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Isabelle THÉRY-PARISOT Lucie CHABAL Sandrine COSTAMAGNO

THE TAPHONOMY OF BURNED ORGANIC RESIDUES AND

COMBUSTION FEATURES IN ARCHAEOLOGICAL CONTEXTS







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Layout Yann BELIEZ

Cover Fabien TESSIER

The contributions should be addressed to:

REVUE P@LETHNOLOGIE

Vanessa LEA, Research associates

TRACES - UMR 5608 of the CNRS

Maison de la recherche 5 allées Antonio Machado 31058 Toulouse cedex 9, FRANCE

hone:	+33 (0)5 61 50 36 98
ax:	+33 (0)5 61 50 49 59
mail:	vanessa.lea@univ-tlse2.fr



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HEATING AND DIAGENESIS-INDUCED HETEROGENEITIES IN THE CHEMICAL COMPOSITION AND STRUCTURE OF ARCHAEOLOGICAL BONES FROM THE NEOLITHIC SITE OF CHALAIN 19 (JURA, FRANCE)

Ina REICHE

Abstract

Bone materials, including numerous fragments of burned bones, are often found at archaeological sites. A comparative study of burned and unburned bone materials originating from the lacustrian site 19 of Chalain Lake, Jura, France, was conducted in order to assess their potential to yield information on past lifeways. The bones were analysed using various complementary physico-chemical methods (SEM-EDX, microPIXE/PIGE, FTIR, XRD, TEM-EDX) taking into account their chemical and structural heterogeneity. Through this "high resolution" approach, it was possible to observe the modifications of the bone mineral phase induced by heating and the processes of soil diagenesis, at the scale of the sample.

Our research has made it possible to distinguish the markers that characterise the diagenesis and heating of the specimens. The markers of heating could be used as quantitative indicators of the heating temperature reached by the bone prior to being buried. In addition, the results show different modification mechanisms depending on the possible heating of the bone prior to burying. Specifically, the concentration profiles determined on cross-sections by PIXE/PIGE make it possible to reveal an increase in exogenous chemical species at the centre of the heated sample, in contrast with the unburned bones which show higher concentrations of exogenous elements at the edge than at the centre. Thanks to the nanoscale structural analysis by transmission electron microscopy (TEM), it was possible to correlate the specific modifications of the elemental composition with the size of the apatite crystals present in the specimens.

This method can now be applied to other archaeological problems such as the investigation of various funerary customs or the taming of fire, in the knowledge that it can be applied to a small number of samples that must consequently be carefully selected from a larger corpus in order to be representative.

Keywords : archaeological bones, heating, diagenesis, Neolithic, physico-chemical microanalysis, FTIR, microPIXE-PIGE, TEM

Heating of archaeological bones and diagenesis

The discovery of an anthropic activity linked to fire or heating-utilised both to modify objects and in the activities of daily life-is of utmost interest in archaeology and prehistory because the mastery of fire represents a crucial step in human cultural and socio-economic evolution. For this reason, it is very important to find meaningful markers in the objects unearthed during archaeological excavations. Bones or objects made from bones, ivory, or Cervid antlers are among the objects most commonly found at prehistoric sites. Bone remains or worked bone objects sometimes bear signs of heating. The temperature rise during the heating of bones translates into a modification of their structure, as well as their chemical and isotopic composition. Physicochemical methods of analysis can be utilised to detect the signs of heating within the material. However, archaeological bones are complex nanocomposite biomaterials, which are modified by diagenetic and taphonomic processes in archaeological sediments. When trying to distinguish the modifications linked to thermal treatments from those related to diagenetic processes, the analysis of archaeological bones proves to be a real challenge requiring complementary techniques both to elucidate the structure and to study the changes in chemical composition at various scales; this distinction is achievable perhaps only at the nanometre scale. Moreover, the alterations are not necessarily homogeneous within the bone specimens.

This paper thus more specifically addresses the study of the diagenesis of the mineral phase of burned archaeological bones at a small scale, utilising high resolution analytical methods in order to detect the heterogeneities induced by the different processes. It presents a physico-chemical analysis of unburned bones compared to that of burned bones from the same archaeological site, that of station 19 of Chalain Lake (3850 - 2900 B.C., Jura, France) (fig. 1).

Present state of knowledge

Brief review of the structure of bone

Biomaterials such as bone, dentine, or ivory are composite materials with a highly hierarchical structure

at the macro-, micro-, and even nanoscale level. However, their physico-chemical and mechanical properties are essentially dependent on the close imbrication of the mineral and organic phases at the nanometric scale (fig. 1.)



Fig. 1 - Schematic structure of a bone at various scales.

Atthemolecular level, the organic phase of bone material is composed of 90% type I collagen (tropocollagen) made rigid by an extremely close-packed filling of crystallites of carbonated hydroxylapatite with the general formula (carb-HAP) that constitutes the mineral phase. Collagen is a protein arranged in a triple helix in which each strand is composed of a chain of amino acids (the most frequent sequence being a glycine, proline, hydroxyproline chain.) The collagen molecules are organised hierarchically: that is, the molecules are assembled in the form of fibrils that in turn form fibres. The organic phase also contains lipids and non-collagenous proteins (like osteocalcin) that provide the interface between the collagenous organic matrix and the mineral phase. It is generally acknowledged that the apatite crystals grow inside the organic framework. These carb-HAP crystals have the shape of platelets whose dimensions are still subject to controversy. Cui et al. (2007) report crystals having dimensions of 50 x 28 x 2 nm³.

Numerous researches have already addressed the heat-induced modifications of bone material. They have considered modifications of the both general appearance of objects and of their structure or chemical composition at the molecular level. In general, a reference standard has been developed based on fresh cortical bone as this is the type of bone that is preserved best in archaeological contexts (Shipman *et al.*, 1984; Baud and Tochon-Danguy, 1985; Stiner *et al.*, 1995; Michel *et al.*, 1996 ; Person *et al.*, 1996; Reiche *et al.*, 2007; Lebon, 2008).

Modifications to the general appearance of bone

A colour change is the most obvious modification caused by heating. When a fresh bone is heated, its colour evolves from beige-light brown, through black, to gray and finally, white. An increase in the friability and porosity of the objects occurs at the same time at this colour change. The modified bones can also assume other colours such as blue, green, red, and black. The colours, except the blue, are not necessarily due to heating of the bone, but rather to the presence of crystalline phases containing iron, manganese, or copper (Reiche & Chalmin, 2008; Shahack-Gross et al., 1997.) Two heat treatment stages can generally be distinguished: carbonisation and calcination. Carbonisation consists of the formation of black carbonised products at temperatures between 280 and 650°C. Calcination refers to a heat treatment at higher temperatures (650°C and higher) that yields grey or white residues. In addition, Stiner et al. (1995) have defined more elaborate heating stages, distinguishing a total of six heating stages ranging from non-heated (0) to calcination (6).

Changes in the structure and chemical composition

The changes on a macroscopic scale are associated with changes at the micro- or even nanoscale level in the structure and chemical composition of the material during heating. To start, the material is fissured and loses water. Starting at 170°C, the organic fraction

changes and volatilises (Chadefaux & Reiche, 2009) which, from a macroscopic point of view, translates into a brownish colour. At around 400°C, a large portion of the organic matter has been carbonised. The bone then assumes a black colour. The carbonisation products are eliminated as the temperature rises to 650°C. The modifications in the mineral phase are observed starting at 500°C, but they do not result in a colour change. They consist of both the transformation of the carb-HAP and of the improvement in the crystallinity of the bone's apatite, that is to say an increase in the size and an improvement in the regularity of the crystals. The carbonates separate from the phosphates in the carb-HAP, thus yielding a purer apatite and some calcite. At around 600°C, the carbonates and hydroxyl ions are lost through the liberation of CO₂ and water, leading to the formation of β -tri-calcium-phosphate (β -Ca₃(PO₄)₂) and lime (CaO) in addition to the apatite.

Employing various investigation techniques, such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), electron paramagnetic resonance (EPR), or transmission electron microscopy (TEM), it has been possible to establish reference standards that allow the detection of modifications to bones during heating and the determination of the temperatures achieved (Person et al., 1996; Reiche et al., 2007; Piga et al., 2008; Lebon et al., 2008.) The determination of the temperature is based either on the calculation of the crystallinity index (CI or SF), or on the average size of the crystal (by XRD or TEM), or on the shift of specific peaks after the splitting of vibrational peaks in the FTIR spectrum. The establishment of this reference standard has now made it possible to estimate the approximate heating temperature reached by a bone before its burial. It should be stressed that in general these analyses approached the object as a whole and did not take into account the possible heterogeneities induced within the material by the various heating procedures. Several heating stages can sometimes be observed on the same ancient specimen. Moreover, it should be noted that the diagenetic processes in the soil can also lead to the modification of the crystallinity of ancient bones. This is why it is difficult to distinguish the changes in crystallinity due to these diagenetic processes

from those linked to heating at low temperatures (less than 300°C.) Nevertheless, a study of the form and size of the crystals by TEM gives a more accurate determination of heating, at least for temperatures above 500°C, because the morphology of the crystal formed upon heating is characteristic and allows us to precisely distinguish a heat induced recrystallisation from one obtained as a result of low temperature geochemical processes. It is obvious that other parameters such as the colour of the specimen or of other burned objects found within the same archaeological context must also be considered to confirm the results obtained.

Description of the study site

Over thirty archaeological sites were discovered during the excavations and prospections carried out by P. Pétrequin and his team at Chalain Lake (fig. 2). This lake is located in the Combe d'Ain, a closed alluvial valley in the Franche-Comté region, at the foot of the folded Jura (Jura plissé). It displays long chronological sequences of littoral villages dating from approximately 3850 to 850 BC. Seven Neolithic villages in the chronological range of 3850-2650 BC have been undergoing excavation for around the pas thirty years by P. Pétrequin's team (Pétrequin &Pétrequin, 1988; Pétrequin, 1997; Pétrequin *et al.*, 1998). Chalain Lake is located at about 500 m altitude (fig. 3), which is the upper limit for the extension of cereal cultivation; it thus presents an example of a specific adaptation by Neolithic civilisations to a harsh climate. The sites corresponding to the archaeological villages of Chalain are not distributed along the entire perimeter of the lake. The majority of the villages are located on the lake's west side, which has the advantage of long-lasting sunlight and proximity to the cereal fields. The sites are separated from the lakeshores by a line of swamps and the littoral platforms are relatively wide. The occupations are concentrated in the two bays nearest to the arable fields (Pétrequin & Pétrequin, 1988; Pétrequin, 1997; Pétrequin et al., 1998). The Chalain sites are in their original topographical positions, with a progressive drying linked to the lowering of the lake level starting in 1904 (P. Pétrequin, pers. comm.)

These sites are marginally located relative to the epicentres of cultural developments and in terms of population density during the period under study. In fact, only two littoral zones of Neolithic sites, 12 km apart as the crow flies, are known in this region (those of Chalain Lake and those of Clairvaux Lake.) They constitute a coherent whole in terms of lifeways and methods of exploiting the environment. The amalgamation of research on the sites of Chalain



Fig. 2 - a) Map of the Jura region, France with Chalain Lake and b) map of the archaeological sites of Chalain Lake, Jura, France. Reproduced with the permission of P. Pétrequin.



Fig. 3 - Photograph of Chalain Lake, I. Reiche, 1998.

(abbreviated CH) and Clairvaux is therefore justified and allows comparisons of the overall development of Neolithic communities, particularly in demographic and agricultural terms. The geographic situation is favourable because the region is well demarcated and its dendrochronological datings thus allow precise determinations of the ages of contemporary or successive villages.

Multidisciplinary research projects have been undertaken at these locations in order to take advantage of the archaeological, biological, chemical, and geological information contained in the objects found (Pétrequin, 1997.) Research on the modifications associated with heating and diagenesis of bone remains are integrated in the context of these investigations.

At Chalain, the bones are exceptionally well preserved throughout the site. According to archaeological observations (P. Pétrequin, pers. comm.), the bone fragments originating from the preparation of meals (butchering and cooking) were discarded in the dumps in front of the only entrance to the houses on pilings, built on flood prone ground (fig. 4.) This refuse (including the bone remains) therefore fell onto humid soil or into shallow water. They were quickly covered by the vegetal litter brought in by humans to stabilise and reclaim the exterior soils during low water periods. After the villages were abandoned, the lake's level rose again and lake chalk was deposited. These conditions favoured the preservation of the remains in an anoxic environment, quite submerged or below the level of the water table. This burial environment is specifically favourable to the preservation of organic materials.



Fig. 4 - Pile house built on Chalain Lake following the building methods of the era. Reproduced with the permission of P. Pétrequin.

Bone materials studied

Despite the exceptional preservation of archaeological remains at lacustrian sites, the bones found can be more or less modified depending on the conditions to which they have been subjected before or after their disposal (Pétrequin *et al.*, 1998):

Type 1: a large quantity of bones was burned as a consequence of deliberately set or accidental fires at the time the littoral dwellings were abandoned or as a result of specific methods of utilisation of bone material (meat cooking, glue manufacture, etc.) These bones were found in the form of small undefined fragments.

Type 2: some unburned bones are characterised by a remarkable state of preservation free of superficial attack and with good conservation of the organic and mineral matter.

Type 3: bones that have undergone another form of degradation apparently have nearly intact organic material. Nonetheless, the bone mineral material is modified to such extent that the bones can be flexible. This is especially the case for bones found in waterlogged environments.

During the excavation campaign conducted in 1998,

some fragments of burned and unburned bones from the emerged station 19 were entrusted to us for analysis. Having been preserved in similar burial conditions, the various burned and unburned specimens allow us to study the influence of heating on the diagenetic processes and therefore to assess their potential to yield information and the representivity of these two types of remains.

Four fragments of unburned bones, labelled AB_ CH19nb1 - 4, corresponding to conservation type 2 and three fragments of burned bones (type 1), labelled ABB_CH19b1-3, were analysed (fig. 5, table 1.) They originate from layers H and K of station 19 of Chalain which are dated to 3040–2970 BC. These layers correspond to villages with raised floor houses with sediments of anthropic dung that contain a small percentage of calcium carbonates deposited by the lake (fig. 6).

"High resolution" physico-chemical approach

The ensemble of this work led us to apply a broad range of complementary analytical methods since in order to evaluate the state of preservation of complex and very heterogeneous bone materials and reliably interpret the results obtained, it is essential to acquire quantitative information on the object itself. This information concerns its chemical composition and the spatial distribution of its constituent elements, its crystallinity, and the morphology of its crystalline phases. To this end, it is necessary to employ a large number of complementary techniques that allow the detection of the changes in the bone structure and trace elemental composition from the microscale to the nanoscale.

The data obtained on the archaeological samples are then compared to those obtained on a fresh reference bone in order to assess the state of preservation. If the archaeological bone shows characteristics similar to those of the fresh bone (made up of the same phases, homogeneous distribution of the component elements, low degree of crystallinity of the carb HAP), it is presumed to be well preserved.



Fig. 5 - Photographs of the bones from Chalain Lake 19 studied: a) unburned specimens and b) burned specimens.

Scanning electron microscopy (SEM), paired with a system of analysis by energy dispersive X-ray spectroscopy (EDX), is utilised to observe and analyse



Fig. 6 - Stratigraphy of station 19 of Chalain Lake. Reproduced with the permission of P. Pétrequin.

Archaeological layer	laboratory reference	Nature of the sample (1)	state of conservation at the naked eye
Н	AB_CH19nb1	undetermined	unburned, brown
К	AB_CH19nb2	undetermined	unburned, brown
К	AB_CH19nb3	left humerus of wild boar	unburned, brown
К	AB_CH19nb4	undetermined	unburned, brown
НК	ABB_CH19b1	undetermined	burned, white
НК	ABB_CH19b2	undetermined	burned, black in the centre and blueish at the border
НК	ABB_CH19b3	undetermined	burned, black in the core and gray at the border
	Archaeological layer H K K K HK HK HK	Archaeological layerlaboratory referenceHAB_CH19nb1KAB_CH19nb2KAB_CH19nb3KAB_CH19nb3KAB_CH19nb4HKABB_CH19b1HKABB_CH19b2HKABB_CH19b3	Archaeological layerlaboratory referenceNature of the sample (1)HAB_CH19nb1undeterminedKAB_CH19nb2undeterminedKAB_CH19nb3left humerus of wild boarKAB_CH19nb4undeterminedHKABB_CH19b1undeterminedHKABB_CH19b1undeterminedHKABB_CH19b2undeterminedHKABB_CH19b2undeterminedHKABB_CH19b3undetermined

(1) Rose-Marie Arbogast is acknowledged for the determination of the analysed bone samples.

Tab. 1 - Characteristics and denominations of the bone specimens chosen for this study.

the inclusions precipitated in the bone matrix during their modification. This technique makes it possible to distinguish the constituent elements of the bone material and the chemical species present in the inclusions, without, however, allowing the detection of the trace elements.

The trace element content is obtained through a very sensitive spectroscopic analysis by X-rays and gammarays generated by a proton micro-beam (microPIXE/ PIGE) utilising the *Accélérateur Grand Louvre d'Analyse Elémentaire* [Grand Louvre Elemental Analysis Accelerator] (AGLAE) of the LC2RMF. This technique also shows the spatial distribution of the elements (major, minor, trace) through the measurement of concentration profiles.

TEM permits a direct structural analysis of monocrystals of nanometre size. This technique is used to complement XRD and FTIR methods that allow the structural and molecular analysis of the overall bulk specimens. In addition, the splitting factor SF (IR)¹, commonly utilised in archaeology to estimate the state of preservation of the samples and a possible heating, is calculated from the FTIR spectra.

The analytical conditions of the techniques are described in detail in the thesis and in the articles: I. Reiche (2000), Reiche *et al.* (1999, 2002a and b, 2003, 2007).

Results of observations and physico-chemical analyses at different scales

The unburned bones from the Neolithic village of Chalain 19

The bones from Chalain 19 are well preserved in the sense that they are composed of slightly crystallised carb-HAP and of a collagenous fraction. The SF (IR) crystallinity index of the four specimens, calculated according to the reference standard of Reiche *et al.* (2003), varies between 2.3 and 2.5, thus remaining very close to that of fresh bone (2.0) (fig. 7.) The elemental composition of the four unburned bone specimens, measured by microPIXE/PIGE, is shown in table 2. In all bones, the Ca/P ratio is higher than in the reference fresh bone; the bones also contain numerous trace elements such as iron, manganese, aluminium, silicon, sulphur, and fluorine in concentrations higher than those measured in the fresh bone.

The XRD and FTIR analyses demonstrate the presence of calcite $(CaCO_3)$ and secondary phases such as boehmite (AlO(OH)). Moreover, as shown by the SEM-EDX analysis, all unburned bone samples contain localised iron sulphide based microcrystals in the pores or the fissures. These

¹- The calculation of a crystallinity index derived from the IR spectrum is commonly utilised in archaeometry for the purpose of showing the bone modifications during diagenesis (Termine and Posner 1966; Shemesh 1990; Weiner *et al.* 1993; Michel *et al.* 1996; Sillen and Parkington 1996; Wright and Schwarcz 1996). This index is based on the splitting of the peaks corresponding to the vibrations of the phosphate groups v, around 560-600 cm⁻¹ obtained in the absorbance mode. This index or "splitting factor" (SF) is calculated from the intensities of the absorbance peaks at 605 and 565 cm⁻¹: SF = (A(605 cm⁻¹) + A(565 cm⁻¹))/ A(base)

The SFs measured by Sillen and Parkington (1996) vary between 2.80 for an untreated modern cow bone to 5.33 for the same bone once it has been burned. *This SF index represents only an overall indication of the crystallinity*. In reality, other adsorption peaks can be superimposed on the phosphate peaks (those of sulphates, for example), but here we will utilise the SF solely to estimate the progress of crystallinity in cases that do not require a more accurate measurement by transmission electron microscopy.

	AB_CH19nb1		AB_CH19nb2	AB_CH19nb3		AB_CH19nb4		MB (modern sheep bone)	
	mean concentration (ppm)	Range (ppm)	mean concentration (ppm)	mean concentration (ppm)	Range (ppm)	mean concentration (ppm)	Range (ppm)	mean concentration (ppm)	Range (ppm)
F	2800±100	2400-3400	non analysé	2000±100	1000-4000	3600±100	2100-6300	550	0-1200
Na	7200±900	6000-8420	3000±300	2800±330	1400-3900	5700±900	4100-9050	8500	7600-9100
Mg	1500±30	900-1700	-	1000±170	650-1250	1500±320	960-2500	7800	7200-8700
AĨ	630±320	140-1640	-	640±120	-	850±350	200-3800	-	-
Si	-	-	1700±160	6300±220	3800-10000	2900±	180-12100	-	-
Р	176000±1000	168000-182000	160900±480	152000±1000	146000-157000	178000±1400	171000- 204000	179 000	178000-180000
S	4300±300	2100-18400	2300±100	8100±220	2300-32400	3900±600	2200-18200	1600	1200-1900
CI	450±15	120-690	160±60	200±80	100-230	220±60	70-410	750	650-820
Ca	404000±2800	379000-417000	427000±1300	419000±3800	380000-431000	390500±2300	353000-	384000	382000-386000
Mn	220±10	170-260	360±10	280±10	200-380	370±20	300-580	-	
Fe	5600±200	4000-7300	6200±180	10700±300	5200-33800	7100±250	3100-18800	-	
Zn	220±10	160-330	260±10	300±10	200-400	160±800	90-350	130	120-140
Sr	190±10	130-230	130±10	150±10	130-170	140±10	90-190	250	180-280
Ca/P	2,3	3	2,7	2,8	3	2,2		2,1	2,1-2,2

Tab. 2 - Average elemental composition and concentration range of unburned bones from site Chalain 19 and of modern sheep bone, analysed via PIXE/PIGE. - = below the method's detection limits.



Fig. 7 - FTIR spectra of unburned bones (AB_CH19nb1_4).

microcrystals, measuring approximately one μ m and having a stoichiometry varying between FeS_{1.5} and FeS₂, generally form agglomerates between 5 et 20 μ m in size (fig. 8.) This observed pyrite has a particular framboidal or botryodial form. The average diameter can be set at 10 μ m, which is consistent with other pyrite-based "raspberries" observed in various geochemical surroundings (Wilkin *et al.*, 1996).

The spatial distributions of the chemical elements were measured on transversal cross-sections of bone specimens. The results for the concentration profiles of the bone fragments are given for sample AB_ CH19nb3 (fig. 9a-b.) Several types of concentration profiles were measured on the bone cross-sections: flat (homogeneous distribution of concentration); irregular; U-shaped (displaying an enrichment starting at the periosteum and the endosteum);



Fig. 8 - Electron micrograph (in backscattering electron mode) showing one of the numerous iron sulphide inclusions in the pores of bone AB_CH19nb3. The size of the aggregates of microcrystals is generally between 10 and 20 μ m and that of the individual microcrystals is between 1 and 2 μ m. The average stoichiometry of the iron sulphides in this specimen corresponds to FeS₂.

reverse U-shaped (displaying lower concentrations at the periosteum and endosteum relative to the centre); decreasing from the periosteum; increasing form the periosteum.

In general, the unburned bones are characterised by a good preservation of the mineral matter, even though numerous trace elements such as fluorine, iron, manganese, aluminium, silicon, and zinc have been incorporated, and others (sodium and magnesium) leached out during diagenesis. In the latter case, the exogenous chemical species are substituted or adsorbed at the surface of the bone phases. The preservation of the organic phase in



Fig. 9 - a-b : Concentration profiles of the constituent elements of an unburned bone (AB_CH19nb3) measured by microPIXE/PIGE at the micro-beam line extracted from the AGLAE accelerator.

these specimens has been evaluated through the presence of specific peaks in the FTIR spectrum (fig. 7.) On the whole, their width and ratio match those of fresh bone. The organic phase thus seems well preserved, even though a portion of the nitrogen from the collagen has likely been leached out.

The burned bones from the Neolithic village of Chalain 19

The burned bones from Chalain are made up of well crystallised HAP and contain very little organic matter according to the FTIR spectrum and the powder X-ray diffractogram (fig. 10a-b.) They are heavily modified relative to fresh bone. Unlike unburned bones, the samples display an almost complete loss of the histological structure and a high porosity as measured by SEM. The calculated crystallinity indexes are higher than that of a fresh bone (2.0) and those of unburned bones from the same site (2.3-2.5) with average values of 4.5 for CH19b1, 2.8 for CH19b2 and 3.2 for CH19b3.

In addition to their constitutive elements, the bones contain trace elements from the burial environment (tab. 2). Iron, manganese, zinc, strontium, and barium were detected at higher concentrations than those



Fig. 10 - FTIR spectrum of a burned bone (edge and centre of specimen ABB_CH19b3) and b. powder X-ray diffractograms (a) ABB_CH19b2, b) ABB_CH19b3, and c) ABB_CH19b1).

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characterising modern bones. The bones are slightly enriched in calcium. Traces of calcium carbonate $(CaCO_3)$ are moreover detected in some specimens.

The spatial distribution of the elements was studied in the case of a burned bone, ABB_CH19b3. The concentration profiles of calcium and phosphorous show a preferential loss of calcium relative to phosphorous towards the edge of the bone. A new type of elemental distribution, showing increasing concentrations from the edge towards the inside of the specimen was observed for a number of exogenous chemical elements such as fluorine, sulphur, iron, and manganese (fig. 11a-c).

Moreover, inclusions of pyrite of FeS_{2} stoichiometry were detected in the fissures, equivalent to those observed in unburned bones from the same site. These



Fig. 11 - a-b-c : Concentration profiles of the constituent elements of a burned bone (ABB_CH19b3) measured by microPIXE/PIGE at the micro-beam line extracted from the AGLAE accelerator.

inclusions are also present in the form of agglomerated microcrystals. These framboidal aggregates measure about 20 μ m and the individual crystals that make them up measure from 1 to 2 μ m (fig. 12.)

Since one of the burned bones more specifically showed an unusual elemental distribution of the exogenous elements having an inverse U-shape, TEM observations were carried out on a sample showing traces of homogeneous heating (ABB CH19b1, fig.13a), as well as at the edge and at the core of a sample showing heterogeneous heating (ABB CH19b3, fig.13b-c.) It is hoped that these phenomena of specific enrichment can be linked to a highly variable crystallinity within the bone. The electron micrographs show large size crystals with polygonal morphology in the homogeneously burned sample (ABB_CH19b1) and near the surface of the heterogeneously burned sample (ABB_CH19b3), while at the core of the latter sample, irregular or needle-shaped crystals were detected. In addition, crystalline phases rich in F, probably in CaF, form, were detected very locally.



Fig. 12 - Electron micrograph (in backscattering electrons mode) showing a pyrite (FeS_2) inclusion in a fissure of the burned specimen ABB CH19b3.



Fig. 13 - Electron micrograph a) of a burned specimen (ABB_CH19b1), b) of the edge, and c) of the core of object ABB_CH19b3.

Discussion

Comparison between unburned and burned bones from station 19 of Chalain Lake

The flood environment of Chalain 19 can be considered as hydrologically fluctuating. Periods of submersion alternated with periods of emersion, indicating a large flow of material as the authors Hedges & Millard, 1995 propose for this type of conditions. During submersion, a seasonal influx of dissolved material in the water and dissolution/hydrolysis, as well as recrystallisation/ repolymerisation processes, take place; during emersion, on the other hand, leaching of elements and precipitation of secondary phases by supersaturation occur. Erosion, if it occurs, should lead to relatively porous, recrystallised, and carbonated samples because the site is found in an environment rich in lake chalk.

The signs of change in the case of unburned bones are the partial modifications of their mineral and organic phases. They display numerous exogenous chemical species adsorbed or substituted on the bone material. On the other hand, burned bones are strongly modified in their organic phase and the mineral phase also underwent transformations induced by heating prior to burial; they show a relatively "pure" recrystallised mineral phase free of preserved organic material. On the one hand, in comparison with unburned bones, the exogenous chemical species are trapped to a larger extent in the pores, created by the degradation of the organic material and the recrystallisation of the apatite during heating, in the form of various inclusions like pyrite and calcite; on the other hand, the high crystallinity of the apatite of burned bones makes them less subject to the dissolution-recrystallisation process, which limits the introduction and adsorption of exogenous elements in the bone's apatite.

The formation of pyrite, observed in both types of burned and unburned bones, takes place directly after the disposal of the bones in the surface sediments. The archaeological layer in question was thus covered by a deposit of calcium carbonate by sedimentation. During the first years of burial, the environment must have been sufficiently reductive with an abundance of organic material rich in sulphur and iron to facilitate the formation of pyrite. As already observed in many cases (Turner-Walker, 1999), the pyrite remains stable afterwards and is found in the form of "raspberries" in these bones, even after the emersion of the site, and the subsequent oxidation, as was the case at Chalain 19 starting in 1904 due to the gradual drying associated with the lowering of the lake level. This corresponds rather well with the environmental situation determined through the study of climatic fluctuations reconstructed from the fluctuations of the lakes levels

	ABB_CH19b1	ABB_CH19b2	ABB_CH19b3		MB (modern sheep bone)		
	mean	mean	mean				
	concentration (ppm)	concentration (ppm)	concentration (ppm)	concentration range (ppm)	Concentration moyenne ppm)	concentration range (ppm)	
F	510±100	4520±100	1670±100	600-2500	550	0-1200	
Na	5110±600	9500±900	8800±900	6950-11800	8500	7600-9100	
Mg	900±300	1600±260	-	-	7800	7200-8700	
AĪ	-	1700±170	-	-	-	-	
Si	2500±220	-	-	-	-	-	
Р	186000±2800	178000±2000	180000±1260	172000-189000	179 000	17,8-18,0 %	
S	1280±170	1760±130	2670±180	800-4250	1600	1200-1900	
CI	1220±120	290±80	570±90	200-810	750	650-820	
Ca	396000±6700	399000±5200	405300±3200	389000-417000	384000	38,2-38,6 %	
Mn	20±10	200±10	110±15	25-180	-		
Fe	380±30	1120±10	3500±100	310-8020	-		
Zn	40±5	710±10	440±20	110-1740	130	120-140	
Sr	120±10	200±10	240±20	10-330	250	180-280	
Ca/P	2,1	2,2	2,3		2,1		

Tab. 3 - Average elemental composition of the burned bones from Chalain 19 determined by PIXE/PIGE. - = below the method's detection limits.

sample	colour	estimated temperature by the colour of the sample (°C)	estimated heating temperature by SF(IR) (°C)	estimated heating temperature by analysis of the X-ray diffractograms (°C)
ABB_CH19b1	white	700-940	800-940 (4,5 ± 0,1)	700
ABB_CH19b2	brown black blueish	400	$300-400 (2,8 \pm 0,1)$ 500-600 (3,2 ± 0,1)	300 550
ABB_CITI3D3	DIACK GIAY	300-800	$500-000(3,2\pm0,1)$	530

Tab. 4 - Estimate of the heating temperature of archaeological specimens derived from powder X-ray diffractograms, from the reference standard established by infrared spectroscopy (Reiche *et al.*, 2007), and from the colour of the specimens.

and the correlation of these data with those of the concentration of residual ${}^{14}C$ in the atmosphere (Arbogast *et al.*, 1996). According to this work, the installation of Neolithic villages is closely tied to the climatic fluctuations; the dynamics of occupation of the shores are consequently regulated by the fluctuations in the lake level.

We can conclude that the unburned and burned bones display common characteristics that are linked to the geochemical environment in which they are buried. One example is the presence of pyrite in both cases. They also display differences in the degree of preservation that are due to heating of one type in contrast with the other. Burned bones are more porous because they have almost no organic material left; consequently numerous inclusions are trapped within their structure. Relatively few chemical species are adsorbed or substituted in the apatite crystals due to the increase of their size caused by heating. Unburned bones are less porous and still contain some organic material. The exogenous chemical species are found adsorbed or as inclusions in the still intact channels.

Estimation of the heating temperatures sustained by the burned bones before burial determined through an evaluation of the crystallinity of the bone apatite

According to our reference standard (Reiche, 2000; Reiche et al., 2007), the bone ABB CH19b1 was heated to at least 700°C, ABB CH19b2 to 300°C, and ABB CH19b3 to 550°C. The bones therefore reached relatively high temperatures prior to burial; these temperatures match quite closely those achieved in fireplaces or in a natural fire (Bennett, 1999). The reference standard is, however, limited to temperatures above 300°C because the changes in the mineral phase alone are not detectable below this temperature (Chaudefaux & Reiche, 2009) For this reason, bone ABB CH19b2 could also have attained its crystallinity as a result of modification processes in the soil. Nevertheless, its superficial appearance and its colour are also indicative of heating. The bones acquire a white colour staring at 700°C, confirming that only bone ABB_CH19b1 was heated to at least 700°C.

The crystallinity of the apatite of the burned bones shows that they underwent various processes before and after burial. Bone ABB_CH19b1, heated to at least 700°C, would have had to be in direct contact with the heat source, meaning it was burned directly, to reach its level of recrystallisation. For specimen ABB_CH19b2 and b3, several processes can be hypothesised. Either the bones were buried and then heated in the course of a fire, or they were thrown into a fireplace that could reach these temperatures. In effect, Bennett (1999) has shown that sand and clay layers can reach temperatures of 400-500°C at a depth of 5 cm during fires in a fireplace or in open, natural fires.

Heterogeneity of the effects of heating on bones demonstrated by the measurement of microPIXE/ PIGE concentration profiles

The microPIXE/PIGE analyses show the distribution of chemical elements in bone remains at the major, minor, and trace level. Various types of distribution can thus be observed depending on the element considered and the conditions of the bone before burial (burned or unburned.) In fact, inverse U-shaped elemental distributions, that is with a concentration of the element considered higher in the core of the sample than at the outside edges, have been observed only very rarely on cross sections of ancient bones. Two phenomena can in principle lead to such a profile. In the case of a constituent element of the bone, like sodium or magnesium, their leaching during diagenesis can give rise to these profiles. With regard to exogenous elements, the explanation of the phenomenon is more complex. It would seem that generally this type of profile is only observed on burned bones that display heterogeneities of the heat-induced changes. Due to the finite rate of heat diffusion, especially in the cases of short duration heating, the heat does not homogeneously modify the structure and the chemical composition of the bone specimen. Its surface reaches higher temperatures than its core. Consequently, the loss of organic material and the heat induced recrystallisation of the bone apatite are more pronounced at the edges than at the centre of the object. The differential loss of the combustion products limits the growth of the apatite crystals. The crystals

attain larger sizes towards the edge of the sample than at the centre. For this reason, the exogenous chemical species like fluorine, iron, or sulphur found in the interstitial water at the burial site can attach themselves preferentially to the smaller crystals at the centre, which offer a much larger specific surface (100-200 m².g⁻¹), than to the large crystals at the edge of the porous specimen. Moreover, the small crystallites at the centre display a higher solubility than the large crystals and this allows them to react to a larger extent by dissolution/recrystallisation with the chemical species of the surroundings. However, unburned bones could also display this type of profile for the exogenous chemical elements, especially if during the object's history, the burial or conservation conditions changed and dissolution/ recrystallisation phenomena occured preferentially at the surface of the object. The observation of inverse U-shape concentration profiles is not sufficient by itself, therefore, to prove heating of the bone specimens; it can nonetheless corroborate other indications of heating such as the observation of high crystallinity.

The observation of the same trends of elemental distribution in the burned bones of another lacustrian Neolithic site corroborates these results. In the course of our research, we have had at our disposal three bone samples collected at another Neolithic site at Gletterens (Neuchâtel Lake, Switzerland) thanks to Denis Ramseyer (*Service Archéologique Cantonal* [Canton Archaeological Service], Fribourg). These bones, dated to approximately 3000 years BC, probably belong to small vertebrates. This settlement is entirely trapped in a thick fine sand layer and the remains remained in a humid layer at the level of a water table, which explains the good preservation of the specimens (Reiche *et al.*, 2002).

Conclusions and archaeological prospects

This study has demonstrated the archaeological importance of the analysis of burned bones at a microscale, and even nanoscale, for understanding diagenetic processes and for the assessment of their potential to yield information. Burned bones are also well preserved in archaeological contexts, but display specific diagenetic modifications; they can thus be utilised also to infer archaeological information. The Rose-Marie Arbogast method, consisting of using the number of bones in an archaeological layer to estimate the relative roles of hunting and animal husbandry in the diets of Neolithic humans is also applicable when burned bones are utilised (Arbogast, 1997; Arbogast & Pétrequin, 1997).

Moreover, our results demonstrate that when trying to understand heat-induced transformations, it is important to take into account the heterogeneities of the structure and chemical composition caused by heating. The study, through various analyses at the core and at the edge of the specimens, makes it possible to gather information not only on the possible heating of the objects and on the temperatures reached, but also to refine the information obtained, specifically concerning the precise heating method (long or short duration, direct flame, fire, multiple combustions in a fireplace, etc.), since this approach makes it possible to determine and to precisely localise the heatinduced modifications undergone by the specimen.

It would be interesting to develop a heated bone reference standard that would take into account the various thermal treatments and which would also allow a more refined detection of the heat-induced modifications of the organic phase at lower temperatures (Chadefaux & Reiche, 2009). It would be possible to conduct a series of bone heating experiments in order to model the relationships between the various types of modifications (size and morphology of the crystals, distribution of chemical species, crystallinity index, and colour). This new reference standard could lead to a refinement of the study of various archaeological problems, such as that of funerary practices by incineration, or that of the domestication of fire.

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Author

Ina Reiche

Laboratoire du Centre de recherche et de restauration des musées de France (LC2RMF) UMR 171 CNRS, Palais du Louvre, 14 quai François Mitterrand - 75001 Paris ina.reiche@culture.gouv.fr

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