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Proton Beam Characterization of Bone Remains from the Middle Mesoamerican Formative

L. Couoh [(a)], J. L. Ruvalcaba Sil [(b)]

(a) Instituto de Inv. Antropológicas y Fac. Fil. y Letras, Universidad Nacional Autónoma de México, MEXICO.
(b) Instituto de Física, Universidad Nacional Autónoma de México, MEXICO.

Abstract. In this work, bone remains of ten individuals from a multiple burial discovered in Tixtla, Guerrero, Mexico, were studied using ion beam techniques. The chronology of the burial corresponds to the Middle Formative Horizon (1200 to 400 BC). The spatial distribution of the bones and their conservation indicated that the bone remains were buried at different times in the tomb. Since collagen contents decrease as a function of the age, the bone antiquity may be estimated from the N/Ca atomic ratios from Ca and N contents measurements by simultaneous Proton Induced X-rays Emission (PIXE) and Backscattering Spectrometry (BS) using the $^{14}N(p,p)^{14}$ N resonance at 4 MeV. Elemental concentrations of Al, Ca, P and Fe, among others, were measured by PIXE using an external beam setup to determine bone alterations and possible diagenesis. For these purposes, complementary analyses by ionoluminescence (IOL) induced by 3 MeV proton beam were carried out as well. The aim of this study is to measure the bone elemental composition and the effects of the deterioration processes on the dating results. PIXE and BS results pointed out to three groups of N/Ca atomic ratios with an estimated antiquity of 3050 to 3380 years. This result agrees with the archaeological age (3005-2409 years). Although some alterations have been observed in the bone remains and metallic concentrations were significant, it was possible to estimate the bone antiquity for non-altered samples.

Keywords: Bone remains, collagen dating, RBS, PIXE, IOL, Tixtla.

INTRODUCTION

In the archaeological site of Tixtla, Guerrero, Mexico, it was found a multiple burial composed at least by ten individuals. According to the spatial distribution and to the different state of conservation of human bones, it is suggested that the individuals were not deposited inside the tomb at the same time. (Fig. 1) [1].

When the bones remains are deposited in the specific soil matrix (archaeological context), they suffer changes in their entire structure at physical and chemical level. This process is known like diagenesis and is due to a wide range of factors. The collagen degradation from the organic bone matrix could be induced by different organisms present in the substrate. Nevertheless, there is an intrinsic loss caused by the course of time, which involves a reduction content of C and N related to collagen.

Since N has a high non-Rutherford backscattering cross sections for 4 MeV protons (about 80 times bigger than the Rutherford one at 165° from the beam direction at lower energies), Backscattering Spectrometry (BS) can be used to measure the loss rate of N with relation to Ca for a relative dating. The reference used is a recent dry bone with 30% collagen in weight; this means a N/Ca atomic ratio of 0.6, considering the composition of $C_4 H_{5.7} O_{1.3} NS_{0.05}$. On the other hand, PIXE and ionoluminescence (IOL) can complete the detection of elements incorporated to the inorganic portion of bone, those are indicators of *postmortem* alteration. Then we can establish the affecting factors, besides the time, of collagen decomposition using proton beam analysis.

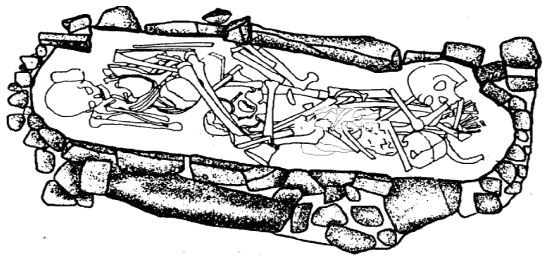

FIGURE 1. Spatial distribution of the human remains in the Tixtla tomb. Ten individuals were distinguished.

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SAMPLES

Bone remains of ten individuals from a multiple burial discovered in Tixtla, Guerrero, Mexico were analyzed. The samples were taken from compact bone of diaphysis of a long bone and skull, one from each individual. The method used for cleaning was mechanical with alcohol ethylic to 99.8% by ultrasonic by periods up to 10 minutes. Once the cleaning and drying processes were completed, the samples were powdered on agate mortar, later those were pressed to 4 ton/m^2 to obtain pellets of 1 cm in diameter.

EXPERIMENTAL

When materials are rich in calcium, like the case of bones, the X-ray counting of this element frequently saturate the light elements X-ray detector. A selective absorber for calcium has been previously developed (Ag thin film + He jet) in order to increase the limit detection and improve the sensitivity [2]. This absorber has been used for the analysis of the light elements. On the other hand, a LEGe detector with a 76 μm Al absorber was used for X-rays of higher energy.

All experiments were carried out in the Pelletron Accelerator Laboratory IF-UNAM. A 3 MeV proton beam for PIXE in air and a 4 MeV proton beam for BS in vacuum were used. The PIXE spectra were analyzed with AXIL and PIXEINT programs while BS spectra were fitted by RUMP code. RUMP scattering cross sections data were modified in order to fit the BS spectra [3, 4]. Nitrogen and oxygen non-Rutherford cross sections for 4 MeV protons at 165° were taken from ref. [5]. For BS simulations, calcium and phosphorous concentrations were obtained from PIXE analysis.

Concerning analysis with IOL, this technique was applied to a reduced number of representative samples. The experimental set up includes a coupled fiber optic spectrometer and the irradiation was done using a 3 MeV proton in vacuum as described in ref [6].

RESULTS AND DISCUSSION

For all the analyzed samples, the BS spectra are similar (Fig. 2) and the results of the N/Ca atomic ratio were lower than 0.1. Table 1 shows the results of BS for all the samples. They may be grouped in three sets: A, B and C, according to the N/Ca atomic ratios. Each group was compared with a calibration curve, previously obtained using ancient bones remains of known antiquity [7-8]. In this way, it may be determined a relative dating for each group (Fig. 3). For the group A, the antiquity estimated corresponds to 3050±100 years, for the second group B, it is 3245±100 while for the group C, it is 3380±100 years. The amounts of N measured in all the samples are very low by comparison of the typical value of 0.6 in dry modern bone. However, dating calculated by BS agrees with the archaeological estimated dating in the Middle Mesoamerican Formative (3200-2400 years before present.). Since there is a difference in the N/Ca ratios, the results corroborated that the ten individuals were not buried at the same time but in three different periods.

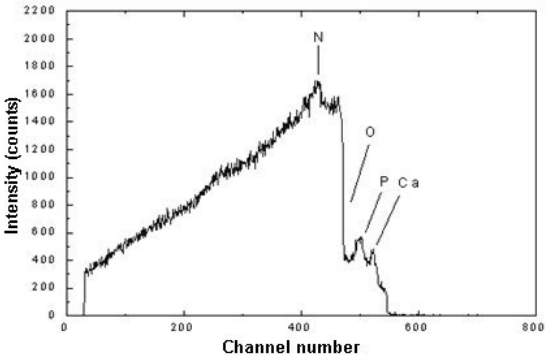

FIGURE 2. Typical BS spectrum of the bone. 4 MeV protons, 165° detection angle.

TABLE 1. BS grouping from N/Ca atomic ratios

Sample	N/Ca ±0.03	Group
1	0.022	
2	0.022	
3	0.022	A
9	0.022	
10	0.022	
5	0.018	
6	0.016	B
7	0.018	
4	0.012	C
8	0.014	

The quantitative analysis of the representative elements of the soil such as Al and Fe, provide information about bone deterioration. Figure 4 shows the elemental concentrations for both elements, as well as the contents of Ca and P. In the ten samples, Al and Fe were detected. Al is present in higher amounts from 4 up to 13% but the contents of Fe ranged from 1 to 3%. In modern bones the proportion of Ca to P is 2:1. In hydroxylapatite, Ca and P contents are 42% and 14% respectively, therefore the P/Ca concentration ratio is 0.33. In all the analyzed samples, it was observed a decrease in the amounts of Ca and P. Sample 5 is the closest to the expected concentration of these elements in hydroxylapatite (HAp). It presents a ratio of 0.32 (P: 12.5% and Ca: 38%). However, we observed a high content of Al (13%). On the other hand, samples 8 and 9 have less metal incorporation in the bone and their P/Ca ratio is 0.37. Therefore, these three samples (5, 8 and 9) are the best preserved. In contrast, the most deteriorated samples are 3, 7 and 10 and they show a considerable decrease of Ca and P, while the quantities of Al and Fe are higher.

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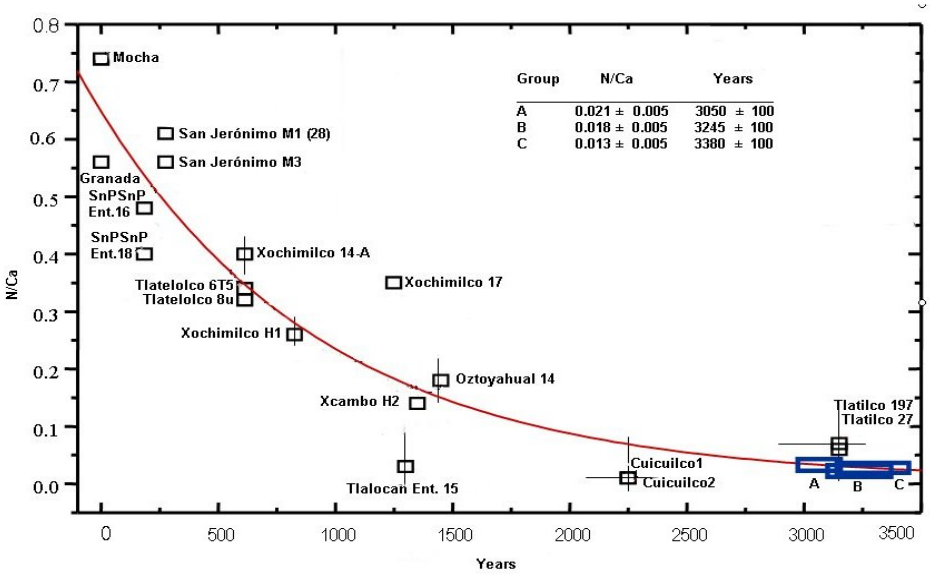

FIGURE 3. Calibration curve for N/Ca atomic ratios for bone remains from BS using a 4 MeV proton beam [8]. The measurements of the groups A, B and C are included.

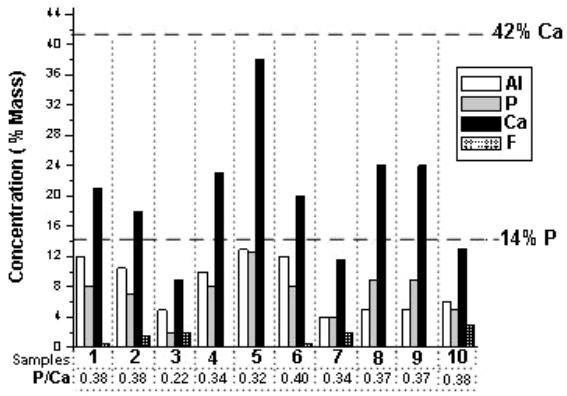

FIGURE 4. Elemental concentrations measured by PIXE for Al, P, Ca and Fe. The horizontal lines represented the normal amounts of P and Ca in HAp.

It is interesting to point out that the incorporation of Al and Fe seems to not affect N preservation, as we can see in sample 8, this is the less deteriorated sample and it has the same N/Ca ratio (0.022) than sample 3, and this is the most degraded one.

On the other hand, in IOL spectra (Fig. 5), three broad emission bands centered on 495 nm, 580 nm and 610 nm were observed in the samples. The first band has not been identified or related to a particular emission, although the other emissions correspond to the luminescent activation of ions Mn^{2+} in HAp and $CaCO_3$, respectively [6, 9].

The presence of Mn in these bones remains is the result of the diagenetic process, occurred during time the bone is buried in a specific soil matrix, and it is not incorporated in the osseous tissue during the individual life due to physiological reasons [9].

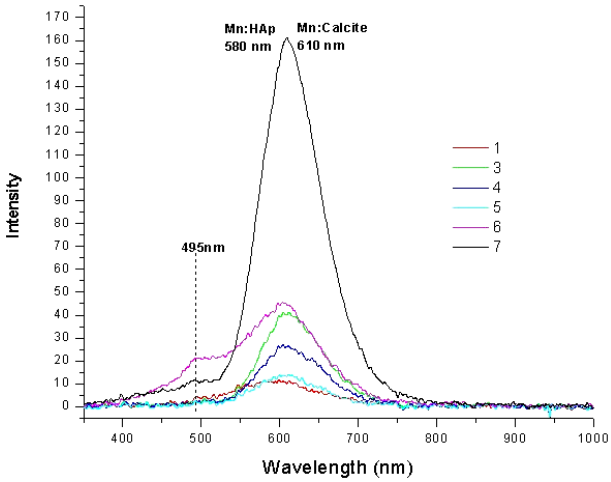

FIGURE 5. IOL spectra for the ten samples.

The IOL analysis indicates the presence of Mn in form of ions Mn^{2+}. Therefore, it is very probable that the most part of this element is found incorporated in the mineral osseous tissue due a ionic interchange process between Ca^{2+} and Mn^{2+} [9].

From IOL intensities, for samples 3, 4, 6 and 7 (Table 2), we observed that there is an important component of calcite in the bone. We may expect an increase in P/Ca concentration ratios, which will modify in consequence the N/Ca atomic ratios and the dating. Nevertheless, for samples 1 and 5, the IOL

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intensities are low and we cannot expect significant changes in these ratios and in their dating. Since all the samples, exception sample 3, have similar values of N/Ca and P/Ca ratios, we may consider that calcite and the soils components has not been completely incorporated into the bone matrix but mainly aggregated to the bone.

TABLE 2. Results of N/Ca atomic ratios, P/Ca elemental concentration ratios, Al and Fe contents measurements by PIXE-BS. IOL data are shown as well.

Sample	N/Ca ±0.03	P/Ca ±0.05	Al (%) ±20%	Fe (%) ±10%	IOL	
					Intensity (a. u.)	Wave length (nm) Activator in Matrix
1	0.022	0.38	12	1	10	580 Mn: HAp 610 Mn:$CaCO_3$
3	0.022	0.22	5	2	40	610 Mn:$CaCO_3$
4	0.012	0.34	10	<1	25	610 Mn:$CaCO_3$
5	0.018	0.32	13	<1	15	610 Mn:$CaCO_3$
6	0.016	0.40	12	1	20 40 45	495 Not identified 580 Mn: HAp 610 Mn:$CaCO_3$
7	0.018	0.34	4	2	160	610 Mn:$CaCO_3$

CONCLUSIONS

The results indicate that it is necessary to carry out analyses of elemental composition for determining the state of preservation of bones remains, it is very important the P/Ca elemental concentration ratio, as well as Al and Fe detection as diagnostic elements to establish soil incorporation to the osseous matrix and diagenesis processes. These measurements are very important for N/Ca atomic ratios determination and the validity of the relative dating.

From IOL detection, it is possible to establish the presence of calcite and others minerals in a sample or in specific regions without diffraction studies. The combined use of PIXE and IOL can provide useful data to determine if exist any intramineral or diagenetic alteration in bone.

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