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Organic staining on bone from exposure to wood and other plant materials

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ABSTRACT

Determining the depositional environment and the postmortem alterations to a set of remains are necessary aspects of a forensic investigation to explain the circumstances surrounding the death of an individual. The present study examines organic staining as a method for reconstructing the depositional environment of skeletal remains and the taphonomic agents with which they came into contact. Organic staining results largely from tannins leaching from plant materials and therefore can be seen on bone deposited in wooden coffin environments or on terrestrial surfaces.

The present study examines the hypothesis that the degree of staining observed on skeletal elements would increase as the length of exposure to the organic matter increased and that different plant materials and environments would leave different patterns or colorations of staining. The sample consisted of 165 pig (*Sus scrofa*) femora divided into four groups exposed to differing experimental conditions, including burial in direct contact with soil or burial in a simulated coffin environment, immersion in water with wood samples, and surface deposition with plant matter contact. The bones were removed once a month from their experimental environments and the level of staining was recorded qualitatively using the Munsell Soil Color Chart.

In all of the experimental environments, staining was present after two months of exposure, and the color darkened across the bone surface with each episode of data collection. The results from the present study indicate that staining can manifest on bone within a relatively short time frame once skeletonization occurs and a variety of colorations or patterns of staining can manifest based on the plant material. The present research also demonstrates the potential of organic staining to aid in estimations of the postmortem interval as well as a depositional environmental reconstruction through plant species identification.

1. Introduction

Analysis of color staining, as well as other taphonomic processes, is essential to reconstruct possible environments in which a set of remains was deposited. In both forensic and archaeological contexts, determination of the deposition interval, original environment of deposition, and the environmental factors that have affected the remains are main goals of a taphonomic analysis [1–6]. One source of postmortem alteration that can manifest on bone surfaces is organic and inorganic staining as a result of contact with natural or artificial materials. The coloration of fresh, defleshed bone is naturally a yellowish-white to beige color, which can then transform into various colorations based on exposure to sunlight, soil, water, plant matter, fire, or metallic materials [5,7–16].

Analysis of bone staining aids in the reconstruction of the depositional environment and helps identify the material that contacted the bone. Green algal staining, for example, suggests that the remains were placed in a moist, shaded, or underwater environment, while bleaching or pale colorations suggest long-term sun exposure [5,6,13,17–21]. A combination of staining agents can affect a single set of remains, which allows a reconstruction of the environment and the relative position within the environment. In circumstances where remains are deposited on the ground surface, bones may display darker staining on the underside from contact with the soil or decomposing organic materials underneath, while displaying

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lighter colorations on the uppermost surface from exposure to sunlight [17–20].

The purpose of the present research is to aid in the determination of the forensic significance of a set of remains, in which analyzing organic staining may allow the distinction of cemetery remains from those that are more recent and would be relevant to greater forensic investigation [22,23]. An additional goal is to determine the utility of organic staining for post-mortem interval (PMI) estimation. An archaeological application of the present research is the determination of the relative socio-economic status of a buried, deceased individual and the economic behaviors of a community when combined with other artifacts, including coffin hardware and grave goods. Determining the species of coffin wood utilized based on the bone staining may allow the estimation of the relative cost of the burial container [24–27] and the use of local or imported wood material [26,28].

2. Previous research

Staining that often manifests earlier in the taphonomic process may occur naturally from the decomposition of soft tissues or the rupturing of red blood cells, i.e., hemolysis [13]. Other kinds of staining can occur from contact or exposure to various environmental agents. Skeletal elements exposed to marine environments may display saltwater bleaching on the bone surface, while terrestrial surface remains can become bleached and weathered from exposure to ultraviolet light [5,7,8,20,21,29]. Bones buried in soil environments often display soil staining, with the color varying depending on the soil matrix. Those buried deeper are often lighter in color when compared to those in the A horizon (topsoil) or even the ground surface due to decreasing decaying organic matter with increasing depth [23].

Staining due to contact with organic matter often occurs as a result of tannins that leach out of plant materials and contact the bone surfaces [15,22,23]. Tannins are a secondary metabolite made by plants that aid in the precipitation of proteins from a solution [30–33]. Tannins are found in various portions of a plant, such as in the wood, roots, and leaves, but the amount of tannins within the plant varies based on the species [30,34]. All wood types are known to contain tannins, but woods lighter in color tend to contain a lower number of tannins than darker wood types [30]. Contact between tannins and a bone's surface is a primary reason for the color change from a normal beige color [13,35] to the brown-stained appearance from coffin wood or the green to tannish staining from plant matter [13,22,23,36].

If skeletal elements are exposed to plant matter on the ground surface, living plants can begin to grow on the porous material, or decomposing plant matter can leave staining on the bone surface [5,12,13,23,37,38]. Plant matter also can leave etchings or grooves on the bone surface due to the production of humic acid as a plant decays, which contacts the bone [5,6,8,9,12,13,20,23,37–41]. The wood material of a plant can cause staining to manifest on a bone surface after extended periods of contact. This staining is displayed on remains within wooden coffin burials from the organic tannins within the wood leaching into the groundwater that has pooled at the bottom of the coffin [15,23]. Other elements associated with the wooden burial container, such as coffin hardware or grave goods, may leave traces of inorganic staining upon archaeological osseous remains if contact occurs [42]. In both archaeological and forensic contexts, inorganic staining can result from contact with metallic objects, such as jewelry, that can stain the bone various colorations depending on the material used to manufacture the object [13].

2.1. Coffin wood

As previously stated, one plant component in which tannins are concentrated is the wood, which has commonly been utilized within the U.S. in coffin construction since the 17th century [26]. Early European settlers rarely used coffins to bury the deceased, and it was not until the colonies grew in size and wealth that simple coffins began to be utilized [24,26]. By the middle to late 1800s, metal caskets became popular to hinder grave robbing as well as the destruction of remains from soil or animals [42,43]. Even when the coffin styles shifted to an increased use of metal containers, wooden shipping crates were often utilized to protect the decorated metal coffin and were typically buried with the coffin inside to dispose of the cheap wood material [26,44]. The wealth of the family or the individual was often reflected in the choice of the wood utilized in the construction of the burial container [26,43,45]. Hardwoods and wood types imported to construct coffins were commonly of a greater expense than softwoods or those found locally [26]. Cemetery excavations throughout the United States have provided remnants of wood within the burial plots that were often identifiable to species [25,27,28,42,44,46–53]. Pine was utilized most often to construct burial containers possibly due to the inexpensiveness of the wood.

3. Materials/methods

The present study took place simultaneously at the Boston University Outdoor Research Facility (ORF) in Holliston, Massachusetts, and at the Boston University School of Medicine. The sample consisted of 165 commercially available pig (*Sus scrofa*) femora divided into four experimental environments (Table 1).

For three samples (Samples 1–3), the epiphyses of the femora were removed with a reciprocating saw in order to manually extract most of the fat residing within the medullary cavity. The skeletal elements were then completely de-fleshed through scraping and the use of dermestid beetles. Completely skeletonized bones were chosen for this portion of the present research due to the extended time necessary for decomposition of soft tissue to complete, allowing organic material to contact the bone directly. The bones were degreased, using an 80–20% dichloromethane and acetone solution to prevent or remove staining resulting from decomposition or hemolysis and then submerged in tap water to remove any blood surface staining that would interfere with the staining from the experimental organic material. Once the bones were cleaned, string was utilized to classify them into groups and to help differentiate between and within groups. Each set (in

Table 1
Sample breakdown for each experimental environment.

Sample environment	Sample number	Number of bones	Number of bones per material	Interval of exposure
Buried wooden boxes	1	40	4	January 2016–February 2017
Plant matter	2	20	5	April 2016–April 2017
Plastic containers	3	90	Wood: 8 (4 per box) Tannic acid/control: 5	April 2016–April 2017
Buried in soil	4	15	15	September/October 2013–2016
		Total	165	