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STUDIES OF ARCHAEOLOGICAL BONE STRUCTURE BY DIFFERENT ANALYTICAL METHODS

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Preservation of the structure of archaeological bones was studied by examining their physicochemical properties. The mechanical properties, and conductivity porosity, crystallinity and content of organic material were evaluated. These parameters were established to be suitable for characterizing changes in the course of time of archaeological bone structure. The obtained results revealed that the elastic modulus and conductivity changes depended on the porosity of bones; and the increase in crystallinity was correlated with a loss of organic matter.

Key words: *archaeological bones, diagenesis, mechanical properties, crystallinity, conductivity, porosity.*

INTRODUCTION

Bone is a major component of the archaeological and palaeontological records [1]. From the point of view of chemistry, it contains information at many different levels (isotopic, molecular, biochemical and structural). Bone consists of major components – the mineral component bioapatite – and organic matter, mainly proteins. The main changes that occur to the bone by interaction with the burial environment are: breakdown and leaching of collagen by microbiological attack; alteration and possible leaching of the inorganic mineral matrix; infilling with soil mineral deposits [2]. The compounds of mineral and organic matrix are affected by diagenesis influencing the porosity, mechanical properties and crystallinity of bone material structure [3–5].

Various techniques have been used for studying the physicochemical properties of bone structure. In this work Fourier transform infrared spectroscopy (FTIR) with the ATR (Attenuated Total Reflectance) sampling mode and X-ray powder diffractometry (XRD) were used for identifying the chemical composition of bone samples; mechanical testing was used for evaluation of the mechanical properties of bones; thermogravimetric analysis (TG) was used for quantifying the organic compounds, flame atomic absorption spectroscopy (FAAS) was used for quantifying the calcium content and electrochemical impedance spectroscopy (EIS) was used for measuring the conductivity of archaeological bones.

EXPERIMENTAL

Characterization of archaeological samples. Osteological material was obtained from two archaeological complexes. Zvejnieki archaeological complex was located over a small hill on the northern side of the bank of Burtnieku Lake. Archaeological artifacts and bone remains represented Late Mesolithic (8150-6500 BP) and Neolithic (6500-4200 BP) periods. This complex was one of the most significant hunter – fisher – gatherer cemeteries in northern Europe. Burial soil environment was mainly gravel type [6].

The second set of bone remains was taken from St. Peter's churchyard. It characterized Riga city urban population in medieval period (13^{th}–17^{th} century). The cultural layers were found with very complex stratigraphy during excavations in 2004. The burials and urban cultural layers had been mixed with different kinds of objects because of the small size of cemetery area and its intense use during the period of five centuries. From a geological point of view the lower horizons exposed the dark cultural layer mixed with blue clay in places, but in the higher layers there was only sandy soil [7].

To describe the preservation status of bone structure, 6 samples from the individual skeletons were collected from St. Peter's churchyard and 8 samples were collected from Zvejnieki archaeological complex.

Specimen preparation. A diamond saw was used to cut out the layer of the middle part of compact bone tissue (tibia). The tubular sample was obtained for the analysis. Specimen preparation for different measurement techniques was given below:

a) *ATR-FTIR, XRD and TG*. The dried sample was ground by Retsch MM301 ball mill and thereafter homogenized using oscillation frequency of 30 Hz and homogenization time of 300 s. The fraction of particle size below 40 μm was used for measurements.

b) *FAAS*. To quantify calcium content in the inorganic fraction, bone matrix ashes obtained in thermogravimetric (TG) analysis were dissolved in concentrated nitric acid and diluted with deionized water.

c) *Mechanical testing*. Compression testing was performed on dry tubular specimens. The radial direction (orthogonal to the fiber direction) of samples was chosen. Each sample was 8 mm wide and 1.25 mm thick (±0.25 mm). Thickness of samples varied due to manual cutting and polishing of the bone pellet.

d) *EIS*. Samples were prepared as for mechanical testing. As bone is a dielectric material, the prepared specimens were before conductivity measurements soaked in isotonic physiological liquid (0.1*M* NaCl) for filling of the pore space of compact bone.

XRD. The powder X-ray diffractometer (*Bruker D8 Advance*), with a Cu K_α X-ray source (λ = 1.54056 Å) and a scintillation counter as the detector was operated at 40 kV and 40 mA. Each sample was scanned from 23 to 43° 2θ in 0.02° step.

ATR-FTIR. IR spectra were registered on *Nicolet 6700* FTIR spectrometer with the "Smart Orbit" diamond micro-ATR accessory. The spectrometer had *DLaTGS* Detector, *Vectra* Aluminum Interferometer and sealed and desiccated optical bench with CsI optics. In order to protect the spectrometer from atmospheric moisture it was constantly purged with dry air. Smart Orbit is pre-

sented itself a horizontal single-bounce ATR microsampling accessory with angle of incidence of 45° and active sampling area diameter of 1.5 mm. It has a diamond ATR crystal (index of refraction 2.418). The spectral range available with Smart Orbit was 10000–55 cm^{-1}.

The following spectrometric parameters were used: resolution 4 cm^{-1}, spectral range 225–4000 cm^{-1}, number of scans 256. The interferometer of the spectrometer was controlled by the fixed wavelength helium–neon laser (632.9 nm). Thermo Electron *OMNIC* software for FTIR spectrometer was used to collect and process the IR spectrum.

TG analysis. Thermogravimetric analysis was performed on TG/DTA 6300 apparatus. The bone samples during heating were recorded from 30 ^oC to 800 ^oC at a heating rate of 10 ^oC min^{-1} in nitrogen atmosphere (N_2 flow 50 cm^3 min^{-1}) [8].

FAAS. The quantitative determination of Ca in solutions was done using *Perkin Elmer AAnalyst 200* FAAS system. Main instrumental conditions for Ca element determination were: multielement hallow cathode lamp for Ca, Mg, Al, basal emission wavelength 422.7 nm with the Deuterium lamp background correction and air – acetylene flame. To avoid ionization process 1% of KCl was added to the all extract solutions.

Mechanical testing. Compressive tests were done by using the *Zwick Roell* universal testing machine *BDO-FB020TN*. All specimens were tested at constant deformation speed of 3 mm min^{-1} and 20 °C temperature. A needle of 1.0 mm diameter was used for applying the penetration force on the bone specimens.

EIS measurements. The EIS analysis was performed using an *Autolab PGSTAT 30* potentiostat. Electrical impedance was measured using a three electrode system. Cell parameter: distance between anode (Pt – plate) and cathode (Pt – bone) – 1.0 cm. The calomel reference electrode was used. A bone pellet was attached to the platinum plate electrode using parafilm. To avoid air burbles in the electrode system it was vacuumed. Measurement parameters – the applied potential –0.25 V in a frequency range of 0.01 Hz to 35 kHz.

RESULTS AND DISCUSSION

X-ray powder diffractometry was primarily used for detection of major inorganic compounds in the bone samples. As seen from the X-ray powder diffraction pattern, the hydroxyapatite was observed corresponding to the reflections [002], [211+122], [300], [202] in all bone samples (Fig. 1).

As it was observed from the X-ray powder diffraction patterns, the peaks of older archeological samples were narrower in comparison to the younger ones in which the samples displayed nearly amorphous diffractograms. X-ray method was also suitable for estimation of *crystallinity*. The crystallinity index (*CI*) [9] was calculated from XRD diffraction pattern using formula (1):

$$CI = \Sigma\{H[202], H[300], H[112]\}/H[211], \quad (1)$$

where H is a peak height, cm.

The lowest values of crystallinity indices were calculated for the bone remains of St. Peters churchyard (ranged from 0.043 to 0.087) and the highest of Zvejnieki complex (ranged from 0.105 to 0.200). This could indicate that an increase of crystallinity reflected an increase in crystal size with less lattice

strain and/or decrease of the amount of the amorphous organic matrix in the older bone samples as a result of the diagenetic processes.

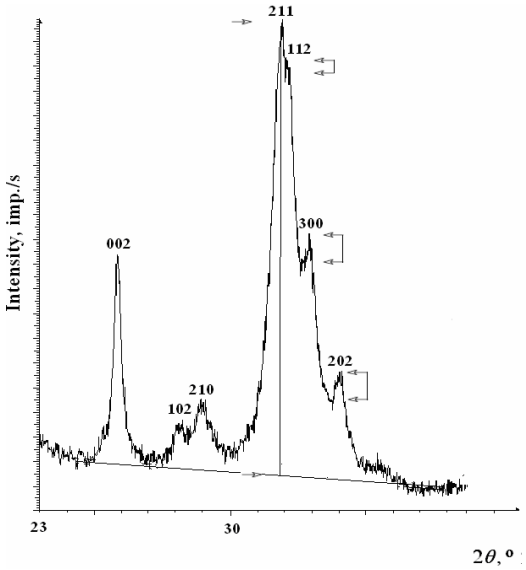

Fig. 1. X-ray powder diffraction pattern of bone sample from the Zvejnieki complex.

The obtained ATR-IR spectra (Fig. 2) showed that the bands at approximately 560 and 600 cm^{-1} and around 960 and 1020 cm^{-1} were related to the phosphate group inherent in the hydroxyapatite structure. The bands at approximately 872 cm^{-1} and 1411 cm^{-1} indicated the presence of carbonate. It could be concluded that hydroxyl (OH^-) and phosphate (PO_4^{3-}) anions in the hydroxyapatite mineral have been partially substituted by carbonate (CO_3^{2-}) anions. The amide I band at 1640 cm^{-1} represented the main protein of the bone collagen. The amide II and III bands are barely detectable at the positions of bands 1546 and 1440 cm^{-1}, respectively. The obtained data of IR spectra were in a good agreement with literature data [10–13].

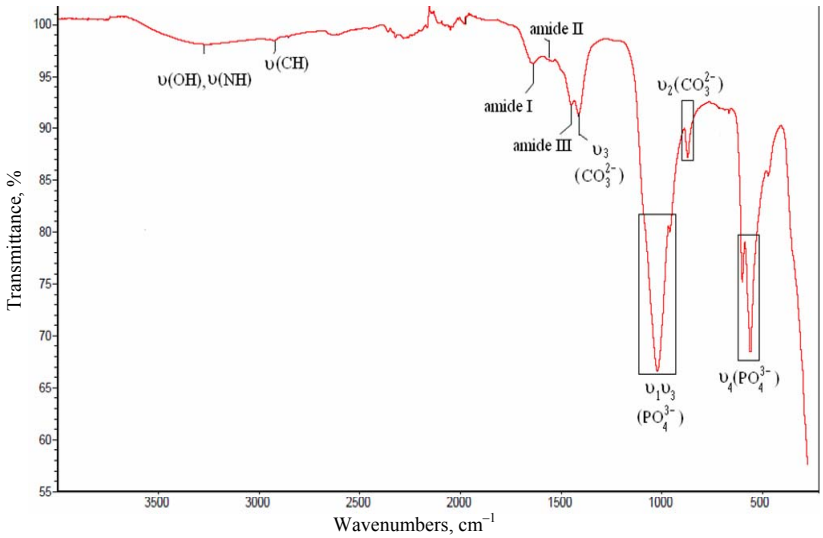

Fig. 2. ATR-FTIR spectrum of bone sample from the Zvejnieki complex.

According to the obtained X-ray diffractograms and IR spectra the main inorganic mineral in the analyzed bone samples was carbonated hydroxyapatite. The ATR-FTIR method could be successfully applied to identify proteins in organic matrix of bone tissue material. The IR spectrum enabled identifying a protein, which could be collagen according to the background knowledge.

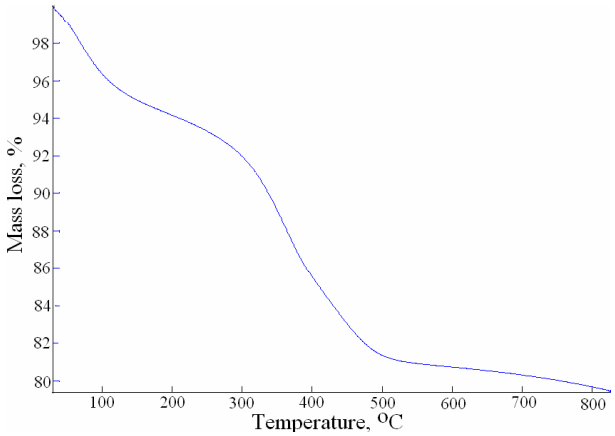

Fig. 3. Typical TG analysis curve obtained from bone sample of the Zvejnieki complex.

Two significant weight loss processes were observed in the bone specimens during the *thermogravimetric analysis* (Fig. 3). The TG curve showed that the incorporated water evaporated at temperatures from 30 °C to about 200 °C, and the organic substances decomposed from 250 °C to 550 °C. The weight loss from 600 to 800 °C was insignificant (Fig. 3) and this was an indication that the organic compounds were completely removed. The calculated results showed that bone specimens obtained from Zvejnieki archaeological complex contained approximately 6% of water, 12% of organics and 82% of inorganic matter, and St. Peters Churchyard bone material contained approximately 8% of water, 24% of organics and 68% of inorganic matter. It should be mentioned that the applied methods characterized mainly the total content of organic matter and did not give detailed information about specific organic substances in the bone structure.

As it was expected, the decrease of organic content of the composite material was higher in geologically older bone samples. Typically, the fresh, dry compact bone, from which soft tissues and fat had been removed, contained 20–30%

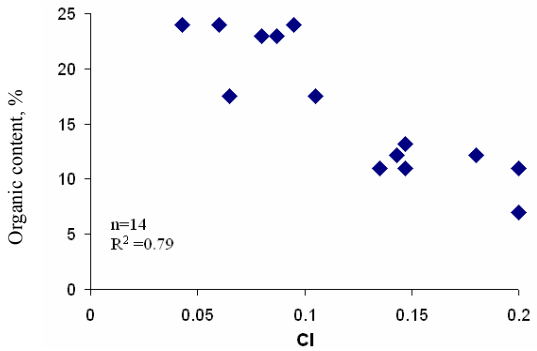

Fig. 4. Correlation between the crystallinity index and the organic matter content in the all analyzed bone specimens.

of organic matrix. The relationship between the organic matter content in the bone samples and the calculated crystallinity index values was investigated. The crystallinity index values were in good correlation with the organic matter content in bone samples (Fig. 4).

The mechanical properties of the compact bone are influenced by the fraction volume and mineral content [14]. The compact bone structure is made up of Haversian canals, vascular canals, and/or lacunae. The mentioned canals are formed as pores or cavities in archaeological bones over time [15, 16]. To calculate the porosity, the dried specimens were soaked in deionized water for 3 days and then weighed. The pore volume (V_p) was calculated using formula (2):

$$V_p = \frac{m_{wet} - m_{dried}}{\rho_{water}} , \tag{2}$$

where m_{wet} – mass of wet specimen, g;
m_{dried} – mass of dried specimen, g;
ρ_{water} – the density of distilled water, g/cm^3.

The total volume (V_t) was calculated using formula (3):

$$V_t = \frac{1}{4} D^2 \pi L , \tag{3}$$

where D – diameter of specimen, cm;
L – length of specimen, cm.

Porosity (P) was calculated according to formula (4):

$$P = V_p / V_t . \tag{4}$$

Porosity values of the bone specimens of St. Peters churchyard ranged from 0.24 to 0.28 (24–28%) and they for the Zvejnieki archaeological complex ranged from 0.30 to 0.55 (30–55%). The cortical bone is defined as a bone with less than 30% porosity (typically 5–10%) [15]. The results showed that the pore volume of the Zvejnieki specimens was higher, indicating differences in bone microarchitecture due to the loss of collagen and/or mineral part and due to the pore space formed from the Haversian canals and vascular canals from which the bone structure is made up.

Calcium is an indirect indicator for characterizing the mineral part of the bone structure. Ca content of bone specimens of St. Peters Churchyard ranged from 275 to 292 g kg^{-1} and of those of Zvejnieki archaeological complex ranged from 256 to 277 g kg^{-1}.

The mechanical behavior of the bone structure was evaluated as the function relating the porosity and Ca content. In order to calculate the elasticity modulus, compressive force was applied to the all bone samples. Young's modulus (modulus of elasticity) was calculated using the following formula (5):

$$E = \frac{F l_o}{\Delta l A_o} , \tag{5}$$

where F – the force applied to sample, N;
l_o – the original length of the sample, cm;
Δl – elongation, cm;

A_o – the original cross-sectional area, through which the force is applied, cm^2.

Better mechanical properties (higher elastic modulus values) were directly related to the higher calcium content and lower porosity (Fig. 5 and 6).

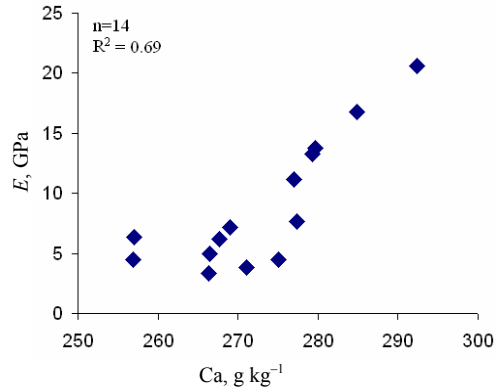

Fig. 5. Correlation between the elasticity modulus (*E*) and Ca content in inorganic mineral fraction in the all analyzed bone specimens.

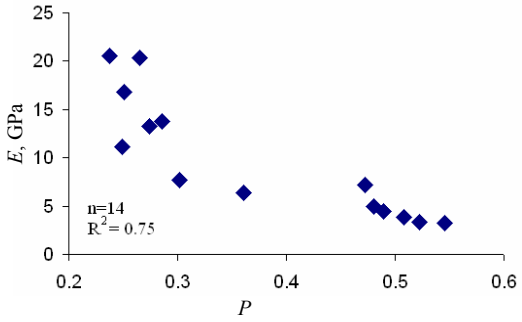

Fig. 6. Correlation between the elasticity modulus (*E*) and porosity (*P*) in the all analyzed bone specimens.

The results indicated that the change in porosity exerted a stronger influence on the mechanical properties of the bone than Ca content in bones.

Conductivity is strongly affected by the bone microstructure, and the impedance spectra usually contain features that could be directly related to the changes in microstructure caused by the variation in the chemical composition [17, 18]. Bone is a good insulator, when it is dry. However, if an electrolyte can penetrate into the bone material, it becomes conductive. The admittance plot method can be used to characterize the conductivity properties of solid electrolytes.

Figures 7 and 8 demonstrate that ion charge transfer in a liquid electrolyte is larger in the analyzed Zvejnieki osteological material. The obtained results showed that conductivity in Riga city urban population bone samples was 1–2 orders of magnitude smaller in comparison to the conductivity of Zvejnieki bone samples. As it could be seen from the values of porosity presented in Fig. 7 and Fig. 8, porosity also positively correlated with the conductivity. Changes of the conductivity properties revealed the preservation status of bone structure.

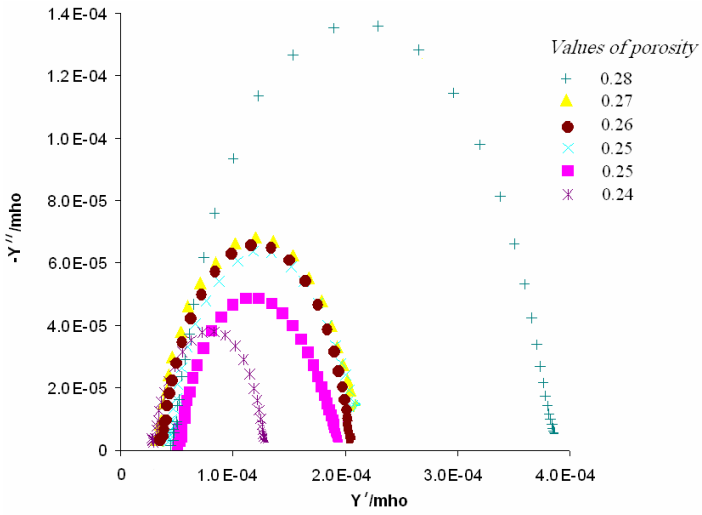

Fig. 7. Admittance plots characterizing the bone conductivity properties of St. Peter's churchyard archaeological complex.

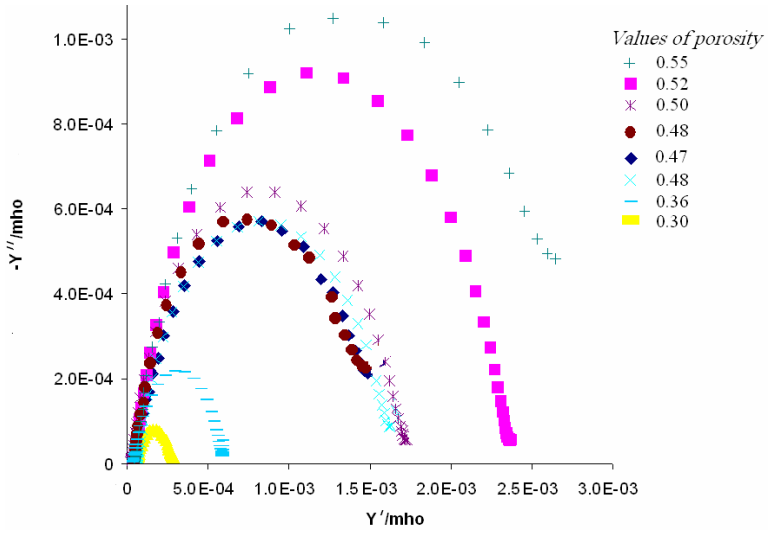

Fig. 8. Admittance plots characterizing the bone conductivity properties of the Zvejnieki archaeological complex.

According to the studies of physicochemical properties, the structure of the bone material taken from St. Peter's churchyard archaeological complex is better preserved in comparison to the samples of Zvejnieki complex. The used analytical methods were successfully used for characterization of the current status of the bone structure for the further interpretation of chemical analysis.

CONCLUSIONS

The structure properties of the compact bone studies are a sensitive indicator to alteration of the mineral and organic phase, illustrating the preservation status of the archaeological bones. The results of this work have demonstrated the occurrence of the good correlation of physicochemical properties on the geological age of the samples.

The methods used in this work are more suitable to archeological samples with higher porosity. The application of these methods may help to understand better the processes of diagenetic environment in which the archaeological bone is situated.

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R E F E R E N C E S

1. Berna, F., Matthews, A., Weiner. S. (2004). Solubilities of Bone Mineral from Archaeological Sites: The Recrystallization Window. *J. Archaeol. Sci.*, *31*, 867–882.
2. Hedges, R.E.M. (2002). Bone Diagenesis: An Overview of Process. *Archaeometry*, *44* (3), 319–328.
3. Hedges, R.E.M., Millard, A.R. (1995). Bones and groundwater towards the modelling of diagenetic processes. *J. Archaeol. Sci.*, *22*, 155–165.
4. Trueman, C.N.G., Behrensmeyer, A.K., Tuross, N., Weiner, S. (2004). Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: diagenetic mechanisms and the role of sediment pore fluids. *J. Archaeol. Sci.*, *31*, 721–739.
5. Olesiak, S.E., Sponheimer, M., Eberle, J.J., Oyen, M.L., Ferguson, V.L. (2010). Nanomechanical Properties of Modern and Fossil Bone. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *289*, 25–32.
6. Zagorskis, F. (1987). *Zvejnieku akmens laikmeta kapulauks*. Rīga: Zinātne.
7. Spirģis, R. (2006). Arheoloģiskie izrakumi Rīgas Sv. Pētera baznīcas kapsētā. *Arheologu pētījumi Latvijā 2004. un 2005. gadā.*, 45–54.
8. Katakam, S., Krishna, D.S.R., Kumar, T.S.S. (2003). Microwave Processing of Functionally Graded Bioactive Materials. *Mater. Lett.*, *57* (18), 2716–2721.
9. Person, A., Bocherens, H., Saliége, J.F., Paris, F., Zeitoun, V., Gérard, M. (1995). Early Diagenetic Evolution of Bone Phosphate: An X-ray Diffractometry Analysis. *J. Archaeol. Sci.*, *22*, 211–221.
10. Ou-Yang, H., Paschalis, E.P., Mayo, W.E., Boskey, A.L., Mendelsohn, R. (2001). Infrared Microscopic Imaging of Bone: Spatial Distribution of CO_3^{2-}. *JBMR*, *16* (5), 893–900.
11. Chadefaux, C., Anne-Solenn, L.H., Bellot-Gurlet, L., Reiche, I. (2009). Curve – Fitting Micro – ATR – FTIR Studies of the Amide I and II Bands of Type I Collagen in Archaeological Bone Materials. *e – PS*, 6, 129–137.
12. Stathopoulou, E.T., Psycharis, V., Chryssikos, G.D., Gionis, V., Theodorou, G. (2008). Bone Diagenesis: New Data from Infrared Spectroscopy and X-ray Diffraction. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *266*, 168–174.
13. Abdel-Maksoud, G. (2010). Comparison Between the Properties of "Accelerated – Aged" Bones and Archaeological Bones. *MAA*, *10* (1), 89–112.
14. Wu, J.S.S., Lin, H.C., Hung, J.P., Chen, J.H. (2006). Effects of Bone Mineral Fraction and Volume Fraction on the Mechanical Properties of Cortical Bone. *J. Med. Biol. Eng.*, *26* (1), 1–7.
15. Martin, R.B., Sharkey, N.A., Burr, D.B. (1998). *Skeletal Tissue Mechanics*. New York: Springer-Verlag.
16. Rho, J.Y., Kuhn-Spearing, L., Zioupos, P. (1998). Mechanical Properties and the Hierarchical Structure of Bone. *Med. Eng. Phys.*, *20*, 92–102.
17. Ličina, V., Gajovič, A., Moguš-Milankovič, A., Djerdj, I., Tomašič, N., Su, D. (2008). Correlation Between the Microstructure and the Electrical Properties of $ZrTiO_4$ Ceramics. *J. Am. Ceram. Soc.*, *91*, 178–186.
18. Zhang, X., Koon, G.N., Anil, K. (2008). Monitoring acid-demineralization of human dentine by electrochemical impedance spectroscopy (EIS). *J. Dent.*, *36*, 1005–1012.

ARHEOLOĢISKO KAULU STRUKTŪRAS PĒTĪJUMS AR DAŽĀDĀM ANALĪTISKĀM METODĒM

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K O P S A V I L K U M S

Kaulaudi ir heterogēns kompozītmateriāls, kurš veidots no organiskās un neorganiskās matricas. Izmaiņas arheoloģisko kaulu struktūrā notiek savstarpējā iedarbībā ar augsnes apbedījuma vietu, vidi un tajā mītošajiem mikroorganismiem. Arheoloģisko kaulu fizikālķīmiskās īpašības novērtētas ar dažādām analītiskām metodēm, nosakot kaulaudu mehāniskās īpašības, porozitāti, kristāliskumu un organiskās matricas zudumus.

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