Environmental Impact on Fossil Record for Palaeoecological Reconstruction Studies

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Abstract. Palaeoecological studies have an important role in understanding past environmental, dietary and/or societal changes however require the authentic signature of fossil materials. Therefore, a significant part of these studies concerns the isolation of the material authentic matrix. Bone hydroxyapatite from different animal species from the archaeological site of Dispilio in Kastoria Lake basin in northern Greece has been subjected to mineral analysis in order to detect if there are suitable for palaeoecological studies. Calcium, phosphorus, oxygen and hydrogen are the main components of bones resulting rigidity, hardness and compressive strength of their structure. However different bone structure resulting different calcium-phosphate phases and different compositions, including Ca/P ratios. These disparities may be attributable to different physiological characteristic, conditions under which the bones were formed or burial environment. Trace element analysis (Ca/P, Sr/P, Fe/Mn) concluded that treated fossil bones retained their biochemical signal without any strong influence by soil remains however without suggesting that no chemical alteration have been occurred.

1. Introduction
“Fossil bones” do not always constitute “archaeological bones”, with respect to reconstruction terms, as many alternations are taking place through their passage from the biosphere to the lithosphere and their finally fossilization. In order to reconstruct information related to physiology or diet habits, environmental conditions or migration episodes, a significant part of palaeoecological studies have been focused in the isolation and the preservation of the authentic chemical structure of bones. On the other hand, chemical, mineralogical and histological alterations during diagenesis process could also be a considerable information reservoir leading to an interpretative tool for taphonomic characteristics and diagenetic environment. One of the main objectives of this study is to read the reliability or not of skeletal samples for palaeoenvironmental and palaeoecological reconstructions with mineralogical methods.

2. Trace elements in bone and teeth samples
Calcium, phosphorus, oxygen and hydrogen are the main components of bones resulting rigidity, hardness and compressive strength of their structure. However different bone structure resulting
different calcium-phosphate phases and different compositions, including Ca/P ratios. These disparities may be attributable to different physiological characteristics, conditions under which the bones were formed or burial environment. Treated with acetic acid fossil bone samples from prehistoric settlement of Dispilio in Kastoria (North Greece) exhibited Ca/P mean weight ratio about 2.14 and molar ratio about 1.40 (Table 1). Considering that corresponding values for stoichiometric hydroxylapatite are 2.15 and 1.65 respectively it seems that fossil bones have not undergone notable alteration. The observed slight divergence could possibly base on the fact that bone apatite, in contrast to pure hydroxylapatite, presents a number of elements and molecular species which might substitute the ions of Ca$^{2+}$, PO$_4^{3-}$ or OH$^-$ in the lattice resulting in slight differences to bone ratios [1-4]. Measured weight ratio values for modern deer were between 2.07 and 2.19 (Table 1) with mean value 2.13 which coincided with that of fossil bones. Moreover, literature data [5] for modern Vulpes vulpes and measured human bones exhibit mean Ca/P values about 2.1 and 2.07 respectively. These values are relatively close to those of hydroxylapatite indicating the biochemical stability of hydroxylapatite deposit environment, without excluded any fluctuations due to different skeletal samples. On the other hand, measured data of raw fossil bone samples (untreated samples) exhibited mean Ca/P ratio 2.33 (Table 1), a value that turns away its print from both modern bones and hydroxylapatite. So considering that Ca/P ratio of treated samples is close to that of hydroxylapatite, it could be concluded that the pretreatment of the samples (acetic acid) managed a sufficient secondary calcite removal.

Table 1. Ratios of trace elements for Roe deer (capreolus-capreolus), Wild boar (Sus Scrofa), Aurochs (Bos Primigenius) and Bear (Ursus) fossil bone from the excavation of prehistoric settlement of Dispilio, modern deer teeth (M2) from central Greece, soil sediment 2m core from Dispilio excavation and modern human teeth (M2/3) from Chalkidiki, Lamia and Athens

|                       | Treated bones | Capreolus capreolus | Raw bones | Modern deer | Sus Scrofa (treated bones) |
|-----------------------|---------------|---------------------|-----------|-------------|---------------------------|
|                       | n=23 samples  | n=20 samples        | n=4 samples | n=27 samples |
| Ca/P                  | min=1.64      | max=2.51            | mean=2.14 | min=1.95     | max=2.81                  | mean=2.33 | min=2.07 | max=2.19 | mean=2.13 | min=1.78 | max=2.44 | mean=2.11 |
| Sr/P                  | min=0.71      | max=1.99            | mean=1.25 | min=0.4      | max=1.32                 | mean=0.94 | min=0.93 | max=1.17 | mean=1.1  | min=0.55 | max=1.87 | mean=1.21 |
| Fe/Mn                 | min=0.85      | max=472             | mean=25.82| min=0.36     | max=59.2                 | mean=12.94| -        | -        | -        | -        | -        | -         |

3. Soil chemistry and ionic interaction with soil solution
The contamination from the surrounding soil through the interaction of bone/teeth samples with the soil solution it should be further considered. Silicon, iron, manganese and aluminum are enriched in sediments but normally absent in living bone. So their incorporation is possible through secondary processes in burial environment as new crystals of phosphate mineral formed during diagenesis. Soils reflect the parent rock material since are the result of biological, chemical, and physical weathering. Inorganic and organic signature of any soil type influences the growth of plant roots supplying them with minerals. Moreover, diagenetic fluids reflect the soil profile in which they circulate [6,7], therefore,
soil chemical profile was constructed. Sediment core related to the taphonomic conditions of fossil bones is characterized by ten horizons from the surface to 2.00m with 0.20m depth step. High concentrations of Fe$^{2+}$ and Mn$^{2+}$ were detected which related by a strong correlation coefficient ($r^2=0.87$) (Figure 3). In more detail (Table 1) Fe/Mn ratio ranged from 2.84 to 4.06 (mean 3.6). Their ratio remained relatively constant with respect to depth however the two horizons of 1.40 and 1.80 differentiated displaying lower values (Figure 1). These layers accompanied by a grey-dark black color as well as by diatom bloom (Figure 2), in contrast to rest layers which characterized by a brown-grey color, implying an elevated primary productivity [8,9]. Previous studies have come up on conclusions regarding the soil zonation in Dispilio excavation based on micromorphological analysis of soil.

Detailed, soil layer of 2.01-1.80 m corresponds to lacustrine sediments and anthropogenic materials that have deposited through physical processes within the lake. This soil layer represents a coastal lacustrine environment of high energy with strong waves and currents [10] in direct interaction with the open lake. The strong presence of anthropogenic material confirms the occupation of the site by humans with constructions above the water [11]. The soil layer of 1.80-1.00 m is characterized by alternate depositions from waving to stagnant water corresponding to an environment with undisturbed ponds, rich in reeds, matching to the present picture in the coastal zone of the lake in front of the excavation site [12]. The interaction with the well-mixed open lake and the physical deposits charged the soil with large amounts of silica allowing high biological productivity (which is evident, as previously reported, on the color shade of soil) and the undisturbed environment gave a boost to the development of diatoms as identified by microscopic and elemental analyzes of soil core under this study. Finally soil layer of 1.00-0.40m correspond to a period without strong interaction with the lake water except wet seasons [11]. The picture of Kastoria Lake with a multiple interaction with sediments overtime is supported by the general observations that been documented by Magny et al. [13] about the instability in European lake-level in Holocene rather driven by climatic oscillations.

Soil samples as well as raw and treated bone samples pictured in Figure 3. In this diagram two conditions of soil are presented. The first (black long dash dot line) concerns the entire soil layers while the second (black solid line) excludes soil layers of 1.80 m and 1.40 m as have been influenced by the diatom bloom and disrupt the territorial ratio of Fe/Mn.

The resulted correlation is much stronger ($r^2=0.96$) with the trend line to end at the bone area. Furthermore, attention was paid on the slopes correspond to each sample group as presented to Figure 3. Entire soil layers exhibited slope “2.5” while without soil layers of 1.80 m and 1.40 m (diatom bloom) exhibited slope “3.4”.

**Figure 1.** Left: Fe/Mn ratio (XRF measurements) along core depth (m) where diatom blooms detected in 1.4m and 1.8m soil layers; Right: Correlation between XRF measurements of Fe and Mn in soil core from excavation in prehistoric Dispilio settlement correlation equation and coefficients. [---]: treated bone samples; [---]: untreated bone samples; [---]: entire soil layers; [---]: without soil layers 1.80m and 1.40m as have been influenced by the diatom bloom.
On the other hand, raw and treated bone samples exhibited slopes “2.4” and “0.7” respectively. It is evident that the slope of treated bones separates them from the rest of groups where present similar slopes. Moreover, based on Coulomb’s law $F = \frac{q^* q^{'}}{D * r^2}$ [$F$: force of attraction or repulsion, $q$: soil surface electrical charge, $q^{'}$: soil solution electrical charge, $r$: the distance of charge separation (cm), $D$: dielectric constant (78 for water at 25 °C) (16)] the attraction or repulsion strength increases with increased electrical charge and decreased distance of separation between soil surface and soil solution. The first circumstance described by trivalent cations where due to their greater charge are held more tightly in soil surface than divalent or monovalent. The second circumstance described by cations with the same valence but smaller radius resulting in strong bonds because of the shortest distance between positive and negative charges. As a result, it is expected a greater cation concentration in soil particle surface and lower concentrations to soil solution [14]. Therefore, raw bone samples present similar slope with soil slope reflecting soil particle contamination while the completely deferent slope of treated bones implies the effective treatment of samples for bioapatite extraction. The fact that Fe/Mn slope of soils does not retained in bones however trendlines are met, possible reflects the process though which plants enriched in trace elements from soils and then transferred to the bones through diet. A competitive relationship has been reported between Fe²⁺ and Mn²⁺ in plants where Fe²⁺ prevents Mn²⁺ accumulation, or vice versa, either during uptake by the roots, or during translocation from the roots to the leaves or other above ground parts [15-17]. This is also evident in this study where the correlation factor between Fe²⁺ and Mn²⁺ ions is really weak ($r^2=0.05$) influenced by higher Fe²⁺ concentrations correspond to lower Mn²⁺ concentrations and inverse. Unlike raw fossil bones exhibit a better correlation ($r^2=0.37$) and a slope closer to that of soil implying their influence from soil remains. Finally, a negative correlation between Ca²⁺ and Fe²⁺ was detected, however wasn’t strong ($r^2=0.4$), implying that Fe²⁺ concentration isn’t governed by calcite dissolution [18].

In mammal physiology strontium is considered to substitute the essential element of calcium and concentrate in skeletal bioapatite [19]. The interaction between plants and soil reservoir and the passage of trace elements to bones are also indicated by Sr/Ca ratio. Many studies [20-24] have been focus on major and trace components of inorganic phase of bone as they constitute reliable tracers for the quality and quantity of ingested food with the trace element of Sr to gather a great interest. About the 99% of strontium corresponding to vertebrates found in bones with less than 10% originate from water, as plants
take up strontium concentrations mainly by soil reservoir. The biochemical path of strontium begins from soil to plant and then to bones of herbivores through their diet. Plants absorb strontium along with calcium in proportions roughly equal to its presence in the environment therefore Sr/Ca ratio of plants should respond to the respective Sr/Ca ratio of soil. Discrepancies often attributed to different kind of plant and the part of the plant consumed or studied. Higher Sr concentrations accumulated through woody vegetation in contrast to grasses, therefore, browsers (e.g., animals eating leaves, shoots, etc.) exhibit higher concentrations of strontium than do grazers (e.g., animals eating grasses, etc.). Sr/Ca ratio ranges between 0.71 and 1.98 (mean 1.25) for treated fossil bones while soil samples characterized by higher ratio ranging between 1.35 and 3.48 (mean 2.15) (Table 1). A reduction of Sr/Ca ratio through the system soil-bone it is observed which is consistent with the fact that strontium amount decreases up the food chain as animals preferentially retain calcium while excreting strontium; however only a ratio of ingested strontium accumulate in bones and teeth of herbivores as this trace element is not fully excreted by organisms [25]. Modern deer bones exhibit Sr/Ca ratio 1.10, close to the ratio of treated fossil bones (1.25) as well as the ratio of raw samples (0.93). Based on this observation it could be assumed that biogenic signal is retained in treated fossil bone in Displio excavation however without suggesting that no chemical alteration have been occurred. The good condition and conservation of bones have also been documented by Nellie Phoca-Cosmetatou et al. [26].

4. Conclusions
Major pathways that diagenetic mechanisms take place are the precipitation of secondary calcite salts or mineral phases in the carbonate matrix of hydroxyapatite, burial contamination from the surrounding soil through the interaction of bone/teeth samples with the soil solution and recrystallization of biogenic apatite to larger crystallized form. Mineralogical analysis managed to address sufficiently the first two mechanisms. Through Ca/P ration in raw and treated bones was concluded the sufficient removal of secondary calcite. Moreover, through Fe/Mn ratio identified the soil particle contribution to raw material and their efficient pre-treatment. The trace element analysis based on Fe/Mn, Ca/P and Sr/Ca ratios concluded that treated fossil bones retain their biochemical signal without any strong influence by soil remains. However mineralogical analysis exhausted its limits in detecting recrystallization processes.

References
[1] Farley D., Boivin G., 2012. “Bone mineral quality”, In: Dionyssiotis Y (ed) Osteoporosis, InTech, available from http://www.intechopen.com/books/osteoporosis/bone-mineral-quality, pp 1–32
[2] Rey C., Combes C., Drouet C., Glimcher MJ., 2009. “Bone mineral: update on chemical composition and structure”, Osteoporos Int 20: 1013-1021
[3] Skinner HCW, 2013. “Mineralogy of bones”, In: Selinus O, Alloway B, Centeno JA, Finkelman RB, Fuge R, Lindh U, Smedley P (eds) Essentials of medical geology, revised edn. Springer, Dordrecht, pp 665–687
[4] Wopenka B., Pasteris J. D., 2005. “A mineralogical perspective on the apatite in bone”, Materials Science and Engineering: C, Volume 25, Issue 2, 28 April 2005, Pages 131-143
[5] Maurer A. F., Person A., Tütken T., Amblard-Pison S., Ségalen L., 2014. “Bone diagenesis in arid environments: An intra-skeletal approach”, Palaeogeography, Palaeoclimatology, Palaeoecology, Volume 416, Pages 17-29
[6] Pate F. D., Hutton J. T., Norrish K., 1989. “Ionic exchange between soil solution and bone: toward a predictive model”, Applied Geochemistry, Volume 4, Issue 3, Pages 303-316
[7] Trueman C. N. G, Behrensmeier 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”, Journal of Archaeological Science, Volume 31, Issue 6, Pages 721-739
[8] Francke, A., Wagner, B., Just, J., Leicher, N., Gromig, R., Baumgarten, H., Vogel, H., Lacey, J.H., Sadori, L., Wonik, T., Leng, M.J., Zanchetta, G., Sulpizio, R., Giaccio, B., 2016.
“Sedimentological processes and environmental variability at Lake Ohrid (Macedonia, Albania) between 637 ka and the present”, Biogeosciences, 13 (4), pp. 1179-1196

[9] Wagner, B., Lotter, A.F., Nowaczyk, N., Reed, J.M., Schwalb, A., Sulphizio, R., Valsecchi, V., Wessels, M., Zanchetta, G., 2009. “A 40,000-year record of environmental change from ancient Lake Ohrid (Albania and Macedonia)”, Journal of Paleolimnology 41, 407-430.

[10] Allen, P.A. & Collinson, J.D. 1986. “Lakes”, 63-94, In: Reading, H.G. (ed.), Sedimentary environments and facies. Oxford: Blackwell

[11] Karkanas, P., 2002. “Micromorphological studies in greek Prehistoric sites: the new insights in the interpretation of the archaeological record”, Geoarchaeology: an international journal, 17/3, 237-259

[12] Chatzitoulousis, S. 2008. “Woodworking technology at the Neolithic lakeside settlement of Dispilio, Kastoria (in Greek with English abstract)”, Anaskamma, 1:93-123

[13] Magny, M., 2004. “Holocene climate variability as reflected by mid-European lake-level fluctuations and its probable impact on prehistoric human settlements”, Quaternary International, 113, pp. 65-79

[14] Pate F. D., Hutton J. T., 1988. “The use of soil chemistry data to address post-mortem diagenesis in bone mineral”, Journal of Archaeological Science, Volume 15, Issue 6, Pages 729-739

[15] Heenan D. P., Campbell L. C., 1983. “Manganese and iron interactions on their uptake and distribution in soybean (Glycine max (L.) Merr.)”, Plant Soil 70:317–326

[16] Moosavi, A. A. and Ronaghi A., 2011. “Influence of foliar and soil applications of iron and manganese on soybean dry matter yield and iron-manganese relationship in a Calcareous soil [online]”, Australian Journal of Crop Science, Vol. 5, No. 12: 1550-1556

[17] van der Vorm, PDJ, Van Diest A., 1979. “Aspects of the Feand Mn nutrition of rice plants. I. Iron-and manganese uptake by rice plants, grown under aerobic and anaerobic conditions”, Plant Soil, 51: 233-246

[18] Heenan D. P., Campbell L. C., 1983. “Manganese and iron interactions on their uptake and distribution in soybean (Glycine max (L.) Merr.)”, Plant Soil 70:317–326

[19] Naeher S., Gilli A., North R. P., Hamann Y., chubert C. J., 2013. “Tracing bottom water oxygenation with sedimentary Mn/Fe ratios in Lake Zurich, Switzerland”, Chemical Geology, Volume 352, Pages 125-133

[20] Elias, R. W., Hirao, Y., and Patterson, C. C., 1982. “The circumvention of the natural biopurification of calcium along nutrient pathways by atmospheric inputs of industrial lead”. Geochimica et Cosmochimica Acta, v. 46, no. 12, p. 2561-2580

[21] Balter V., Bocherens H., Person A., Labourdette N., Renard M., Vandermeersch B., 2002. “Ecological and physiological variability of Sr/Ca and Ba/Ca in mammals of West European mid-Wurmian food webs”, Palaeoecogr. Palaeoclimatol. Palaeoecol., 186, pp. 127–143

[22] Kiedorf U., Stoffels D., Kiedorf H., 2014. “Element Concentrations and Element Ratios in Antler and Pedicle Bone of Yearling Red Deer ( Cervus elaphus) Stags-a Quantitative X-ray Fluorescence Study”, Biological Trace Element Research, Vol. 162, Issue (1-3), 124-133

[23] Safont S., Malgosa A., Subirà M.E., Gilbert J., 1998. “Can trace elements in fossils provide information about palaeodiet?”, Int. J. Osteoarchaeol., 8, pp. 23-37

[24] Sillen A., 1992. “Strontium–calcium ratios (Sr/Ca) of Australopithecus robustus and associated fauna from Swartkrans”, J. Hum. Evol., 23, pp. 495–516

[25] Reitz E.J. and Shackley M, 2012. “Environmental Archaeology, Manuals in Archaeological Method, Theory and Technique”, DOI 10.1007/978-1-4614-3339-2_12, Springer Science + Business Media, LLC

[26] Nellie Phoca-Cosmetatou et al., 2008. “The terrestrial economy of a lake settlement the faunal assemblage from the first phase of occupation of middle Neolithic Dispilio (Kastoria, Greece)”, Anaskamma 2: 47-68