after the cremation process. These images could provide additional evidence to determine what happens to the bone that causes it to breakdown. Additional cremations can also be performed for shorter time ranges to create a standardization of the bone breakdown at each stage of the cremation process as well as cremating fleshed specimens to see the variation in the process when flesh is added. Further investigating the trend seen in the magnesium sample can lend more insight into bone composition and structure. Research can also expand into other disciplines. Forensic anthropologists can analyze the bones to see what can be deciphered from them after cremation, environmental scientists can find ways to safely dispose of the alkaline solution, and mortuary scientists can further explore its use for full body cremations.

## 5. Conclusion

Three pig femur bones were cremated using the alkaline hydrolysis cremation process to varying degrees of success. The second femur had portions of bones which were able to be crushed without much force but did not achieve the complete cremation that was expected. The third femur reached full cremation where the bone, once dried, was crushed using only a strong squeeze of a hand. The analysis of the calcium, iron, and magnesium metals in the basic solution showed interesting results. The calcium and iron concentrations steadily increased throughout the entire AHC process but they decreased around the fourth hour of cremation. The results of magnesium were the opposite of what was expected. The magnesium concentration reached a maximum peak within 60 minutes of cremation and steadily decreased over the next three hours. This could be due to magnesium not being used internally within the bone structure as the literature suggested.

The successful cremation of two femur bones in four hours shows the ability to reduce the commercial process to a small scale apparatus that can be used in a lab setting. The results of the metal analysis show that the removal of calcium and iron are just one factor that contributes to bone breakdown.

## 6. Acknowledgements

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## 7. References

1. Pettitt, P. From funerary caching to the earliest burials of early *Homo sapiens*. In *The Paleolithic Origins of Human Burial*; Routledge: Milton Park, Abingdon, Oxon, 2011; 57-77.
2. "Statistics" 2016. National Funeral Directors Association. <http://www.nfda.org/news/statistics>. (accessed Oct 30, 2016).
3. Kim, M. "How Cremation Works" 31 March 2009. HowStuffWorks.com. <http://science.howstuffworks.com/cremation.htm> (accessed Oct 18, 2016).
4. Schvaneveldt, J.D. *Utah State University Extension*. **1989**, Paper 451, 1-3.
5. Rumble, H., Troyer, J., Walter, T., Woodthorpe, K. *Mortality*. **2014**, 19(3), 243-260.
6. Olson, P.R. *Science, Technology, & Human Values*. **2014**, 39(5), 666-693.
7. Baumen, P.A., Lawrence, L.A., Biesert, L., Dichtelmüller, H., Fabbriizzi, F., Gröner, A., Jorquera, J.I., Kempf, C., Kreil, T.R., von Hoegen, I., Pifat, D.Y., Petteway Jr, S.R., Cai, K. *Vox Sanguinis*. **2006**, 91, 34-40.
8. White, T.D., Black, M.T., Folkens, P.A. Bone Biology and Variation. In *Human Osteology*; Brown, L., Anderson, K., Eds.; Academic Press: Burlington, 2011; Third Edition, 25-42.
9. Maciejewska, K., Drazaga, Z., Kaszuba, M. *International Union of Biochemistry and Molecular Biology*. **2014**, 40(4), 425-435.

10. Ren, F., Lu, X., Leng, Y. *Journal of the Mechanical Behavior of Biomedical Materials*. **2013**, 26, 59-67.
11. Vallet-Regí, M., González-Calbet, J.M. *Progress in Solid State Chemistry*. **2004**, 32, 1-31.
12. Boivin, G. *Medicographia*. **2007**, 29(2), 126-132.
13. Castiglioni, S., Cazzaniga, A., Albisetti, W., Maier, J.A.M. *Nutrients*. **2013**, 5, 3022-3033.
14. Lemberg, R., Wyndham, R.A. *Biochem J*. **1936**, 30(7), 1147-1170.