FORENSIC TAPHONOMY: COPPER AND ALUMINUM STAINING ON SKELETAL MATERIAL

By

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ABSTRACT

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George Mason University, 2021

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In forensic investigations, when an unknown decedent is found, the postmortem interval is a critical data point in establishing identification as well as reconstructing circumstances surrounding the death event. When the body reaches the fourth stage of decomposition, advanced decay, the soft tissues have been completely broken down. The decomposition of soft tissues leads to skeletonization, where only the hard skeletal tissues remain. These materials are then subject to diagenetic processes, including discoloration. The most commonly encountered stains on bones are from soil, organic materials, or metals. Staining on bone from metal compounds can be caused by numerous circumstances wherein various types of metal from clothing, projectiles, or other personal artifacts comes in contact with the remains. Because several factors influence the rate of decomposition, the postmortem interval between death and skeletonization can vary widely. Furthermore, the methods for determining the time between skeletonization and discovery are limited. The following study explored the potential for estimating the postmortem interval via copper and aluminum staining patterns on skeletal remains. The specific goal of this research was to determine whether the discolorations can assist in estimating time since skeletonization and reconstructing the depositional environment. Additionally, the two types of metals were compared to establish any distinct staining pattern or discoloration on bone that is unique and can be presumed as belonging to either copper or aluminum. In the experiment, seven deer tibias were buried in a temperature-controlled environment. Pieces of copper and aluminum were affixed to each tibia. Once a week, for 20 weeks, each bone was examined for signs of discoloration from the metals. Munsell soil color charts were used to quantify the observed skeletal color changes, and a qualitative scoring system was used to measure the degree of staining each week. The staining on bone caused by copper was predominantly green with some yellow and grey variation and became more pronounced over time. Aluminum staining was largely white and exhibited a lesser extent of color change. The data analysis suggested both types of staining possess a rate of color change whose variability is correlated with time. The results of this study will contribute to the identification and assessment of discolorations on skeletal remains, which can potentially help reconstruct the depositional scene and estimate the time since skeletonization.

INTRODUCTION

In forensic investigations, when an unknown decedent is discovered, the post-mortem interval is a critical data point in establishing identification. The estimation of time since death is crucial in the evaluation of human remains and the determination of an unknown individual’s identity. Furthermore, the location and environment in which a deceased person is found can provide insight regarding the sequence of events leading up to body disposition; when deposited in an outdoor setting and exposed to non-skeletal material for a prolonged period of time, skeletal remains can undergo numerous modifications, such as changes in color. Therefore, the overarching question that drove the following study is this: can metal staining on bone help estimate time since skeletonization and/or contribute to the reconstruction of the depositional environment?

After a body has decomposed to the point where only skeletal material is present, one component of the postmortem interval is the time since skeletonization. One of the goals of the following research was to improve upon current methods for the estimation of time between skeletonization and discovery of the decedent by providing a system for assessing discolorations on bone caused by prolonged contact with inorganic materials. The primary objectives were to identify, document, and assess the skeletal staining patterns caused by pure aluminum and pure copper samples by using the Munsell color system to compare and quantify the changes in color over a period of several months.

Based upon previous studies that investigated organic staining on skeletal material, it was expected that discoloration due to the metals would begin three months upon first contact and the stains would darken in appearance over time. Data were collected to establish any distinct staining patterns or discolorations on bone that is unique to either copper or aluminum; furthermore, statistical analyses were performed to determine whether such trends in changes in color are consistent and can be used in the evaluation of deposition environment and time since skeletonization.

Importance of this Research

The human body undergoes significant postmortem modifications; immediately upon time of death, countless taphonomic processes begin, and the remains are altered in ways that can be scrutinized in order to ascertain identity and evaluate the environment in which the body was deposited. Complete assessment of human remains requires several aspects of inquiry—one of which includes the estimation of the postmortem interval (PMI). Although there are numerous methods for calculating PMI, every estimate is made with some degree of uncertainty. In general, a shorter postmortem interval will result in a narrower estimated time range, while the longer the postmortem interval, the greater the estimated time range and often the greater the likelihood of error (Dix & Graham, 2000). By the time the remains have reached skeletonization, wherein only the hard tissues of the skeleton remain, any PMI estimate from that point on will inherently be associated with a large degree of uncertainty due to the amount of and variability in time required to reach the skeletal stage. Ultimately, postmortem interval assessments may narrow down the pool of potential identities in a case with an unknown decedent. Additionally, developing more precise PMI estimation methods can aid investigations by corroborating or refuting witness/suspect statements. Currently, the methods for estimating the time between skeletonization and discovery are limited; the results from this study offer a method to improve upon the accuracy of such approximations by offering an approach to linking skeletal staining color and time.

Another component to forensic death investigations involves the depositional environment of human remains. Reconstructing the nature and position of the objects that were in close proximity to the decedent after time of death is important in understanding the circumstance of disposal as well as potentially assisting in establishing motive and offender behavior (Janaway, 2008). Personal belongings, artifacts, or textiles often accompany a buried or deposited body, and such objects can produce discolorations on skeletal material. The staining patterns on bone from aluminum and copper observed in this study may be applicable to medicolegal situations where similar changes in color are detected. Distinguishable trends in bone discoloration over time may be useful to infer a metal artifact's close proximity at the time of deposition, even if it is no longer present when the decedent is discovered due to human intervention or natural deterioration. In addition, certain patterns may be associated with a particular metal type, particularly with regards to the distribution and shape of the staining, which may contribute contextual information related to the investigation.

Background Information

Decomposition

During the process of decomposition, there are five recognizable stages: fresh, bloat, active decay, advanced decay, and skeletal (Dix & Graham, 2000). Due to its potential to assess PMI, determining the decomposition stage of a set of human remains at the time of discovery has become an increasingly important component of medicolegal death investigations over the past several decades, driving interest in understanding the decomposition process (Wescott, 2018). Although considerable variation in the progression of each phase exists based on regional, seasonal, and microenvironmental conditions, certain characteristics distinguish each stage (Carter et al., 2007):

- The fresh stage immediately succeeds death, when the corpse undergoes a reduction in temperature, stiffening of the extremities, and pooling of the blood in the direction of gravity.
- During the bloat stage, a shift from aerobic to anaerobic bacterial species within the gastrointestinal tract leads to the excretion of gases that cause the body to swell.
- Much of the body mass is lost through insect and bacterial activity in active decay, and the soft tissues begin to liquify.
- Putrefaction is nearly complete during advanced decay, and staining from bodily fluids may be present on the surrounding environment.
- The final stage, skeletonization, is characterized by the presence of primarily skeletal and connective tissue and the lack of diverse insect colonization.

The rate of decomposition is influenced by multiple factors, including temperature, humidity, pH, body weight, and the presence of clothing. The interval between death and skeletonization can vary widely depending on the climate and ecological zone, which significantly affect the duration of the postmortem decay process. Several methods for quantifying the morphological changes in the stages of decomposition have been proposed. One technique is the total body score (TBS) system, which assigns numerical value to the identifiable, progressive characteristics seen in the head/neck, torso, and extremities during each stage of decomposition (Megyesi et al., 2005). Similarly, the degree of decomposition index (DDI) provides a value between 0 and 5 based on the stage of decomposition for each body element present (Fitzgerald & Oxenham, 2009). Generally speaking, the most important environmental factor in the rate of decomposition is the temperature accrued over the postmortem interval (accumulated degree days, or ADD). Consequently, Megyesi et al. (2005) developed a technique for calculating the number of calendar days since death by taking into account the temperatures to which the remains were exposed. The advantage of ADD is that not only does it incorporate chronological time and temperature, it can also be used across different climatic regions and seasons.

Another common analytical method that can reflect the stage of decomposition relies on entomological evidence. Within the first 72 hours after death, entomological activity is one of the most accurate indicators for determining elapsed time since death (Wells, 2019). After 72 hours, decay of the remains is further fueled by insect activity. In general, particular species of insects found on the body are associated with a particular stage of decomposition (Joseph et al., 2011). Although the research that evaluates postmortem modifications has advanced throughout the past few decades, improved accuracy of estimating time since death remains an important goal for forensic investigators.

Taphonomy

The comprehensive evaluation of human remains involves the assessment of taphonomic processes. The discipline of forensic taphonomy encompasses all of the changes the human body undergoes following death (Huculak & Rogers, 2009). These postmortem alterations can affect the preservation and recovery of the human remains. In outdoor settings, the most common modifications are caused by such natural agents as soils, plants, animals, and weather (Schultz et al., 2003). One of the responsibilities of forensic archaeologists and anthropologists is to recognize and document the specific changes produced by each of these agents in order to understand and explain the visual nature of the discovered remains. This type of analysis can ultimately contribute to other aspects of a death investigation, such as PMI, indications of human intervention, or secondary depositional locations (Pokines & Symes, 2013). Beginning in the 1970s and 80s, protocols for processing outdoor death investigation scenes have been extensively developed, which can aid in reconstructing sequence of events between deposition and discovery or distinguishing postmortem versus antemortem injuries (Dirkmaat & Cabo, 2016). Pattern identification and distinguishing features left by taphonomic agents have been the focus of numerous studies that have benefitted the analysis of postmortem alterations to the human body.

The chemical, biological, or physical alterations that osseous remains undergo are known as diagenesis (Brooks, 2016). Some of these processes include avian feeding and dispersal, carnivore or rodent gnawing, and subaerial weathering. Yet one of the most commonly observed diagenetic processes is staining from organic compounds. According to Huculak & Rogers (2009), determination of the deposition interval, original environment of deposition, and the environmental factors that have affected the remains are the main goals of a taphonomic analysis. The scrutiny of color staining on skeletal remains is essential to reconstruct possible environments in which a set of remains was deposited (Pokines & Symes, 2013). Fresh, defleshed bone is naturally a yellowish-white to beige color, but exposure to sunlight, water, or fire can produce a change in appearance. However, soil, organic compounds, and metals are the primary causes of osseous discolorations, and staining patterns emerge as a result of physical contact between such materials and the surface of bone over an extended period of time (Schultz et al., 2003). Skeletal remains may lie in close proximity to metal substances for a variety of reasons. Depending on a combination of how aggressive the depositional environment is to osseous material and how long the remains have been exposed to those conditions, the buttons or zippers on dress fastenings, or personal adornments such as watches or other jewelry, may produce staining on the bones due to the corrosive nature of the metals (Janaway, 2008).

Corrosion

Corrosion is the deterioration of metal due to the chemical reactions that occur between it and the surrounding environment. This process stems from a refined metal's tendency to convert to a more stable form; metals in their manufactured states return to their natural oxidation states via reduction-oxidation reactions (Goffer, 2007). Metal becomes oxidized by its surroundings—most often by the oxygen in air or water. There are three groups of metals, based on their susceptibility to corrosion: corrosion-resistant metals; metals that, after initial rapid corrosion, form a layer of stable corrosion products and, thus, become resistant to further attack; and metals that corrode rapidly but do not form a layer of protective corrosion products (Cukrowska et al., 2005).

Copper falls within the second metal corrosion category—it creates a protective corrosive layer and can maintain an extensive metallic core for hundreds of years (Goffer, 2007). Completely stable, corroded copper possesses a thin, green to green-blue layer on the surface of the metal, commonly known as patina (Morris, 1981). Copper corrosion occurs due to several chemical reactions. The first step in the development of the patina is oxidation to form copper oxide, which has a red or pink color. Copper atoms further react with oxygen molecules from the air or other sources, transforming the copper oxide from red/pink to black. Finally, the reaction with carbon dioxide and hydroxide ions in atmospheric water create a blue-green patina (Morris, 1981). The extent of humidity and the level of sulfur-related air pollution have a significant impact on how fast the patina develops. Similarly to copper, aluminum oxidation creates a protective layer over the surface of the metal. Aluminum has a high affinity for oxygen. The oxidative process between aluminum and oxygen results in aluminum oxide, which appears as a powdery white or dull, grey coating (Shahack-Gross et al., 1997). Aluminum oxide protects the metal from further decay. Should the film of aluminum oxide dissolve or meet another form of disturbance, further deterioration will take place.

Corroding metal in contact with skeletal remains results in metal staining and/or adhered corrosion products to the bone. Within a forensic context, copper and iron are the two most common metals that produce osseous discolorations (Cukrowska et al., 2005). If the environment contains high levels of moisture, metals will corrode much faster, and burial environments will obviously contain moisture related to the decomposition process (Janaway, 2008). The rate of corrosion depends on a number of different factors, including the composition and structure of the metal artefact, the chemical nature of the burial environment, and the interval of burial (Morris, 1981).

Munsell Color System

In the early twentieth century, Albert Munsell developed a system of color notation in which each color is broken down into three dimensions: hue, chroma, and value (Pearson, 2000). The hue is the quality by which each color is distinguished from one another and is divided into five main sections: red (R), yellow (Y), green (G), blue (B), and purple (P) (Munsell, 1912). There are classifications in between as well; for example, YR lies between red and yellow. Each distinct hue has four subdivisions: 2.5, 5, 7.5, and 10. Therefore, there are 40 hues in total, each with its own alphanumeric identifier. A 5 marks the middle of a hue, and a 10 marks the boundary between one hue and the next (Ruck & Brown, 2015). Chroma is the strength or intensity of a color; it distinguishes a strong color from a weak one. Another term for chroma is saturation, and in the Munsell system, this is numerically represented. Chroma values range from 0 for very light or weak hues to 30 for very strong hues. The third quality is the value, or lightness, of the color. The value is used to distinguish darker and lighter shades of a hue and varies from 0 (black/darkest) to 10 (white/lightest) (Ruck & Brown, 2015). Munsell system notation uses the following order: hue first, followed by value and chroma, with the latter two separated by a forward slash (e.g., 5GY 7/8).

Previous Studies

In 2018, Pollock et al. performed a study that addressed the staining process on skeletal remains caused by four different organic materials: soil, wood, blood, and plant matter. Remains were left in each of the four environments for a period of one to three years, with data collection and observations performed every month. The Munsell system was used to identify color changes on the bones throughout the study. The intent of the research was to examine organic staining as a technique for establishing the taphonomic agents with which skeletal remains come into contact in their depositional locations. Pollock et al. (2018) hypothesized that the degree of observed staining would increase as the length of exposure to each type of organic material increased and that each environment would yield different patterns or colorations of staining on the bones. After a period of two months, staining was observed in all four of the experimental environments. The discolorations also darkened in appearance across the bone surface with each subsequent examination. The team concluded that because of the variation in staining patterns between samples, remains could be presumed to have been exposed to various wood species, buried in a soil environment, and/or exposed to plant matter (Pollock et al., 2018). The bones buried in wooden containers displayed more uniform staining on the external bone surface, while the remains exposed to plant matter displayed more sporadic staining across the surface. Furthermore, the extent of variety in staining color was found to be an important differentiator when assessing possible burial environment: diffuse staining, in which a wide range of colors are observed, suggested that the depositional environment included direct soil contact, while uniform organic staining with limited colorations most likely indicated deposition in a location with an abundance of wood.

The findings from Pollock et al. (2018) influenced the expected outcomes in the following research: staining was present after two months of exposure from each of the four organic materials; therefore, in the present study, it was hypothesized that it would take at least three months of direct contact with metal samples for discolorations to appear due to the different decay rates of inorganic versus organic substances. Additionally, the conclusions Pollock et al. (2018) reached may be similarly applied to the patterns and color variations observed in the following study.

Nearly two decades ago, a team of scientists discovered manganese dioxide and iron dioxide from surrounding dolomite on human skeletal remains (Cukrowska et al., 2005). The nature of the effects on the bones deviated from typical staining. Instead, the manganese dioxide and iron dioxide appeared to coat and, at times, penetrate the surface of the bones, resulting in obscured fine detail and hindering identification and interpretation of surface modifications caused by different taphonomic agents (Cukrowska et al., 2005). The findings described in the article depict a unique circumstance in which metallic elements from surrounding rock formations impacted the skeletal remains to such a degree that they ultimately obstructed other diagenetic analyses. These reports are an important consideration for this study because they depict the effects of some metal types on skeletal remains and the subsequent impact on an investigation.

Additionally, Morris (1981) demonstrated that the preservative actions of metallic copper can be strong enough to maintain fairly large organic items, because dense concentrations of copper salts are toxic to the biological agents of putrefaction. In 1981 in South Africa, Morris discovered green staining on the cranium and forearm of human skeletal material. Six out of eleven sets of remains with the green discolorations were also found with copper artifacts (Morris, 1981). After surveying archaeological literature, Morris asserted that the specific locations of the bone staining correlated with ornament type: mastoid-mandibular condyle staining was found to be caused by earring artifacts, while wrist discoloration was due to bangle type adornment. The results of Morris' research suggest green discoloration of skeletal remains can be a useful indicator of the presence of copper ornaments even if none exist at the time of the body's recovery.

MATERIALS AND METHODS

Experimental Design

Seven adult deer tibias between 10 and 12 inches long were allowed to deflesh naturally in an outdoor environment. The deposition time for each bone is unknown, but they all share similar general taphonomic histories, and, upon procurement, were visually comparable in terms of lack of soft tissue and other visual qualities. The bones were placed in a plastic bucket and soaked in a solution of Clorox® (Oakland, CA) and Comet® and bleach powder cleaner (Lancaster, PA) for approximately 12 hours in order to remove any pre-existing stains from decomposition. The solution consisted of 12.5% Clorox®, 3% Comet® cleaner, and 84.5% water. The bones were taken out to dry overnight. Each bone was labeled with a number between 1 and 7. Photographs with a scale were taken of all seven bones prior to any metal contact (Figure 1).

***Figure 1**: Seven deer tibias were soaked in bleach then labeled 1 through 7*

Laboratory-grade metal strips of copper and aluminum from Carolina Biological Supply® (Burlington, NC) measuring 3/4 x 5¼ x ⅛ inches (1.9 x 13.3 x 0.31 cm) were cut into squares measuring approximately 2 x 2 cm. One copper square and one aluminum square were placed on opposite sides of the proximal end of each bone and pressed firmly to mold to the bone's shape. Each metal square was fastened to the bone using an 11-inch Hyper Tough® (Bentonville, AR) white, nylon plastic cable tie. The cable tie was tightened in order to prevent slippage between the bone and metal piece. The areas of contact between the bone and metal sample were outlined with a black, fine-tipped Sharpie brand permanent marker (i.e., two outlines per bone) in order to assure consistent placement of the metal samples after each observation. The outlined boxes on each bone were labeled as either 'Cu' or 'Al.' Also for the consistent placement of the copper and aluminum against each tibia, the side of each metal square that faced outward from the skeletal material was marked with the Sharpie permanent marker (Figure 2).

***Figure 2**: Tibia 4 with copper and aluminum samples attached with plastic cable ties. The squares of contact and metal sides not facing bone were marked.*

In order to simulate a realistic skeletal remains decomposition environment, all seven bones were buried in GardenPro® (Mooresville, NC) topsoil, which was purchased in a 40-pound bag from Lowe's. The initial conditions of the soil were measured with a HoldAll light, water, and pH meter from Lowe's, and the same conditions were similarly monitored on a weekly basis for the duration of the experiment. The bottom of a Sterilite® (Townsend, MA) 12-gallon clear tote with a latching lid was covered completely with a layer of soil about 2 inches deep. Then the bones were placed in the bin on top of the soil layer and buried beneath approximately 6 inches of additional soil (Figure 3). The soil was firmly tamped down. The tibias were left to sit indoors with an average temperature of 21° C based on the thermostat and observed every Sunday afternoon for a period of twenty weeks.

Figure 3*: The tibiae were buried under topsoil in an indoor environment for 20 weeks.*

For each weekly observation, first, the soil pH and moisture conditions were measured. The HoldAll meter was inserted into the soil roughly 6 inches deep and left to sit for one minute, after which the pH and moisture values were recorded (Figure 4). Next, the soil covering the bones was removed by hand and placed in a white Glad® (Oakland, CA) trash bag. The bones were removed from the bin, and one at a time, the zip ties were loosened, and the metal squares were removed. Each tibia was examined for metal stains, and photographs were taken using an iPhone 7 rear camera (12-megapixel, $f/1.8$). Photos were always taken under 40-watt bulb lighting and against a black background, with the camera held approximately 5 inches directly above each tibia. Observations for each bone were recorded, then the aluminum and copper samples were reattached to the tibias using the zip ties, with the previously drawn outlines used to guide placement. The side of the metal square in contact with the bone was also always consistent, as the opposite side of each square was marked with a Sharpie®. The bones were placed back in the plastic bin, and the soil was scooped from the trash bag and into the bin until the bones were buried under approximately 6 inches of soil.

Figure 4: *The soil moisture and pH conditions were measured with a HoldAll meter.*

Munsell soil color charts were used to quantify the skeletal color changes that were caused by the metal samples. Discolorations were identified using the Munsell color system and corresponding values were recorded. For each of the seven tibias, the area surrounding the points of contact with the metal samples as well as directly underneath were examined closely. When a tibia exhibited staining, the Munsell color chart was placed beside the area of discoloring, and the value that matched the stain color was identified and recorded. In addition, a method for qualifying the degree of staining each week was created, where 0 indicated no staining, 1 indicated faint staining, 2 indicated light staining, 3 indicated moderate staining, 4 indicated heavy staining, and 5 indicated severe staining.

Data Analysis:

The recorded Munsell data values were transformed to Cartesian coordinates using the protocol from Ruck and Brown (2015) for the intention of statistical testing. First, the hues for each data entry were converted into angles. As there are 40 hues total within the Munsell color system, an arbitrary hue (5R) was chosen to be the origin, 0°. The remaining 39 hues are consequently assigned degree values, each 9° apart. For further calculations, degrees were converted to radians (Equation 1).

Together, the hue and chroma of each datum entry form a pair of polar coordinates along a plane. Converting these polar coordinates to the x and y Cartesian coordinates was accomplished with simple trigonometric functions (Equation 2 and Equation 3). The z coordinate, or height in the third dimension, for each datum point was simply the recorded value for the Munsell color (Equation 4). The distance (*d*) between a given week's coordinates (x_2, y_2, z_2) and the coordinates of the first week (x_1, y_1, z_1) was calculated using Equation 5. Each *d* value represents the degree of staining for that week compared to the week staining was first observed, with larger *d* values indicating greater changes in color, and smaller *d* values indicating lesser changes in color. Therefore, the expectation was for *d* values to increase with time.

Equation 1: $1 \text{ rad} \times 180/\pi = 57.296°$

Equation 2: $x = \sin(\text{Hue}) \times (\text{Chroma})$

Equation 3: $y = \cos(\text{Hue}) \times (\text{Chroma})$

Equation 4: $z = \text{Value}$

Equation 5: $d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}$

The assignment of values for the qualitative 0 to 5 scoring system each week were determined by the visual nature of the stain in comparison to previous weeks' appearance. If no discoloring was apparent, a score of 0 was given. If staining was just beginning to appear, a score of 1 was given. Scores of 2 through 5 were differentiated based on photographs of the bone from the week prior: if the stain appeared darker, larger, or there were additional stains present, then the score for that week was the previous week's score increased by 1.

The data were analyzed primarily through scatter plots and linear regressions. A scatter plot was generated for each metal's *d* values, degree scores, hues, chromas, and values over time in weeks. Each plot was fit with a linear regression and coefficients of determination (R^2) for the regression. The coefficients of determination for each of the three Munsell qualities were compared both within and between metal types to identify the quality whose change was most influenced by time. In order to determine if one metal resulted in significantly more staining than the other, several summary statistics were compared between copper and aluminum, including the average week at which staining first appeared, the average *d* value in the final week of data collection, and the average degree score at the final week. Additionally, in evaluating the Euclidean distance equation as a method for quantifying the change in staining color over time, for each metal, the coefficient of determination for the *d* value plot was compared to the average coefficients of determination for the three Munsell qualities. This comparison reveals the ability of the distance equation to account for each of the changes seen by all three of the Munsell qualities over time. The qualitative score data was also compared over time: final scores for each metal were averaged and compared to in order to see if one metal type resulted in a greater extent of staining than the other, and coefficients of determination for each scatter plot were analyzed to determine the overall change in discolorations throughout the weeks.

RESULTS AND DISCUSSION

Results

Before burial or any contact with copper or aluminum samples, the starting color of all 7 tibias was grayish orange (10YR 7/4). Within the first two weeks, four tibias had developed initial very pale green (10G 8/2) stains from the copper contact. Between weeks 3 and 5, those four stains progressed to pale green (5G 7/2) and light green (5G 7/4). From weeks 5 to 10, Tibias 1, 2, and 4 transformed to darker greens, while at week 5 Tibia 7 darkened to brilliant green (5G 6/6) and remained at that shade until week 11. Tibia 5 saw initial staining at week 7 and changed from light greenish gray (5G 8/1) to light green (5G 7/4) within the next three weeks. In the second half of data collection, changes in color due to copper were less frequent, and the soil moisture likewise declined. Between weeks 11 to 20, both staining colors on tibias 4 and 7 were consistent, with brilliant green (5G 6/6) coloring on tibia 4 and moderate green (5G 5/6) on tibia 7. In the same time period, staining colors transformed every few weeks on tibias 1, 2, and 5, ranging from light green to pale or moderate yellowish green to grayish yellow green (5GY 7/2). No staining due to copper was observed on tibias 3 or 6 during the course of data collection.

***Figure 5**: Tibia 1 copper discolorations at weeks 1, 10, and 20 (left to right)*

***Figure 6**: Tibia 2 copper discolorations at weeks 3, 10, and 19 (left to right)*

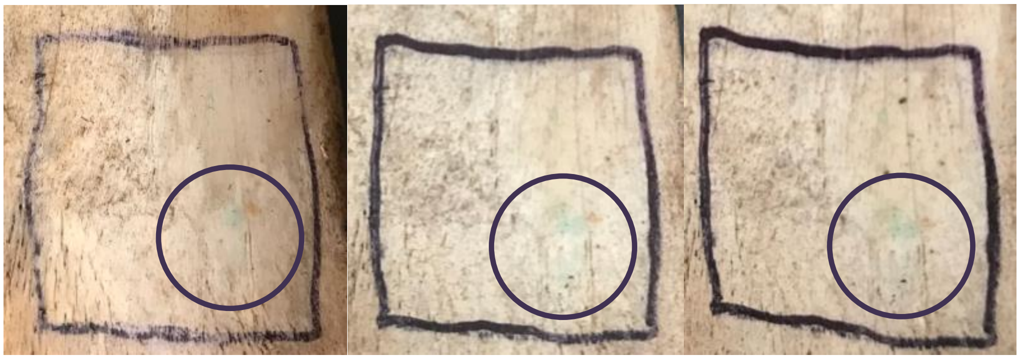

***Figure 7**: Tibia 4 copper discolorations at weeks 3, 13, and 20 (left to right)*

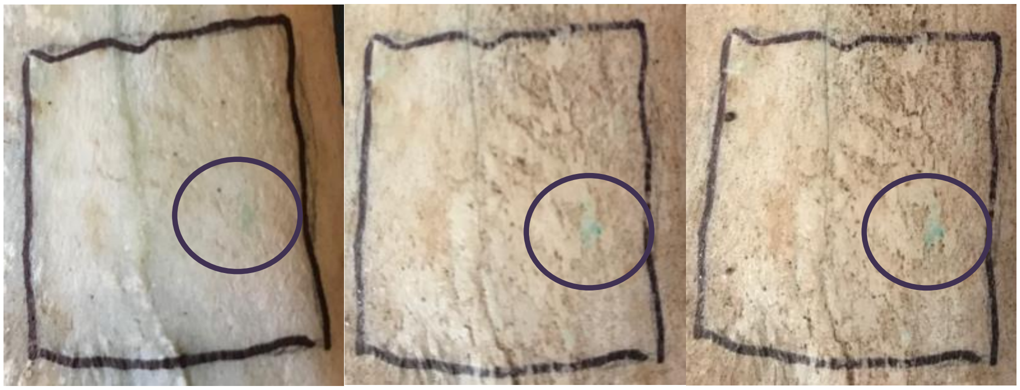

Figure 8*: Tibia 7 copper discolorations at weeks 1, 11, and 19 (left to right)*

Aluminum staining was visible at week 3 for tibia 7, week 4 for tibias 2 and 3, week 5 for tibia 4, at week 9 for tibia 6, and week 11 for tibia 5. The discoloration for each was identified as bluish white (5B 9/1), and the only change from that color was at week 12 for tibia 4 to a light bluish gray (5B 7/11). From then, discolorations were consistent for all six bones until the final week. Tibia 1 did not exhibit any signs of aluminum staining throughout the study.

Figure 9*: Tibia 2 aluminum discolorations at weeks 1, 11, and 20 (left to right)*

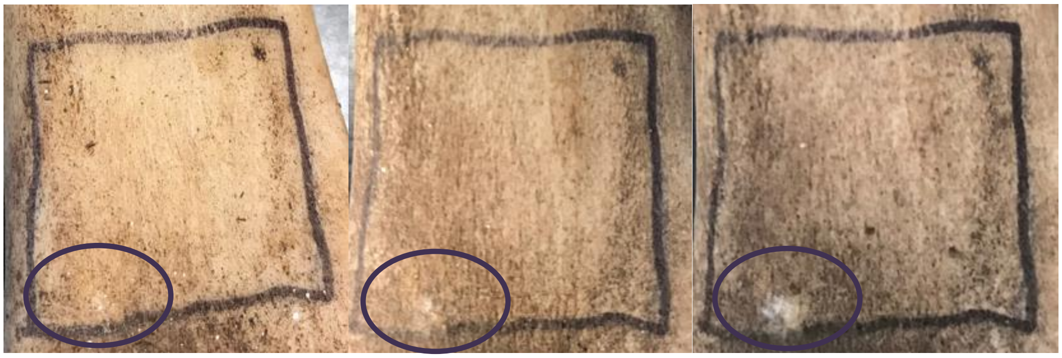

Figure 10*: Tibia 3 aluminum discolorations at weeks 4, 9, and 20 (left to right)*

Figure 11*: Tibia 4 aluminum discolorations at weeks 5, 11, and 20 (left to right)*

Discussion

For both metal types, the Euclidean distance (*d*) values were plotted against time in weeks, with the *d* values recalibrated so as to compare each value to the first appearance of staining, rather than the starting color of each tibia (Figures 12 and 13). This was done because the natural bone color is qualitatively different from those produced by the metal stains. As only three different Munsell colors were observed from the aluminum discolorations, only two distinct *d* values were recorded throughout the course of observations, which suggests that time is weakly associated with the variation in discoloration when examining the hue, chroma and value of the Munsell colors as a whole. After fitting the scatter plot with a linear regression, the coefficient of determination (R^2) was 0.0021 for the aluminum group, meaning only 0.21% of all the variation in the *d* values around its mean can be explained by time. However, the copper samples exhibited a stronger relationship between the change in Munsell values and time, with 10% of the variance in *d* explained by time.

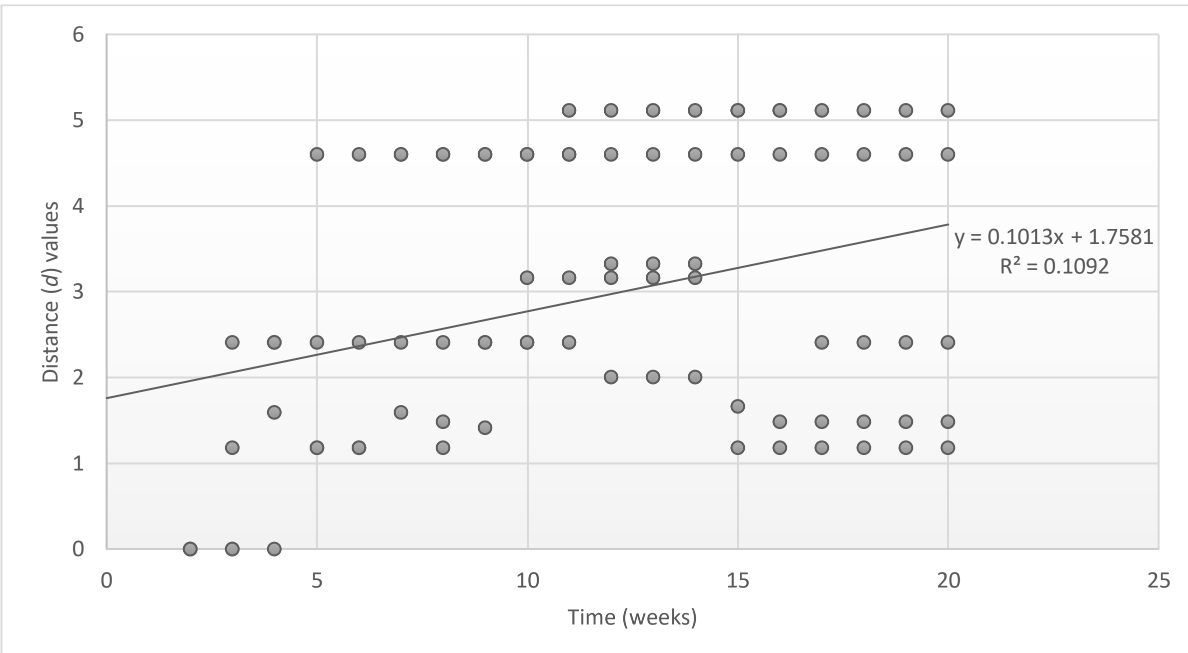

Figure 12*: Change in d values for Cu samples over time*

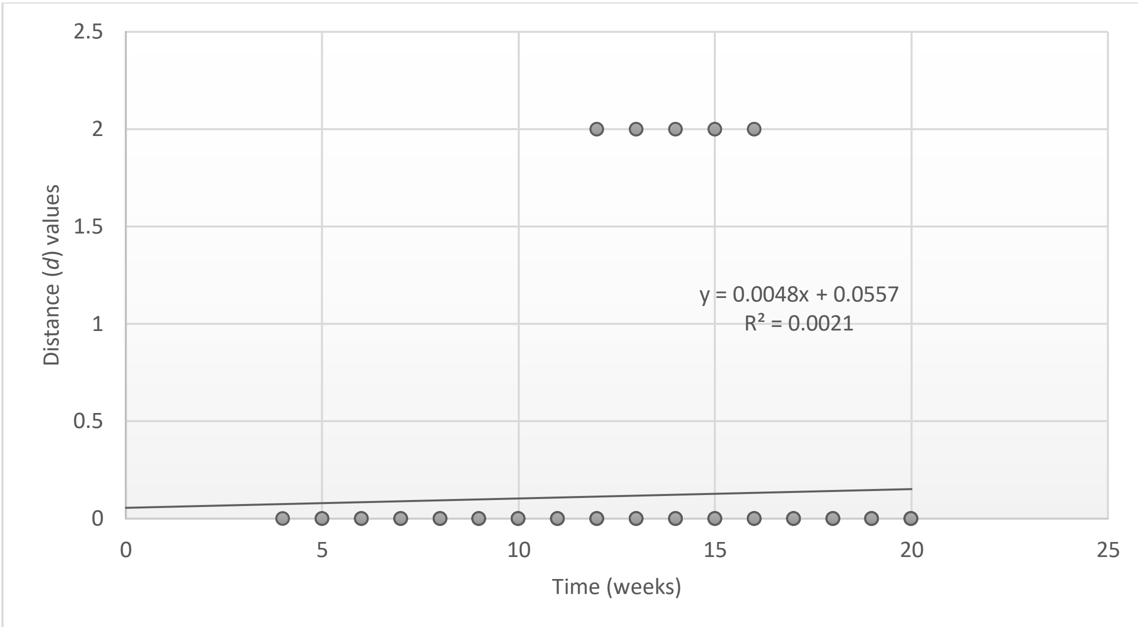

***Figure 13**: Change in d values for Al samples over time*

The range of possible variation within each of the shades is important when comparing the general coloring of the copper and aluminum stains. Overall, the copper staining fell under the green hue, with occasional shifting into partially yellow and gray hues, whereas all of the aluminum staining remained within the white hue for the entirety of data collection. The potential variation that exists within the hues exhibited by the copper stains is far greater than the potential variation for the white aluminum stains. This lack of inherent mobility within the color white itself— especially when compared to the mobility within greens and yellows—could be a factor in the discrepancies between the unexplained staining variation from each of the two metals.

The qualitative change (0-5 scale) in the degree of staining observed for each metal type over time was plotted and a linear regression was calculated (Figures 14 and 15). The R^2 value for the aluminum graph was 0.5312 and 0.64 for the copper graph, indicating that 64% of the variability in the degree of copper staining and 53% of the variability in the degree of aluminum staining is correlated with time. Over the course of data collection, the overall staining produced by copper resulted in a wider range of values than the overall staining produced by aluminum. Degree-of-staining scores ranged from zero to four under copper, while only between zero and three under aluminum. Based on the R^2 values, copper staining on bone is more strongly correlated with time by approximately 11% when compared to staining caused by aluminum, which may be explained by the differences between the variation of discoloration between the two staining patterns: while both the aluminum and copper scores exhibited a linear progression, the copper stains showed a greater extent of variation as the weeks progressed.

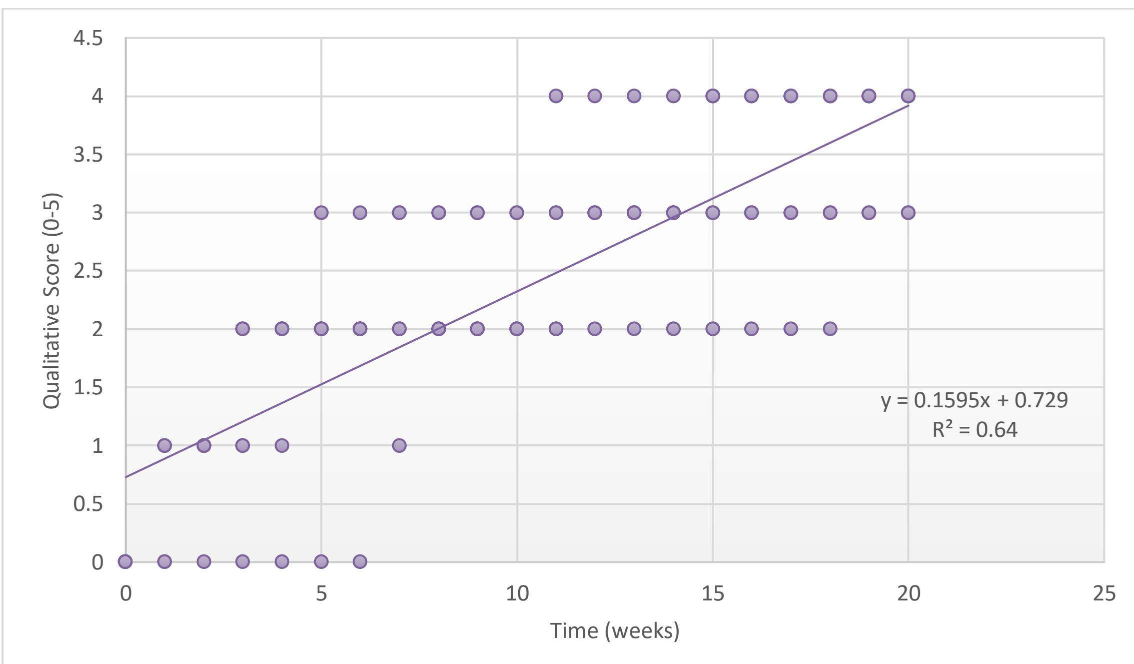

Figure 14*: Change in degree of staining for Cu samples over time*

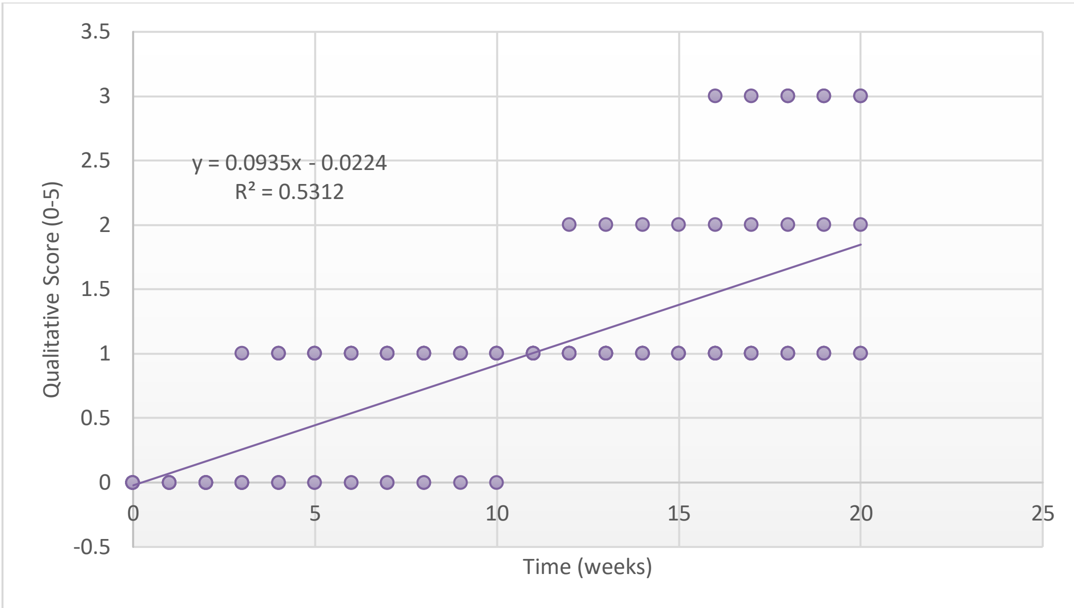

Figure 15*: Change in degree of staining for Al samples over time*

The Munsell color score for each datum entry was divided into its three scored qualities (hue, chroma, and value), and each of them were plotted against time for the aluminum group and the copper group. Figures 16 and 17 depict the change in hue over each week. The hue conversions to degrees were plotted, instead of the actual Munsell hues, in order to more accurately represent the full spectrum of potential hues. Based on the coefficients of determination from the linear regressions, time is more closely correlated with aluminum's staining hue than copper's, with time accounting for 50% of the variability in hue change from aluminum and only 12% of the variability in hue from copper. Interestingly, the appearance of a stronger correlation between hue and time for aluminum stemmed from the lack of a wide range of hues in the discoloring. Because there were only two different hue values throughout the course of data collection, the model for the scatter plot was a better representation for the average values of the aluminum hues than for the copper hues. However, based on the plot, the residuals are not randomly scattered around zero but rather display a pattern around the expected dependent variable. This undermines the quality of the linear fit and suggests that, despite the relatively high R^2 value, the regression does possess some bias, which would likely be due to the fact that aluminum staining hue only changed twice in the study.

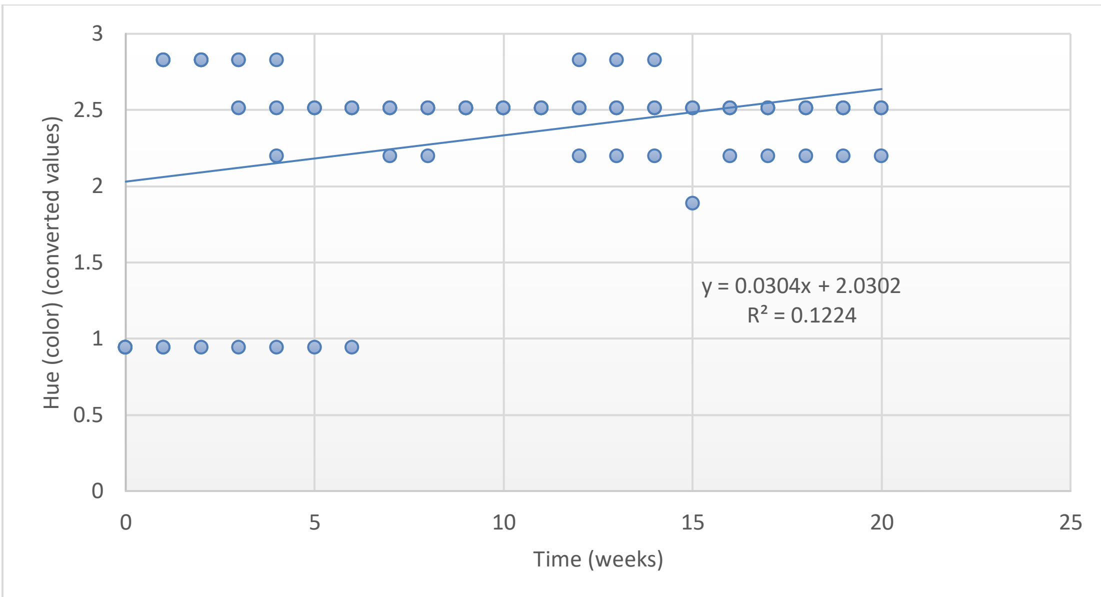

Figure 16: *Change in hue for Cu samples over time*

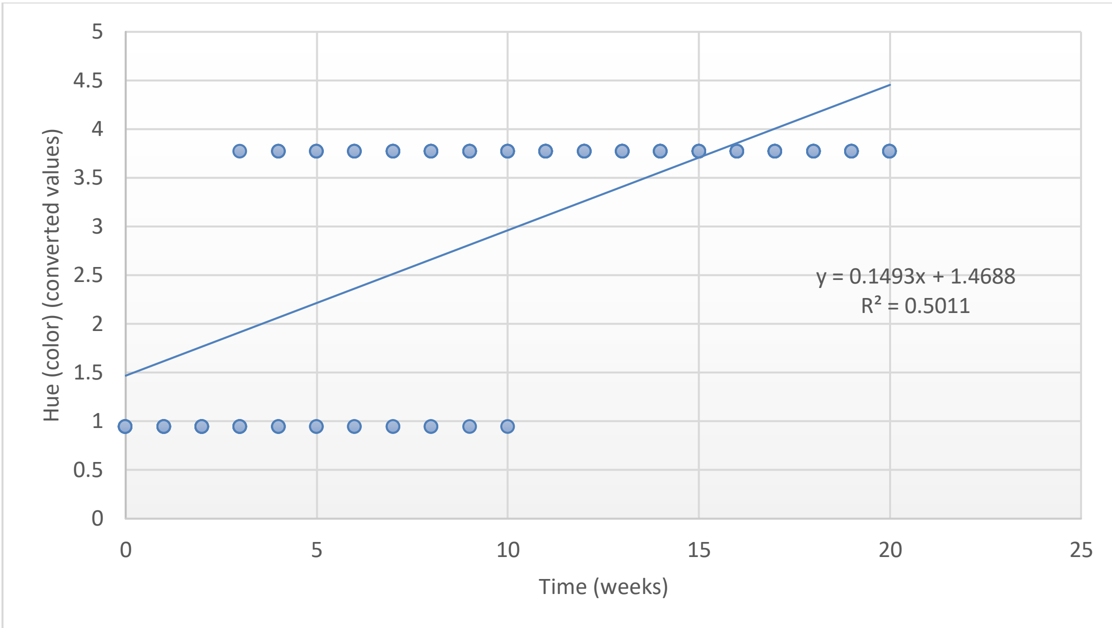

Figure 17: *Change in hue for Al samples over time*

Value was the quality between the two metals' staining that showed the most similarity in terms of the proportion of variability associated with time. Figures 18 and 19 portray the progression of value against weeks elapsed and a linear regression with the coefficient of determination. The percent of value variability explained by time is 39% and 26% for aluminum and copper, respectively. Because value is the light or dark quality of a hue, this indicates that throughout the study, the variations in this component of the discolorations were accounted for by time roughly equally. However, the overall trend for each of the metals was dissimilar: the value in the copper stains saw a decrease with time, meaning the colors got darker, whereas the aluminum values increased with time, indicating the colors got lighter. The staining from the copper pieces showed a wider range of hues, which could explain the overall lightening—for example, a transformation from a more yellowish hue with a higher value (lighter) to a more greenish hue with lower value (darker); although within each of their respective hues the value decreased, as a whole, the discoloration got darker when transitioning between yellow and green. This could be the primary reason the average rate of change in the copper chroma was negative. Conversely, the pattern for aluminum staining began with white, with a low value score (darker), then saw a slight change to a blue tint with a higher value (lighter). The overall change from darker to lighter in value was because of the transition across hues.

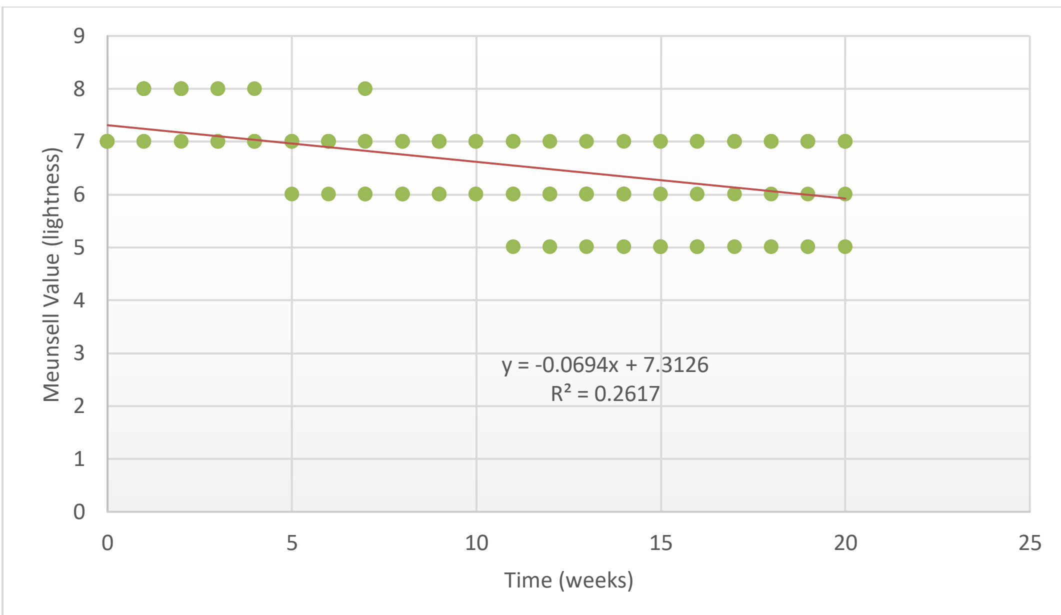

Figure 18*: Change in value for Cu samples over time*

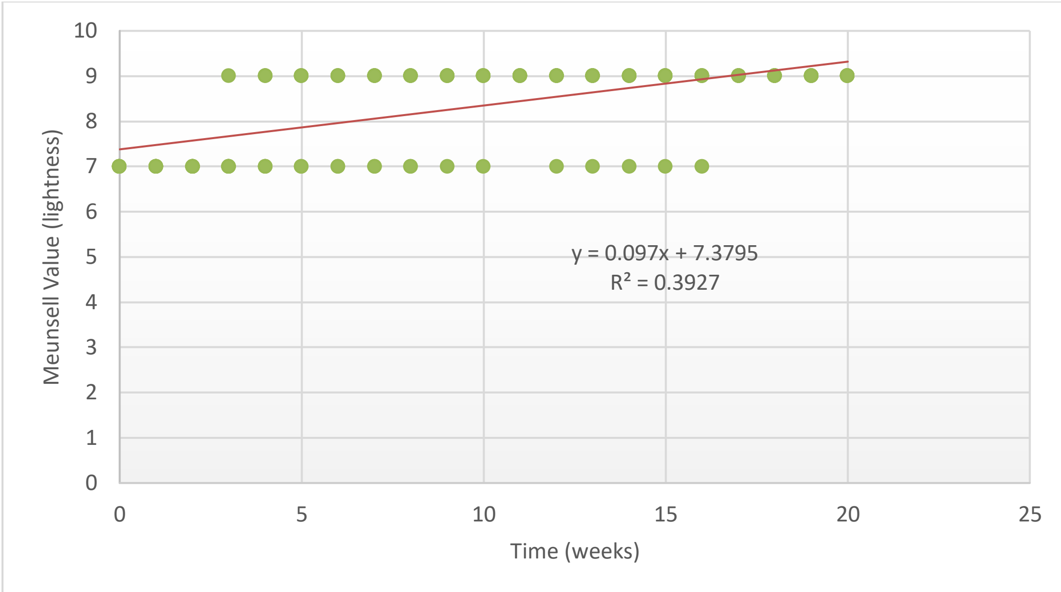

Figure 19*: Change in value for Al samples over time*

Similar to the value data between the two metals, the directional pattern of chroma scores were opposite, with copper becoming more saturated and aluminum becoming less saturated as the number of weeks increased. Overall, the chroma variability explained by time was 27% for aluminum and only 5% for copper. This phenomenon is comparable to the trends seen in the change in the staining values: the transitions between hues lead to shifts in overall intensity, wherein the color may progress from a more saturated lighter color to a less saturated darker color, or vice versa. As the aluminum staining saw shifts from white hues to bluer hues, the overall saturation decreased, whereas the copper staining developed more saturation as the hues changed from yellow- and gray-greens to more vibrant greens. Nonetheless, even though the copper discolorations exhibited a greater variety in chroma than the aluminum, the variance in aluminum can be better accounted for by time, as suggested by the R^2 numbers (Figures 20 and 21).

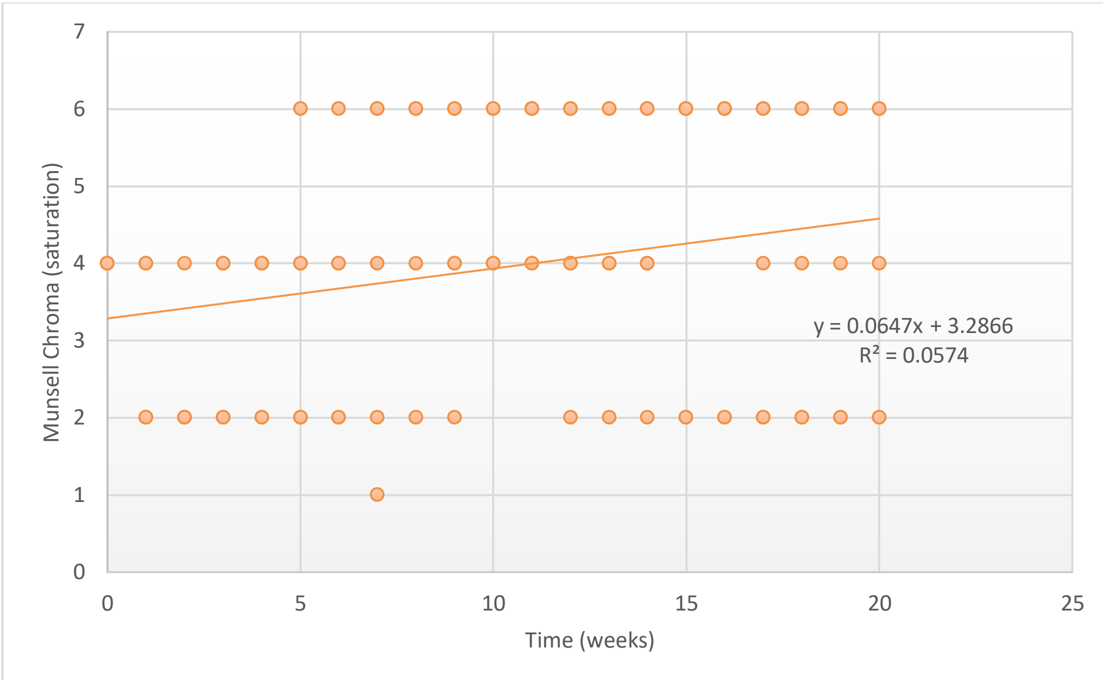

Figure 20: *Change in chroma for Cu samples over time*

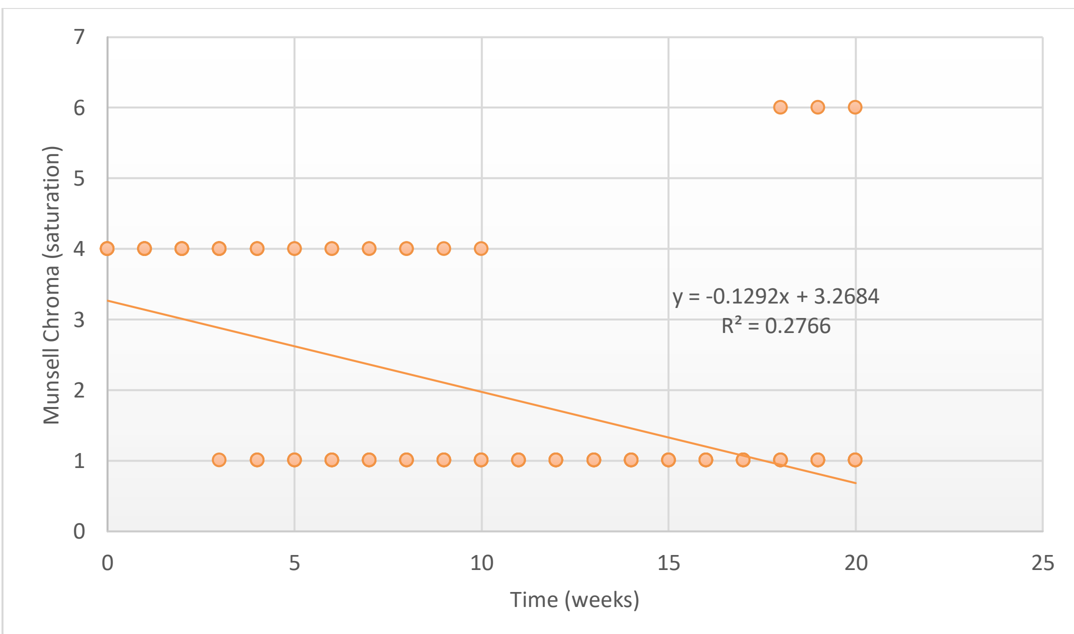

Figure 21: *Change in chroma for Al samples over time*

Due to the absence of observable staining from copper on Tibia 3 and 6 and from aluminum on Tibia 1, the data sets for these three groups were left out when plotting each of the scatter plots. The entries for each of these samples for the entirety of data collection was the initial bone color and including these numbers in the graphs would have resulted in a biased linear regression and coefficient of determination. One possible reason for the lack of staining in these three instances is the way in which the metal squares were positioned on the tibiae. In order to maximize the area of contact between the two surfaces, all metal samples were fastened to the proximal end of each bone. Yet, due to the natural curvature of the tibia on the proximal end, some metal samples needed to be pressed against a concave area of the bone. The metal pieces on concave tibia locations were not held as tightly to the bone with the cable ties as the pieces on convex areas. Staining, then, may be dependent on the amount of force or relative distance between the two surfaces in contact.

The range of time in which metal staining first appeared on the tibias varied between groups. More than half of the copper samples that produced discolorations did so after the first week of skeletal contact, with the other two copper samples first showing stains at week 2 and week 7. Aluminum staining began more sporadically, occurring between weeks 3 to 11 (Figure 22). The wide timeframe in which staining first appeared could be due to a myriad of reasons, including the extent of surface contact between the metal squares and the bone, the moisture content of the soil, the differences between the corrosion properties of the two metal types, or the location of corrosion on the metal pieces. A majority of the staining positioning on the tibias was along the edges of the metal contact, so a particular edge that was corroding may not have had enough contact to actually transfer any degree of color until later weeks. In addition, the soil conditions were fairly dry to begin with. The highest water content the soil possessed was 3 out of 10, and with time, the soil became dust-like in its dryness, measuring a 0 out of 10 on the moisture meter (Figure 23). Because corrosive reactions are driven by water and oxygen, this likely slowed down the oxidation that occurred on the metal pieces. Soil pH would not have affected the staining in this study, as the pH of the soil was 7 throughout the whole data collection period (Figure 23).

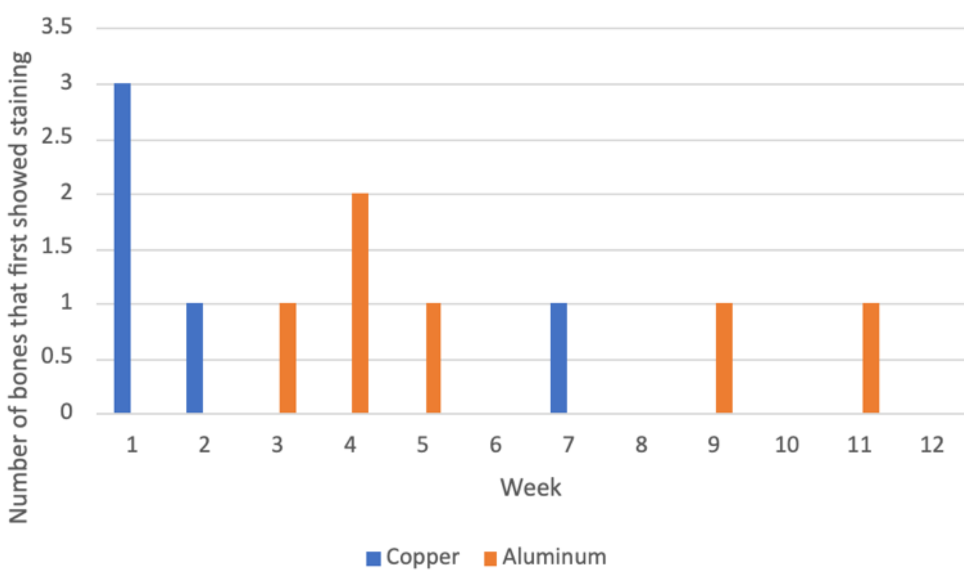

Figure 22*: First staining appearance from copper and aluminum by week*

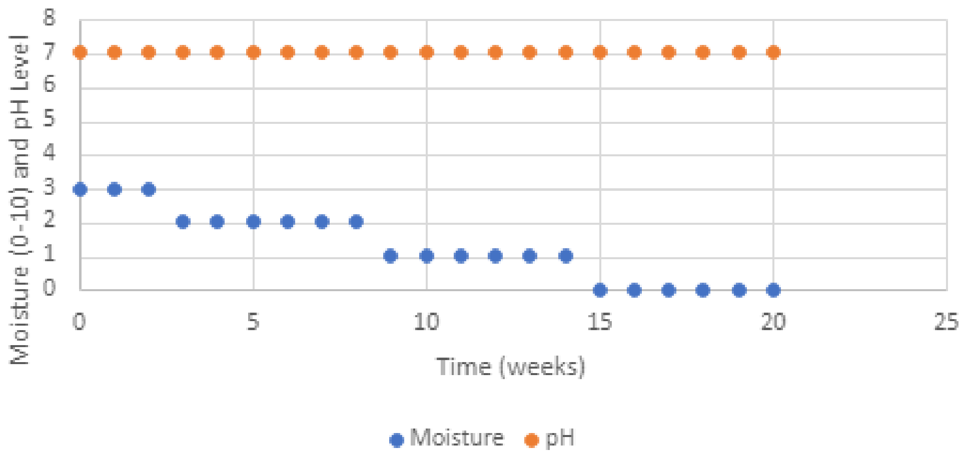

***Figure 23**: Change in soil moisture and pH conditions over time*

Table 1 shows several summary statistics between the aluminum and copper groups. The average week at which staining was first observed for copper was 2.5 weeks for copper and 6 weeks for aluminum, which suggests the onset of oxidative reactions for aluminum are typically much longer than copper. Such information may be helpful if aluminum or copper staining is utilized to estimate the postmortem interval; bones with aluminum staining can be estimated to have been in close contact with aluminum for at least six weeks past skeletonization, while copper-stained bones can be estimated to have been in close contact with metal for a minimum of two weeks. The average final *d* values differed between groups as well, with aluminum's at 0.33 and copper's at 2.95, which indicates that the copper squares produced a greater overall change in bone color by the end of the experiment. The average final degree of staining depicts a similar pattern: over the course of several months, copper tends to cause a greater extent of discoloration on bone from the products of oxidation. In the final week of observation, the average copper stains were scored between moderate (3) and heavy (4), but only faint (1) to light (2) under aluminum.

***Table 1**: Average copper and aluminum values for the week staining first appeared, the final d value of staining, and the final staining degree score*

	First Staining Appearance	**Final *d* Value**	**Final Degree of Staining (0-5 Score)**
Copper	2.5 weeks	2.95	3.6
Aluminum	6 weeks	0.33	1.8

In general, the implementation of the Euclidean distance equation to evaluate the change in color over time from metal stains is an effective method, provided the Munsell values from the first sign of staining are used, instead of the initial bone color. Yet, it is worth noting that the R^2 values calculated from the linear fits based on *d* values over time was much lower than the average R^2 for hue, chroma, and value when looking at aluminum, and nearly identical when comparing the same numbers for copper (Figure 24). Consequently, the use of *d* values to assess the change in color over time seems better suited for staining with a wider variety of Munsell values; the Euclidean distance equation does not account well for changes in color when such changes are circumscribed, as seen with aluminum staining. Aluminum created discolorations on bone that remained predominantly white throughout the study, whereas copper showed a mix of yellow, green, and gray stains. When the overall staining patterns display a greater range in hue, value, and chroma, using *d* values to determine the proportion of variation explained by time is comparable to separating each of the three Munsell qualities and calculating the R^2. But when skeletal staining is a color with inherently less variability, such as the whitish hue produced by aluminum, the correlation between *d* and time will potentially be greatly underestimated when compared to the average R^2 for the same set of data.

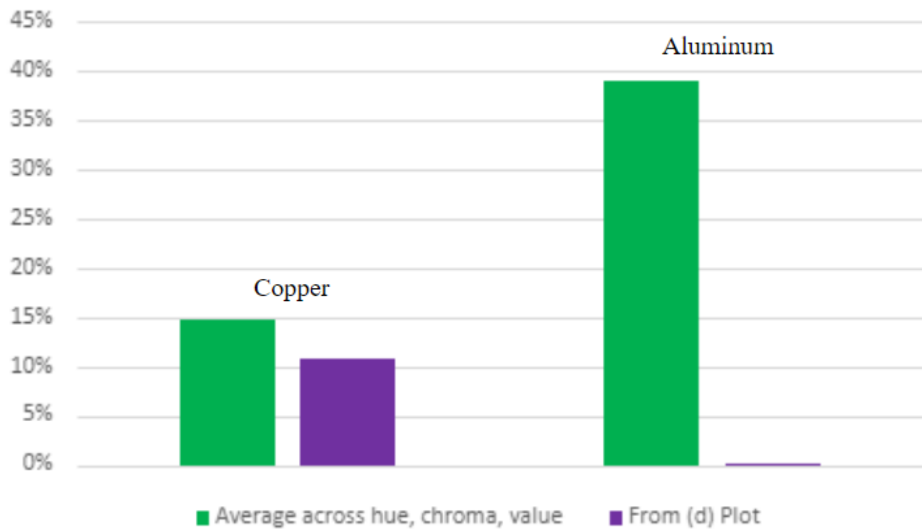

Figure 24: *Percentages of average R^2 values for the three Munsell qualities (hue, chroma, value) and R^2 value of the d values for the scatter plots of aluminum and copper*

When looking at the two metal types separately and comparing the three Munsell qualities' change over time with one another, one quality each for copper and aluminum was most correlated with time: for copper, it was the value (lightness), whose variation was most explained by time, at 26%, and with aluminum, it was hue at 50% (Table 2). However, as previously mentioned, the aluminum stains only saw a change in Munsell color twice, which is very low variability. Considering the residuals of the aluminum hue, chroma, and value plots, the bias factor is strong. Since the variability was small to begin with, the association between rate of change and time may appear larger than it actually is. However, data collection was only five months in the current study. Therefore, aluminum staining may produce far greater variation in color with an increased duration of contact with skeletal material.

Table 2: *Coefficients of determination (R^2) in percentages for each of the Munsell components' change over time for each metal*

	Hue	***Value***	***Chroma***
Copper	12%	26%	5%
Aluminum	50%	39%	27%

Overall, the results in the study underscore the idea that upon the discovery of skeletonized remains that have been stained by metal, copper would be expected to offer a more detailed contribution to the estimation of the postmortem interval, since copper staining is more evident than aluminum staining under similar conditions when viewed within a timeframe of around five months. The utilization of the Euclidean distance equation to quantify the change in color over time from metal stains is an appropriate method, provided the Munsell values from the first sign of staining are used instead of the initial bone color. Within a timeframe of five months of contact with either copper or aluminum, each resulting staining color on bone is distinguishable to the extent that identification of these colors suggest contact with the metal. The results also indicate a beneficial application to the estimation of time since skeletonization; the creation of a predictive formula to approximate time based on the observed staining color could be used to enhance the PMI estimate. Rates of change over time for each Munsell quality were calculated in each of the linear regressions, as well as for the change in *d* values, and they could be applied to construct a more general approximation equation.

Several limitations were present in this experiment. One of the most prominent relates to time; because data collection was only for five months, any changes in aluminum or copper staining color past that time frame were not evaluated, meaning any skeletal assessments garnered from the data in the present study are limited to a similar range of time. The sample size was another area of restriction. A total of 7 tibias were used, and not every sample yielded discoloration. Additionally, the human error component may have influenced the results to a certain extent. As the stain colors were identified visually using the Munsell charts, the possibility exists that the stains were not matched to their exact Munsell colors. Lastly, reduced staining may have been caused by the extent of surface area actually in contact with bone; the tibias possessed curvatures to their shape and pressing the metal pieces against each bone provided contact, but not across the entirety of each metal square.

A diverse scope of future, related experiments exist. Possible variables to change include testing different types of metals to compare staining colors; changing the soil conditions, such as pH, moisture, depth of bone burial; using bones with alternate morphologies or larger metal samples in order to maximize area of contact; or observing changes in discoloration for longer periods of time. One metal in particular that warrants skeletal staining data is iron, as this is among one of the most common types of metal discovered with osseous remains. Because iron corrodes in the form of rust, which is typically orange in color, skeletal discolorations from iron might likewise possess changes that are associated with time. The soil moisture conditions are another variable to examine more closely: changing the water content of the soil might produce different discoloration trends, and the data collected from such conditions could apply to various geographic regions with tropical or more humid climates. Regardless of the direction future studies choose to follow, the important aspect is growing these compilations of staining data. This experiment suggests that when metal corrodes in close proximity to bone, the resulting discolorations on the skeletal material have the capacity to contribute a meaningful analysis to the depositional environment and PMI.

CONCLUSION

The intent behind this experiment was to study the staining on skeletal material caused by extended physical contact with samples of pure copper and aluminum and to address the usefulness of the discoloration trends in death investigation evaluations such as the PMI and depositional environment. Based on the data collected, the changes in color over time for these inorganic stains are sufficient to impact such forensic assessments. Within the first five months of contact with either metal, the resulting stain colors are distinct from one another; identification of either blue-green or white colors on skeletal remains may suggest contact with copper or aluminum, respectively, and further analysis or testing might confirm contact with either. However, the transition between white hues caused by aluminum were minimal in comparison to the color changes across green, yellow, and grey hues seen by the copper stains. Consequently, the narrower range of color shown by aluminum staining may restrict its potential usefulness compared to copper staining within a forensic context.

The data suggest a positive impact on PMI estimation—at least, in the approximation of time since skeletonization. One option would be to develop a comprehensive predictive equation using the rates of change from the linear regressions for the *d* values, the individual Munsell qualities, or a combination thereof. For copper, the value, or lightness, was the color quality that changed most with time, but for aluminum, it was the hue. Therefore, another option would be to create a less comprehensive formula that focuses on a single quality for the corresponding metal type. From such equations, upon identification of skeletal staining from suggested aluminum or copper contact, the time since skeletonization could be evaluated. In such estimations, it is also important to factor in the relative onsets of metal staining after initial skeletal contact: aluminum's corrosion rate is slower than copper's, which can imply a longer initial period before the appearance of staining on bone.

In cases where the decedent goes undiscovered for months or even years, the time since skeletonization makes up a large portion of the overall postmortem interval and estimating that portion with increased accuracy would benefit the overall PMI. Although more inorganic staining data needs to be collected to augment these initial findings, overall, the changes in color produced by copper and aluminum on bone possess the capacity to benefit postmortem forensic assessments. As the collection of raw data expands for inorganic staining on bone, the potential impact on death investigations likewise expands.

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