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BLUE BONE ANALYSES AS A CONTRIBUTION TO THE STUDY OF BONE TAPHONOMY IN SAN JOSECITO CAVE, NUEVO LEON, MEXICO

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Blue-stained bones, collected along a single stratigraphic level in San Josecito Cave, Nuevo León, México, were analyzed in order to better understand the diagenetic processes that pertained to their formation, and in order to ascertain if those processes could be used in inferring past environmental conditions. Using XRD, two minerals were identified as composing the fossil bone, hydroxylapatite and calcite. INAA, ICPS, XRFS, and colorimetric methods were used to quantify trace elements, including Cu, Sr, Zn, F, and Cl (in descending order). The findings point to the presence of a physical phenomenon produced by transition metal ions impurities, that in turn seems to be associated with physical and chemical processes occurring inside the cave, rather than with outside paleoenvironmental conditions.

Cave deposits often occupy much of the space eroded out of bedrock. They affect processes enlarging the space, supplement erosional records of cave history, provide means of absolute chronology, and provide evidence of regional environmental change (Jennings 1985). Caves constitute highly efficient sediment traps; their stability within the changing landscape facilitates geologic concentration and attracts diverse organisms in search of shelter (Collcutt 1979).

Caves frequently contain rich fossil deposits because they serve as refuge for wild animals, and are converted into focal points for bone accumulation at death. Bone deposits in caves usually are not the result of long-distance transport, but reflect local environments (Harris 1977). The catchment size, or area sampled, for cave faunas is partially dependent upon the size of the internal drainage system (small compared to open fluvial systems), the home range and prey selection of predators or scavengers serving as vectors for bone accumulation (particularly microvertebrates preyed on by owls), and pit fall entrapment of individuals living near the cave entrance. Therefore, cave paleocommunities do not represent a complete spectrum of the past biota, but instead a regional thanatocoenose (death assemblage; Shipman 1981) produced by several types of sampling (Graham 1986; Guilday 1962). In this respect, it is essential to consider in detail how collections of fossil organisms have been accumulated. A comprehensive taphonomic analysis is critical to address the least-known aspect of paleoecology, i.e., the role of fossil samples as components of ecosystems (Gifford 1981).

San Josecito Cave (Fig. 1) is one of a few localities in México containing a detailed fossil record for the late Pleistocene (Kurtén & Anderson 1980). This cave and its paleontological materials have been the focus of several studies in the past 12 years (Arroyo-Cabrales 1994; Arroyo-Cabrales & Johnson 1997, 1998; Arroyo-Cabrales *et al.* 1989, 1993, 1995). A major goal of these studies has been to understand the taphonomic history of the bone fossils and to reconstruct a sound paleoecological interpretation. In order to address the taphonomic history, studies of sediments (Rolong *et al.* 1994) and specific features on bones (Arroyo-Cabrales and Johnson 1997) have been undertaken.

The current research focuses on the authigenic evolution of secondary minerals on the bat and rodent bones in the cave. This study is important for three major reasons. Mineralogically, from the fossilized bones, it is possible to infer chemical and physical conditions from both the actual molecular structure and the diagenetic processes produced by sedimentary environments. These effects are shown by the inclusion of organic and inorganic materials into the buried bones. Taphonomically, from the recognition of the diagenetic processes that the bone underwent, it is possible to propose what has happened since the animal died, and to infer local environmental conditions around the cave at different geolog-

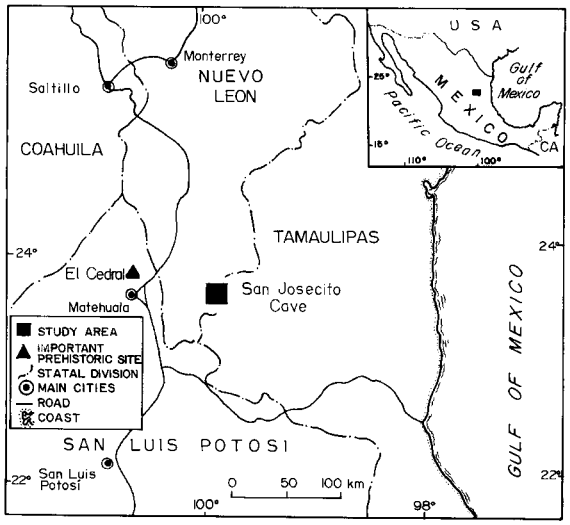

Figure 1. Location of San Josecito Cave, Nuevo León.

ic times. Archaeologically, results of the study may allow the environmental reconstruction for a region and period in which the earliest purported human evidence in México (approximately 33,000 years BP) has been found at El Cedral, San Luis Potosí, located less than 100 km to the west of the cave (Lorenzo & Mirambell 1986; Fig. 1).

STUDY SITE

San Josecito Cave is located on the western flank of Sierra Madre Oriental, at the edge of the Mexican Plateau, in the southern part of the Mexican state of Nuevo León (Fig. 1). Paleontological quarrying of the cave deposits by personnel from the California Institute of Technology (1935-1941) yielded a large vertebrate fossil assemblage (Stock 1943; Kurtén & Anderson 1980; Arroyo-Cabrales 1994). More recently, controlled excavations by a collaborative endeavor between the Museum of Texas Tech University and the Instituto Nacional de Antropología e Historia, México (INAH) has added to the fossil record, geochronology, and microstratigraphy of the cave (Arroyo-Cabrales 1994; Arroyo-Cabrales *et al.* 1995; Mead *et al*. 1999; Rolong *et al*. 1994). This material has been the basis for important taxonomic and systematic studies of several animal groups, including shrews, bats, birds, carnivores, and ungulates (see Arroyo-Cabrales & Johnson 1998 for a comprehensive listing). Radiocarbon dates from the different strata have provided evidence of an age range between 45,000 and 11,000 years BP (Arroyo-Cabrales *et al.* 1995). Several cave faunal assemblages are from the Wisconsinan Pre-Pleniglacial (Arroyo-Cabrales 1994).

In 1990, Mexican-US renewed excavations were undertaken with a strict stratigraphic control. Two test units were opened, excavated stratigraphically, and all sediments were water-screened. Samples from most strata were collected for various studies in laboratories in México and the United States (Arroyo-Cabrales & Johnson 1998).

Materials from one of the strata (770) showed a very characteristic blue-greenish stain. An early report pointed to the presence of copper (Arroyo-Cabrales & Johnson 1997) as the cause for the coloration. However, the small amount of copper present in the bone warranted further chemical and mineralogical analyses to explain the staining. Furthermore the original copper source has not been identified. A more comprehensive understanding of the "blue bone" from San Josecito Cave is now documented.

PREVIOUS STUDIES ON FOSSILIZED BONE FROM STRATUM 770

Previous assays of the "blue bone" included X-ray diffraction and energy-dispersive X-ray (EDAX) techniques (Arroyo-Cabrales & Johnson 1997). The diffraction patterns pointed to the presence of two mineral fractions, a principal one that has the same interplanar spacing values of the hydroxylapatite [$Ca_5(PO_4)_3OH$]. In the same sample, another mineral related to the carbonate group (calcite, $CaCO_3$) was identified; this mineral formed after the bone underwent an internal mineralization process.

From the energy-dispersive X-ray (EDAX) fluorescence test, qualitatively the main constituents were phosphorus (P) and calcium (Ca). Trace elements were copper (Cu), zinc (Zn), bromide (Br), and strontium (Sr). This analysis pointed to the presence of copper as the staining mineral, while zinc was only providing the metallic shine, and bromide was intrusive.

EXPERIMENTAL ASSAYS

The stratum 770 sample is composed of small vertebrate bones including lizards (*Barisia imbricata*; Mead *et al*. 1999), bats (*Desmodus rotundus*, *Leptonycteris* sp.), and rodents (*Neotoma* sp., *Peromyscus* sp.) (Arroyo-Cabrales 1994). Some bones were compressed strongly, probably due to sediment load (Arroyo-Cabrales & Johnson, 1997). Most bones are stained completely, including the overall shaft, and a subsample from blue bone fragments was chosen for analysis (~10 g).

The initial sample analyses were conducted at the Geology and X-Ray laboratories of the Subdirección de Laboratorios y Apoyo Académico, Instituto Nacional de Antropología e Historia. Observations were done using both a stereoscope and a petrographic binocular microscope.

Two mineral components were observed with the stereoscope. One had a fine and homogeneous texture with blue-green tones, and some lighter concretions of different composition. This same observation was enhanced using a scanning electron microscope (Fig. 2). Such concretions were tested with hydrochloric acid, and the reaction supported its identification as carbonate, and characterization as calcite ($CaCO_3$) by DRX and SEM probes.

The blue-greenish bone component was sorted carefully and ground to obtain fractions with a diameter range between 20-30 µm. The powder was put in a polished glass to get an even distribution of material. The sample was assayed with

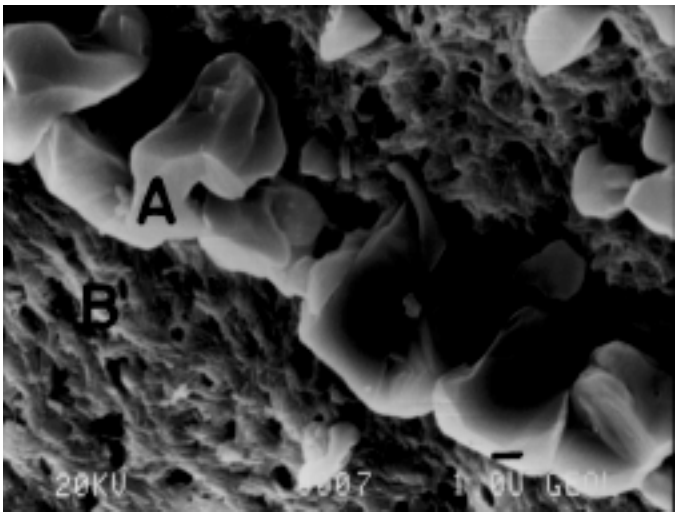

Figure 2. SEM image of blue-greenish bone showing calcite crystals (A) over phosphasized bone (B). Scale bar on lower right side equals 1 µ.

copper X-rays (Cuλ = 1.54 Å), and scanning conditions of 20 Kv - 20 mA, signal counting each second, scintillation detector (Sc), 1° collimator, and Ni filter. The diffraction pattern was compared to the Diffraction Standard Manual for powder samples (Berry *et al*. 1974). From a diffractogram, two minerals were identified: hydroxylapatite, $Ca_5(PO_4)_3OH$; and calcite, $CaCO_3$ (Fig. 3a).

Comparative analyses were assayed with yellowish bone from level 740-750 (Fig. 3b). The results indicated that the main constituent in both samples is hydroxylapatite, but their megascopic color is not the same. Microscopically, crystalline arrangements in blue-green bone showed alternate anomalous reddish-yellowish-bluish interference colors of second order, not common for hydroxylapatite (Fig. 4).

To address these anomalies, sample analysis using X-ray fluorescence spectrometry (XRFS) was undertaken with model JEOL-JSDX-60P3A spectrometer, Cr anode, TAP analyzer crystal and flux detector. The search for fluorine used the comparative fluorite (CaF_2) standard. This element was not quantifiable in the problem sample due to the small sample size and low level of F concentration. However, Energy Dispersive Analyses by X ray fluorescence (EDAX) in scanning electron microscope JEOL-JSM-35C, with detector crystal Si-Li, berilium windows and Tracor Northern analyzer system, and colorimetric method revealed the level of chlorine (500 ppm), and fluorine at less than 1 ppm.

Complementary chemical analysis by instrumental neutron activation (INAA) in light-blue bones was performed in a reactor with Am-Be source and 6.1 x 106 n/cm². Concentrations of Cu were <4 ppm in a pallid blue-greenish sub-sample. However, inductively coupled plasma spectrometry (ICPS), in a Thermo Jarrell Ash Atom Scan 16 spectrometer, showed Cu concentrations between 21.08-38.8 ppm for a dark blue-greenish sub-sample, strontium at 4.94 ppm, and Zn less than 5 ppm; a yellowish bone subsample from above stratum 800 resulted

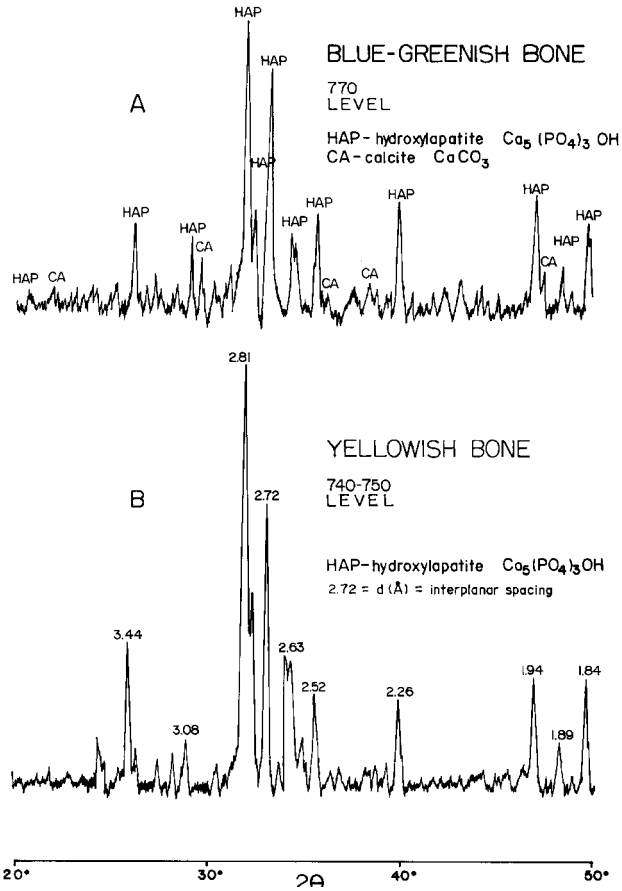

Figure 3. X-Ray diffraction patterns of fossilized bones collected from different stratigraphic levels in San Josecito Cave; although the bones produce the same diffraction response for hydroxylapatite (HAP), they do not show the same color effects.

in Cu concentrations of 54.95 ppm, Sr at 247.4 ppm, and Zn at 5.94 ppm (Table 1).

PROBABLE CAUSES OF THE COLORATION IN THE BONES

The phenomenon of blue coloration in the bones was analyzed based on diagenetic processes, due to the fact that the studied samples present an almost total degree of replacement. The minerals are colored upon absorbing certain wavelengths of light. Therefore, the observed color is the result of the combination of wavelengths that arrive to the eye (Hurlbut and Klein 1985; White 1997). Within the factors that could cause the coloration of the analyzed bones, the presence of the mineral vivianite and forms of odontolite, fossil turquoise, or fluorapatite may be suspected, but other phenomena also could be causing the blue-green coloration. The first cases were discarded based on the results of X-ray diffraction, while the second was rejected by the lack of concentrations of Cu greater than 7% total weight (Palache *et al.* 1951). The third case was rejected due to the low fluorine concentration (<1 ppm); several authors have shown that fluorine concentrations on the

Table 1. Synthesis of chemical analyses in fossilized bones from Stratum 770 (blue), and from above stratum 800 (yellowish) in San Josecito Cave, Nuevo León, México.

Technique	Trace elements	Total bulk	
		Blue	Yellowish
X-ray fluorescence spectrometry (XRFS)	F	Not detected	
Instrumental neutron activation analysis (INAA)	Cu	4 ppm[1]	
Scanning microscopy with X-ray analysis (EDAX)	Cl	500 ppm	
Inductively coupled plasma spectrometry (ICPS)	Cu	21.08-38.8 ppm[2]	54.95 ppm
	Sr	4.94 ppm	237.4 ppm
	Zn	< 5 ppm	5.94 ppm
Colorimetric method	F	< 1 ppm	

[1] Pallid blue-greenish sub-sample [2] Dark blue-greenish sub-sample

order from 2% and 3.73% were required for fluorapatite (Chang *et al.* 1996; Fleet & Pan 1997; Hurlbut & Klein 1985; Palache *et al.* 1951).

Results of the different analyses assayed during this study provided the basis for another explanation of the blue-green color, indicating that the color is due to the presence of transition trace metal ions. It is suggested that a chemical element in sufficient concentrations to form molecules is not the unique cause of color in minerals. Chemical impurities in small quantities can be incorporated into defective structures named transition metal ions, and intensely absorb the light to give the mineral an intensive color sensation (Nassau 1978; White 1997). Among the elements that can provide intensive colorations to the minerals are Cr, Mn, Fe, Co, Ni, and Cu. Which element is providing the intense coloration cannot be predicted as the same element in different structural environments presents different transitional energy and causes different coloration (Blackburn & Dennen 1988; Hurlbut & Klein 1985).

The fossilized bones from stratum 770 are blue-green stained due to impurities within the transition metal ions. Particularly, copper in concentrations between 21 and 38.8 ppm is the main cause for this phenomenon. Similar cases, where Cu promotes blue-green color are turquoise, azurite, malachite (Nassau 1980) or allophane and other amorphous aluminum silicates (White 1997).

DISCUSSION

The minerals identified in the rodent bones from stratum 770 of the San Josecito Cave are hydroxylapatite and calcite. The first occurs as a product of the local authigenic mineralogical progression, while the second is a response of carbonate precipitation from saturated solutions and/or by Ca exsolution from the bone.

The authigenic mineralogy of the bat bones, which constitutes the focus of the present study, was accomplished in three stages. First, during mineral nucleation, apatite was developed in the bones. This development was due to contact with Ca^{2+} and Mg^{2+} rich solutions with the bones. Those solutions previously had to cross bat guano. With these characteristics, the environment was modified, and reducting conditions formed in the humid space. Second, the authigenic mineralogy evolved to a most stable phase (hydroxylapatite) by enrichment of the environment with OH^- ions. And third, the final phase was identified with the taphonomic study, and corresponds to a demurrage where the hydroxylapatite has incorporated chemical element ions as Cu, Zn, Br, Sr, Cl, F into its crystalline structure, producing optical property distortions.

The first stage of mineralization, considered as nucleation, is deduced from observations made on buried bones, in caves all over the world (Courty *et al.* 1989). The bone, in the current phase, presents a blue-green coloration, associated with the influence of impurities of transition metal ions, produced by the absorptive capacity of the light elements mainly as copper. The source of origin of the ions incorporated into the bones probably is the calcareous rocks that constitute the cavernous structure (Table 1), but only in stratum 770 were blue-greenish bones are present. This was possibly also caused by lithostatic change in a focused humid environment (as shown by the compressed blue bones).

CONCLUSION

This study has addressed one question regarding the presence of stained bluish bone of small vertebrates in cave sediments. The small vertebrate bones exclusively come from a very thin stratigraphic unit. It seems reasonable to constrain the explanation of the color presence to a single sedimentary period, between ca. 28,000 and 11,500 years BP (Arroyo-Cabrales *et al.* 1995).

The results of the analyses point to a color phenomenon due to the influence of transition metal ions. Furthermore, the abundance of small vertebrates, mainly lizards and bats, accounting for the presence of large amounts of guano, could contribute to the chemical processes accounting for the blue staining of bone. Finally, it is shown that research on paleontological and archaeological bones would gain from an in-depth analysis of the different diagenetic taphonomic features

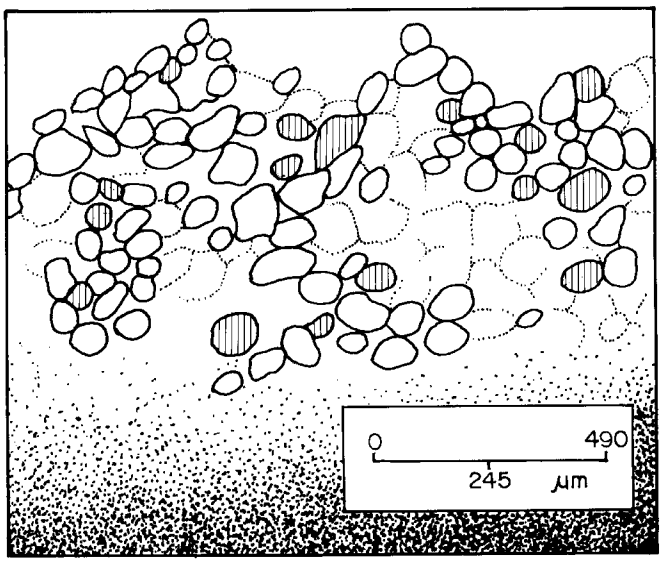

Figure 4. Schematic view of the thin section of fossilized bone from stratum 770 at San Josecito Cave; under petrographic microscope, vertical shadowed bars represent hydroxylapatite minerals with second order interference colors.

present in them.

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