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Biological mineral content in Iberian skeletal cremains for control of diagenetic factors employing multivariate statistics

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ABSTRACT

The aim of this study was to define a strategy for a correct selection of bone samples by employing inductively coupled plasma optical emission spectroscopy (ICP-OES) for reconstructing the biological mineral content in bones through the determination of major elements, trace elements and Rare Earth Elements (REE, lanthanides) in skeletal cremains of ancient Iberians (III–II B.C), discovered in the Necropolis of Corral de Saus (Moixent, Valencia) between 1972 and 1979. The biological mineral content was determined taking into account diagenetic factors. A control method for a better reading of results was applied. To explore large geochemical datasets and to reduce the number of variables, Principal Component Analysis (PCA) was used, thus, providing a deeper insight into the structure of the variance of the dataset. PCA shows that the elemental profiles of bone and soil samples are clearly different. Bone samples obtained from the outer bone layer were shown to have a different elemental composition; more similar to soil samples than samples of the inner bone layer. PCA scores and loadings plots were preferred to dendrograms obtained using Cluster Analysis, due to the limits of the latter one to appreciate the spatial ordering of samples. Partial least squares discriminant analysis (PLS-DA), a frequently used supervised classification method, was applied to differentiate between degradation states of bone samples. PLS-DA results obtained in this study confirmed that changes derived from different burning conditions were associated with transformations in the mineral part of the bones. Accordingly, carbonized bones can be differentiated from cremated bones. Class assignment of bone samples with uncertain thermal conditions in dependence on their elemental composition has shown to be feasible. Consequently, for biochemical-archaeological studies the analysis and statistical classification of carbonized and cremated archaeological bones, as well as those exposed to unknown thermal conditions together with experiments in modern bones, are recommended.

1. Introduction

The structure and chemical composition of bones can be modified post-mortem while they are buried by many natural factors. Natural alterations (weathering, dissolution, precipitation, microbial attack, mineral replacement, ionic substitution, recrystallization and isotopic exchange) are commonly referred to as diagenesis. Many authors have been intensively investigating how those processes are affecting and limiting the reconstruction of diet, migration, pathology and, in general, our understanding of ancient civilizations (Lambert, 1985; Person et al., 1995; Reiche et al., 1999, 2003; Surovell and Stiner, 2001; Trueman et al., 2004; Tütken and Vennemann, 2011).

In many cases, a previous impact on the structure and chemical composition of the bones is induced by cremation during funerary rituals, cooking habits or other human activities. Therefore numerous authors consider that identifying and understanding heat-induced changes in bones are potentially valuable for resolving forensic and archaeological problems (Clark and Ligouis, 2010; Devlin and Herrmann, 2008; Douglas, 2009; Etok et al., 2007; Lanting et al. 2001; Rogers and Daniels, 2002; Shipman et al., 1984; Schultz et al., 2008; Squires et al. 2011; Walker and Miller, 2005). Many studies have simulated some aspects of the diagenesis evolution by heating fresh bones, and have appreciated changes in crystallinity at certain temperatures and at various time scales over which it occurs (Holden et al. 1995; Lebon et al., 2010; Munro et al. 2007; Person et al., 1996; Stiner et al. 1995).

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Several studies investigating the elemental composition of archaeological, contemporary and experimentally cremated bones have been carried out in the last decades in order to increase the understanding of the chemical behaviour of elements in bones under thermal conditions and the influence of diagenetic processes (Bergslien et al., 2008; Grupe and Hummel, 1991; Herrmann and Grupe, 1988; Kaczanowski et al. 1996; Price and Kavanagh, 1982; Rodríguez et al., 2002; Runia, 1987; Schultz et al. 2008; Subira and Malgosa, 1993). Some studies have shown that diagenetic processes are similar for cremated and non-cremated archaeological bones (Herrmann and Grupe, 1988; Subira and Malgosa, 1993). Experimental works (Grupe and Hummel, 1991) carried out in contemporary bones incinerated at different temperatures indicate that Ca and Sr are suitable for paleodiet studies but Ba and Pb, at temperatures exceeding 800 °C, are not suitable and Zn and Mg seem to have an unintelligible behaviour. On the other hand, the study of the archaeological remains of the S'Illot des Porros Necropolis (Mallorca, Spain) Subira and Malgosa (Subira and Malgosa, 1993) found higher concentrations of Cu, Ba, Zn, and Sr in non-incinerated cortical than in incinerated bones, whereas levels of Ca and Mg were higher in incinerated remains. Although in this study (Subira and Malgosa, 1993) the higher values in non-incinerated bones were not statistically significant, incinerated bones had not been divided into carbonized and cremated bones to verify if the results were influenced by thermal conditions.

During experimental cremation studies, Kaczanowski et al. (1996) appreciated a decrease in concentrations of some trace elements such as Pb and Cd considering their melting and vaporation points (*Tm* and *Tv*, respectively). More recently, inductively coupled plasma-optical emission spectroscopy (ICP-OES) has shown to be suitable for elemental analysis of cremains (Brooks et al., 2006).

The overall objective of the present study was to define a strategy for a correct selection of cremated cortical bone samples through the elemental profile identification of Iberian bones. The samples selected for this study belong to adult individuals and derive from the Iberian Necropolis of Corral de Saus discovered in 1972 (Izquierdo Peraile, 2000), situated in the internal area of Comunidad Valenciana (Spain). The Iberian funerary ritual consisted in the burning of the corpse on a pyre. Burnt bones and ashes were deposited in an urn tomb. In this site, the cremation tombs belong to the period that lasted from the V century B.C. until the late II century B.C. or the beginning of the I century BC.

Therefore, a method for the determination of major elements, trace elements and Rare Earth Elements (REE, lanthanides) in skeletal cremains of ancient Iberians was developed employing ICP-OES. Twenty-four adult individuals were studied. Samples from the inner part of their bones were divided into "carbonized" indicating bones fired in reducing atmosphere, "cremated" for bones fired in oxidizing atmosphere and "unknown" for bones of unidentifiable burning. Furthermore, bone samples from the outer bone layer, unburned animal bones and soil samples were analysed. Finally, we have developed a Principal Component Analysis (PCA) based method for controlling diagenetic factors and a classification model employing multivariate statistical tools to identify bone samples with a preserved elemental composition.

2. Materials and methods

The samples selected for this study derive from the Necropolis of Corral de Saus (Izquierdo Peraile, 2000) situated in the internal area of Comunidad Valenciana, province of Valencia (Spain), 8 km from the village of Moixent. The geographical coordinates of the site are: 38° 51′ 19″ Latitude Nord/0° 51′ 1″ Longitude West. The Necropolis was discovered in 1972 and has been dug by the SIP (Research Service of Prehistory) of Valencia in many excavation campaigns during twenty years. Corral de Saus is one of the most important necropolis discovered in Comunidad Valenciana. The site is 2500 B.P. "Iberian" is an archaeological proxy for Iron Age in the area between Andolusie (SW Spain) and Marseille (SE France).

The Iberian funerary ritual consisted of burning the corpse on a pyre; then, burnt bones and ashes were deposited in an urn containing a dowry which consisted of personal items and gifts given from family and friends. When funeral feasts were organized, remains of food or crockery used were also deposited around the tomb.

Tomb types vary from simple earthen pits to larger buildings, such as cameras with mounds. In Corral de Saus, cremation tombs belong to the period that lasted from the V century B.C. until the late II century B.C. or the beginning of the I century B.C. The analysed bones, dated between the III and II centuries B.C., are from adult individuals. At this time, those cremations were deposited in urns decorated with geometric, floral and figurative style. Each urn contained the remains of just one individual. When it was feasible, the bones of each individual were sampled and divided into "carbonized", indicating bones fired in reducing atmosphere, "cremated", for bones fired in oxidizing atmosphere, "unknown", for bones of unidentifiable burning as well as bone samples from the outer bone layer of "carbonized", "cremated" and "unknown" bones. Samples from the outer bone layer were analysed in order to detect elemental differences between the inner and external part of the bones, induced by diagenetic factors. In addition, unburned animal bones and soil samples obtained from the inside of urns mixed with ashes, carbons and bones were analysed.

2.1. Chemical analysis of bone samples

The bone samples were taken from burned long bones (cortical bones) of twenty-four individuals of Corral de Saus archaeological site (Izquierdo Peraile, 2000). The sampling was carried out taking into account the different grade of burning of the fragments in each individual. Bones and soils mixed with ashes and carbons, were sampled using a micro spoon spatula made of stainless steel, always cleaned before taking a new sample and stored in test tubes of 15 ml. Samples were oven-dried and incinerated in a muffle oven using the following ramps program: I) 30 min at 150 °C; II) 1°/min up to 450 °C; III) 24 h at 450 °C; IV) down to 30 °C. Successively, samples were homogenized with an agate mortar and pestles. We have developed a digestion method and a range of dilutions from the digested solution in order to provide reproducible and comparable results compatible with the sensitivity of the analytical method. The digestion method consisted of the addition of 1.5 ml HCl and 1.5 ml HNO_3 to 0.5 g of sample (bones and soil mixed with ashes and carbons) and two blanks in glass tubes placing them in a water bath at 100 °C for 40 min. Subsequently, the digested solutions were carefully poured into plastic tubes of 15 ml, bringing the volume to 15 ml with purified water. This concentrated solution (A), was used to measure trace elements such as Zn, Cu, Ba, V, Mn, Pb, Cd and lanthanides. To measure Mg and Sr, solution (A) was diluted to 1:250 obtaining solution (B). Solution (C) was obtained to read Ca from diluting solution (A) 1:2000. The concentration of HCl and HNO_3 was maintained constant in all solutions. For the preparation of the standards, a solution of 100 μg/ml of Ca, Mg, Sr, Ba,Cu, Zn, Pb, Mn, Cd, V was employed to obtain a multielemental solution. For the preparation of the calibration standards, 50 ml flasks were used adding 5 ml of HNO_3, 5 ml of HCl, the corresponding volume of standard solution and filling up to volume with pure water. Concentrations ranging between 0 and 20 μg/mg were used