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BRIEF REPORT

Alteration of the metal content in animal bones after 2.5-year experimental exposure to sediments

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Introduction

Fossil and sub-fossil bones accumulated in caves and other sites tend to form large assemblages. One of the primary questions posed by taphonomists that is especially important to archeologists, zooarchaeologists, and forensic scientists is whether a bone assemblage represents a singular episode of accumulation or is a palimpsest—the result of stratigraphic concentration or redepositional mixing. Radiocarbon dating may be used to answer this question; however, this method has three significant disadvantages: it is limited to assemblages not older than 50,000 cal. years BP; it is limited to well-preserved material that has been neither weathered nor contaminated; and it is relatively expensive. The alternative method is geochemical fossil provenance analysis. While chemical composition of the bone may be affected by some environmental and behavioral factors during life of animal or human (Balter et al. 2002; Martínez-García et al. 2005; Allmäe et al. 2012; Martiniaková et al. 2011), the concentration of metals in buried bones are usually much higher than in fresh ones, which indicates advanced post-mortem chemical alterations (Trueman 2007). Diagenetic parameters of bones, such as their chemical composition, are influenced by the environment, including the chemistry of surrounding sediment (Henderson et al. 1983; Wright et al. 1984; Plummer et al. 1994; Johnsson 1997; Dauphin et al. 1999; Trueman 1999; Pawlikowski and Niedźwiedzki 2002; Trueman et al. 2003, 2006; Martin et al. 2005; Smith et al. 2007; Cook and Trueman 2009; Rogers et al. 2010). According to Kohn and Moses (2013), the bone acts as a sink for trace elements, causing the depletion of particular metals in the surrounding sediment. For some trace elements, it has been shown that the element content in the bone does not correlate with its content in the sediment (Nelson and Sauer 1984; Denys et al. 1996), which suggests that the chemical composition of archeological bone is affected by factors other than only the chemical composition of the surrounding sediment. Those additional factors may include not only moisture, porosity, pH, redox conditions, climate, and time (Pate and Hutton 1988; Nielsen-Marsh and Hedges 2000b; Smith et al. 2007; Trueman 2007) but also the valence and ionic radii of accessible competing cations due to different fit to the crystal lattice of the bone apatite (Kohn and Moses 2013). Accordingly, Trueman et al. (2006), Denys et al. (1996), and Iliopoulos et al. (2010) have demonstrated that various stratigraphic layers at the same site contain bones with different contents of trace elements. This phenomenon, called a "geochemical fingerprint," can be used to identify the layer from which a particular bone originates.

Geochemical study of bone assemblages deposited in alluvial deposits (MacFadden et al. 2007) showed that bones representing different faunal communities, but accumulated together, bear variable chemical traces, probably related to different primary depositional environments. Studies of metal compositions, both of macro-elements and trace elements, in assemblages of animal bones and teeth from several caves in Poland (Krajcarz 2009, 2013; Krajcarz and Cyrek 2011) exhibited not only differences between layers but also some intra-layer inconsistency, which may also be related to post-depositional mixing. This observation has been confirmed by inconsistency in the results of radiocarbon dating observed in

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Pleistocene fossils from the caves of Poland (Lorenc 2007, 2013). Mentioned observations introduce a very important issue of the redeposition of bones, as the observed inconsistency may be an effect of post-depositional mixing of sediments and their components, such as fossil bones. If we assume the possibility of such redeposition of bones from layer to layer, the geochemistry of bones becomes a crucial point in the perceiving of these events, especially if hypothesized that bone geochemistry may record the primary signal of the sediment, where the particular bone was originally deposited. For the first time, a similar approach was applied by Pavlish and Alcock (1984); however, they analyzed the chemical composition of the sediment attached to the bone, instead of a bone itself. As that may be a more accurate approach, it cannot be used in case of bones stored in archeological and paleontological collections, already washed and cleaned of sediment.

To engage into further interpretations a hypothesis of pre-redepositional environment recorded in fossil or archeological bones, we have to recognize two important aspects of bone diagenesis. First, how quickly after primary deposition the chemical signature of bone is formed in sediment? This is crucial to know if bone might have taken a chemical signature during time interval before the redeposition occurred, and if that alteration was strong enough to create a "geochemical fingerprint" of the original environment. Trueman et al. (2004) showed that 15 years may be enough to alter the concentration of some metals in bones, but their study was limited to surficial exposure of bones to atmospheric conditions. Second, how stable is geochemical signature once recorded in a bone while staying in its primary deposition site, and may it change after the movement to another sediment? This is important to understand the possibility of recording the original environment by bones which underwent the redeposition.

The experiment presented in this paper was performed by author to initiate the study on the first of the abovementioned questions. The objective of this experiment was to check if relatively short time (2.5 years taken here) is enough to create the geochemical signal in bones buried in variable soils, that is detectable with available analytical methods. Additional goal was to observe the initial directions of chemical alterations at the very early stage of bone diagenesis. All bone samples were cut from a single bone specimen. The samples were buried in containers, each containing one of 17 types of sediment of different lithology, representing soil types usually found at archeological sites. The containers were kept at constant temperature (23–25 °C) and air humidity (60%); one group of containers was kept air-dry, and these are referred to as "dry" hereafter; the second group was regularly soaked with deionized water (reverse osmosis, electric conductivity 0.05 μS, at a rate of 50 ml/50 cm^2 per week, which represents 520 mm of annual precipitation and is similar to the mean annual precipitation in Central Europe), and these are called "moist" hereafter. In nature, rainwater is slightly acidic due to being charged with CO_2. However, the deionized water used in the experiment is not an exact replication of natural precipitation and was used only to keep the sediments moist. There were no holes in the bottom of the containers, so the water only escaped by evaporation. The experiment started in 2011 and was finished in 2013, after 970 days. After 2.5 years, the bones were excavated from the containers. The visual appearance of the bones varied after excavation (Fig. 1). Most of the samples stored in dry containers were almost unaltered and exhibited a similar color and similar preservation state to that prior to their burial. Samples stored in regularly water-soaked containers were visibly altered; they were usually cracked and dark-colored.

The inductively coupled plasma mass spectrometry (ICP-MS) method of chemical analysis was chosen for this study. This method is widely used in studies of fossil bones (e.g., Cook and Trueman 2009, Trueman et al. 2004, Kocsis et al. 2010), which allows to use the results for comparisons. Moreover, this method is quite cheap and requires relatively small sample size (0.25 g) to achieve reliable results, in contrast to other methods. In particular, AAS requires at least 2 g of bone, while XRF requires thick sample to ensure the correct

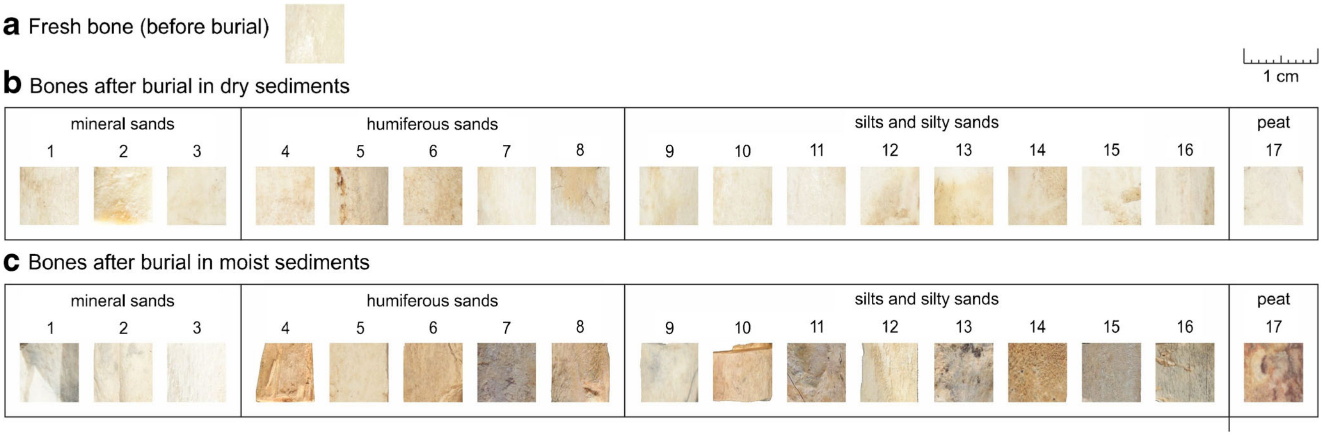


Fig. 1 Appearance of the surfaces of bone fragments after removal from sediment. Alteration of color in humiferous sediments and alteration of color and surface morphology in organic sediment (code 17) are noteworthy

measurement of light elements, and electron microprobe requires the work-consuming preparation of the polished sections. In this study, the analysis was focused on general specter of metals, including also quite abundant metals in bones, such as Sr and Ba, not particularly on REE (rare earth elements, lanthanides), which are usually investigated.

Materials and methods

Sediments

Seventeen randomly selected sediments from different geological and geomorphological locations in Poland were used (Table 1), including soils common at archeological sites, i.e., humic and non-humic variants of sands, silts, and sandy silts. Peat and gyttja were added for comparison. An approximately 0.5-kg portion of each sediment was collected from the field outcrop and was subsequently homogenized by mixing. A volume of approximately 350 cm^3 of each sediment was placed into a separate plastic container similar to flowerpots used for plant seedlings in horticulture. The remaining portions of the sediments were used to analyze the lithological and geochemical parameters of each sediment.

Bones

One fresh femur bone of subadult cattle (*Bos taurus*) was used in the experiment. The bone was bought in a slaughterhouse several days before the experiment and stored in a fridge. The bone shaft was fragmented into 38 pieces of similar size (ca. 1 × 1 × 1-cm cubes) by a diamond-coated rotary saw. Soft tissues and cancellous bone were mechanically removed with a diamond-coated bit. Each of 17 samples was placed in the middle of a sediment-filled plastic container approximately 3 cm above the bottom and 3 cm below the surface (referred as "dry" sediments). The other 17 samples were placed in similar containers with the same types of sediments, but "moist," as explained in paragraph "Experimental conditions." Five remaining samples were not buried but kept in a refrigerator (+5 °C) as comparative material; these are referred to as the "fresh" samples hereafter.

Chemical analysis

The chemical composition of the sediments was determined via ICP-MS. An approximately 1-g homogenized sample from each dried, raw sediment was taken prior to the start of the experiment, and sediment was taken from the middle of the container after the experiment. The samples were powdered in a ball-mill pulverizer (agate cups and balls) and analyzed at Bureau Veritas Minerals Laboratory (Vancouver, Canada) according to the MA250 analytical packet (protocol for Ultra Trace ICP-MS chemical analysis of multi-acid digested samples). A 0.25-g fraction was heated in fuming HNO_3-$HClO_4$-HF and taken to dryness. The residue was dissolved in HCl. The use of strong oxidizing acids (HNO_3 and $HClO_4$) ensured the removal of organic matter, while the use of HF enabled the dissolution of silicates, which allowed for the near-total dissolution of the mineral fraction. The solutions were analyzed by ICP-MS, and the content of 59 elements (including 53 metal and 6 nonmetal species) was determined. The list of these elements, their detection limits, and the accuracy and the reproducibility of the measurements are presented in Online Resource 1.

The bone samples excavated after the experiment were dried, ultrasonically cleaned in deionized water, and dried again at 40 °C. "Fresh" samples were processed in the same way. Fragments approximately 0.25 g in mass were cut with a diamond-coated rotary saw, powdered and analyzed in the same way as the sediment samples.

The following elements occurred in the studied bone samples in low concentrations, either below or close to the lower detection limit: Ag, Al, Au, Be, Bi, Co, Cs, Dy, Er, Eu, Fe, Hf, Ho, Gd, In, Lu, Nd, Pr, Re, Sm, Sn, Ta, Tb, Th, Tl, Tm, U, V, W, Y, Yb, and Zr; P was above the upper detection limit (Online Resource 1).These elements were excluded from further analysis.

Parameters of sediments

The detailed methods used to establish the lithological parameters are presented in Online Resource 2.

The $CaCO_3$ content in sediment was measured as the weight loss after 10% HCl treating until the end of visual reaction (at least 24 h), on centrifuged, decanted, and dried sample mixtures. The carbon and nitrogen content was measured in a Vario MicroCUBE CHNS elemental analyzer in HCl-treated aliquots of sediment. The pH of sediments was measured with an HQ40d electronic pH meter in deionized water (pH in water) and separately in 1 M KCl solution (pH in KCl) to determine the cation-exchange capacity of the sediment (Thomas 1996). The porosity was calculated using the equation $P = (\rho_p - \rho_{db})/\rho_p \times 100\%$, where ρ_p is the particle density and ρ_{db} is the dry bulk density of the sediment (Myślińska 2006). The ρ_p was assessed using the pycnometer method well established in soil science (Myślińska 2006), and ρ_{db} was measured using a metal cutting probe ring. The grain size composition of sediments was analyzed by laser diffraction in a Fritsch Particle Sizer "analysette 22" at the Polish Geological Institute (Warsaw, Poland).

Statistical processing

Pearson's correlation and Spearman's rank correlation were used to determine the relationships between the parameters of the sediments and the concentration of metals in bones.

Table 1 Sediments used in the experiment

Code	Type of sediment	Geological context	C_{org} %	N %	$CaCO_3$%	pH in H_2O	pH in KCl	Porosity %	Grains < 63 μm %
1	Coarse-grained quartz sand	Dune sand	0.03	0.00	0.00	5.89	5.23	30.17	0.01
2	Medium-grained quartz sand	Dune sand	0.03	0.00	0.23	5.81	4.80	29.41	0.00
3	Coarse-grained limestone sand	Limestone regolith	0.03	0.01	33.87	7.92	9.26	30.05	0.90
4	Humiferous medium-grained quartz sand	Soil horizon A developed on dune sand	0.46	0.01	0.41	5.38	3.65	31.22	1.03
5	Humiferous medium-grained limestone sand	Soil horizon A developed on limestone regolith	0.29	0.01	15.15	7.57	7.73	61.58	1.16
6	Humiferous coarse-grained quartz sand	Soil horizon A developed on dune sand	0.11	0.01	0.27	6.02	3.93	32.55	0.51
7	Medium-grained ferruginous sand	Soil horizon B developed on dune sand	0.24	0.01	3.22	5.13	4.20	46.57	0.59
8	Humiferous medium-grained sand with plant remains	Proluvium of temporary stream	3.09	0.18	2.96	6.11	5.60	58.84	1.83
9	Quartz silt	Loess	0.17	0.01	3.16	8.44	7.99	32.49	89.19
10	Silty sand	Glacial till	1.37	0.14	0.00	7.97	6.99	38.66	37.47
11	Humiferous quartz silt	Soil horizon A developed on loess	1.03	0.07	0.00	6.17	5.36	52.71	88.84
12	Humiferous silty sand	Soil horizon A developed on glacial till	2.80	0.26	0.68	7.52	7.33	32.34	27.63
13	Humiferous sandy silt	Sapropel in water reservoir	1.39	0.03	0.33	6.01	5.13	55.03	62.85
14	Humiferous sandy silt	Alluvial flood mud	2.76	1.69	0.19	5.32	4.38	26.69	69.68
15	Humiferous silty sand	Artificial sediment (industrially reworked gyttja)	11.49	0.36	5.00	6.39	5.91	65.42	32.50
16	Humiferous silty sand	Artificially reworked gyttja	15.19	0.40	2.49	7.43	7.21	58.89	25.90
17	Peat with plant remains	Peat bog	29.92	0.31	0.00	3.68	3.17	59.78	0.00

Pearson's correlation provides a complete description of the association when the variables are bivariate normal, but because this was not the case for most of the measured parameters, Spearman's rank correlation was used. This correlation, which applies to ranks and provides a measure of a monotonic relationship between two continuous random variables, is useful with ordinal data and is robust to outliers. In the sediment-bone relationship, synergistic processes may occur, such as different rates of the dissolution or precipitation of an element's compounds in relation to the concentration in the environment. Therefore, the correlations between sediment parameters and bone parameters may be nonlinear, which makes Spearman's correlation a much more appropriate measure than Pearson's correlation, which is usually used in natural science.

Chemical composition is a closed dataset, where the concentration of a given element has an influence on the concentrations of all others. Such a dataset is therefore constrained (Reimann et al. 2008) and requires a transformation to remove the constraint and make the data usable for statistical analysis. The CoDaPack software, version 2.02.04 (Comas and Thió-Henestrosa 2011), was applied to transform the data using the centered log-ratio (CLR) transformation.

Multivariate PCA (principal component analysis) was applied to the data transformed by CLR to check the main direction of chemical alterations and the variability of the alterations. Statistical calculations were performed using PAST software, version 2.17b (Hammer et al. 2001).

Presentation of chemical alteration

The chemical alteration of the metal composition of the bones after the experiment was presented as an anomaly in the chemical composition. The anomaly of a given element ($A_{element}$) was calculated as the difference between the measured chemical concentration of the given element in the experimentally buried bone ($CExp_{element}$) and its concentration in fresh bone ($CFresh_{element}$) according to the following formula:

$$A_{element} = (CExp_{element} - CFresh_{element}) \cdot 100\% / CFresh_{element}$$

where $CExp_{element}$ and $CFresh_{element}$ are given in the same analytical units (weight ppm or weight %). According to this formula, the anomaly will be positive if the concentration of a given element increased during the experiment (with +1% anomaly representing an increase by 1% of original content of a given element, +2% an increase by 2%, etc.) and negative if it was depleted (to a minimum of −100%, representing the total elimination of a given element). The anomaly will be 0% in the case of no alteration. The anomaly was calculated in a similar way for sediments by using their chemical composition before and after the experiment.

Results

The total concentrations of metals in bones were altered in the experimental diagenesis in various ways. Generally speaking, in moist sediments, the total metal content in bone changed far more intensively than in dry sediments (Fig. 2, upper row). The common feature independent from moisture is increase of Ca and decrease of Na and Mg (Fig. 2). The bones buried in moist sediments, regardless of other lithological parameters, usually exhibit the decrease of Na, K, Rb, Mg, Ni, Cu, Ga, and also nonmetals (As, Se, and Te) and slight increase of Ca. In dry sediments, concentrations of Ba, Sc, Ni, La, Ga, and Te usually decreased in bones. In moist not humic sands and silts (codes, 1, 2, 3, 7, 9), most of metals decreased in bone, followed by increase in surrounding sediment (Fig. 3). The exception are Ca, Cr, Li, Mn, and Sc, which increased in bone. In moist humic sands with low humus content (codes, 4, 5, 6, 7), Cd, Cr, Mn, Sc, and Sb increased, while most of the other metals decreased. In moist humus-rich sediments (codes, 8, 10–17), the intensive increase of Cd, Ce, Cr, Li, Mn, Mo, Nb, Pb, S, Sb, and Sc is typical, regardless of grain size composition, porosity, or acidity. Slight increase of Ca, Ba, and Sr, but clear decrease of Mg and alkali metals (except of Li, being close do the lower detection limit) also occur. The chemical alterations of sediments were in most cases less significant than of bones and usually reverse (Fig. 3). Details regarding the changes in the concentration of each metal are shown in Online Resource 2 and for the chosen sediments in Fig. 3.

The correlation between metal content in bones after the experiment and the initial parameters of sediments are quite weak (Table 2; more complete results are provided in Online Resource 4). The greatest number of particular correlations is observed in the case of the organic matter content in the sediment. However, only Ca, Cd, Mo Sc, and Zn exhibit high correlation indexes with organic C or N content in the sediment.

The results of PCA are given in Online Resource 5. The most important components cover 47.527% (component 1) and 14.09% (component 2) of variance, respectively. The highest loadings (> 0.1 or < −0.1) are observed in component 1 for Mn, As, Sc, Rb, Cd, Ni, K, Se, Na, and Mg (respectively, −0.4989, 0.4427, −0.388, 0.3736, −0.3488, −0.1919, 0.1554, 0.1513, 0.145, 0.1105) and in component 2 for Ce, Mn, Te, As, Sc, Sb, Cu, Mo, Cd, Rb, S, and Ti (respectively, −0.4915, −0.3961, 0.353, −0.2743, 0.2692, 0.2448, −0.2356, 0.2272, −0.2081, −0.1781, 0.1239, 0.105). This makes Mn, Ce, As,

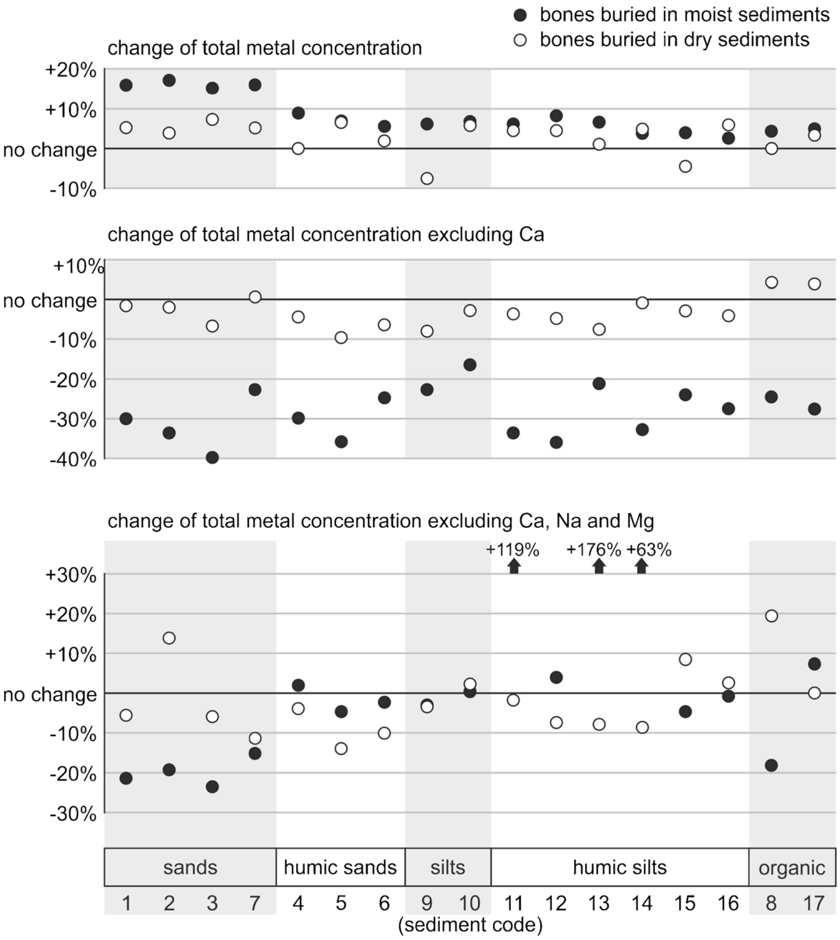
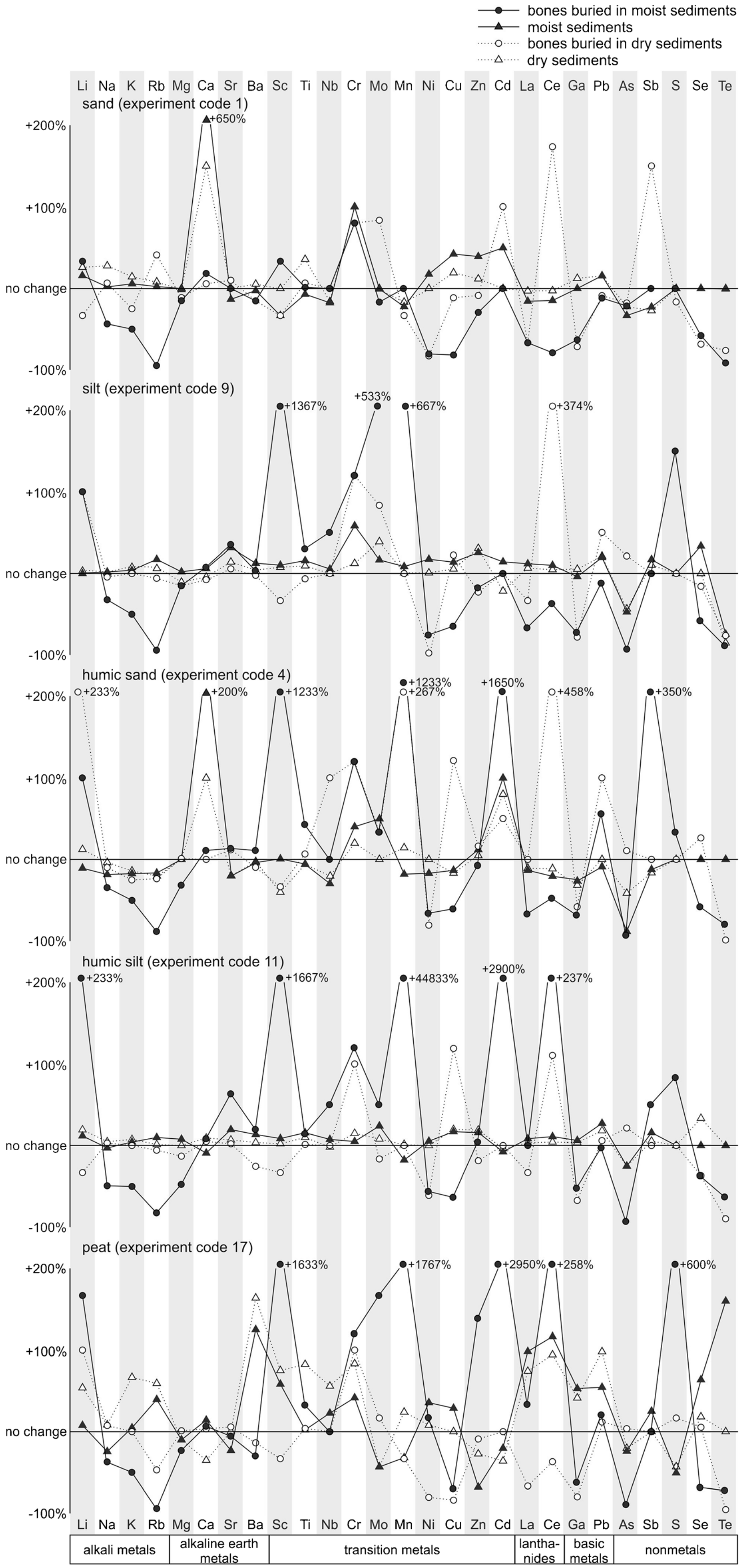


Fig. 2 Chemical anomalies of total metal content in experimentally buried bone samples. Middle and lower diagrams present the alteration of total metal content with exclusion of the most abundant metals (Ca, Na, and Mg). Anomalies represent the percentage change of element content in relation to its primary concentration in fresh bone

Fig. 3 Chemical anomalies of element concentrations in chosen experimentally buried bone samples, representing different lithological types of sediments



Sc, and Te the most important elements responsible for the chemical variability of the studied bones.

Discussion

Samples used in the experiment were small in size, which may be a reason for the increased chemical alteration due to the relatively large surface staying in contact with the sediment. However, fragmented bone material is common at archeological sites, especially those where bones were in use, such as butchery sites, food preparation points, bone craft workshops, and waste pits.

The time duration of this experiment was not long, especially when compared to fossil materials, which have spent hundreds or thousands of years in sediment. However, the time interval was similar to the 2-year burial time tested by Howes et al. (2012), which was enough time to produce structural and bulk chemical changes in bones, and shorter than the 15 years reported by Trueman et al. (2004), which was enough time to create an alteration of some metal contents in the bones exposed to weathering on the soil surface. Nevertheless, the bones in this study clearly show visible, macroscopic signs of multidirectional alterations. These changes vary between sediments and differ between the dry and water-soaked sediments. The primary differences are the macroscopic ones: changes in the color, smoothing, occurrence of mineral precipitations, fragility, roughness, and cracking. What is especially significant are the distinct chemical alterations in the composition of metals in the experimentally buried bones.

The total concentrations of metals were altered in the experimental diagenesis in various ways. In moist sediments, the total metal content typically increased (Fig. 2, upper row), which is strongly related to the increase of calcium, the most abundant metal in bone. This may be partially related to the decay of the organic components of bone in a moist environment or the removal of other metals. The most advanced change of Ca content is marked in mineral sands, including non-carbonate quartz sands (sediment codes 1 and 2), which indicates that the increase of calcium occurred due to the concentration of its primary content and not adsorption of environmental calcium, which was proposed for some types of soils (Nielsen-Marsh and Hedges 2000). After excluding Ca from the calculation of the anomaly of total metal content, the clear decrease is visible, especially in moist sediments (Fig. 2, middle row). This phenomenon disappears after removal of the next most abundant metals, Na and Mg (lower row on Fig. 2).

Importance of lithological parameters in the formation of metal composition of buried bone

Statistical correlations between the lithological and geochemical parameters of sediments and the content of metals in

Table 2 Spearman's rank correlation between main lithological parameters of sediments and concentration of metals in experimentally buried bones (shown only for bone fragments kept in moist sediments; for dry conditions and p values, see Online Resource 4). Indexes over 0.5 or below −0.5, and with $p < 0.05$ are italicized. K was removed from correlation due to lack of variability

Sediment parameter	Concentration of element in bone																									
	Alkali metals			Alkaline earth metals				Transition metals										Lanthanides		Basic metals		Nonmetals				
	Li	Na	Rb	Mg	Ca	Sr	Ba	Sc	Ti	Nb	Cr	Mo	Mn	Ni	Cu	Zn	Cd	La	Ce	Ga	Pb	S	Se	Te	Sb	As
C org (%)	0.4	0.1	0.2	−0.1	*−0.8*	0.4	0.4	*0.7*	*0.5*	0.1	*0.5*	*0.7*	0.3	*0.6*	*0.6*	*0.8*	*0.6*	0.5	*0.5*	*0.6*	*0.6*	*0.8*	0.4	0.4	*0.6*	0.0
$CaCO_3$ (%)	*−0.5*	0.3	−0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	−0.2	−0.2	0.2	−0.1	−0.2	*−0.6*	−0.4	−0.4	0.0	0.1	−0.2	−0.1	0.4	−0.4
pH_{H2O}	0.0	0.4	−0.1	0.0	−0.1	*0.5*	0.3	0.2	0.2	0.1	0.0	0.3	*−0.5*	*−0.6*	−0.3	−0.4	*−0.6*	−0.2	−0.2	−0.3	−0.4	−0.1	0.3	0.1	−0.1	−0.4
pH_{KCl}	−0.1	0.2	−0.4	−0.1	0.0	0.5	0.2	0.1	0.0	0.1	−0.1	0.2	*−0.5*	*−0.5*	−0.1	−0.4	*−0.5*	−0.3	−0.3	−0.2	−0.4	0.0	0.3	−0.1	0.0	−0.3
Porosity (%)	−0.2	0.3	0.2	0.1	*−0.5*	0.1	−0.1	*0.6*	0.2	0.1	0.4	0.4	0.1	0.1	0.2	0.5	0.2	0.0	0.2	0.2	0.1	*0.5*	0.0	*0.7*	0.3	0.1
N (%)	0.2	−0.1	0.2	−0.3	*−0.7*	0.3	0.2	*0.6*	0.3	−0.1	0.4	*0.5*	0.4	*0.6*	*0.6*	*0.9*	*0.7*	0.5	*0.5*	*0.6*	*0.5*	*0.8*	0.2	0.4	*0.6*	0.1
S (%)	0.2	0.1	0.0	−0.1	−0.2	0.1	0.0	0.4	0.2	−0.2	*0.6*	0.3	0.1	0.3	0.3	0.5	0.4	0.2	0.3	0.4	0.4	*0.5*	0.0	0.3	0.4	0.1
Clay + silt (%)	0.4	0.1	0.3	−0.1	−0.4	*0.7*	*0.5*	0.1	0.3	*0.5*	0.3	0.4	0.3	0.2	0.4	0.3	0.2	0.3	0.4	0.1	0.2	0.4	*0.7*	0.1	0.2	−0.3

experimentally buried bones (Table 2) may be used to track the influence of the soil type on the diagenetic metal composition.

Organic matter

The content of organic matter in sediments, measured as either the content of organic carbon or the content of nitrogen, demonstrates a correlation with the concentrations of Mo, Sc, and Zn in experimentally buried bones and a weak correlation with Cd, Ce, Cr, Cu, Ga, La, Ni, Pb, Sc, Ti, and Zn (Table 2, also Online Resource 3). This observation, together with the lack of correlation with acidity, suggests that these metals occur in used sediments bound in humus compounds and underwent relocation from organic matter to bone during burial. Ca shows an inverse relationship, demonstrated by the negative correlation between the Ca content in bone and the organic matter in the sediment. This result indicates that Ca was removed from bone in organic sediments. Surprisingly, there is no clear correlation between the Ca content in bone and the acidity of the sediments, which suggests that the dissolution of calcium compounds in bone is not a direct function of the acidity of the sediment but is instead a complex process. In sediments with the lowest content of organic matter (especially the clean sands, sediment codes 1, 2, and 3 in Table 1), the increasing concentrations of Ca, Cr, Sc, and Sr in bones are prominent, while the concentrations of Ba, Zn, Pb, and alkali metals are depleted.

Porosity

The negative correlation between the porosity of sediments and the Ca content in buried bones (Table 2) indicates that calcium is more intensively removed from the bone in the porous sediments. Porosity is, however, correlated with the content of organic matter in the sediments used in the experiment; therefore, this relationship may be an artificial effect in this experiment.

Grain size composition

The amount of the fine fraction (silt and clay fractions, < 63 μm) in sediments correlates with concentrations of Sr and slightly with some other trace metals (Ba, Nb) in bones (Table 2). This result may suggest that the main environmental sources of Sr and geochemically related metals available for incorporation into the buried bone were clay minerals.

Carbonate content

The carbonate content in sediment is negatively correlated with the concentration of lanthanides and some other metals (Y, Li, Co; Table 2). Unlike one can predict, there is no correlation between the carbonate content in the sediment and the Ca content in bone. Seemingly, the carbonates in sediment had only a weak influence on the geochemistry of the buried bone.

Acidity

Strong negative correlations were observed between the pH of the sediment and the concentration of some heavy metals in the bone (Mn, Pb, Zn, Ni, Cd, Co; Table 2), which suggests that these metals were concentrated in the bone in acidic environments. Howes et al. (2012) showed that acidic soils promote the structural ordering in bone apatite, and this process may stay behind the accelerated incorporation of bivalence cations during apatite recrystallization. Conversely, Ba and Sr exhibit positive correlations with the pH (Table 2). Kohn and Moses (2013) noted that those metals are less privileged in Ca substitution due to relatively large ionic radii; however, this does not explain the reverse correlation in comparison with bivalence transitional metals. Possible explanations for these relationships are that alkaline soils are the source of accessible Ba and Sr or that the alkaline soils prevent the dissolution of Ba- and Sr-bearing apatite.

Metal content in the sediment

Nelson and Sauer (1984) showed that for some metals (Mn and Zn), the element content in the bone is not correlated with the element content in the surrounding sediment. Denys et al. (1996) reported similar observations for Mg, Al, and Si. In the experiment presented in this paper, the correlations calculated between the concentrations of particular metals in sediment and in the diagenetically altered bones are prominent in the case of some heavy metals only (Online Resource 4), including Ba, Ce, Cu, Mo, Nb, Pb, Sr, and Zn. Those mostly 2-valence metals are known substituents of Ca in apatite (Ptáček 2016), which may explain the relation between their availability in soil and incorporation into the buried bone. Some examples of the relationships between the concentrations of the chosen metals in sediments and in bones are shown in Fig. 4 and reveal that mostly moist conditions are responsible for this effect, which was previously theorized by Millard and Hedges (1996). Dry sediments usually caused weak alterations; however, some elements regularly increased (such as Ce, Cu, Mo) or decreased (Ga, La, Ni, Sc, Te) in dry conditions.

In the case of Ca, no clear correlation exists between concentration in sediments and in bones. The same may be observed for Na (as shown in Fig. 4, but similar relationships are also exhibited by Rb and Mg). These metals probably migrated from the bone to the environment with infiltrated water, as salts of these metals are water soluble. This migration was likely the reason for the lack of correlation. In contrast, heavy metals and Sr were relocated from the burial sediment to the bone. This is clearly shown in the Mn case, which exhibited the highest increase in bones together with a decrease in sediments. Kohn and Moses (2013) proved, by experimental exposure of bones to the trace metal solutions, that the bone acts as a sink for trace elements, causing depletion in the

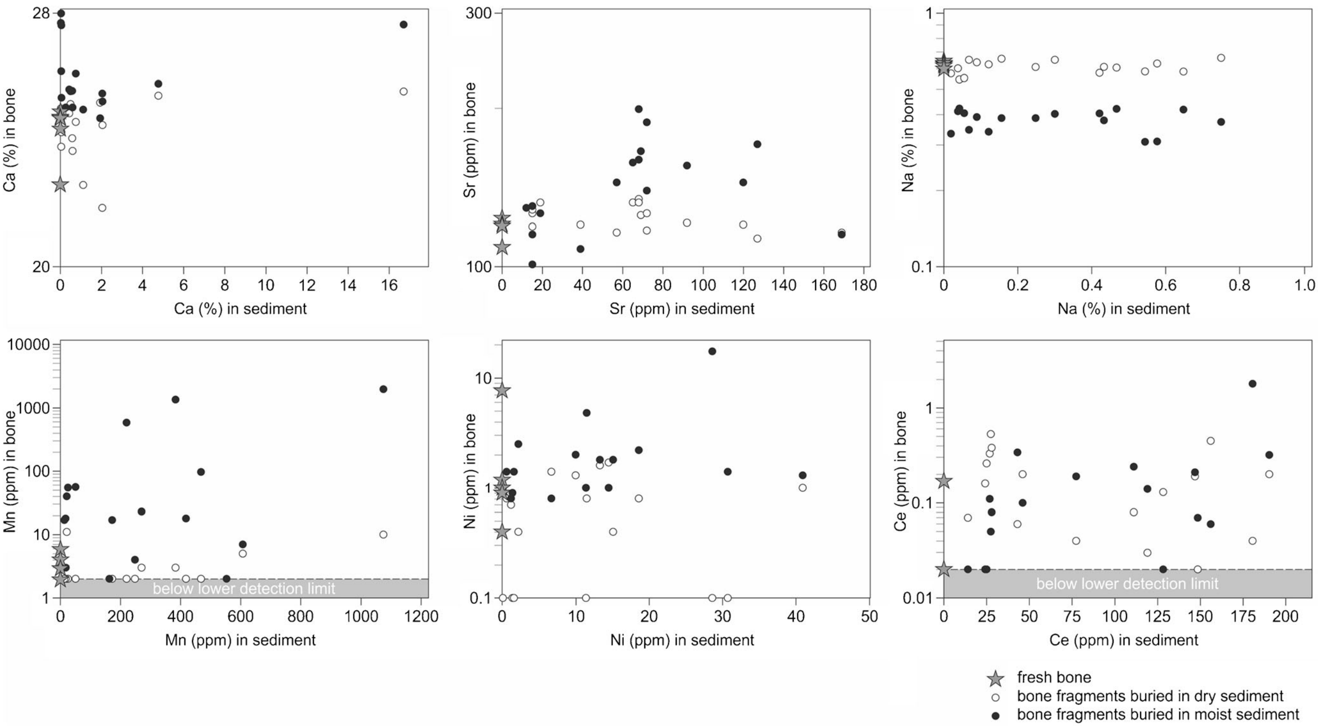


Fig. 4 Concentration of chosen metals (Ca, Sr, Na, Mn, Ni, Ce) in experimentally buried bones in relation to concentration of the same element in the sediment of burial environment. Original concentrations in fresh bones are also shown. Note the logarithmic vertical scales. Symbols (white and black dots and gray stars) use the same code as in Fig. 5

surrounding environment. This explains why their final concentration in the bone is likely related to their occurrence in the environment. In this experiment, most of the analyzed elements exhibit the expected behavior of reverse change of concentration in the bone and in sediment. The exception is Ca, which usually increased in both materials. This is probably due to two competitive processes acting simultaneously: the decay of the organic component of the bone, causing the relative increase of Ca in the bone, and dissolution of part of the apatite, causing the withdrawing of Ca from the bone to the soil. A similar explanation may be correct in the case of Cd, Cr, Li, Sc, and Sr in some sediments (see Fig. 3).

The Fe concentration in sediments correlates with the content of some metals in bones (Mo, Ni, Zn, lanthanides, and weakly with Cu, Co and others); nevertheless, the Fe concentration in the experimentally buried bones is below the detection limit. This result indicates that although Fe plays a minor role in the permineralization of bone during the early stage of diagenesis, the compounds of Fe present in soil may constitute an important reservoir of metals accessible for the fossilization process.

Importance of sediment moisture in formation of metal composition of buried bone

Most of the diagenetic effects observed in this experiment—both geochemical and macroscopic alteration—occurred only in bones buried in moist sediments. The chemical composition of the bones kept in dry sediments remains nearly unaltered (Fig. 5). Among the bones buried in moist sediments in the experiment, the strongest alterations are observed in some of the humiferous sediments, especially in peat, humic silts, and humic sands. These alterations are observed mainly in the increased concentrations of heavy metals such as Zn, Cd, Pb, Co, Mn, Nb, lanthanides, and also Ca. Light metals, with tendency to forming water-soluble salts (Na, K, Rb, Mg), decreased in moist sediments, probably due to dissolution and removal by infiltrating water.

Jaggers and Rogers (2009) reported similar observations of stronger macroscopic changes in their experimental burial. Other studies on modern and fossil bones (Grupe and Piepenbrink 1989; Soudry and Nathan 2000) showed that fungi play an important role in the relocation of some metals (such as Al, Ba, Mg, Sr, U, Zn) from the sedimentary environment to the bone. This might explain stronger chemical alterations during the experiment in moist sediments, which is more suitable for fungi growth. According to Kohn and Moses (2013), the wetting-drying cycles may enhance trace element movement into the bone, even without the biological activity, because different capillary parameters of the bone and soil cause the movement of trace element-depleted water from the bone during drying, while wetting recharges the bone with trace element-loaded soil water. Additionally, Millard and

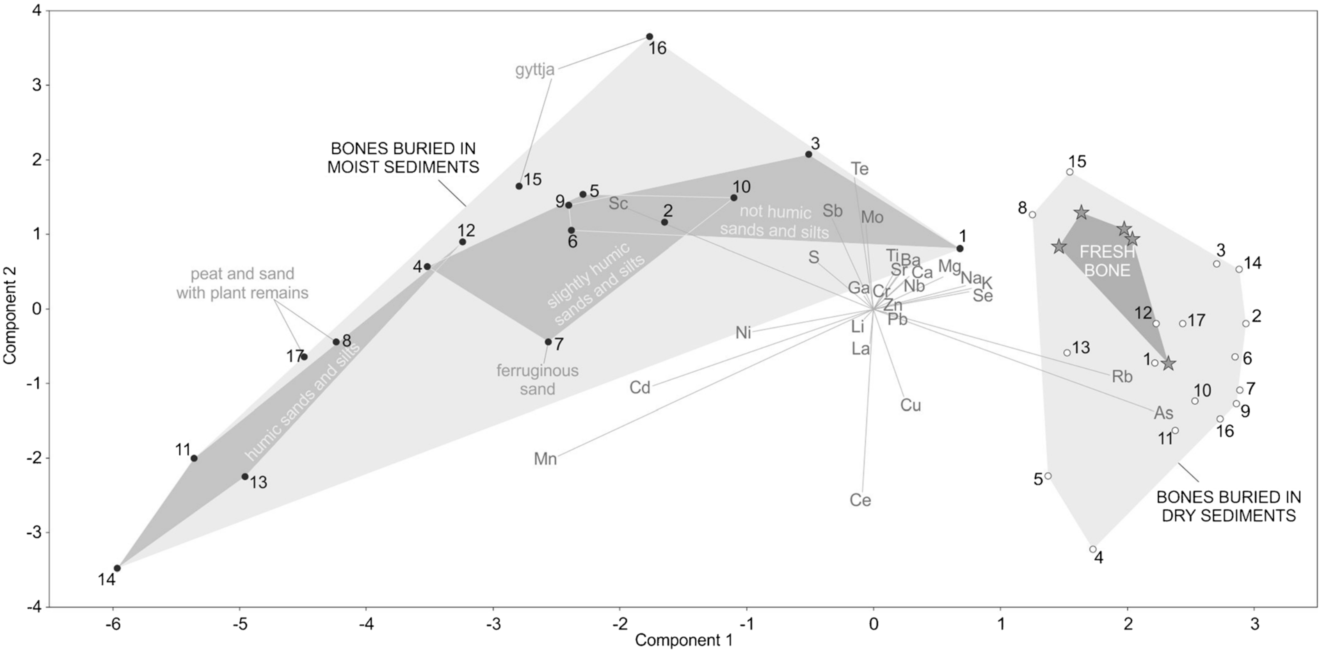


Fig. 5 Principal component analysis (PCA) of the chemical composition of experimentally buried and fresh bones. Symbols use the same code as in Fig. 4

Hedges (1996) noticed that frequent drying and rewetting might be an important process of metal transport into the bone.

Directions of chemical alteration

According to PCA results (Fig. 5), component 1 is the one responsible for chemical differences between bones kept in moist and in dry conditions. In moist sediments, especially in those kept in the soils rich in organic matter, the most important changes include the increase in concentrations of Cd, Mn, Ni, and Sc and a decrease of As, Se, and light metals (K, Na, Mg, Rb). In dry conditions, the chemical alterations in bones are relatively weak along the component 1 axis. Component 2 appears to be responsible for differences caused by different types of sediments in the bones kept in both moist sediments and dry sediments. This variability is more important in the case of dry conditions, and changes of Ce, Cu, Mo, and Te concentrations play a major role here.

The layout of samples kept in moist sediment shown in Fig. 5 reveals the parallel orientation of the humus content in sediment to the bi-plot vectors of Ca, Cd, K, Na, Ni, Mg, Mn, and other minor vectors. This indicates the positive relationships between organic matter in the soil and concentrations of Cd, Mn, and Ni in the bone, which is confirmed by Spearman's rank correlation of organic matter content and Cd and Ni (Table 2).

The following phenomena in the chemical alteration of the buried bone, observed in this experiment, may be useful in recognizing the provenance of bones in case of multilayered sites with differentiated lithology:

- Decrease of Ca content and increase of Mo, Sc, and Zn in organic sediments
- Decrease of Ca and increase of Sr and Ba in sediments rich in trace metals
- Increase of Sr and Ba in alkaline sediments
- Increase of transition metals (Cd, Co, Mn, Ni, Zn) and Pb in acidic sediments
- Increase of Sr in fine sediments (clays, silts)

The important observation concerns the lanthanides (REE), commonly regarded as diagenetic indicators (Denys et al. 1996, Trueman et al. 2004, 2006, MacFadden et al. 2007, Cook and Trueman 2009, Iliopoulos et al. 2010, Kocsis et al. 2010, Kohn and Moses 2013). In opposition to Holocene and Late Pleistocene fossils cited in the literature, the bones buried in the experiment show only minor enrichment in REE, limited mostly to La and Ce, along with Pr and Nd in a few samples. Concentrations of all other lanthanides are below the detection limit. Additionally, some samples exhibit depletion of La and Ce, especially those buried in moist, clean sands. Kohn and Moses (2013) showed that after 18 months of exposition to the trace element-bearing solution, the concentration of particular lanthanides in the bone might be high, but only in the limited surficial part. At a depth of approximately 20 µm, the concentration of most of the REE was lowered close to or below the detection limit.

Conclusions

The experimental approach to bone diagenesis presented in this study is a rarely undertaken type of research due to the long duration of the experimental burial phase. The results presented here offer data that may be used in determining the relationships between the chemical composition of archeological bones and the lithology of strata.

The chemical alterations of the metal compositions in the experimentally buried bones presented here may serve as an initial database for the chemical alterations associated with particular types of sediments. These results are significant for the development of the geochemical provenance of bones, especially those found at multilayered sites with multiple and lithologically diversified strata.

The results achieved in experiment show that the most significant lithological features of soil with respect to the chemical alteration of metal concentrations in bone are organic matter content and the moisture of the sediment. Both of these factors acting together are necessary to significantly alter the chemical composition of bones. The chemical compositions of bones kept experimentally in either dry non-humiferous sediments, moist non-humiferous sediments or dry humiferous sediments were not altered sufficiently to be chemically distinguishable from the fresh, unaltered bone, after the time interval used in this experiment. Soil acidity is also an important factor. The other features of sediments, such as their grain size composition, porosity, carbonate content, and metal concentrations, play only minor roles in the final metal composition of buried bones, at least during the early stages of diagenesis. However, the most important conclusion is that 2.5 years is enough to alter the chemical composition of the bone to the detectable level, if environmental conditions are appropriate (i.e., the sediment is moist and rich in organic matter). This means that even those bones that underwent redeposition at very early stage of their diagenesis may already bear the traces of the original depositional environment. The separate question is about the stability of this initial signature after redeposition, and this indicates the most important research direction in the usage of the geochemistry of fossil bones in the reconstruction of the site formation processes.

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