Diagenetic analysis and historical interpretations.  
Case studies from eastern Romania

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Abstract. Interdisciplinary investigations of the human osteological remains help us to understand the genetic diversity, the mobility or the paleodiet of the past communities and offer us insights on the diagenetic processes. The present study aims to assess the results of chemical, mineralogical and microscopic analyses performed on different human osteological remains selected from archaeological sites situated in Eastern Romania in order to understand the diagenetic transformations involved in the site formation processes and to estimate their influence on the historical interpretation.

Rezumat. Analiza interdisciplinară a fragmentelor osteologice umane contribuie la înțelegerea diversității genetice, a mobilității și paleodietei comunităților din trecut, dar și la evidențierea proceselor diagenetice care au afectat structura materialului osteologic. Studiul prezent își propune să integreze date privind compoziția chimică, mineralogică și structurală a unor fragmente osteologice selectate din situri arheologice ce provin din estul României pentru înțelegerea proceselor diagenetice care au afectat formarea siturilor arheologice și modul în care acestea influențează reconstituirea istorică.

Keywords: diagenetic parameters, eastern Romania, human bones, ATR-FTIR analysis, SEM-EDX analysis.

Introduction

The transformations which affect the human bones in the burial environment are of archaeological, paleontological and forensic interest. The preservation of the bone can be estimated by measuring the so-called diagenetic parameters. Understanding the mechanisms, which control the bone diagenesis, is very useful for gaining insights into how the

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archaeological and taphonomic record was formed. One of the key issues in the bone diagenetic studies over the last twenty years was represented by the enquiries into understanding its bio-molecular preservation, especially, for predicting DNA survival. In addition, we regularly use chemical indicators (trace elemental compositions, stable and radioactive isotopes) as a key source of information for dating, climatic reconstructions, identifying past diet and mobility. For this, we must be able to distinguish if we are measuring the original composition and trace the degree of alteration and the extent to which modifies the proxy indicators we are targeting. Another area where diagenetic investigations can offer valuable information is in initiating long-term preservation strategies for archaeological heritage.

Bone structure varies according to the length of scale at which the structure is examined. Bone is thus a hierarchically organized material. Understanding structure is the key to better understanding diagenesis and the information embedded in the structure.

The basic constituents are mineral, organic matter (the organic matrix) and water. The relative proportions of these constituents can vary considerably between bones. The mineral phase of the bone is represented by the carbonate hydroxylapatite \([Ca_{10}(PO_4,CO_3)_{6}OH)]\) which can be described as a more deformed version of the geogenic mineral hydroxylapatite, having some of the initial carbonates replaced by the phosphates. The average mineral content of a particular bone is under strict biological control. Most bones have mineral contents that range from 60 to 70 weight percent. Furthermore, the mineral phase continues to form after it is initially deposited. The forming mineral phase replaces some of the water in the material. During diagenesis, the mineral crystals of bone increase in size and in the atomic order.

The organic phase of the modern bone usually constitutes about 20 % by weight of the material. The major constituent (about 90 % by weight) is the protein type I collagen. In fact, type I collagen is the most abundant protein in the vertebrates. Collagen is composed of polymers of amino acids and therefore comprises hydrogen, nitrogen, oxygen and carbon atoms. The carbon and nitrogen content from the collagen and bone mineral fraction in ratios determined by the food source utilized by the organism. The nitrogen content, also, is dependent on the trophic

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6 WEINER 2010.
7 For further details see TĂTAR et alii 2014.
10 WEINER 2010, 102.
11 WEINER 2010, 87.
12 KEENAN 2016, 1945.
chain. Strontium isotopic ratios preserved in bone are dependent on the age and on the chemical composition of the underlying rock and are therefore a strong indicator for the geographic origin.\(^{13}\)

Collagen loss can be the result of enzymatic hydrolysis promoted by collagenase activity, creating pathways that facilitate microbial invasion. Microbial attack in specific areas produces focal microscopic destruction, during which collagen loss follow bone demineralization, leading to reduction in bone strength. The extent of these changes can vary dramatically depending on the time and conditions of burial. They are especially influenced by factors such as humidity, pH and temperature. While physico-chemical deterioration is accelerated by extreme pH or high temperature, microbial activity is optimized in conditions close to neutral pH.\(^{14}\)

The remaining 10% by weight of the organic matrix of bone is a complex assemblage of other proteins, proteoglycans (proteins associated with polysaccharide chains), and various lipids. Among the noncollagenous proteins (NCPs) is a group of relatively acidic proteins that are thought to play a direct role in mineralization. After type I collagen, the second most abundant protein is osteocalcin (also referred to as bone Gla protein). It is important to note that some of these proteins (including osteocalcin, but excluding collagen) are intimately associated with the mineral phase and cannot be extracted, unless the mineral phase is dissolved. This has important ramifications with regard to diagenesis, where the mineral phase apparently affords such proteins relative protection from breakdown.\(^{15}\)

Bone diagenesis or bone decomposition is considered to consist in dissolution, precipitation, mineral replacement and recrystallization. Bone diagenesis in soil is characterized by destruction of histological integrity, alteration in bone porosity and mineral crystallinity, and loss of protein content.\(^{16}\)

The analysis of the diagenetic processes was initiated as a consequence of the initial enthusiasm registered during the ’80s for the use of trace elements content for paleodiet reconstruction.\(^{18}\) Contamination of bone in the ground takes both physical and chemical forms. The infiltration by foreign materials of the bone tissue is caused by its highly porous structure. Mainly, the contaminants can occur because of precipitation from groundwater (calcium can be introduced through precipitation from groundwater) or as physical incorporation of materials into the bone structure (quartz can be added as solid grains but,

\(^{13}\) KING et alii 2011, 2222.
\(^{14}\) MELLO et alii 2017 with references therein.
\(^{15}\) WEINER 2010, 105.
\(^{16}\) KEENAN 2016, 1943–1944 with references therein.
\(^{17}\) NIELSEN-MARSH, HEDGES 2000.
\(^{18}\) Lately on, most of the authors agreed that dietary studies should be based on isotope analysis, since the trace element contents of prehistoric bones is highly variable and subject to diagenesis (For details see BURTON, DOUGLAS PRICE 2002, 159–167).
also, rootles or fragments of charcoal)\textsuperscript{19}. Elemental distribution tends to vary in an unpredictable manner within individual sections and from one bone to another. These variations are recognized to be caused by the diagenetic alterations, but it is difficult to estimate accurately the potential influencing factors found within the soil composition. However, soil chemistry must to some degree control the floras present which makes essential the evaluation of as many aspects of soil chemistry\textsuperscript{20}.

Bones registering high values for the diagenetic parameters have reduced amounts of collagen and reduced histological index and increased crystallinity, which is caused by the transformation of bioapatite into the more thermodynamically stable apatite\textsuperscript{21}.

Different techniques have been applied to human osteological remains in order to characterize taphonomic alterations, evaluate preservation states and understand diagenetic alterations. Spatially worked out analyses of the molecular and structural composition of bones, which offers a glimpse into the wide heterogeneity of composition and complex hierarchical structure of bones were applied to ancient material\textsuperscript{22}. The chemical composition, the degree of crystallinity and of organic content is widely estimated by using Fourier Transform Infrared Spectroscopy (FTIR) applied to bulk samples. Recently, Attenuated Total Reflection–Fourier Transform Infrared Spectroscopy (ATR-FTIR) mode has been used for assessing both qualitative and quantitative information on ancient human bones and for estimating the diagenetic transformations\textsuperscript{23}. The main benefits of using FTIR spectroscopy in ATR mode resides in the minimal sample preparation ensuring faster analysis of the inner and outer side of the sample and reducing the influence of sample preparation on the results, although there are some constraints on the size and shape of the sample.

Due to the changes in bone structure after death (even after a very short period), including the secondary infiltrations of remineralization, microscopic study is essential to understand this wide range of transformations\textsuperscript{24}.

The groundwater and soil may introduce elements, which are included in different ways into the bone structure. They may reside in pores, voids or microcracks in the bone matrix, form complexes with the organic component, adsorb onto the surface of hydroxyapatite matrix and hence may be transported into the bone structure via diffusion processes by ionic

\textsuperscript{19} DOUGLAS PRICE et alii 1992, 514.

\textsuperscript{20} The ion exchange process registered in different soil types is an important aspect for understanding the whole diagenetic process starting from dissolution to recrystallization. For further details see BELL 1990, 86–87 with references therein.

\textsuperscript{21} SMITH et alii 2005, 107.

\textsuperscript{22} HOLLUND et alii 2012; LEBON et alii 2014; DAL SASSO et alii 2016.

\textsuperscript{23} DAL SASSO et alii 2016, 169 with references therein.

\textsuperscript{24} BELL 1990, 85–100.
exchange\textsuperscript{25}. For an accurate estimation of elemental concentrations spot chemical analysis are necessary.

After describing the human bones sampled, the methods we have chosen to measure the selected parameters, we explain briefly the significance of the defined diagenetic parameters, and summarize the results obtained. The relationship between diagenetic parameters and the possibility to use them for extracting different types of historical information are then discussed.

\textbf{Materials and methods}

Archaeological human bones samples were selected from a larger database of material collected from a number of sites, which were selected for DNA analysis throughout the project \textit{Genetic Evolution: New Evidences for the Study of Interconnected Structures. A Biomolecular Journey around the Carpathians from Ancient to Medieval Times} (GENESIS).

The samples were selected from a variety of different burial contexts from eastern Romania. The age of the human bones elected for this study ranges from c. 4100 BC to 10\textsuperscript{th} century AD. The site location is presented in Figure 1 while the number of samples from each site and the archaeological contexts are given in Table 1.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{archaeological_sites.png}
\caption{The location of the archaeological sites from where the bone samples were selected.}
\end{figure}

\textsuperscript{25} CARVALHO, MARQUES 2008, 32 with references therein.
Table 1. The identification, location and chronology of the analysed human bone samples.

<table>
<thead>
<tr>
<th>No.</th>
<th>ID</th>
<th>Archaeological site</th>
<th>Archaeological context</th>
<th>Chronology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S22</td>
<td>Vorniceni (Botoșani)</td>
<td>A ritual pit (Pit 40) containing a disarticulated human bone identified in section S22 (▼1.10–▼3.10m)</td>
<td>4100–3800 BC</td>
</tr>
<tr>
<td>2</td>
<td>PM1</td>
<td>Popeni (Vaslui)</td>
<td>Half of a tumulus containing a woven structure (4.90 × 2.90m), the remnants of a wooden structure, a burial pit, two lumps of ochre and the anthropological remains of an adult (▼3 m)</td>
<td>2800–2500 BC</td>
</tr>
<tr>
<td>3</td>
<td>T72</td>
<td>Tarnița (Bacău)</td>
<td>Single flat grave containing a flexed human skeleton and two vessels identified in S IV (▼0.30–▼0.80m)</td>
<td>1400–1150 BC</td>
</tr>
<tr>
<td>4</td>
<td>M15</td>
<td>Isaiia (Iași)</td>
<td>Grave M15 of the Sarmatian necropolis was identified in 2003 in the southern part of L8 (▼1.80m)</td>
<td>1st–2nd century AD</td>
</tr>
<tr>
<td>5</td>
<td>M13</td>
<td>Isaiia (Iași)</td>
<td>Grave M13 of the Sarmatian necropolis was identified in 2003 in the southern part of L8 (▼1.75m)</td>
<td>1st–2nd century AD</td>
</tr>
<tr>
<td>6</td>
<td>M18</td>
<td>Isaiia (Iași)</td>
<td>Grave M18 of the Sarmatian necropolis was identified in 2004 in the southern part of L8</td>
<td>1st–2nd century AD</td>
</tr>
<tr>
<td>7</td>
<td>M1</td>
<td>Capidava (Constanța)</td>
<td>Grave M1 was identified in 2010 in section X extramuros (▼0.80m)</td>
<td>10th century AD</td>
</tr>
<tr>
<td>8</td>
<td>M3</td>
<td>Capidava (Constanța)</td>
<td>Grave M3 was identified in 2010 in section X extramuros (▼0.90m)</td>
<td>10th century AD</td>
</tr>
<tr>
<td>9</td>
<td>M4</td>
<td>Capidava (Constanța)</td>
<td>Grave M4 was identified in 2010 in section X extramuros (▼0.95m)</td>
<td>10th century AD</td>
</tr>
</tbody>
</table>

Sample S22 was selected from the archaeological site located at Vorniceni village (commune Vorniceni, Botoșani county) (Figure 1) which is situated on the terraces belonging to the Jijia river basins. The human remains discovered at Vorniceni (Botoșani county) are represented by 12 disarticulated bones that were identified in different ritual pits, which contains large amounts of richly decorated pottery, and animal bones. The human bones were attributed based on the associated artefacts identified in the pits to the Cucuteni A-B communities. The human bone which was sampled for diagenetic and aDNA analysis was classified based on the anthropological analysis as being a long bone (femur) coming from an adult male, age 40.

27 Identification done by G. Miu, according to DIACONESCU 2012, 19.
Sample PM1 comes from a skeleton identified in a western half of a tumulus partially explored in a recently rescue excavation in Popeni (Găgești commune, Vaslui county) (Figure 1) within the Elan basin\(^{28}\). The kept half of the tumulus covers a funerary structure, which contains a human skeleton in a left crunched position. The left hand was flexed with the palm at the skull level while the right hand was slightly lodged in the pelvis area and the legs were strongly bent on the left side\(^{29}\). The tumulus is located at the western extension of the Yamnaya burial barrow groups (Early Bronze Age) which are noticeable even nowadays in the area. The osteological remains were in a very poor state of preservation and the sample for diagenetic analysis was selected from the femur.

The osteological remains from where the T72 sample was selected belong to a funerary context located in the Tarnița village (Oncești commune, Bacău county) (Figure 1) which consists in a pit containing a human skeleton in a left supine position. The left hand was flexed and sustains the skull while the right hand was slightly embedded on the pelvis and the legs were strongly flexed on the left side. Based on the associated grave goods (two pottery vessels) the funerary remains were attributed to the Late Bronze Age Noua culture from the eastern part of the Siret basin\(^{30}\). The osteological remains were in a poor state of preservation and the samples for the aDNA\(^{31}\) and for diagenetic analysis were taken from the humerus.

Samples M13, M15 and M18 were taken from the Sarmatian necropolis identified in the Isaia village (Răducăneni commune, Iași county) (Figure 1) located on the lower terraces of the Prut river\(^{32}\). The human bones from M13 belong to a young woman (age 25–30) being relatively well preserved. As grave goods, glass beads and a bone pendant are present\(^{33}\). M15 represents the richest grave, which includes the badly preserved remains of an old woman (age 60–65). The rich funerary inventory consists in glass beads, amber and lapis lazuli beads, a spindle whorl, an iron fibula and a bronze mirror\(^{34}\). M18 contains the remains of an adult woman (age 40–45) buried with no grave goods. The human remains buried in M18 are in a very poor state of preservation\(^{35}\). For M8, M13, M15 the bone sample for diagenetic analysis was taken from the humerus, while for M18 the femur bone was selected.

The human bone fragments M1, M3 and M4 come from the Middle Age necropolis identified at Capidava (Topalu commune, Constanța county) (Figure 1) which is situated on one of the Danube terraces (B terrace). The osteological remains identified in M1 were in a supine position with the hands assigned on the pelvis. Based on the anthropological analysis,
the remains belong to a female (age 40–45) buried without any grave goods. M3 contains a skeleton in a supine position with his hands upon his chest attributed to a woman (age 40–45). As grave goods, a bronze ring decorated with a pentagram was found. The human remains discovered in M4 belong to a young male (age 30) and has a bronze hoop earring and two bronze button pendants as grave goods. The human remains found in M1, M3 and M4 were in a good state of preservation and the samples for diagenetic analysis were taken from the femur.

All the human bones we have sampled were selected to represent as much as possible the same state of diagenesis for the selected bone. Each bone was rinsed in tap water and carefully brushed, to remove all possible contamination layer. After the cleaning procedure, all the samples were washed in distilled water and dried in a clean environment. From each bone, a small sample (1x1 cm) was sectioned using a low speed diamond saw. Prior to analysis, the samples were cleaned in an ultrasonic bath with ethanol for a few minutes and air dried overnight.

The spot chemical analysis of the inner and outer surfaces of all samples, in terms of major and minor elements, and the microscopic analysis for detailed structural analysis were determined by Environmental Scanning Electron Microscopy – Energy Dispersive X-ray (ESEM-EDX) analysis. The sectioned bone fragments were fixed on copper supports and their inner and outer surfaces were examined using an Environmental Scanning Electron Microscope (ESEM) type Quanta 200, operating at 20 kV with secondary electrons in Low vacuum mode. The Quanta 200 microscope is equipped with an Energy Dispersive X-ray (EDX) detector for qualitative and quantitative analysis and elemental mapping.

The same bone sample was analysed using a Bruker Vertex 70 FTIR Spectrometer equipped with a ATR Golden Gate diamond crystal. The distribution of the main components and structure of the mineral and organic content of the bone fragments were monitored from the absorbance ratios (4000–600cm⁻¹) in order to avoid variations of raw intensities due to the quality of the contact between ATR crystal and the sample.

Results and discussion

The classical indices that we have monitored by ATR-FTIR analysis consisted in the use of carbonate and phosphate vibration bands to evaluate the mineral composition and of the Amide I band to estimate the degree of collagen preservation in the human osteological remains selected from different archaeological sites from eastern Romania (Figure 1). The relative carbonate content was inferred from the peak intensity of the absorbance of band \( \nu_3 \text{CO}_3 \) band at 1415 cm⁻¹ while for the phosphate content we have used the intensity of the

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36 PINTER et alii 2011, 388.
ν₃PO₄ band at 1035 cm⁻¹. In addition to these, we have referred to the intense band extending between 900 and 1200 cm⁻¹ that covers the ν₁ν₃PO₄ domain composed by the symmetric (ν₁) and anti-symmetric (ν₃) P-O stretching vibrations. Based on the sub-bands included in this large peak we can distinguish between the phosphate groups present in the apatite environment (1020–1100 cm⁻¹ spectral area) and the non-apatite phosphate environments (1100–1200 cm⁻¹ spectral domain)³⁸.

For the evaluation of the degree of crystallinity, the classical approach is to use the so-called splitting factor measured from the two anti-symmetric bands of phosphate (ν₄PO₄) at 565 and 603 cm⁻¹. The type of detector we have used for ATR-FTIR analysis did not allow us to estimate the ν₄ domain, the 600–400 cm⁻¹ spectral domain being outside the detection range. For a rough estimation of the crystallinity, we have looked at the peaks from 1030 to 1020 cm⁻¹ and 1060 to 1075 cm⁻¹ in relation to the baseline between 900 and 1200 cm⁻¹.⁴⁰

In order to complement the results of the ATR-FTIR analysis of the composition of the inclusions in bone pores and of the remaining bone structure we have performed chemical spot investigations by using EDX analysis.

Towards distinguishing between the factors influencing the diagenetic parameters under evaluation in this study, we have grouped our samples in the graphical presentation of the results by chronology and geographical distribution.

In Figure 2 the mineralogical and collagen content of the oldest bone we have selected are presented. The peak intensity of the Amide I spectral area, which is representative for the collagen, has very low values, sample S22 has the highest intensity, while for sample T72 the Amide I band is almost non-visible. The spectral bands specific to the carbonates content show very similar aspects for all the samples listed in Figure 2. The main differences between the Chalcolithic (S22), Early Bronze Age (PM1) and Late Bronze Age samples (T72) consists in the intensity of the ν₁ν₃PO₄ spectral domain, sample PM1 having the highest intensity. Due to the burial conditions, flourine from the water-bearing soil and sediments substituted into the original structure of PM1 bone sample. A sharp peak present at 1096 cm⁻¹ is characteristic of apatite in which fluorine is substituted in hydroxyl sites forming francolite.

Even if a small amount of fluorine occurs in the original hydroxylapatite and its low solubility contributes to the sharpening of the shoulder at 1096 cm⁻¹, the clear separation of this distinctive peak indicates significant diagenetic change of the hydroxylapatite structure.⁴¹ If we look at the degree of crystallinity, we observe the same pattern as for

³⁷ WRIGHT, SCHWARCZ 1996, 935.
³⁸ LEBON et alii 2010, 2267.
³⁹ WEINER, BAR-YOSEF 1990, 191.
⁴⁰ LEBON et alii 2010, 2268 with references therein.
⁴¹ WRIGHT, SCHWARCZ 1996, 939.
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Figure 2. ATR-FTIR spectra of the outer (ext) and inner (int) surface of the bone samples from Vorniceni (S22), Popeni (PM1) and Tarnita (T72).

Figure 3. Chemical composition of the outer (ext) and inner (int) surface of the bone samples from Vorniceni (S22), Popeni (PM1) and Tarnita (T72).
ν1,ν3PO₄ spectral domain, which shows an increase in the peak intensity for the PM1 sample reflecting the extension of the diagenetic transformations.

Based on ATR-FTIR analysis we could not see any major differences between the inner and outer part of the bone samples in terms of mineralogical transformation, collagen loss and the increase of crystallinity.

For understanding, the mechanisms of contamination, which operates from the surface in, we undertook spot elemental determinations of the inner and outer surfaces of the bones by EDX analysis (Figure 3). The chemical composition presented in Figure 3 shows the uptake of contaminative elements (Fe, Al, K, Mn, Si, S, Cl) into the bone structure. All these external elements present in the burial environment have moved into the bone matrix by several different mechanisms, including exchange with natural bone constituents, deposition in unfilled voids or defects, and adsorption on the surface. Strontium is considered to replace calcium in the hydroxyapatite matrix; Mn, Fe and Si normally fill voids without heterionic exchange, while Ca and Na could leach out of the bone matrix. In the samples presented in Figure 3, Sr has higher value for the outer surfaces of S22 (0.70%) and PM1 (0.88%) caused by the partial replacement of the Ca in the bone matrix. A higher content of Fe (5.03%) and Si (9.19%) was detected in the voids from the inner surface of S22. For samples S22 and PM1 we observed a significant depletion in the Ca content in the inner bone surface (8.9%, 18.36%) in comparison with the outer surface (27.67%, 23.42%) which can be caused by the possible soil contamination.

Another proof for the environmental contamination can be the higher content of P for the outer surface (11.67%) of S22 in comparison with the inner surface (2.92%). The use of the spot chemical analysis allowed us to observe some differences between the inner and outer surface of the analysed human bone samples and to trace the environmental contribution to the diagenetic transformations.

SEM analysis was conducted for the complementary study of the composition and morphology of the inclusions present in human bone pores and of the remaining bone structure. According to the microphotographs presented in Figure 4, we can observe partial diagenetic transformations (a, c) and extensive diagenetic alterations (b) which had changed (d), removed (e) or obscured (f) the characteristic morphology and density associated with adult human bone.

The diagenetic alterations can be estimated based on the evaluation of the degree of mineralization, which relates to the destruction of the smaller crystallites and the appearance of the “demineralized zones” or to the re-crystallization and the appearance of the

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42 LAMBERT et alii 1985, 479 with references therein.
43 EZZO (1994, 8) reports a value of 38% Ca content in modern adult human bone.
Figure 4. Low-magnification (a, b, c) and high-magnification (d, e, f) images of the bone samples from Vorniceni (S22) (a, d), Popeni (PM1) (b, e) and Tarnița (T72) (c, f).

Figure 5. ATR-FTIR spectra of the outer (ext) and inner (int) surface of the bone samples from Isaiia (M13, M15, M18).
“hypermineralized zones”\textsuperscript{44}. Based on the microscopical analysis we could identify in samples S22 (Figure 4/d) and PM1 (Figure 4/e) the presence of hypermineralized zones caused by the dissolution and reprecipitation of hydroxylapatite, while sample T72 (Figure 4/f) show the creation of demineralized zones\textsuperscript{45}.

The bone sample from Popeni (PM1) has a crumble texture (Figure 4/b) exhibiting extensive destruction of the organic matrix of bone (collagen), thus facilitating dissolution and remineralization which corresponds to extensive diagenetic alterations (Figure 4/e). Most of the bone surface of samples S22 and T72 shows islands of intact bone combined with obvious dissolution and re-precipitation of bone mineral reflecting partial diagenetic change caused, mainly, by bacterial attack (Figure 4/d, 4/f)\textsuperscript{46}.

Figure 5 presents the spectra obtained from the bone samples attributed to Sarmatian population from the necropolis identified at Isaiia (Iasi county). The Amide I spectral domain indicating the amount of collagen has a higher intensity in comparison with the samples presented in Figure 2 which indicates a better-preserved collagen content. The peaks reflecting the carbonates have a higher intensity while the peaks specific to $\nu_1,\nu_3\mathrm{PO}_4$ spectral domain show a decrease in intensity when compared to the samples listed in Figure 2. The environmental contamination is reflected by the presence of the narrow band at 712 cm$^{-1}$ specific to the secondary calcite\textsuperscript{47} suggesting a more intense circulation of water within the sediments since bone deposition. The precipitation of secondary calcite caused, also, an increase in the degree of crystallinity, especially for the outer surface of the M18 bone sample.

The incorporation of the contaminative elements into the bone matrix can be detected from the chemical composition presented in Figure 6. For the bone samples selected from the Sarmatian necropolis, we observed some differences between the uptakes of the different contaminative elements into the pores. For sample M15 we noticed some increased values of Mn (2.52%, 3.48%) and Fe (4.65%, 3.85%), and lower values for the Si (0.5%, 0.37%) content. P shows increased values for all samples on the outer surface due to the different sources of contamination present in the soil.

For the P values, we could observe a good correlation between the chemical composition and the ATR-FTIR phosphate peak intensity, which shows more intense peaks for the outer surface of the bone samples reflecting the environmental contributions.

The large voids visible in Figure 7/a (on the right side of the image) were created, mainly, by soil bacteria which appear dispersed and randomly distributed, independent of the

\textsuperscript{44} HEDGES \textit{et alii} 1995, 207.  
\textsuperscript{45} BELL 1995, 58.  
\textsuperscript{46} FERNANDEZ-JALVO \textit{et alii} 2010, 65.  
\textsuperscript{47} SALESSE \textit{et alii} 2014, 45.
Figure 6. Chemical composition of the outer (ext) and inner (int) surface of the bone samples from Isaiia (M13, M15, M18).

Figure 7. Low-magnification (a, b, c) and high-magnification (d, e, f) images of the bone samples from Isaiia (M13-a, d, M15-b, e, M18-c, f).
histological structure of the M13 bone sample. The area affected by bacteria is bounded by a dense, reprecipitated hydroxylapatite (Figure 7/d). The low magnification microphotograph obtained for M15 (Figure 7/b) and M18 (Figure 7/c) show areas of intact bone, while at higher magnification we could identify some small areas of demineralized zones (Figure 7/e, 7/f) which are relatively depleted in the phosphorous content, according to the EDX analysis listed in Figure 6.

Based on the chemical spot analyses we could identify more variation in the demineralized zones than in the unaltered bone structure. Despite obvious demineralization of the bone structure, the integrity of the bone mineral content appears to be preserved. Furthermore, the movement of mineral must be a local phenomenon caused by incipient diagenetic alterations.

The degree of mineralization observed in the SEM images (Figure 7) correlates with the degree of crystallization identified based on the ATR-FTIR spectra and provides information about how physical changes to the bone tissues influence chemical changes and mineralogical transformation and survival.

The spectral domains registered for the human bone samples selected from the medieval necropolis identified at Capidava are presented in Figure 8. Samples M1, M3 and M4 show the highest peak intensity for the Amide I spectral region from all the samples we have analysed (Figure 2 and 5) reflecting a good preservation of the collagen content. Even if all the samples have the same age and very similar contexts, we could observe some differences in the carbonate area peak intensity.

Sample M1 has the most intense peak in the carbonate spectral domain and registers, also, the presence of the secondary calcite band identified 712 cm\(^{-1}\) that reflects some differences in the water circulation within the sediments. In the phosphate spectral region, we observed the existence of more intense peaks for the outer surfaces of the analysed samples, which could be caused by the enrichment in the P content due to the soil contamination. Due to the enhanced carbonate content, sample M1 registers, also, an increase of the degree of crystallinity in comparison with the other bone samples listed in Figure 8. Recent studies revealed that differences in crystallinity do not play a major role during the early stage of bone hydroxylapatite diagenesis and that carbon isotope exchange in bone might be controlled by collagen preservation.

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48 TURNER-WALKER 2008, 17 with references therein.
49 TURNER-WALKER, SYVERSEN 2002, 466.
50 A. Zazzo identified the existence of three different trends in the \(^{14}\)C dating of enamel, dentine and bone apatite: enamel older than dentine or bone apatite; enamel younger than dentine or bone apatite and identical ages in three different fractions (ZAZZO 2014).
The elemental values obtained for bone fragments selected from the Capidava necropolis are shown in Figure 9. The contaminative elements have a different behaviour than in the previously presented samples (Figure 3, Figure 6).

Mn is the only element present mainly in the pores from the inner bone surface in different amounts ranging from 0.38% (M4) to 1.46% (M4). Si, Fe and S have higher values for the outer bone surfaces while Ca and P shows, also, significant higher contents for the outer bone surfaces reflecting a good preservation of the bone matrix and a minimum effect of the environmental contaminants.

![Figure 8. ATR-FTIR spectra of the outer (ext) and inner (int) surface of the bone samples from Capidava (M1, M3, M4).](image)

![Figure 9. Chemical composition of the outer (ext) and inner (int) surface of the bone samples from Capidava (M1, M3, M4).](image)
The elemental values obtained for Ca and P are in agreement with the results obtained by ATR-FTIR analysis, which revealed different mechanism of calcite absorption for the investigated samples. The presence of the contaminative elements in different amounts on the outer and inner bone surface complemented the ATR-FTIR detection of collagen content and helped us to understand the limited extension of the diagenetic alterations.

The SEM microphotographs presented in Figure 10 exhibits typical field of good bone preservation. However, there are gradations of preservations within the analysed bone samples from Capidava such as the very fine cracks (Figure 10/a–c) resulting from microfractures lines originated by soil pressure and products of the diagenetic crystallization process (e.g., calcite crystals revealed by the ATR-FTIR spectra and spot EDX analysis)\(^51\). Thus, newly crystallized structures can sometimes aggregate and partly break up the internal structure of the bone causing the appearance of the hypermineralized zones (Figure 10/d).

It is therefore not surprising that the microscopically well preserved medieval specimens (Figure 10) produced much better Amide I (collagen) values evidenced on the ATR-FTIR vibration bands (Figure 8) than the sample relatively less well preserved EBA bone sample from Popeni (Figure 4/b, e).

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\(^51\) More details on the microenvironmental contributions to diagenesis can be found in Schultz, Schmidt-Schultz 2015.
Conclusions

In this study, we have identified the extent and variation of the main diagenetic parameters of human bones from five archaeological sites from eastern Romania such as the change in protein content, crystallinity and microstructural preservation. In particular, we have shown that measurements of the mineralogical preservation and transformation correlated with the microstructural and chemical analysis give a clear and direct indication for the degree of diagenetic transformations. Also, we could identify that the diagenetic changes are largely independent of the age of the material. This may suggest a different timescale for the protein loss, the destruction of microstructure and the mineral recrystallization. Therefore, diagenetic parameters express different types of information about the interaction of the human bones with the burial environment.

In this study, bone chemistry, mineralogy and microstructure were investigated at the intra-individual scale in order to understand the effects of diagenesis on skeletons buried in different environments from Eastern Romania. Bone Ca/P, degree of mineralization and microstructural transformations revealed that the skeletons selected from five archaeological sites suffered from different diagenetic processes generated by specific environmental conditions prevailing within the immediate surroundings of the skeletons.

Such studies show that if you do not consider diagenesis, the embedded information you obtain can be misleading. If the aDNA was damaged, then erroneous information could be obtained. In addition, diagenesis can seriously alter the accuracy of the isotopic results. The process through which strontium substitutes easily calcium in the inorganic fraction of bone during life continues to apply post-depositional. The same rule applies to carbon isotopes ratio in the bone apatite, often used in conjunction with collagen carbon can be modified by the addition/removal of carbon after burial. Sometimes the carbon/nitrogen analysis in collagen is not possible due to the breakdown of the collagen molecules during diagenesis.

Bone diagenesis is, also, important, when evaluating the reliability of $^{14}$C dates due to the exchange of carbonate between bioapatite and burial environment. The carbon isotopes exchange appears immediately after burial and always causes higher levels of $^{14}$C in the carbonate phase.

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**References**


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