Journal of Archaeological Science 85 (2017) 13–29

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Micromorphological indicators for degradation processes in archaeological bone from temperate European wetland sites

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ARTICLE INFO

Article history:
Received 23 September 2016
Received in revised form
29 June 2017
Accepted 30 June 2017

Keywords:
Taphonomy
Bone decay
Phosphates
Fungi
Bacteria
Ash

ABSTRACT

Micromorphological investigations of archaeological bones make it possible to study decay processes and the associated depositional environment in one go. A selection of micromorphological thin sections from soil samples from three wetland sites in Switzerland, The Netherlands and Norway that contained bone fragments were studied. The goal was to investigate the type and the timing of decay processes to better understand the taphonomy of bones in such sites. Using optical microscopy and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX), a range of biological decay processes and chemical/mineralogical transformations were observed. In two of the sites – Zug-Riedmatt in Switzerland and Hazendonk in The Netherlands – a relatively short exposure to adverse conditions must have occurred: Some of the bones from Zug-Riedmatt show localized collagen decay related to exposure to fresh ashes; others show cyanobacterial tunnelling related to submersion in shallow, clear water. In Hazendonk, bone fragments and fish scales apparently have first been exposed to bacterial decay related to putrefaction. Subsequently, alternations between wet and dry conditions resulted in the dissolution of some of the bone mineral and the formation of Ca, Fe(III) phosphates, probably mitridatite. Fungal decay caused extensive tunnelling of bone and fish scales as well as the secondary phosphates. These processes apparently ended when the bone-rich layer became permanently waterlogged and anoxic. In Stavanger, bone mineral is transformed into mitridatite and possibly other Ca Fe(III) phosphates. Indications that the redox conditions are variable at present suggest that these processes are still active.

1. Introduction

1.1. Degradation processes and the archaeological record

The archaeological record may contain a highly variable range of materials in the form of artefacts, human and animal remains, botanical material and soil features. Because these remains react differently to different environmental conditions, there are large differences in the chance of survival between different materials, and between different types of burial environments. Because of these differences, the archaeological record is intrinsically biased by the differential degradation of artefacts and ecofacts. Those remains that have a large chance of surviving ages of burial – like stone and ceramic objects – are present in most archaeological contexts. Fragile or easily degraded remains on the other hand – like the non-carbonized tissue of plants and soft animal parts – are much rarer, and moreover mostly restricted to specific environments (in essence extremely wet, dry or cold). For archaeologists, it is therefore of primary importance to take into account which types of materials can survive long-term burial in various soil environments (Renfrew and Bahn, 2012; Huisman, 2009).

From experience, a general idea on the effects of the burial environment and the chance of survival of specific archaeological materials has formed, which is generally taught in archaeological training as part of the curriculum (see e.g. Wood and Johnson, 1978). In the last few decades the emergence of the "preservation *in situ*" paradigm drove more targeted research into degradation of specific materials and the role of the burial environment (see Huisman (2009) and Canti and Huisman (2015) for an overview).

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http://dx.doi.org/10.1016/j.jas.2017.06.016
0305-4403/

1.2. Analysing and identifying bone degradation

Many bone decay processes have been identified by analysing polished bone sections with microscopes (Jans et al., 2002, 2004; Jans, 2005; Tjelldén, 2016) or electron microscopes (Bell et al., 1991; Bell, 2012; Tjellden et al. in press; Turner-Walker, 2012), i.e. by histological methods. For this purpose, bone is first cut in longitudinal and/or transversal sections. Subsequently these fragments are usually (but not always; Fernández-Jalvo et al., 2010) embedded in resin, and polished. Polishing is sufficient for electron microscopy or microscopic analyses using incident light. For microscopic analyses using transmitted light, samples are usually ground to a standard thickness of c. 80 μm prior to polishing, although e.g. Jans (2005) ground the samples to 30 μm thickness which is better suited to recognize decay features.

Histological analyses on bone samples have been instrumental in identifying a range of (micro) biological and chemical processes that affect bones in archaeological as well as in forensic research (Bell, 2012; Fernández-Jalvo et al., 2010; Hacket, 1981; Hedges et al., 1995; Hedges, 2002; Hollund et al., 2012; Jans, 2005, Nielsen-Marsh and Hedges, 2000; Smith et al., 2007; Trueman and Martill, 2002, Turner-Walker, 2012; Turner-Walker and Jans, 2008). The method has several disadvantages, however, when applied to bones from archaeological sites: Firstly, in archaeological contexts it can only be done on bone or bone fragments that are large and firm enough to prepare oriented cross sections. This excludes small bones and bones or bone fragments that are degraded to such an extent that they cannot be isolated or mounted – or even recognized macroscopically. Secondly, bones are taken out of their context and burial environment prior to histological preparation. The direct connection between the bone and evidence for past and present burial conditions, i.e. the embedding sediment, is lost in the process. This is especially important for those cases where the present burial environment differs from that in the past – which is a common phenomenon in many archaeological sites. Thirdly: many hand-collected large bones extracted directly from the archaeological sites are air dried and washed with water, removing possible degradation features on their surfaces. Because of the correlation between burial environment and bones, histological study of bone fragments has been employed in several archaeological heritage management studies to assess present-day threats to archaeological sites (Huisman et al., 2008; Huisman, 2009). On the UNESCO world heritage site of Schokland (Huisman and Mauro, 2013), and during research on the middle Neolithic site of Swifterbant S4, the degree of degradation was found to vary to such a degree that it was concluded that much of the decay had taken place as a taphonomical process, i.e. before and shortly after burial.

Soil micromorphologists study polished thin sections from resin-impregnated undisturbed soil samples using microscopical techniques. Microscopic study with transmitted plane polarized light (PPL) or crossed polarizers (XPL) can be supplemented with incident light (IL) and ultraviolet or Blue light fluorescence microscopy (UV resp. BLF), scanning electron microscopy (SEM) and a range of analytical techniques. Undisturbed soil samples are impregnated with resin, thin sections are cut from the impregnated samples, mounted on a glass plate and subsequently polished and lapped to a thickness of 25–30 μm. The combination of minerals, organic materials, their distribution and the soil structure forms evidence for present and past processes and hence for the development of soils and the burial environment (Stoops, 2003; Stoops et al., 2010).

For the study of bone decay a main advantage is that smaller and strongly decayed bone fragments can still be studied, thus not only allowing decay studies in more archaeological sites but also making the study of advanced decay processes possible. The use of ultraviolet and Blue light fluorescence microscopy is especially suitable for studies on bone decay as many phosphate minerals – including bioapatite – have fluorescent properties that may be affected by heating or degradation processes (Karkanas and Goldberg, 2010; Villagran et al. in press). But at least as important may be the potential to identify past, terminated decay processes and combining them with evidence for past, altered burial conditions (Huisman et al., 2009). A main disadvantage, however, is that the orientation of the bones and bone fragments in a thin section is random. This makes it not only hard to recognize type of bones; it is not ideal when decay patterns are to be compared to those from histological sections.

1.3. Bone degradation

From a point of view of degradation processes, bone is one of the more complex materials that can occur in archaeological sites: It consists basically of an intricate combination of some 70% mineral material (carbonated hydroxyapatite or HAP), organic material (mostly collagen but also osteocalcine; both proteins), and 7–8% tightly bound water in a fresh bone. On a microstructural level these components are intimately connected in lamellae of several microns thick, protecting each other due to their intimate association (Collins et al., 2002; Turner-Walker, 2009; Huisman et al., 2009). Several different pathways of (micro)biological, chemical and physical decay or transformation processes in bone are known. Which of these processes occur depends on the burial environment (see e.g. Collins et al., 2002; Turner-Walker, 2009). Pathway 1, following the terminology of Collins et al. (2002), entails the slow chemical degradation of collagen. Evidence for this pathway is rare, as this process is extremely slow in most burial environments. Only (pre-burial) heating and burial conditions with extreme pH are capable to speed up this process enough to have a noticeable impact on the bone structure. Pathway 2 is the chemical deterioration of the HAP. This process is restricted to neutral to acidic environments, as HAP is stable in lime-buffered burial conditions (with pH ~8.2). It is not only exacerbated by low pH, but also by fluctuating hydrological conditions and/or metal-binding humic substances that prevent the establishment of chemical equilibrium between HAP and the burial environment (Collins et al., 2002; Turner-Walker, 2009). Pathway 3 consists of several types of microbial decay. With the potential exception of tunnelling by cyanobacteria (see below), initial HAP dissolution following pathway 2 is instrumental in facilitating the (much faster) processes of microbial decay (Collins et al., 2002).

Microbial bone degradation comes in several types, which were first distinguished by Hacket (1981). He identified four types of decay patterns that are related to different agents: Linear longitudinal, lamellate and budded microfocal destruction sites ("mfd's") are attributed to decay by bacteria (see also Jans et al., 2004). From the discussion in Trueman and Martill (2002) it becomes clear that it is likely that different types of bacteria are involved successively to produce these decay patterns. The bacterial decay is generally linked to putrefaction processes that can only proceed when soft body tissue is still present (Jans, 2005; Fernández-Jalvo et al., 2010). The fourth type, Wedl tunnelling, is attributed to fungal decay (Hacket, 1981; Trueman and Martill, 2002; Bell et al., 1991). Because it depends on initial dissolution of HAP, fungi can degrade bone only as long as the environment is moist (but not waterlogged), oxygenated and the pH is neutral to acidic (i.e. not lime-buffered) (Huisman et al., 2009). In addition to these decay patterns, bone from underwater environments can show another type of tunnelling that is restricted to the outer surface layers of the bone. This tunnelling is most commonly attributed to decay in marine or fresh water by cyanobacteria (Bell et al., 1991; Turner-Walker, 2012; Bell,