ORIGINAL PAPER

Bone diagenesis in dry tropic forest necrosols

Timisay Monsalve-Vargas[1] · **David Arboleda**[1] · **Santiago Vélez**[1] · **Santiago-Alonso Cardona-Gallo**[2]

Abstract

Orobajo Cemetery, a contemporary community cemetery originally located in the floodplains of the Cauca River in Antioquia, Colombia, was exhumed and reinterred in a new site as part of the environmental and cultural protection program associated with the construction of the Ituango Hydroelectric Dam. Bioanthropological, biochemical, and soil sciences methodologies were used throughout the exhumation to recuperate the skeletonized remains of the deceased and evaluate the age and sex of the interred individuals. Individuals of both sexes and every stage of the life cycle were found buried at the cemetery, and their skeletal remains showed bone deterioration and destruction. Thus, the environment in which the deceased were buried was also studied, analyzing the biophysical and biochemical mechanisms of fungal biodeterioration and the chemical composition of the deterioration, as well as the soil of the cemetery. Social context and burial practices were studied to understand their relation to the soil composition in the cemetery. Samples were taken from 31 of the 180 skeletonized remains exhumed at the site. Polarized light microscopy and scanning electron microscopy were used to analyze the samples. The samples showed chemical and physical deterioration as well as fungal biodeterioration with visible loss of minerals, and biodegradation of the organic bone matter, resulting in the destruction of the bone. The study concludes that the high rates of bone deterioration and the resulting fragility of the skeletal remains are due to the burial environment in dry tropical forest soils and the biotic and abiotic agents through which biophysical and biochemical mechanisms produce diagenetic alterations at the microscopic level of the bone.

Keywords Burial · Bone matter destruction · Bone deterioration · Biodeterioration · Cemetery · Polarized light microscopy · Scanning electron microscopy (SEM) · Taphonomy

Introduction

Buried bodies are subject to the environmental conditions of the burial soil; chemical, physical, and biological effects differ depending on the type of burial deposit and the relationship between social mortuary practices and the natural environment which can affect biodeterioration processes and the subsequent degradation of the bone. Lawrence (1979), defines the term *fossildiagenese* as the "events that take place after the final burial of organic remains" (245). Biotic, abiotic, and even social factors produce effects, and the processes characteristics to mortuary practices influence the conservation or degradation of the bones after burials. When bone remains are found in highly degraded or fragmented states, with microstructural or biochemical alterations, it is difficult to carry out taxonomic identification (Greenlee and Dunnell, 2010). Moreover, the environments produced in post-mortem biogenic processes (Piepenbrink, 1986) make the reliability of the dating and the characterization of the bio-osteological, archeological, or forensic context more difficult. On the other hand, the objective of the article was to identify the alteration, not the causes of the said alteration.

In the northwest of Antioquia, a department located in the Central Range of the Colombian Andes, in the township of Orobajo, 180 ossified remains of individuals of both sexes and every life stage were exhumed from the community cemetery as part of the environmental and cultural restitution program associated with the construction of the Ituango Hydroelectric Dam. Bioanthropological, biochemical,

✉ Santiago-Alonso Cardona-Gallo
scardona@unal.edu.co

1 Grupo Estudios Biosociales del Cuerpo-EBSC, Departamento de Antropología, Universidad de Antioquia, Sede Medellín, Carrera 80-65-223, Apartado postal 050034 Antioquia, Colombia

2 Departamento de Geociencias y Medio Ambiente, Universidad Nacional de Colombia, Medellín, Colombia

and soil sciences methodologies were used to recover the remains, evaluate them for information regarding sex and age at time of death, and relocate them to a new burial site. The cemetery, located about 100 to 200 m away from the town's residences, is approximately 1,316m² in area. The bodies were buried in wooden boxes that later degraded like bone. Typically, the deceased were buried with articles of clothing and, in some cases, their blankets. Yet often, the bones of two individuals were found mixed together during excavation, a sign that burial sites were reused with a second body from a later period buried on top of the previous. The inconsistency of burial practices and records and the presence of unmarked tombs resulted in the cemetery's legal classification as "unconventional." The cemetery is a community and they do not have books, death certificates, or similar documentation that informs about the deceased buried there. Oral tradition provides the only information that was obtained.

The bone remains recovered in this study showed high levels of deterioration in organic and inorganic matter, resulting in the appearance of generalized destruction. The objective of this investigation was to explain the deterioration of the bony remains exhumed from dry tropic forest necrosol in a rhizosphere environment. The study showed that the generalized destruction of the bony microstructure was due to the abundance of fungi in the necrosol, which caused biogenic alterations, and the sand and gravel texture of the soil, which led to the diagenetic alteration observed in the bones. Temperature, high humidity, and microorganism accelerate the degradative reactions.

Burial environment

Climatic conditions, pH, temperature, and the morphological composition of the ground are all extrinsic factors that assist or codetermine the type of diagenetic alterations in bone matter (Gordon and Buikstra, 1981; Child, 1995a, 1995b; Fernández-Jalvo et al., 2010). *Taphonomy* studies process of fossilization that result from the burial environment. This environment is a complex structure of biotic, abiotic, and social phenomena, and their effects on the bone, which contribute to its conservation or deterioration. A holistic analysis of diagenetic alteration in bones requires evaluating thanatopraxic interventions (embalming or other preservation practices prior to burial), the deposition of the body, the depth of the tomb, and the container in which the body was interred, all of which affect the preservation of the buried bone.

The burial environment is structured in two levels: the atmosphere and rhizosphere. The rhizosphere, a zone of interaction between plant roots and the microorganisms in the soil, consists of two types of substrates: soil and bone. Depending on the environmental conditions, the soil will

present acidic or alkaline properties that, when added to the amphoteric property of water, vary the pH, which can play an important role in the elements present in the soil. The bioclimatic zone and humidity regimen will have similarly direct effects on the conservation or degradation of the bone; the presence of water and humidity makes chemical, biological, and biodegradable reactions more efficient (Nielsen-Marsh & Hedges (2000). The acidity or alkalinity of the soil is an abiotic phenomenon that affects the stability of organic and inorganic bone matter, accelerating or decelerating the hydrolysis of molecules like collagen (Gordon and Buikstra, 1981; Hanson and Buikstra, 1987; Nielsen-Marsh et al., 2007; Turner-Walker, 2008).

Vegetation in the bioclimatic zone interacts with fungi in the rhizosphere and soil microbiome, favoring the reproduction of fungus not only in the soil substrate but also in the bone. Moreover, meso or macro soil porosity favors the growth and dispersal of plant root hairs and the presence of microorganisms. Sand and gravel soils are highly porous, drain efficiently, and have little capacity for water retention. Easy drainage through the soil and mineralized bone tissue improves the preservation of the bone. If the soil has a slightly acidic pH, probability of the skeleton leaching minerals is increased, and assists in the fossilization of the bone, leaving the bone silhouette in the soil (Kendall et al., 2017; Turner-Walker, 2008).

Oxygen, water, pH, and temperature—and the cultural use of the burial ground—influence the type, stability, and competition of microorganisms in the soil. Necrosols, grounds used for human burial, lack natural layers in the soil. However, as they are filled with the deceased, they are rich in human-made matter like the remains of coffins and clothing articles, as well as bones, and thus have a high content of phosphorus, iron, and calcium, and other minerals (Vélez et al., 2019). Microbiotas are abundant in necrosols and, over time, they stabilize and adapt to the cemetery's biotope; microbiota in a synergetic relationship with biostratinomic agents like plants and animals contribute to the more efficient deterioration and biodegradation of bone and the human skeleton in a rhizosphere microenvironment (Turner-Walker, 2019; Vélez et al., 2019).

The high porosity of sand and gravel necrosols favors the gaseous phase of oxygen within the substrate, which improves the flow of oxygen, promotes the growth of fungi, and supports the presence of aerobic microorganisms and necrophagous insects. In turn, the presence of these microorganisms triggers oxidative processes in the organic bone matter (Bell, 1995; Child, 1995a, 1995b; Hanson and Buikstra, 1987; Pfretzschner, 2004).

Diagenetic alteration begins in the external zone of the bone that is in direct contact with the soil (Hackett, 1981; Kendall et al., 2017). Hanson and Buikstra (1987) showed that the presence of fungi results in two main diagenetic

changes: first, the organic matrix is decalcified, and second, the metabolic activity of the microorganisms affects the makeup of the bone tissue.

Fungal biodeterioration

Fungi are single-cell or multicell chemotrophs. Hyphae and mycelia are characteristic of multicell fungi. The cell wall of fungi incorporates substances solubilized through external enzymatic action by diffusion. This enzymatic secretion degrades organic matter and substrates of vegetable or animal origin, among other subproducts, that are later transported and absorbed by the microorganism. Metals like potassium, magnesium, iron, and calcium, as well as non-metals like hydrogen, carbon, nitrogen, oxygen, and phosphorus, are essential elements needed to construct or activate enzymes and maintain the growth of the fungi.

Bone is a substrate that has a nutrient and chemical composition that favors the metabolic activities of fungi by offering easy access to the elements and minerals needed for energy production. In relation with other microorganisms, fungi increase the absorption of nutrients from the substrate like in the mycorrhizae. Depending on the nature of the fungi complex, fungal growth and reproduction increases in high-temperature—between 25 and 40° C—bioclimatic zones when they do not have oxygen (Arenas, 2014; Ehrlich et al., 2008; Estrada and Ramírez, 2019).

Biophysical mechanisms of biodeterioration

Bioerosion is a term used to describe the general process of deterioration, or the change in the properties of hard-composition matter like bone, which can be caused by the action of microorganisms in the environment. Its principal effects are derived from the dynamic interaction between the biophysical and biochemical mechanisms that demineralize the bone—eliminate the inorganic part or the biominerals of the bone—and consequently decalcify the matrix.

Fungi mechanically wear down the bone and advance in a network-like manner; they adhere to the surface of the substrate and send thin hyphae through the cracks in the bone, penetrating the natural spaces where they establish new colonies. There, the fungi continue excavating microscopic galleries, and the bone is disintegrated by the thin hyphae that continue to grow even as they reach deeper into the interior (Child, 1995a; Sterflinger, 2000; Ehrlich et al., 2008; Ehrlich et al., 2009; Bindschedler et al., 2016; Gadd, 2017).

In the weathering or decomposition of rocks and mineral-based substrates, fungi dissolve the mineral, producing metabolic acids and complexing agents. The hyphae initially dissolve the mineral and produce metabolites through a biochemical mechanism that simultaneously gives the fungus the ability to mobilize metals for its reproduction, movement, and maintenance (Child, 1995a; Ehrlich et al., 2009; Fomina et al., 2010).

Biochemical mechanisms of biodeterioration

Buried bone is incorporated into the biogeochemical cycle through fungal action. Fungi assist in the decomposition of bone matter, transforming the organic matter of the bone and the metabolic subproducts that result into secondary minerals (Bindschedler et al., 2016).

The first biochemical process in biodeterioration consists of the dissolution of the hydroxyapatite, through the mechanisms of acid metabolites and complexing agents (Fomina et al., 2010). Fungi produce acid in the substrate, dissolving and solubilizing the apatite compounds present in the bone through leaching (Silverman & Muñoz, 1970; Ehrlich et al., 2009; Turner-Walker, 2019).

Metals and minerals in solution are moved between the soil substrate and the bone substrate through the process of diffusion. When the parts of the bone are separated through leaching or dissolution, a zone of high concentration of the compounds freed from the bone are generated to form cations or anions. In the soils of cemeteries, minerals like calcium, iron, and phosphorus are made available (Vélez et al., 2019). In these conditions, the metals and minerals available in the ground react with the cations and anions freed from the bone and the fungi serve as the mediators in reaction processes (Bindschedler et al., 2016; Gadd and Dyer, 2017; Rashid et al., 2016). Iron, through the process of chelation, can be transported on organic matter to form siderophores.

Another biochemical process of deterioration depends on the soil texture and its capacity for mineral infiltration. Through processes like percolation, soil particles can be absorbed into the bone. For example, when humic and fulvic acids are absorbed into the bone, they stain it and produce generalized deterioration due to the presence of functional groups in the humic substances (Hackett, 1981; Hollund et al., 2011). Like acids, ground metals and minerals like iron (Fe), aluminum (Al), potassium (K), and magnesium (Mn) can be mobilized and deposited in the buried bone (Lambert et al., 1984). Humates and amorphous organic matter, as well as metals and minerals, contain metabolic subproducts of ground and soil microorganisms that attach to the bone through the biochemical mechanisms of immobilization (Child, 1995a; Bindschedler et al., 2016). Fungi solubilize the phosphate crystals of bone calcium to extract complex proteoglycans and biodegrade organic bone matter. They also free enzymes, like collagenase, to biodegrade bone proteins (Child, 1995a; Ehrlich et al., 2009).

Social and environmental context

Bioclimatic zone

The Orobajo Cemetery measures 1316m^2 (7°01.671′N and 75°47.536′W) in the depression formed by the canyon of the Central Range of the Colombian Andes (Suter et al., 2011; Caballero et al., 2016) with a dry tropical forest ecosystem (Fig. 1). Annual rains fluctuate, though always less than 100mm, and there are three or more months of drought when the evapotranspiration potential is greater than the precipitation. The annual average temperature is reported to be greater than or equal to 17°C, with two dry seasons (Espinal, 1985; Pizano et al., 2014). The edaphic environment was characterized by an ustic humidity regime and isohyperthermic soil temperature regime of 22 °C.

Ground and soil properties

The cemetery in Orobajo had two distinct layers of natural soil with different morphologic characteristics. The first layer, the A_p layer, is a dark grey-brown color, whereas the second layer is a very dark brown color; the A layer was easily distinguished because of its different color. Both layers, however, were classified as Coluvic Regosols, referring to their colluvium-alluvial origins (IUSS Working Group WRB., 2015). A_p designates a superficial mineral horizon with structure and presence of humified organic matter mixed with the mineral fraction, which is also influenced by tillage or other human actions (Rojas 2009). The soil was a mix of sand and gravel with a granular structure and biogenic pores (Fig. 1). It is a sandy loam soil according to laboratory analysis.

In contrast, the burial grounds in the cemetery had a vastly different soil composition. The soils of the cemetery were a light yellow-brown color, with a mix of human-originated matter, sand, pebbled gravel, and rock fragments. In the lower layers, the soil of the tombs consisted of filling material mixed with buried human remains, wood fragments, nails from the coffins, and pieces of cloth derived from clothing articles. At the micromorphological level, the soil presented fine, medium, and thick compounds (Velez et al., 2019). The thick compounds included abundant angular mineral grains like fragments of K biotite (Mg,Fe^{2+}) (Al,Fe^{3+}) $Si_3O_{10}(OH,F)_2$. Likewise, the soil contained dark-colored humified organic matter and biogenic structures like coprolites and pedogenetic aggregates with colloidal organic matter in the sandy matrix of the soil (Velez et al., 2019). The biomass of the region was composed of dry tropic forest vegetation, creating a rhizosphere environment of 180cm deep (58–182 cm, Table 1) (Vélez et al., 2019) (see Fig. 1).

Chemical properties of the soil

The soils of the cemetery of Orobajo presented high values of effective cation exchange capacity and high concentrations of sulfur (S), mobile iron (Fe), and phosphorous (P). Concentrations of these minerals were higher in the lower layers of the cemetery, demonstrating a relationship between the depth of the tomb and the increase in S, mobile Fe, and P. The highest values of magnesium (Mn), copper (Cu), and zinc (Zn) also corresponded to the deepest tombs. The pH range of the soil in the tombs oscillated between slightly acidic and slightly basic (Vélez et al., 2019).

Cemetery characteristics

Located along the Cauca River, Orobajo is a 14- to 16-h mule ride away from the closest neighboring town, Sabanalarga, or an hour and a half by canoe along traditional river navigation routes. The population, highly impoverished, survives on fishing, marginal farming, and artisanal alluvial gold mining (Vélez et al., 2019). The poverty of Orobajo and its distance from the surrounding communities is reflected in the community's burial practices.

Ethnographic findings date the Orobajo Cemetery back to the middle of the twentieth century. Located about 150 m away from the residences, the burial grounds were demarcated by a circle of stones (Fig. 1). Many of the tombs, however, were unmarked. A space in the southwest section of the cemetery was dedicated to the burial of infants and children. Due to the community's burial and tomb-marking practices, the cemetery falls into the legal classification of non-conventional or irregular cemetery.

The cemetery offered no spaces for thanatopractice activities, and no special treatment was carried out on the bodies of the deceased before burial. In the depositional period of the cadaver of a recently deceased individual, the body was placed in an artisanal coffin made from the wood of old canoes, doors, or other pieces of wood found around the village. The cadaver was placed in the wooden coffin and then deposited directly into the ground (Fig. 1). Therefore, only small pieces or traces of wood were found around the skeletal remains during the process of excavation and recuperation. In some cases, wood from one coffin was found on top of another, illustrating the practice of burying a second person in the tomb of a previously buried individual at a later date (Vargas et al., 2022).

The deceased were typically buried with clothing and blankets. Sometimes, however, a recovered skeleton was found without any burial goods. During interviews conducted as part of the ethnographic research, community members stated that it was likely the exhumed body had been wrapped in a shroud of a cotton-based material called *coleta*, a material that disintegrates rapidly when it comes
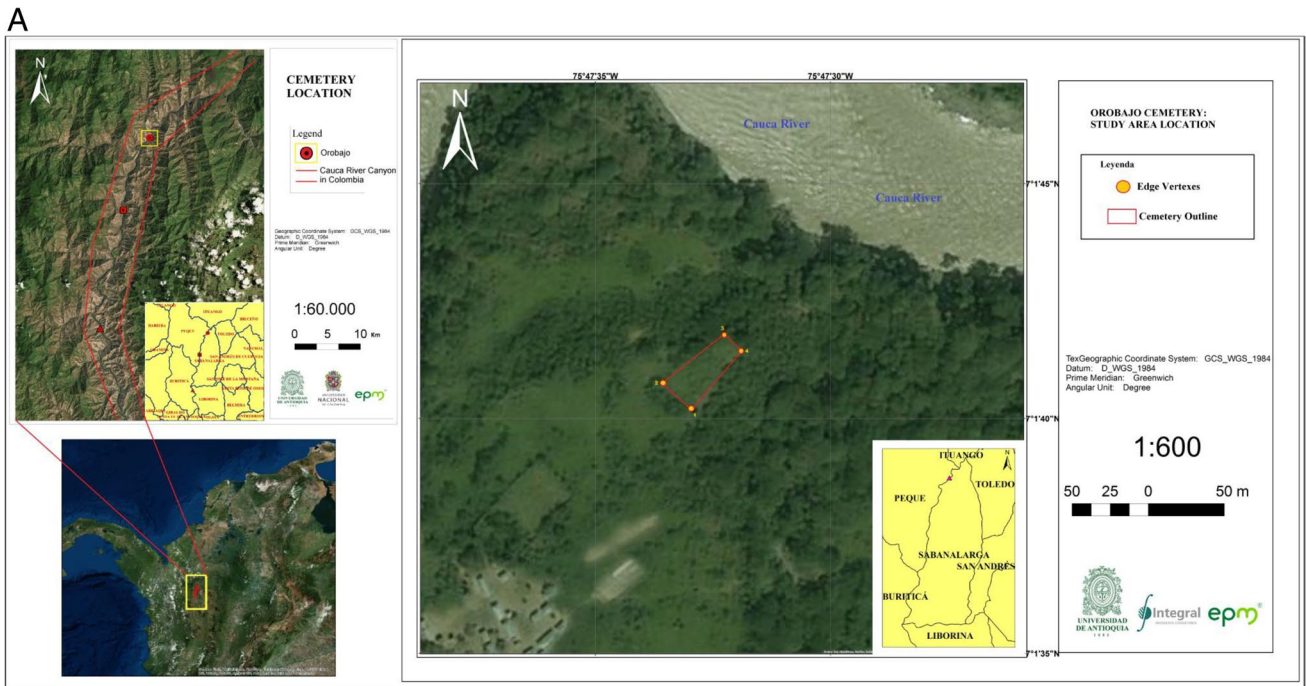
Fig. 1 The location of the cemetery in Orobajo, Colombia. **A** Map of the contemporary community cemetery in Orobajo, Colombia, located along the floodplains of the Cauca River. **B** The landscape context and Ap designates a mineral horizon

Table 1 Description of the sample

Code	Characteristics of the body				Characteristics of the tomb		
	Life cycle stage	Sex	Body position	Orientation	Depth (cm)	Wooden coffin or casket	Burial goods/shroud
ORO001	Subadult	Female	Dorsal decubitus	Southwest/northeast	81	Yes	Cloth, paper, aluminum
ORO002	Adult	Male	Dorsal decubitus	Southwest/northeast	119	Yes	No shroud or burial goods
ORO003	Adult	Female	Dorsal decubitus	Southwest/northeast	123	Yes	Articles of clothing, textile, paper, aluminum
ORO004	Adult	Male	Dorsal decubitus	Southwest/northeast	115	Yes	No shroud or burial goods
ORO014	Adult	Male	Dorsal decubitus	Southwest/northeast	110	Yes	No shroud or burial goods
ORO019	Adult	Female	Semi-flexed	North/South	116	Yes	Textile, round metal buttons
ORO020	Undetermined	Undetermined	Irregular	No orientation	130	No	No shroud or burial goods
ORO022	Subadult	Female	Dorsal decubitus	Southwest/northeast	120	Yes	Shoes and scraps of cloth
ORO030	Fetal	Female	Dorsal decubitus	Southwest/northeast	84	Yes	Articles of clothing and blanket
ORO033	Subadult	Male	Irregular	No orientation	130	Yes	No shroud or burial goods
ORO036	Adult	Undetermined	Dorsal decubitus	Southwest/northeast	110	Yes	Textile and plastic buckles
ORO041	Subadult	Male	Dorsal decubitus	Southwest/northeast	102	No	No shroud or burial goods
ORO046	Subadult	Undetermined	Dorsal decubitus	Southwest/northeast	120	Yes	No shroud or burial goods
ORO050	Subadult	Undetermined	Irregular	No orientation	98	No	No shroud or burial goods
ORO052	Adult	Male	Dorsal decubitus	Northeast/southwest	110	Yes	Clothing articles, blanket
ORO064	Adult	Undetermined	Dorsal decubitus	Southwest/northeast	90	Yes	No shroud or burial goods
ORO078	Subadult	Female	Dorsal decubitus	Southwest/northeast	135	Yes	Clothing articles, paper, aluminum, pillow
ORO091	Adult	Female	Dorsal decubitus	Southwest/northeast	110	Yes	Dental prosthetic, gold tooth crown, crucifix
ORO100	Subadult	Undetermined	Dorsal decubitus	Southwest/northeast	150	Yes	No shroud or burial goods
ORO102	Subadult	Female	Dorsal decubitus	Southwest/northeast	90	Yes	No shroud or burial goods
ORO103	Adult	Undetermined	Dorsal decubitus	Southwest/northeast	128	Yes	No shroud or burial goods
ORO106	Adult	Female	Dorsal decubitus	East/west	117	Yes	Clothing articles, quilt, textile
ORO110	Adult	Female	Dorsal decubitus	Southwest/northeast	114	Yes	No shroud or burial goods
ORO113	Adult	Undetermined	Dorsal decubitus	Southwest/northeast	110	Yes	No shroud or burial goods
ORO117	Adult	Male	Dorsal decubitus	Southwest/northeast	114	Yes	Clothing articles, textile
ORO122	Adult	Female	Dorsal decubitus	Southwest/northeast	110	Yes	Clothing articles, textile, quilt
ORO127	Subadult	Male	Dorsal decubitus	Southwest/northeast	86	Yes	No shroud or burial goods
ORO168	Adult	Female	Dorsal decubitus	Southwest/northeast	103	Yes	No shroud or burial goods
ORO173	Adult	Male	Dorsal decubitus	Southwest/northeast	125	Yes	Textile

in contact with water and soil. Information was obtained in the field by oral tradition.

The burials, for the most part, demonstrated a southwest to northeast orientation, with the head of the deceased orientated towards the mountains and the feet towards the river. The excavated skeletons were buried primarily in a supine position; those that demonstrated an irregular position belonged to secondary burials.

Tomb spaces were reused because the cemetery was extensively utilized. When tombs were reused, the remains of the first burial were not fully exhumed, and the bones of the previously deceased became part of the fill matter for the new tomb. Thus, during the excavation of the cemetery, bone pieces of previous burials were found separated from the full skeleton, buried on top of or next to the skeletonized remains of the more recent burial. These mixed bone remains were called *revueltos* (scrambles) by the community.

Social context

The community in Orobajo does not carry out practices for the preservation of the corpse after death. Mortuary practices consist of dressing the deceased in cotton textiles, placing them in coffins made from recycled wood and depositing them in tombs. Tombs consist of a lateral chamber dug into the ground, which are later demarked with temporal objects like vegetation and rocks.

Upon coming in contact with the ground, the wood used to make the coffin and the textile with which the body was covered rapidly disintegrate. Previous archeological research shows that in dry soils with little water retention, the deterioration of wood is caused more by the action of fungi than by bacteria (Björdal and Nilsson, 2002). In general, fungi generate a more accelerated and complete decomposition than bacteria (Björdal and Nilsson, 2002; Huisman and Klaassen, 2009), reaching speeds as much as 100 mm per year (Huisman and Klaassen, 2009).

In contrast, textile conserves fairly well in water-soaked contexts or in extremely dry environments like the deserts of Egypt. However, in temperate climates, where the soil is damp, the air humidity is relatively high and there is year-round precipitation, cloth disintegrates rapidly in the presence of bacteria, and, even more so, there is fungi that quickly degrade the material, turn it brown, and cause it to lose its integrity and disappear (Huisman et al., 2009).

Materials and methods

Sample

From a total of 180 skeletonized bodies exhumed from the Orobajo Cemetery, bone samples were taken from 31 individuals of both sexes in all stages in the life cycle: fetal, subadult, and adult. Table 1 details the characteristics of the exhumed bodies and tombs.

Methods

The samples were collected in sections of 3 to 5 mm with a Marathon brand micromotor, with an external support of distilled water. The samples were then prepared for polarized light microscopy and analysis. First, the bone was embedded in polyester resin and cured for 24 h. Then, the resin was refined using a microcutter (Metkon, microcut 152) and a diamond jewelry saw, polishing and buffing the samples until they reached a thinness of 30 and 40 microns (Metkon, Forcipol 102).

For the scanning electron microscopy (SEM), the samples were washed in 70% ethyl alcohol, and embedded in polyester resin, and the polished fragments were brought to the microscope for SEM. The analysis was carried out using a high vacuum JEOL JSM 6490 LV equipment (Universidad de Antioquia). Data was gathered in regard to four categories: presence and type of microscopic focal destruction, presence of the matter from inclusion and infiltration, and the intensity of birefringence (Jans, 2005). The methods employed for studying destructive changes to bone caused by microorganisms, infiltrations, and staining after death

were taken from studies by Garland (1989, 1993), Hackett (1981), and Hollund et al. (2011, 2018).

The Oxford histological index (OHI) represents the amount of bone preserved; its value of OHI was calculated according to the method proposed by Hedges and Millard (1995:203). The intensity of the birefringence was evaluated in accordance with the scoring system proposed by Jans (2005:348): 1 denotes normal intensity, 0.5 reduced intensity, and 0 signifies obliterated. The topography and morphology of the generalized destruction was described with the terminology used by Garland (1989: 223–226) and Hollund et al. (2018:8). For the description of the alternation, each bone sample was divided in periosteal circumferential laminar, interstitial laminar, osteon laminar, and endosteal circumferential laminar.

Light microscopy was used with the objective of evaluating the type of destruction in the microstructure of the cortical bone and its histological characteristics, the staining of the material, and the intensity of birefringence in the organic matter of the bone. SEM was employed to evaluate the topography and the structure of the deterioration, to characterize the micro-morphologic composition, and to identify the chemical composition of the material of inclusion and infiltration.

Sand and gravel soils have a high capacity for infiltration and percolation of water. This characteristic of alteration has been associated with minerals, dragging and weathering, and the humic substance at the deposit site that infiltrates and deposits over the bone tissue and penetrates its microstructures as such as lacunae, resorption cavities, osteon canals, and Volkmann canals in all levels of the periosteal, interstitial, osteon, and circumferential endosteal lamellar bone (Garland, 1989; Hollund et al., 2011; Jans et al., 2002).

Results

In the process of exhumation, the destruction and loss of integrity of the bones in the skeletonized remains was noted at a macromorphological level. The range of depth of the tombs was between 62 and 152 cm in a rhizosphere environment. Then each of the bones of the exhumed skeletons (Fig. 2, OR0002) were analyzed using microscopic techniques to evaluate the macroscopic and the qualitative characteristics of the degradative change in the bone tissue and to identify the mechanisms that produced said change. A summary of the diagenetic changes in given in Table 2.

Characteristics of histologic deterioration

The topography of the bone of the analyzed sample presented two types of superficial morphology and relief. The first type of morphology observed in the sample was
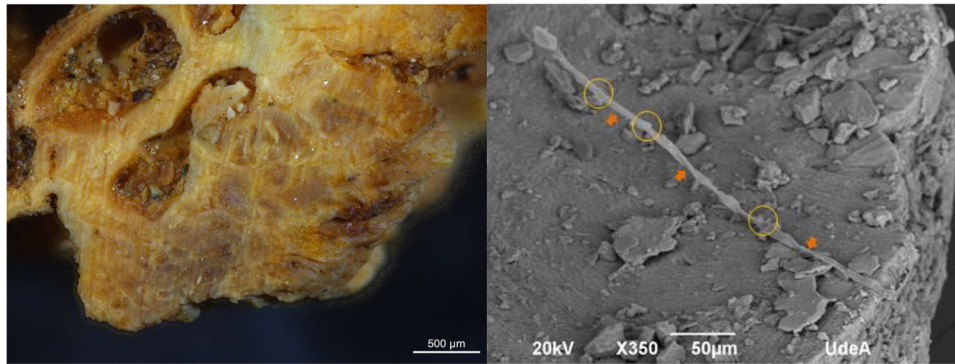
Fig. 2 ORO002: Recognizing the macromorphological destruction and loss of integrity of each bone in the exhumed skeletonized remains. Sample ORO002 presents a regular bone topography without apparent massive destruction, but with signs of metabolic pro-duction and mineral neo-formations like calcium crystals (circles) attached to hyphae (arrows), which illustrates the originary relationship between the hyphae and the crystals

anfractuous, rough, and crumbling with topographic alteration. The second type of morphology presented consistent topography and regular texture in the cortical and circumferential periosteal lamellar bones, but when exploring deeper in the bone, towards the interstitial lamellar and osteon, it was falling apart (Fig. 3, ORO091). The bony micro-taphonomy of the sample corresponds with the generalized destruction and the loss of microstructural characteristics of the bone tissue and the amorphous appearance that has already been described in the literature (Garland, 1989, 1993).

Inclusions

The material included in the bone sample from Orobajo is within that already characterized by Garland (1989) as non-osseous derived material between the available bone spaces. It is comprised of organic matter like fungi and plant roots or inorganic matter like minerals and their compounds. Consequently, in a rhizosphere micro-environment, roots in the soil substrate perforate and penetrate the bone matter, leaving behind root extensions with alveolar micromorphology rhizomes that deteriorate the cortical and trabecular bone (Fig. 4, ORO002.3). As noted previously, the stable microbiota in cemetery soil act in synergistic relation with this microenvironment.

Biodeterioration

Fungi is reproduced on the surface of the bone substrate. The sample ORO173 (Fig. 5) presented spherical particles, 7μ in diameter. Because the morphology and size correspond to that of spore bodies, the finding gives evidence of fungal reproduction on the bone. Furthermore, hyphae are present in the bone substrate. Young hyphae present homogeneous tubiform filaments and fixated rhizoids with appressoria that adhere to the surface of the bone (Fig. 6 ORO002.7). The biometry of the hyphae was viable, reaching a size of approximately 12 μ in diameter inside the tubiform filament (Fig. 7 ORO004.3). The sample showed all the states in the life cycle of the fungal microorganisms. In the oldest zones of fungal expansion, the remains of aged hyphae were found, with smooth surface textures and protruding hexagonal and rhombohedral microstructures with possible organic matter covering the surface (Fig. 8 ORO052.1). Sample ORO002 presented a regular bony topography without apparent mass destruction, but with signs of metabolic production and mineral neoformation like crystals attached to the hypha, which illustrates how the hyphae created the calcium crystals (Fig. 6).

Inclusion and infiltration of ground matter and minerals

Due to the type of soil of the burial environment of Orobajo, all of the samples showed staining or pigmentation in the range of colors from red-yellow, light brown, brown, dark orange, ochre or rust, with high levels of pigment saturation on the material, which has the effect of deteriorating the bone (Fig. 9 ORO003.1).

Under light microscopy, dark amorphous stains were observed. The staining seen in the osteons, the Haversian canals, and the lacunae generated the appearance of greater thickening of the bone and alteration of the normal morphology. The staining was greatest in the periosteal circumferential laminar bone and in natural bone cavities like the osteons and lacunas (Fig. 10 ORO014). Minerals like iron and magnesium precipitated into the bone. The round agglomerations with translucent borders were indicators of staining caused by the presence of iron (Fig. 11 ORO102).

Table 2 Diagenetic changes

Code	Type of bone	BI	BI structure	Destructive changes	Infiltrations and inclusions	Type of microorganism
ORO001	Rib	N/A	N/A	Generalized	Microorganism, plants. Minerals. Staining.	Fungi
ORO002	Femur	N/A	N/A	Generalized	Microorganism, plants. Minerals. staining	Fungi
ORO003	Rib	N/A	N/A	Generalized	Microorganism. Minerals. staining	Fungi
ORO004	Ulna	Obliterated	Cortical	Generalized	Microorganism. Minerals. Staining.	Fungi
ORO014	Humerus	Reduced	Cortical	2	Minerals. Staining.	Fungi
ORO019	Radius	Reduced	Periosteal, interstitial	2	Staining	N/A
ORO020	Femur	Obliterated	Cortical	Generalized	Staining	N/A
ORO022	Radius	N/A	N/A	Generalized	Minerals. Staining	
ORO030	Femur and rib	Obliterated	Cortical	Generalized	Staining	N/A
ORO033	Femur	N/A	N/A	Generalized	Minerals. Staining	Fungi
ORO036	Femur	Reduced	Periosteal	4	Staining	N/A
ORO041	Radius	N/A	N/A	Generalized	Microorganism. Minerals. Staining.	Fungi
ORO046	Humerus	Obliterated	Cortical	Generalized	Staining	N/A
ORO050	Tibia	Obliterated	Cortical	Generalized	Microorganism. Staining	Fungi
ORO052	Rib	N/A	N/A	Generalized	Microorganism. Minerals. Staining.	Fungi/bacteria
ORO064	Ulna	Normal	Periosteal	3	Minerals. Staining	N/A
ORO078	Tibia	Obliterated	Cortical	Generalized	Staining	N/A
ORO091	Parietal	N/A	N/A	Generalized	Microorganism. Minerals. Stain	Fungi
ORO100	Humerus	Reduced	Periosteal, osteon.	4	Microorganism. Minerals. Stain	Fungi
ORO102	Humerus	Reduced	Periosteal	4	Minerals. Stain	N/A
ORO103	Ulna	N/A	N/A	Generalized	Microorganism, plants. Minerals. Stain.	Fungi
ORO106	Radius	Obliterated	Cortical	4	Minerals. Stain	N/A
ORO110	Femur	Normal	N/A	4	Stain	N/A
ORO113	Humerus	Normal	N/A	5	Stain	N/A
ORO117	Ulna	Obliterated	Periosteal, osteon.	3	Stain	N/A
ORO122	Humerus	Obliterated	Periosteal, osteon.	4	Stain	N/A
ORO127	Humerus	Obliterated	Cortical	Generalized	Microorganism. Minerals. Stain	Fungi
ORO168	Ulna	Obliterated	Cortical	Generalized	Stain.	N/A
ORO173	Femur	N/A	N/A	Generalized	Microorganism. Mineral. stain	Fungi

BI birefringence (part of structure affected)

Fig. 3 ORO091: The second type of sample had consistent topography and surface morphology in the cortical (**A**) and the periosteal circumferential laminar bone. Nonetheless, the interstitial bone and osteon laminar bone were crumbled (**B**) (BF photo 40×, SEM micrograph 2800×)
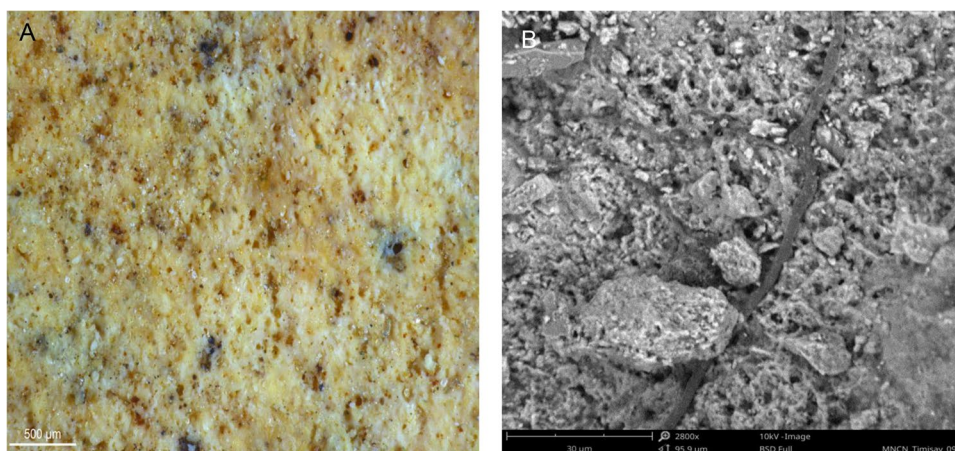
Fig. 4 ORO002.3: In a rhizosphere microenvironment, roots (1, 3) from the soil substrate perforate and penetrate the bone matter, sending extensions (2) with alveolar (3) rhizome morphology out into the bone, which deteriorates the cortical and trabecular bones
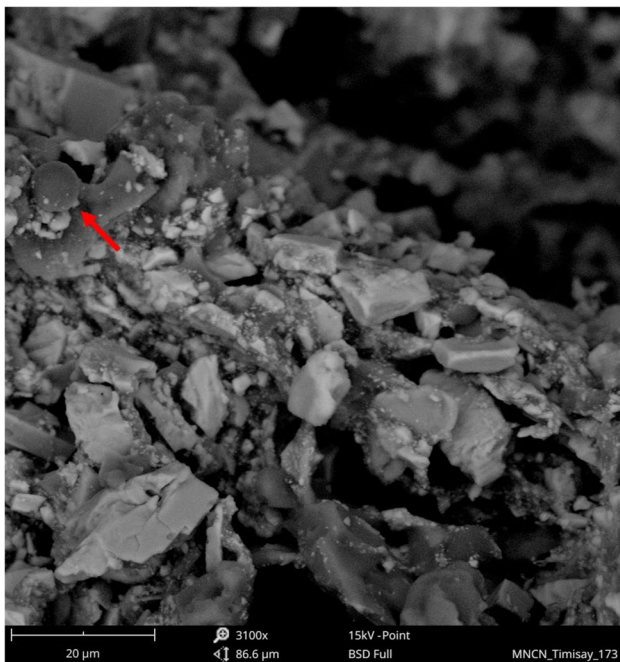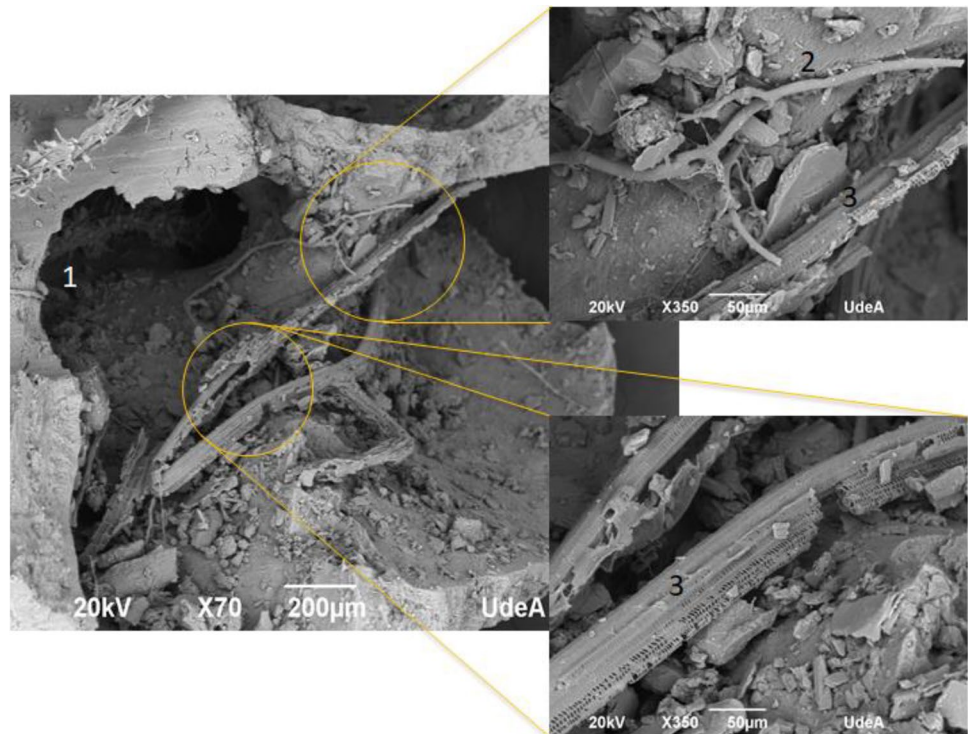

Fig. 5 ORO173: Fungi reproduce on the surface of the bone. Small spherical particles of 6.5-μm diameter were found in the sample. Due to their size and morphology, they are thought to be spore bodies. (Emmons et al., 2020)

Fig. 6 ORO002: Young hyphae have homogeneous tubiform filaments and fixation rhizoids with appressoria that adhere to the bone surface

Chemical composition of the deterioration

Due to the processes of percolation, granular exogenous mineral compounds were incorporated into and replaced the matrix of the bone matter, as Garland (1989) stated. Compounds such as those derived from silicon, silicon dioxide, wollastonite, and albite, and others like pyrite, potassium bromide, alumina, and indium arsenide, were found. Table 3 shows the data about the chemical composition in the energy-dispersive X-ray analysis of the samples and the wide variety of elements and exogenous chemical compounds found in the bone as a result of precipitation. Likewise, the same occurs with the compounds that are metabolic genomic subproducts of fungi like Prokaryotic Genome Annotation Pipeline
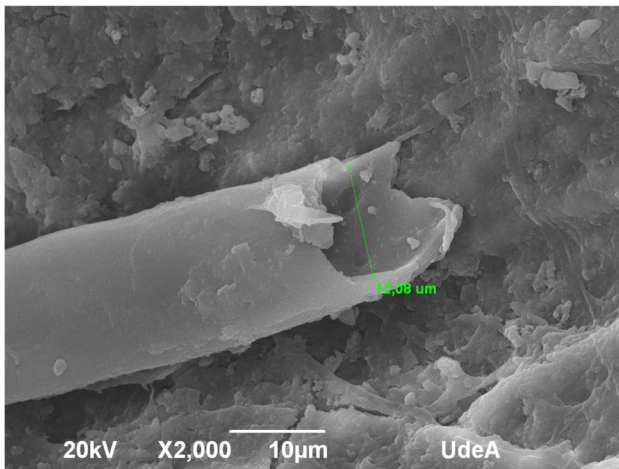
Fig. 7 ORO004.3: The biometry of the hyphae varied, reaching a size of approximately 12 μ in internal diameter in the tubiform filament. Microorganisms found in the sample corresponded to all life stages of the fungus

(PGap) and secondary mineral compounds like calcium carbonate (Fig. 12 ORO002.1). See Table 3 for a description of the compounds of inclusion and infiltration.

Degradation of organic matter

The presence of organic matter in the bone was evaluated using birefringence to evaluate the orientation and the intensity of the collagen fibrils. The samples presented degradation of the organic matter in the bone. This degradation was expressed in the loss of the orientation pattern of the collagen fiber and/or the reduction of the intensity of the birefringence. The interstitial and/or endosteal bone was less affected in terms of loss or degradation of organic matter, as can be seen in Table 2 of the diagenetic change (Fig. 13 ORO122).

Discussion

The burial environment is a system of dynamic interactions on two levels: atmosphere and soil. In tropic zones, the soils are more efficient in the degradation of organic and inorganic matter, thus skeletonized remains degrade more rapidly in these zones than in temperate or cold zones (Kendall et al., 2017; Turner-Walker, 2008). In dry tropic forests, chemical and physical interactions take place in the rhizosphere, which can reach great depths in the soil. As a result, the community of microorganisms will drive the type and speed of diagenetic alteration that occurs in the bone after burial. In a rhizosphere environment, the microbiome is the first and most important element in the production of bone diagenesis (Hedges, 2002).

According to Hedges (2002), microbial attack and the production of early diagenesis is not linked to the time of burial, but rather with the environmental conditions. The rate of deterioration changes in accordance with the soil properties. Sand and gravel soils accelerate the degradation of bone, making them more crumbly and porous (Hedges, 2002), like those excavated from the cemetery at Orobajo. Because sand and gravel soils are highly porous, have little or no water retention capacity, and drain efficiently, microorganisms reproduce quickly in the presence of oxygen, aerobic conditions, and adequate humidity (Hanson and Buikstra, 1987).

The analysis of the bone samples found microorganisms from drained soils (Hedges, 2002): terrestrial fungi like Rhizopus and Mucor that cause the loss of organic matter and the deterioration of collagen through the mechanisms explained

Fig. 8 ORO052.1: In the zones of the fungus' earliest expansion, the remains of aged hyphae were found (schematized drawing). They had smooth surface textures and hexagonal and rhombohedral protuberances, with the presence of fluorinated apatite or flourine- and carbonate-enriched apatite from the synthesis of bone bioapatite (circles). (Mowna Sundari et al., 2018; Emmons et al., 2020)
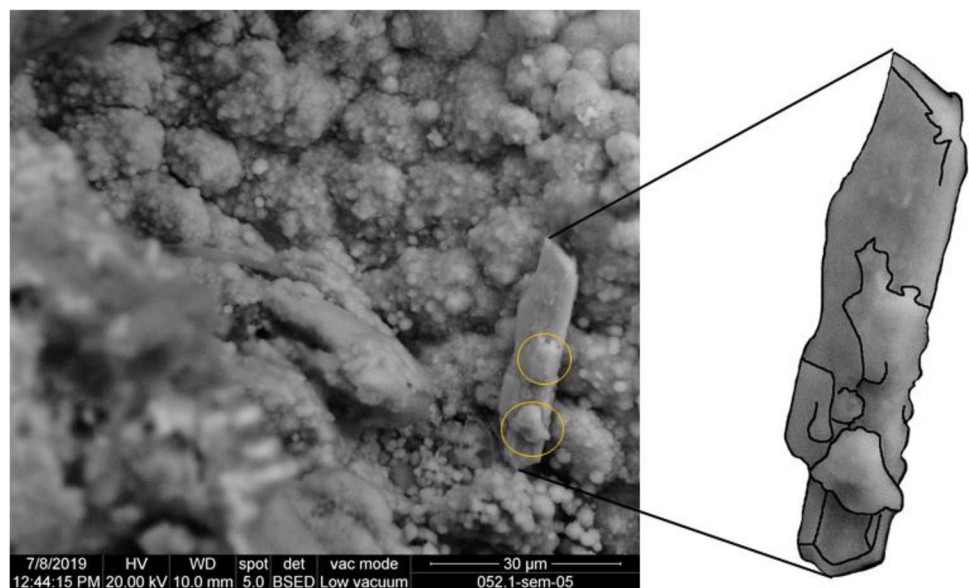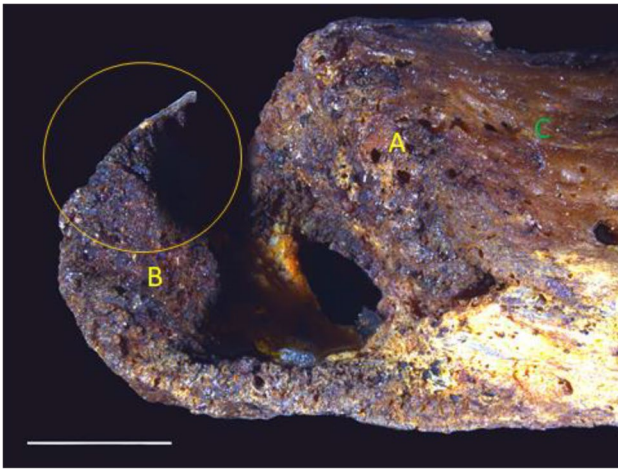
Fig. 9 ORO003.1: Discoloration, staining, and pigmentation were observed in a range of colors from yellow-red (A), to brown, dark (B) orange, ochre, or rust (C). The high levels of pigment saturation in the bone proved to be dark amorphous staining and deterioration when analyzed under light microscopy (circle). Scale bar 2cm

by Marchiafava et al. (1974). The bone matter exhumed in the present study appeared complete in the ground, with structural integrity. However, when removing the bones from the soil, they fractured, presenting the porous and crumbly morphology of matter with postmortem taphonomic alteration, with

off-white and irregular borders. The alterations were evaluated through an analysis of birefringence, where the decrease in its intensity was observed. Additionally, the hydroxyapatite crystals showed an accelerated and extensive loss of orientation (Gebhardt, 1906; Weiner et al., 1997; Weiner et al., 1999), losing of the Malta cross shape and the lines parallel to the axis of the periosteal circumferential laminar bone.

Fungi recycle organic matter and produce metabolic products like organic acids, which precipitate secondary minerals like calcium carbonate oxalates into the bone substrate. These minerals are then deposited in the ground by leaching processes or remain immobilized in the destroyed bone tissue, like microaggregates of calcium carbonate or organic matter (see Fig. 8 ORO052.1). The fungus mobilizes the minerals that are microaggregated in the soil substrate, producing siderophores of coordination and complexation or chelators (Bindschedler et al., 2016).

The formation of chelators by microorganisms implies that the ions or molecules are joined with the metallic ions like calcium. As a result, the chelators change the concentration of calcium in the bone matter. Normally, chelators are organic molecules, proteins, polysaccharides, and/or nucleic acids produced by the metabolic processes of microorganisms (see Table 3). In addition to the aforementioned processes, the accelerated biodeterioration of bone is also caused by the deposit of secondary minerals and microaggregates produced by the fungus and the mobilization and

Fig. 10 ORO014: Pigmentation was evidenced with greater saturation in the periosteal circumferential lamellar bone (**A**) and in natural bone cavities such as osteonals and lacunae (arrows **A**). SEM micrograph shows loss of bone morphology (**B**); only few lacunae were visible (inset **B**). BF and SEM microscopy show the extent of bone deterioration (**A**, **B**, inset **B**)
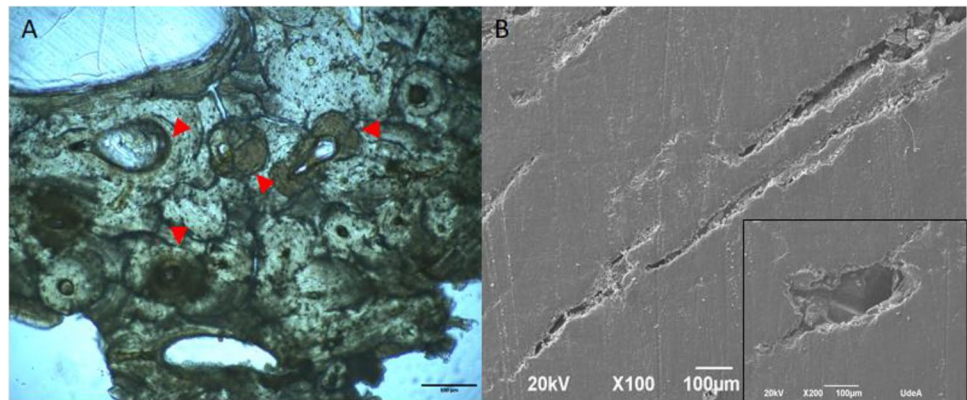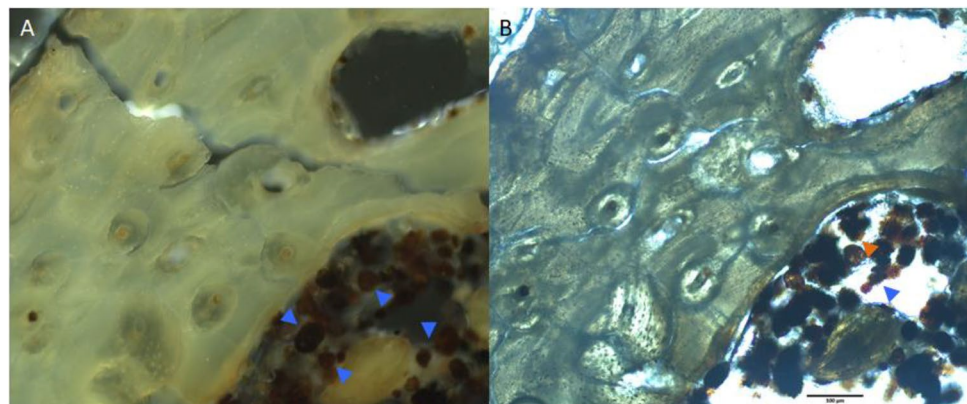

Fig. 11 ORO102: Minerals like iron (Fe$^+$) and magnesium (**A**, blue arrows) precipitated into the bone and formed round agglomerations with translucent borders, an indicator of pigmentation due to the presence of iron oxide (arrows **B**). Both images are the same sample. Image **A** was taken in a dark field with reflected light, image **B** in a bright field

Table 3 Chemical composition of the samples

Code	Elements	Endogenous compounds			Exogenous compounds			Metabolic products
		$CaCO_3$	MgO	P_2O_5	SiO_2	Ca-Wollastonite $CaSiO_3$–ECa_3 $[Si_3O_9]$	Others	PGap $C_3H_7O_6P$
ORO001	C, O, Ca, P, Mg, Si, Br, Al, Fe, Na	Yes	Yes	No	Yes	Yes	KBr, Al_2O_3, $NaAlSi_3O_8$	Yes
ORO002	C,Ca,O,Mg, Al,Si, P, K, Ti,Fe, As, Br, Mn	Yes	Yes	No	Yes	Yes	Al_2O_3, $KAlSi_3O_8$, KBr, InAs, $NaAlSi_3O_8$	Yes
ORO030	Si, P, Ca	No	No	No	Yes	Yes	No	Yes
ORO033	C,O, Ca, P, Na, Mg, Al, K	No	Yes	Yes			CaO, Na_2O, Al_2O_3, K_2O	No
ORO041	C, O, Mg, Na,P, Ca, Si	Yes	Yes	Yes	Yes	Yes	$NaAlSi_3O_8$	Yes
ORO052	C, O, Na, Mg, Si, P, S, Ca, Fe, Pt	Yes	Yes	Yes	Yes	Yes	$NaAlSi_3O_8$, FeS_2	Yes
ORO173	O, Ca, Ti, P S, Si, Na, Mg, Mn, K, Y, Al							

The blank cases correspond to non-acquired information from the microscope scans
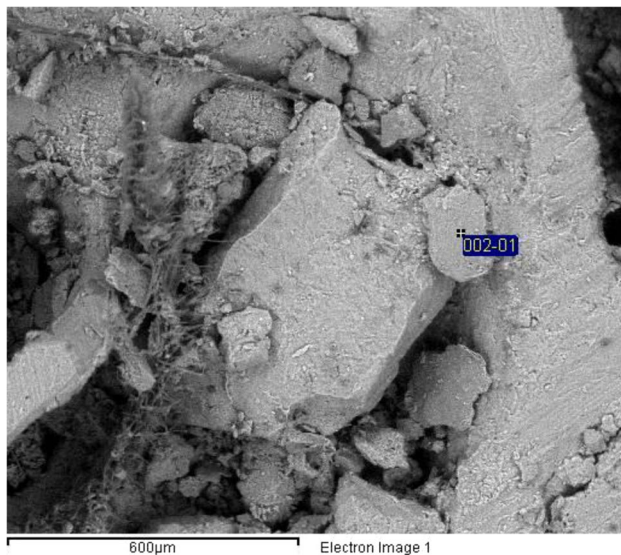

Fig. 12 ORO002.1: Compounds comprising metabolic subproducts from the fungus and secondary mineral compounds like calcium carbonate

microaggregation of minerals from the ground around the bone. These accumulations of calcium carbonates or oxalates, minerals, and exogenous compounds in the bone change the microenvironment and collapse the tissue.

Fungus has the capacity of forming large three-dimensional networks to aggregate particles, which happens in both bone and soil substrates and increases the reaction area on the surface of the bone Marchiafava et al. (1974). The structure of hard matter is altered through mechanic action disintegrating in three stages, as is presented in the samples from Orobajo.

In the first stage, pre-penetration, the oppressors adhere to the substrate. In the second stage, the thin hyphae penetrate the substrate through the weak spots in the matter, like the natural cavities of the bones, expanding towards the interior

of the substrate where new colonies develop. This process was evident in the samples, where the young and graceful hyphae extended throughout the entire bone structure (Fig. 6).

Specifically, the type of fungi found in the sample of Orobajo, Colombia, fits the model established by Sterflinger (2000) in regard to the form of growth and the bone morphology that favors their development during the diagenetic process. Hanson and Buikstra (1987) noted that the propagation of microorganisms was facilitated by the demineralization of the bone tissue. Finally, the changes caused by internal pressures weakened the material to the point of breaking. According to Sterflinger (2000), the process of penetration and rupture means that the substrate is gradually lost and that the fungus penetrates progressively, producing bio-pits. This model explains not only the generalized destruction described by Fernández-Jalvo et al. (2010), but also the extensive loss of hard tissue reported by Piepenbrink (1986), which, when bone morphology and biometry were analyzed, do not correspond to the tunnels of Wedl (1864). The samples from Orobajo have a crumbly texture in the periosteal surface of the cortical bone as described by Fernández-Jalvo et al., (2010).

Oxalic acid is one of the principal acids produced by fungi and one of its most important functions is to liberate the nutrients from the mineral or the mineral-based substrate, like bone. In the environment, compounds like oxalate ion ($C_2O_4{}^{2-}$), or oxalate salt (CaC_2O_4 or $Ca(COO)_2$) are present due to the processes of precipitation of micro-genetic secondary minerals, like oxalates, carbonates, and phosphates that are deposited in the tunnels produced in the bone substrate by fungus and hyphae Marchiafava et al. (1974).

Therefore, there are microenvironments developed in saturation conditions which favor the dissolution of the calcium carbonate ($CaCO_3$) in the bone. Oxalates are part of the multiple functions of fungal metabolism; thus, they are present in the bone, like calcium carbonate ($CaCO_3$). Freed calcium ions
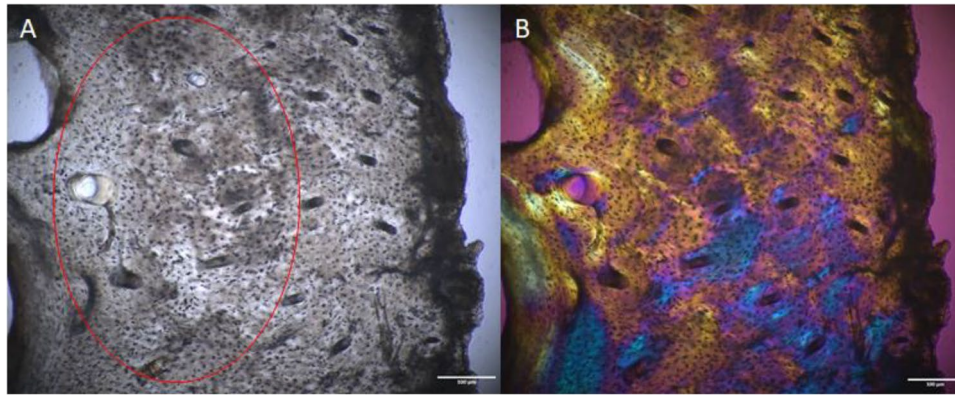
Fig. 13 ORO122: The interstitial and/or the endosteal bones were the least affected (**A**, circled area) in terms of the loss or degradation of organic matter. Birefringence of the bone shows little presence of bone crystallinity (blue color, **B**) surrounded by possible dissolution (yellow and purple colors) rather than recrystallization of the bioapatite. Lost of bioapatite equilibrium disrupts the crystal lattice and results in dissolution of the lattice structure (**B**). (Augmentation 20×)

(Ca^{2+}) are fundamental for the apical growth of the hyphae and the production of calcium oxalic metabolites. Marchiafava et al. (1974) showed the relationship of the hyphae's age and the size and quantity of metabolic products of the fungus.

As the type of secondary metabolism depends on the environment, fungi that grow in calcium (Ca)-rich substrates like bone present high concentrations of Ca^{2+} and CO_3^{2} in solution, and the colonies of aged hyphae will produce great quantities of metabolites. The large-scale production of metabolites by the aged hyphae consumes the organic matter excreted by the fungi or the residuals generated by the hyphae in decomposition, as the fungal polymers of the cell wall, like the chitin, have a great capacity for combination with Ca^{2+} (Bindschedler et al., 2016; Child, 1995a; Ehrlich et al., 2009; Fomina et al., 2010; Gadd, 2017; Gadd and Dyer, 2017).

The metabolic production of the fungus is diffused in the bone tissue (Piepenbrink, 1986) and, as calcium Ca^2 has the capacity to combine with chitin, the fungal polymers freed at the death of the fungus or the decomposition of its hyphae form microaggregates with polysaccharides, highly adhesive proteins, and fungal metabolites (Eldor, 2014). In the conceptual model of the natural hierarchy of the aggregate of the soil reported by Tisdall and Oades (1982), the microaggregates range between 2 and 20 μm.

The organic matter in the soil, like decomposing hyphae, are aggregated with the metabolic subproduct resultant of fungal activity like PGaP and secondary minerals like calcium carbonate. These aggregates are precipitated around the bone matrix, overloading it with these amorphous particles (Fig. 8 ORO052.1).

Some of the samples presented a solid appearance in the cortical of the circumferential periosteal laminar bone and, although they had lost the birefringence, the microstructures and the type of osteons derived, typical in the tissue of immature bone on a subadult individual, were observed. However, this type of sample presented alterations in microstructure as a result of staining and thickening of osteons, canaliculi, or lacunae or deteriorations of the periosteal circumferential laminar bone. Thus, the bone morphologically appears like bone because only the bone matter is identified or because the destruction is at deeper layer than the cortical. Another type of bone in the sample showed that the fungi biodegrade the collagen internally; the fungi destroyed the morphology of the bone, and it therefore presented generalized destruction in the bone microstructure.

The destruction of the bone tissue in the deeper layers could not be seen with the use of a light microscope. However, by using SEM techniques, the generalized internal destruction of the tissue was observed in very small holes created when the hyphae penetrated the deepest layers of the bone substrate. Light microscopy revealed staining in all the matter, calling attention to the earth tones, possibly due to the infiltration of humic and fulvic materials during the process of chelation and/or microaggregation, or generated by the metabolic products of the fungus as Hollund et al. (2018) report and seen in the sample from Orobajo. In methodological terms, the use of various types of microscopy techniques added to the theoretical information allowed for the explanation of a wide panorama of diagenetic processes in the bone.

In Orobajo, cultural mortuary processes did not include cadaver preservation techniques. Decomposition, therefore, was facilitated by the proliferation of fungi and rapid degradation of the materials with which the body was covered once they entered in contact with the ground. As the soil was sandy or gravel-based, percolation levels were high, which accelerated the propagation of fungi that deteriorate the wood of the coffin and the items used to cover the body much more quickly.

In the area, there is little fluvial precipitation and there are long dry periods, which lead to the greater destruction of the materials (Huisman and Klaassen, 2009). Thus, the cadaver's decomposition results from exposure to the external agents in the surrounding medium. The findings from the

exhumations—fragmented and degraded bone pieces incrusted with roots, bones that appeared whole in the ground but collapsed upon their removal, few remaining coffin pieces or textile fragments, and the discoloration of bones and burial goods alike—corroborate previous research in the field.

In one of the samples, spherical objects were found, which were thought to be bacteria spores based on morphology and biometry. None of the other samples presented bacteria or the effect of bacteria in the morphology of the bone microstructure. A possible explanation for this result is that the bacterial presence is due to the production of the secession of microorganisms after the exhumation and the contamination of the sample. However, it would require amplifying the analysis to verify the hypothesis.

Diagenetic alteration product of bacteria was not found. Thus, it is considered that upon burying the cadaver and reaching the phase of skeletonization, the organisms in the rhizosphere environment like fungi competed with decomposition bacteria in an accelerated process of succession of microorganisms. The acidic environment favored the fungi over the bacteria. The widespread proliferation of fungi in different phases of the life cycle and metabolic production, from hyphae to young and aged mycelia, were present in all the samples, showing that the environmental conditions were propitious for accelerated decomposition and diagenetic alteration, seen as the generalized destruction of the buried bone.

Conclusion

In the burial environment of dry tropical forests, biotic and abiotic agents determine diagenetic alteration on the microscopic level of the bone through biophysical and biochemical mechanisms. Skeletonized remains buried in a rhizosphere microbiome constituted by sand- and gravel-based soils offer the necessary conditions for the reproduction of fungi. Fungal alteration of the bone results in the generalized destruction and biochemical wearing of the minerals and the biodegradation of the organic matter in the bone which, in turn, produces metabolites that act as a secondary mechanism for the destruction of the bone tissue. Thus, destruction of bone by fungi can be identified by the crumbling morphology of the degrading bone.

Necrosols, soils in which human remains are buried, are rich in micro and macronutrients apt for the proliferation of adaptive and dominant microorganisms, like fungi (Całkosiński et al., 2015). Like in any microbial biotope sustained under the principal of the balance of dominance, microorganisms are structured for and specifically adapt to the rhizosphere of necrosol environments.

Declarations

References

Arenas, G. (2014). *Micología médica ilustrada. Generalidades de los hongos. Dermatofitosis.* (McGraw-Hill Interamericana, Ed.). México.

Bell LS (1995) Post mortem microstructural change to the skeletonPhD Thesis. University College London, University of London

Bindschedler S, Cailleau G, Verrecchia E (2016) Role of fungi in the biomineralization of calcite. Minerals 6(41):1–19. https://doi.org/10.3390/min6020041

Bjordal CG, Nilsson T (2002) Waterlogged archaeological wood — a substrate for white rot fungi during drainage of wetlands. Int Biodeterior Biodegradation 50:17–23

Caballero JH, Rendón A, Gallego JJ, Uasapud NV (2016) Inter-Andean Cauca River canyon. In: *Landscapes and landforms of Colombia. World geomorphological landscape*. Springer, Cham, pp 155–166

Całkosiński R, Płoneczka-Janeczko K, Ostapska M, Dudek K, Gamian A, Rypuła K (2015) Microbiological analysis of necrosols collected from urban cemeteries in Poland. *Biomed Res Int*. https://doi.org/10.1155/2015/169573

Child AM (1995a) Microbial taphonomy of archaeological bone. Stud Conserv 40(1):19–30 Retrieved from http://www.jstor.org/stable/1506608

Child AM (1995b) Towards an understanding of the microbial decomposition of archaeological bone in the burial environment. J Archaeol Sci 22:165–174

Ehrlich H, Koutsoukos PG, Demadis KD, Pokrovsky OS (2008) Principles of demineralization: modern strategies for the isolation of organic frameworks: part I. Common definitions and history. *Micron* 39(8):1062–1091. https://doi.org/10.1016/j.micron.2008.02.004

Ehrlich H, Koutsoukos PG, Demadis KD, Pokrovsky OS (2009) Principles of demineralization: modern strategies for the isolation of organic frameworks part II. Decalcification. *Micron* 40:169–193. https://doi.org/10.1016/j.micron.2008.06.004

Eldor P (2014) *Soil microbiology, ecology, and biochemistry* (fourth Ed). Academic Press is an imprint of Elsevier

Emmons AL, Mundorff AZ, Keenan SW, Davoren J, Andronowski J, Carter DO, DeBruyn JM (2020) Characterizing the postmortem human bone microbiome from surface-decomposed remains. PLoS One 15(7):e0218636. https://doi.org/10.1371/journal.pone.0218636

Espinal LS (1985) Geografía ecológica del Departamento de Antioquía (zonas de Vida (formaciones vegetales) del Departamento de Antioquía). Revista Facultad Nacional de Agronomía Medellín 38(1):5–106

Estrada GI, Ramírez MC (2019) *Micología general* (Centro Edi), Manizales

Fernández-Jalvo Y, Andrews P, Pesquero D, Smith C, Marín-monfort D, Sánchez B, Alonso A (2010) Early bone diagenesis in temperate environments part I : surface features and histology. Palaeogeogr Palaeoclimatol Palaeoecol 288(1–4):62–81. https://doi.org/10.1016/j.palaeo.2009.12.016

Fomina M, Burford EP, Hillier S, Kierans M, Gadd GM (2010) Rockbuilding fungi. Geomicrobiol J 27(6):624–629. https://doi.org/10.1080/01490451003702974

Gadd GM (2017) Geomicrobiology of the built environment. Nat Publ Group 2(3):1–9. https://doi.org/10.1038/nmicrobiol.2016.275

Gadd GM, Dyer TD (2017) Bioprotection of the built environment and cultural heritage. Microbial 1:1–5. https://doi.org/10.1111/1751-7915.12750

Garland AN (1989) Microscopical analysis of fossil bone. Appl Geochem 4(3):215–229

Garland, A. N. (1993). An introduction to the histology of exhumed mineralized tissue. In *histology of ancient human bone: methods and diagnosis proceedings of the "Palaeohistology workshop" held from 3-5 October 1990 at Gottingen*. Berlin Heidelberg: Springer-Vedag. https://doi.org/10.1007/978-3-642-77001-2

Gebhardt W (1906) Ueber funktionell wichtige anordnungs- weisen der gr6beren und feineren bauelemente der wilberl- tierknochens. Roux Arch Entw Mech 20:187–322

Gordon CC, Buikstra JE (1981) Soil pH, bone preservation, and sampling bias at mortuary sites. Am Antiq 46(3):566–571

Greenlee DM, Dunnell RC (2010) Identification of fragmentary bone from the Pacific. J Archaeol Sci 37(5):957–970

Hackett CJ (1981) Microscopical focal destruction (tunnels) in exhumed human bones. Med Sci Law 21(4):243–265. https://doi.org/10.1177/002580248102100403

Hanson DB, Buikstra JE (1987) Histomorphological alteration in buried human bone from the lower Illinois Valley : implications for palaeodietary research. J Archaeol Sci 14:549–563

Hedges REM (2002) Bone diagenesis : an overview of processes. Archaeometry 3:319–328

Hedges REM, Millard AR (1995) Measurements and relationships of diagenetic alteration of bone from three archaeological sites. J Archaeol Sci 22:201–209

Hollund HI, Blank M, Sjogren K-G (2018) Dead and buried ? Variation in post-mortem histories revealed through histotaphonomic characterisation of human bone from megalithic graves in Sweden. PLoS One 13(10):1–26. https://doi.org/10.1371/journal.pone.0204662

Hollund HI, Jans MME, Collins MJ, Kars H, Joosten I, Kars SM (2011) What happened here? Bone histology as a tool in decoding the post-mortem histories of archaeological bone from Castricum, the Netherlands. *Int J Osteoarchaeol*. https://doi.org/10.1002/oa.1273

Huisman DJ, Joosten I, Nientker J (2009) Textile. In *Degradation of archaeological remains*. Sdu Uitgevers b.v, Den Haag

Huisman DJ, Klaassen RKWM (2009) Wood. In *Degradation of archaeological remains*, Sdu Uitgevers bv Den Haag

IUSS Working Group WRB (2015) World reference base for soil resources 2014, update 2015: international soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106:192.)

Jans MME (2005) Histological characterisation of the degradation of archaeological bonePhD Thesis. Institute for Geo and Bioarchaeology

Jans MME, Kars H, Nielsen-Marsh CM, Smith CI, Nord AG, Arthur P, Earl N (2002) In situ preservation of archaeological bone: a histological study within a multidisciplinary approach. Archaeometry 44(3):343–352

Kendall C, Høier Eriksen AM, Kontopoulos I, Collins MJ, Turner-Walker G (2017) Diagenesis of archaeological bone and tooth. *Palaeogeogr Palaeoclimatol Palaeoecol*. https://doi.org/10.1016/j.palaeo.2017.11.041

Lambert JB, Simpson SV, Szpunar CB, Buikstra JE (1984) Copper and barium as dietary discriminants: the effects of diagenesis. Archaeometry 26(2):131–138

Lawrence DR (1979) Diagenesis of fossils—Fossildiagenese. Paleontol Encyclopedia Earth Sci:245–247. https://doi.org/10.1007/3-540-31078-9_46

Marchiafava V, Bonucci E, Ascenzi A (1974) Fungal osteoclasia : a model of dead bone resorption. Calcif Tissue Res 14(1):195–210

Mowna Sundari T, Anand AP, A., Jenifer, P. et al (2018) Bioprospection of basidiomycetes and molecular phylogenetic analysis using internal transcribed spacer (ITS) and 5.8S rRNA gene sequence. Sci Rep 8:10720. https://doi.org/10.1038/s41598-018-29046-w

Nielsen-Marsh CM, Hedges RE (2000) Patterns of diagenesis in bone I: the effects of site environments. J Archaeol Sci 27(12):1139–1150

Nielsen-Marsh CM, Smith CI, Jans MME, Nord A, Kars H, Collins MJ (2007) Bone diagenesis in the European Holocene II : taphonomic and environmental considerations. J Archaeol Sci 34(9):1523–1531. https://doi.org/10.1016/j.jas.2006.11.012

Pfretzschner H (2004) Fossilization of Haversian bone in aquatic environments. C R Palevol 3:605–616. https://doi.org/10.1016/j.crpv.2004.07.006

Piepenbrink H (1986) Two examples of biogenous dead bone decomposition and their consequences for taphonomic interpretation. J Archaeol Sci 13:417–430

Pizano C, Cabrera M, García H (2014) Bosque seco tropical en Colombia; generalidades y contexto. Instituto de Investigación de Recursos Biológicos Alexander Von Humboldt, Bogotá (Colombia) Ministerio de Ambiente y Desarrollo Sostenible

Rashid MI, Mujawar LH, Shahzad T, Almeelbi T, Ismail IMI, Oves M (2016) Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. Microbiol Res 183:26–41. https://doi.org/10.1016/j.micres.2015.11.007

Rojas R (2009) Guía Para la descripción de suelos (No. FAO 631.44 G943 2009). FAO, Roma (Italia)

Silverman MP, Muñoz EF (1970) Fungal attack on rock: solubilization and altered infrared spectra. Science 169(3949):985–987

Sterflinger K (2000) Fungi as geologic agents. Geomicrobiol J 17(2):97–124. https://doi.org/10.1080/01490450050023791

Suter F, Martínez JI, Vélez MI (2011) Holocene soft-sediment deformation of the Santa Fe–Sopetrán Basin, northern Colombian Andes: evidence for pre-Hispanic seismic activity. Sediment Geol 235((3-4)):188–199. https://doi.org/10.1016/j.sedgeo.2010.09.018

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. J Soil Sci 33:141–163

Turner-Walker G (2008) The chemical and microbial degradation of bones and teeth. Adv Human Palaeopathol 592

Turner-Walker G (2019) Light at the end of the tunnels? The origins of microbial bioerosion in mineralised collagen. Palaeogeogr Palaeoclimatol Palaeoecol 529:24–38. https://doi.org/10.1016/j.palaeo.2019.05.020

Vargas TM, Uribe CL, Bedoya SV, Zapata MLQ, Cardona-Gallo SA (2022) Cemetery relocations in Hidroituango: an interdisciplinary study. An Acad Bras Cienc 94(1):e20201098. https://doi.org/10.1590/0001-3765202220201098

Vélez S, Monsalve T, Quiroz ML, Castañeda D, Cardona-gallo SA, Terrazas A, Sedov S (2019) The study of necrosols and cemetery soils. DYNA 86(211):337–345. https://doi.org/10.15446/dyna.v86n211.80757

Wedl C (1864) Über einen im Zahnbein und Knochen keimenden Pilz. In *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften* (p. 171–193.).

Weiner S, Arad T, Sabanay I, Traub W (1997) Rotated plywood structure of primary lamellar bone in the rat: orientations of the collagen fibril arrays. Bone 20:509–514

Weiner S, Traub W, Wagner H (1999) Lamellar bone: structure–function relations. J Struct Biol 126:241–255