

Review

Bone Diagenesis and Extremes of Preservation in Forensic Science

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Abstract: Understanding the composition and diagenetic processes of the deposition environment is pivotal to understanding why bone undergoes preservation or diagenesis. This research explores the complex nexus of diagenesis at the extremes of preservation, via the interdependent chemical, and short- and long-term microbial processes that influence diagenesis. These processes include dissolution, ion exchange, hydrolysis, recrystallisation, waterlogging, acidity and alkalinity, soil composition, redox potential, bacterial activity, and microbiome composition. Diagenetic processes are discussed in relation to typical sites and sites with extremes of preservation. Understanding site conditions that impact diagenetic processes is critical to understanding the visual features presented in recovered skeletal material, ensuring an appropriate post-mortem interval is assigned, and for subsequent post hoc analysis of bone.

Keywords: archaeology; bone; burial; decomposition; chemistry; microbiology; diagenesis

1. Introduction

Forensic investigations encounter human remains across a wide spectrum of decomposition and therefore require an understanding of the mechanisms throughout decomposition and their impact on analytical interpretations. Whilst there is a wealth of research into the rapid decomposition of soft tissue in shorter forensic timescales, longer time periods for greasiness, drying, weathering and diagenesis of bone are difficult to investigate and consequently see fewer experimental and longitudinal research studies (Hedges, 2002; Stokes et al., 2020; Tibbett & Carter, 2009; Wescott, 2018). Furthermore, the diagenetic processes that impact the degradation of a buried bone or object over time involve a complex interaction between many biological, chemical and physical processes as well as characteristics of the bone and the environment buried in through deep time intervals (Kendall et al., 2018; Lecher et al., 2023; Turner-Walker, 2007). The state of preservation presented depends upon how protective or destructive chemical and microbial processes act on the bones. Following the initial burial and subsequent early decay, diagenesis becomes slower and more complex as it follows interdependent chemical and biological mechanisms discussed in this paper. Diagenesis may also be more destructive toward certain materials; for example, the perishable organic content within bone is susceptible to rapid organic decomposition (Good, 2001; Janaway, 2011; Keenan, 2023) whereas the inorganic phase of bone can survive for many centuries. Bone is approximately 70% inorganic mineral (predominantly hydroxyapatite), with the remaining 30% made of organic content, predominantly collagen (Rosa et al., 2022). Both components are integrated together, resulting in the histological structure of the osteon



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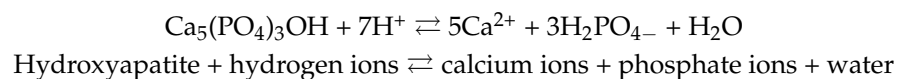
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or Haversian system (see [Rosa et al., 2022](#) for details on bone composition and histology terminology). The composition of bone means that bone apatite is one of the most stable phases of calcium phosphate so it can survive for a significant time, especially if conditions are anaerobic or desiccated with an alkaline to neutral pH ([Caple, 2004](#); [Child, 1995](#); [Pate et al., 1989](#); [Turner-Walker, 2007](#)). Therefore, this paper focuses on bone in its entirety as a critical resource to focus on when investigating decomposition, diagenesis and preservation throughout forensic and archaeological time intervals. There remains a need to pull apart the chemical and microbial nexus of diagenesis and understand their interaction together when producing new tools for visualising preservation conditions and for understanding or estimating the likelihood of good or poor preservation for subsequent investigation.

2. Chemical Diagenetic Processes

2.1. Dissolution and Collagen Breakdown

Dissolution is primarily concerned with the leaching of inorganic content from a bone due to the acidity of the external environment ([Keenan, 2023](#); [Smith et al., 2007](#); [Turner-Walker, 2007](#)). The pH level of soil is the logarithmic scale of how acidic or alkaline the soil is (meaning, the concentration of hydrogen (H^+) ions) ([Caple, 2004](#)). pH level impacts buried bones, such as acidic soils breaking down calcium phosphates in the hydroxyapatite of buried bone, resulting in the continuous dissolution of bone and preservation of collagen until equilibrium is achieved ([Smith et al., 2007](#); [Turner-Walker, 2009](#)):



Essentially, acidic soils break up the hydrogen ions in bone and push the equation to the right as dissolution continues, releasing calcium and phosphate ions into soil. Hydroxyapatite reacts by consuming some of the released hydrogen and pushing the reaction back to the left for further dissolution of bone ([Turner-Walker, 2009](#)). These exchanges are determined by the redox potential of soil for oxidation–reduction reactions. This is where electrons are transferred between chemical species, with one atom losing an electron (the reducing agent oxidises) and the other atom gaining that electron (the oxidising agent reduces) ([Caple, 2004](#)). Redox determines the ability to gain or lose electrons through ionic exchange in soil and subsequent elemental cycling (Figure 1).

Soil has homeostatic functions to regulate a stable state and resist the effects of fluctuations in soil properties such as extreme pH levels. Soils are buffered by a cation exchange that bonds H^+ ions in acidified conditions or releases H^+ ions in alkalised conditions to maintain a stable pH level ([Caple, 2004](#)). However, the pH level of soil can be catastrophic to either bone or collagen with an inverse relationship; low acidity dissolves the inorganic material rapidly and leaves behind the collagen jelly bone, whilst high alkalinity degrades the organic content ([Collins et al., 2002](#); [High et al., 2015, 2016](#)). [Gordon and Buikstra \(1981\)](#) demonstrated this effect in archaeological scenarios, with worse preservation in bones from acidic gravesites than from alkaline soils due to the H^+ buffer exchange in acidic soils. Additionally, adding lime and slaked lime to bodies as part of traditional funerary practice would increase the pH level due to the reduction of H^+ ions, accelerating collagen degradation ([Anbu et al., 2016](#); [Collins et al., 2002](#)).

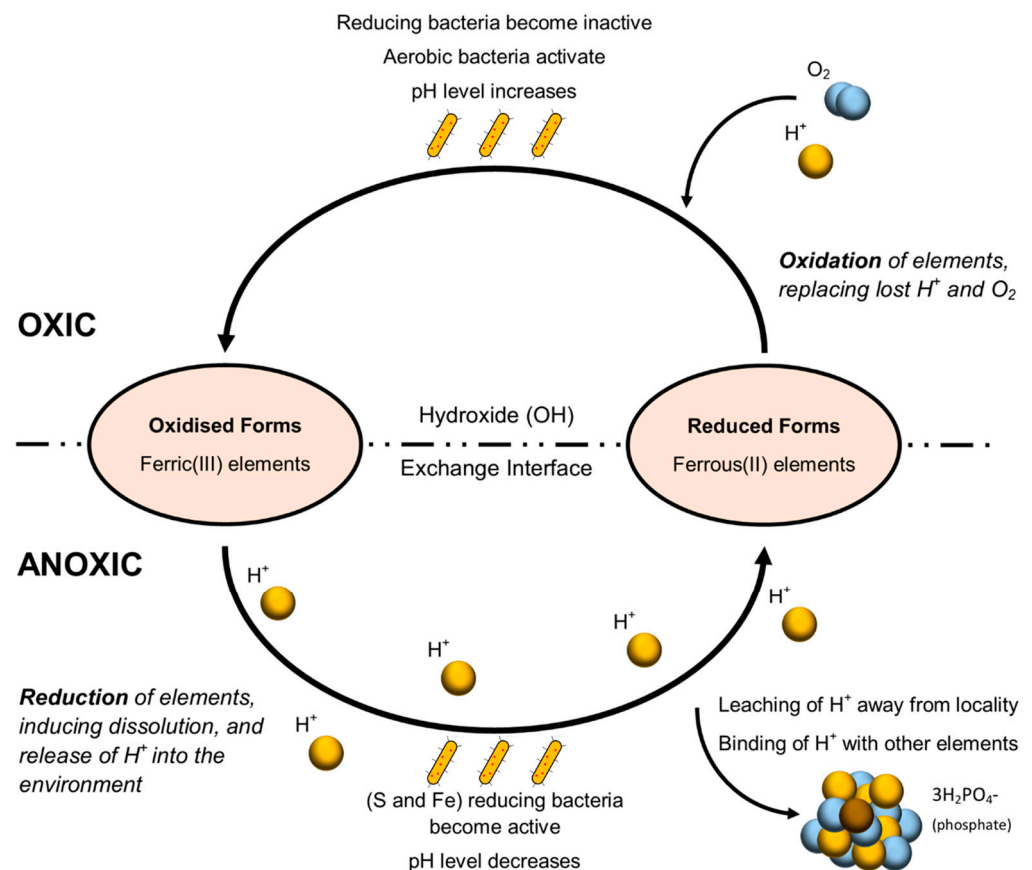


Figure 1. Redox cycle, showing the increased dissolution and leaching caused by anoxic conditions. Designed using information from (Rosa et al., 2022; Turner-Walker, 2009).

2.2. Accelerated Hydrolysis

Hydrolysis is the breakdown of the molecular covalent bonds of an object due to water effects. Collagen loss accelerates hydrolysis and the formation of cracks and porosity, allowing amino acids within the environment to infiltrate the bone (Collins et al., 1995, 2002; Hedges, 2002; Lecher et al., 2023). Hydrolysis is critical to chemical deterioration because this function exponentially exposes more collagen and internal surfaces to further diagenetic change (Collins et al., 2002; Hedges, 2002; Latham & Madonna, 2012; Turner-Walker, 2007). This may be through further dissolution or by recrystallisation; additional apatite from the deposition environment such as fluorine and carbonate can be incorporated into the molecular structure of bone mineral as part of a new, protective barrier (Hedges, 2002; Loy et al., 2023). Waterlogged conditions may be identified by extraneous minerals deposited from percolating groundwater and incorporated within the histology, particularly iron and manganese due to their abundance and insolubility in soil (Booth, 2017; Loy et al., 2023).

There are four types of groundwater movements to consider (Figure 2): dry or no water, diffuse or waterlogged, flow with periodic or seasonal wet–dry cycles, and recharge with rewetting from frequent changes in the water level (Hedges & Millard, 1995). Groundwater is arguably the most influential cause of diagenesis and dissolution because groundwater is the medium in which many other chemical and biological diagenetic processes occur (Hedges & Millard, 1995; Nielsen-Marsh et al., 2000; Turner-Walker, 2007). Landscapes with unstable groundwater tables such as flow and especially recharge movements are destructive because the water occupying pores in a buried bone are drawn out by hydraulic movement and replaced with ‘fresh’ water that soon becomes saturated with elemental content leached from the bone, resulting in rapid destruction as the cycle repeats (Hedges & Millard, 1995; Lecher et al., 2023; Nielsen-Marsh et al., 2000; Nielsen-Marsh & Hedges, 2000;

Turner-Walker, 2007). In contrast, bones buried in soil with limited water movement and relatively high concentrations of inorganic minerals will theoretically see good survival over an indefinite time interval because the bones are not subject to groundwater dissolution effects (Lecher et al., 2023; Turner-Walker, 2007, 2009).

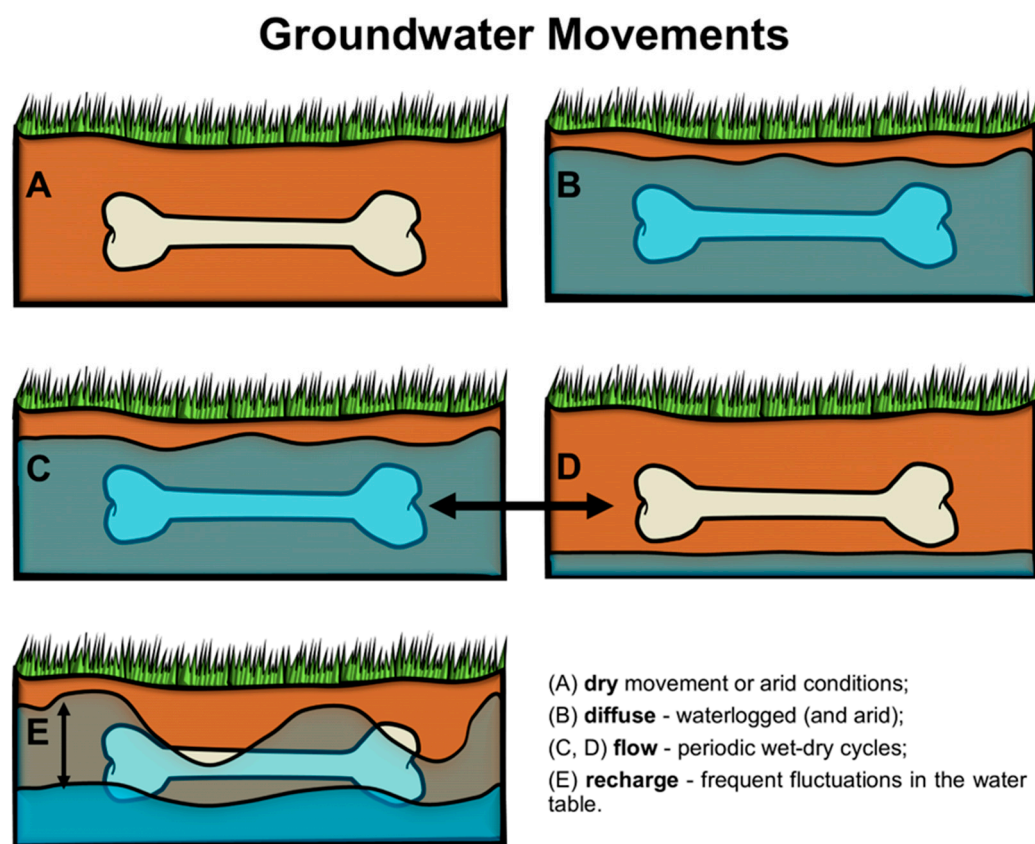


Figure 2. Four types of groundwater movements: minimal water movement observed when dry (A) or waterlogged (B), intermediate water movement occurring periodically or seasonally (C,D), and maximum water movement with frequent rewetting under recharge movement (E).

2.3. (Re)crystallisation

Crystallinity refers to the structure and patterns of molecules forming a solid substance (Keenan & Engel, 2017). Diagenetic alterations begin at the molecular level with demineralisation, where the elemental structure is reduced, removed, and substituted by extraneous elements to then become recrystallised (Hedges & Millard, 1995; Nielsen-Marsh et al., 2000; Pate et al., 1989). As material dissolves, the object becomes structurally weaker and elemental content is leached between the bone and environment, which leaves traces in soil or contaminations in the bone that can be visualised with chemical techniques (Caruso et al., 2021; Loy et al., 2023; Pate et al., 1989; Wilson et al., 2008). The molecular structure becomes increasingly unstable and intensifies the susceptibility to other diagenetic processes of the deposition environment (Pate et al., 1989; Piepenbrink, 1989; Scaggion et al., 2024; Trueman et al., 2004; Turner-Walker, 2007). Whilst this has a relatively negligible impact on organic material, the crystallinity of inorganic material is reorganised, increasing porosity and microstructural changes until macroscopic destruction and eventual disintegration are achieved (Caruso et al., 2021; Nielsen-Marsh et al., 2000). This effect is exacerbated in juvenile remains, as their higher proportion of organic content and developing microstructure results in poorer inorganic survival compared to adult remains (Caruso et al., 2021). Inhibiting the dissolution and porosity of buried bone through recrystallisation is critical to preservation and potential fossilisation (Hedges et al., 1995; Hedges & Millard, 1995;

Keenan & Engel, 2017). Although the threshold is unclear, pH levels below five promote demineralisation whilst remineralisation is promoted in alkaline soils (Child, 1995).

3. Short-Term Microbial Diagenesis of Bone

The timings for short- and long-term periods are not defined and, in this paper, generally allude toward initial interment and proliferation from soft-tissues and associated byproducts whereas long-term periods relate more toward skeletonisation and where the body is in closer equilibrium or stability with the environment buried within. The biological conditions of a deposition site refer mostly to the microbial community of the environment and their actions in soil or toward buried bone (Forbes, 2011; Guareschi et al., 2023). Bones on the ground surface are exposed and quickly degraded by a range of taphonomic processes, whereas buried, anoxic and submerged bones see these processes in reduced capacity but still degenerate within several years (Blanchette et al., 1989; Eriksen et al., 2020; Guareschi et al., 2023). Bacteria promote the breakdown of bone via bioerosion; the act of microorganisms destroying histological structures to access and decay organic content (Booth et al., 2022; Good, 2001; Lander et al., 2023; Turner-Walker & Jans, 2008). In bone, this breakdown leaves characteristic patterns called microfocal destruction (MFD) sites categorised by the hypermineralised cuffs present in the morphological damage of cross-sections (Guareschi et al., 2023; Lander et al., 2023; Turner-Walker & Jans, 2008). The extent of bioerosion, chemically induced porosity and general diagenesis are typically examined by assigning an Oxford Histological Index (OHI) score based on the amount of intact bone presented in histological cross-sections (Hedges et al., 1995; Millard, 2001), although additional parameters are available (summarised in Table 1). Most bioerosion is suggested to occur during early diagenesis by utilising the histological structures of bone (Damann & Jans, 2017; Jans, 2012), although cyanobacteria colonise the external surface before tunnelling through histological pores with expansive action (Damann & Jans, 2017; Turner-Walker & Jans, 2008). For fungal attack to occur, the environment must be aerobic, neutral to acidic pH level, and moist but not waterlogged (Huisman et al., 2017). When in environments with few nutrients available, some fungi such as ectomycorrhizal fungi may target the bone mineral apatite for nutrition (Huisman et al., 2017). Although these studies have focused on destruction, organic molecules and proteins on the bone surface can delay diagenesis by passivating the chemical mechanisms occurring on these areas (Collins et al., 2002; Nielsen-Marsh et al., 2000). Regardless of the extent of bioerosion already present, bacteria invade bone via the Haversian systems and other networks in bone microstructure, evidenced by their concentration around osteocyte lacunae rather than the endosteal and periosteal surfaces (Bell et al., 1996; Booth, 2017; Booth et al., 2022; Hedges, 2002; Jans et al., 2004; Mein & Williams, 2023).

Table 1. Common methods for determining bioerosion and diagenesis (in bone).

| Method | Parameter | Study | Description |
|--------------------|------------------------|--|--|
| Visual Observation | Macroscopic weathering | (Behrensmeyer, 1978; Gordon & Buikstra, 1981; Stokes et al., 2020) | Scores of 0–5 on the extent of fragmentation, degeneration and cracking of the bone surface. Assigned macroscopically. |

Table 1. Cont.

| Method | Parameter | Study | Description |
|--|--|--|--|
| Light Microscopy | Oxford Histological Index (OHI) | (Hedges et al., 1995; Millard, 2001) | Score of 0–5 to determine the percentage of destruction in a histological section: 0 OHI = <5% intact; 1 = <15%; 2 = <33%; 3 = >67%; 4 = >85%; 5 = >95% preserved. |
| | Porosity and microscopic focal destruction (MFD) | (Hackett, 1981; Nielsen-Marsh et al., 2007) | Categorising small, medium and large pores to determine the mode of degeneration: accelerated collagen hydrolysis, catastrophic mineral dissolution, fungal attack, and bacterial attack. MFD is a feature observed during microscopy and OHI. |
| | Birefringence intensity (BI) | (Jans et al., 2002) | Supplements light microscopy by matching birefringent collagen against damaged areas, scored at 0 (no BI), 0.5, or 1 (full BI). |
| Fourier-Transform Infrared Spectroscopy (FTIR) | Ratios and indices | (Schwarcz & Nahal, 2021; Thompson et al., 2009; Wright & Schwarcz, 1996) | The comparative amounts of P (900 cm^{-1}), carbonate and amide ratios (1250 to 1650 cm^{-1}), and the C:P ratio (1415 cm^{-1} to 1035 cm^{-1}) on an FTIR-ATR spectrum. |
| Scanning Electron Microscopy (SEM) | Crystallinity index (splitting factor) | (Thompson et al., 2009; Weiner & Bar-Yosef, 1990) | A measure of the crystalline lattice structure, which is affected by diagenetic and thermal alteration. Calculated using the peak heights at $(565 + 605)/595$. |
| | Histotaphonomic Characterisation | (Bell, 2011; Bell & Jones, 1991; Longato et al., 2015; Turner-Walker & Jans, 2008) | Increasing porosity and damage of bone sections under SEM. Supplemented with elementary elemental analysis for mapping areas of extraneous material or dissolution. |

Diagenetic Bacteria

Microbiomes of burial environments can be complex and susceptible to many external environmental changes (Lozupone & Knight, 2007; Ursell et al., 2012; Xu et al., 2017). For simplification, Turner-Walker and Jans (2008) identified three broad categories of bacteria involved in the diagenesis of bones (aerobic soil bacteria, cyanobacteria, and sulphate-reducing bacteria). These generalised categories also relate to significantly different environments; aerobic bacteria thrive in aerated soil with loose structure whereas cyanobacteria thrive in waterlogged and aquatic environments (Eriksen et al., 2020; Turner-Walker & Jans, 2008).

There has been debate over the source of most diagenetic bacteria, with the modern consensus being that buried bone and decomposing soft-tissue are the main source rather than the burial environment (Booth, 2017; Child, 1995; Ley et al., 2008; Mein & Williams, 2023; Procopio et al., 2021; White & Booth, 2014). For example, Jans et al. (2004) observed a significant reduction in bioerosion in scavenged and butchered remains (isolated from the abdomen) compared with articulated remains. In a preliminary study, Mein and Williams (2023) observed more diagenetic markers in whole carcass samples compared to limb and defleshed samples when counting diagenetic lacunae for short-term post-deposition rather than OHI in long-term post-deposition. These differences were due to the proximity to acids and putrefactive gut flora released by the stomach as it decomposed (Booth, 2017,

2020; Child, 1995; Mein & Williams, 2023) but, due to the experimental setup discussed by Miszkiewicz et al. (2024), caution and further analyses are required before forensic applications. As such, Turner-Walker et al. (2023) only observed histological tunnelling in bones directly contacting soil and not in bones from whole bodies without soil contact, suggesting that diagenetic tunnelling bacteria is not always sourced from the gut. Morales et al. (2018) also observed similar extensive bacterial attack in samples without gut proximity. Countering these studies, Schotsmans et al. (2024) showed differences between histological degradation in humans and pigs following soft-tissue decomposition, demonstrating that previous studies employed incomplete or unvalidated methodologies and that there was no basis for the proximity to gut putrefaction, resulting in advanced bioerosion. The extent of bioerosion of diagenesis is also potentially more advanced in adult human bone than in animals (Grine et al., 2015; Jans et al., 2004), but extensive bioerosion has been found extensively in animal remains (Morales et al., 2018), and therefore, differences may be due to butchery practices and cleaning of animal remains rather than preferential bioerosion (Grine et al., 2015; Schotsmans et al., 2024; Turner-Walker et al., 2023; White & Booth, 2014). Differences have also been observed across newborn, stillborn, foetal and juvenile remains (Booth, 2017, 2020; White & Booth, 2014) likely due to dietary differences creating a simpler and less efficient microbiome (Booth, 2017, 2020; Koenig et al., 2011; Ley et al., 2008; White & Booth, 2014). Laboratory experiments can produce unrepresentative bioerosion due to the removal of natural microbiomes and the impact on chemical diagenesis. For example, Balzer et al. (1997) inoculated and incubated bones resulting in biomass but no MFD, and Dixon et al. (2008) sterilised and incubated bones with *Prevotella intermedia* (anaerobic microbe common in soil), resulting in MFD patterns unrepresentative of the random dispersal and internal invasion in natural MFD observed by Fernández-Jalvo et al. (2010). Overall, short-term diagenesis appears influenced by the decomposition and microbiome of organic material rather than the biome of the deposition site, and it may present itself differently in forensic casework depending on the post-deposition time and location. It is therefore important to employ a range of analytical techniques and to consider the body, location, deposition environment and subject species as sources of diagenetic bacteria whilst the research investigating exogenic (soil-sourced) and enteric (gut-sourced) bioerosion continues (Booth et al., 2024).

4. Long-Term Microbial Influences

Long-term microbial diagenesis or preservation become dependent on the deposition site and chemical interactions. Soil aeration, groundwater activity, light, pH levels, salinity, and temperature are all critical factors as they open the pathways for bioerosion, determining which microorganism species are present and their abundance (Booth, 2017; Child, 1995; Hale & Ross, 2023; Kendall et al., 2018). Bacteria may switch their metabolic activities when the environment becomes unsupportive but will restart growth and bioerosion when conditions become favourable (Peters et al., 2014).

4.1. Waterlogging Influence

Wetting dry soil boosts microbial activity but waterlogging and poorly draining soils slow microbial activity and favour anaerobic communities by limiting the oxygen content needed for aerobic microbial action (Carter et al., 2010; Eriksen et al., 2020; Latham & Madonna, 2012; Tibbett & Carter, 2011; Troutman et al., 2014; Turner-Walker, 2019). Waterlogged environments are particularly restrictive to biological mechanisms, and flooding can 'restart' bioerosion by replacing bacteria with potentially less-efficient decomposers (Booth, 2017; Huisman et al., 2017; Turner-Walker, 2009). Collagen decay, cyanobacterial tunnelling and fungal tunnelling cease in permanently waterlogged and anoxic environments (Huis-

man et al., 2017; Turner-Walker, 2009, 2019). These features can be useful when determining previous burial conditions; Huisman et al. (2017) observed localised destruction of each form of bioerosion to infer that the bone transitioned from being in a damp environment with periods of oxidation to becoming waterlogged and anaerobic.

4.2. pH Level Influence

There is a close interaction between the microbial community and the elemental content of soil for buffering the pH level and encouraging preservation, evidenced clearly by microbial sulphuric reduction. Sulphate-reducing bacteria acidify soil by oxidising the S into SO_4^{2-} to convert it into sulphuric acid (H_2SO_4) and release H^+ , significantly accelerating the chemical and geological process in soil (Caple, 2004; Kalev & Toor, 2018; Sposito et al., 1999; Tiedje et al., 1984). Without oxygen available to oxidise S, anaerobic bacteria convert S into hydrogen sulphide (H_2S) at a slower rate. Introducing organic matter into soil simulates microbial activity, which exponentially increases the amount of biomass, and in turn, increases the number of varied metabolic processes available for ecosystem activity and subsequent decomposition (Lander et al., 2023; Margesin et al., 2017). Simple organic compounds present in humus are also oxidised by sulphate-reducing bacteria, producing H_2S in the process and encouraging dissolution (Hollund et al., 2012; Lander et al., 2023). However, high humic levels can inactivate enzymes, inhibiting the autolytic and putrefactive processes of decomposition (Hollund et al., 2012). Consequently, Booth and Mercer (1964) and Booth et al. (1962) observed remarkably good preservation of iron nails in the corrosive soils at Hungate, postulated as due to tannins inhibiting enzyme activity of the sulphate-reducing bacteria that are destructive toward iron objects.

Acidic soils see higher proportions of fungal populations whereas alkaline soils see higher proportions of bacterial populations (Kibblewhite et al., 2015). Regarding diagenetic markers, acidic soils should therefore favour fungal tunnelling whereas alkaline environments favour MFD bacterial bioerosion (Hackett, 1981; Nielsen-Marsh et al., 2007). Neutral pH or slightly acidic and alkaline environments allow the development of diagenetic microbiomes (Child, 1995; Collins et al., 2002; Kendall et al., 2018), whereas microbial activity is reduced at extreme pH levels (Latham & Madonna, 2012; Troutman et al., 2014). A neutral pH level therefore has a theoretically high abundance of bacteria, although the source of these bacteria influences whether they are decomposers or protective (Booth, 2017; Kendall et al., 2018). Irrespective of the pH level and associated microbial activity or lack of, chemical processes of acidic dissolution or alkaline degradation will still proceed.

4.3. Soil Composition

Soil provides a potentially protective environment for buried material. Microbial activity may be limited by the toxicity of metal ions in soil (Janaway et al., 1996). Sulphate-reducing bacteria create H_2S from humus, which is toxic to other microorganisms and may have a preservation effect by simplifying the microbial community or inhibiting the growth of degenerative bacteria (Hollund et al., 2012). The direct influence of soil type on diagenesis is insignificant (Booth, 2016; Brönnimann et al., 2018; Forbes et al., 2005), although peat and clay soils generally preserve bones better because they become waterlogged, element-rich and allow for anaerobic conditions more easily than loamy and sandy soils (Hedges & Millard, 1995; Kibblewhite et al., 2015).

4.4. Oxidation Influence

Where arid conditions allow aerobic microbes to rapidly decompose matter, constant waterlogging suppresses these microbes so that most activity is by anaerobic microbes (Holden et al., 2006). The sphagnum mosses and anaerobic, acidic and organic-rich conditions found in peat bogs and deep burial layers create an environment inhospitable to most

bacteria, leading to exceptional preservation of hair, skin and other organic material (Booth, 2016; Bryan et al., 2024; Holden et al., 2006; Kendall et al., 2018; Mula, 2023). Shifting to aerobic conditions allows aerobic bacteria to flourish and rapidly degrade buried bones (Booth, 2016; Turner-Walker & Jans, 2008). This impact may be exacerbated by the elemental profile, where the oxidation of Fe^{2+} accelerates microbial metabolism and thus organic decomposition (Hall & Silver, 2013).

4.5. Microbiome of Preservation

The microbiome for PMI estimation has seen increasing research but the reliance of the microbial community on environmental conditions and time causes difficulty in identifying and scoring the relevant microbes (Díez López et al., 2022; Moitas et al., 2023). Furthermore, there are some sex-specific differences; Bell et al. (2018) and Javan et al. (2016) observed *Rothia* and *Streptococcus* in males and abundances of *Clostridium* and *Pseudomonas* in females. The combination of prenatal and postnatal factors, genetics, medicine, diet, lifestyle, and a myriad of other factors result in a unique microbiome that could be recognised from human remains but also complicate the assessment of post-death processes and time intervals (Dawson et al., 2024; Franceschetti et al., 2024; Zheng et al., 2022). Generally, *Firmicutes* (*Bacillota*) are the first colonisers of a body and show high abundance in the early decomposition stages but rapidly reduce upon rupture of gases (Dong et al., 2019). *Acidobacteriota* also decrease sharply after rupture and subsequent alkalinising of the local environment and soil. *Gammaproteobacteria*, especially from flies, are abundant in early stages and produce gases (DeBruyn & Hauther, 2017; Dong et al., 2019). As late decomposition proceeds, *Bacteroidota* and *Enterobacterales* accumulate following rupture and may be identifiable (Dong et al., 2019; Metcalf et al., 2013). Overall, the transition of microbes post-mortem becomes distinct from those environments without decomposition present (Burcham et al., 2024). If exploring microbiomes for PMI estimation, the investigator must consider developing consistent, comparable and deployable protocols for sampling, extraction and handling processes from the body and soil beneath it (Díez López et al., 2022; Metcalf, 2019).

Soil contains a diverse microbial community, with varying efficiency of preservation and degradation. For example, bacteria that exploit bone proteins are common in most soil types and deposits, but Vraný et al. (1988) observed degenerative enzymes *Gelatinase* and *Collagenase* in samples extracted from sewage, spruce growth, meadow, vegetable field and a garden; however, biological function of these enzymes may be poor in low temperatures typical of burials (Child, 1995). Furthermore, the microbial community structure during soft-tissue decomposition is clearly distinguishable but becomes more homogenous with the deposition environment as time and decomposition proceed (Booth, 2017; Child, 1995) meaning such bacteria may not be identifiable in archaeological contexts. Despite this, Xu et al. (2017) compared the microbial community of the No. 1 Wangshanqiao Chu tomb in Jingzhou, Hubei Province, which saw undisturbed areas with good preservation, and poor preservation in the areas disturbed by robbery and subsequent flooding. The disturbed area was dominated by *Proteobacteria* (*Pseudomonadota*), which has the potential to be more destructive than the *Actinobacteria* (*Actinomycetota*) that dominated the undisturbed area (Xu et al., 2017). Some studies aimed to identify microbial communities relating to archaeological soil environments. Margesin et al. (2017) examined the microbial community of soil samples extracted from a refuse pit and fireplace annexe of a 6th-century BC house at the site of Archaic Monte Iato, Sicily, to identify anthropogenic activity. However, only these two locations were sampled and without comparative controls (Margesin et al., 2017), meaning that they only examined differences between refuse pit and fireplace rather than the impact on the archaeological soil microbiome caused by refusing and fire making. In

contrast, [Peters et al. \(2014\)](#) compared archaeological samples from Kabardinka, Russia, with unmodified reference samples extracted from outside the site, resulting in clearer distinctions between ‘normal’ and ‘anthropogenic’. Finally, [Orr et al. \(2021\)](#) and [Taylor et al. \(2023\)](#) compared the chemical and microbiological characteristics of excavations at Vindolanda that contain exceptionally well-preserved organic artefacts, showing domination of *Firmicutes*, *Proteobacteria*, *Campilobacterota* and *Bacteroidota* alongside changes in Fe, P and S, relating to occupation periods and not burial depth. These studies promoted the potential of microbial soil analysis in archaeology for distinguishing locations of an excavation site.

5. Diagenetic Interaction

Chemical and microbial diagenetic processes interact to determine the preservation or destruction of organic and inorganic objects (summarised in Figure 3). For example, [Hollund et al. \(2012\)](#) compared the histological destruction in bones from different burial conditions at Castricum, The Netherlands, observing less bioerosion in bones buried in clay soils that were generally anaerobic and humic-rich, thus being inhospitable to most bacteria. Limestone caverns contain most hominin fossils because they shelter from weathering, with neutral pH and limited water movement ([Pokines & Baker, 2012](#)). Destructive environments typically have a lower buffer capacity, resulting in an inability to maintain constant, protective conditions, particularly around buried material where pH levels, mineralogical leaching and microbial activity are variable and destructive ([Jans, 2012](#)). As such, Figure 4 shows that a recharge environment (frequent rewetting cycle) does not become saturated because bacteria and leached elements are removed, resulting in continuous dissolution. In contrast, waterlogged environments become saturated with bacteria and elemental content leached from the object, resulting in a state of equilibrium where elements are not being dissolved or absorbed. The pH level may exacerbate these effects, such as through acidic corrosion or inhibiting microbial activity. Irrespective of the site conditions, chemical and microbial markers of decomposition and diagenesis may be considered long-term evidence but can be present within just several months of burial and require consideration in forensic casework ([Booth, 2020](#); [Hale & Ross, 2023](#); [Mein & Williams, 2023](#)).

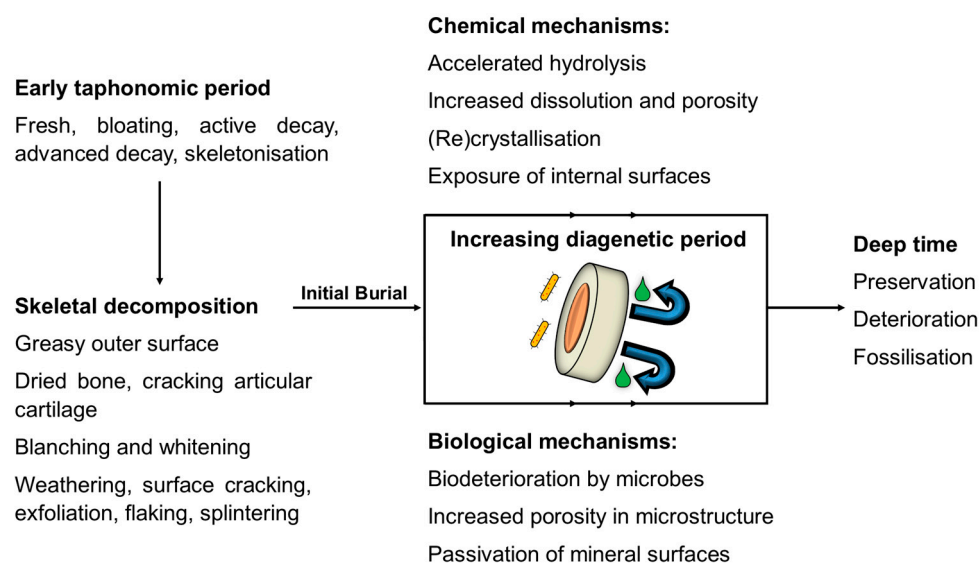


Figure 3. Mechanisms of diagenesis of fresh bone, starting from the early taphonomic (soft-tissue) period and initial burial, progressing through chemical and biological processes toward deep time survival. Depending on the mechanisms present, material may be preserved, deteriorated, or fossilised.

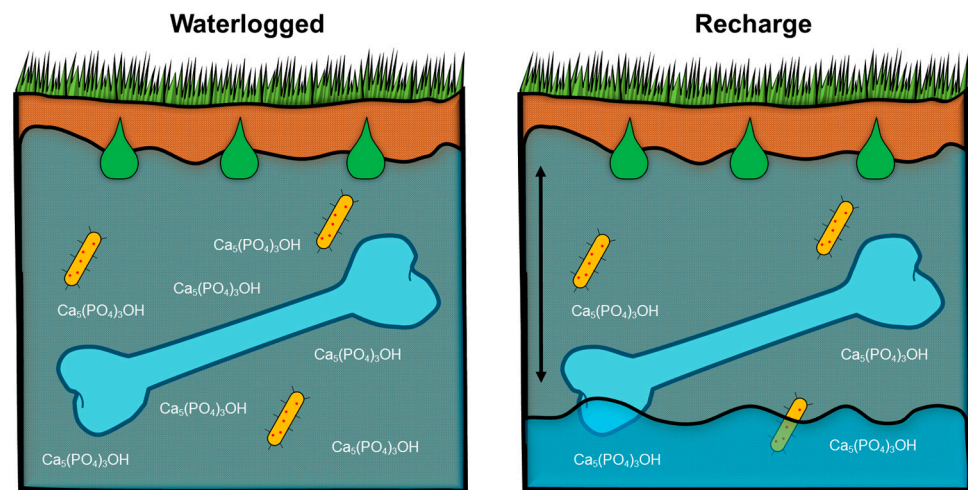


Figure 4. Combined diagenetic effects, demonstrated with waterlogged and recharge water movements. The waterlogged environment becomes saturated with hydroxyapatite from bone, whereas the recharge environment leaches the hydroxyapatite away.

Understanding the interaction between the diagenetic processes and their impact on preservation is essential, particularly when strategising DNA analysis, because diagenetic alteration can determine what analytical tools are available, their subsequent interpretations, and contaminations to account for (Nielsen-Marsh et al., 2007; Sponheimer et al., 2019; Tătar et al., 2014). Sample screening often seeks to detect the quality and quantity of collagen preservation prior to expensive and destructive radiocarbon, stable isotope and DNA testing. For example, Sponheimer et al. (2019) demonstrated the need to screen for bone collagen with spectroscopy before destructive sampling because the highly variable preservation across environments may produce negative results. Bones can also be screened with FTIR to identify environmental contamination and chemical degradation when selecting samples for successful DNA extraction (Scaggion et al., 2024; Tătar et al., 2014). Lebon et al. (2016) and Schwarcz and Nahal (2021) used FTIR-ATR to determine the preservation of collagen and the age of bone, typically achieved via the C:N ratio. A phosphate peak width of 85% is shown to be reliable for informing the structural information of inorganic and organic components of bone (Scaggion et al., 2024). Alternatively, (Durga et al., 2022) showed that minimal amounts of bone can be examined with thermogravimetric analysis to determine the loss and volume of collagen in bone, but not hydroxyapatite. Finally, laser-scanning confocal microscopy has shown some promise in identifying diagenetic change by the different fluorescence in fresh samples compared to five-year-old and ancient samples and may be a valuable method in the future (Smith et al., 2022). When analysing dietary signals in bone, the pre-mortem elemental content and post-mortem soil contamination must be distinguished to ensure valid data (López-Costas et al., 2016). Consequently, there is a necessity to consider environmental and diagenetic parameters before analysing bones.

Soil chemistry and microbiology are fundamentally linked and interact to determine the properties and composition of soil. The dissolution and leaching of buried content into soil is targeted when surveying a site for locating graves and interpreting areas of activity (Cannell et al., 2020; Danielisová et al., 2022; Williams et al., 2021). Additionally, human intervention can influence these to cause substantial alterations in the elemental composition of soil. For example, the annual ploughing of the abandoned church site in Furuland, Norway, resulted in the truncation of topsoil sediment that surfaced skeletal material and dispersed the elemental content leached from the suspected buried graveyard and complicated the identification of the burial zone (Cannell et al., 2018). Conversely, the limited extent of weathering and post-deposit activity at Tel Burna resulted in an elemental

composition in 'fresh' topsoil that reflected closely to the composition of soil sampled from archaeological soil horizons below the topsoil (Šmejda et al., 2018). Ongoing weathering and land-use may disguise the anthropogenic input with other elements or redistribute the anthropogenic soil away from the initial deposit location. These examples show the risk that human activity poses on the survival and analysis of burial sites. However, some sites show extreme levels of preservation and destruction due to the specific conditions encountered and require careful consideration in casework.

6. Extremes of Preservation

The type of environment may have a predisposition toward the chemical and biological diagenetic processes affecting the burial, thus could inform the assumptions on preservation likelihood (Tibbett & Carter, 2009). For example, the structure and texture of soil influences the moisture it can hold, and consequentially, microbial mobility and nutrient diffusion are altered (Troutman et al., 2014). Light and porous soils such as sand generally facilitate the dispersion of water and oxygen facilitate diagenesis whereas dense soils such as clay and wetlands absorb water better and may lead toward the waterlogged and anoxic conditions that allow excellent preservation (Carter et al., 2010; Kibblewhite et al., 2015). Similarly, coarsely textured soils allow gas and moisture to be rapidly removed from the soil, resulting in desiccation (Delannoy et al., 2016). Tumer et al. (2013) demonstrated this experimentally when observing more advanced decomposition in samples buried in loamy and organic soils than in clay and sandy soils, but the quality of data was limited because they only examined one sample per soil for both sets of exhumations. Similarly, Forbes et al. (2005) observed an accelerated formation of adipocere in meat samples kept in moist, warm sands and silts and not in the clay or sterilised soils, concluding that these differences were due to soil type, but the microbially sterile and anaerobic environment would have substantially slowed diagenesis in the clay and sterile soils. In contrast, Booth (2016) observed no significant differences in the microscopic degradation or frequency of bacterial bioerosion in 301 bones from clay, gravel, sand and silt soils across 25 archaeological sites, showing that environmental factors other than soil type are required for well-preserving or destructive conditions. Forensic casework may see a wide range of diagenetic markers depending on the site conditions, and these may cause difficulty in interpreting the site and age of the remains.

Wetlands and waterlogged environments see excellent preservation due to the limited water movement resulting in anoxic conditions and stable elemental exchange. Acidic wetlands can preserve organic material excellently, although inorganic materials will demineralise (Mula, 2023; Pokines & Baker, 2012). These environments are susceptible to climate change and human intervention, such as the lowering water tables at Star Carr resulting in oxidation and acidification of the S-rich soil leading to rapidly dissolving bones (High et al., 2015, 2016), or the altered moisture content and microbial activity of the exposed portions of the anoxic Rose and Globe Theatres putting the site at risk (Caple, 1994; Holden et al., 2006). Depending on the conditions of the wetlands, organic matter and DNA may be excellently preserved for extensive time periods, resulting in a number of bog bodies that were initially assigned and investigated as forensically relevant until being later deemed archaeological (Chapman et al., 2020). Remains mummified from bog environments will therefore require bespoke adjustments when using existing PMI estimation methods (Mula, 2023). Submerged or aquatic burials are typified by sunken ships, such as the excellent preservation of Mary Rose and Vasa due to anoxic water preserving the wood, but their salvage resulted in oxidation and subsequent acidification of contaminating S (Sandström et al., 2002; Sandstrom et al., 2005). Similarly, the forensic analysis of Brienzi in Brienz originally identified the remains as modern sheep due to the adipocere and organic content

presented but was later identified as human remains from the 1700s (Thali et al., 2011). The complexity of waterlogged environments and factors ensuring recovery and long-term survival lead to English Heritage recommending waterlogged reburial policies for the in-situ preservation of these environments (Caple, 1994; Holden et al., 2006), and careful, bespoke approaches being essential for forensic cases. For example, Gawliński et al. (2023) comment on casework involving a bag of human remains deposited in a lake for 24 years and the poor approach taken toward recovering individual items, resulting in many skeletal elements being uncovered and submerged.

Permanently arid environments preserve organic material excellently due to the absence of moisture inhibiting bacterial decay and hydraulic flushing, resulting in desiccation (Monsalve-Vargas et al., 2022; Pokines & Baker, 2012; Tibbett & Carter, 2011). This allowed Shahack-Gross and Finkelstein (2008) to analyse the animal dung that survived at an Iron Age site in the Negev Highlands, Israel, for isotopic analysis of livestock practices. Arid preservation is usually associated with hot climates, but humidity encourages organic decay. This resulted in the poor preservation at Chaminade II, a tropical Middle Stone Age site in the Rift Valley of Africa that informed the diversified evolution of African populations (Wright et al., 2017). Arid environments pose unique considerations due to the potential desiccation and shrinkage in fragile specimens. Weathering is a primary concern in arid environments due to light, dry sediment eroding surfaces through wind action. As such, Simpson and Byard (2020) focused on difficult evaluations of weathered remains recovered over seven years from arid conditions in South Australia, with contextual interpretations and personal effects providing much support where post-hoc testing was unavailable.

The geometry of cave systems can see closed ecosystems with variations in arid and humid conditions that result in unique preservation. For example, arid caves across Nevada contain mummified material in excellent condition, including weaved textiles and the oldest North American mummy, from Spirit Cave (Edgar et al., 2007). Pollen was well-preserved in the arid area of caves in Murcia, Spain, but not in humid or wet areas (Navarro et al., 2002). Similarly, Byard et al. (2020) saw adipocere formation and decomposition smell in remains wrapped tightly in leather and deposited in a desert cave, resulting in an indeterminate period of death.

Glacial environments can provide optimal levels of preservation of all materials and objects due to the absence of bacterial activity, oxygen, water movement, and restricted scavenging (Pokines & Baker, 2012). For example, a frozen Alaskan mummified body with visible tattoos was dated to 400 BC (Smith & Zimmerman, 1975), and several frozen mummified mammoths were discovered in Siberia with preserved wool and stomach contents (van Geel et al., 2011). This can confuse forensic investigations; for example, Ötzi was initially considered a missing mountaineer before carbon dating showed an age over 5000 years old (Kutschera & Rom, 2000). Environmental preservation and special recovery are required because thawing and refreezing can rot and disintegrate the internal structures (Matthiesen et al., 2014). Experimentally, however, frozen remains can show some structural change under SEM but without statistical significance or predictable and systematic patterns (Hale & Ross, 2017; Tersigni, 2007), and repeated, controlled freeze-thaw cycles cause swelling and minor surface cracking that does not extend beyond the initial few cycles but may be exacerbated with moisture and outdoor conditions (Pokines et al., 2016). Climate change also has significant impact, such as melting glaciers revealing WWI soldiers and mass disaster victims from forensic time intervals (Gaudio et al., 2019; Gaudio & Gobbi, 2022).

Funerary practices and disposal methods will also influence the preservation of bone and can exhibit atypical features. Coffins essentially trap the body and grave contents in an enclosed space, potentially causing skeletal erosion due to the pooling of acidic groundwa-

ter (J. Pokines & Blanton, 2022), reduced scattering (Alfsdotter et al., 2022; Maurer et al., 2014), and adjusted weathering procedures due to the delayed onset inside the coffin and eventual bone damage caused by the breakdown of the coffin (Buekenhout et al., 2018). Generally, coffins may delay the initial onset of diagenesis, but the eventual destruction of coffins results in closely relatable and insignificant differences between coffin-buried and soil-buried bodies with other factors such as bone type and individual age having more impact (Bertoglio et al., 2021; Maurer et al., 2014). Vivianite is typically observed in archaeological sites with reducing, anaerobic conditions (McGowan & Prangnell, 2006; Taylor et al., 2019) but has been observed in forensic intervals. For example, naturally formed vivianite encrusting was observed on the skulls of the American military in Vietnam 28 years after death due to soil conditions and nearby iron-rich buried goods (Mann et al., 1998), or with nearby metal objects such as coffin nails, tools and weaponry (McGowan & Prangnell, 2006). Reburial of remains will confound the assessment of diagenesis because the body has been disturbed and relocated into a new environment with potentially drastically different conditions. Depositional practices and changes in the environmental history of bone can be identified via OHI and microscopy to identify arrested bioerosion, variable staining and MFD, and significant destruction from exposure and reinterment (Booth & Madgwick, 2016; Thompson et al., 2024). As discussed throughout, preservation requires a delicate balance in environmental conditions and changing these risks significant and immediate destruction of buried objects. Burning removes organic content and alters the molecular structure of bone due to the extreme heat, resulting in significant transformation (discussed in detail by Ellingham et al. (2015) and Thompson (2015)). In particular, the FTIR ratios and crystallinity index increase with burning (Snoeck et al., 2016; Thompson et al., 2011). Removing the organic composite via burning impedes many analytical techniques, but isotopic analysis can still investigate the inorganic composite with good success (De Coster et al., 2024; Snoeck et al., 2022). However, bodies disposed of with fire might not achieve the temperature and time required for this destruction, resulting in comparatively less bone alteration after discolouration (Carroll & Squires, 2020; Thompson, 2015) and potentially speeding decomposition due to the removal of body tissue and exposure of internal organs attracting insects, as observed by Wang et al. (2019) in several forensic burning cases. Finally, disposing of a body in **corrosive** substances can lead to the total destruction of the body in rapid timescales, depending on the strength and type of corrosive used, and will suggest a much longer post-mortem interval (Fulginiti et al., 2019; Hartnett et al., 2011). A body disposed of via strongly acidic or alkaline conditions will be impacted and visualised by clear manifestations of the associated chemical and microbial mechanisms discussed throughout.

7. Conclusions

Understanding the composition and diagenetic processes of the deposition environment is pivotal to understanding why bones see archaeological survival. Upon death, establishing a new protective environment and reducing the impact of diagenetic processes becomes critical to preservation. Although burying bones may provide a new protective environment, they will be subjected to chemical and biological factors that vary widely across deposition environments (Child, 1995; Hedges & Millard, 1995; Holden et al., 2006; Keenan, 2023; Kendall et al., 2018; Loy et al., 2023). These interactions are interdependent, where most biological mechanisms require chemical degeneration of the bone to provide access to biologically destroy the internal portion, which then exposes internal surfaces for additional chemical degeneration (Child, 1995; Keenan, 2023; Kendall et al., 2018; Lander et al., 2023; Turner-Walker, 2007). Chemical processes such as hydraulics, oxidation and pH level determine the efficiency of microbial decomposition due to their sensitivity to aerobic

or anaerobic and acidic or alkaline conditions (Child, 1995; Collins et al., 2002; Kendall et al., 2018). Once material is exposed from preserving burial conditions, degradation becomes rapid; appropriate recovery, packaging, recording, conservation and protection procedures are a necessity for good recovery and survival of skeletal material.

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