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Macromorphological and Microscopic Effects of Temperature in a Controlled Environment on the Process of Decomposition in Bone

By

A. Marie Cato

A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

In

Applied Anthropology

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Macromorphological and Microscopic Effects of Temperature in a Controlled Environment on the Process of Decomposition in Bone

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This thesis has been examined and approved by the following members of the student's committee.

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Committee Member

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Abstract

This research explores the idea that the processes and rate of bone decomposition are affected by differential temperatures after death. Previous research supports changes in the molecular structure of bone due to different weather conditions, in addition to gross macroscopic changes, but there remain issues in understanding how these possible changes affect decomposition overall. In this research, I will explore how the freeze-thaw cycle affects decomposition, and what the relationship between weathering on bone and the natural decomposition process is. My hypothesis is that the freeze-thaw cycle will induce further decay, steady higher temperatures will speed up decomposition, and freezing will slow decomposition. My control sample will act as a measure to evaluate the role that weather (temperature) plays in decomposition.

Acknowledgements

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Introduction

The post-mortem interval (PMI) is one of the most important features of a death investigation. The time passed since a person's death can shape the direction of how an investigation will be conducted. Conversely, many factors can affect the PMI of an individual. One of the many factors that will be addressed in this study are the effects that different weather patterns have on bone decomposition. Bone decomposition is one characteristic of overall decay, like the decomposition of flesh and other parts of the body. The rate at which bones begin to decay can inform the forensic scientist about the possible time of death. If the PMI has been affected by outside factors, that do not occur naturally within the human body, that can affect the accurate estimate of time since death (PMI). This study is an extension of my earlier study done on weathering and trauma ("The Effects of Sharp and Blunt Trauma on Bone in Summer and Winter Weathering Patterns"). This study further explores the macroscopic and microscopic changes that occur in decomposition in bone.

There is a plethora of literature out there addressing the effects that warm temperatures have on the decompositional process of bone and soft tissue. This is, in part, due to the person who first began to collect data on human decomposition, Dr. William M. Bass, who created the "body farm" at the Anthropological Research Facility at UT-Knoxville. When he started to publish on his findings concerning the human decomposition process, other researchers began to investigate different aspects of decomposition in warmer climates (Hiller et al. 2003; Janjua et al. 2008; Keough et al. 2015; Hiller et al. 2003; Fernandez et al. 2013; and Ross et al. 2011). As this topic grew in popularity, other researchers began to explore the changes that may occur in other climates and in different environmental conditions (Hale and Ross 2016; Turnip 2017; Tersigni 2007; Calce et al. 2007; Boaks et al.2014; Cross et al. 2010; and Bartlett 2015). Even though the

literature has expanded immensely on this topic, there are still areas of this research that have lacunae where new findings can have a positive impact on how forensic scientists can assess changes that occur when bone begins to decompose.

This research utilizes a laboratory-controlled environment to simulate weather temperatures that can be found in the natural environment. The femoral samples used in this study all come from swine (*Sus scrofa*) carcasses that were collected from a local butcher shop (Clancy's Meat & Fish, 4307 S. Upton Ave., Minneapolis, MN). This was a six-week study that observed the macroscopic and microscopic changes that occurred throughout the study. This study and its findings are important for articulating the in-depth comparative differences that occur in bone when exposed to freezing, heat, and freeze-thaw cycles in an experiment simulating weather-related effects on decomposition in bone

Theoretical Framework

My study follows the theoretical framework of the scientific method. I will formulate a hypothesis that I then plan to test in a laboratory setting. The scientific method is as follows: making an observation, thinking of questions, creating a hypothesis, developing testable predictions, gathering data, and developing a theory. My observations come from my previous study and the examination of research articles by those who have done similar research on the topic. There is not much research out there on the freeze-thaw cycle itself, but researchers have found that bone decomposition speeds up in warmer weather and slows down in colder weather. It is still widely unknown how bone reacts when it has been frozen and thawed in a cycle, which is what I plan to clarify. If I can get a clear understanding of how bone decomposes and reacts to the freeze-thaw method, then this research can perhaps help with the estimation of postmortem interval when studying remains that have been subject to these conditions.

Background and History

This research will help provide a better understanding of how bone reacts to different weather conditions (hot/warm, cold, freeze-thaw), which in turn can give a more accurate determination of the postmortem interval of bodies recovered in these conditions. My study will follow the theoretical framework of the scientific method and the foundation of research done within the topic of human decomposition.

Background Information

The background information provided here is to show, very broadly, details concerning the structure of bone, the identification of skeletal trauma, taphonomy, and other variables that affect decomposition. This background will provide a much clearer understanding of the inner workings of bone, as well as the environmental variables that play a part in this research.

Bone is a very complex material and can show significant variation between species and individuals. White and colleagues (2012) state that bone is one of the strongest biological materials in existence, in terms of bearing weight and overall imperviousness. This is illustrated by the fact that even after cremation at high and prolonged temperatures bone fragments are still very much present after the procedure, as it takes a great deal of heat before bones become ash. Bone is composed of a protein called collagen and a mineral known as hydroxyapatite. This composition allows bone to be able to reshape and react to any stress, as does any living tissue. Bone is a part of the musculoskeletal system, so it routinely resists compression, tension, shear, and bending (Klein 2014, White et al. 2012, DiGangi and Moore 2013, Clines et al. 2014). When looking at the gross level of bone in an adult skeleton, there are two different types of bone present-cortical and trabecular bone. Cortical bone is found on the inside of the bone's inner surface near the medullary cavity, wherein lies the marrow, as well as on the external surfaces of

bone. Trabecular or spongy bone is located on the inside of the bone; in long bones, it is found closer to the epiphyseal ends of the bone. The differences in the compositions of bone are their density; cortical bone is much denser and more compact than trabecular bone, which is very thin and web-like in texture.

Numerous studies detail the inner structure of the bone (Jowsey 1966; Currey 2002; White et al. 2012; DiGangi and Moore 2013, among others). Additionally, DiGangi and Moore (2013) in their section on histology go into further detail when discussing the structure and modeling of bone. They note the different forms of bone and how they perform in the body. They discuss the three primary types of bone that can be deposited during the remodeling process. Primary lamellar bone is organized in a circumferential pattern between the periosteal and endosteal layers of bone (DiGangi and Moore 2013, 365). The next is plexiform bone, which is a significant focus of this study; it is often seen in larger, faster-growing mammals than humans, including cows, sheep, or deer. It is very rarely seen in humans, and if present, it occurs in younger individuals. It is characterized by having a rectangular, brick-like shape as it stems from mineral beds that grow perpendicular and then parallel to the outer edge of the bone surface (DiGangi and Moore 2013, 365). The third type is primary osteonal bone, which is comprised of circular or concentric layers of lamellae bone surrounding a vascular canal. DiGangi and Moore (2013) and Tersigni-Tarrant (2012) both note that much of the information about histology in anthropology is used in terms of aging and sexing individuals at their time of death.

Currey (2002), Jowsey (1966), and DiGangi & Moore (2013) all discuss the Haversian structure. Even more, Currey (2002) specifically notes that lamellar bone exists in a separate form as Haversian systems or secondary osteons (15). Haversian systems (Haversian bone) form when “bone-destroying cells, called osteoclasts, move forward in a concerted attack on the bone tissue” (Curry 2002; 14). He describes their shape as a “cutting cone,”, noting that “osteoclasts

are not derived from cells that occur locally, but instead come from cells circulating in the blood. As the cutting cone advances, it leaves a cylindrical cavity of diameter about 200 μ m behind" (Curry 2002; 14). The Haversian bone is created as a result of remodeling; the end result is bone that has cylindrical layers and a central cavity where one or two blood vessels or nerves run. Haversian systems are secondary, and they replace bone that has already previously existed; this is why Haversian bone is only found in older remains rather than younger individuals.

Bell (1990) addressed the issue of diagenetic change in human skeletal materials. She used a qualitative assessment of bone density changes using a scanning electron microscope (SEM), while Tersigni-Tarrant (2012) analyzed bone changes using light microscopic analysis. Both researchers looked at both standard and pathological changes that may cause misinterpretation. Bell (1990) explained that there needs to be extensive research into osteonal canals and osteocyte lacunae to fully understand how these different networks of bone play a role in the changes that occur in human bone after death. She does not directly state which type of bone she is working with, but she does note that the samples were taken from adult human femora and tibiae. She is most likely working with both cortical and trabecular bone. The results indicate that the changes are not random and correlate to the natural structure of lamellar bone, which confirms the view of other authors cited in her study. Moreover, her research recognizes that dismissing structural changes due to diagenesis can affect the perception of pathologies.

Another study conducted by Bell et al. (1996) evaluated the potential speed of postmortem changes in the microstructure of skeletal remains by examining human material that was drawn from different environmental contexts. The environments that the remains were derived from were wet coastal, dry/cold, dry, mild/wet/coastal, and the intertidal zone of salt water. Their results found that post mortem alterations occur very soon after death; however, how quickly this happens still needs to be investigated. Whereas, Keough et al. (2017) show that

early stages of decomposition in pigs and humans vary, so the rate at which these alterations occur needs to be taken into account.

Although my study does not involve any form of trauma, understanding temperature changes in bone and their resulting damage will provide helpful information for cases that involve trauma. Kroman and colleagues (2013), Forensic Anthropology (2013), and Boer et al. (2016) provide information on the biometrical properties of bone, as well as the basic concepts of bone trauma. Trauma can be inflicted by external forces such as blows or projectiles, but also occurs more naturally through falls or sudden compression. It can occur earlier in life (antemortem), at or around the time of death (perimortem), or after death (postmortem). Another study by Bell and colleagues (1996) evaluated the potential speed of post mortem changes in the microstructure of skeletal remains by examining human material that was drawn from different environmental contexts. Their results found that post mortem alterations occur very soon after death with remains in wet coastal areas, with dry/cold mild/wet/coastal environments exhibiting surface decomposition of soft tissue, while in dry environments the decomposition was turned to skeletalization, and the intertidal zone of salt water showed complete defleshing of remains. However, how quickly this happens still needs to be investigated.

Other studies conducted by Mann and colleagues (1990), Rodriguez and Bass (1985), and Megyesi (2001) provide insight into the many different variables that play a part in decomposition. The key variables they note are temperature, humidity/aridity, rainfall, soil PH, trauma to the body, access of the body to insects, burial and depth, carnivore and rodent activity, size and weight of the body, surface the body is on, clothing, and embalming. Overall, they show that the rate of bodily decay is variable and that temperature plays a large role in that variation. The current study hopes to elucidate the varying role played by temperature in the process of decomposition. In many forensic cases, bone may display both weathering damage and trauma.

Depending on the type of damage that is present, it can be difficult to discern the two. So, understanding how bone changes in differing weather conditions can bring about a better understanding of the differences between trauma and postmortem damage. Ubelaker (1997) also discusses the importance of the applications of taphonomy in forensic anthropology. Taphonomy is the study of what happens to organisms after death. This discipline works hand in hand with forensic anthropology and has direct application to this study of bone decomposition and temperature. Ubelaker (1997) states, "In the taphonomic process, weathering represents the response of bone to its immediate environment, *e.g.*, soil, sun, etc. as opposed to carnivore modifications, trampling, fluvial transport, and geochemical changes" (79). Damann and colleagues (2015) further discuss this importance, especially in the case of bacterial development associated with dead bodies. It was observed that "partially skeletonized samples maintained a presence of bacteria often associated with the human gut, whereas the bacterial composition of dry skeletal remains maintained a community profile similar to soil communities" (849). The study of taphonomy applies to forensic areas of interest concerning the estimation of the postmortem interval, environmental reconstruction, reconstruction of postmortem events, and distinguishing evidence of alterations caused by other taphonomic factors. The ability to separate these factors is vital during criminal investigations.

Although there are many biological aspects of bone that I will touch on that may play a part in the decompositional process of bone, I will also briefly mention the biological effects of soft tissue on bone decomposition in this study. Since soft tissue is present in this study, it is a factor that may or may not affect the decomposition of bone. This study looks at the effects of drying, decay, freezing, and thawing on tissue. The presence of soft tissue may invite bacteria to the sample that may or may not have a significant impact on the study results.

<u>Early Studies</u>

One of the first researchers to study decomposition was Dr. William M. Bass, who in 1971 created the Anthropological Research Facility at UT-Knoxville (the first "body farm") to allow anthropologists to assess how different variables, such as body weight, burial vs. surface disposal, sun/shade, clothing types, and many more, affected the decomposition process (Sharanowski et al. 2019). Researchers like Mann & Meadows (1990) continued with further research on body decomposition. Heynes' (1991) review shows that using animal and human remains in comparison for study can be used to show postmortem changes; it also highlighted the importance of the taphonomic processes in creating further changes in human and animal bone. As this research gained more popularity and different environmental circumstances in criminal cases emerged, understanding how bodies decompose in different weather conditions became essential in identifying an accurate time-since-death. Much of the earlier literature was concerned with decomposition in warmer climates, with Galloway and colleagues (1989), Shean and colleagues (1993), Mann and Bass (1990), Mann and colleagues (1990), and Tappen (1994) being some of the first to conduct research in this area. Komar (1998) was, arguably, the first to begin looking at bone decomposition in natural cold weather climates. It is only recently that freeze-thaw has been studied in much more depth (Pokines 2016), but there have been other attempts in the past, including research by Micozzi (1986) on the effects of colder temperature on decomposition in bone.

Cold Weather Studies

Cold weather studies did not gain popularity until much more recently. Although there are studies that appear as early as Micozzi (1986), around the time when studying decomposition in bones first began, much of the bulk of the research on this topic occurred in the 2000s. One of the first modern studies of frozen human bone comes from Tersigni (2007) who provides information about how bone reacts in cold weather and what that can tell a researcher about the rate of decomposition in cold weather conditions. This study led to further work in the field, and more studies are attempting to gain further information on how this process exactly works. The effects of freezing on bone have been studied by a number of researchers (Tersigni 2007; Hale and Ross 2016; Turnip 2017; Pokines et al. 2016; Meyer et al. 2013; Roberts et al. 2015; and Micozzi 1986). Most have reached similar conclusions, including that there are changes in bone density and that breaks or cracks appear in bone that severely damage the surface, and potentially the internal composition when these surface cracks are present.

Additionally, there has been new information presented when looking at freeze-thaw cycles. The observation of the freeze-thaw cycle is a much more recent area of forensic anthropological research in areas where freezing temperatures coexist with increases in temperature to above freezing. One common finding in much of the literature (*e.g.*, Pokines et al. 2016) is that the damage exhibited in remains is a speedy process that usually happens within the first few weeks of the freeze-thaw cycle. Much later, there aren't many changes that occur to the bones as the cycle continues. Further, Pokines and colleagues (2016) found that freeze-thaw samples exhibited macroscopic penetrative cracks that pierced through to the bone marrow cavity and that also caused damage to the internal bone composition. There is still little literature on this topic, but the findings that have been presented to date indicate notable differences can

occur in the freeze-thaw condition versus changes in remains subjected only to freezing conditions.

The observation of changes that occur microscopically is an essential piece of this study Tersigni (2007) discusses microscopic changes of bone following freezing. She examined her materials at various levels of magnification. Statistical analysis of the histomorphometric values indicated there were no significant differences in the size of the Haversian canals following freezing. Tersigni (2007) notes that the changes she found were not statistically substantial enough to suggest that freezing causes these damages, but she did stress that the damages found should be taken into account. There needs to be further research to determine the exact cause of these damages and what effects freezing actually has on bone.

Moreover, studies by Micozzi (1986) and Pokines and colleagues (2016) on the freeze-thaw phenomena both show accelerated rates of decay. Micozzi (1986) especially saw, in the microscopic examination, that extensive decay was present. Additionally, these remains were more susceptible to invasion by microorganisms from the outside, which can also accelerate the decaying process. Micozzi (1986) points out that the freeze-thaw cycle in postmortem decomposition occurs from the "outside-in" (predominantly decay), meaning that decomposition starts on the outside and works its way to the inner surface. This is different from other remains where postmortem decomposition occurs from the “inside-out,” indicating a different process in decomposition.

Warm Weather Studies

There is much more research done on the decomposition of remains in warmer weather; part of this has to do with the fact that significantly more remains have been found in warmer weather climates. Standard features that are common in most research on remains in a warm climate are drying of soft tissue and discoloration of bone and soft tissue (Dautartas et al. 2018; Parks 2011; Suckling et al. 2016; Mann et al. 1990; Tappen 1994; Hiller et al. 2003; Janjua et al. 2008; Keough et al. 2015; and Fernandez et al. 2013); all note visible macroscopic changes in warm weather samples.

The macroscopic changes that these authors collectively observed occur soon after exposure to warm or hot temperatures; specifically, they may include discoloration, skin slippage, appearance of greasiness, cracking and flaking of cortical bone, color change, charring (black in color), calcine (grey/white/blue/ash-brown color), brown burn (brown discoloration), heat border (off-white yellowish border located between charred and unaltered bone), heat line (a thin, whitish line directly adjacent to the heat border), delineation, greasy bone, joint shielding, predictable cracking (small heat fractures parallel to heat border), minimal cracking, delamination, heat-induced fractures, and drying of soft tissue (mummification); all are dependent upon the amount of heat to which the bone is exposed. Hiller et al. (2003) and Fernandez et al. (2013) recorded microscopic changes that occur in extreme heating instances. Although my study has a maximum temperature of 36 degrees Celsius (96.8 degrees Fahrenheit), the changes exhibited in these maximum heating situations is important to note to see if they occur in any other forms of weathering. Their findings show that the crystalline structure of bone changes (hydroxyapatite). In Fernandez et al. (2013) the hydroxyapatite can withstand temperatures up to 800°C before the large crystalline structures are no longer identifiable. Hiller et al. (2003) explain this phenomenon as having to do with the sintering process when heating

that causes the hydroxyapatite crystals to form a specific shape and size. On the other hand, Fernandez et al. (2013) describes this phenomenon as happening due to the compounds derived from the hydroxyapatite thermal hydrolysis, which results in retraction, fracture, Haversian canals bursting, and cluster formation. Overall, extreme heat has a diminishing effect on the microscopic structure of bone, to the point where the structure is no longer recognizable. In conclusion, high temperatures can be severely damaging to bone.

Parks (2011) provides an outline for the different stages of decomposition that can be expected to result from temperatures occurring in the southwestern United States. She identifies the fresh stage, early decomposition stage, advanced decomposition stage, mummification stage, skeletalization stage, skeletal bleaching stage, and skeletal exfoliation stage. She identified time frames associated with each stage, giving a detailed description of each. Many of the macroscopic changes listed above were identical to the changes listed by Parks (2011) in her research. The outline provided by Park (2011) assists with the determination of the PMI and identifying the changes that can be found within a certain period of time for each stage of decomposition.

In addition to the heat-related changes that Janjua and colleagues (2008) noted as occuring in bone, they also support the notion of using femora as a reliable source for clearly exhibiting patterns of weathering. This is especially important and significant to note in the case of this research because all samples used were femora. This suggests that the use of this bone will provide accurate information pertaining to warm-temperature weathering, as well as other forms of weathering on the bone.

Other Decompositional Studies

There are many other studies out there that are concerned with the effects of both warm and cold weather studies, but are very specialized. Many of these studies would be much harder to replicate and cannot be broadly applied to any one area of warm or cold weather studies. Although, these studies do provide valuable information for understanding decompositional patterns in different circumstances that may help explicate the patterns that I see in my samples.

Boaks and colleagues (2014) investigated the process of decomposition. They found that following soft tissue decomposition collagen was lost at the periosteal and endosteal surfaces. They further note that bacterial activity could have led to further destruction of the bone, leading to significant changes over a 2 to 12-month period. Much of their research was not directed towards PMI estimation, but was useful for detecting changes in the concentration of protein in bone. A study by Walden and colleagues (2018) showed that barium, calcium, iron, potassium, magnesium, zinc and phosphorus demonstrate elemental changes during the early stages of decomposition. Even though they did not note any changes affected by temperature this study does speak to changes in bone at the microscopic level.

A number of additional specific studies focus on buried bodies and the rate of decomposition in warm weather climates (Kelly et al. 2009; Rodriguez & Bass 1985; Eline et al. 2011). Although these studies are not specifically relevant to my research, they do suggest that buried bodies show similar decompositional patterns to that of non-buried remains (albeit at a much slower rate); this then affects the determination of time since death. These patterns include maggot activity, decomposition of soft tissue, bloating, etc. Megyesi and colleagues (2005) further discuss the importance of PMI in the case of human remains and how it can be disrupted due to temperature. Ceciliasona and colleagues (2017) studied postmortem changes within a closed environment; they found that estimating PMI in outdoor and indoor conditions is

different, but overall yields similar results. Humidity can, however, play as significant a role as temperature in the process of decomposition. Sharanowski and colleagues (2019) found that more moisture in the air indicates a higher probability of rotting, while aridity often leads to mummification. Additional specific studies focus on buried bodies and the rate of decomposition in warmer weather patterns (Kelly et al. 2009, Rodriguez & Bass 1985, Eline et al. 2011). Sharanowski et al. (2019) show that there are specific reasons for why we may see mummification of remains and rotting of others; humidity is a significant factor in why we see these differences occur, as more moisture in the air means a higher probability of rotting than in a much more arid environmental condition.

These specific studies may not have any direct link to the research in this study but do provide further information about different factors that can play a part in decomposition, as well as how the process of decomposition advances. They also illustrate situations that are commonly encountered within forensic anthropological research and practice.

Methodology

This study followed methodology inspired by Tersini (2007), Ross (2011), and Turpin (2017). These researchers help set a foundation for the type of research I conducted and what elements to use to achieve desired results. Four swine (*Sus scrofa*) femora were collected from Clancy's Meat & Fish, 4307 S. Upton Ave., Minneapolis, MN and transported to Mankato, MN on June 28th, 2018 at 10:30am. The femora were then placed in a controlled lab setting at three different temperatures for study for a period of six weeks. One bone was placed in an incubator at 36 degrees Celsius, one in a freezer at -1.1 degrees Celsius, and one in a standard lab setting temperature of 21 degrees Celsius. The fourth bone alternated between the freezer (-1.1 degrees Celsius) and sitting out in the lab at a temperature of 21 degrees Celsius on a weekly basis. This six-week study was undertaken to observe the microscopic and macroscopic changes in the bone brought about by the decompositional process. To understand and document the microscopic changes, thin sections of the bone were taken from the proximal end on the day the bone entered the lab, and then again after the six-week experiment had concluded. The purpose of this will be to have before and after microscopic photos of the bone, prior to environmental destruction.

The bones were examined for four different types of damage: freeze-thaw cycle damage (alternating freezer and lab setting), warm weather (incubator) damage, freezing weather (freezer) damage, and lab setting damage (for control). The freeze-thaw cycle involved the bone being frozen for a week and thawed out for a week; altogether, the bone was frozen for three weeks of the time, while the other three weeks the bone was in a state of thawing. Although this was a short period of time, this is appropriate for the assessing the initial differences in decompositional changes. Moreover, the incubated femur remained in the incubator for the full six weeks, and the same goes for the lab temperature and freezer femora.

Over the six-week study, the bones were examined macroscopically before microscopic examination began. Once a week for six weeks, the bones were examined on all sides (left, right, proximal, and distal), while also looking exceptionally close at the articular ends of the femora. Observations were recorded starting June 2nd and persisted through to August 6th. Thin sections were collected the following week of August the 14th. The observations were recorded during the early afternoon. Photos were taken on July 24th, July 30th, and August 6th of 2018 of all examined sides of bone. Approximately 25 photos were taken during the last three weeks of the experiment of the macroscopic changes. The bones were then taken to processing for thin sections.

After the bones had undergone their various treatments for the full six-week study, the samples were transported to lab space in the Department of Geology for cross-sectioning and preparation for thin sectioning. The bones were cut on the proximal ends of the femora, to ensure that I was collecting both compact and cancellous bone. A tile saw was used to take bigger sections from the shaft of the femora to use for collecting thin sections. There were two cross-sectioned samples collected from each category of bone treatment (*i.e.,* heat, cold, freeze-thaw, control), leaving me with eight half-inch samples. After the samples were cut, they were polished down on a potter's wheel facing their cross-sectional surfaces. The wheel was prepared with water and by sprinkling a 600-level granite (a very finely grained granite similar to sandpaper) on the wheel’s surface. There was no set amount of granite used during this process since the process comes down to getting a flat surface on either side of the cross-sections. Depending on how well the sections were cut with the tile saw, one sample might need more or less processing than another. The sections were held with light pressure against the wheel for sanding while being moved back and forth at a 90-degree angle. The wheel was sprayed down with water, and more granite was added as needed. This was done until the bone’s surface appeared smooth and

glass-like. The samples were then taken to be cleaned and placed in an incubator at 34 degrees Celsius.

After processing, the samples were cleaned with Alconox, a powerful cleanser, and a toothbrush. This cleanser was mixed with water to remove any granite that remained on the surface or in the crevices of the bone. I had to be sure that no granite particles were remaining in or on the surface of the bone. The samples were also placed under a microscope and tweezers were used to pick out any remaining particles the Alconox treatment may have missed. There was still a fair amount of soft tissue in and around the bone; the bone marrow was left in place, while the soft tissue around the bone was removed with pinching pliers. The samples were then dried in a 34 degrees Celsius oven on aluminum trays (aluminum foil). After 36 hours, the impregnation process began.

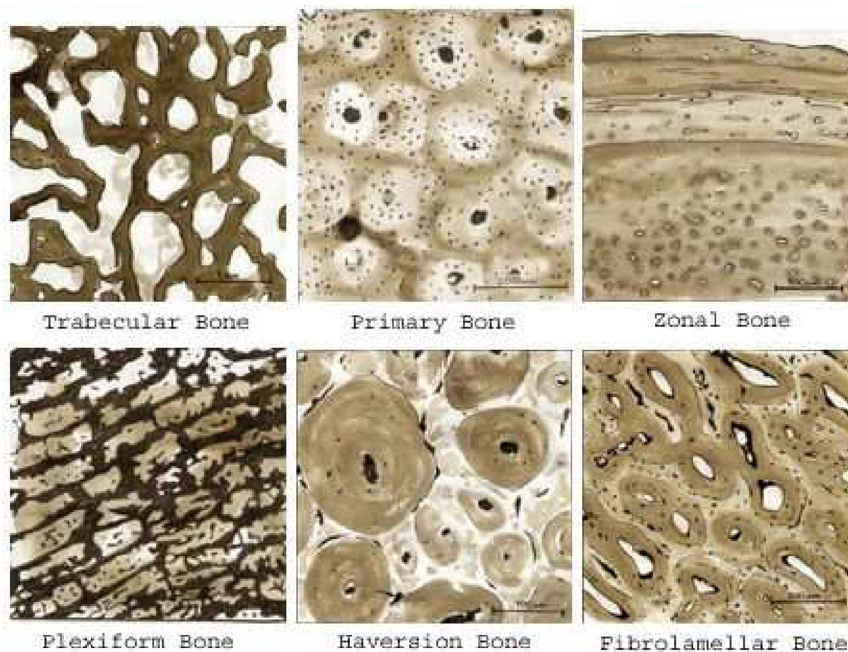
The impregnation process draws epoxy into the spaces on the inside of the bone through a vacuum. A makeshift vacuum was made out of rubber tubing and a large glass jar. There are tiny pebbles (desiccant, which acts to suck moisture out) at the bottom of the jar and a large plastic tray inside the jar. Moreover, a high vacuum sealant (shin-etsu silicone) was used on the rim of the glass jar to help keep a solid seal. The glass jar was attached to a pressure valve to monitor the pressure being added. Before the samples were added to the vacuum, the epoxy was spread over the surface and around the edges of each sample. The epoxy used was Hysol Loctite; this epoxy contains one-part hardener and one-part resin. In order to create the right amount of epoxy to bone ratio, there needs to be enough epoxy created to fill up the tray or "work" area that the bones are placed in. There was no measurable amount added; there was only enough resin added to cover the surface area of the sample (approximately 3-5 millimeters in height). After the epoxy was added to the samples, they were placed back in the 34 degrees Celsius oven for 48+ hours to allow the epoxy to dry.

Once the epoxy was completely dried, the samples were taken back to the wheel to be polished down with 600 granite grit. The desired effect is to have the epoxy fill all the openings within and on the bone for the smooth surface to stick onto the glass slides. For many of the samples that were treated with epoxy, there was an abundance of epoxy on the surface that had to be removed before placing them on the glass slides. For many of the samples that had been weather treated, #61 and #200 granite was implemented in order to remove the large amount of epoxy that covered the surface. This was in an effort to speed up the processing time of removing the epoxy. Once the epoxy was polished down close enough to the surface of the bone, tin oxide and rapid polish granite was used to polish the surface of the bone. After all the samples were treated, they were placed to dry in a 36 degrees Celsius oven for 24hrs.

After the samples were dry, more epoxy was mixed and used to place the samples on the glass slides, and they were put into a 34 degrees Celsius oven to dry for 48+ hours. When the samples were dry on the glass slides, they were taken to the thin sectioning machine to cut thin sections on the glass slides. This was the procedure prior to the breaking of the thin sectioning machine in the Geology Department. After the loss of this machine, the remainder of the samples were shipped to National Petrographic Service, for further epoxy and thin sectioning processing. When the samples were shipped back, they were then analyzed on a Meiji microscope and photos were taken using a Canon EOS Rebel T2i. The Scanning Electron Microscope (SEM) was also used to take photos for a more in-depth analysis.

Results

When the experiment concluded, over 50 photos were taken of the microscopic changes that occurred over the six-week period. The parts of bone that will be discussed are cortical bone, trabecular bone, and plexiform bone. It is important to note here that plexiform bone is not found in humans and is exclusive to animals such as cow or sheep. The Haversian structure is a feature of cortical bone that can be viewed at a microscopic level. Since there were two different sets of data collected for the samples, the results section will be split into addressing the macroscopic and microscopic results. It should be noted that the bones were not placed into their simulated weather conditions until three days after I received them; however, this did not seem to affect the bones much at all, at least not macroscopically. Prior to taking them into the lab, they were kept in a refrigerator unit set to 37□. At the time the bones were placed in their simulated weather conditions, they still had a fresh appearance to them (pearly white and bloody). There was still a layer of muscle and some tendon on the bone at the time of placement, but the bone surface was visible.



(Figure 1: Example of different forms of bone)

There were two samples taken from each weather simulation category (control, heat, freeze, and free-thaw) as can be seen in Figure 2. Each of these samples seen below is approximately 2 ½ to 3 millimeters in width, which varies from sample to sample. As shown in Figure 2, the medullar cavity varies in size depending on the sample. Measurements in the table below were taken from samples in order from upper left to lower right. All measurements are of the maximum diameters in each direction in millimeters. In the photo, the thin sections are oriented with their anterior aspect towards the top of the photo and their posterior section to the bottom.

(Figure 2: From upper left to lower right: Frozen sample, Pre-control sample 1, Freeze-thaw sample, Control sample, Pre-control sample 2, Heat-simulated sample)

	Frozen	Pre-Control 1	Freeze-thaw	Control	Pre-Control 2	Heat-simulated
Sample 1	Height: 4.3 Width: 3	Height: 4.3 Width: 3.2	Height: 3.9 Width: 3	Height: 4.1 Width: 3.1	These samples are duplicates of Pre-control 1	Height: 4 Width: 3
Sample 2	At the end of study, the second sample was not available	Height: 4.6 Width: 3	Height: 4 Width: 2.6	Height: 4.5 Width: 3	These samples are duplicates of Pre-control 1	Height: 4.6 Width: 2.9

(Figure 3: Sizes of samples listed in order from top left to bottom right)

The samples were oriented in the fashion presented in Figure 2; the photos were taken with the area with the smallest surface area orientated in the north position, and the area with the largest surface area orientated in the south position. The photos were taken of each sample around the entire available surface of the bone, including the anterior, posterior, medial, and lateral sides, as well as those areas of the cross-section in between. Depending on the sample and side, this did affect how many photos I was able to take of the samples. One photo was taken of all the sides listed above. The purpose of taking photos of all sides was to see the distribution of changes throughout the bone and the different kinds of changes that can be observed throughout the bone. For the most part, the majority of the changes exhibited were consistent through all samples.

Macroscopic

Pre-Experiment Control

These samples were not subjected to weather treatment and did not show any discernible signs of decomposition or damage prior to the start of the experiment. The samples were immediately taken to be sectioned for microscopic examination and were then disposed of. These samples appeared as any bone would immediately following death, having a pearly white appearance with fresh tissue on the surface of the bone. These samples were removed at the very start of the experiment and were not used for the rest of the experiment.

Control

After the first week, the control samples that were kept at the laboratory temperature of 21 degrees Celsius were observed to have started to decay; the soft tissue had become oily. Although these samples exhibited changes each week, it should be taken into consideration that the lack of insect activity would have a great effect on the rate and manner of decomposition.

The bone and tissue started to turn a brown color, and a strong odor began to emanate from them. Moreover, the blood vessels appeared to have dried up. After the second week, I observed that not much had changed with the soft tissue. The odor was still quite potent. The bone started to turn a greyish-white color and began to look very dry in texture. On the epiphyseal ends of the bone, the marrow seemed to start showing signs of decomposition.

After the third week of processing, the decomposition of both the soft tissue and bone began to look more extreme. The bone was now a grey/ashy color and the soft tissue started to look very discolored (brown/black/white in color). Further, an indentation began to form on the surface of the marrow at the cross-section at the end of the femur. The bone marrow was dark in color and looked granular in texture; it looked as if the bone marrow tissue wanted to escape its orifice. At weeks four, five and six, few additional changes were observed in the bone; instead

there was a progression of the changes observed in earlier weeks at this temperature. The marrow was a brownish color, and the internal surface began looking wider (larger) as the weeks progressed. This may be attributed to the bone marrow receding from the medullary cavity.

Warm Weather Simulation

Once the warm weather simulation samples were placed into the incubator at 36 degrees Celsius, these samples began to react very quickly. After the first week of being put into the incubator, the soft tissue began to gain an oily appearance and started to loosen from the bone. The smell immediately became quite pungent and could be smelled outside of the laboratory. Moreover, the bone began to turn brown. Following the second week, the strong odor persisted. The tissue went from an oily and wet texture to very stiff. Small brown/black dots started forming over the surface of the soft tissue. The bone itself did not appear to have changed much. In the third week, the bone began turning a dark brown/black color. The marrow during this stage still appeared to be more intact than that in the control samples. In the fourth week, the bone marrow and surrounding bone began to turn a black color and overall had a darker brown color to it than previously. In the fifth and sixth weeks, few changes were noted in the surrounding soft tissue. When thin sections were prepared for microscopic observation, the bone marrow was soft and fragmented in consistency. The medullary cavity of the bone where the marrow resides was now much more open in comparison to the other samples; *i.e.* the marrow had shrunk in size significantly. The marrow was also so soft that much of it fell out the medullary cavity during processing.

Frozen

The frozen bone showed minimal changes in terms of the decomposition of the bone and tissue itself during the entire six-week process, but there seem to be some notable changes that occurred in the bone during the freezing process. After the first week in the freezer (set to -1.1 degrees Celsius), crystals or frost had formed on the surface of the bone, on the bone marrow, and on the surface of the soft tissue. The bone still retained its pearly white appearance, and the blood vessels were frozen. Following the second week, the bone itself appeared to be completely frozen; as did all the blood and tissue. There was no distinct smell. The bone still retained its pearly white appearance, but an indentation began to appear on the surface of the bone marrow at the cross-section. This indentation may have been due to the bone marrow decomposing and collapsing away from the medullary cavity. After the third week, flaking of the soft tissue was present, as was a slight odor. The bone marrow indentation seemed to grow progressively a bit deeper and the marrow itself began to separate from the inner surface of the bone.

Moreover, after the fourth week, the bone marrow started changing in color, becoming darker. The soft tissue over the bone had taken on a very red appearance, but there were not many extreme changes to the bone. In the fifth week the bone showed a significant amount of frost collection; otherwise, minimal changes were noted from the previous weeks. The sixth week showed very few differences. When cutting into the bone, the marrow was very malleable as it started to thaw, similar to the fresh marrow seen originally in the bones immediately following death. The medullary cavity where the marrow resides was a small and tightly closed space. It looked as if the soft tissue inside had been undisturbed and was not subjected to putrefaction as seen in the warmer weather samples.

Freeze-thaw

In the first week after the samples were brought to the lab, they were first placed in the freezer at -1.1 degrees Celsius. After the first week of freezing, the bone mimicked the same results as the other frozen bone, with crystals forming on the soft tissue and bone, but with the bone retaining its prior pearly white appearance. After the first week of being placed under the fume hood at 21 degrees Celsius (week 2), changes quickly became apparent. Both the bone and soft tissue appear to have thawed and turned slightly brown in coloration. The soft tissue looked slightly oily and tough in texture. The bone began to become a white-greyish color; the color was unevenly distributed over the surface of the bone. This could be attributed to the freeze-thaw cycle causing some form of moisture and bacterial build up, which could lead to a mold or fungus growing on and within the bone. Further, the marrow started to show an indentation on the surface.

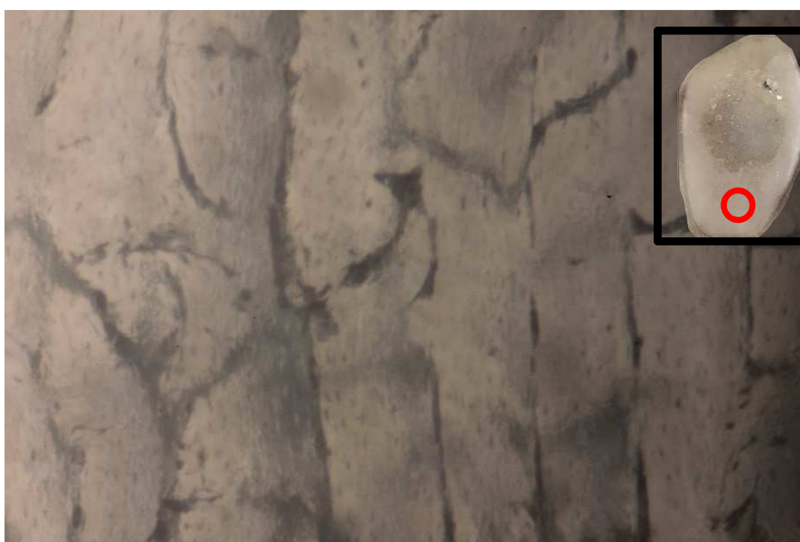
(Figure 4: Freeze-thaw Bone Sample)

The bone was placed back into the freezer for the third week, with the bone continuing to retain its whitish-grey color. A slight odor started to emanate from the samples, but the soft tissue had not changed in appearance much since being put back into the freezer, although the cross-sections of the bone showed the bone marrow to have turned brown/black in coloration, and an accumulation of frost/ice crystals had formed around the inside surface of the medullary cavity. There also seemed to be a clear line of demarcation between the cancellous bone, cortical bone, and the marrow (Figure 4). The photo gives a representation that shows darkening and frost separating these different parts of the bone. During the fourth week, the bone was left out again under the fume hood, and the following changes were observed. The bone itself was black near the medullary cavity of the bone but much lighter on the surface, which was in contrast a grey color. The odor was much more pronounced. The bone marrow had turned a much lighter color from that of the previous week and frost was apparent on both the marrow and the surfaces of the bone. The fifth week in the freezer did not yield many changes from the previous week, but there was a lot more frost build-up on the cross-sections and much more discoloration of the bone and soft tissue. After the sixth week under the fume hood the bone did not show any further changes, but when the thin-sections were made the bone marrow was transparent and had a firm consistency.

Microscopic

Pre-Experiment Control

These samples were not exposed to any simulated weather treatment and were removed prior to the start of the experiment. The samples show little to no damage to the internal structure. These samples serve as an example of how the plexiform and Haversian bone look prior to the commencement of decomposition. They provide a comparison for any changes that appear in the temperature-treated samples of the experiment.

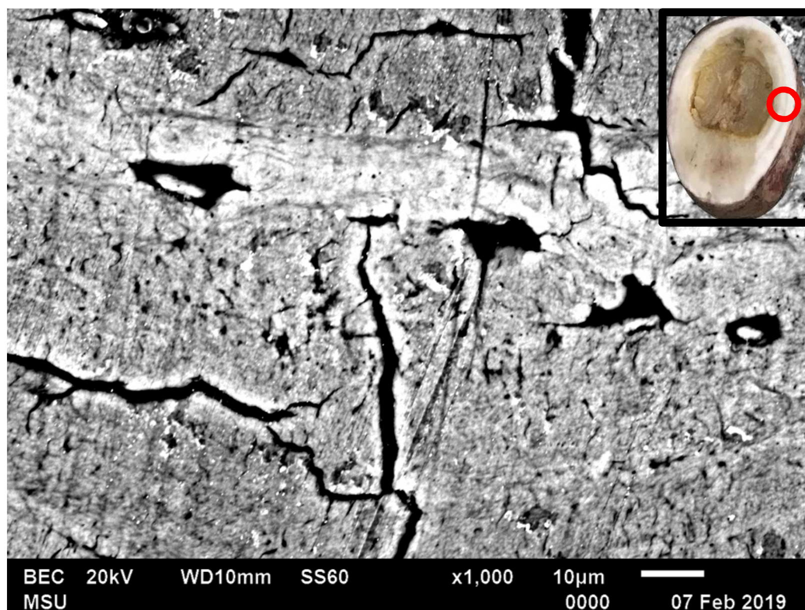


(Figure 5: Pre-Control Bone, cross-section, posterior aspect – Plexiform Structure)

Control

Two samples were taken from the laboratory control samples for examination under a standard microscope and the Scanning Electron Microscope (SEM). In both samples, the plexiform structure of the bone was still present. Also present were tiny lacunae or openings a within the bone. These small openings covered the surface of the bone and appeared in clusters rather than being evenly spread out. There was significant spacing between the openings within the bone. In some areas, like the middle portions of the samples, the spacing in between the

parallel brick plexiform structures seemed to have increased relative to those of the pre-experimental control samples. These samples looked very similar to the pre-experimental samples with these minimal changes.

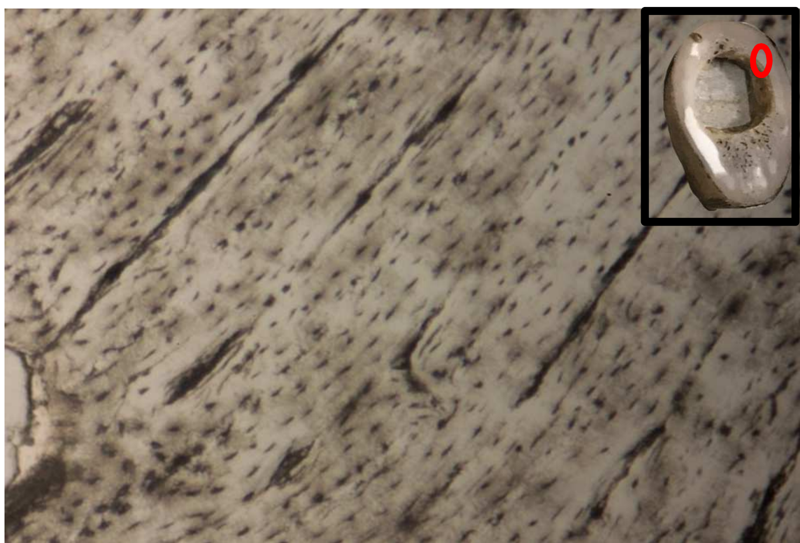


(Figure 6: Control Bone Sample 2 – cross-section, mediolateral aspect)

In the SEM photos taken at 1000x magnification, some fragmented areas on the surface of the bone are revealed, as well as some penetrative lacunae. The surface appears very textured. Preparation marks are present, but discernible as such. The lacunae on the surface are very short in length and do not appear very wide. These lacunae did not cover the entire surface of the bone. The openings were mostly to be seen on the posterior aspect of the cross-sectional samples (where there is much more surface area); however, there are some images of the posterior section where lacunae are not present.

<u>Warm Weather Simulation</u>

Few changes were noted in these samples, relative to the laboratory control samples, but there appears to be slightly more damage. These samples were exposed to warmer temperature levels (approximately 20-degrees Fahrenheit warmer) than the laboratory control samples. The same small openings appear in these two samples of bone as seen in the laboratory control samples, but are much more clustered and greater in number than the laboratory control samples. Also, the plexiform structure seems to have diminished within these samples; the structure is not as pronounced as it was in the laboratory control samples. The lines of demarcation (that express its brick-like structure) of the plexiform structure are still present, but they have become more difficult to identify because the structure has started to dissipate (Figure 7).



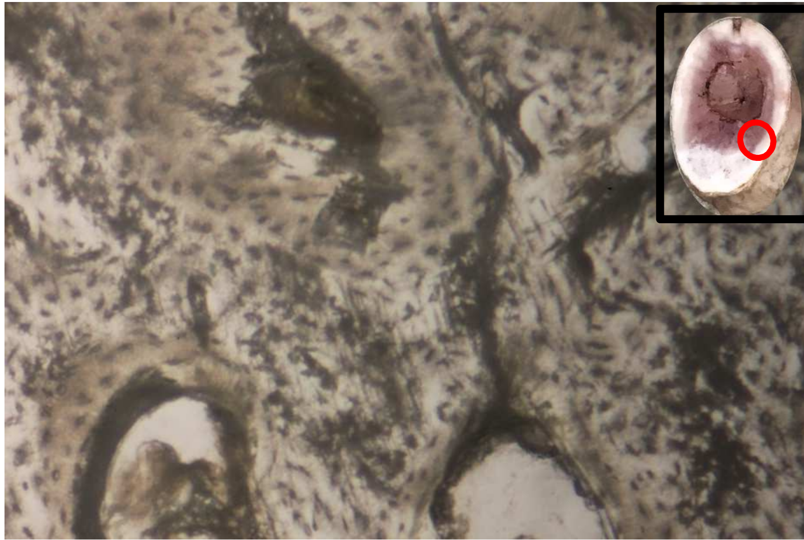
(Figure 7: Heat-simulated Bone, proximal/mediolateral aspect of Sample 1 – Plexiform Structure)

In addition to the small openings present in the bone, there are some definite areas of separation that have appeared in irregular regions of the bone. They seem to appear as a recession, areas of opening or missing areas of the bone that is small, but noticeable. They take

different shapes and sizes, but are not large to the point where they take up a significant amount of surface area.

Freeze

The results for the frozen bone come from only a single sample, but there are some differences associated with the frozen sample. Similar to the heated samples, the plexiform structure has been damaged and is almost unrecognizable. Even more noticeably, the plexiform structure looks almost as if it has been distorted; this seems to be more prevalent in the bottom portion of the sample, where there is more surface area of the bone present. The straight brick lines of the plexiform structure appear to take a different shape, and instead of coming out in straight lines they appear twisted (Figure 6). Further, the small dark openings are much more prevalent here and cover much more surface area than in the control and warm weather samples. There are some small regions in the anterior and the mediolateral aspects of the bone cross-section where the small dark openings do not cover as much surface area as they do in the posterior region. Also, the clear separations or recessions of bone (i.e. the lacunae within the bone) appear much larger in the frozen bone sample, especially in the areas where there is much more surface area (*i.e.* the posterior aspect of the cross-section sample).

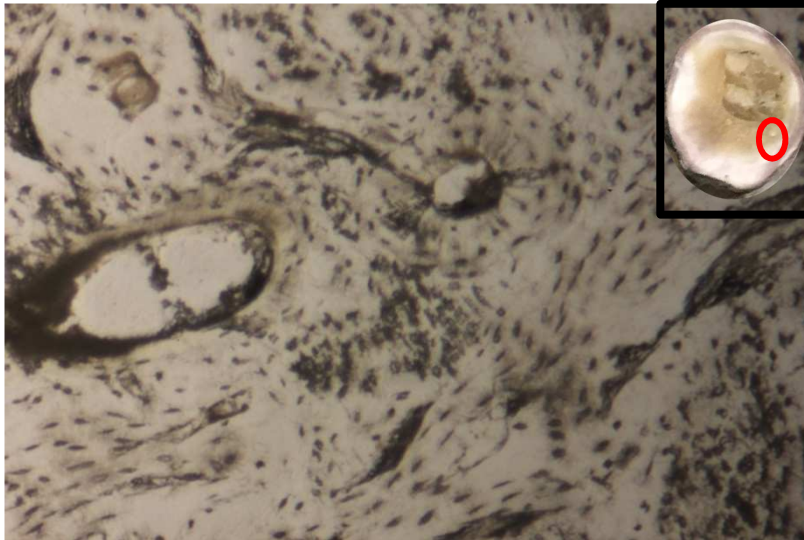


(Figure 8: Frozen Bone, posterior/mediolateral aspect, Sample 1 – Haversian Structure)

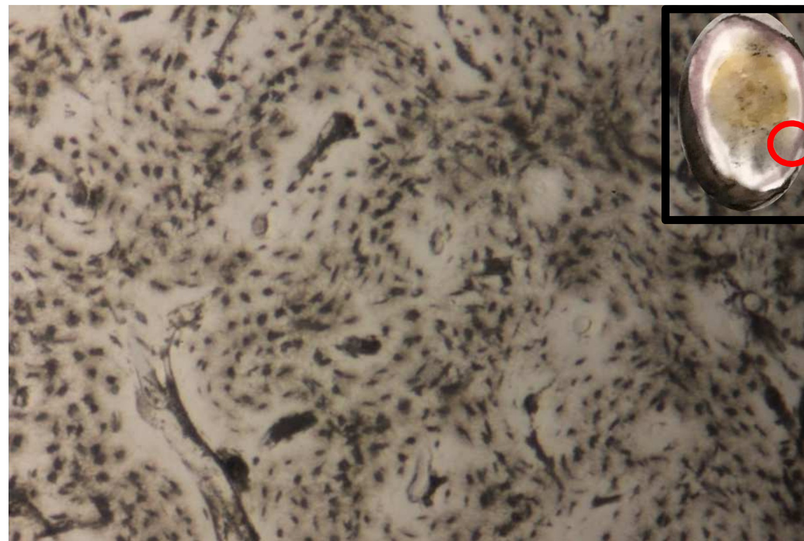
Freeze-thaw

In the samples from this simulated weather treatment category, there are two different types of bone present: cortical, the type of bone that contains Haversian systems; and plexiform, the quickly-forming bone found only in nonhuman animals, in both of the samples. I think it would be more appropriate to discuss the different bone structures separately since the patterns are very different and the changes are expressed differently.

In sample one, the plexiform bone structure is very much distorted, much more so than in any other sample, including the sample that remained frozen for the duration of the experiment. Many of the lines of demarcation are either not present, with those remaining being very warped. The lines of demarcation that cover the internal surface in the second sample seem much larger. Additionally, the small dark lacunae and elongated openings are densely clustered throughout all the inner surfaces of the bone that show a larger surface area; however, in areas where there is only a small surface area of bone (the anterior and mediolateral regions of the sample), the openings are spread around much more evenly.



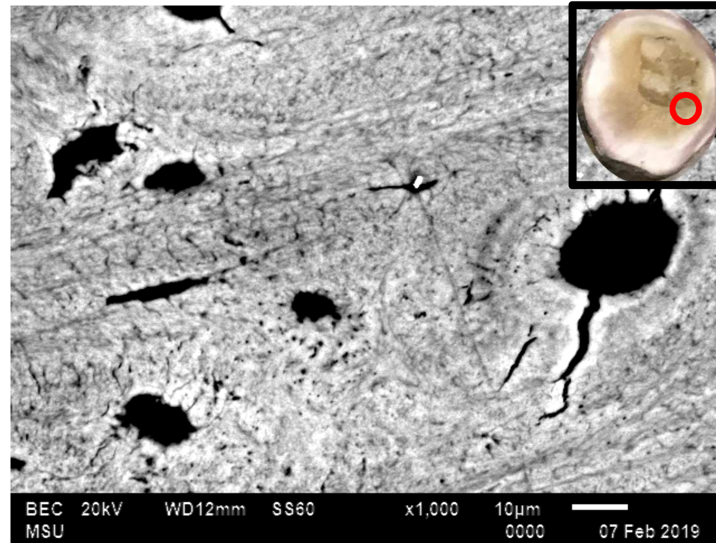
(Figure 9: Freeze-thaw Bone Sample – medial/lateral aspect)



(Figure 10: Freeze-thaw Bone, posterior/mediolateral aspect, Sample 2– Haversian Structure)

The Haversian bone structure in both samples also appeared damaged, as did the plexiform bone structures, but not to an almost unidentifiable state. On the other hand, the circular shape of the Haversian canals is still visible. One significant difference that can immediately be noted, not just in the Haversian canals, but also in other areas of the bone, is the increase in separation in the bone; there appear to be areas in which moisture is trapped inside

the bone or large openings have been created on the internal structure. It is difficult to note the difference due to the transparent appearance of these areas of separation. These lacunae appear in the middle of some of the Haversian canals. There are also many other areas where circular pockets and lacunae (especially in sample one) appear over the surface of the bone.



(Figure 11: SEM Freeze-thaw Bone Sample)

The SEM photos that were taken at 1000x magnification reveal a very dense surface surrounding the areas of separation on the samples. There were enormous breaks over the surface area of the bone. Many of the pits were gaping and cover a significant amount of the surface area of the bone, which presents much less texture overall. There is a significant number of marks indicative of the preparation of the samples, but are they are distinguishable as such. The photos give an overall detailed image of the amount of damage seen in the bone.

Discussion

The samples in this research provide a variety of inferences that can be drawn from the resulting changes that occurred within the bones, both macroscopically and microscopically. The decomposition rates, as well as the specific form the decomposition took, varied relative to the temperature to which they were exposed. These differences were seen both macroscopically and microscopically.

Macroscopic

The control samples here, both pre-experiment and those present during the experiment, were to serve as an example of how bone looks at the time of death and how bone decomposes under non-extreme temperatures. The control samples were observed for the duration of the experiment. These bones exhibited the characteristics of decomposing soft tissue and bone (drying of soft tissue, darkening of bone and tissue, strong odor, oily soft tissue, bone turning a grey color); these replicate the results in many similar studies (Dautartas et al. 2018; Parks 2011;, Suckling et al. 2016; Mann et al. 1990; Tappen 1994; Hiller et al. 2003; Janjua et al. 2008; Keough et al. 2015; and Fernandez et al. 2013). These results appeared to be a gradual process over the six-week period of the study, where changes could be carefully identified and recorded.

The macroscopic results are similar to those found in the studies mentioned above. There are many more studies on warmer weather samples in the literature for comparing and contrasting results mentioned in my literature review that can be applied. In the warmer weather decomposition research, the characteristics that authors (Hiller et al 2003, Janjua et al 2008, Keough et al 2015) note are similar to the findings in this research. There is drying of soft tissue, discoloration of bone and soft tissue, and the oily appearance of the soft tissue. These are characteristics of an acceleration of decomposition that can be observed in warmer temperatures.

In addition, there are evident changes in the way that the bone marrow in each of these samples has either decomposed or changed throughout the experiment (*e.g.* changes in texture and consistency). There does not appear to be any literature that focuses specifically on bone marrow decomposition in either the forensic science or medical research literature, but it is important to note because in this experiment there were significant changes to the bone marrow that may be indicative of bony remains subjected to certain types of weathering.

Bone marrow is a solid soft tissue found in the medullary cavity of bone; it is also found in the cancellous portions of bone (DiGangi and Moore 2013; White et al. 2012; and Currey 2002). There are two different types of bone marrow, yellow and red bone marrow. Yellow bone marrow contains a higher amount of fat than red bone marrow and is responsible for the partial development of white blood cells. Red bone marrow obtains its color from the red and white blood cells that arise in this region. Red bone marrow is found in the adult bones of the torso (vertebrae, hips, breastbone, ribs, and skull), while yellow bone marrow is located in the long bones (DiGangi and Moore 2013; White et al. 2012; and Currey 2002). Due to the fact that the samples for this experiment were long bones, it is safe to say that it is the yellow marrow that was encountered in these samples.

My results also indicate how the bone marrow starts to decay over time, which was further seen during thin sectioning of the samples. In the warm weather simulated samples, the bone marrow was very soft and malleable in texture; it practically fell out of its orifices as the samples were made into sections (and eventually was removed for further processing of the samples). This could most definitely be due to the acceleration of decomposition that occurs in warmer weather conditions. These samples were exposed to 36 degrees Celsius (~96.8 degrees Fahrenheit) for an extended period of time, so in extremely warm conditions it appears that this is how bone marrow decays. Studies by Bell and colleagues (1996), as well as Keough and

colleagues (2017) investigated the rate of decompositional changes in pig and human bone, respectively, noting that they happen very soon after death, especially in warmer conditions. This aligns with results from my own six-week study. They also positively correlate to Parks (2011) assessment of decompositional stages, showing signs by the end of the experiment of advanced decay-mummification. This stage is characterized by a leathery appearance of the skin as well as the growth of mold on the body (Parks 2011). This appears to correlate with the dark spots that are seen in my samples, but as Park (2011) does not provide any photographic evidence of the mold found in their study, I cannot accurately say whether the characteristic mold they encountered was identical to the mold within my study.

By the way of contrast, in areas where temperatures are usually 10 to 20 degrees lower on average, decomposition would be a much more gradual process. The same can be said for all of the characteristics that are shown in the heat-simulated samples; given the temperature and the number of changes that have occurred over these six weeks for these samples indicates how temperature can have an expedited effect on decomposition. In addition, the effects that this had on yellow bone marrow are also dramatic. Bone marrow is a solid tissue, and in these samples, the tissue has turned into soft round fragments. The heating of solid fats usually turns them into a liquid (Ruth et al. 2010), but what seems to be happening here is solid soft pieces of tissue are present. It may be that the fat has liquified and escaped the medullary cavity, which is possibly why the outer soft tissue has such an oily appearance, leaving the inner soft tissue dry.

On the other hand, for frozen samples, the opposite can be noted. Here there appears to be a reduction in how quickly bones decay on the surface. Studies by Tersigni (2007), Hale and Ross (2016), Turnip (2017), Pokines and colleagues (2016), Meyer and colleagues (2013), Roberts and colleagues (2015), and Micozzi (1986) have all shown similar results. Although there are fewer studies on frozen bone compared to warmer weather studies, the authors noted

above describe the changes that occur in frozen bone. Many of the characteristics that they discuss appear in my frozen samples; they discuss features of frozen bone such as changes in bone density and breaks or cracks on the bone at the surface. As mentioned before, bone is composed of collagen and hydroxyapatite, and this protein and mineral, respectively, play a large part in the changes that occurred in these samples. The cracks and breaks that are noted in other studies and observed in this study may be due to the collagen in the bone freezing.

Hydroxyapatite is already a hard mineral, while collagen is a much softer protein that decays very quickly; collagen is even seen to break down in living individuals (Klein 2014, White et al. 2012, DiGangi and Moore 2013, Clines et al. 2014). So, due to the cold temperatures, we can presume that the collagen is frozen here (because of it being a living soft tissue), which would decrease the amount of elasticity within bone causing it to crack and break. When it comes to the discoloration of the soft tissue, there still needs some exploration as to why we see the darker discoloration. Another possibility would be the likelihood of freezer "burn", which is the damage of food due to dehydration and oxidation by air reaching the food. The samples were not sealed for preservation and were taken out of the freezer for examination every week; this exposure to air could have likely caused dehydration and oxidation to occur, causing damage to the soft tissue.

On the other hand, Boaks et al. (2014) and Walden et al. (2018) discuss other measures that could result in further decomposition in these remains. They mention bacterial activity and elemental changes in barium, calcium, iron, potassium, magnesium, zinc and phosphorus during the early stages of decomposition. These elements could have much do with some of the changes that are discernable between the control and weather-treated samples. This can especially be true for the warm weather samples. Although temperature was not accounted for in their studies, it can certainly be applied when observing the rates of change that occurred. Sharanowski and

colleagues (2019) also show that humidity is a significant factor in whether more "rotting" or mummification occurs; more moisture in the air means a higher probability of rotting than in a much more arid environmental condition.

The one confirmation that can be made about the macroscopic results of this study is that they align with what others have found (Tersigni 2007; Hale & Ross 2016; Turnip 2017; Pokines et al. 2016; Meyer et al. 2013; Roberts et al. 2015; and Micozzi 1986). That is, in frozen remains, damage (penetrative cracks, discoloration, breakage) appears soon after the remains are placed in frigid temperatures and changes become less frequent as time continues on, versus damage and deterioration happening slowly over time in warmer weather or milder temperatures. The damages that are found in week one and two of the frozen remains are changes that would typically be found in later weeks (3&4) of remains exposed to warmer weather or milder temperatures. This suggests that decomposition in bone is drastically different in colder temperatures than in warmer temperatures.

The freeze-thaw samples were fascinating in their results as they had similar features to the frozen and warmer weather samples. The samples, in total, were left under the fume hood for the three weeks of the experiment and in the freezer for the other three weeks, alternating between the freezer and fume hood each week. The most significant macroscopic difference that was apparent in these remains that was not present in the others was the texture of the bone marrow. It had a jelly-like consistency, which could be the result of the heating of the yellow bone marrow and the limited time given for the liquified fat to escape the medullary cavity due to the freezing after the thawing process (Ruth et al. 2010). I presume at some point during the thawing process, any of the fat that did liquify during the thawing process may have escaped the medullary cavity and created some of the oily appearance that is observed in these samples, as well as the oily appearance mentioned in other research (Dautartas et al. 2018; Parks 2011;

Suckling et al. 2016; Mann et al. 1990; Tappen 1994; Hiller et al. 2003; Janjua et al. 2008; Keough et al. 2015; and Fernandez et al. 2013). However, to fully understand this change, further investigation would be needed.

Further, the discoloration seen in the freeze-thaw remains is another drastic change and the time frame is a factor to be taken into consideration. Micozzi (1986) and Pokines and colleagues (2016) mention the speed at which changes happen in freeze-thaw remains, and this is reflected in these samples. The samples show significant changes occurring on the surface within the second week, first trial, of the freeze-thaw cycle. The same discoloration and oily and tough texture that was found in the warmer weather remains of week three became readily apparent in Wk 2 for the freeze-thaw samples. The bone additionally started to turn a whitish grey color. This was not present in the warm weather or the frozen bone samples: this may be due to the decomposition of bone that began to speed up when taken out of the fume hood and then was preserved in that state of decomposition when put back into the freezer. This preservation of damage could have caused further damage to persist, especially with a high moisture content present. However, further investigation is needed to understand why these characteristics are present.

Microscopic

The control samples in the experiment served the same purpose for both the macroscopic and microscopic analysis. These samples represent what plexiform and Haversian bone look like, and how they decompose, without exposure to any temperature extremes. This is in contrast to the samples that were exposed to differing maximum and minimum temperatures meant to model diverse weather conditions.

As mentioned by Currey (2002), Jowsey (1966), and DiGangi and Moore (2013), plexiform and Haversian bone will be discussed in detail as these are the two types of bone that can be seen in these samples. Plexiform bone is a type of bone structure that is not found in humans (Figure 1). Plexiform is a rapidly forming type of bone found in the limb bones of animals that grow quickly, as opposed to some of the other types of bone found in mammals that take much more time to develop. Although this form of bone is not present in humans, it is still important to note the effect of differing temperatures/simulated weather on this type of bone as exposure to the different temperatures still affected the underlying bone structure. Haversian bone structure is commonly found in cortical bone, the dense outermost surface of the bone. Cortical bone structure is found in humans and it is important to note these changes as they are likely similar to the changes that human bone might also exhibit.

The microscopic samples exposed to the 36 degrees Celsius temperature (~96.8 degrees Fahrenheit) in the incubator for the entirety of the six-week experiment looked very similar to the control samples of the fume hood at the conclusion of the experiment. In the cross section, the sample's coloration and cavities look very much the same. The reason why these samples may look so similar is likely due to the temperatures to which they were exposed. They were both placed in warmer settings, but with a twenty-degree difference. The difference in degrees may have played a part in the acceleration of decomposition. The changes seen in the warmer

weather samples versus the control samples were much more apparent much sooner. Nevertheless, the changes seen in these bone samples indicate only a difference in degree (rate) of decomposition, not in kind (type). This was also seen in the macroscopic results.

There is little research concerning how plexiform bone structure reacts to different weather conditions, and this is especially true in the forensic literature. This is partly due to the fact that many forensic simulations emulate results that can be seen and replicated in human bone. Even though plexiform bone is not explicitly mentioned in the literature, there has been some microscopic analysis of bone in warmer weather studies. Even the studies that do involve animal bone do not specifically note changes to plexiform bone. Hiller et al. (2003) and Fernandez et al. (2013) discuss some of the microscopic changes that occurred in their studies involving extremely high heat temperatures. Fernandez and colleagues (2013) specifically found retraction, fracture, the bursting of Haversian canals, and cluster formation within the bone. Hiller et al. (2003) state that extreme heating of bones causes the hydroxyapatite crystals to form into a specific shape and size, which causes cluster formations. Both authors show that high temperatures can be severely damaging to bone.

In these warmer weather samples, the plexiform structure appeared to no longer be uniform, as if it was deteriorating. This was the most significant change that could be readily seen in these samples and could be characteristic of warm weather decomposition. Bone is made up of organic compounds that begin to decompose shortly after the death of the animal or human. So, over time, bone is expected to deteriorate (Parks 2011). In fact, in conditions like extreme heat, this can change the decomposition process, at least in the rate of decomposition. In this case, the samples show an acceleration. In the macroscopic and microscopic analysis of the remains, it is not as easy to see the changes happening week by week, as a result of the weather simulations. It is not known how or when the plexiform structure began to no longer look

uniform, given the samples were collected at the end of the six-week experiment, but a loss of definition was the result. The changes in the plexiform structure definition could be due to the drying and cracking of the bone that started on the surface and worked its way to the medullary cavity. These changes can be correlated to the changes that we see in the macroscopic results (the small openings, lacunae, and areas of separation), and supported/explained by the findings of Hiller et al. (2003) and Fernandez and associates (2013) that found retraction, fracture, Haversian canals bursting, and the cluster formation in high heat samples.

The control and warmer weather samples show significant drying of the bone that would lead the bone to lose its elasticity through the breakdown of the collagen protein (Klein 2014; White et al. 2012; DiGangi and Moore 2013; and Clines et al. 2014). This drying of the bone marrow in the medullary cavity can give the illusion of deterioration; this can only be said based on observation of the initial appearance to the final appearance. Boaks and colleagues (2014) confirm that the decomposition of bone marrow is significant enough to further accelerate the decomposition of bone (the soft tissue surrounding the bone has an effect on the decomposition of the bone). The samples show that the bone marrow is retracting from the medullary cavity walls, and during the process of making the thin sections it became apparent the texture of marrow was different from sample to sample, as it often fell out of the medullary cavity during the process. The decomposition of the bone marrow and its separation from the inner walls of the bone creates the illusion that there is an expansion of the medullary cavity. At first glance, it is difficult to distinguish between where the bone marrow ends and begins prior to the decomposition process. Alternatively, the bone itself could have been collapsing/deteriorating along with the bone marrow, thus creating a larger space within the medullary cavity. Though, because there was not further examination of the soft tissue, it is uncertain which (or both) circumstances were occurring.

As for the SEM results, the shadowing seen on the surface of the control sample seems to suggest there are raised areas present. The SEM photo provides a very high magnification of the inner surface of the bone and, due to the degree of magnification (1000x) not as much of the internal structure overall can be seen. The SEM photos were only taken of the control and freeze-thaw samples. It should be noted that there are preparation markings from the process of preparing the samples for microscopic observation present. The shadowing that is exhibited are depressions on the surface. These depressions and openings give further support to the bursting of the Haversian canals mentioned by Hiller et al. (2003) and Fernandez et al. (2013).

The samples that were frozen also show the destruction or disorganization of the plexiform bone, like the warm weather simulation samples. Based on this study it is unclear why there are similar changes occurring between the two temperatures. The disorganization is so extreme that the organized, brick-like structure of the plexiform bone is almost undetectable. As was noted for the warmer weather studies, it was difficult to find any studies that discuss plexiform bone changes associated with freezing temperatures. Hale and Ross (2016) discuss in their cold weather study cracking and bone density changes within the bone, as well as state that the internal surface changes when these surface cracks begin to become present. Other researchers (Pokines et al. 2016; Meyer et al. 2013; Roberts et al. 2015; and Micozzi 1986) also acknowledge that there are changes in bone density and the internal surface, but there is no mention of observed changes at the microscopic level. Articles by Turnip (2017) and Tersigni (2007) report findings that support these changes in the medullary cavity and overall density. However, no specifics on why this occurs within frozen bone samples are provided, nor is any microscopic evidence presented showing these specific changes.

Above all, the changes seen in the internal cavity of the bone could be evidence to support the damage that they refer to as changes that occur in frozen bone. The dark areas and

areas of separation in the sample photos may very well contribute to the overall reduction in the weight and density of the bone. Unfortunately, in this experiment, samples were taken for microscopic use only, and the samples were not weighed prior to and after the simulated weather treatment.

Due to the fact that the other literature on frozen remains focused on human bone, the disorganization of the plexiform structure of the bone cannot be adequately explained by referencing the literature. The breakdown of the regular layer structures seen in plexiform bone might be due to moisture within the bone freezing and disrupting these structures on a cellular level. Unfortunately, no cortical bone with Haversian systems was present in this sample (only one sample was prepared for the frozen bone), so, for this project, there is no data concerning the effect of freezing on Haversian systems in the type of bone that would be found in humans.

In the samples from the bone subjected to the freeze-thaw cycle in this experiment, both Haversian and plexiform bone was present; SEM photos were also produced to get a closer look at the internal surface. The presence of cortical bone with Haversian systems in this sample means that the results can be directly correlated to characteristics that we can find in humans. The literature on freeze-thaw effects on bone is very sparse. There have not been many published, intensive studies on the effects of the freeze-thaw cycle on bone. Pokines and colleagues (2016), although they did not report any microscopic findings, did describe penetrative cracks piercing through the medullary cavity as a result of subjecting bone to a freeze-thaw cycle. Even more, Tersigni (2007) provides SEM microscopic evidence of penetrative cracks directly associated with the act of freezing the bone but does not show any effects to Haversian bone. Micozzi (1986) notes that the freeze-thaw cycle of postmortem decomposition occurs from the "outside-in" (predominantly decay), meaning that decomposition starts on the outside and works its way to the inner surface. This is different from other remains

where postmortem decomposition occurs from the "inside-out". I believe my study shows that there are changes that have been made to Haversian canals and plexiform structure due to both the freezing and freeze-thaw cycle.

Plexiform and Haversian bone changes will be discussed separately, as these are different types of bone that show different types of changes in this study. Plexiform bone is laid down very quickly; it is a form of lamellar bone tissue, which is composed of collagen fibers and is mechanically weak (White et al. 2012; Currey 2002; Jowsey 1966; DiGangi and Moore 2013). Although all bone reacts to pressure or stress, more fragile portions of bone may change much more quickly than mechanically stronger areas of bone with more resistance. Consequently, the changes seen in the plexiform bone seem quite dramatic compared to the other simulated weather treated samples. The structure of the plexiform in these samples was very distorted and appeared twisted, very different from its normal parallel line structure resembling a brick wall. Moreover, the small dark lacunae or openings are elongated and densely clustered over the surfaces of the bone (White et al. 2012; Currey 2002; Jowsey 1966; DiGangi and Moore 2013). The changes that are present can be associated with the type of bone and the pressure of being frozen and thawed. The bone was being moved from -1.1 degrees Celsius to 21 degrees Celsius every other week within a six-week period. During the experiment, once the samples thawed, there would be an increase in moisture and bacteria growing on the bone as a result of the increase in temperature (Boaks et al. 2014). Freezing the bone again acts to preserve the moisture build-up, bacteria, and other processes of active decomposition that may have occurred during the thawing process. This phenomenon could be the cause of the acceleration in the decomposition process. In the macroscopic results, the changes that are seen in Week 1 in the freeze-thaw remains are not seen until Week 3 in the warm weather remains. Unfortunately, given the timing of the bone

sampling, the rate of microscopic changes in the bone could not be observed as the samples were only collected at the conclusion of this experiment.

The Haversian bone changes were just as drastic as the plexiform bone changes, but did not result in the complete loss of the identifiable bone structures. Haversian bone is found in cortical bone, which is the dense outer layer of bone that protects the inner layer of bone. The Haversian bone or Haversian canals are the microscopic tubes in the outermost region of the bone that allow the blood vessels and nerves to travel through them (White et al. 2012; Currey 2002; Jowsey 1966; DiGangi and Moore 2013). This bone is composed primarily of hydroxyapatite, an inorganic mineral, with small amounts of collagen for elasticity. The Haversian bone is a system in the inner portion of cortical bone that can be directly observed in the microscopic samples. Due to there being very little literature on microscopic changes to bone associated with freeze-thaw cycles, there is little to compare these results to. However, the different forms of macroscopic damage mentioned in the previous studies (Dautartas et al. 2018; Parks 2011; Suckling et al. 2016; Mann et al. 1990; Tappen 1994; Hiller et al. 2003; Janjua et al. 2008; Keough et al. 2015; and Fernandez et al. 2013) give some idea of the types of damage that may be found.

The changes in the Haversian bone seen in this experiment include increases in separation on the inside of the bone and areas where there appeared to be a build-up of moisture (water) that has been frozen or encased within the bone. There are many areas of circular pockets and lacunae in the bone that seem to sit on top of the Haversian bone patterns. The Haversian bone patterns themselves no longer have their distinguishable circular shape; the circular pattern is still present but not as uniform as that seen in Figure 10. The regular Haversian pattern looks to be breaking down, becoming very sparse and disorganized, as if the pattern is disappearing. This may be due to the decompositional process. There are large cracks or dark lines that cover the

surface area of the bone that may be associated with the advanced decomposition that is occurring in these samples. The pockets appear clear. This may be the result of moisture being trapped in the bone, and then creating the openings as a result of expansion during the freezing process. The SEM photos further showcase the large cracks and presence of openings inside the bone, providing further evidence of the freeze-thaw cycle on bone.

Limitations

One thing to keep in mind with this study is that all the different temperatures used in this study were under a controlled environment. These temperatures were simulated in a laboratory, which does not allow for natural environmental components to affect the decompositional process. This is limiting because this study does not entirely mimic the natural environmental conditions. Insect activity, wind, soil, scavenging, excess heat or moisture are elements that were not present in this study and could have changed the rate or pattern in how these samples would have decomposed. Moreover, due to the equipment that I had available at my disposal, humidity was a factor that I was unable to control for in this study. Also, this study does not show the changes that would have taken place if samples had been covered in soil or snow. This study would not be able to evaluate the changes that could have taken place if remains were buried in snow or buried in soil. These factors would change the consistency of temperature and exposure to temperature.

Another limitation that can affect the application of this study is the fact that there is a form or type of bone within this study that is not found in humans. Since plexiform bone is not found in humans, the patterns that were found in plexiform bone are patterns that cannot be replicated in human remains. The only patterns that forensic scientists could use and possibly see in practice are those from the Haversian bone. All that can be extrapolated from the findings in plexiform bone is that the temperatures the samples were placed in were extreme enough to cause damage that created visible changes within the bone structure. Moreover, Keough and colleagues (2017) show that early stages of decomposition in pigs and humans varies, so the rate at which these alterations occur needs to be taken into account.

I should also note that the bones are not from the same animal as they are all femora (minus the proximal ends) of swine (*Sus scrofa*). Therefore, at least two different animals were

used for this study and the different sides (left and right), were not taken into account when While the animals were likely butchered on the same day, it is not known if they were from the same facility or farm; this is something that could not be assessed because they were derived from a butcher shop that receives animals from many different vendors. So, it is possible that there were fundamental differences in the various samples prior to the experiment based on age, side, weight, nutritional status, sex or other confounding factors.

Furthermore, a possible limitation that could give a more detailed analysis, is the microscopic collection of samples. In a more extensive study, taking microscopic samples each week could assist in showing the gradual changes week by week in how the bone structure is changing. This would be helpful to see how drastic the changes are, microscopically, on a weekly basis and the results could show what initially begins to happen to the bone structure when exposed to extreme conditions. Additionally, the collection of the SEM photos could have been much more consistent. The SEM photos were taken to look at the surface of the bone at a much higher magnification. The SEM photos were taken of the control and freeze-thaw to show the dramatic changes that the freeze-thaw cycle has on bone. The collection of SEM photos for all the samples could have shown the differences in between the control, warm weather, freeze, and freeze-thaw. This would have shown if there were similar changes or very different ones between the samples.

Lastly, the final element missing from this study that could have solidified the evidence provided in the microscopic results is an image analysis. An image analysis of the lacunae and other surface features that are found in the microscopic results could have provided more evidence for how much damage occurred within each temperature treated sample. This could have provided more criteria for a forensic scientist to follow when looking for these changes and the extent to which they occur.

Conclusion

This study provides useful information for the continued understanding of the decompositional process. The purpose of this study is to show, in comparison, the changes that different forms of weather or temperature have on the decomposition of human remains. This study's goal was to simulate temperatures that can be found in a natural environment. Three different weathering patterns were tested, which can provide more information and help better the practices within forensic science when it comes to the identification of human remains. This experiment has gathered data to assess how bone structure changes and attempt to conclude why these changes may be happening. This can be extremely important when bodies are found in remote areas where the environment has had considerable time possibly to change how the post-mortem interval of an individual would be assessed.

The analysis of the freeze-thaw cycle not only provides more information on how this cycle works, but it also shows how these changes in temperature can affect the overall conditions of bone. The comparison of the frozen, freeze-thaw, and warmer weather remains further supports the idea that these temperatures have a different effect on bones and change the way they decay over time. They not only change the way bone decays over time but also how quickly signs of decomposition begin to show in these different temperatures. When recovering remains, it is essential to take into consideration the external forces that may have played a part in the remains looking the way that they do at the time of recovery.

Environmental stressors such as weather can complicate the identification of an accurate (or close to accurate) post-mortem interval (PMI). There are many factors, both inside and outside of an individual's body, that can affect the estimation of time since death. This study analyzes one of the outside effects that can influence the accuracy of PMI estimation. The results of this study can also be used in conjunction with factors to help eliminate possible confusion

between the kind of damage that is seen as a result of weather, and what is seen as a result of trauma. This study may help to positively impact the estimation of PMI by forensic anthropologists. It also adds to the literature to provide a better understanding of bone decomposition (Kroman and colleagues 2013; Forensic Anthropology 2013; Boer et al. 2016).

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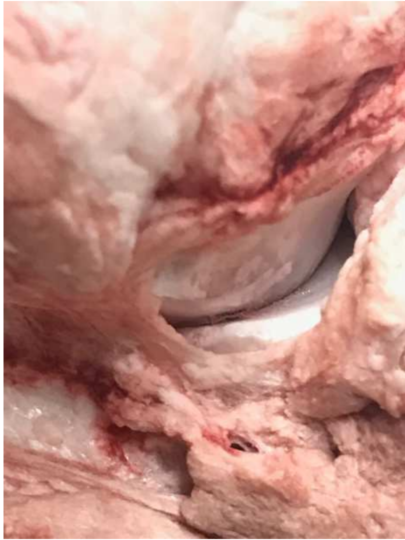
Appendix A

Macroscopic Photo



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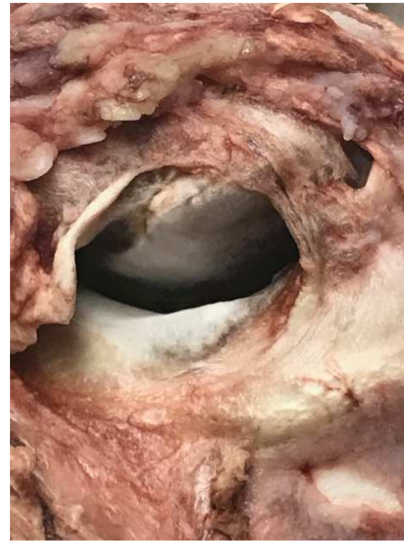
(Frozen Bone Sample – Week 4)

(Frozen Bone Sample – Week 4)

(Frozen Bone Sample – Week 4)

(Frozen Bone Sample – Week 4)



(Freeze-thaw Bone Sample – Week 4)

(Freeze-thaw Bone Sample – Week 4)

(Freeze-thaw Bone Sample – Week 4)

(Freeze-thaw Bone Sample – Week 4)

(Freeze-thaw Bone Sample – Week 4)

(Control Bone Sample – Week 4)

(Control Bone Sample – Week 4)

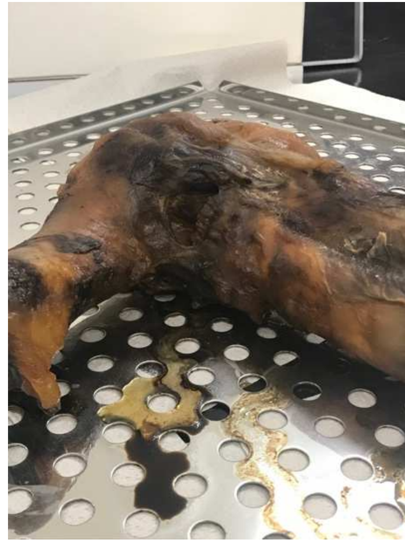
(Control Bone Sample – Week 4)

(Control Bone Sample – Week 4)

(Control Bone Sample – Week 4)

(Heat-Simulated Bone Sample – Week 4)

(Heat-Simulated Bone Sample – Week 4)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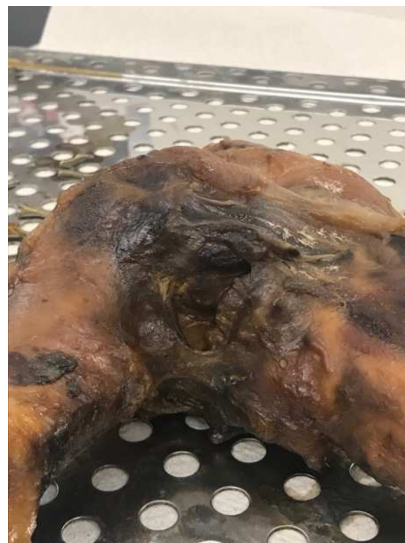
(Heat-Simulated Bone Sample – Week 4)


(Heat-Simulated Bone Sample – Week 4)

(Control Bone Sample – Week 5)

(Control Bone Sample – Week 5)

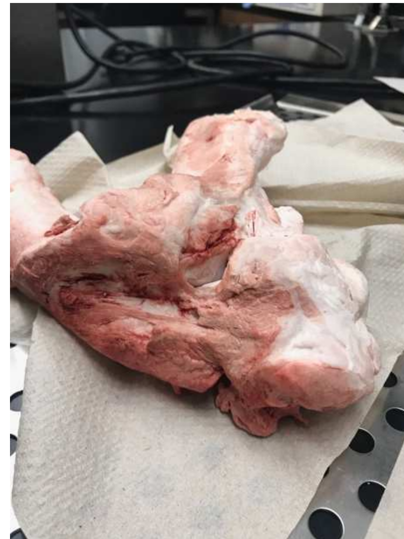
(Freeze-thaw Bone Sample – Week 5)

(Freeze-thaw Bone Sample – Week 5)

(Freeze-thaw Bone Sample – Week 5)

(Frozen Bone Sample – Week 5)

(Frozen Bone Sample – Week 5)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(Heat-simulated Bone Sample – Week 5)

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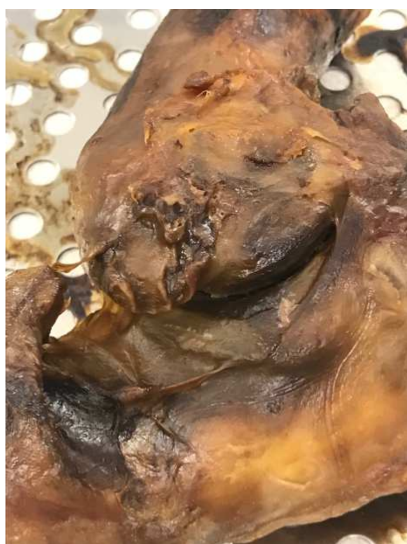
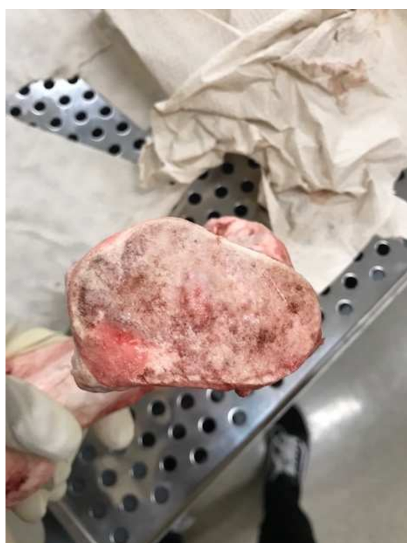
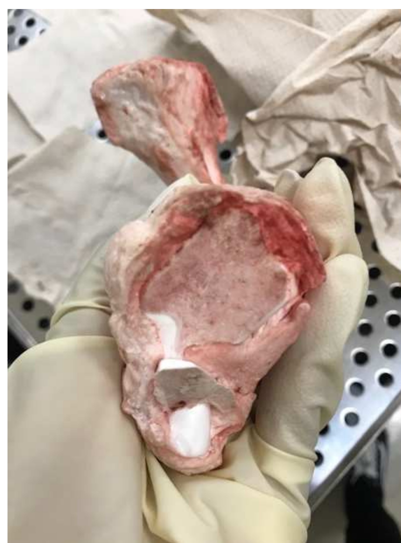


(Heat-simulated Bone Sample – Week 5)

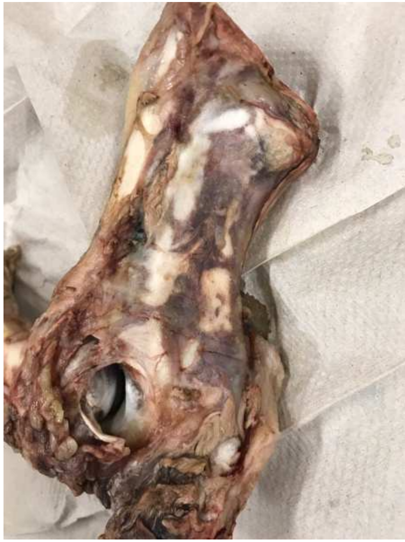
(Frozen Bone Sample – Week 6)



(Frozen Bone Sample – Week 6)



(Frozen Bone Sample – Week 6)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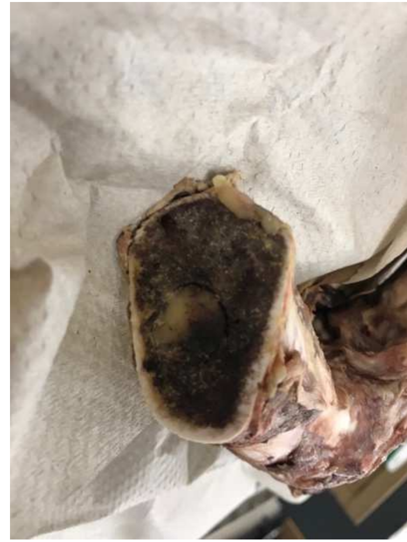
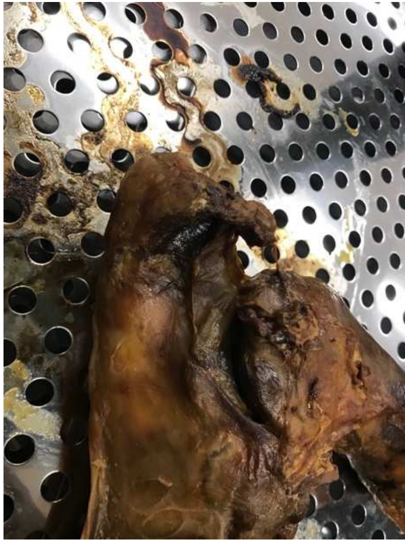

(Control Bone Sample – Week 6)

(Control Bone Sample – Week 6)

(Control Bone Sample – Week 6)

(Heat-simulated Bone Sample – Week 6)

(Heat-simulated Bone Sample – Week 6)



(Heat-simulated Bone Sample – Week 6)

(Heat-simulated Bone Sample – Week 6)

(Heat-simulated Bone Sample – Week 6)

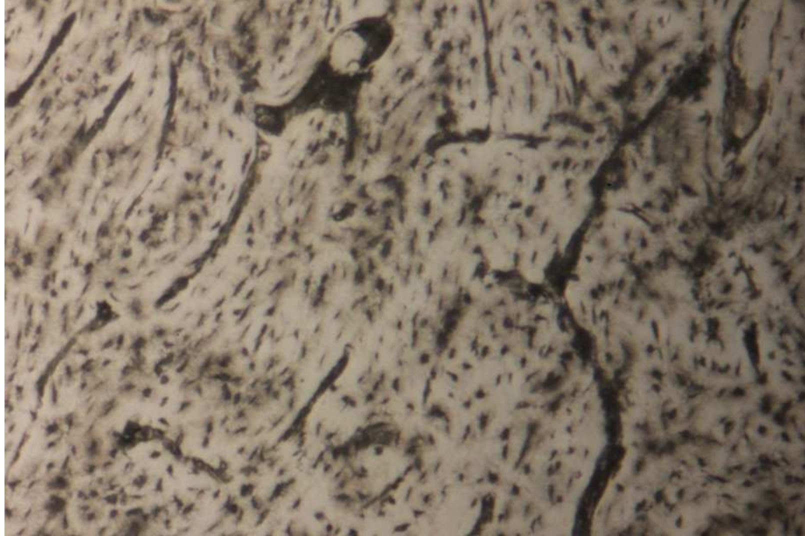
(Heat-simulated Bone Sample – Week 6)

Appendix B

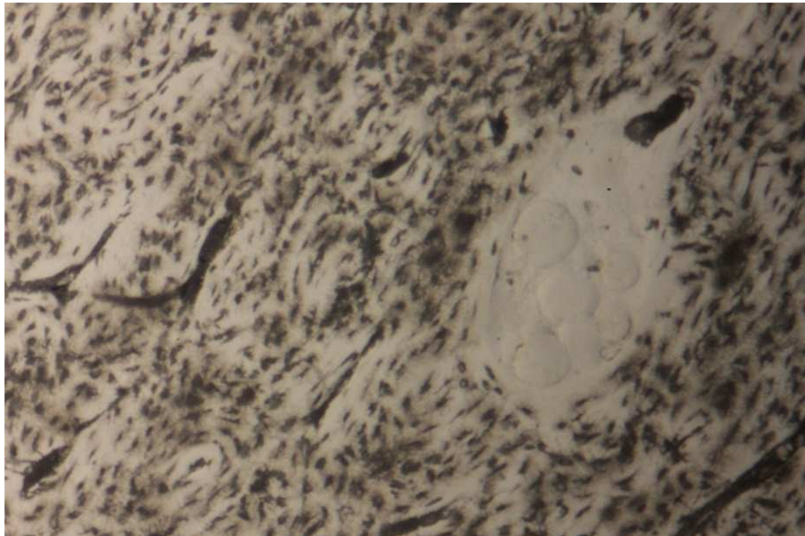
Microscopic Photo

(Pre-Control Bone Sample – Plexiform Structure)

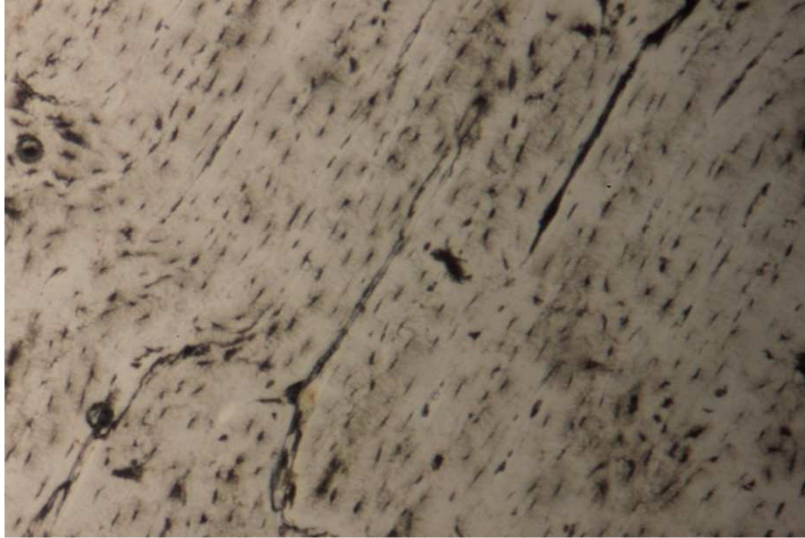
(Pre-Control Bone Sample – Plexiform Structure)



(Frozen Bone Sample – Plexiform Structure)



(Frozen Bone Sample – Plexiform Structure)



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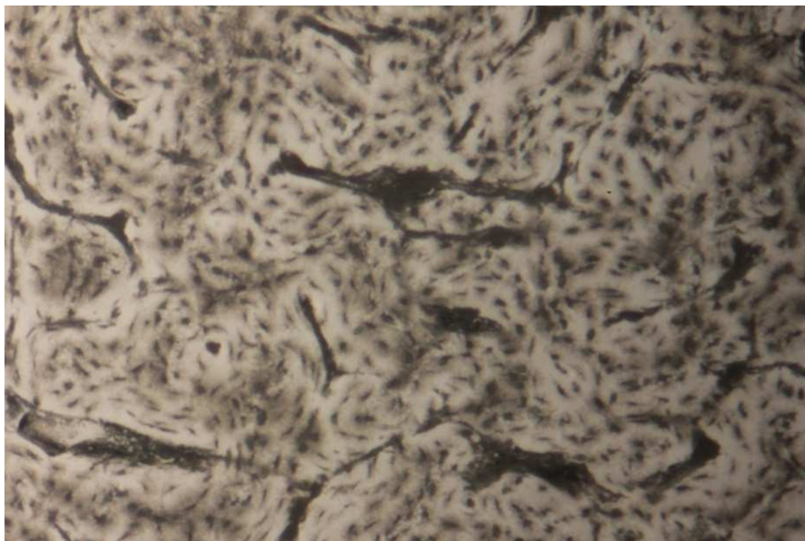

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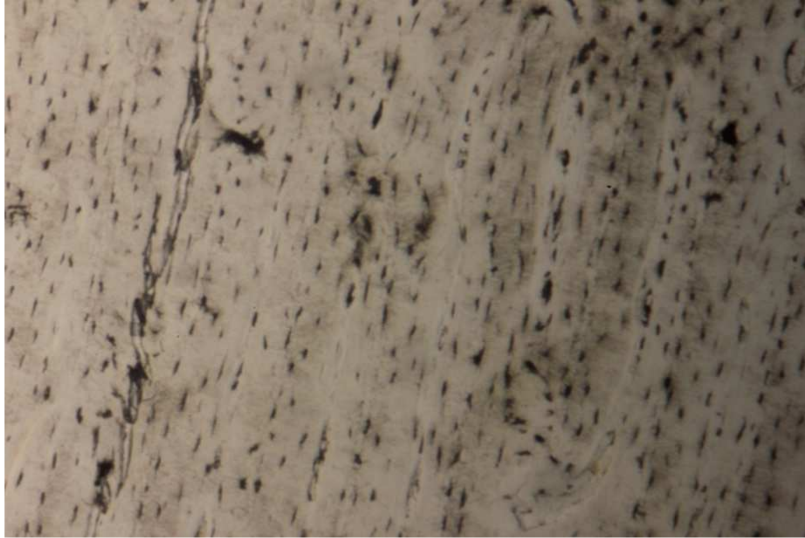

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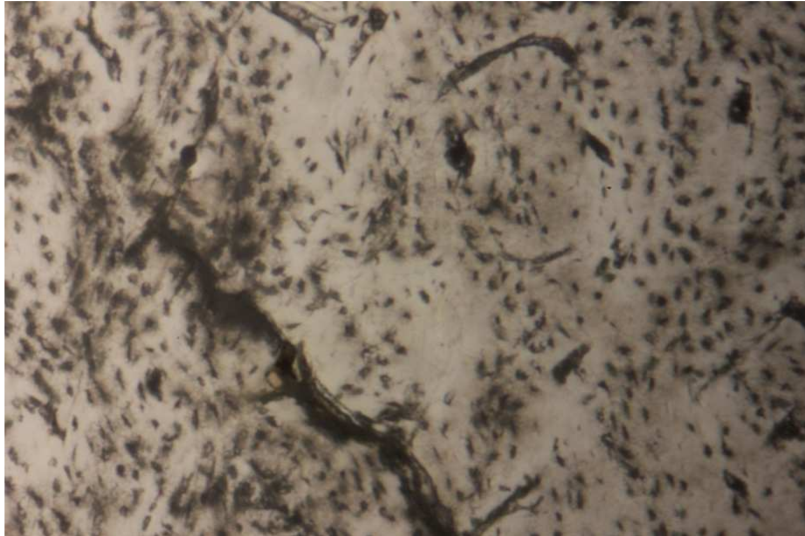
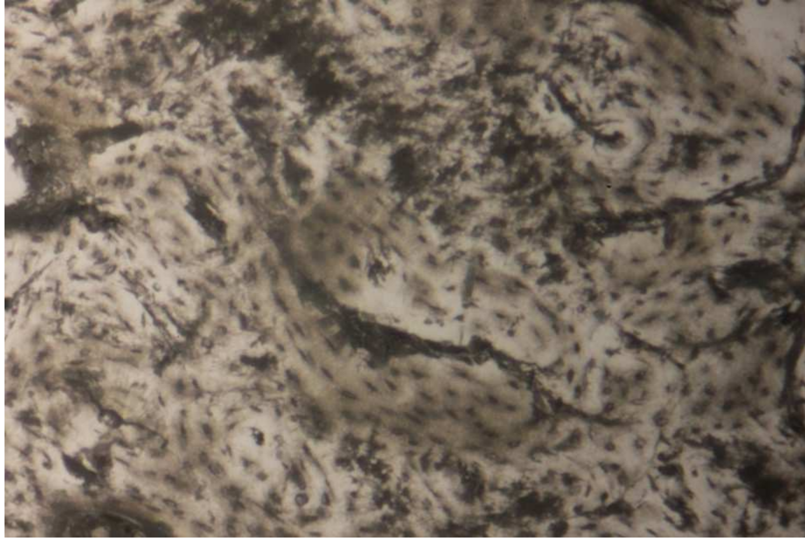
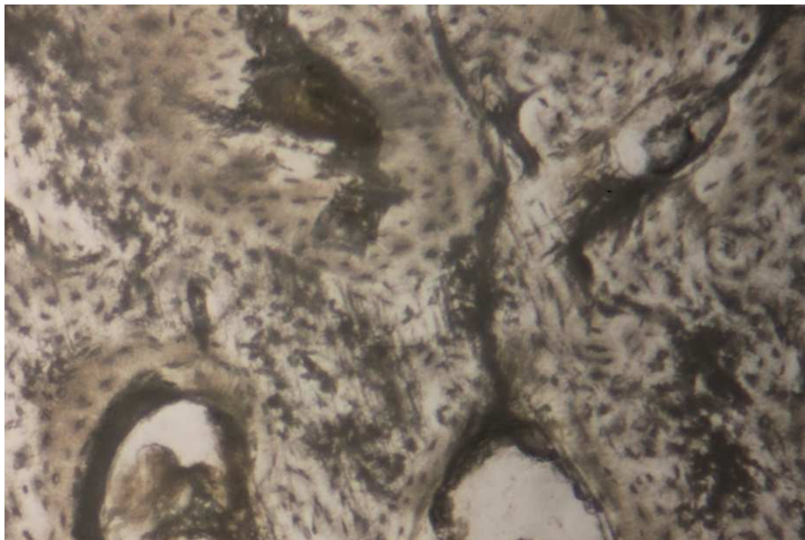


(Frozen Bone Sample – Plexiform Structure)



(Frozen Bone Sample – Plexiform Structure)



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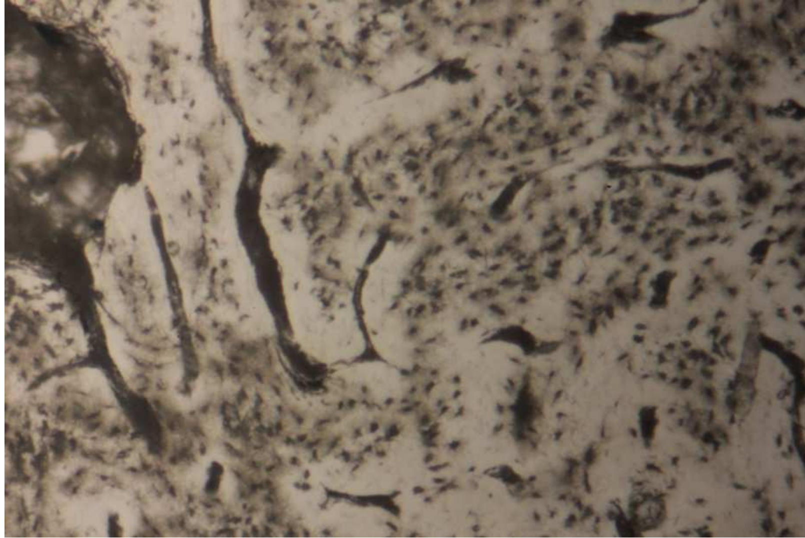
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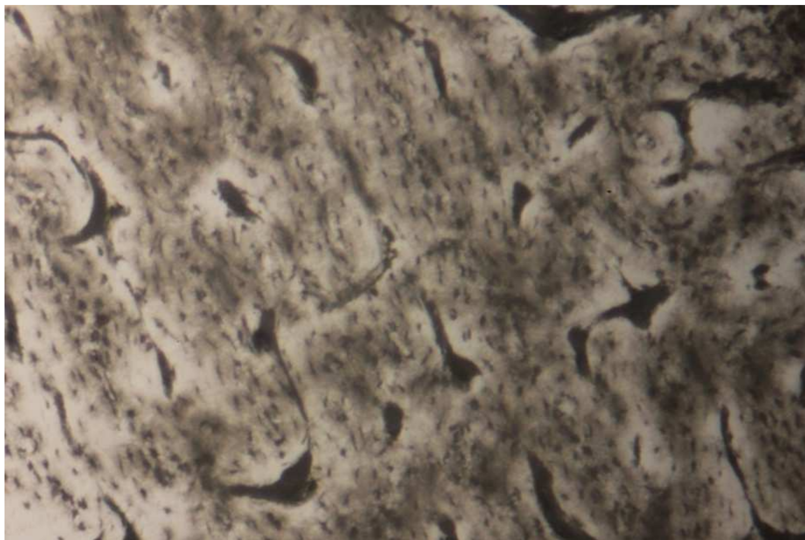
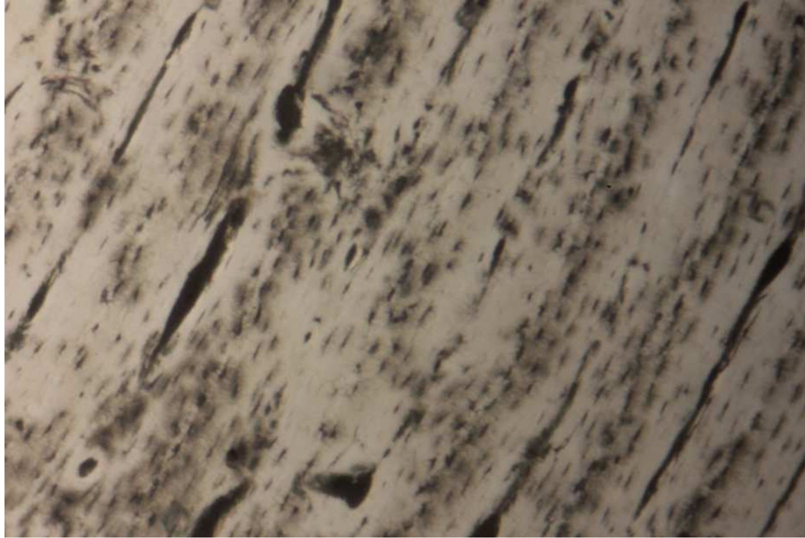

(Control Bone Sample – Plexiform Structure)



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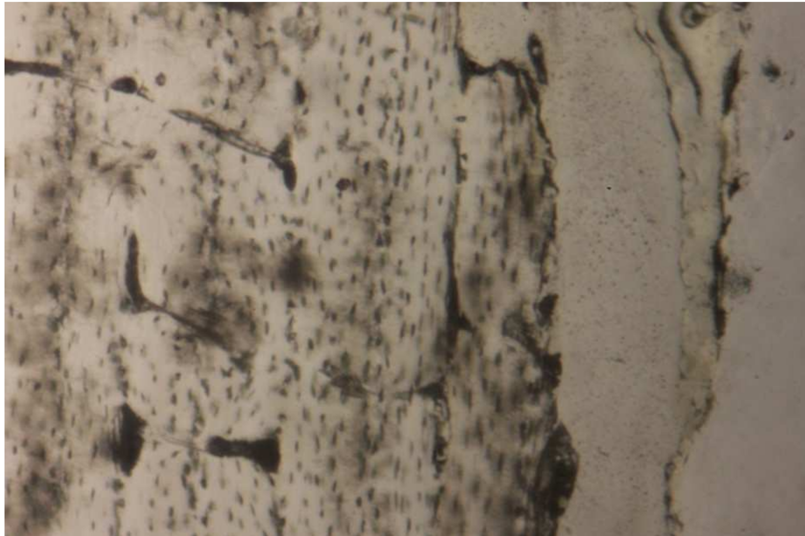
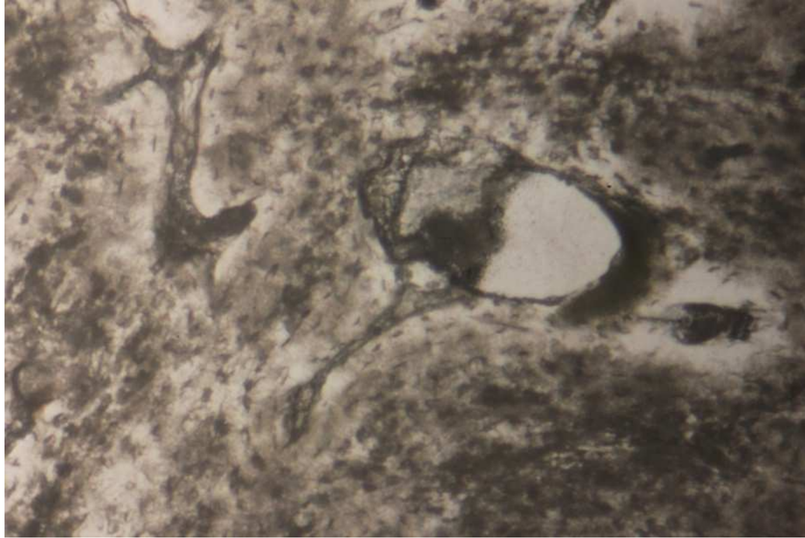


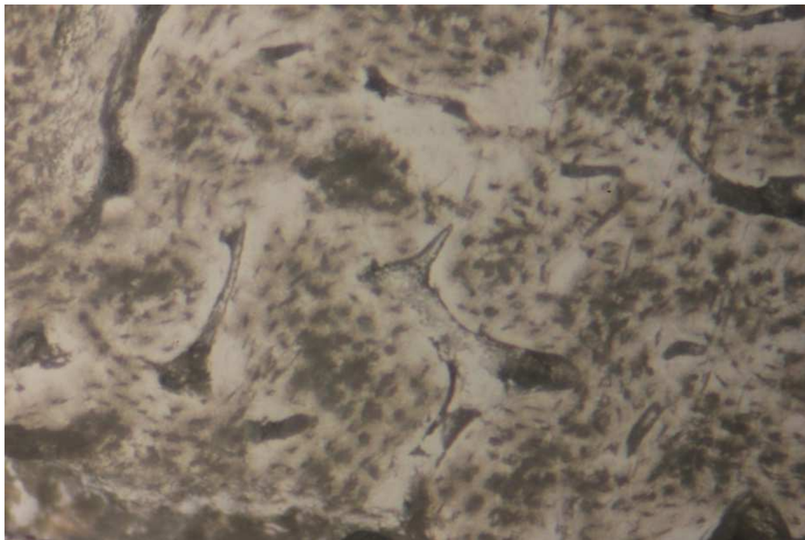
(Control Bone Sample – Plexiform Structure)

(Control Bone Sample – Plexiform Structure)

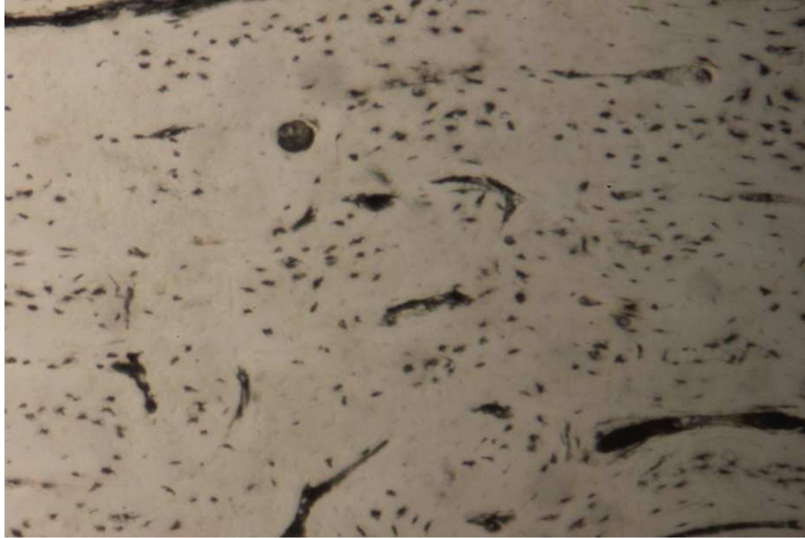
(Control Bone Sample 1 – Plexiform Structure)



(Control Bone Sample 1 – Plexiform Structure)



(Control Bone Sample 2 – Plexiform Structure)



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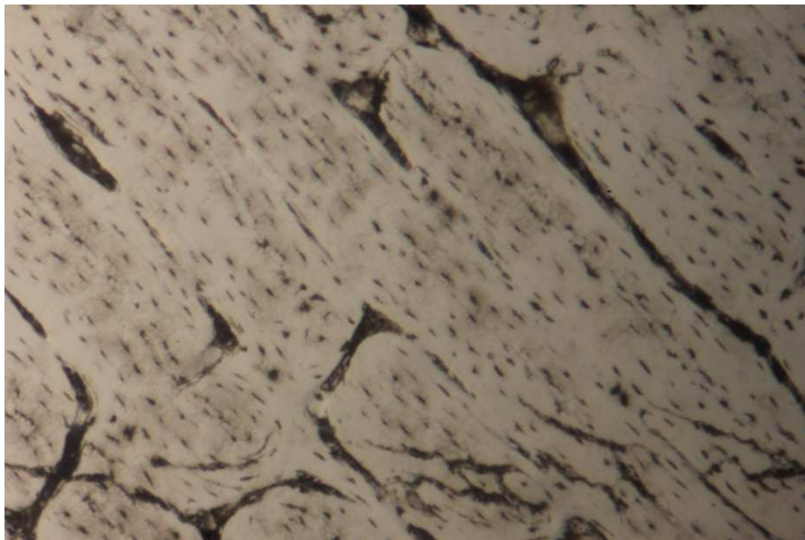

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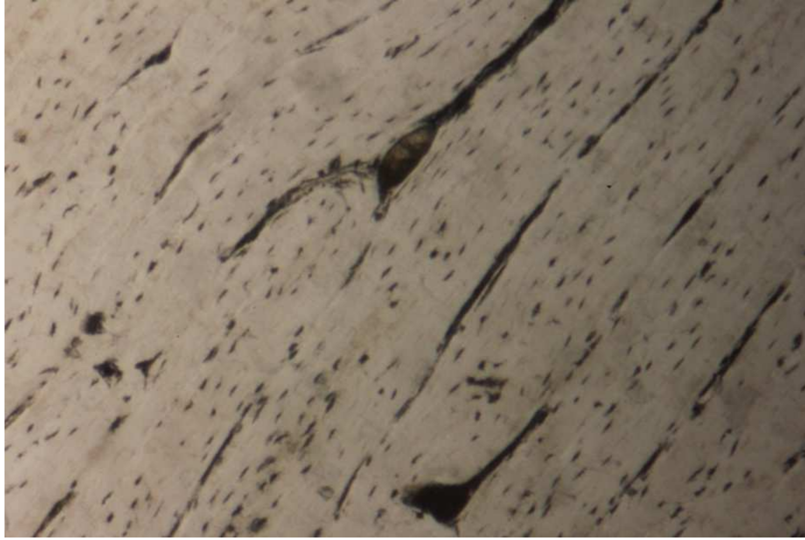

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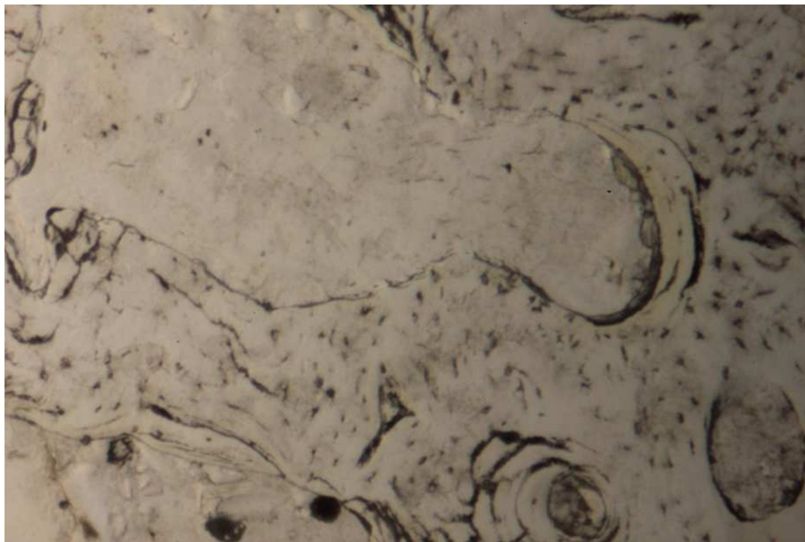
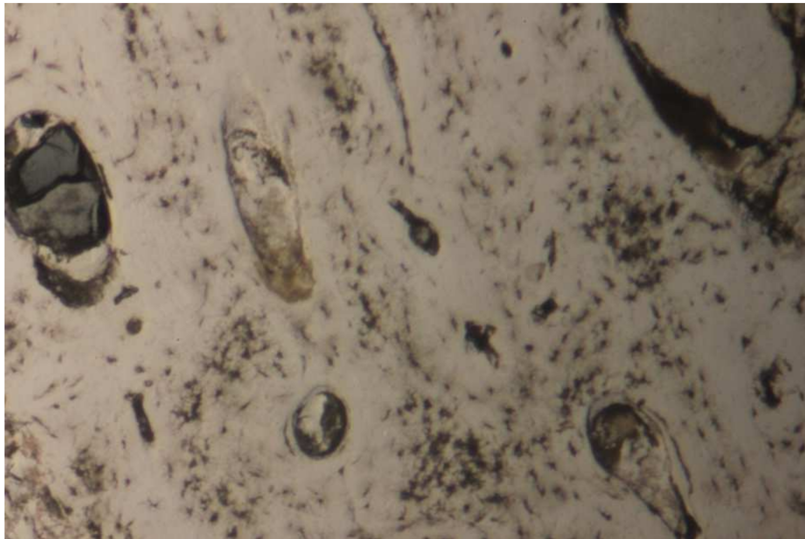


(Control Bone Sample 2 – Plexiform Structure)

(Control Bone Sample – Plexiform Structure)



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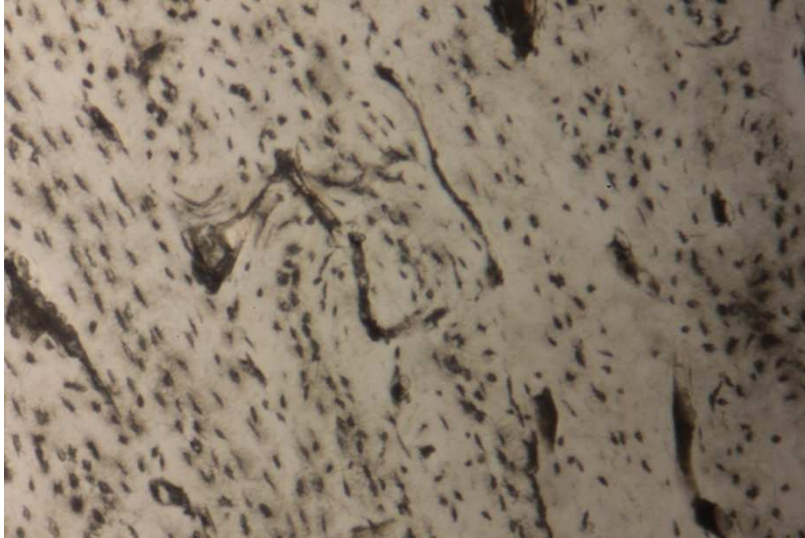

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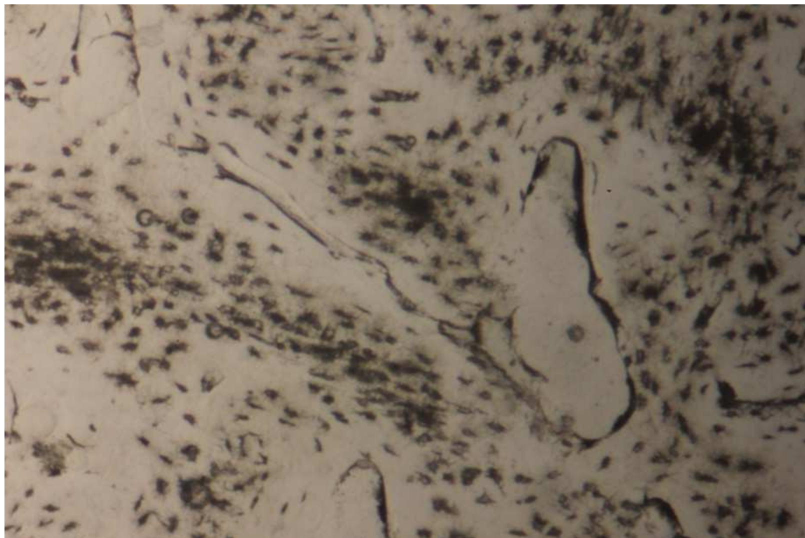

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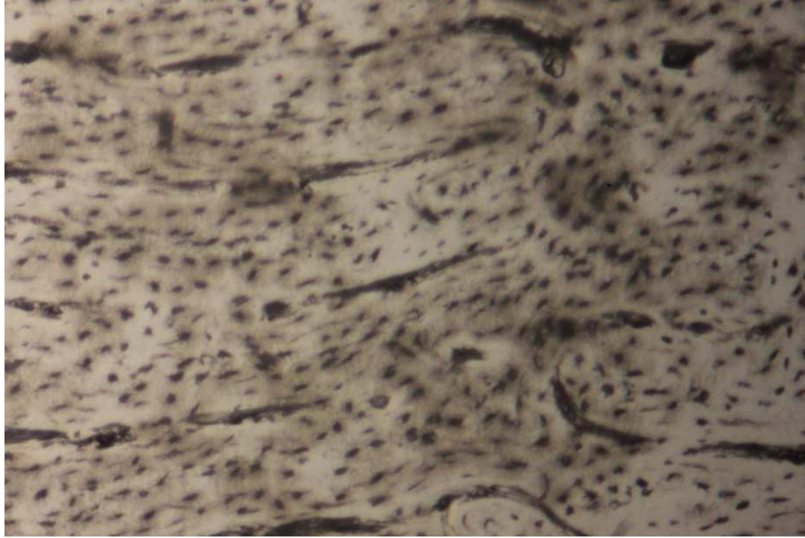

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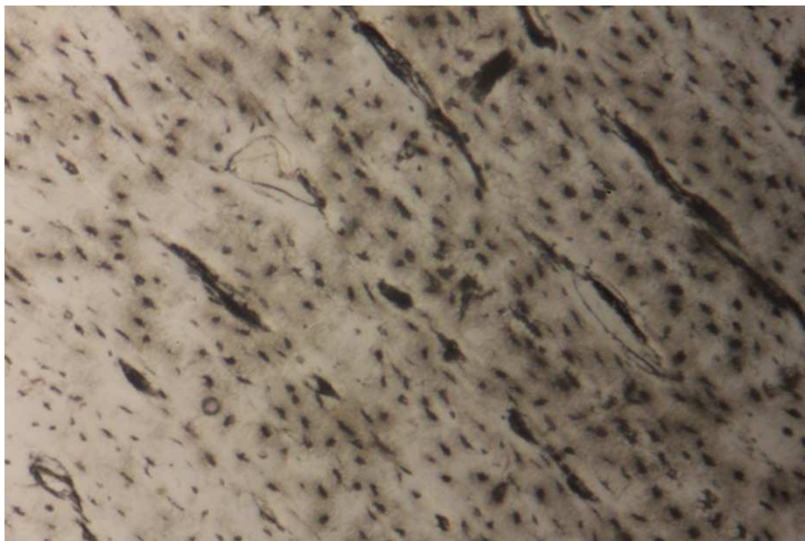

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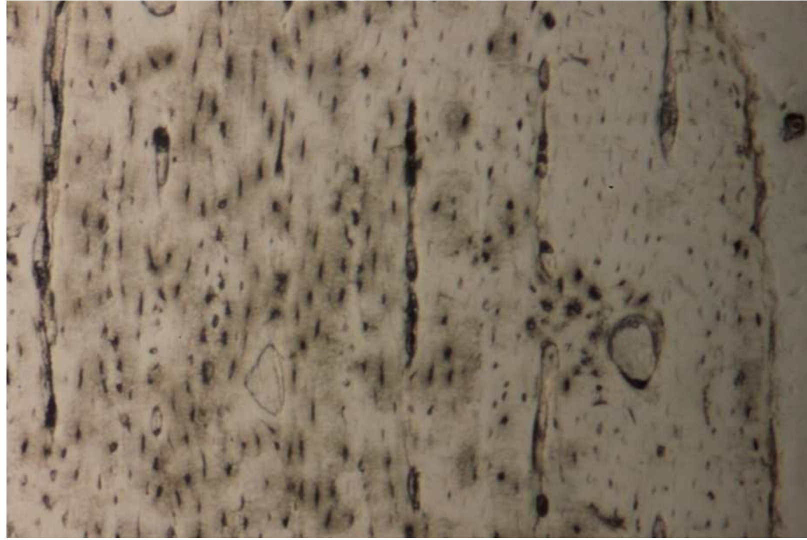

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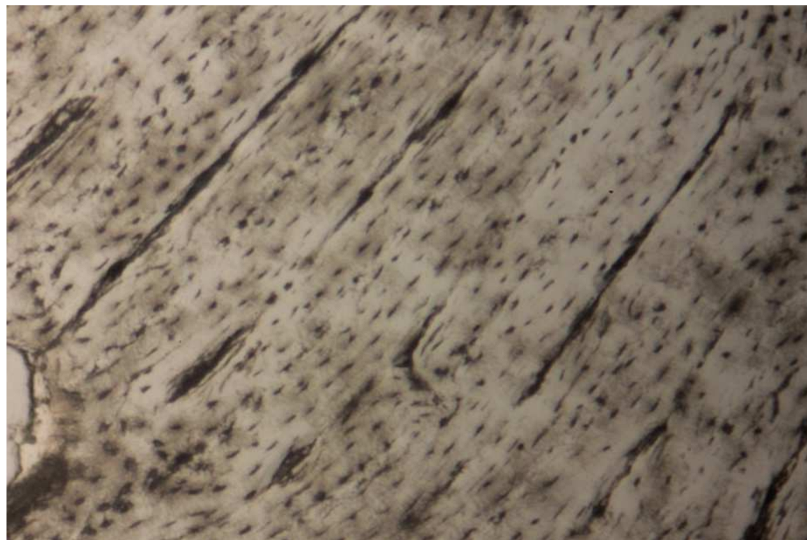
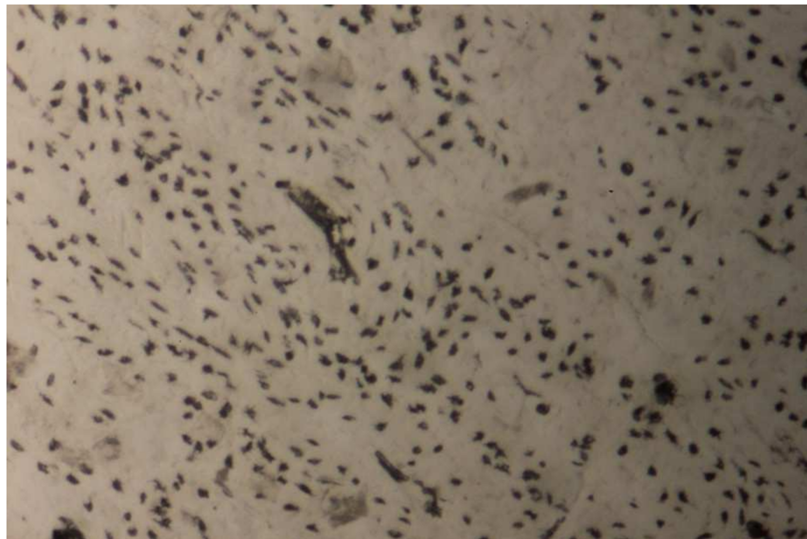


(Heat-simulated Bone Sample 1 – Plexiform Structure)

(Heat-simulated Bone Sample 1 – Plexiform Structure)



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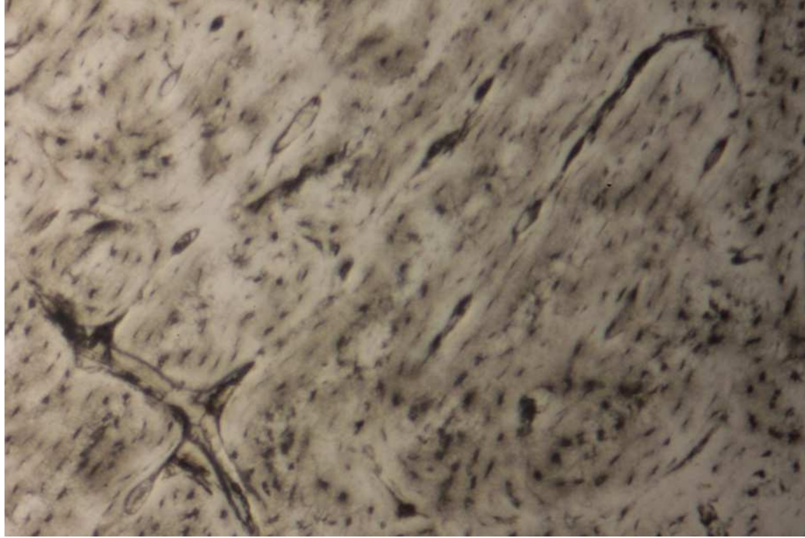

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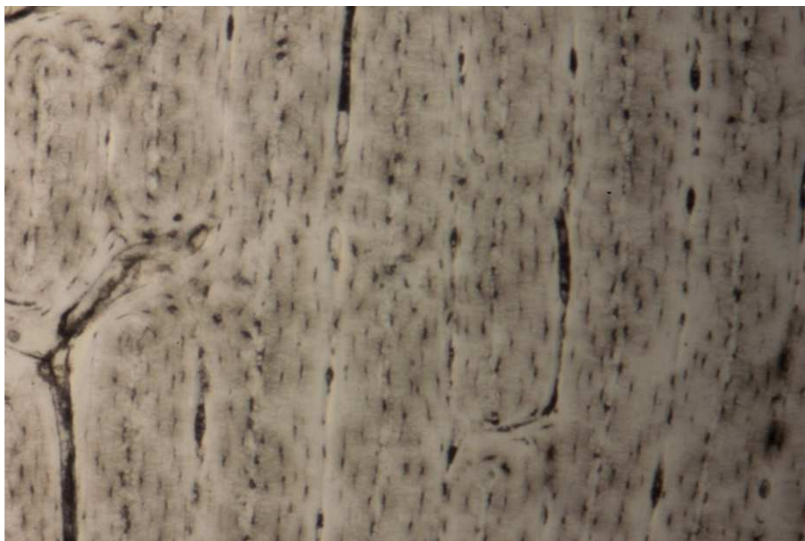

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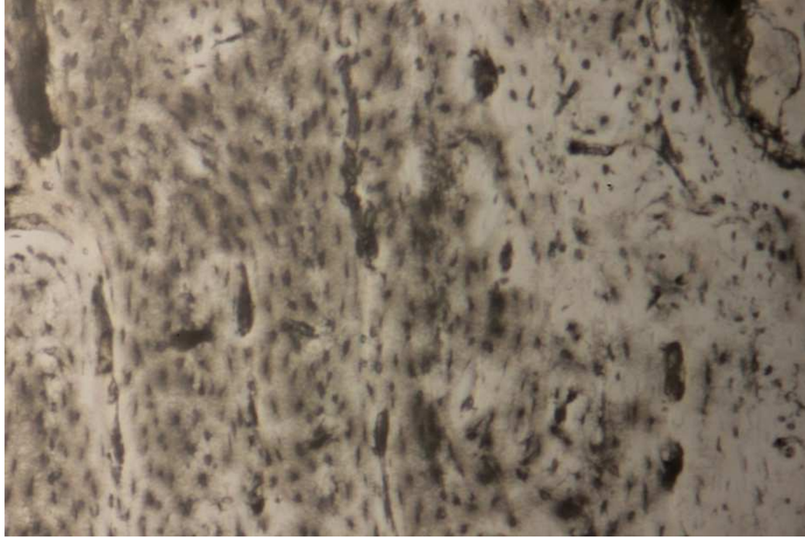

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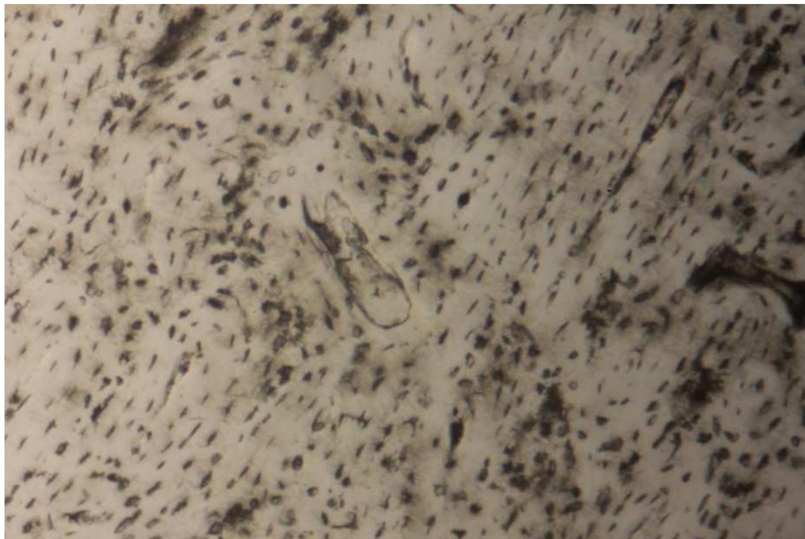

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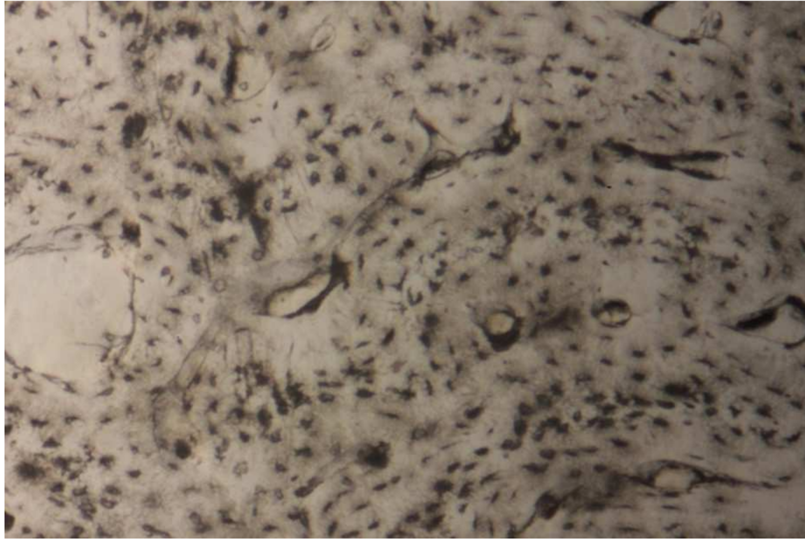

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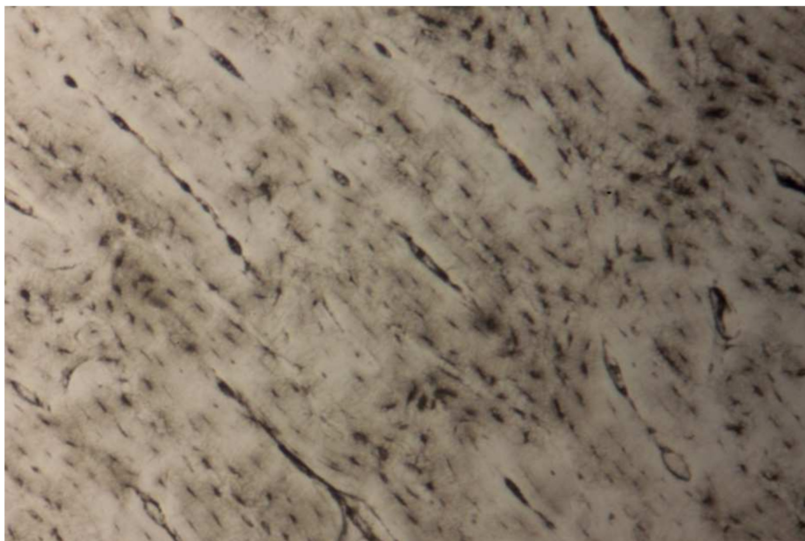

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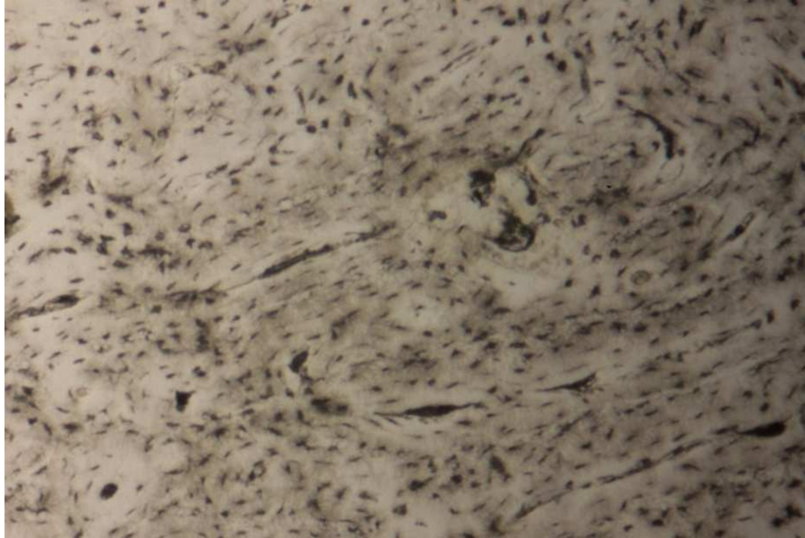
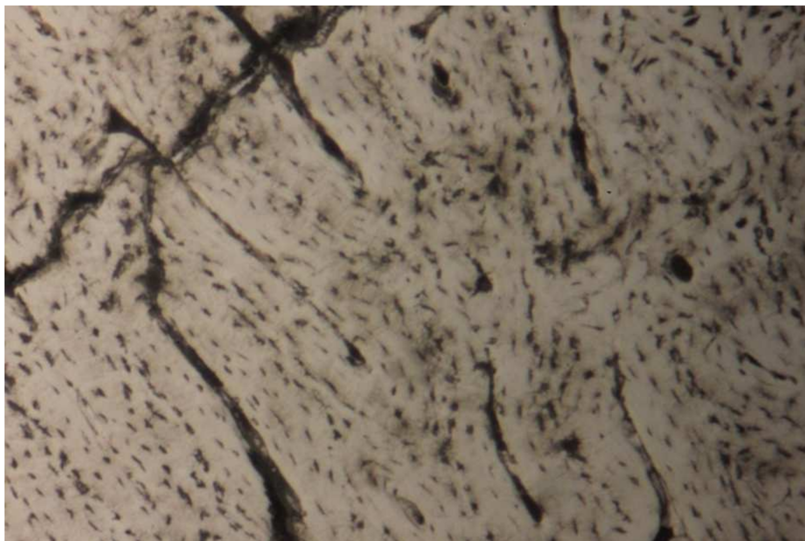


(Freeze-thaw Bone Sample 1 – Plexiform Structure)



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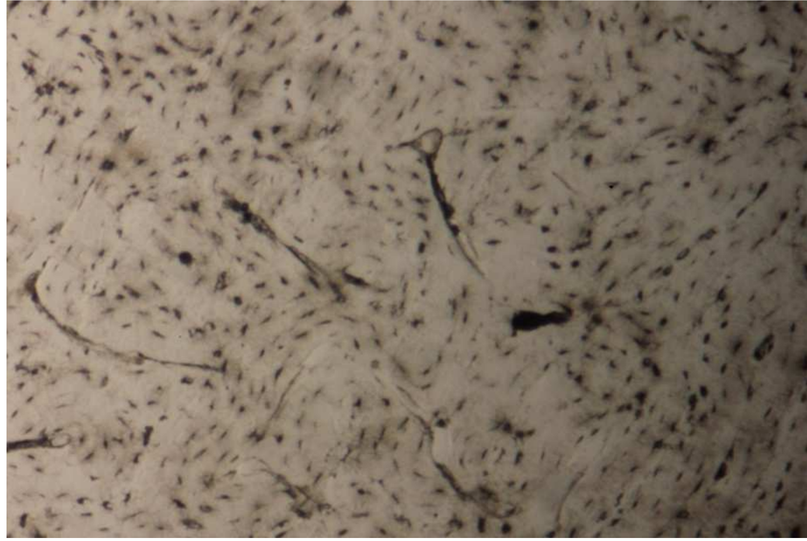

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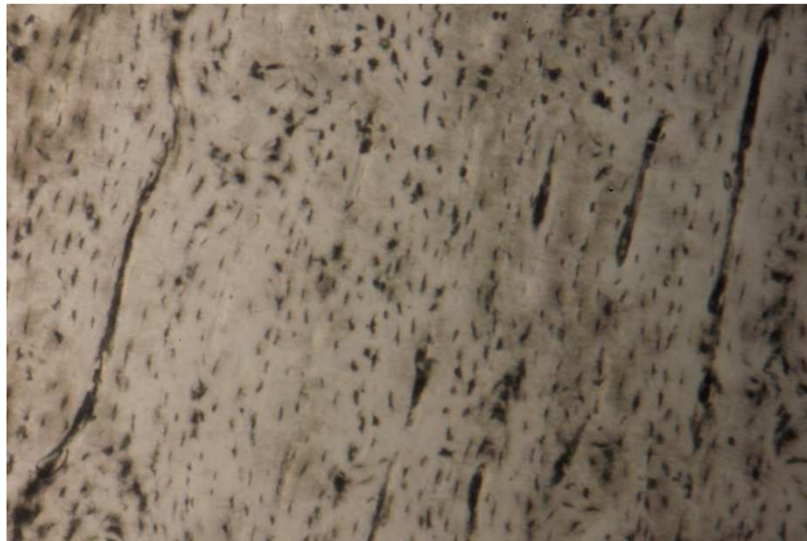
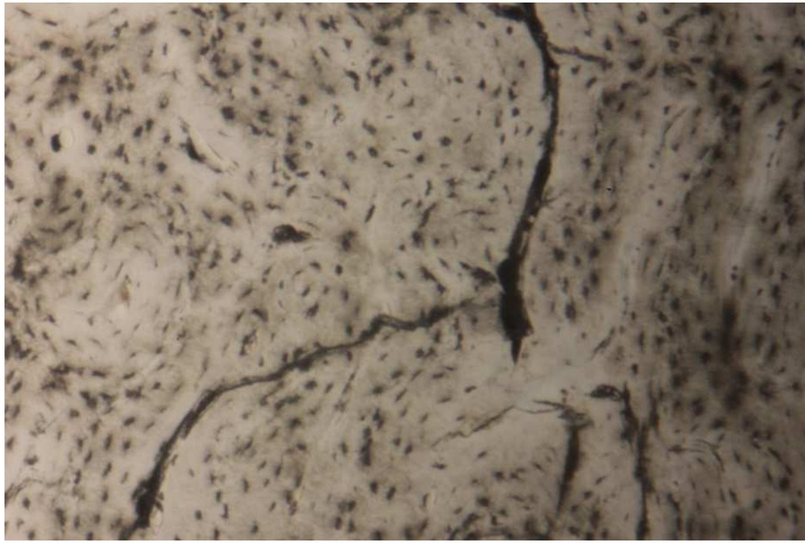


Freeze-thaw Bone Sample 1 – Plexiform Structure)



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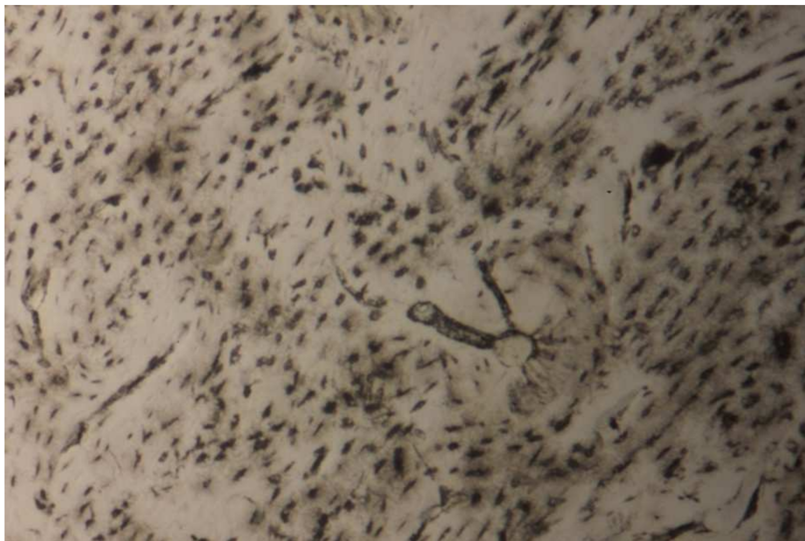

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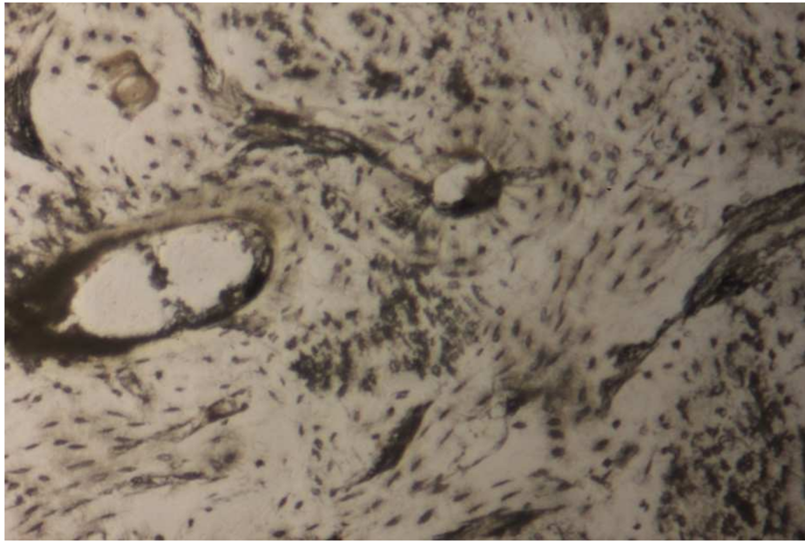

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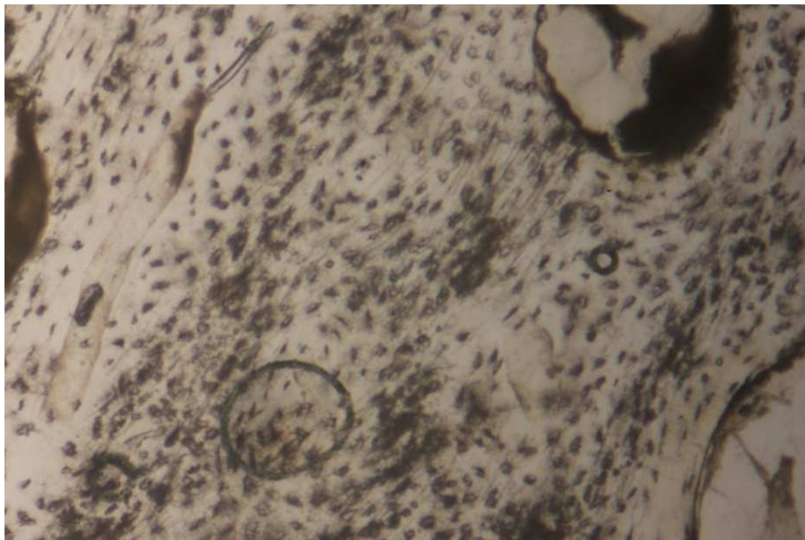

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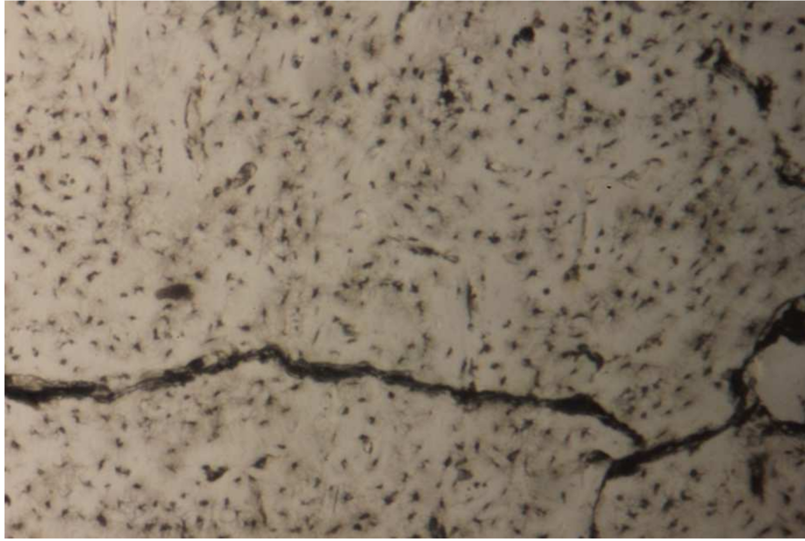

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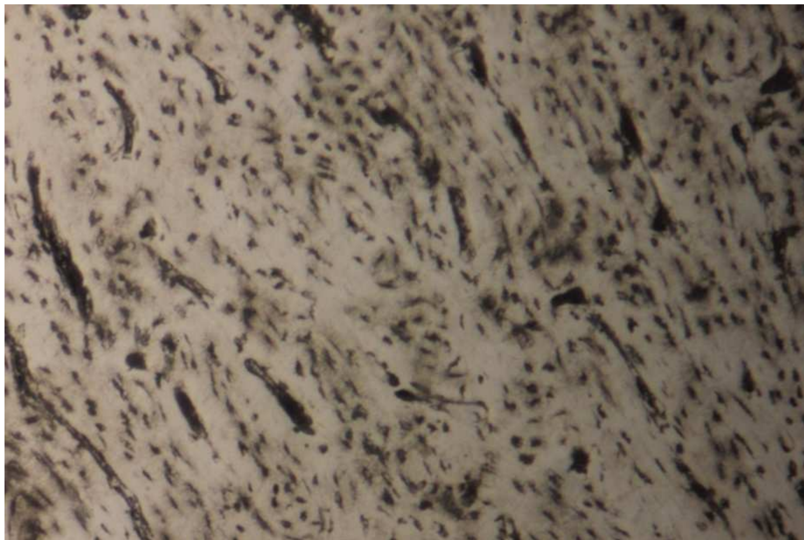
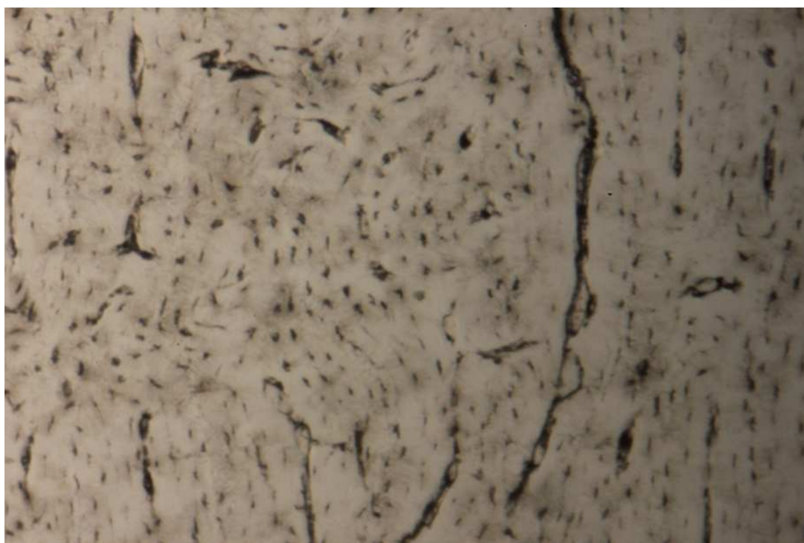
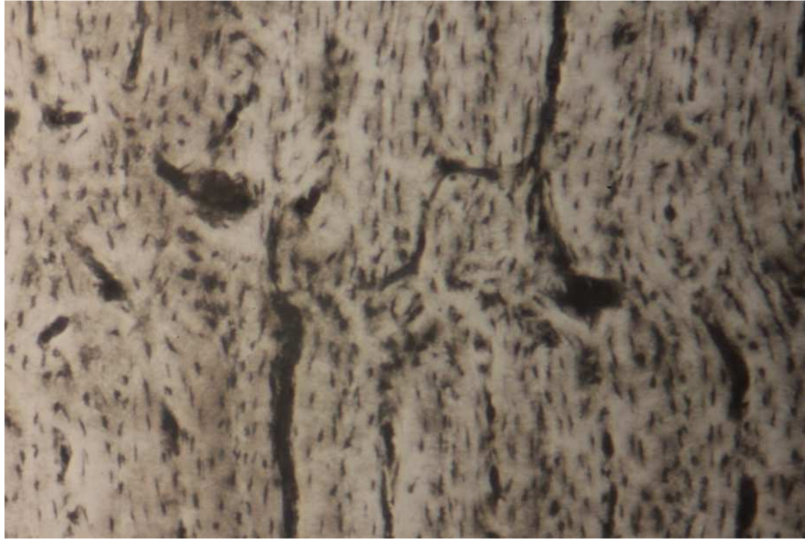


(Freeze-thaw Bone Sample 2 – Plexiform Structure)

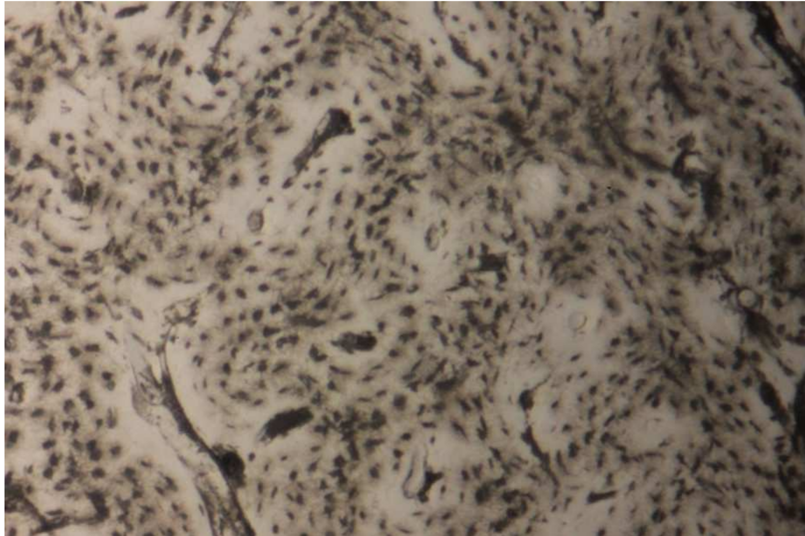
(Freeze-thaw Bone Sample 2 – Plexiform Structure)



(Freeze-thaw Bone Sample 2 – Plexiform Structure)



(Freeze-thaw Bone Sample 2– Plexiform Structure)



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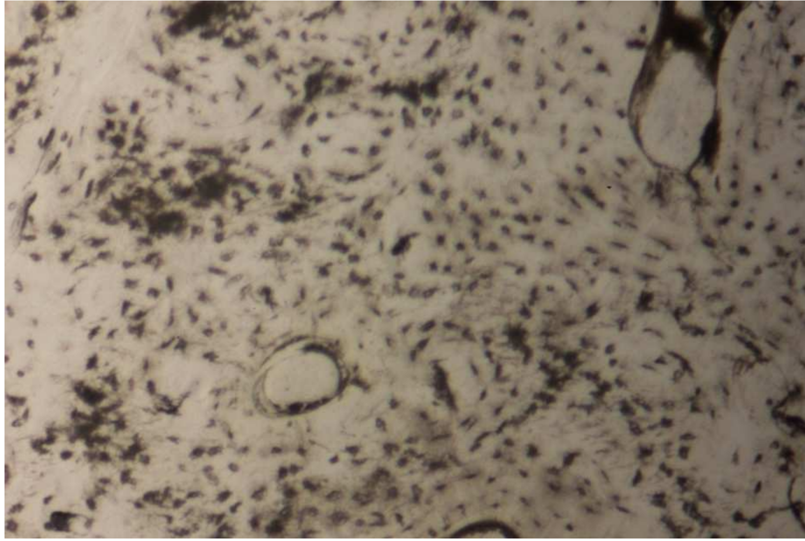

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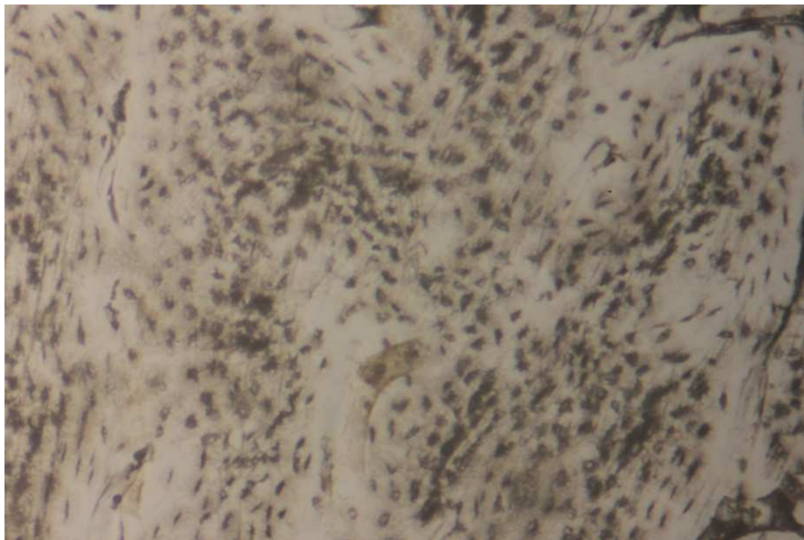
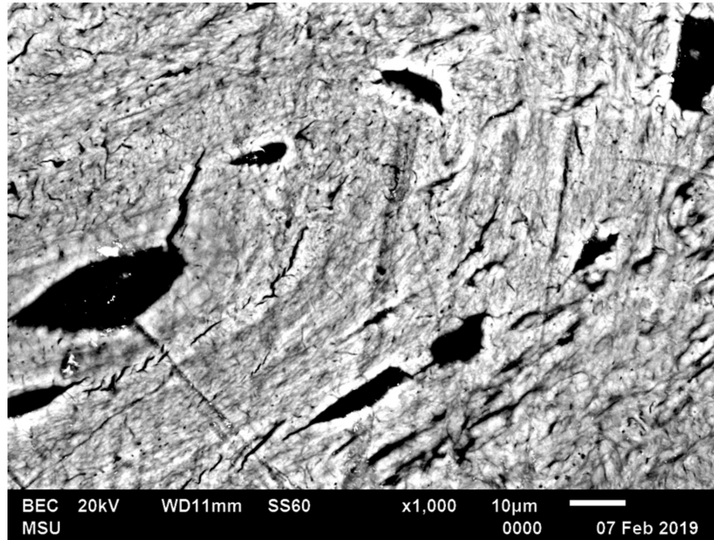


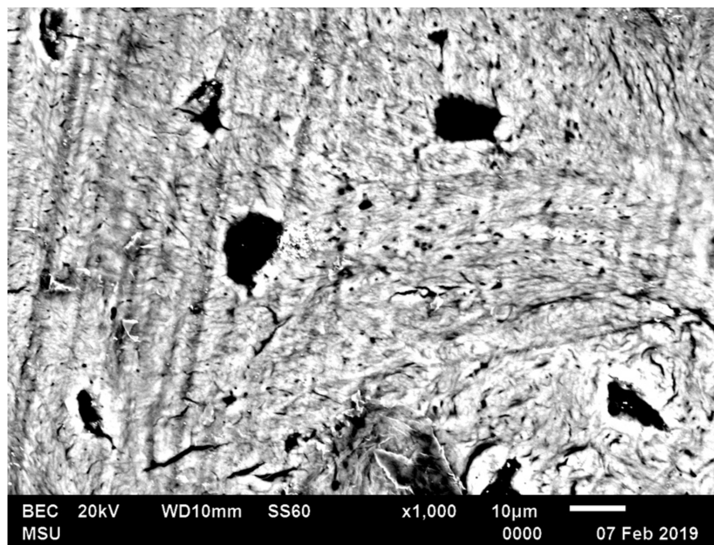
(Freeze-thaw Bone Sample 2 – Haversian Structure)

Appendix C

Scanning Electron Microscope (SEM) Photos



(Control Bone Sample 2)



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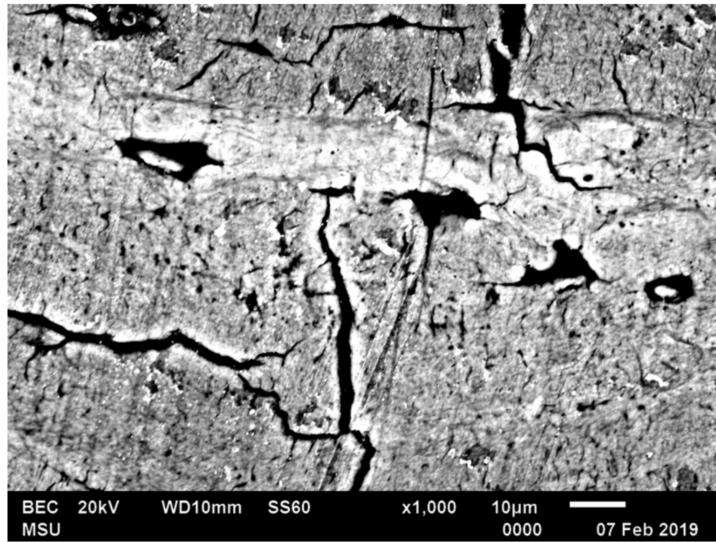

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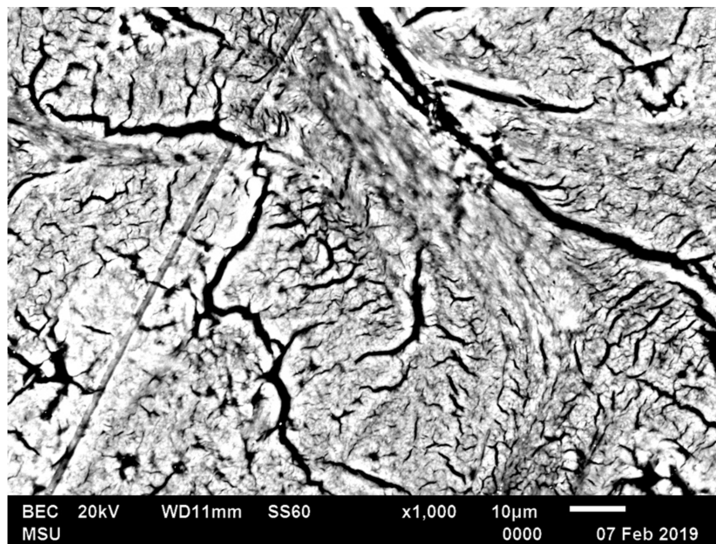

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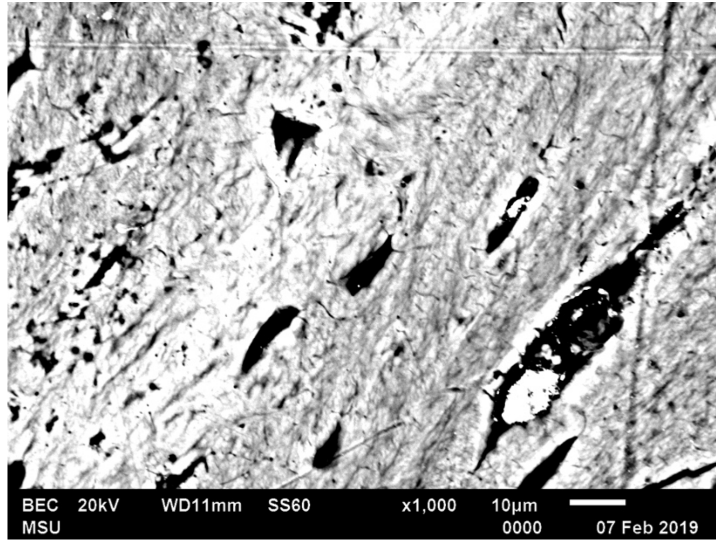

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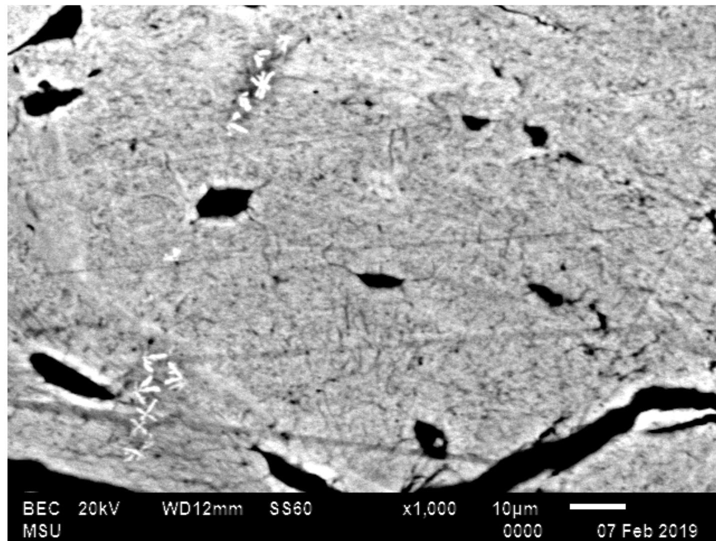

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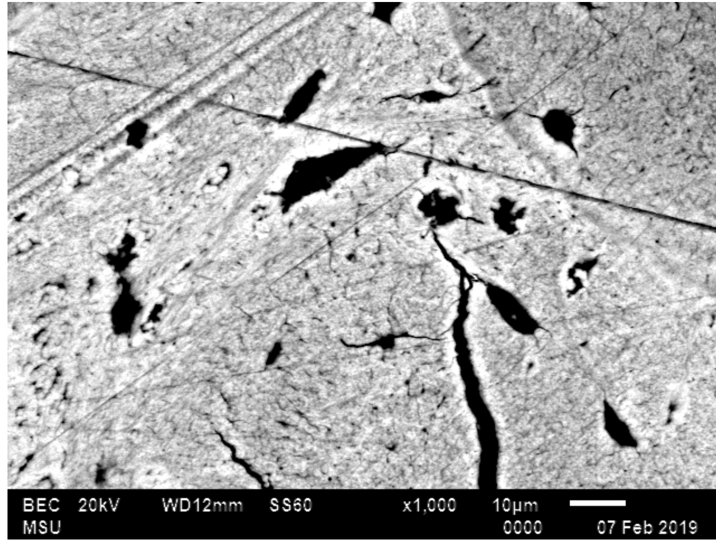
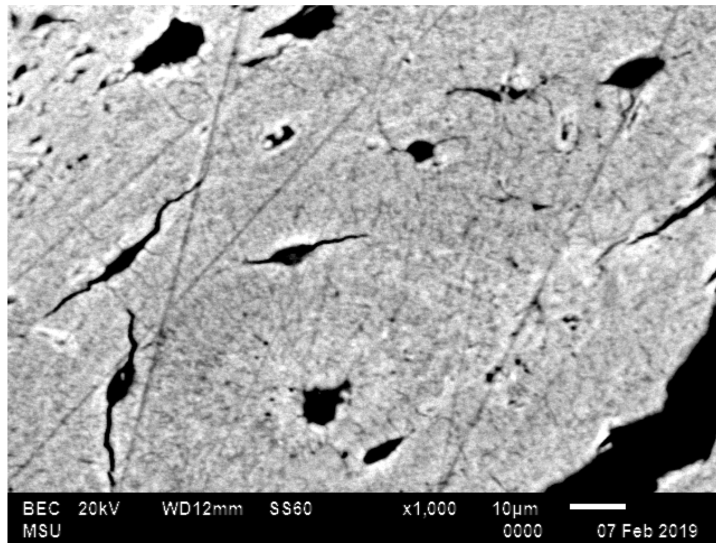
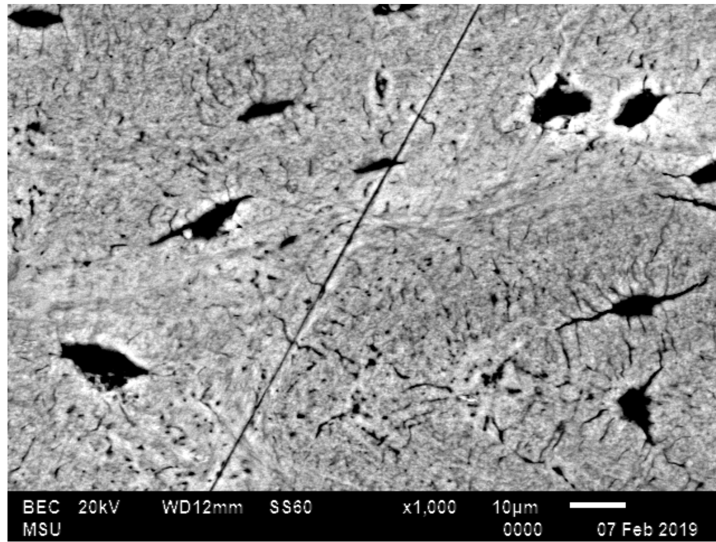


(Freeze-thaw Bone Sample)



(Freeze-thaw Bone Sample)



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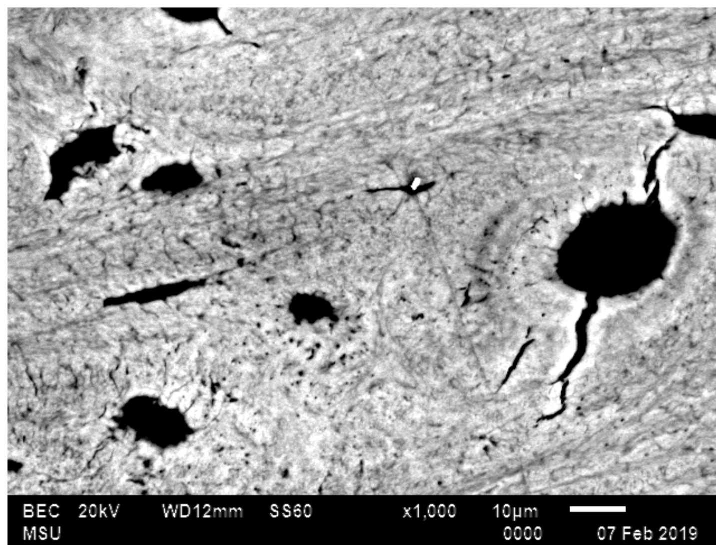

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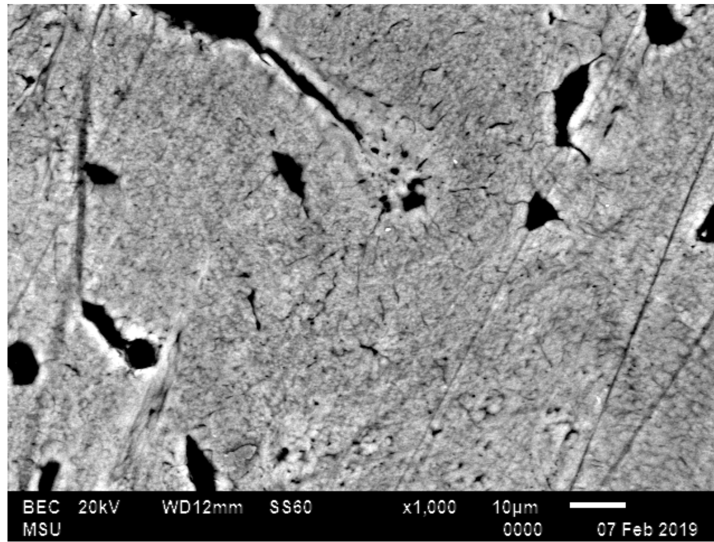

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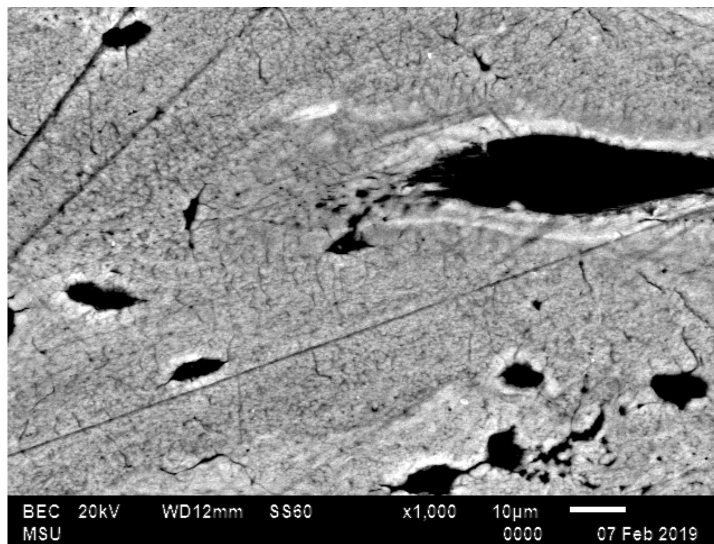

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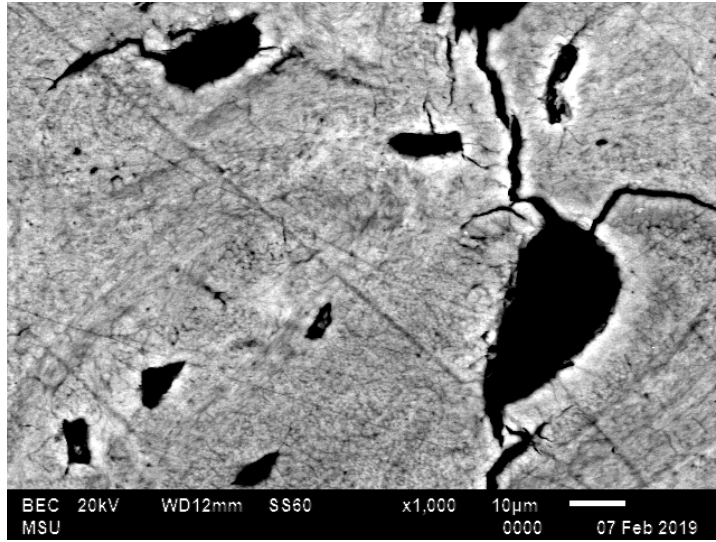

(Freeze-thaw Bone Sample)