

Taphonomic Processes Etch Bones:  
An Experimental Look at Plant Root Etchings on Bovine Bones

by  
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## **Abstract**

Taphonomy is the study that examines the changes that biological remains undergo after death. My project focuses on the aspect of taphonomy that investigates how plant roots interact with skeletal material. This experiment explores the effects of root etching on buried bovine rib bones over a nine-month duration within a controlled greenhouse environment. For the experimental setup, four plant species and two control samples were evaluated. Results show that root staining and mechanical root action can occur within this period; however, conclusive evidence of rootlet etching is inconclusive without scanning electron microscopy (SEM) analysis. Additionally, unexpected differential staining was noted on some of the bones, which was proposed to be due to components within the potting mix. Moreover, the presence of intrusive *Fatoua villosa* highlighted the difficulties posed by uncontrolled environmental factors in medico-legal scenes. The results found within this experiment can advance forensic anthropology by refining timelines for root interactions with remains, suggesting possible uses in medico-legal investigations.



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## Terminology

**biostratinomic changes:** *refer to chemical, biological, or mechanical alterations that occur to organic remains after death or before burial.*

**bovine:** *relating to cattle.*

**diagenetic changes:** *in the context of taphonomy, diagenetic changes refer to chemical, biological, or mechanical alterations that occur to organic remains after burial.*

**extrinsic factors:** *in the context of taphonomy, extrinsic factors are external factors that affect the preservation and decomposition of remains. (e.g., environmental conditions, soil type, scavenging, etc.).*

**mechanical root action:** *root activity that causes physical erosion of bone; in most cases, tunneling into the bone through the easiest avenues, such as into the foramina of the bone.*

**postmortem interval (PMI):** *estimated time since death.*

**root exudates:** *organic acids released from plant roots.*

**sternal end:** *The end of the rib that connects to the sternum.*

**taphonomy:** *the study of processes affecting remains after death.*

## INTRODUCTION

Taphonomy, stemming from the Greek words "taphos" for burial and "nomos" for law, is an interdisciplinary field that emerged from paleontology but has found applications in disciplines like archaeology, paleoecology, and forensic science (Christensen et al., 2019, p. 145). Taphonomy delves into the processes governing the fate of organic remains following death, exploring how environmental factors, scavengers, plant life, and various conditions contribute to the preservation or decomposition of these remnants (Christensen et al., 2019, p. 145). Taphonomic processes are globally ubiquitous and have many variables that will impact preservation, such as types of flora or fauna in the environment or the climate in the region. In the context of forensics, taphonomy refers to the study of what happens to an organism's remains postmortem until the remains are discovered or analyzed. Taphonomy covers the processes and factors that influence the survivability, recoverability, and alteration of biological materials such as bones, soft tissues, and other organic matter.

Forensic anthropology relies on a deep understanding of taphonomy to reconstruct the postmortem history of human remains. This interdisciplinary approach combines the knowledge of biological processes, environmental interactions, and anthropological expertise to contribute to the resolution of medico-legal questions surrounding death investigations involving skeletonized remains. Within medico-legal investigations, forensic anthropologists utilize taphonomy to give context to the postmortem interval (PMI), which defines how long an individual has been deceased (Vidoli et al., 2020, p. 251). Additionally, studying taphonomy is employed within forensic anthropology to

differentiate bone markings induced before death (antemortem), at the time of death (perimortem), and after death (postmortem) (Pokines, 2018, p. 89).

### *History of Taphonomy*

The study of taphonomy was first proposed by professor and paleontologist Ivan Efremov in the 1940s. Efremov believed that the field of paleontology was incomplete, thus leading to false interpretations of ancient ecosystems (Efremov, 1940). He noted that terrestrial fossils were even more challenging to analyze since most were discovered in “alien” landscapes, which essentially means that the fossils had been transported from their original ecosystems, likely due to environmental factors (Efremov, 1940). His solution to this missing piece was to propose a new branch of paleontology called taphonomy, deriving from the Greek words “taphos,” and “nomos”, or “burial laws.” Taphonomy seeks to understand the conditions under which fossils are preserved and embedded, integrating both biological and geological aspects to connect the two (Efremov, 1940). Since fossil records are products of both life and death, Efremov saw this new branch of study as a way to bridge the gap and challenge other paleontologists to look beyond the fossils to include the environment. His philosophy was that we needed to broaden our perspective to note all of the evidence, not just what is easily seen. In addition, Efremov wanted to quantify the taphonomic data to predict future or past fossilization patterns (Efremov, 1940). From the time between Efremov’s work and now, there has been tremendous research within the field of taphonomy. With more subcategories of taphonomic changes, whether they are considered biostratinomic or diagenetic, the field continues to grow and expand (Noe-Nygaard, 1987, p. 7).

Biostratinomy refers to the study of taphonomic processes that occur to organic remains after death but before burial; in comparison, diagenetic processes occur after burial (Christensen et al., 2019, p. 125). The research for this thesis would be an example of a diagenetic process because the methodology involves the burial of bovine bones. Plant root etching is typically a diagenetic process when it occurs underground. However, if the root action takes place above ground before burial occurs, it can be considered a biostratinomic process. In modern usage, forensic taphonomy for medico-legal purposes typically refers to the study of how taphonomic agents influence the preservation and recovery of skeletal remains (Christensen et al., 2019, p. 119).

### *History of Forensic Anthropology*

In broad terms, forensic anthropology can be considered a subfield of physical (or biological) anthropology—the study of human biology and skeletal anatomy—with a medico-legal aspect applied to it (Christensen et al., 2019, p. 1). Forensic anthropology directly employs methods of skeletal analysis in legal and criminal investigations, focusing on the identification of human remains and the construction of a biological profile. The history of forensic anthropology can be divided into four developmental eras between the years prior to the 1940s and the present day.

Prior to the 1940s, there were no established standardizations or formal instructions for forensic applications (Christensen et al., 2019, p. 2). Many practitioners, including anatomists, physicians, and sometimes physical anthropologists, were either self-educated or received informal training, resulting in limited knowledge and minimal engagement with medico-legal issues (Christensen et al., 2019, p. 2). The foundational



methods of the biological profile were first published in 1878 in *The Identification of the Human Skeleton: A Medicolegal Study* by anatomy professor Thomas Dwight. His work included methods that estimated the sex, age, and stature of a human skeleton. For this reason, Dwight was given the title of the father of American forensic anthropology (Ajeesh, 2022, p. 53). After the 1940s and into the early 1970s, recognition for forensic anthropology increased due to the use of anthropological techniques to identify deceased service members from previous wars. The increased use of anthropological techniques further led to a rise in the development of methods within the forensic anthropological community. Additionally, by the 1990s, forensic anthropology gained greater professional recognition and acceptance within the forensic community (Christensen et al., 2019, p. 3). This increased recognition has resulted in forensic anthropology being widely utilized today across various fields; however, it continues to be a relatively new field of study with limited standardization.

### *The Professionalism of Forensic Anthropology*

The professionalism of forensic anthropology began to emerge around the 1970s and 1980s with the establishment of the physical anthropology section within the American Academy of Forensic Sciences (AAFS). The AAFS is a professional society that promotes forensic science, education, and the standardization of methods within the various fields of forensic science (Christensen et al., 2019, p. 3). In addition to AAFS, The American Board of Forensic Anthropology (ABFA) was created in 1977 and serves to provide a certification program in forensic anthropology (Ubelaker, 2017, p. 917). As of this year (2025), the ABFA has created a Multilevel Certification Program, which will

create two distinct levels for practitioners: Analyst and Diplomate. The distinguishing aspect between the two levels is the amount of competency and knowledge an individual possesses. According to the ABFA, while one can take the Analyst exam with a master's degree, passing the Analyst exam and holding a PhD are required to take the Diplomate exam (ABFA, n.d.).

With the growing professionalism of forensic anthropology, new methods of identification have also emerged. Examples of new technologies include DNA analyses and stable isotope analysis. DNA analyses of bones involve taking a bone cutting, and testing the sample for mitochondrial DNA (mtDNA) (Holland et al., 1993, p. 543). Mitochondrial DNA refers to the DNA found within the mitochondria of a cell, which is directly inherited solely from the mother (Vadakedath et al., 2023). The interesting aspect of mtDNA is that since it is only inherited from the mother, the DNA found in children's mitochondria will be an exact replica of the mother's mtDNA; as a result, the same mtDNA is passed down through generations. Therefore, mtDNA is useful for individual identification of victims, or of perpetrators who have left biological materials behind at a crime scene. As for stable isotope analysis, stable isotopes such as carbon, strontium, nitrogen, or oxygen, for example, can be used to determine the geographic origin of unidentified human remains. Stable isotopes can be found in human tissues, such as bone, tooth enamel, or hair, and provide information about an individual's residency based on their dietary patterns and/or migration patterns (Bartelink et al., 2014, p. 128).

As forensic anthropology has become more prominent in investigations and in determining the PMI, the role of taphonomy within forensic anthropology has also expanded. To better understand how extrinsic factors can influence decomposition, Dr.

William M. Bass at the University of Tennessee, Knoxville (UTK) established the first human decomposition research facility in 1981 (Kolpan, 2025, p. 218). Known colloquially as the “Body Farm,” the Anthropology Research Facility (ARF) was created to address the lack of published research on how various factors, such as environmental conditions or human intervention, can impact the rate of decomposition. Within the ARF, donated human remains are put in various depositional scenarios to understand how decomposition is affected by different conditions (Kolpan, 2025, p. 219). Today, there are eight decomposition facilities in the U.S. alone, with additional sites outside the country.

Moreover, forensic anthropology has received increased attention due to efforts aimed at recovering and repatriating the remains of fallen soldiers. The application of forensic anthropological methods became particularly necessary during and after World War II because of the vast number of fallen service members. The scale of this task, with almost 400,000 American service members missing in action in WWII (DPAA, n.d.), highlighted the demand for a systematic approach to search, recovery, and identification efforts. In response, the American Graves Registration Service (AGRS) established the Central Identification Laboratory (CIL) in Hawaii in 1947. Today, the CIL, now part of the Defense POW/MIA Accounting Agency (DPAA), remains one of the world’s largest and most respected skeletal identification laboratories. The methods and techniques typically involved with aiding the identification process include DNA analyses, odontological records, and stable isotope analyses.

### *The Experimental Emphasis & Rootlet Etching*

Within this experiment, the taphonomic focus was on how plant roots can etch skeletonized bovine rib bones, as a proxy for plant root action on human bone in forensic contexts. Plant roots can alter bones in a taphonomic environment through a process known as rootlet etching or root action (White & Folkens, 2005, p. 57). This phenomenon occurs when plant roots come into contact with bones and their acidic secretions, also known as plant root exudates, or by mechanical action resulting in the dissolution or removal of bone material (Schultz, 1997, p. 206). Plant roots can exude organic acids during normal metabolic processes, such as citric, oxalic, malic, acetic, and lactic acids, and many others, which can have a corrosive effect on the mineralized components of bones, primarily hydroxyapatite, an inorganic mineral present in bones (Schultz, 1997, p. 206; Haynes & Wojtal, 2023, p. 515; Koo et. al., 2005, p. 424). As plant roots move across the bone surface, hydrogen ions released by the plant roots react with the salts in the earth to develop a more acidic milieu which dissolves the bone surface, tracking the direction of growth (Pokines, 2018, p. 101). This dissolution of minerals leaves behind dendritic patterns or a meandering web of superficial furrows (Togerson, 2013, p. 84; Butler, 2018, p. 15). These patterns can also be curved and intersected across the bone cortical surface (Byers, 2002, p. 372). From recent literature, it is shown that there is a minimum of a one-year timeframe before etching is noted (Macho-Callejo et al., 2023, pg. 2). In addition to the chemical effects, the physical growth and penetration of roots can contribute to bone etching (Schultz, 1997, p. 206). As roots grow and expand, they may exert mechanical pressure on bones, causing abrasions or surface damage. Roots can

create channels or hollows on the surface of bones as they continue to grow and explore the surrounding soil. These channels may expose the bone to further environmental factors, accelerating the weathering process (Schultz, 1997, p. 206). The presence of plant roots and their interactions with bones are influenced by environmental factors such as soil composition, moisture levels, and the type of vegetation in the region. Different plants may have varying effects on bones depending on their root structure and secretions. However, there is very little research done or a database that can properly quantify the etching variances between different plant species (Macho-Callejo et al., 2023, pg. 2).

#### *The Need for This Taphonomic Research*

Although the fields of anthropology and forensic science have evolved significantly over the last century, there remains a vast, unexplored gap of knowledge. While much research still needs to be conducted, this thesis emphasizes the processes of plant root etching and mechanical root growth. The taphonomic process of plant root etching is a crucial area within forensic anthropology and taphonomy, providing insights into the PMI (Christensen et al., 2019, p. 119; Willey & Heilman, 1987). With more extensive research into plant root etching, forensic anthropologists can estimate how long a body has been exposed to plant activity. This estimate, based on exposure, is particularly useful in cases where environmental factors significantly influence decomposition. Furthermore, by researching taphonomic agents, forensic anthropologists will be better equipped to differentiate between postmortem damage caused by taphonomic agents and trauma occurring antemortem or perimortem. For instance, forensic anthropologists could

analyze root etching patterns to ascertain whether markings left on bones were inflicted by flora, cuts, or resulted from a perimortem disease (Haglund & Sorg, 1997, p. 206). Furthermore, mechanical root action represents another area of research that could benefit from more experimentation and case studies. As previously stated, mechanical root action can cause various taphonomic damages, such as channels, hollows, or cracking, which may be mistaken for antemortem trauma. Additionally, root growth into or between skeletal matter can also be used to estimate the PMI by examining the number of rings in the root, providing an indication of the minimum timespan for which the skeletal remains may have been exposed to taphonomic agents (Willey & Heilman, 1987, p. 1265).

Overall, it can be stated that there is a need for further research and standardization within the taphonomic field, especially for plant root etching and mechanical root action. By updating the literature on how different plants, whether perennial or annual, may affect skeletal matter, we can increase the accuracy of forensic analysis by providing another variable to consider when determining the PMI and understanding decomposition.

## **MATERIALS AND METHODS**

### *Choosing the Plant Materials*

To mimic the taphonomic process of plant root etching, plants with known aggressive root systems were selected to give the project the best chance to work. Four plants were chosen as the experimental variables: Silver Maple (*Acer saccharinum L.*), Butterfly bush (*Buddleia davidii L.*), Bermuda grass (*Cynodon dactylon*), and Spearmint (*Mentha spicata L.*). Each of the selected plants, except for the Spearmint plants, has prolific fibrous roots that can be adventitious. These types of roots branch directly from the base of the stem and spread out as a mass of similar-sized roots (Merriam-Webster, n.d.). This abundance of roots allows a greater chance of rootlet etching. In comparison, Spearmint plants have a different sort of root growth. These plants have a rhizomatous root system, which allows the plant to grow new plants off the horizontal trailing root system. This characteristic is one of the reasons that this plant was chosen for the experiment.

### *Setting the Experiment Up*

There were two of each plant, making a total of eight experimental subjects, along with two control pots, which brings the total to ten pots. The plants used were either small young sprouts or seeds. The controls contained no plants but still received the other control variables such as water, soil, temperature, environment, and so on. These controlled variables ensured that the plants were the only difference to the bones. The other materials that were needed to set up the project included potting soil – Miracle-Gro Potting Mix was used for this experiment – ten 8.80in x 8.25in pots with a 10-inch

diameter, a controlled environment, and ten bovine rib bones cut into 20cm pieces (the cuts were made from the sternal end of the bone). For the controlled environment used during this experiment, I received access to one of Middle Tennessee State University's greenhouses – located behind the Academic Classroom Building – granted by Professor Nate Phillips.

The bovine bones used for this experiment were sourced from a single animal so that the time, duration, and conditions of skeletonization were consistent across all test samples. Rib bones were the best skeletal element to choose due to the abundance that can be sourced from a single animal. Before the setup of the project, the bones being utilized were washed following standard protocol and thoroughly inspected, with notes being taken to record any marks or discolorations that were already present on the bones before the project started. Each bone was assigned a number 1 through 10 and then was marked with three 1x1cm squares on both the front and back surface areas of the bones, as pictured in Figures 1-2 below. The placement of the squares was determined by marking the middle of the bone, approximately 10cm, then measuring 5cm away from the middle on each side to mark the other two boxes.





*Figure 1. Front view of 1x1cm squares with their respective labels.*



*Figure 2. Back view of 1x1cm squares with their respective labels.*

The squares were drawn on with a thin sharpie with the help of a 1x1 cm square stencil made from an index card. As shown above, each of the squares was marked with the numeric of the bone and a letter (a-f) to help distinguish and organize them in my files. Photographs were also taken using a standard laboratory light microscope with a 35 mm DSLR camera mount. The bones were photographed with a set orientation to ensure that the before and after pictures would correlate. Additionally, the initial condition of the bones within the squares was scored following a scoring procedure. Per the scoring

procedure, the bones were categorized based on the amount of cortical bone erosion using a grading scale of 0-5; 0 being the absence of any changes and 5 being a complete obscuring of the cortical surface with a note of extent and position.

Once the bones were recorded and marked, the plants and pots were prepared. Dirt was placed in the pot until approximately 7cm remained from the top of the soil to the rim of the pot. The bones were then placed, and 5cm of soil was added on top. The base of the plant roots was then added, and the remaining soil was added to fill the pot. This process ensured that the experimental bones were placed 5cm below the base of the plant root systems. The control bones were planted in the last two pots, with the bones being the same distance below the surface as the experimental bones. The setup can be seen below in Figure 3.



*Figure 3. Experimental setup with labeled plant pots.*

The experiment then ran for approximately 9 months with watering check-ins every week. Each pot, experimental or control, got the same amount of water which

amounted to a quart or approximately 950 mL each visit. The watering amount was figured to fully soak the dirt within the pot; however, the excess water was drained through the drainage holes at the bottom of the pots. Within the greenhouse, the plants received a constant source of light with natural light in the day and artificial grow lights during dark hours. Additionally, the greenhouse mostly maintained a temperature of around 70 degrees fahrenheit through the duration of the project. The temperature is difficult to manage during the summer months due to the high temperatures (Nate Phillips, personal communication).

#### *Removal of the Bones and the Final Examination Process*

Once the duration of the experimental process was completed, the plants were uncovered. To retrieve the bones in the control pots, the dirt was carefully removed from around the bone, and the bone was extracted from the pot once uncovered. For the bones within the experimental pots, the sides of the pot were gently pressed to loosen the dirt from the sides and extract all the pot contents. This process was to certify that I could photograph and carefully extract the bones from potentially entwined roots. Once uncovered, the bones were quickly rinsed with cool water to remove any loose dirt, and I used my fingers to gently wipe the surface of the bones to ensure that no potential rootlet etchings were damaged by scratching the malleable surface. The bones were then left to air dry at room temperature on paper towels. Finally, to replicate the initial analysis of the skeletal material, the bones were reanalyzed using a standard laboratory light microscope with a 35 mm DSLR camera mount. Careful attention was given to capturing images of any

notable features both inside and outside of the marked squares to conclude the final examination of the bones.

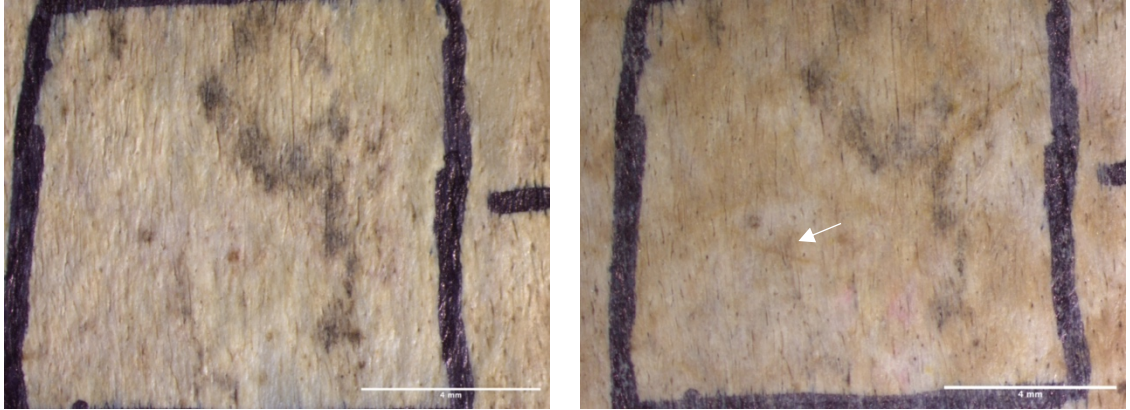
## RESULTS

The results of the bone analysis will be divided into four sections: root staining, purple blotches, mechanical root growth, and the uninvited variable.

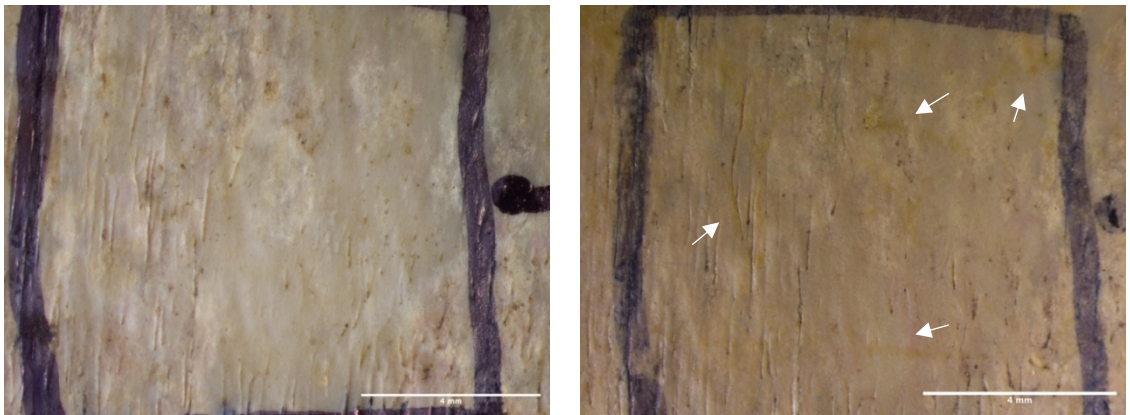
### *Root Staining*

During the initial examination and photography of the bones, it was clear that root staining had occurred in some areas. However, to fully assess the extent of the staining, a comprehensive side-by-side comparison of before and after photos was necessary. The easily identifiable areas are characterized by dendritic patterns and a distinct yellow staining, which is hypothesized to be caused by the roots. This staining is thought to result from tannins in the plant material. Tannins, also referred to as tannic acid, are derived from phenolic acids (Chung et al., 1998, p. 168). These chemical compounds are found in various parts of the plant; however, the specific structure relevant to this experiment is the roots. Tannins can be exuded from root structures into the soil, which in turn imparts a yellowish discoloration to the soil or its surroundings. The primary function of tannins is to act as a defense mechanism for plants against microbial infections (Chung et al., 1998, p. 168). The distinct staining can be observed below on bones 1, 2, 3, 4, and 6; the staining on bone 2 will not be discussed in this section but will be addressed in the ‘uninvited variable’ section.

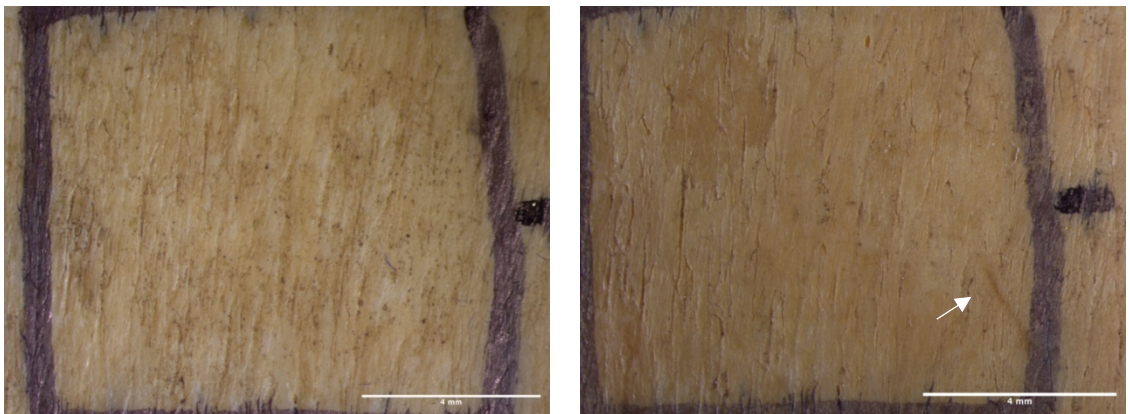




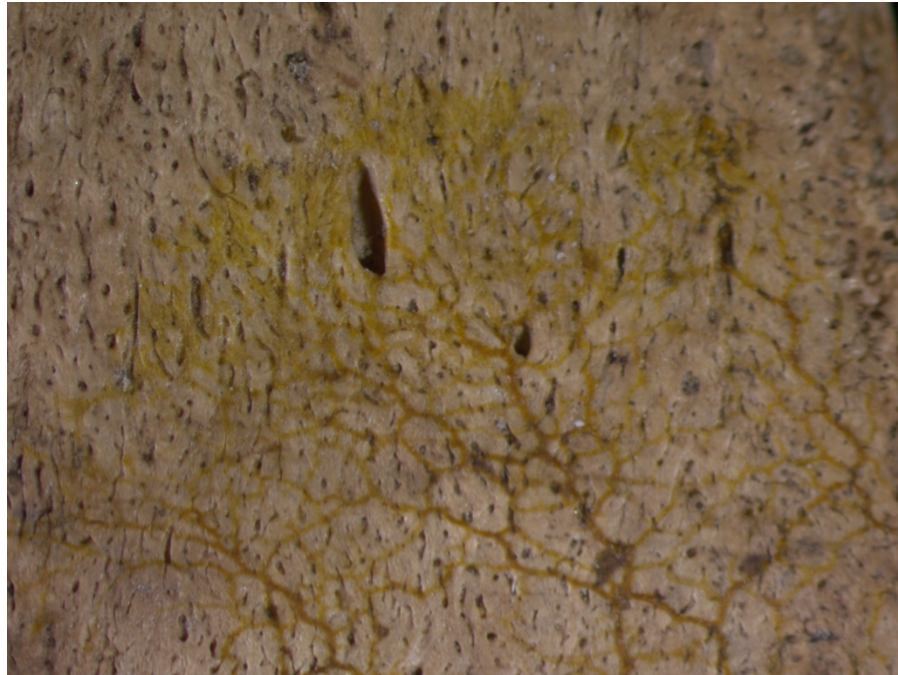
*Figure 4. (left) Before photo of bone 1 square b – 1b; (right) after photo of 1b with presumable root staining marked with an arrow.*



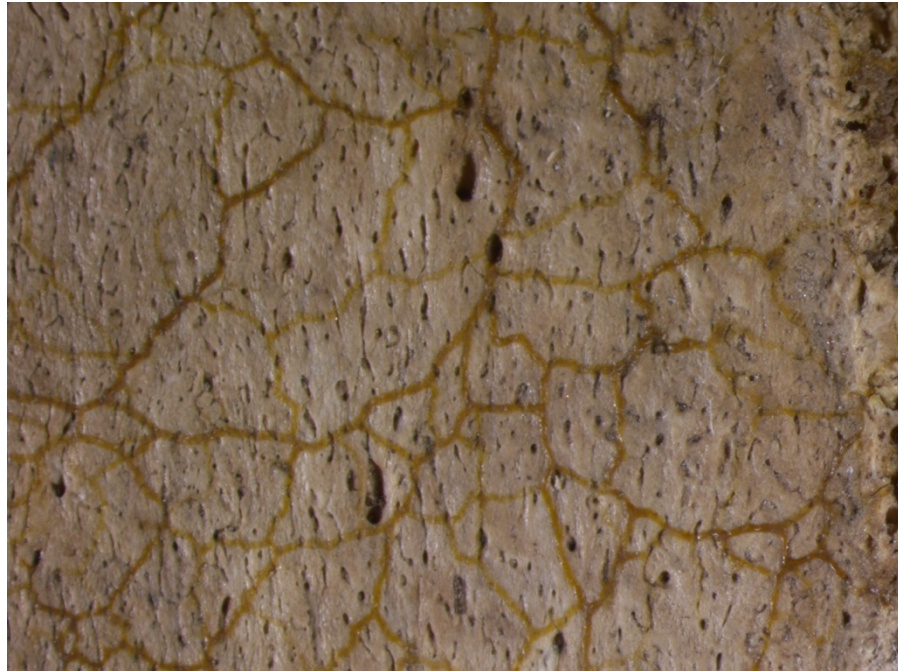
*Figure 5. (left) Before photo of bone 1 square e – 1e; (right) after photo of 1e with presumable root staining marked with arrows.*



*Figure 6. (left) Before photo of bone 3 square f – 3f; (right) after photo of 3f with presumable root staining marked with an arrow.*

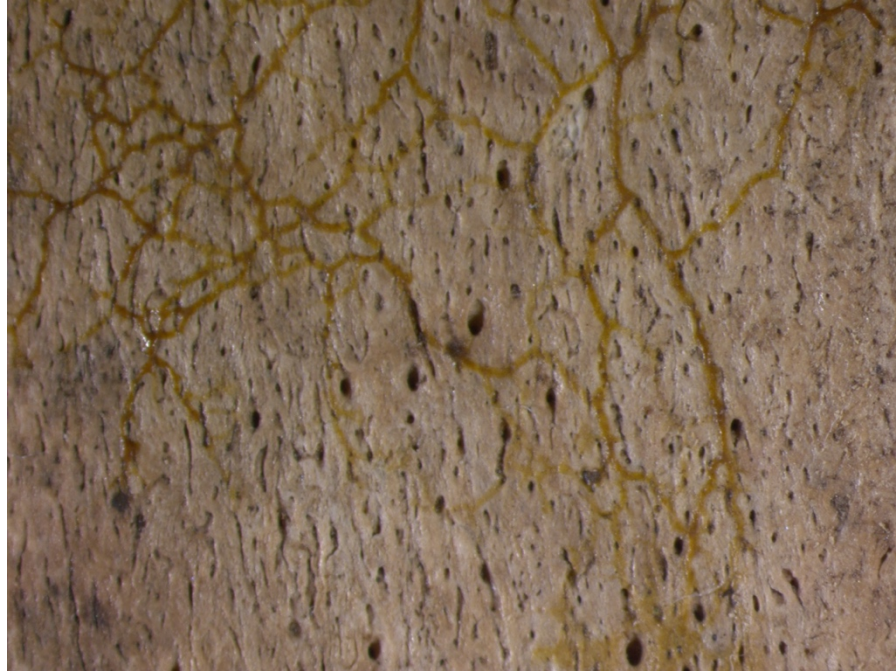


*Figure 7. Dendritic staining found on bone 3 during analysis after exhumation – part 1.*



*Figure 8. Dendritic staining found on bone 3 during analysis after exhumation – part 2.*



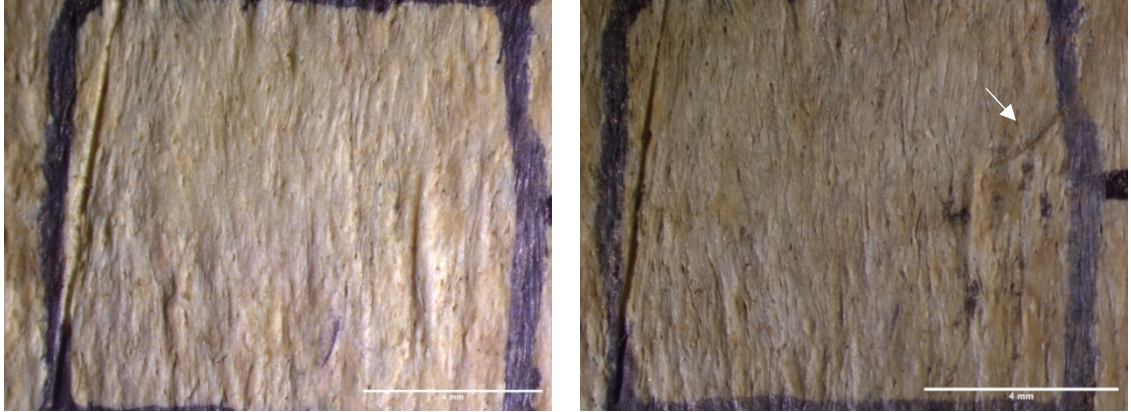


*Figure 9. Dendritic staining found on bone 3 during analysis after exhumation – part 3.*

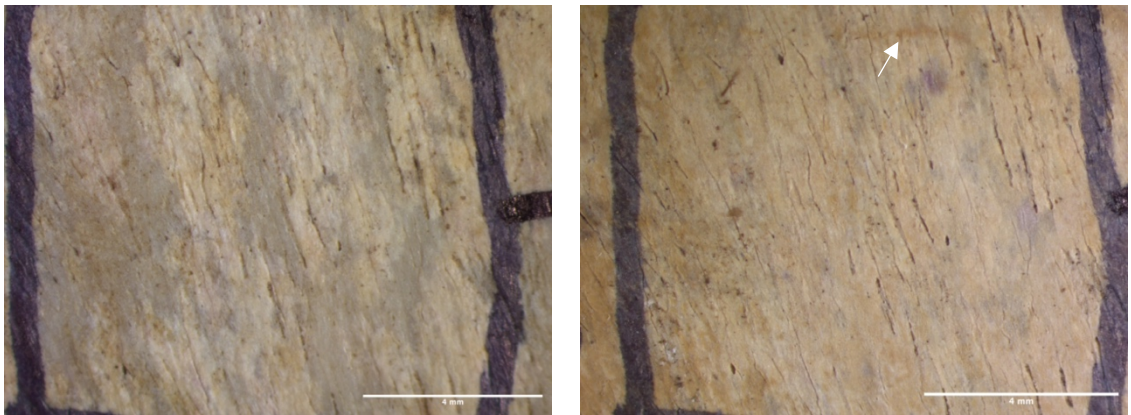


*Figure 10. Dendritic staining found on bone 3 during analysis after exhumation – part 4.*

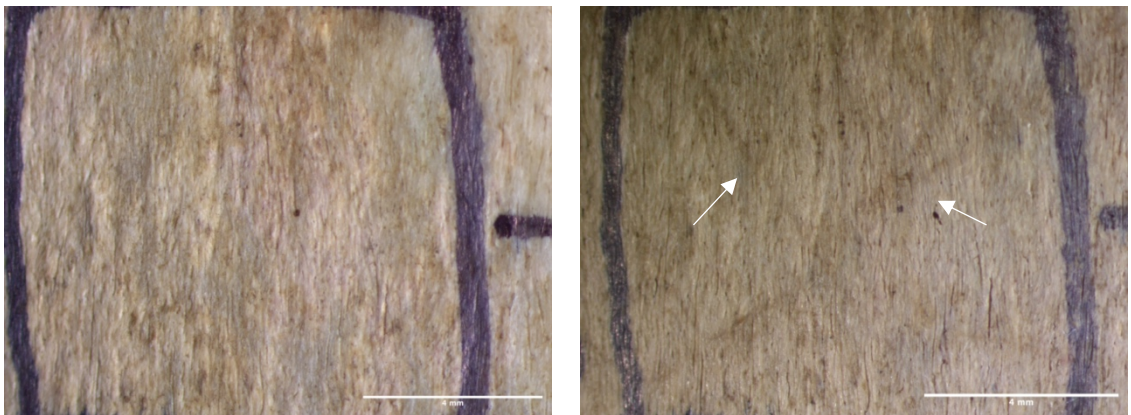




*Figure 11. (left) Before photo of bone 4 square a – 4a; (right) after photo of 4a with presumable root staining marked with an arrow.*



*Figure 12. (left) Before photo of bone 6 square c – 6c; (right) after photo of 6c with presumable root staining marked with an arrow.*

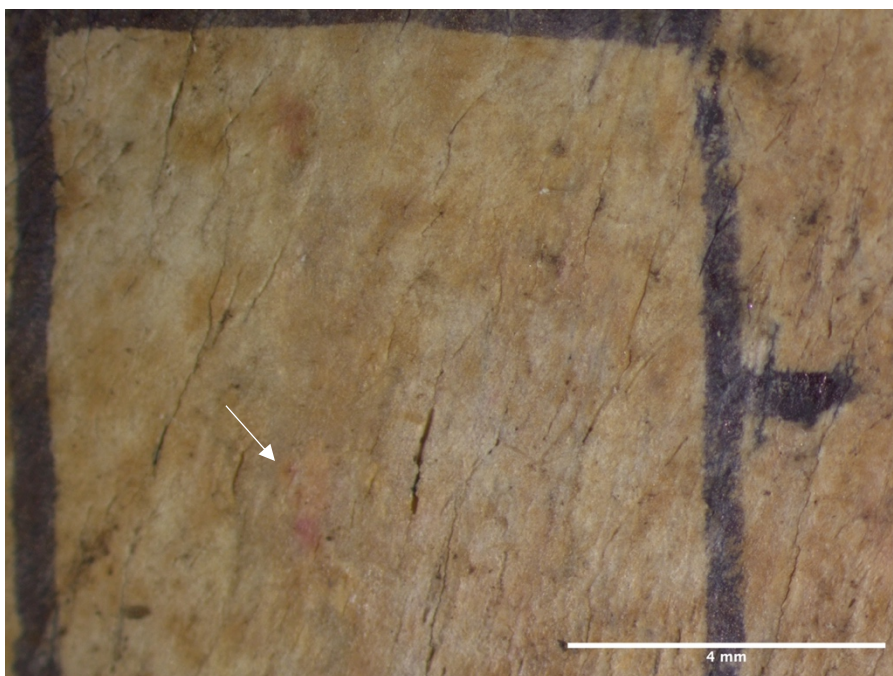


*Figure 13. (left) Before photo of bone 6 square e – 6e; (right) after photo of 6e with presumable root staining marked with arrows.*

As there is discernable soil staining on the bones, as seen throughout the comparison photos, it is difficult to piece out all of the root staining due to the colors blending in. There may have been more markings that I have missed throughout my examination. However, this does not discount the findings overall, and it is shown that there is considerable root staining throughout the experimental samples.

### *Purple Blotches*

In addition to root staining, I encountered peculiar spots ranging from light lavender to indigo, along with some highlighter-pink areas. The intriguing part about these findings is that the distinctive coloration can only truly be seen through the microscope. The coloration looked muted or dull when examining the bones, almost like brown spotting. These spots were noted to be found on both the experimental and one of the control bones. The differential staining was found on bones 1, 4, 5, 6, 7, 8, and 9, as shown in the photographs below. One observation noted is that the staining on bone 5 appears to be ubiquitous throughout the entire surface of the bone, while the other bones show only minimal staining spots.

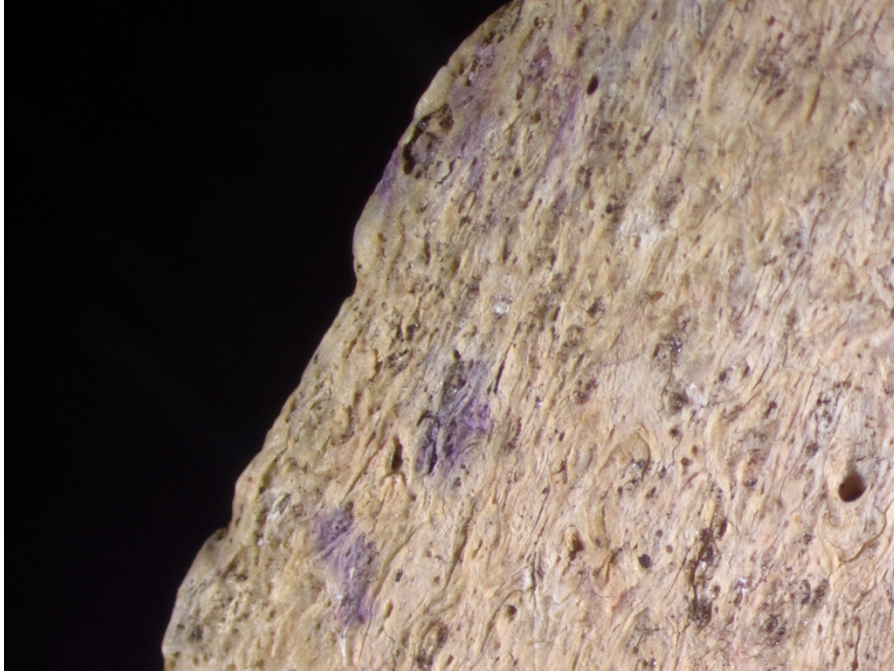


*Figure 14. Differential coloring (pink), marked with an arrow, noted on bone 4 located in square c.*



*Figure 15. Differential coloring (purple and pink) found on bone 5 near the sternal end of the bone.*





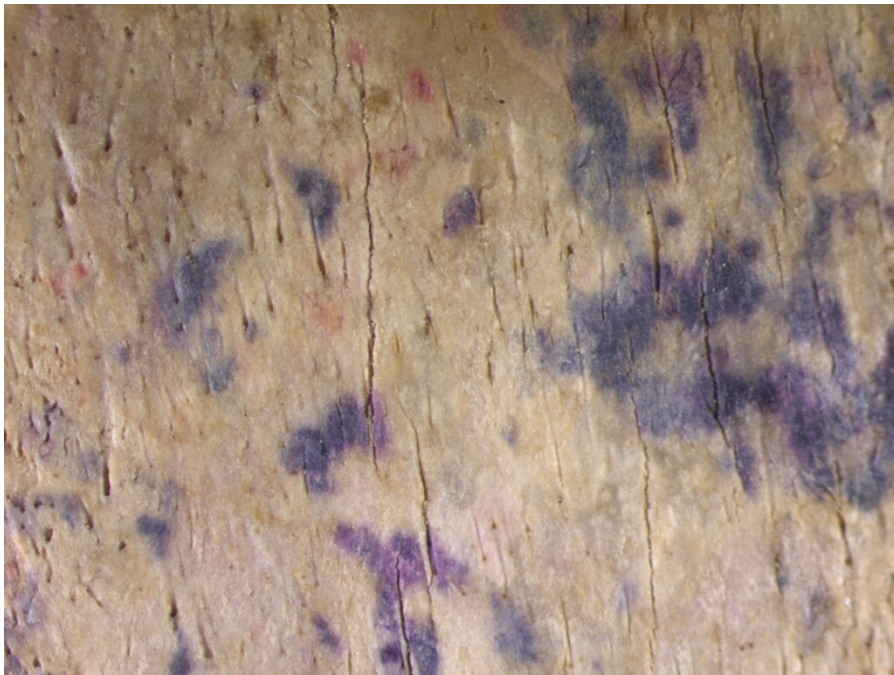
*Figure 16. Differential coloring (purple) found on bone 5 near the sternal end of the bone.*



*Figure 17. Differential coloring (purple) found on bone 5 near the sternal end of the bone.*



*Figure 18. Differential coloring (purple and pink) found on bone 5 on the body of the bone.*

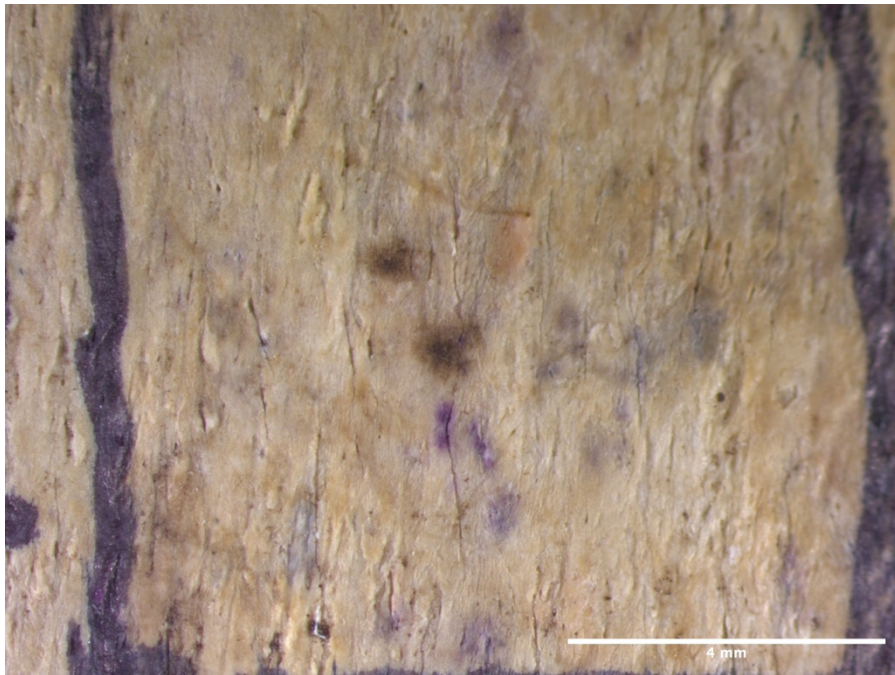


*Figure 19. Differential coloring (purple and pink) found on bone 5 on the body of the bone.*

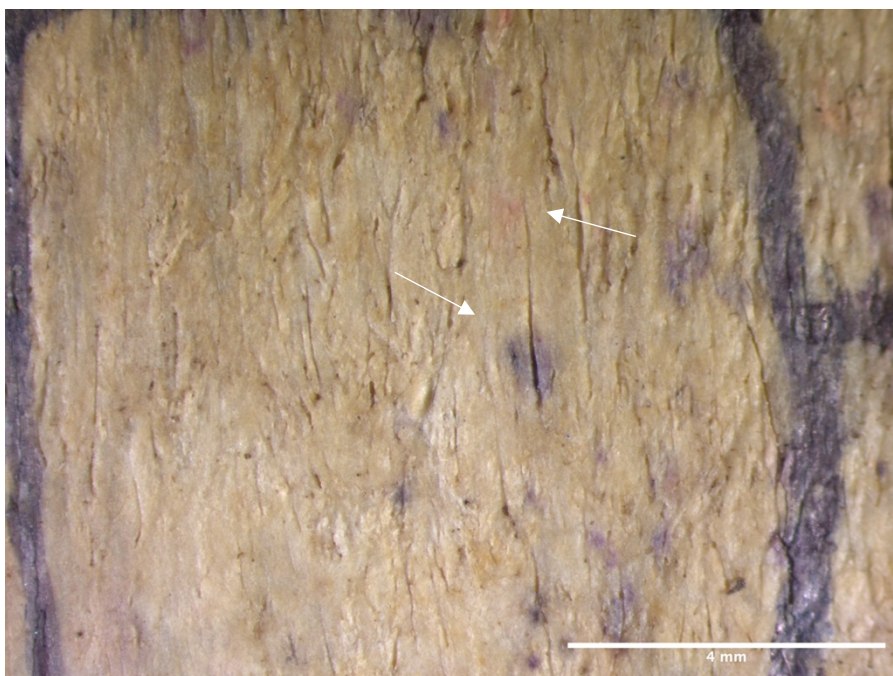




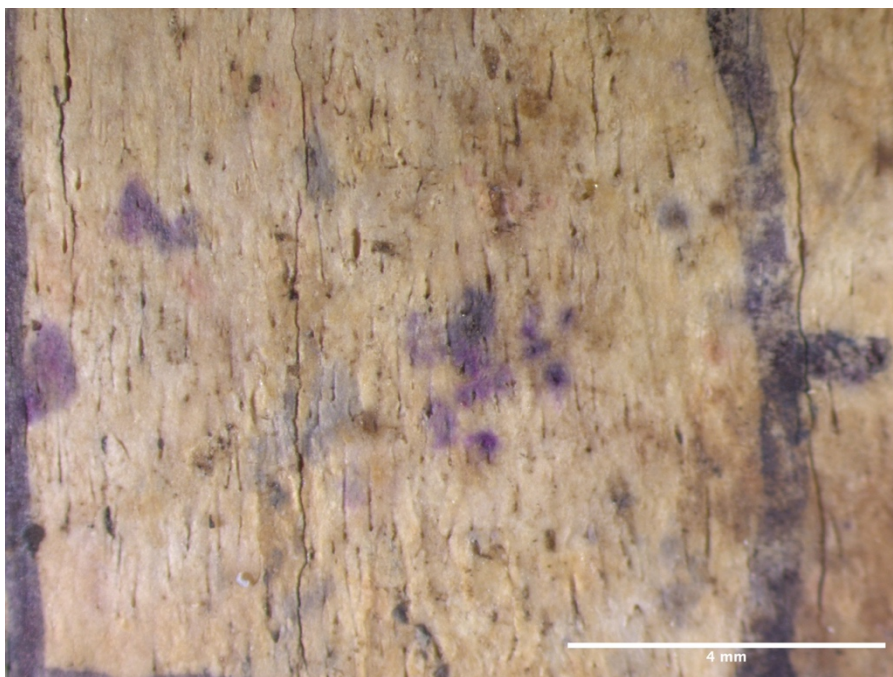
*Figure 20. Differential coloring (purple) found on bone 5 on the body of the bone.*



*Figure 21. Differential coloring (purple) found on bone 5 in square d.*

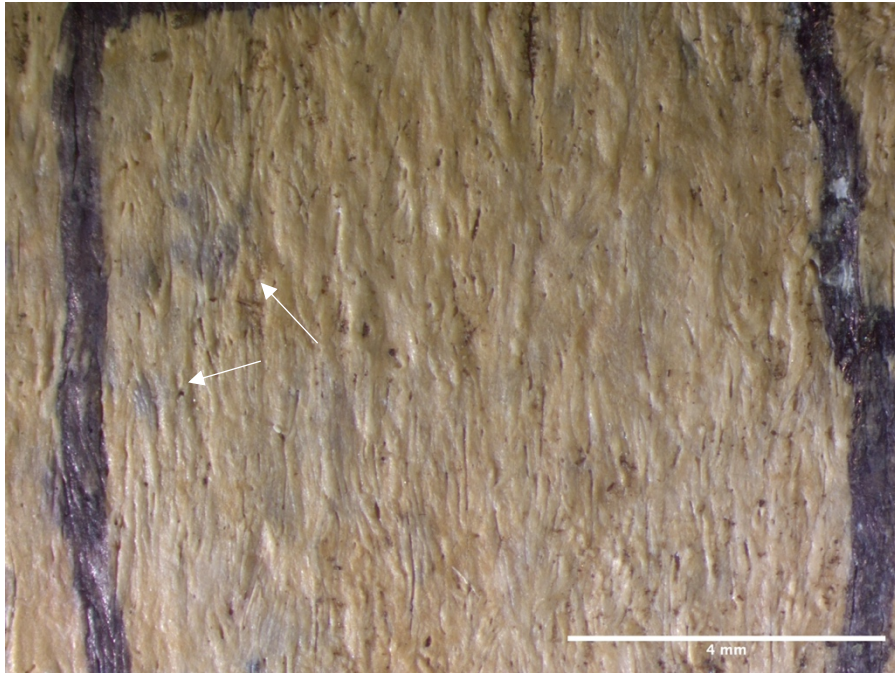


*Figure 22. Differential coloring (purple and pink) found on bone 5 in square e.*

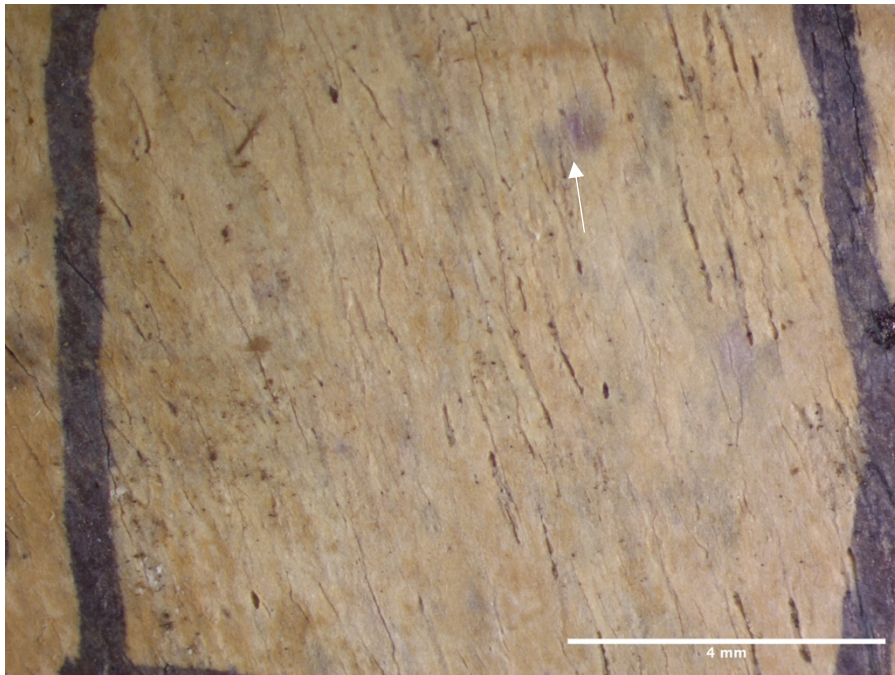


*Figure 23. Differential coloring (purple) found on bone 5 in square f.*



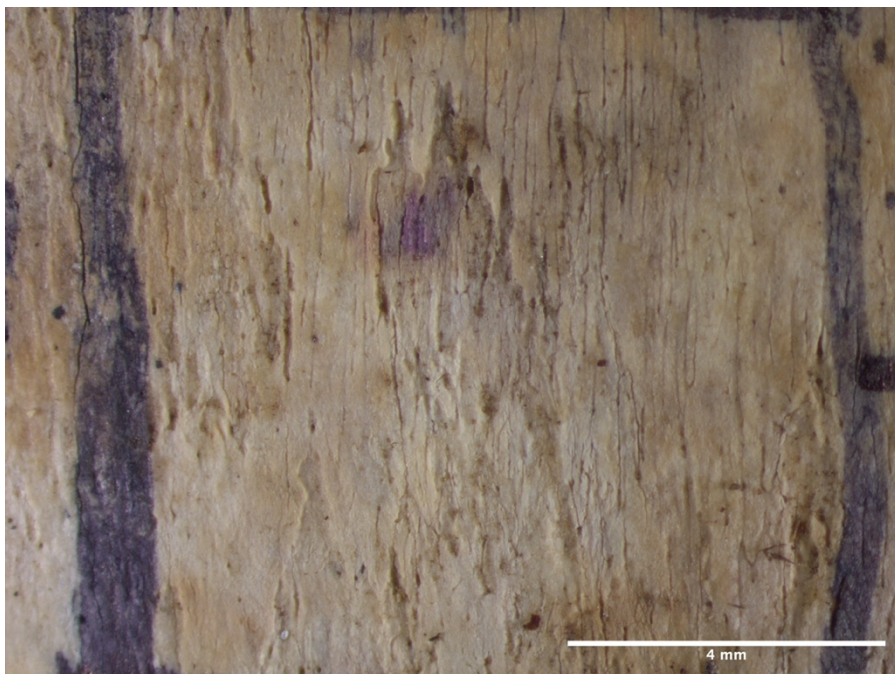


*Figure 24. Differential coloring (purple) found on bone 6 in square a.*

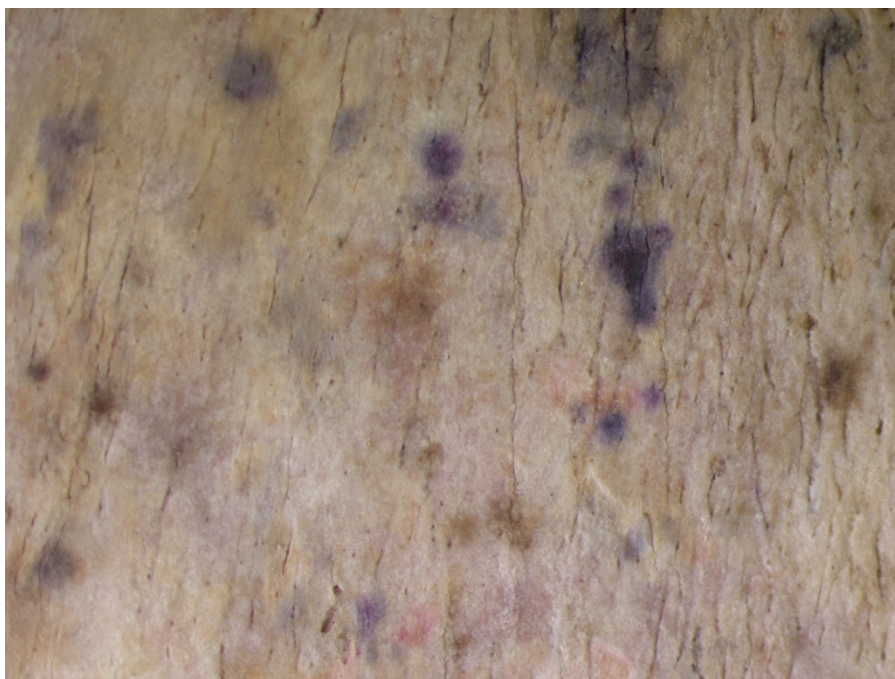


*Figure 25. Differential coloring (purple) found on bone 6 in square c.*





*Figure 26. Differential coloring (purple) found on bone 7 in square c.*



*Figure 27. Differential coloring (purple and pink) noted on bone 8 on the body of the bone.*



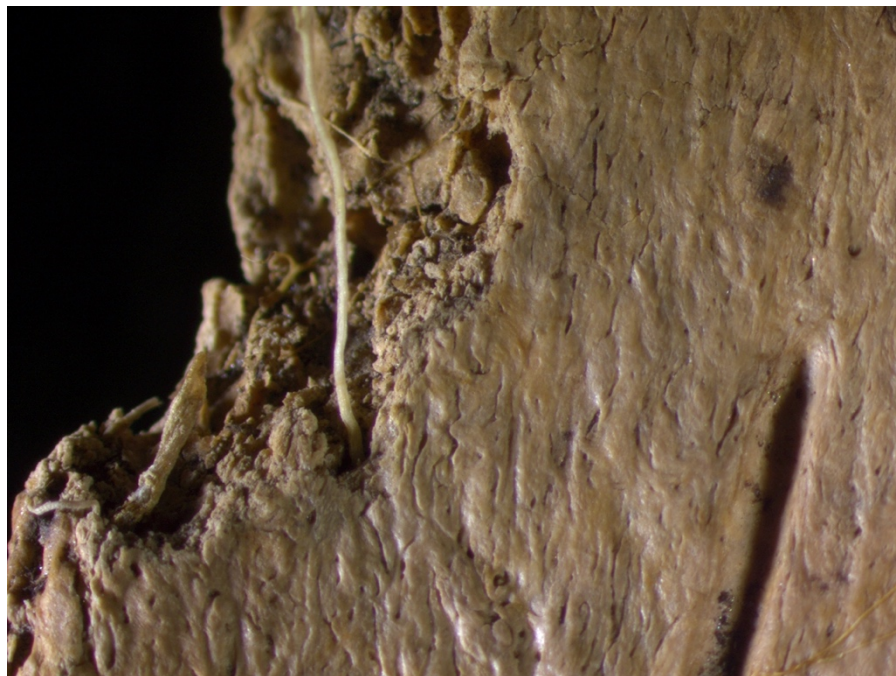
*Figure 28. Differential coloring (purple and pink) noted on bone 9 on the body of the bone.*

The origin of these differential colorings can be hypothesized to stem from certain minerals or materials present in the potting soil. Numerous articles discuss differential staining resulting from various metals or other elements in the soil that have caused similar coloration (Bradfield, 2018; Mann et al., 1998). Furthermore, there is an enzyme – purple acid phosphatase (PAP) – found in both plants and animals, primarily in bone tissue, which has been shown to induce purple staining (Cole & Waldron, 2016). Upon checking the MSDS for Miracle-Gro Potting Mix, the listed components that are in the mix include sphagnum peat moss, composted bark, perlite, ammonium nitrate, ammonium phosphate, calcium phosphate, and potassium sulfate. Though it is unknown if any of these components can or did cause the differential staining, there is a chance that the staining could have resulted from these elements within the potting soil. Further testing of the differential color blotches could allow us to identify what components are present and may be the culprit of the coloration.

### *Mechanical Root Growth*

After exhuming the bones, it was apparent that mechanical root action had occurred on some of the bones. Mechanical root growth refers to the action of roots to embed or travel through the bone surface, mostly taking the path of least resistance into foramina or through the ends of the bone – notably the sternal end instead of the cut end. After analysis, it can be noted that seven out of the eight experimental bones had mechanical root action present. The bones that showed mechanical root growth include bones 1, 2, 3, 4, 6, 7, and 8; the root action of bone 2 will not be shown in this section but instead will be shown in the ‘uninvited variable’ section.

The subsequent photos will illustrate the mechanical root growth observed on the following bones: 1, 3, 4, 6, 7, and 8.



*Figure 29. Mechanical root action located on bone 1 from Acer saccharinum L. near the sternal end of the bone.*



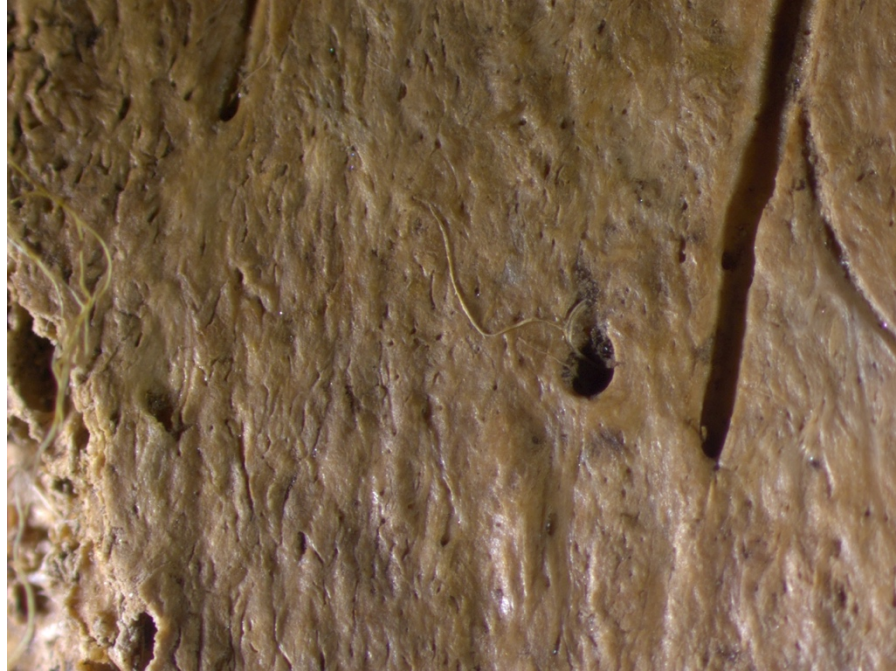


Figure 30. Mechanical root action located on bone 1 from *Acer saccharinum L.* through a foramen of the bone.



Figure 31. Mechanical root action located on bone 1 from *Acer saccharinum L.* on the sternal end of the bone. In addition, purple blotches can be noted in the bottom right corner.



Figure 32. Mechanical root action located on bone 3 from Buddleia davidii L. near the sternal end of the bone.



Figure 33. Mechanical root action located on bone 4 from Buddleia davidii L. near the sternal end of the bone.





Figure 34. Mechanical root action located on bone 4 from Buddleia davidii L. near the sternal end of the bone.



Figure 35. Mechanical root action located on bone 6 from Mentha spicata L.; In addition, there are purple blotches on the lower right side of the bone





Figure 36. Mechanical root action located on bone 6 from *Mentha spicata L.* near square d.

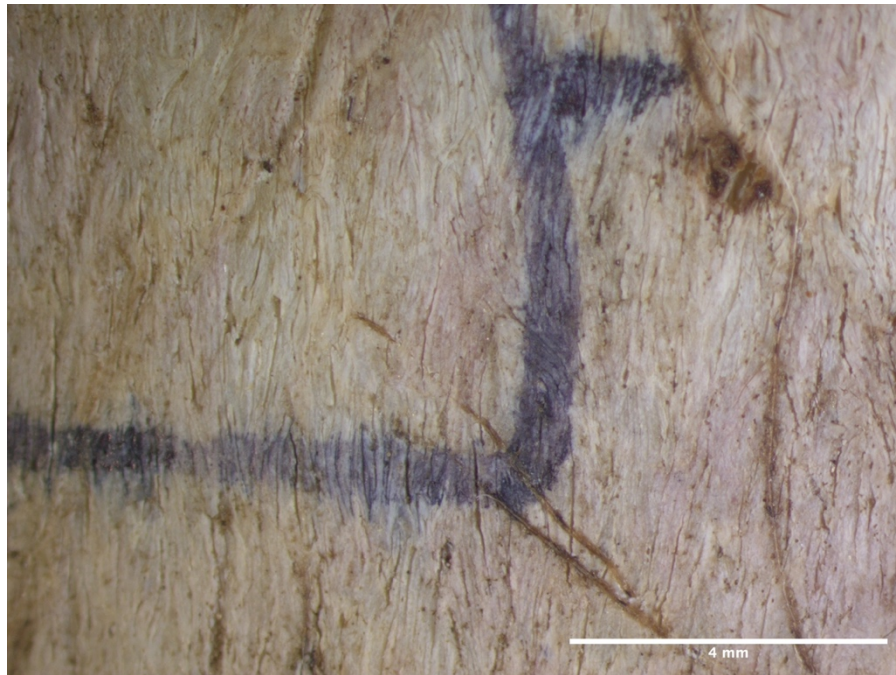


Figure 37. Mechanical root action located on bone 6 from *Mentha spicata L.* going through square d.



*Figure 38. Mechanical root action located on bone 7 from Cynodon dactylon coming out of a foramen.*

For bone 8, the root growth was so extensive that when exhuming the bone, the sternal end of the bone broke off of the body of the rib, leaving two pieces.





*Figure 39. Mechanical root action located on bone 8 from Cynodon dactylon on the broken-off sternal end of the bone.*



*Figure 40. Mechanical root action located on bone 8 from Cynodon dactylon on the broken-off sternal end of the bone.*



*Figure 41. Mechanical root action located on bone 8 from Cynodon dactylon on the broken-off sternal end of the bone.*



*Figure 42. Mechanical root action located on bone 8 from Cynodon dactylon on the broken end of the body.*



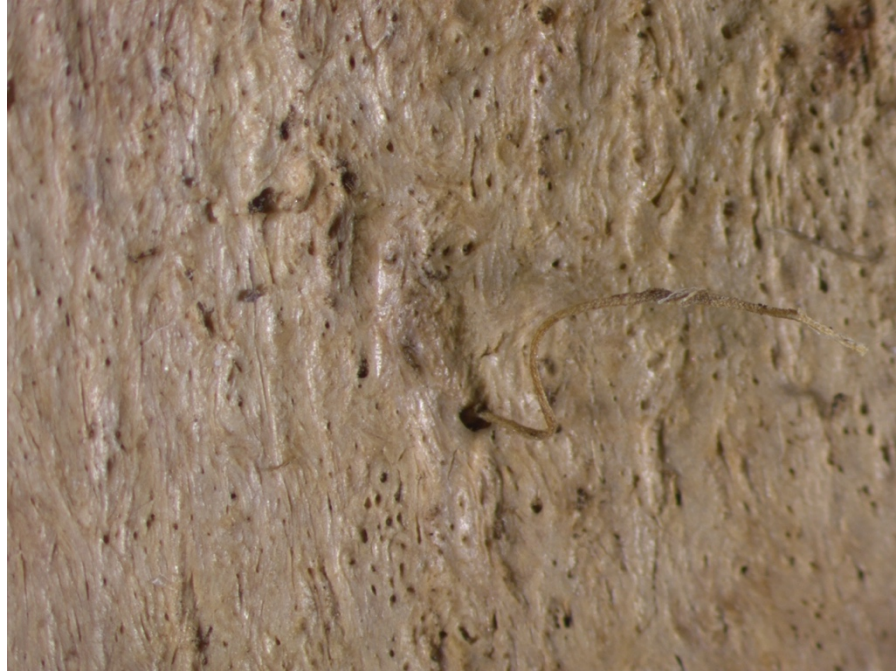


Figure 43. Mechanical root action located on bone 8 from *Cynodon dactylon* on the body of the rib coming out of a foramen.

### *The Uninvited Variable*

Despite trying to control all variables within the experiment, except for the growth of the chosen plants, an unwelcome weed continuously grew and spread throughout the experimental and control pots. When found in time, the weeds were picked out of the pots to ensure that they did not affect the experiment. However, due to a lack of knowledge of the weed at first, the weed was able to grow within one of the experimental pots. To address this, I created a “control” for the weed from a pot where the selected plant never grew, allowing it to grow freely in hopes of distinguishing any root markings that might be present. The bone that was present within the pot is denoted as bone 2. The weed was identified as Mulberry Weed (*Fatoua villosa*), which is known to spread invasively through explosive seeding that can spread out in a four-foot radius.



This explosive seeding explains how it could proliferate so easily throughout the greenhouse that housed the experiment. As for the markings left by the weed in the makeshift “control” pot, the presence of both root staining and mechanical root action is apparent.

The staining present on the bone can be seen on square 2e, as seen below in Figures 19 & 20.

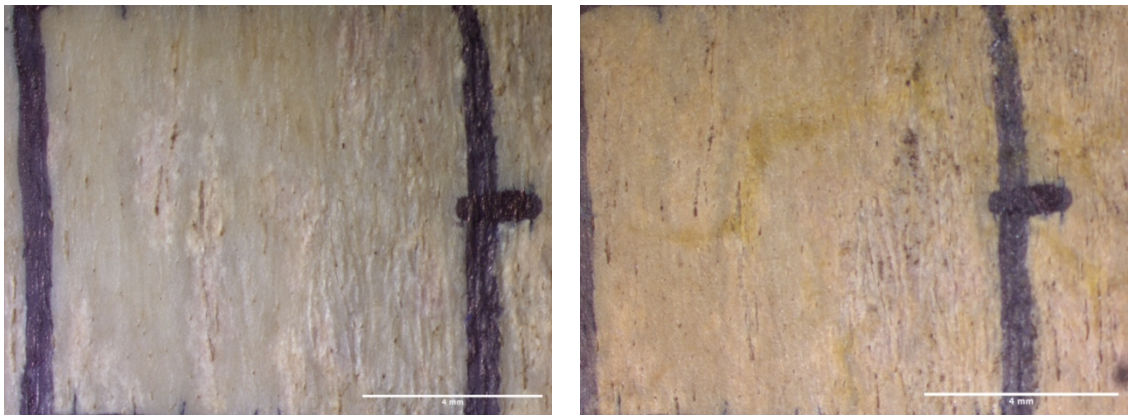


Figure 44. (left) Before photo of bone 2 square e (2e); (right) Root staining from *Fatoua villosa* located on square 2e.

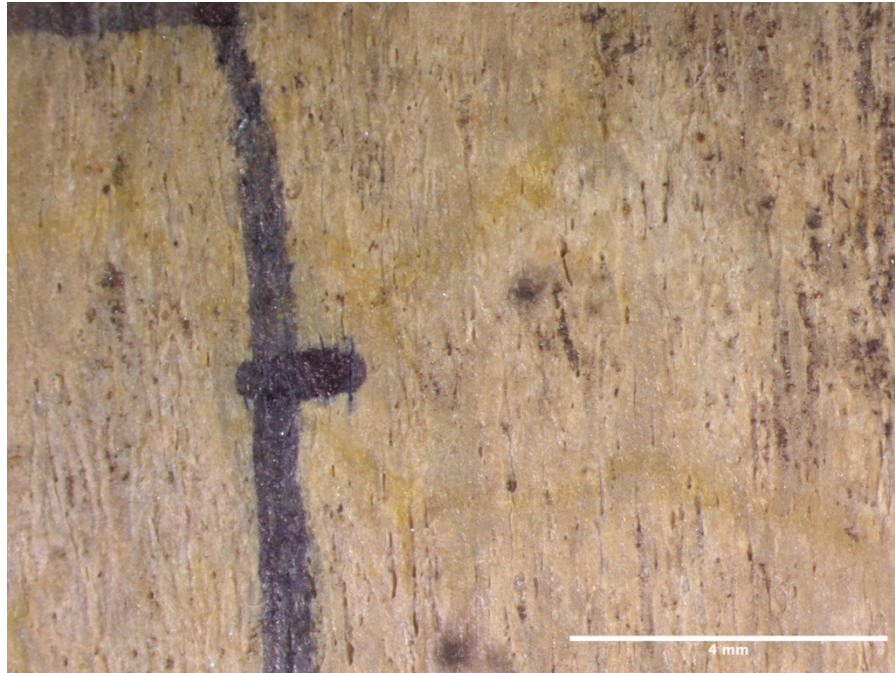


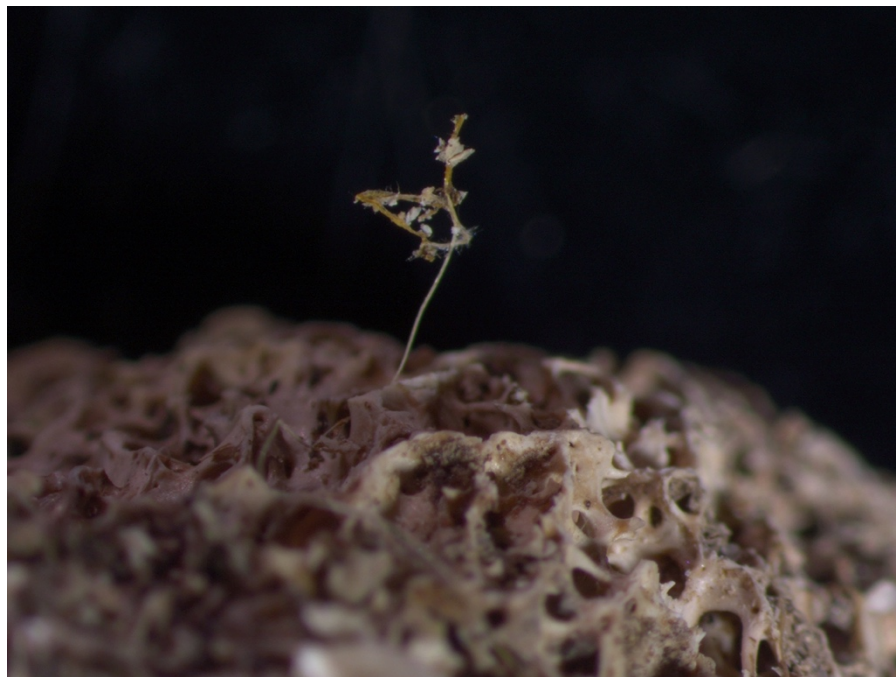
Figure 45. Continued root staining from *Fatoua villosa* found continuing from square e on bone 2.

As seen above in Figure 19, there is distinct yellow staining that was not present before on square 2e. The patterns can be discerned as dendritic patterns that are typical of a root. In addition to this staining, another staining marker was noted to also have dendritic patterns. Although this marking was not situated within the designated areas and there is no prior image to confirm it, all the bones were scanned before planting for any markings like those found, and nothing noteworthy was observed. The marking identified in the analysis after the experiment is illustrated below in Figure 21. The markings shown in Figure 21 are very similar to the ones that are present on bone 3.



*Figure 46. Dendritic staining found on bone 2 during analysis after exhumation.*

In addition to staining, mechanical root action took place and can be seen below in Figures 22 and 23.



*Figure 47. Root action present on bone 2, located on the sternal end of the bone.*





*Figure 48. Root action present on bone 2, located below square d.*

The root action appeared to have occurred in areas where the roots can easily navigate into the bone cavity, such as through the sternal end or an existing foramen.



## DISCUSSION

As noted previously, there is a lack of repeatable knowledge when it comes to how long plant root etching takes to occur on skeletonized remains. For this reason, this research project was built to explore the capabilities of plant root etching in a controlled environment. From the analysis of the results, it can be concluded that nine months is sufficient time for staining markings to develop and for mechanical root action to occur. Without the use of a scanning electron microscope (SEM), it is challenging to ascertain whether etching is present. If any etching exists, it is predicted to be minimal, as there are no visible or tactile grooves. Although this cannot be included in the honors thesis, an analysis of the bone surface will be conducted with an SEM, thanks to a generous contribution from Dr. Belcher at the University of Nebraska-Lincoln. Hopefully, this will enable us to determine whether any etching has occurred for future publication.

A recent literature review has concluded that within other experiments, a one-year minimum time frame is needed to create any etching on the bones, but these studies do not address root staining (Macho-Callejo et al., 2023, p. 4). However, given the results, we know that plant root staining can occur much sooner than the one-year minimum, which is not a fact that is investigated in detail in other publications. By updating the literature, we can continue to further the capabilities of investigators to assess how long skeletal remains may have been exposed to underground plant activity. With this in mind and the information indicated by the results, this project can be of great use to the forensic science and anthropology communities. Using the taphonomic information learned from this project, we can begin to start applying it to medico-legal contexts. For instance, based on the knowledge that nine months is sufficient time for root staining and

mechanical root action to affect bones when exposed, a forensic anthropologist could conclude that a skeletonized victim has likely been exposed to roots for at least nine months.

In addition to the root staining and mechanical root action, by exploring the anomaly of the differential staining left on some of the bones, more research could be done to further help the anthropological and forensic communities. The purple to pink staining is speculated to come from minerals or metals found within the potting mix; however, without further research, it is just a hypothesis. Research that could be done includes chemically testing both the dirt and differentially stained areas, then completing a comparative analysis of the compounds. This future research could be of use to both communities as they may find similar staining and have no explanation for it.

Finally, as for the Mulberry Weed (*Fatoua villosa*), its abrupt and consistent growth provides a perfect example of how taphonomic agents in uncontrolled environments, like crime scenes, can present unknown variables. Shockingly, the root growth from the weed “control” pot showed great examples of root staining and mechanical root action. As a future project, I believe using commonly found flora of crime scenes as the next experimental variables could be beneficial to catalog what a forensic anthropologist may encounter within these cases. Additionally, expanding the taphonomic project to a less controlled environment could be the next step for future studies.

## **LIMITATIONS**

There were quite a few limitations to this experiment, the main issue being the timeframe for the experiment. With a deadline to complete this thesis, the experiment could only run for 9 months instead of a full year at a minimum. In addition, the sample size is underwhelmingly small to yield substantial data. To be more accurate and truly telling of the experiment, a larger sample size is needed; however, due to lack of space, eight plants (2 of each) is what was feasible.

## **FUTURE WORKS**

Regarding future works, different durations are needed to fully explore the limitations and capabilities of utilizing rootlet etching markings within forensic anthropology. For example, an experiment could be set up where each experimental pot is run for a different duration (2, 4, 6, 8 months, etc.) to accurately quantify when staining and etching first begins. For other experiments, having a larger sample size would significantly enhance data collection and the repeatability of the experiments. Additionally, one question to consider for future studies is: Does the amount of space the plant has to grow lead to more etching due to constraints on where the roots can grow? This experiment could be tested by using various-sized surface areas.

Another question that can be investigated is whether the calcium levels change within the bone material after rootlet etching. This question could be potentially looked at by using an X-ray fluorescence unit, which can analyze the amount of calcium present (Gonzalez-Rojas, et al., 2023). The X-ray unit would allow for a before-and-after comparison of calcium levels within the bone material. Additional elements that could be analyzed in future projects would be the chemical composition of the root exudates and how they specifically affect the cortical surface. Furthermore, chemically testing the differential coloring found on the bones and comparing it to a chemical test of the soil could give us an idea of what component is causing the bewildering coloration.

To expand on the taphonomic aspect of the project, switching out skeletal remains for less decomposed or fresh remains could fully test how the rootlet etching or staining is affected by not having immediate direct contact with the bones. Additionally, testing commonly seen plants that would be found in various crime scenes would be extremely

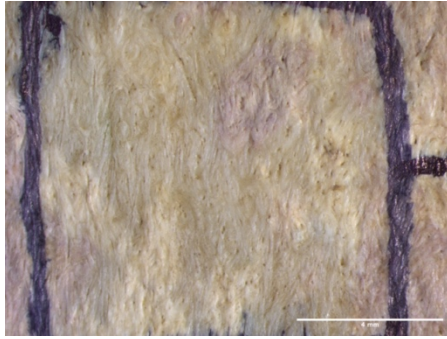


beneficial to catalog the taphonomic changes they induce on the bones within a controlled environment.

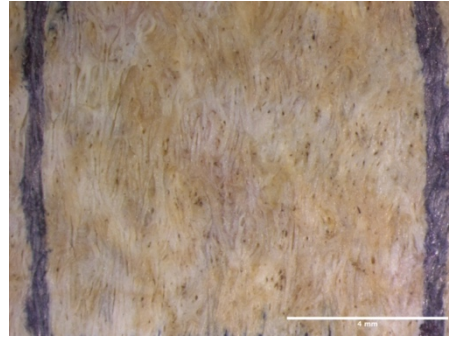
## CONCLUSION

In conclusion, this research has made significant strides in advancing our understanding of plant root etching and its effects on skeletonized remains, particularly regarding the timeline for root staining and mechanical root action. While it cannot be asserted that a nine-month duration is sufficient to produce rootlet etching, it can be concluded that this timeframe was adequate to result in root staining and mechanical root action. Although there are limitations, such as the inability to definitively detect etching without SEM analysis, this experiment provides valuable insights into the timing and extent of root interaction with bovine bones. The upcoming SEM analysis, facilitated through collaboration with Dr. Belcher at the University of Nebraska-Lincoln, will further elucidate whether any etching has occurred. By contributing new knowledge to the existing body of literature, this research has the potential to enhance medico-legal investigations, providing forensic anthropologists with more accurate methods for determining how long skeletal remains have been exposed to underground plant activity. Additionally, the discovery of differential staining on some bones paves the way for future research into the chemical composition of the staining agents, which may offer additional tools for identifying similar occurrences in forensic cases. Furthermore, other future works, such as expanding the taphonomic project to include less decomposed or fresh remains, could give a broader scope to forensic cases not limited to skeletal remains. Ultimately, this project will significantly contribute to the fields of forensic science and anthropology, assisting investigators in applying taphonomic knowledge to practical medico-legal applications.

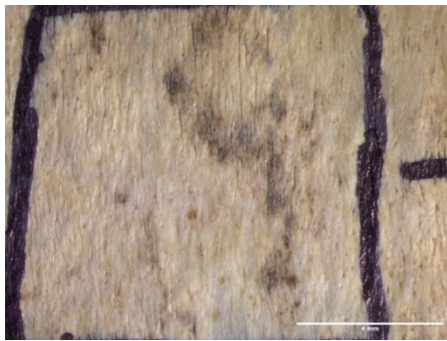
## APPENDIX A



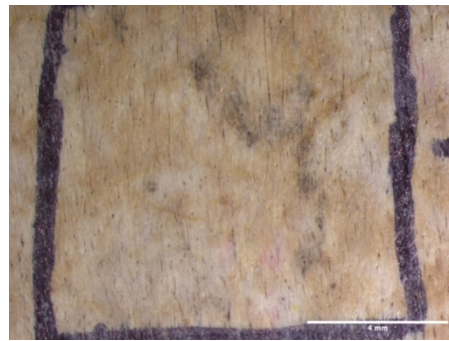
*Figure 49. Before photo of bone 1 square a.*



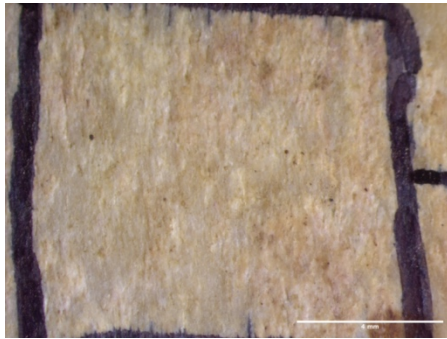
*Figure 50. After photo of bone 1 square a.*



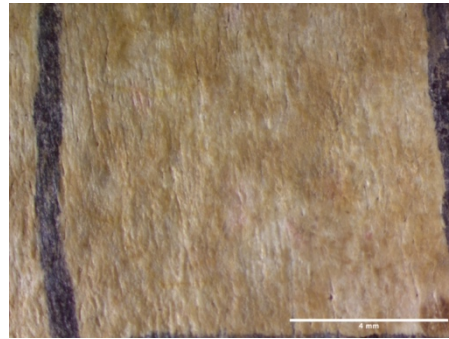
*Figure 51. Before photo of bone 1 square b.*



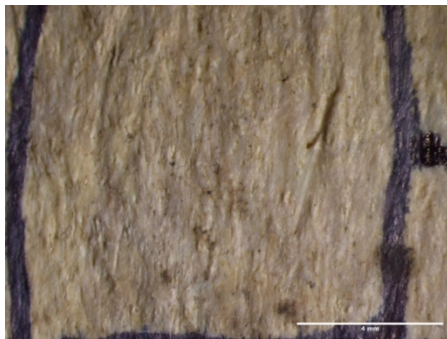
*Figure 52. After photo of bone 1 square b.*



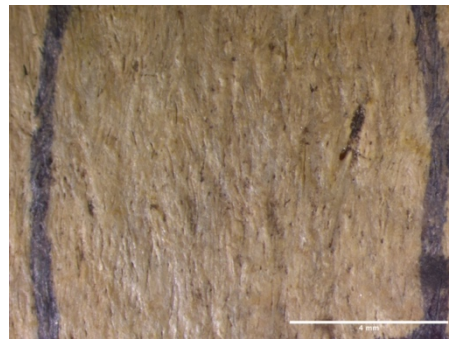
*Figure 53. Before photo of bone 1 square c.*



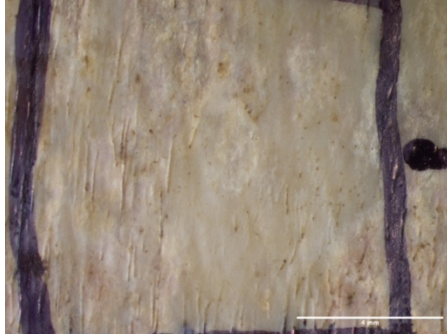
*Figure 54. After photo of bone 1 square c.*



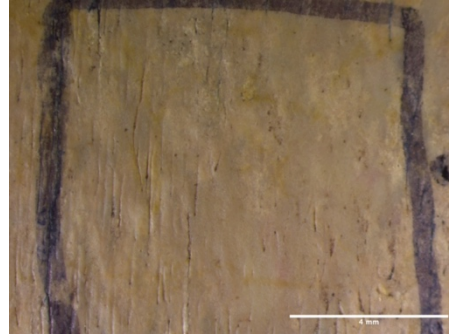
*Figure 55. Before photo of bone 1 square d.*



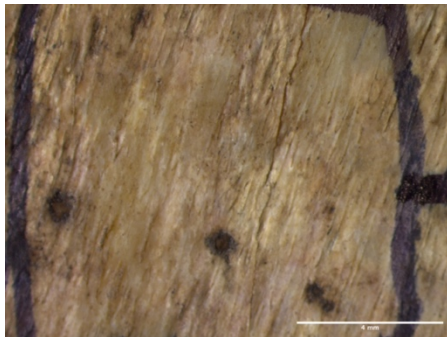
*Figure 56. After photo of bone 1 square d.*



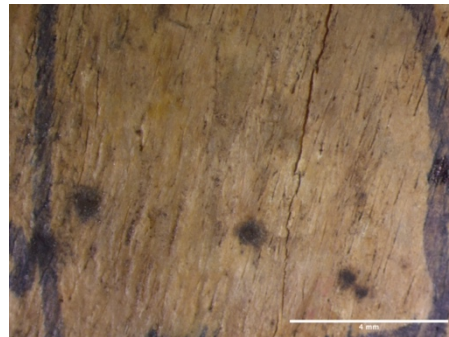
*Figure 57. Before photo of bone 1 square e.*



*Figure 58. After photo of bone 1 square e.*



*Figure 59. Before photo of bone 1 square f.*



*Figure 60. After photo of bone 1 square f.*



*Figure 61. After photo of bone 1,  
mechanical root action on sternal end.*

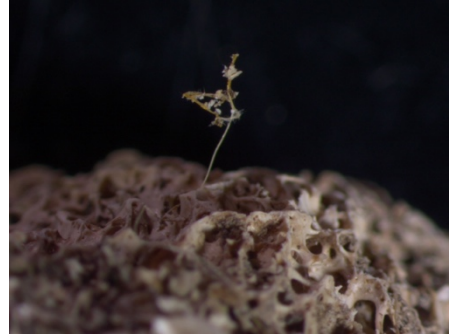


*Figure 62. After photo of bone 1,  
mechanical root action near sternal end.*





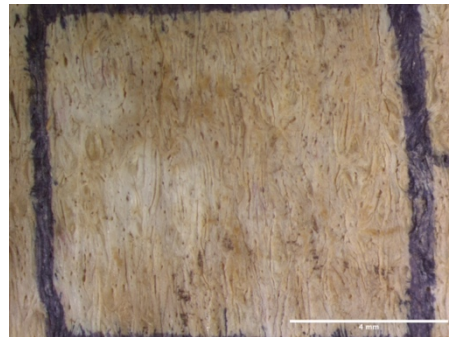
*Figure 63. After photo of bone 1, mechanical root action on sternal end.*



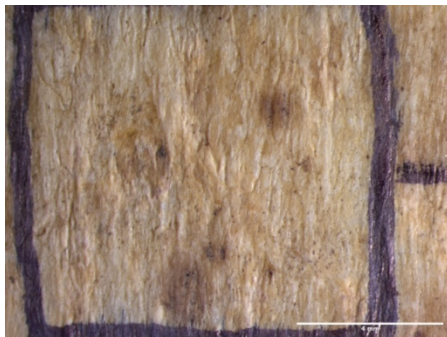
*Figure 64. After photo of bone 2, mechanical root action on sternal end.*



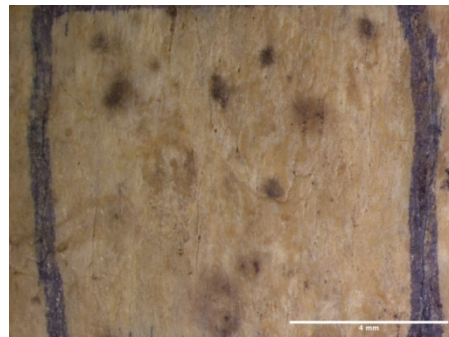
*Figure 65. Before photo of bone 2 square a.*



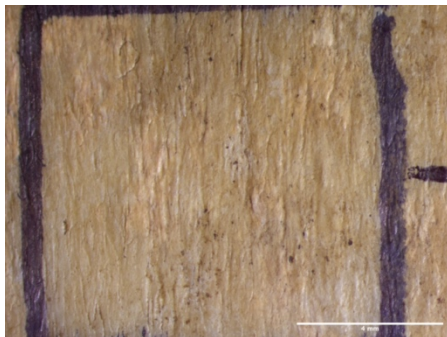
*Figure 66. After photo of bone 2 square a.*



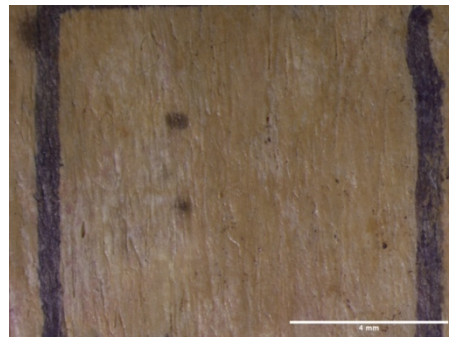
*Figure 67. Before photo of bone 2 square b.*



*Figure 68. After photo of bone 2 square b.*

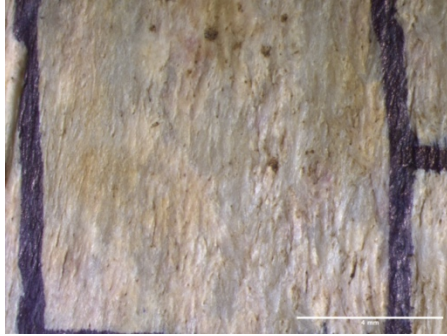


*Figure 69. Before photo of bone 2 square c.*

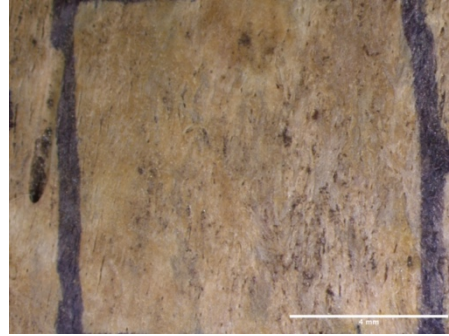


*Figure 70. After photo of bone 2 square c.*

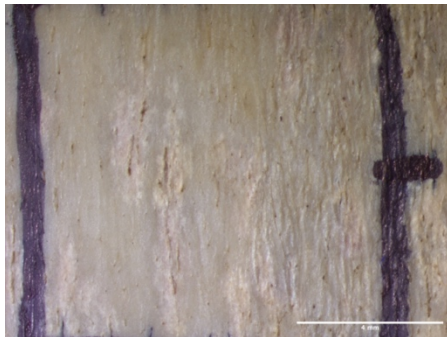




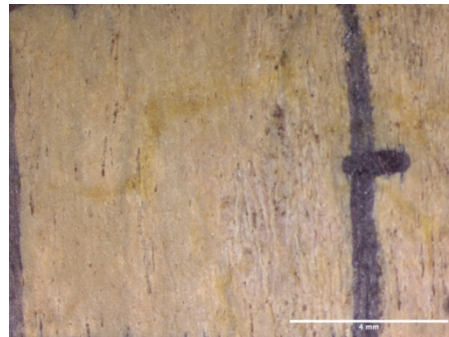
*Figure 71. Before photo of bone 2 square d.*



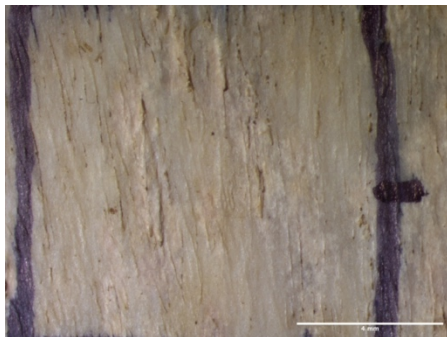
*Figure 72. After photo of bone 2 square d.*



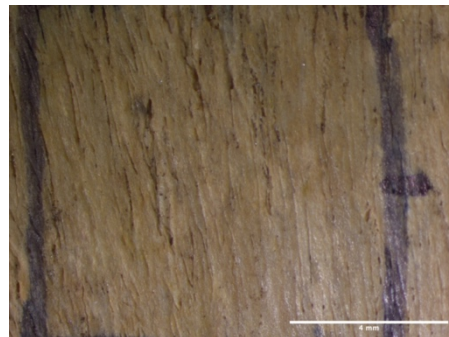
*Figure 73. Before photo of bone 2 square e.*



*Figure 74. After photo of bone 2 square e.*



*Figure 75. Before photo of bone 2 square f.*



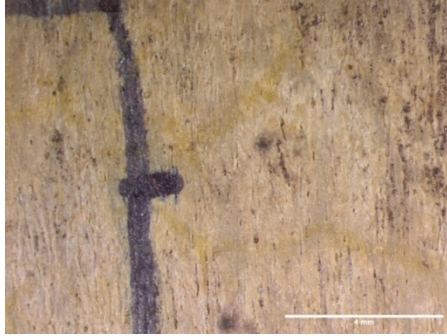
*Figure 76. After photo of bone 2 square f.*



*Figure 77. After photo of bone 2, dendric staining/etching.*



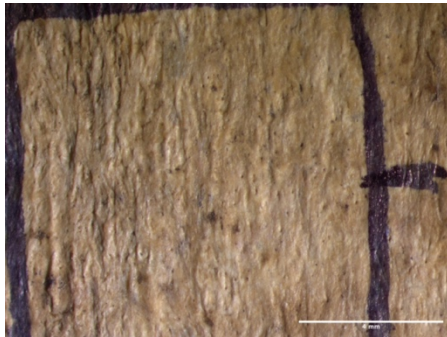
*Figure 78. After photo of bone 2, mechanical root action near square d*



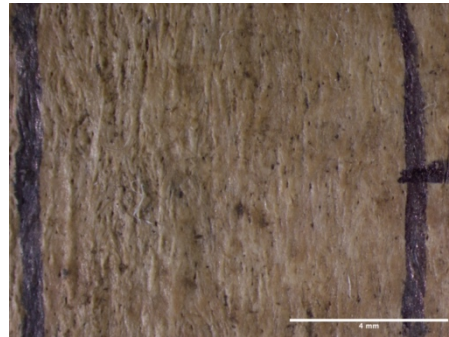
*Figure 79. After photo of bone 2, staining/etching near square e.*



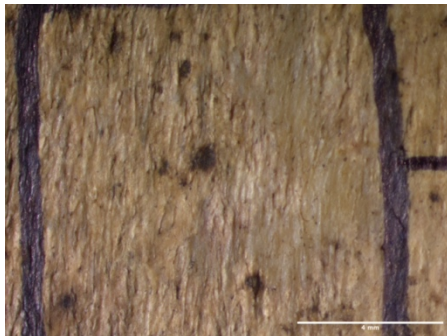
*Figure 80. After photo of bone 3, mechanical root action on sternal end of bone.*



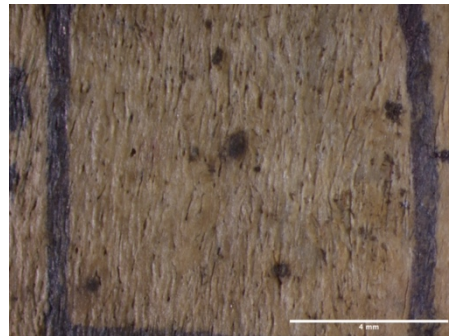
*Figure 81. Before photo of bone 3 square a.*



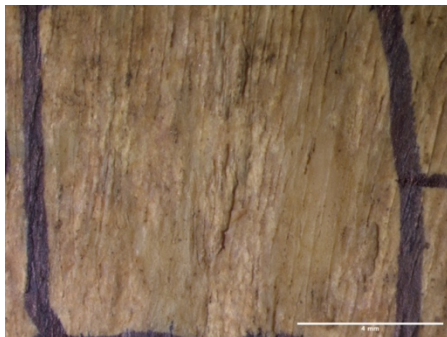
*Figure 82. After photo of bone 3 square a.*



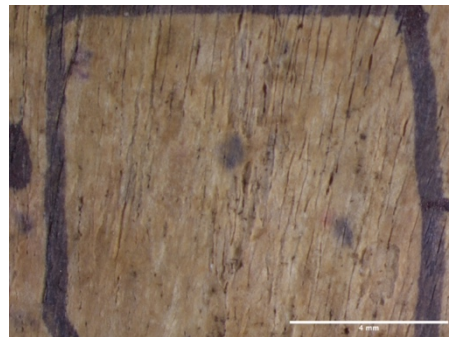
*Figure 83. Before photo of bone 3 square b.*



*Figure 84. After photo of bone 3 square b.*



*Figure 85. Before photo of bone 3 square c.*

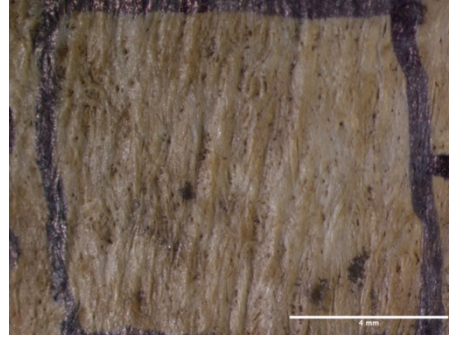


*Figure 86. After photo of bone 3 square c.*

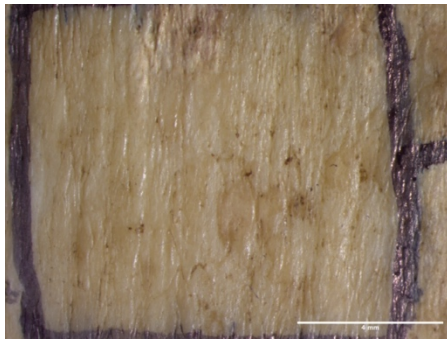




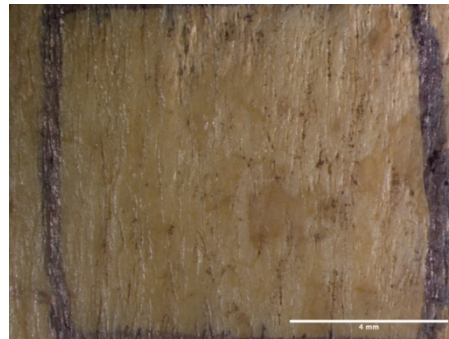
*Figure 87. Before photo of bone 3 square d.*



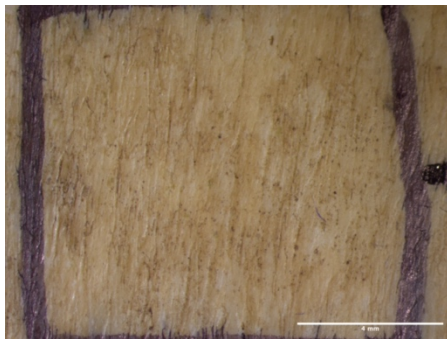
*Figure 88. After photo of bone 3 square d.*



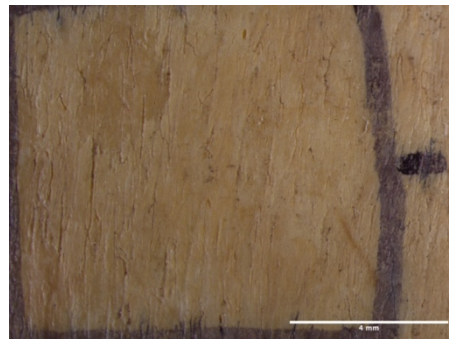
*Figure 89. Before photo of bone 3 square e.*



*Figure 90. After photo of bone 3 square e.*



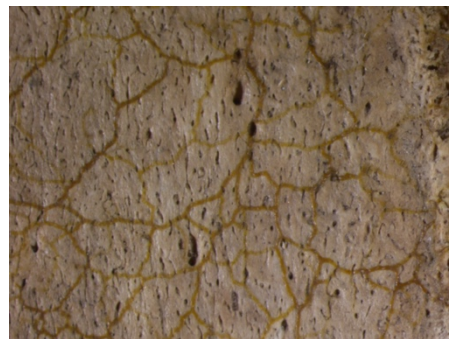
*Figure 91. Before photo of bone 3 square f.*



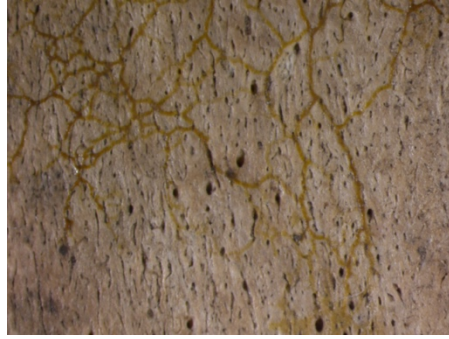
*Figure 92. After photo of bone 3 square f.*



*Figure 93. After photo of bone 3, dendric staining/etching.*



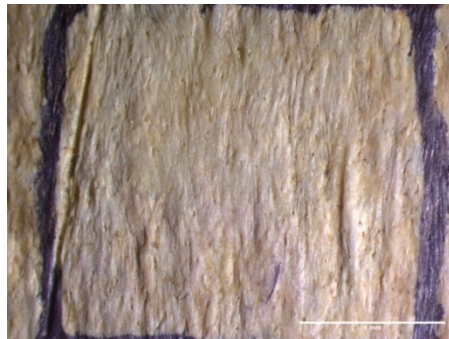
*Figure 94. After photo of bone 3, dendric staining/etching.*



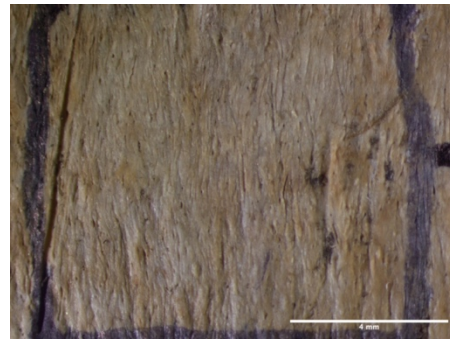
*Figure 95. After photo of bone 3, dendric staining/etching.*



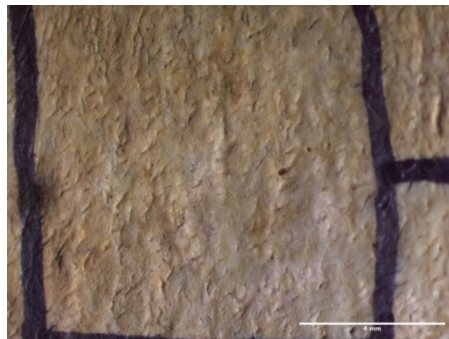
*Figure 96. After photo of bone 3, dendric staining/etching.*



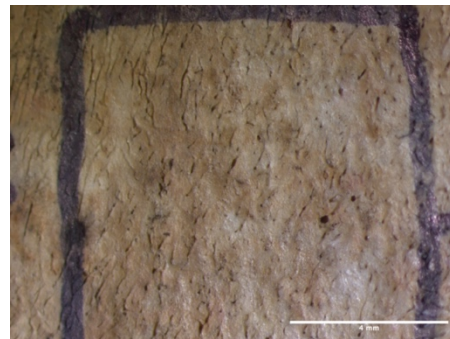
*Figure 97. Before photo of bone 4 square a.*



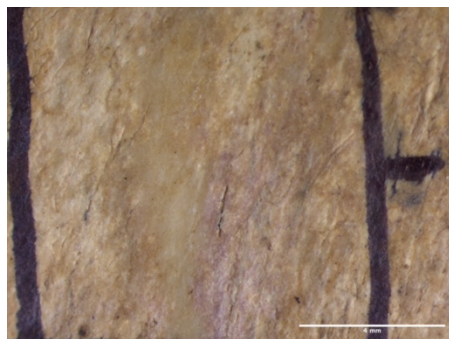
*Figure 98. After photo of bone 4 square a.*



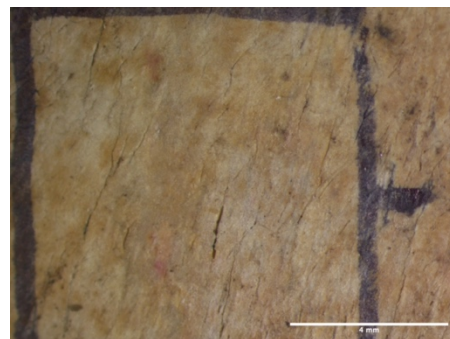
*Figure 99. Before photo of bone 4 square b.*



*Figure 100. After photo of bone 4 square b.*

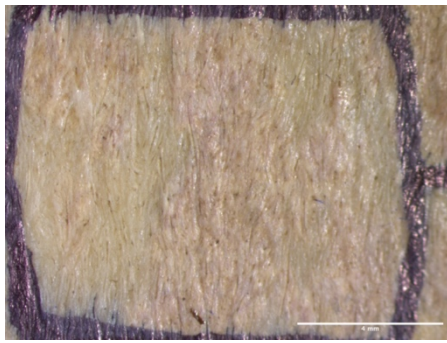


*Figure 101. Before photo of bone 4 square c.*

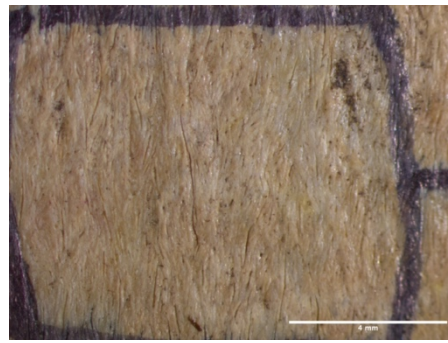


*Figure 102. After photo of bone 4 square c.*

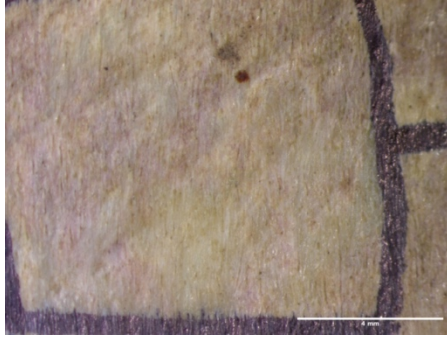




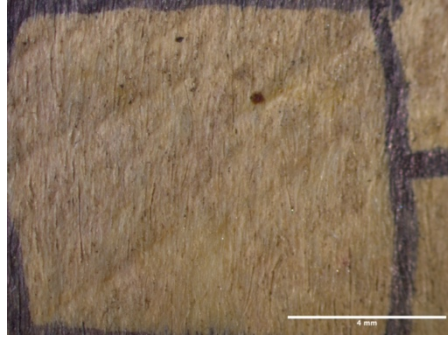
*Figure 103. Before photo of bone 4 square d.*



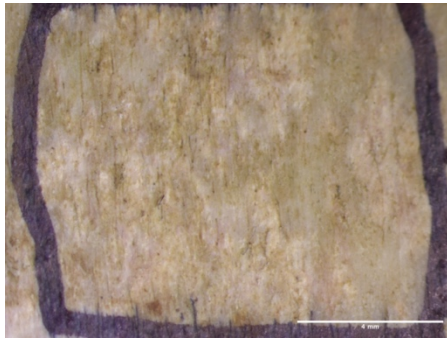
*Figure 104. After photo of bone 4 square d.*



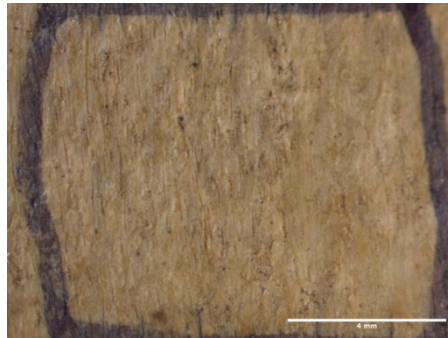
*Figure 105. Before photo of bone 4 square e.*



*Figure 106. After photo of bone 4 square e.*



*Figure 107. Before photo of bone 4 square f.*



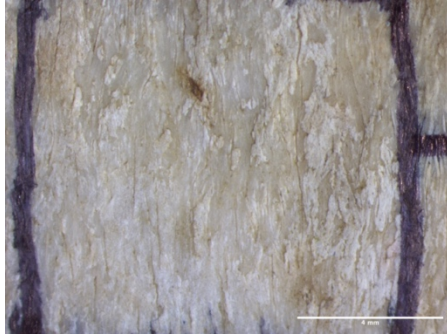
*Figure 108. After photo of bone 4 square f.*



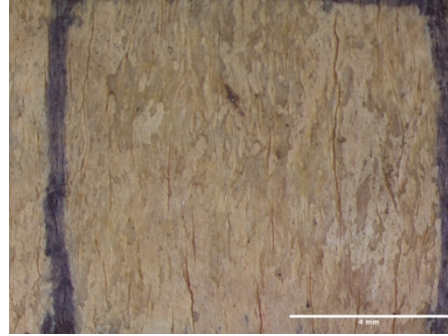
*Figure 109. After photo bone 4, mechanical root action on sternal end of bone.*



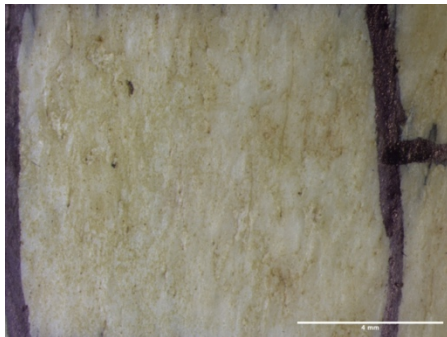
*Figure 110. After photo bone 4, mechanical root action on sternal end of bone.*



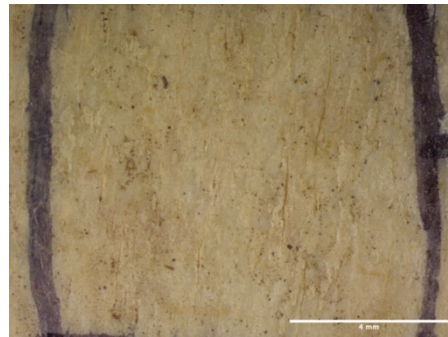
*Figure 111. Before photo of bone 5 square a.*



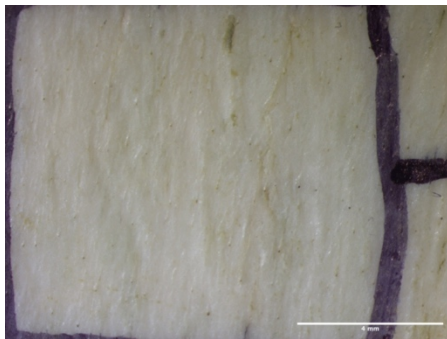
*Figure 112. After photo of bone 5 square a.*



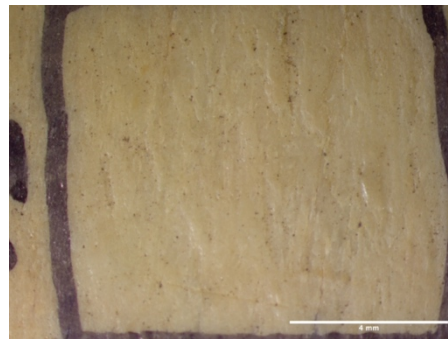
*Figure 113. Before photo of bone 5 square b.*



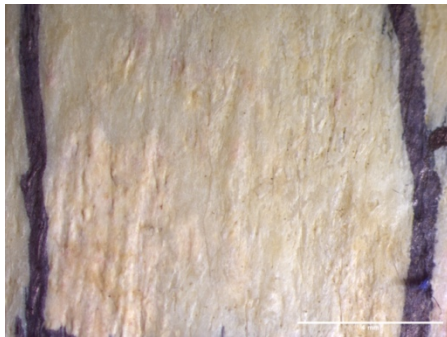
*Figure 114. After photo of bone 5 square b.*



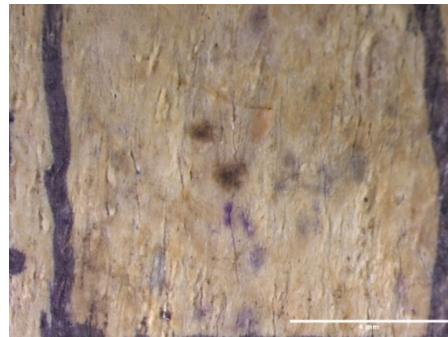
*Figure 115. Before photo of bone 5 square c.*



*Figure 116. After photo of bone 5 square c.*

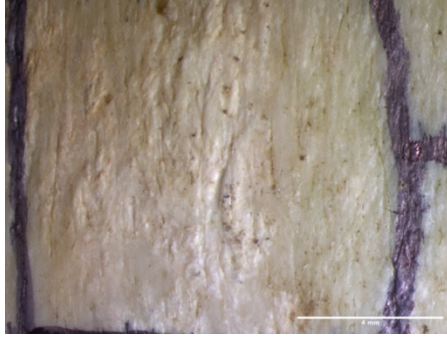


*Figure 117. Before photo of bone 5 square d.*

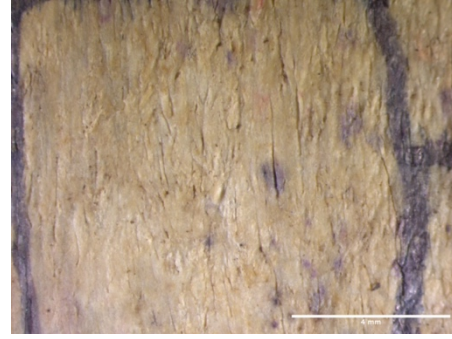


*Figure 118. After photo of bone 5 square d.*

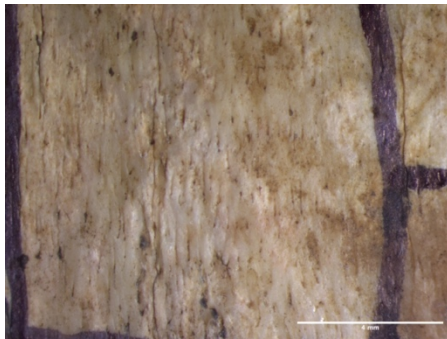




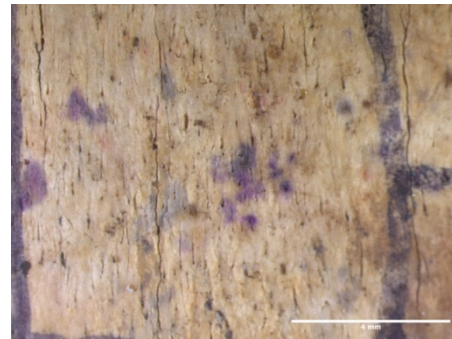
*Figure 119. Before photo of bone 5 square e.*



*Figure 120. After photo of bone 5 square e.*



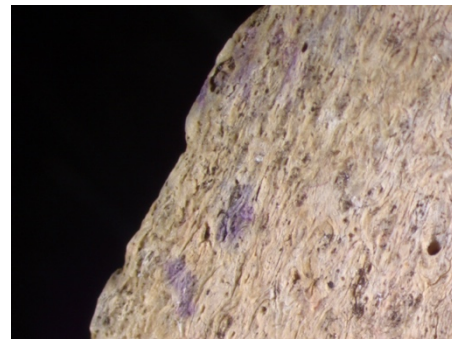
*Figure 121. Before photo of bone 5 square f.*



*Figure 122. After photo of bone 5 square f.*



*Figure 123. After photo of bone 5, purple/pink differential staining .*



*Figure 124. After photo of bone 5, purple/pink differential staining .*



*Figure 125. After photo of bone 5, purple differential staining .*



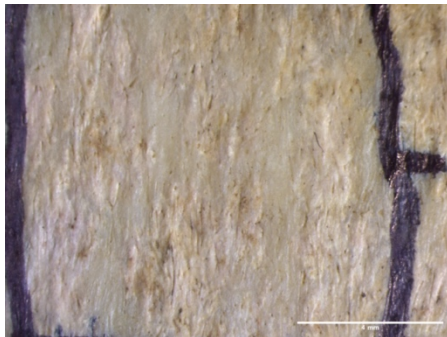
*Figure 126. After photo of bone 5, purple/pink differential staining .*



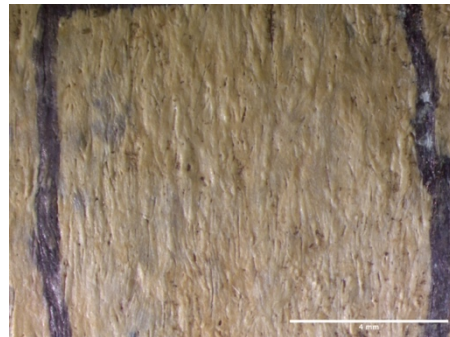
*Figure 127. After photo of bone 5, purple/pink differential staining .*



*Figure 128. After photo of bone 5, purple differential staining.*



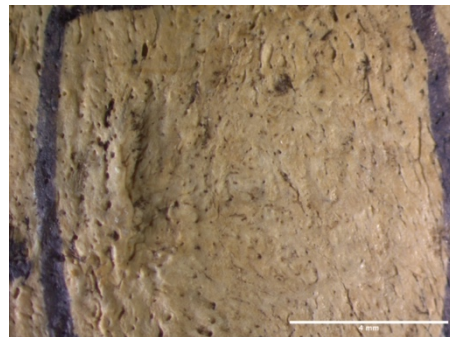
*Figure 129. Before photo of bone 6 square a.*



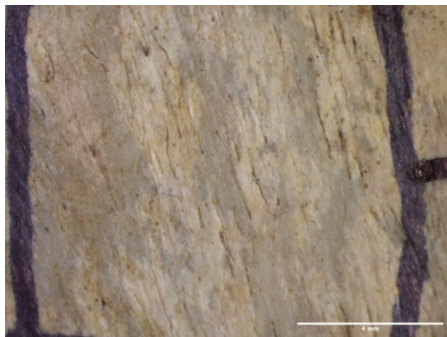
*Figure 130. After photo of bone 6 square a.*



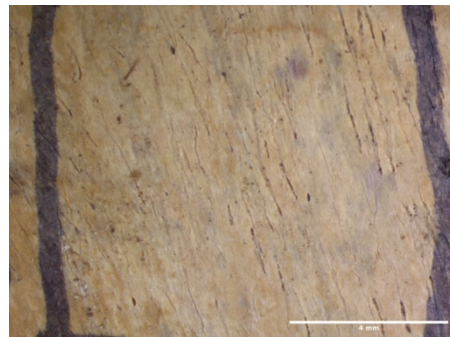
*Figure 131. Before photo of bone 6 square b.*



*Figure 132. After photo of bone 6 square b.*

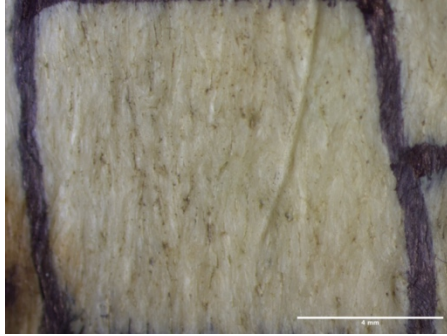


*Figure 133. Before photo of bone 6 square c.*

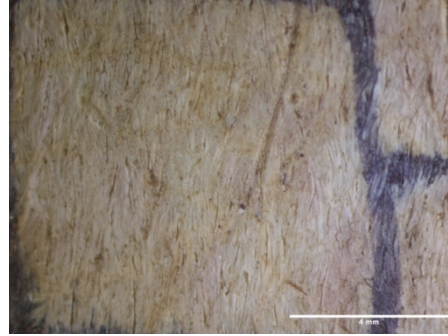


*Figure 134. After photo of bone 6 square c.*

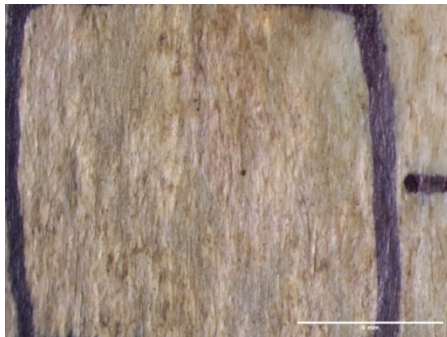




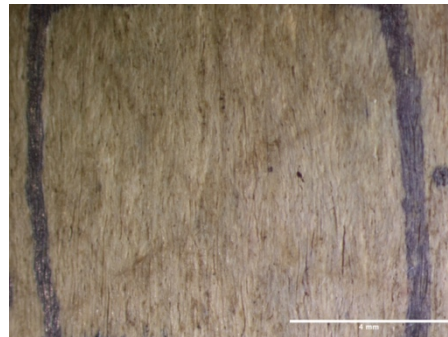
*Figure 135. Before photo of bone 6 square d.*



*Figure 136. After photo of bone 6 square d.*



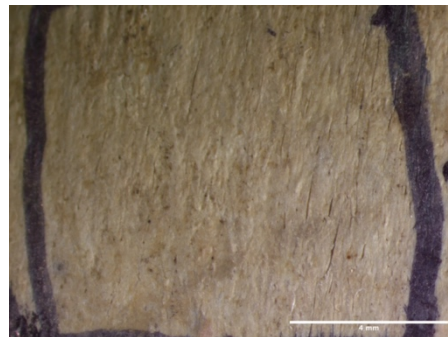
*Figure 137. Before photo of bone 6 square e.*



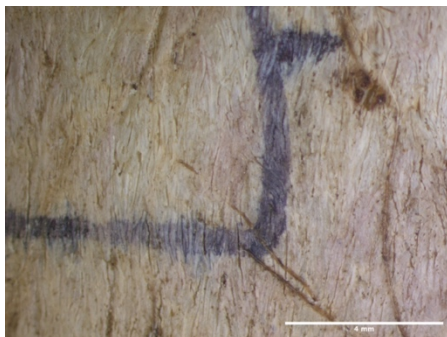
*Figure 138. After photo of bone 6 square e.*



*Figure 139. Before photo of bone 6 square f.*



*Figure 140. After photo of bone 6 square f.*



*Figure 141. After photo of bone 6,  
mechanical root action near square d.*



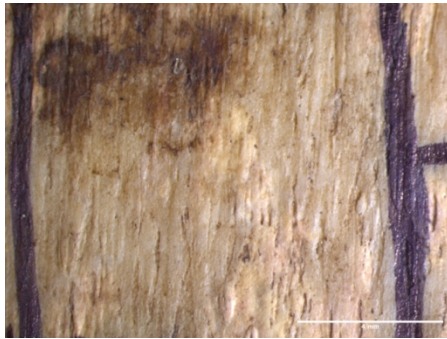
*Figure 142. After photo of bone 6,  
mechanical root action near square d.*



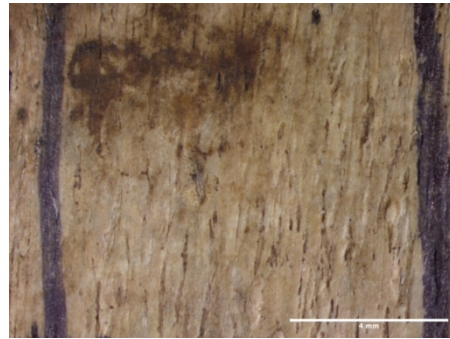
*Figure 143. After photo of bone 6,  
mechanical root action on sternal end of  
bone.*



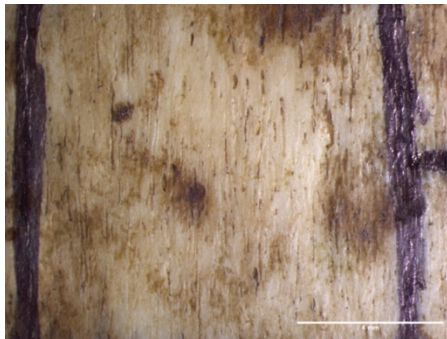
*Figure 144. After photo of bone 7,  
mechanical root action near sternal end of  
bone.*



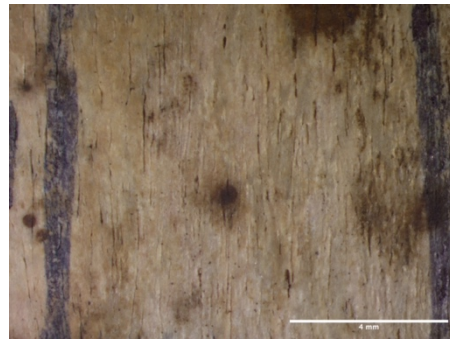
*Figure 145. Before photo of bone 7 square a.*



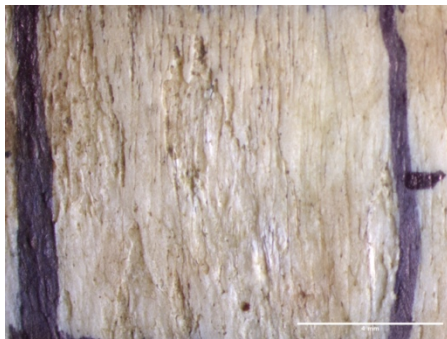
*Figure 146. After photo of bone 7 square a.*



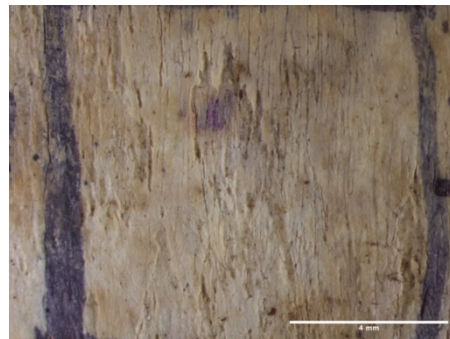
*Figure 147. Before photo of bone 7 square b.*



*Figure 148. After photo of bone 7 square b.*

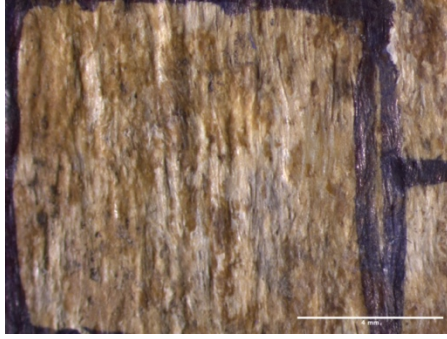


*Figure 149. Before photo of bone 7 square c.*

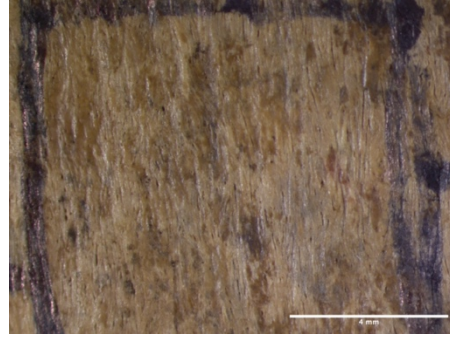


*Figure 150. After photo of bone 7 square c.*

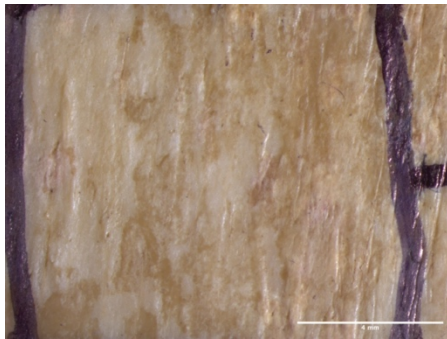




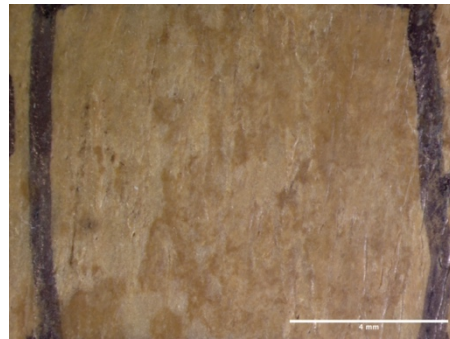
*Figure 151. Before photo of bone 7 square d.*



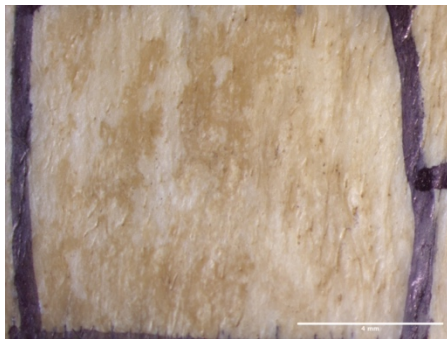
*Figure 152. After photo of bone 7 square d.*



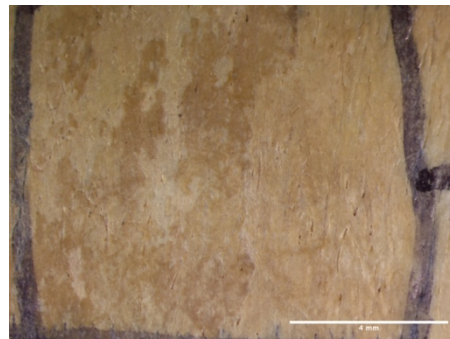
*Figure 153. Before photo of bone 7 square e.*



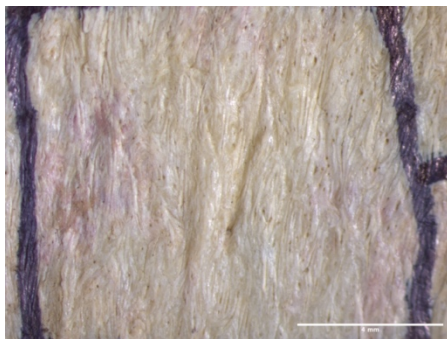
*Figure 154. After photo of bone 7 square e.*



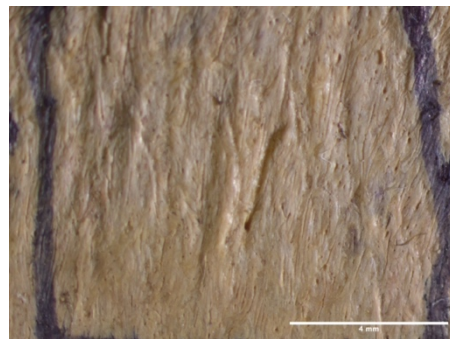
*Figure 155. Before photo of bone 7 square f.*



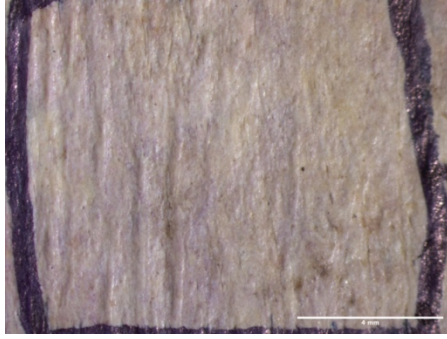
*Figure 156. After photo of bone 7 square f.*



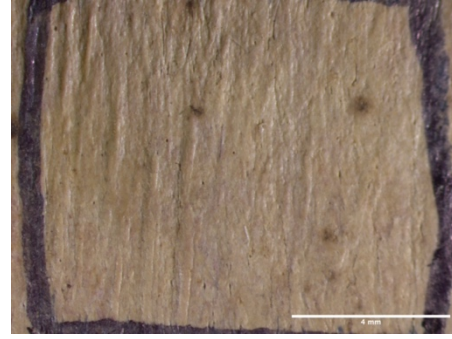
*Figure 157. Before photo of bone 8 square a.*



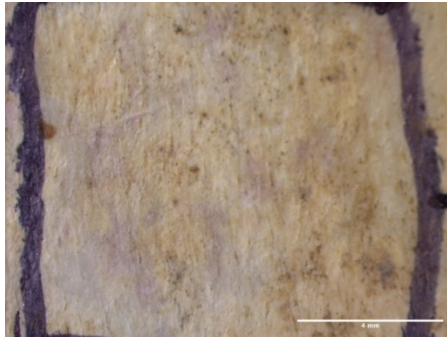
*Figure 158. After photo of bone 8 square a.*



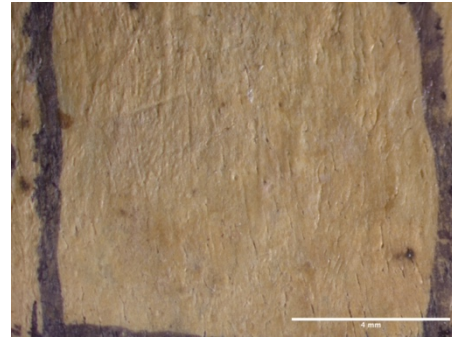
*Figure 159. Before photo of bone 8 square b.*



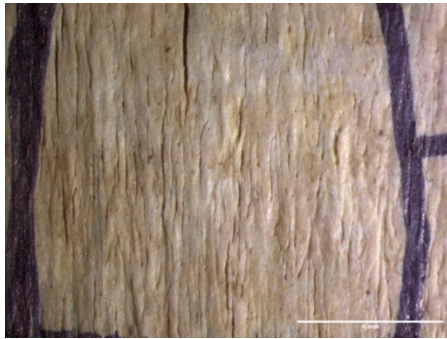
*Figure 160. After photo of bone 8 square b.*



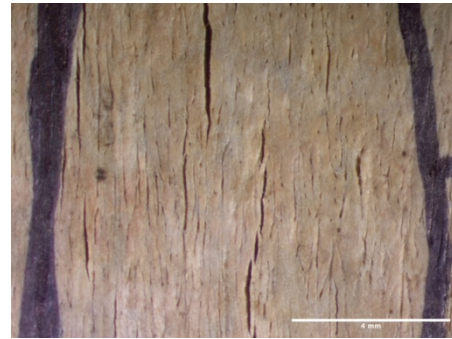
*Figure 161. Before photo of bone 8 square c.*



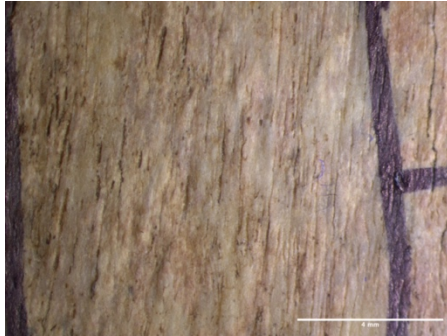
*Figure 162. After photo of bone 8 square c.*



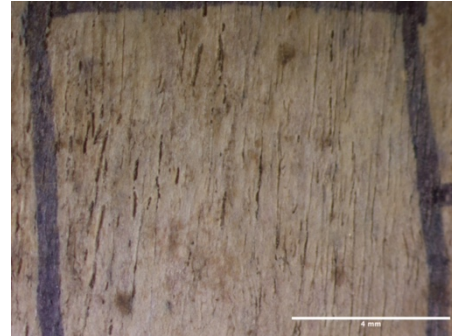
*Figure 163. Before photo of bone 8 square d.*



*Figure 164. After photo of bone 8 square d.*

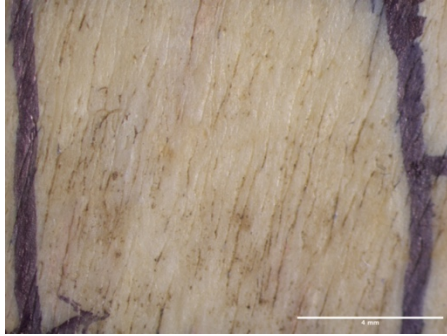


*Figure 165. Before photo of bone 8 square e.*

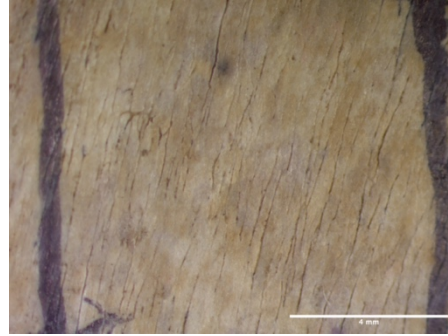


*Figure 166. After photo of bone 8 square e.*





*Figure 167. Before photo of bone 8 square f.*



*Figure 168. After photo of bone 8 square f.*



*Figure 169. After photo of bone 8, mechanical root action on sternal end of bone (broken- off piece).*



*Figure 170. After photo of bone 8, mechanical root action on sternal end of bone (broken- off piece).*



*Figure 171. After photo of bone 8, mechanical root action on sternal end of bone (broken- off piece).*



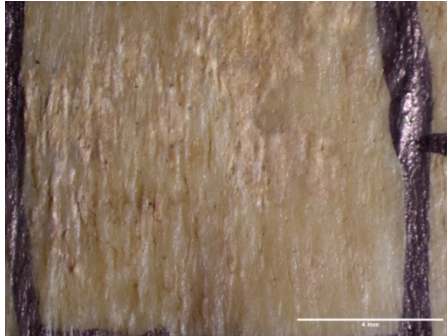
*Figure 172. After photo of bone 8, mechanical root action on sternal end of bone.*



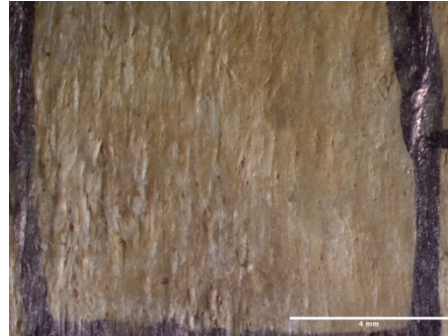
*Figure 173. After photo of bone 8, mechanical root action on body of bone.*



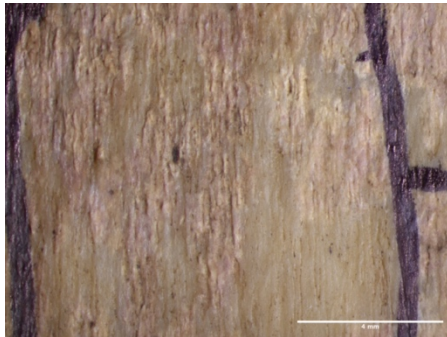
*Figure 174. After photo of bone 8, purple/pink differential staining.*



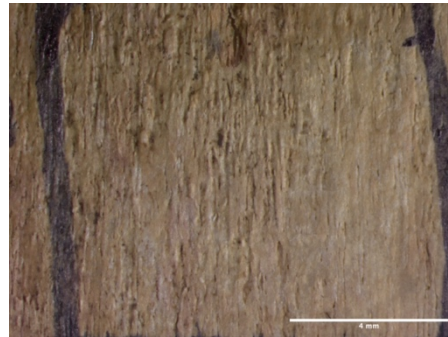
*Figure 175. Before photo of bone 9 square a.*



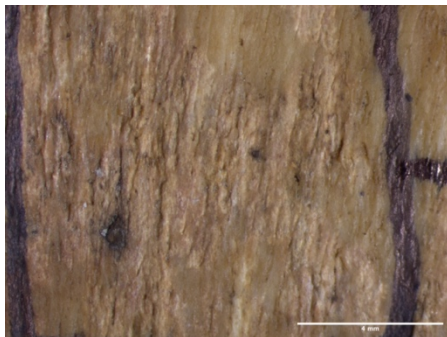
*Figure 176. After photo of bone 9 square a.*



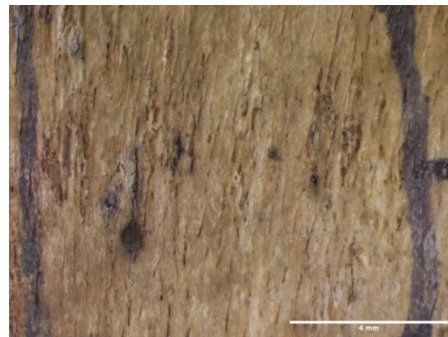
*Figure 177. Before photo of bone 9 square b.*



*Figure 178. After photo of bone 9 square b.*

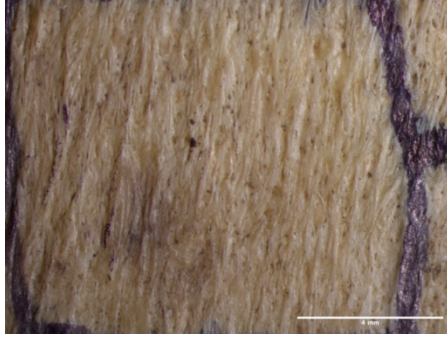


*Figure 179. Before photo of bone 9 square c.*

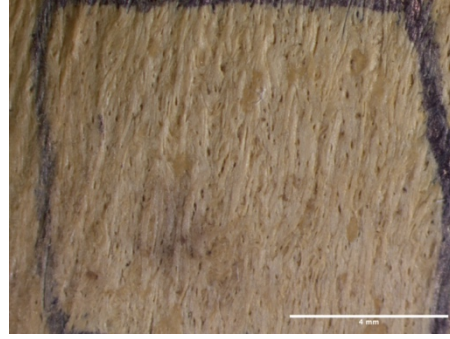


*Figure 180. After photo of bone 9 square c.*

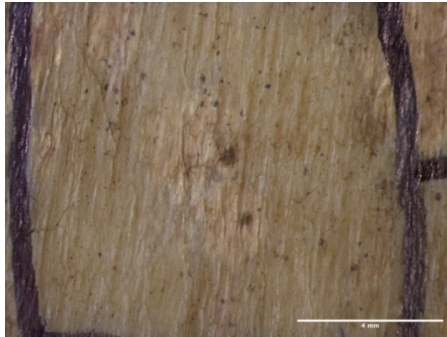




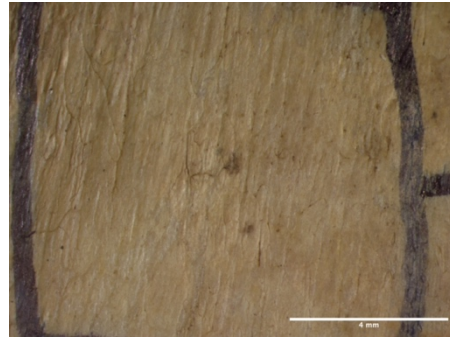
*Figure 181. Before photo of bone 9 square d.*



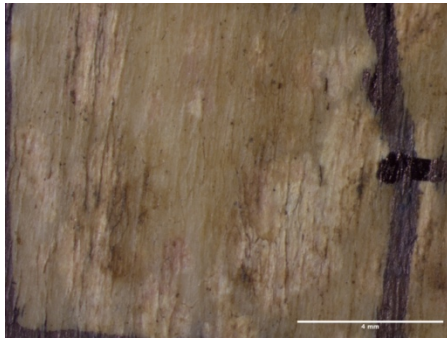
*Figure 182. After photo of bone 9 square d.*



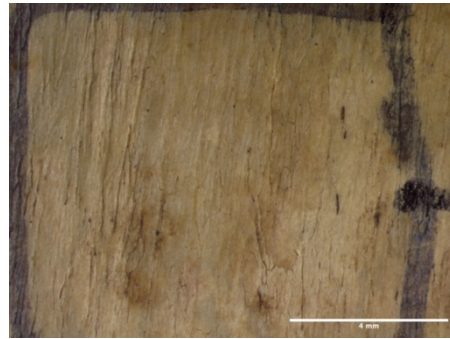
*Figure 183. Before photo of bone 9 square e.*



*Figure 184. After photo of bone 9 square e.*



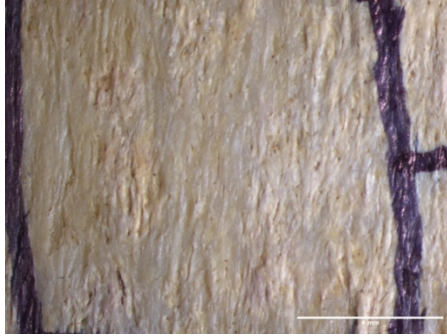
*Figure 185. Before photo of bone 9 square f.*



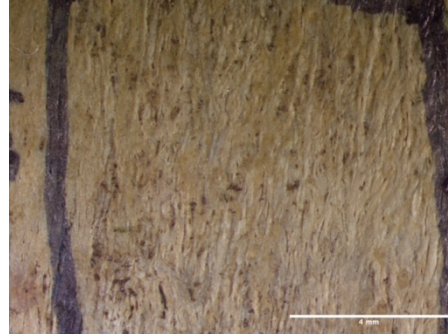
*Figure 186. After photo of bone 9 square f.*



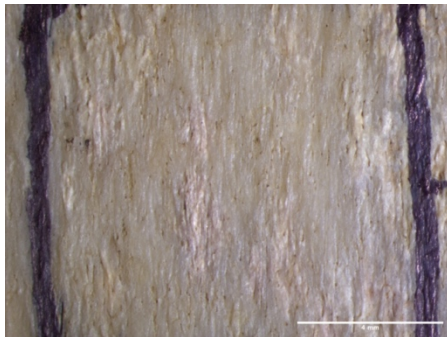
*Figure 187. After photo of bone 9, purple/pink differential staining.*



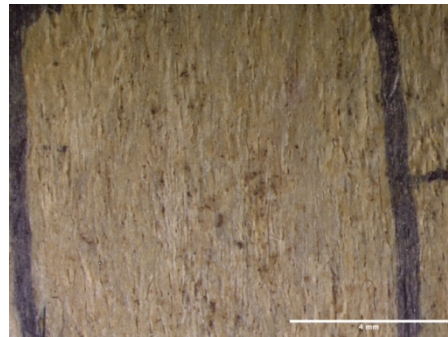
*Figure 188. Before photo of bone 10 square a.*



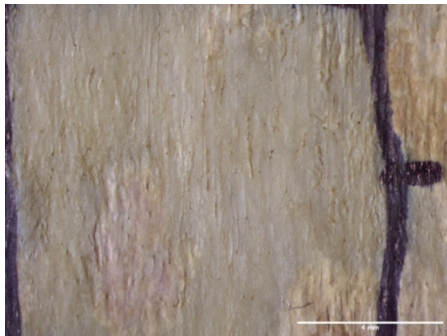
*Figure 189. After photo of bone 10 square a.*



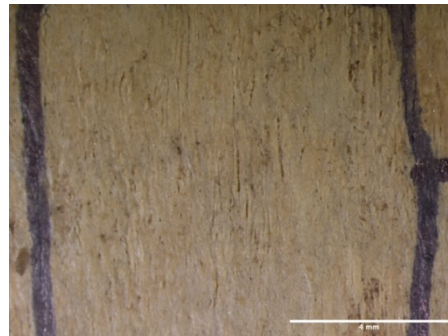
*Figure 190. Before photo of bone 10 square b.*



*Figure 191. After photo of bone 10 square b.*

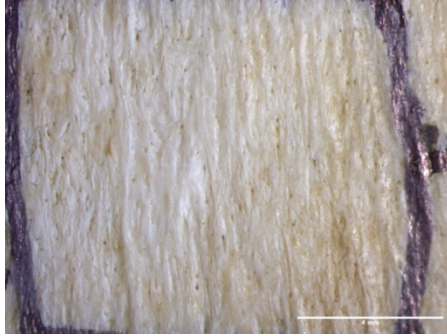


*Figure 192. Before photo of bone 10 square c.*

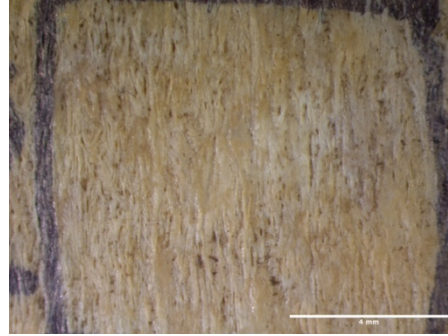


*Figure 193. After photo of bone 10 square c.*

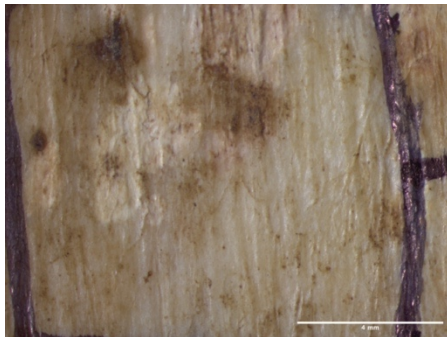




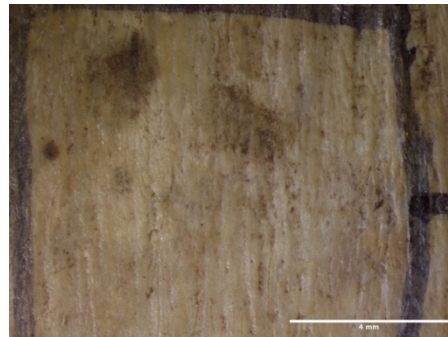
*Figure 194. Before photo of bone 10 square d.*



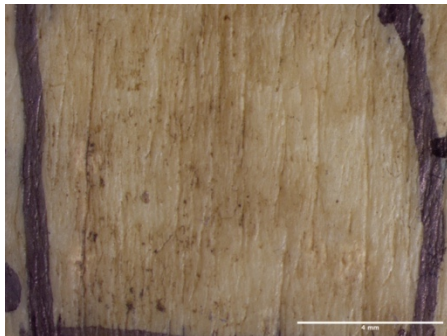
*Figure 195. After photo of bone 10 square d.*



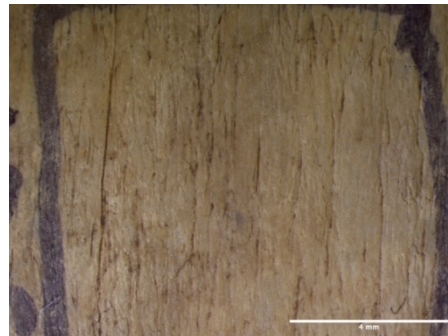
*Figure 196. Before photo of bone 10 square e.*



*Figure 197. After photo of bone 10 square e.*



*Figure 198. Before photo of bone 10 square f.*



*Figure 199. After photo of bone 10 square f.*

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