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Quantitative evaluation on ante-mortem lead in human remains of the 18th century by triaxial geometry and bench top micro X-ray fluorescence spectrometry

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Abstract

The aim of this work is to demonstrate the suitability of the commercial bentchtop micro X-ray fluorescence (μ-XRF) system M4 Tornado, to evaluate the differences on lead distribution in the different bone and tooth structures. *Ante-mortem* and *post-mortem* Pb accumulation was also assessed and the lead amount in the different tissues was compared. Micro-XRF based in polycapillary systems is a relatively new technique with capabilities to provide multielemental maps and quantitative measurements. Another advantage of the technique is being non-destructive and requiring only a small amount of sample.

In this work we measured the lead concentration in human remains, bone and tooth of an 18th century young male subject, around 30 years old, and compared the results obtained using the μ-XRF with a setup with triaxial geometry. Accuracy of the microanalytical system for pressed pellets and cross sections of bone and tooth were also certified.

The μ-XRF setup provided analytical point spectra, line profiles and elemental maps for Pb and Ca distribution in bone and tooth. The quantitative calculations were accessed by the fundamental parameters and compared mode methods. The accuracy and the detection limits were checked using standard reference materials for Ca, Zn, Sr and Pb. Furthermore, unusual extremely high amounts of Pb in cortical bone, tibia and fibula, were observed, reaching 120±10 μg.g^{-1}, while the trabecular region reached 250±20 μg.g^{-1}. Rib presented the highest levels, 560±30 μg.g^{-1}. In tooth structure the highest amount of Pb was found in pulp and root with 130±50 μg.g^{-1}. Low levels of Pb in the surrounding soil have been found.

Keywords: Lead concentration; human bone; micro-X ray fluorescence; triaxial geometry; tooth;

1. Introduction

X ray fluorescence technique is known to be a powerful technique for measurement of trace elements in a variety of solid matrices, including biological and environmental samples[1-5]. In the past 20 years much research has been done measuring trace elements and elemental constitution of human bone, many of them using X-ray Fluorescence. Spatial distribution of trace elements at the micrometer scale, were obtained in bone, by a benchtop monochromatic microbeam X-ray fluorescence setup[6]. PIXE and nuclear microprobe were also used for elemental distribution[7-10]. The concentration of lead in tibia (cortical bone) and calcaneus (trabecular bone) was measured by an *in vivo* X-ray fluorescence technique in active contemporary workers with a system based on the non-invasive 109Cd excited K X-ray fluorescence technique[11-14]. Chemical analyses and atomic absorption spectroscopy allow measurement of the elemental concentration, but they have the disadvantage of being destructive[15]. Lead concentrations in several cortical and trabecular bones in deceased smelter workers has been obtained by electrothermal atomic absorption spectrometry[16]. Laser ablation inductively coupled plasma mass spectrometry has also been used for quantitative measurements of lead in bone[17]. Electron probe micro analyses offer a spatial resolution in the micrometer and sub-micrometer range, but suffer from low sensitivity for some elements such as Sr, U, Ba, Zn and Pb, which are often present in fossilized bone[18, 19].

PIXE and X-ray micro fluorescence analyses are both multielemental, non-destructive and the detection limits are at the µg level. The spatial resolution reachable depends on the experimental set-up and is in the range of μm^2 to several mm^2. Synchrotron microprobe has also been used in bone analysis[20].

A potentially important and little explored application of micro X-ray fluorescence (µXRF) is the measurement of the spatial elemental distribution in forensic applications, namely the elemental distribution of lead or other toxic metals in gunshot residues[21,22]. Such studies are required to develop and understanding the biological fate of ingested heavy metals or poisoning compounds following environmental or occupational exposure, or following deliberate administration during drug therapy. In the case of lead, this data would be valuable for the application of bone lead measurements as a biomarker and determining Pb long-term ingestion or acute intoxications. Whilst

qualitative or semi-quantitative data are sufficient for some applications, rigorous quantitative analysis is desirable for clinical, biomedical and forensic samples[23].

Bone and tooth are the hardest structures of the human body. Both have been considered good biomarkers for man elemental exposure being the main targets for the deposition of heavy metals, and good indicators for long range exposure. Bone consists of cortical (substantia compacta) as well as trabecular bone (substantia spongiosa). The high porosity of the spongy bone and its open morphology makes it more susceptible to *post-mortem* alteration[24, 25]. Tooth consists of three main components: enamel, dentine and pulp. In human remains, lead can be both from *post-mortem* and *antemortem* origin. Inner compact tissue might represent in vivo accumulation and trabecular one corresponds to uptake during burial. Physical and chemical changes can occur in bone during its burial period. As bone is fossilized, the natural process of diagenesis serves to alter the bone composition from its *ante-mortem* state. These processes and their rate of reaction mainly depend on direct environmental conditions such as groundwater, soil composition, soil pH, redox potential and temperature.

In this work the spatially resolved elemental analysis in human remains, bone and tooth has been achieved by the micro-analytical commercial M4 Tornado (Bruker) with capability for elemental distribution at a spatial resolution of 25 µm. This is a benchtop nondestructive method and the quantification process is easy to handle with appropriate calibration. The quantitative calculations were checked by a second equipment with triaxial geometry and very well documented for many kinds of samples[2-5]. The results obtained with the two systems in terms of Pb *antemortem* accumulation from environmental exposure during life and *post-mortem* uptake from the burial place, are discussed and explored through the distribution patterns of Pb along the tooth, and bone. The obtained values show strongly increased levels of lead especially in spongy bone. However, the compact bone also showed unusually high values for this element, which may indicate *ante-mortem* Pb-exposure. Furthermore, in the tooth, the highest values of Pb where found in the pulp and root, confirming *ante-mortem* Pb-exposure, considering that low levels of Pb in soil were found.

2. Experimental

2.1. Sample collection and preparation

In this work we report the analysis of several bones and teeth of one particular subject, who was part of a collection of 83 individuals recovered from the inside of a chapel, dating from 18th-19th centuries[26]. This was a young male, around 30 years old, laying about 1 m deep. The bone material consisted of several ribs, foot bone, thighs, skull, femur, fibula and tibia both compact and trabecular bones. Several teeth were also collected. In order to evaluate possible contaminations from the burial surroundings, we also analyzed the soil.

Each bone and tooth were rinsed in tap water and carefully brushed, to remove completely the soil. After this cleaning process the samples were washed in distilled water, dried in a clean environment at room temperature.

From each bone a few grams from the inner compact and trabecular area bone were taken, by means of a polyester tool. Prior to analysis each sample was powdered in a polyester mill and the obtained fine powder was pressed into pellets 1.5 cm in diameter and 1 mm thick, without any chemical treatment. A minimum of three pellets of each sample, and a minimum of 3 samples of each type were taken to minimize the effects of inhomogeneity. Each pellet was glued on a Mylar film, on a sample holder and placed directly under the X-ray beam, for elemental determination, as described in Guimarães et al[3].

During the grinding sample preparation, special care has been paid to contamination, as well as during the whole procedure. All the material used is made of polyester, to avoid any contact with metals. For microanalysis, transversal sections of femur and longitudinal slices of tooth, around 1mm thick, were obtained with a microtome equipped with a diamond saw and the samples were placed directly on the x-ray microbeam.

2.2. Experimental setup

2.2.1. Benchtop microanalytical system

In this work we used a commercial benchtop spectrometer, the M4 Tornado by Bruker (Germany) for elemental mapping. The X-ray tube is a micro-focus side window Rh tube powered by a low power HV-generator and cooled by air. A poly-capillary lens is used to obtain a spot size down to 25 μm for Mo-Kα. The X-ray generator was operated at 50 kV and 300 μA and a composition of filters was used to reduce the background (100 μm Al/ 50 μm Ti/ 25 μm Cu). Under these conditions we could almost eliminate

the energy radiation below 9 keV of the bremsstrahlung. This, together with the preferential reflexion energy for the poly-capillary, the experimental setup allows a quasi-monochromatic beam between 9-15 keV, much better than the white incident beam originated from the X-ray tube. The detection of the fluorescence radiation is performed using a thermoelectrically cooled Silicon-Drift-Detector with energy resolution of 142 eV for Mn-Kα.

Measurements were carried out under 20 mbar vacuum conditions. The vacuum system avoids back diffusion and improves detection limits. This equipment was used to perform mappings of cross sections of the bones and tooth samples. Spectra deconvolution and fitting were performed using WinAXIL software package (Canberra, Belgium) and quantification was performed through compare mode[27]. This method makes use of SRM with the same matrix and similar elemental composition as the unknown sample to determine the sensitivity for each element and configure the quantification procedure.

2.2.2. Triaxial geometry

One of the used spectrometers to quantify Pb in bone consists of a high power X-ray tube with a tungsten anode, water cooled, equipped with a changeable secondary target of molybdenum. This arrangement makes it possible to obtain a monochromatic source and to select the secondary target in order to get the best excitation conditions for a special element. The X-ray tube, the secondary target and the sample are in a triaxial geometry. With this arrangement we decrease the background, taking the advantage of the effect of the partial polarization of the incident x-ray beam from the tube, and so improving the detection limits[28]. The characteristic radiation emitted by the elements present in the sample was detected by a Si(Li) detector, with a 30 mm^2 active area and 8 μm beryllium window. The energy resolution is 135 eV at 5.9 keV and the acquisition system is a Nucleus PCA card. The X-ray generator was operated at 50 kV and 20 mA and a typical acquisition time of 1000 s was used. The beam size on the sample is around 1.5 cm x 2.0 cm. Quantitative calculations are made through the fundamental parameters method. Experimental parameters were obtained through calibration, using standard reference bone materials.

3. Accuracy tests

3.1. Pressed pellets

The accuracy of the benchtop microanalytical system has been checked by analyzing pressed pellets of, SRM NIST-1400 bone ash (9.1 μg g^{-1} of Pb), NYS RM 05-02 bovine bone (16.1 μg g^{-1} of Pb) and NYS RM 05-04 caprine bone (31.5 μg g^{-1} of Pb) pellets and the values are presented in Table 1. The detection limits were also obtained and are presented in the same table. As one of the main goals of this work is to study the performance of this microanalytical system for Pb evaluation, we checked other bone reference materials with lower amount of Pb, NYS RM 05-01, bovine bone with 1.09 μg g^{-1} and NIST SRM 1486, bone meal, with an amount of Pb 1.4 μg g^{-1}. It was not possible to detect Pb for the first material and for the second one it was impossible to quantify. Considering that this technique is multielemental, besides Pb, the accuracy and the detection limits have been also evaluated for Ca, Zn and Sr, elements of foremost importance for their relationship to Pb[29]. Burial soil has also been analyzed and the accuracy of the system for this complicated matrix has also been checked by SRM IAEA-soil 7. The results are presented in Table 2.

The accuracy of the triaxial geometry system for bone samples was checked by analysis of two pressed pallets of standard reference materials; caprine bone NYS RM 05-04 and caprine bone NYS RM 05-02, with 30 and 16 μg g^{-1} Pb concentration respectively. The accuracy and detection limits were also calculated and the results are presented in Table 1.

Considering that the amount of Pb in the present bone samples was much higher than the one in the reference material, the accuracy of the systems for such high values needed further requirement. Taking into account the lack of such certified reference materials in the market, another bone sample previously analyzed has been used as reference. In a previous work[2], bone samples have been studied by 109Cd-based X-ray fluorescence. Pressed pellet samples with an amount of Pb of 330 μg g^{-1} have been used. Together with these pellets, reference calibrated phantoms of plaster of Paris reference material with (200 μg Pb/g bone mineral) have also been used.

3.2. Mapping

Transversal cross-sections from the tibia and longitudinal cross-sections from the teeth were cut by a microtome with a diamond saw, 1 mm thick. Each sample was mounted directly on a table 360 mm x 260 mm, which was attached to a stage translatable along

XY, and analysed directly by μXRF. The scanning step size was 25 μm. For each point, the counting time was 3.76 s.

Each sample was analyzed over a period of 12 h, to accumulate sufficient data points for high resolution mapping. The analysis was fully automated and unattended. The counting time and the scanning spatial resolution are freely selected according to the required resolution. A CCD camera allows visualize the studied area. The data output was arranged into a table giving the X-ray intensity of specific X-ray peaks representing element signals each measured point defined by its X and Y coordinate (μm).

The data were converted using the software's function into a data matrix, from which XY contour maps (2-dimensional maps) of the data were generated for each element. In Fig. 1 and 2 the elemental maps for Pb and Ca both in tibia and tooth cross section respectively, are presented.

4. Results and Discussion

4.1. Quantification on pressed pellets

From the results presented in Tables 1 and 2 for accuracy in bone and soil pressed pellet samples we can conclude that very good agreement is obtained with the microanalytical equipment. From Table 1 we also conclude that good agreement between reference bone material and the values obtained with the triaxial system is observed. Furthermore, when we compare the results obtained by the microanalytical system, with the obtained by the triaxial one, the most important finding is that the uncertainty of the obtained values is higher in the microanalytical system. In Fig. 3 we display one spectrum of a bone obtained with both setups, to better evidence the background obtained by the two systems. There is a reduction on the scattered radiation in the microanalysis system in the interest region and especially on the low energy side, with the vanishing of the Ar from the air, due to vacuum conditions. This is essential when light elements are important like P in tooth. Moreover, the use of the polycapillary also contributes to improve the background to peak ratio.

The elemental distribution Ca and Pb can be observed in Fig. 1 for tooth longitudinal cross section and in Fig. 2 for tibia transversal cut, respectively for Ca and Pb. Calcium is more or less uniformly distributed in both samples, Pb appeared to be enriched in the inner part of the tooth, pulp and root. Concerning bone samples an increase of Pb is

visible on the outer surface. In the inner spongy bone we distinguish small spots with enhanced level of Pb.

From the literature we can find studies devoted to assess *post-mortem* Pb in bones buried in contaminated environments and on the other side we can find research works dedicated to the *ante-mortem* Pb distribution and quantification. In this case we can find studies obtained either by *in vivo* analysis or from autopsy of deceased people.

Buried bones in contaminated environments were studied by M. L. Carvalho[1,2,30] in contaminated soils and a lead coffin by X-ray fluorescence based techniques. Femoral sections of a woman skeleton buried in lead sarcophagus were also studied[10]. Another important study on Pb uptake from the burial soil was the study of a mining population buried in a very high Pb contaminated soil close to the mining area[9]. In all studies the levels of Pb in the outer parts of the bone or in the trabecular regions were tremendously increased evidencing strong *post-mortem* enrichment of Pb. The highest concentrations correspond to large pores and voids in the bone structure, while compact tissue with harder structure is less susceptible to Pb uptake. The exchange mechanisms between the archaeological bone and its neighborhood were obvious. The concentration profiles obtained from the outside of the bone to the inner part confirm that Pb penetrated into the bone matrix. The diffusion of the metal was from the outside to the inner structure of the compact bone, which remained almost non-affected. The ratios between concentration values in the different structures reached one to several orders of magnitude, depending on the degree of contamination of the mortuary environment. The amount of Pb deeply decreased from the affected areas to the inner compact bone showing that the Pb in bones was obtained by *post-mortem* uptake.

Concerning *ante-mortem* Pb distribution it can be obtained either by *in vivo* analysis or from autopsy in deceased people. The most relevant work on *in vivo* analysis can be found in a summarized work by D. Chettle[14]. Furthermore three works on deceased people deserve to be referred[8, 16, 29]. U. Lindh[8] carried out by micro-PIXE with a spatial resolution of 5 μm, the analysis of two human femurs; one belonged to a worker exposed to lead in heavy metal industry, and the other worker was from the same environment but not exposed to lead. The mean lead concentration in the poisoned and the reference case in compact bone was 70 and 30 μg g^{-1}, respectively. The poisoned case exhibited two peaks in the distribution, at distances of approximately 0.2 and 1.8 mm from the periphery, respectively.

The work of L. Gerhardsson[16] aimed to compare bone lead concentrations in several cortical and trabecular structures in long-term exposed lead smelter workers, and to relate the measured concentrations to the corresponding ones in non-exposed workers. He analyzed, by electrothermal AAS, seven bones (trabecular: sternum, vertebrae, iliac crest, rib; cortical: femur, forefinger, and temporal bone) in 32 male, long-term exposed to lead and 10 non-unexposed male, reference persons. Furthermore this study presents values for Sr and Zn in the same bones. Todd[29] studied by electrothermal AAS adult human tibia for lead concentration determination. The goal of this work was to determine whether there were any differences between core and surface tibia lead concentrations. Lead concentrations in the nine tibiae ranged from 3.1 to 27.9 μg lead/g of dry bone. They concluded that the studied human tibiae showed a greater surface tibia lead concentration than core tibia lead concentration by a factor of the order of 2.

The indication of preferential accumulation in trabecular (spongy) rather than cortical bone by a factor around 1.4 in contaminated people was observed by Gerhardsson[16]. A factor of 2 has been observed by Chettle[14] by in vivo analysis of calcaneus and tibia.

In the present work the concentration values obtained in pressed pellets for the several bones are presented in tables 3 and 4 respectively for M4 Tornado and triaxial system for Ca, Zn, Sr and Pb. The agreement between the results obtained with both systems is remarkable. However, as already noticed for accuracy authentication, the uncertainties are higher for microanalytical system, of the order of 20% while for the triaxial system the uncertainty is around 10%. Nevertheless, from these values we can deduce that quantification for the benchtop microanalytical system with reasonable accuracy for Ca, Zn, Sr and Pb in pressed pellets is possible. The highest values for Pb were found in spongy bones: ribs, followed by the skull, foot bone, femur, fibula and tibia. Finally the inner part of thick compact bones, fibula and tibia present the lowest concentrations. The ratio between the content of Pb in both spongy and compact area of the same bone is around 2.

The relation between the amount of Pb in these bones is similar with the one obtained by Gerhardsson[16] in workers exposed to Pb contamination for around 30 years. However the values obtained in the present work are much higher than the mean values in that work. In Fig. 4 we can compare a spectrum from soil and compact bone. It is obvious that Pb in soil is much lower than in bone. From this result and considering that the ratio Pb content in spongy and compact bone is of the order of 2 we can deduce that the subject under study was *ante-mortem* contaminated.

Concerning the values of Sr they are more or less constant in all the studied bones, as well as the levels of Ca. Unlikely, Zn presents high variation in the different bones.

4.2. Quantification in microanalysis elemental distribution

For complete calibration of the benchtop system it is necessary to guarantee that quantification directly on the samples, without any sample preparation, is possible and accurate. This is actually the main goal of this study. To accomplish this purpose we used the slices of tibia bone and tooth and carried out quantitative calculations on several points. The results obtained for tibia are displayed in Fig. 5. They are in very good agreement with the corresponding ones obtained in pressed pellets. The lowest concentration values are obtained in the inner part of the tibia compact bone and enriched levels are observed on the direction of the spongy bone. A tendency to accumulation in the outer surface is also evident, in agreement with the results observed in the distribution mapping. Furthermore we also could confirm a factor of 2 for the ratio between the minimum and maximum value, in agreement with the attained values in pressed pellets. Determination on the micro distribution of Sr and Pb have been carried out by Bellis[6] using a prototype benchtop XRF system based on focused monochromatic microbeam X-ray fluorescence with a low power source coupled to doubly curved crystal (DCC) optics. However in this work the authors do not performed quantitative calculations. Only elemental maps have been obtained. Elemental distribution is not enough in most cases, as referred, and quantitative analysis is crucial. The conclusions of the present work are however similar to those obtained by Bellis. Lead appeared to accumulate in a thin band near the periosteal surface. Elevation in scattered discrete spots was also observed at the endosteal surface, and to a lesser extent within the core, also in agreement to the findings of Todd[29], who found that Pb measurements performed on human tibiae showed that Pb was enriched at the tibia surface relative to the core and that lead concentrations were significantly lower toward the ends of the tibia sections.

For tooth quantitative elemental micro analytical calculations we obtained the values for P, Ca, Zn, Sr and Pb. The values are presented on table 5. Two different parts can be considered; the pulp and the hard enamel, dentine and root. Pulp presents a completely different matrix in what concerns P, Ca, Sr and Zn, although presenting the highest values for Pb accumulation. This pattern is in agreement with lead *ante mortem*

intoxication[31-33]. Lead circulates in very high concentrations in blood of intoxicated people and might accumulate preferably in pulp region highly irrigated. Phosphorus and Ca are more or less constant in enamel, dentine and root. Zinc is enriched in root following the behavior of Sr and Pb.

5. Conclusions

From this work we could conclude that micro X-ray analytical technique based on a polycapillary system is a powerful technique for Pb determination in hard and soft tissues at the level of 3 $\mu g\ g^{-1}$ with 20% uncertainty. Furthermore we also demonstrate the capabilities for elemental distribution with micrometer resolution and quantitative calculations for other elements of biological interest, like P, Ca, Zn and Sr. These are obvious advantages when comparing this system to other XRF based techniques, like synchrotron radiation. Furthermore, the other benefits of the system, being non-destructive and very low amount of sample being necessary make it the preferred choice instead of AAS, ICPMS, LA-ICP-MS when non-destructive process is required. In addition, our results indicate a tendency of higher accumulation of lead in spongy bones. Finally the inner part of thick compact bones, fibula and tibia present the lowest concentrations. The ratio between the content of Pb in both spongy and compact area of the same bone is around 2. This was the ratio found by Chettle[14] when analyzing the bones of exposed individuals. The ratio of Pb in buried bones in contaminated environment is much higher, from 1 to several orders of magnitude[1, 2, 9, 10]. Moreover, to support the *ante-mortem* contamination explanation, we believe that it is unlikely that a soil about 50 $\mu g.g^{-1}$ of Pb[34] was the cause for contamination. We were dealing with a case of severe lead poisoning of a rather young man. An important source of Pb intake might have been linked to smelter exposure. In addition, the properties of lead, its corrosion resistance and formability, made it extensively used in plumbing, building, and ship construction existing in the area.

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Figure captions

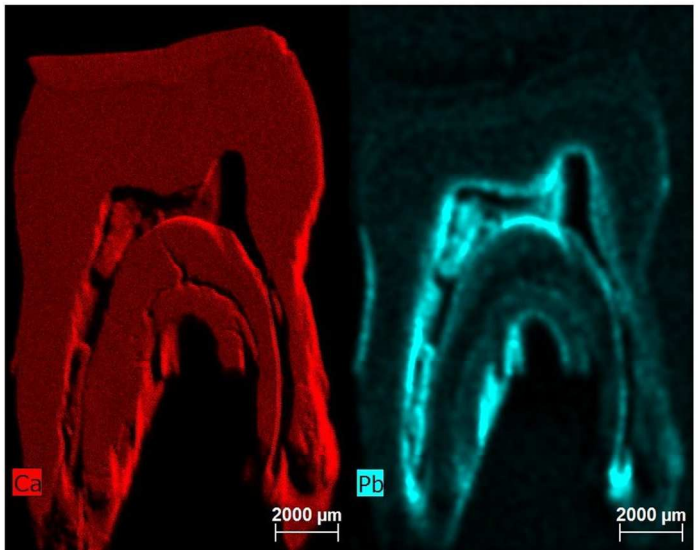
Fig. 1. Calcium and Pb mappings obtained using the microanalytical system on a tooth longitudinal cross-section

Fig. 2. Calcium and Pb mappings obtained using the microanalytical system on a tibia transversal cross-section

Fig. 3. Bone spectra obtained using both microanalytical and triaxial systems

Fig. 4. Comparison of the spectra obtained for soil and compact bone samples

Fig. 5. Lead distribution along a slice of tibia bone obtained using the M4 Tornado from the external part to the inner bone.



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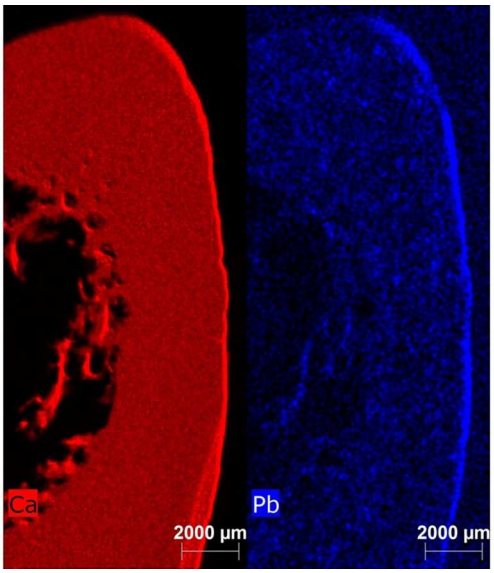

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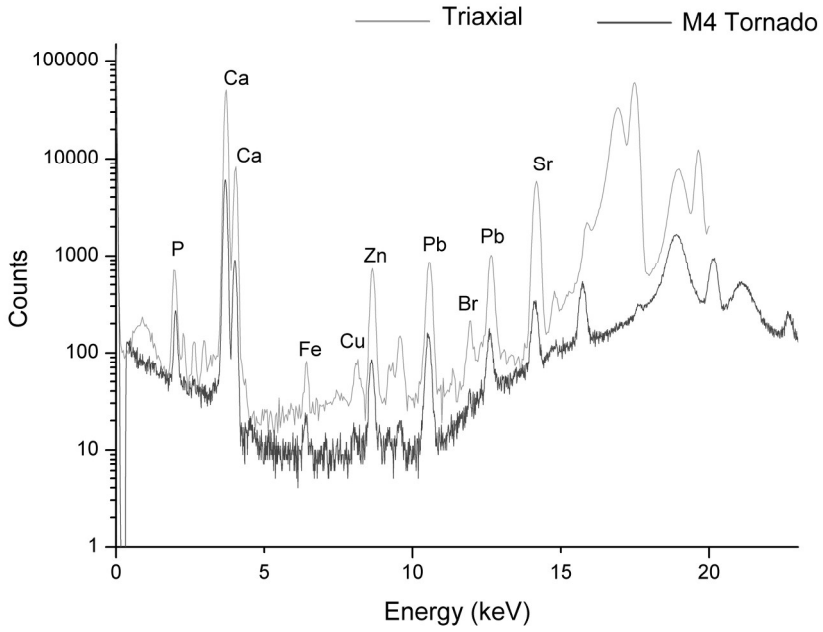

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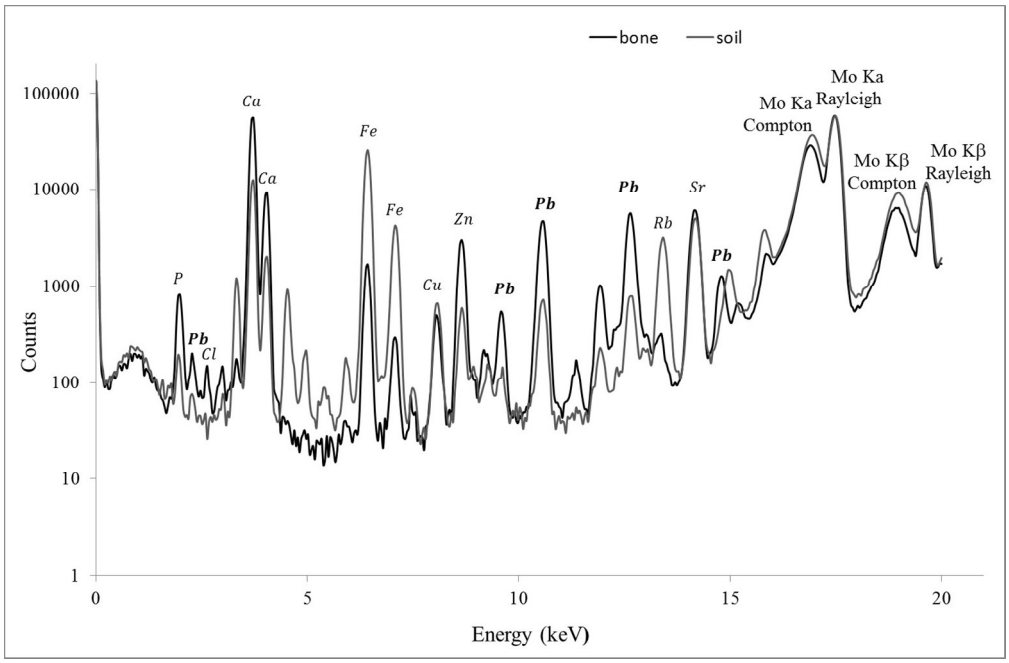

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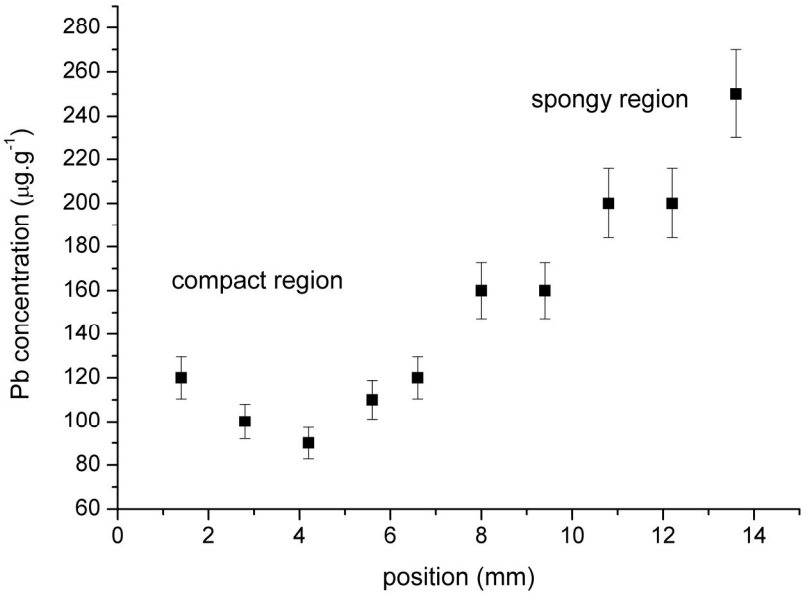

190x133mm (300 x 300 DPI)

Table 1. Accuracy and detection limits in μg g^{-1} obtained for the the microanalytical system in bone samples (N= 4)

	NYS RM 05-02 Bovine Bone					NIST SRM 1400 Bone ash					NYS RM 05-04 Caprine Bone		
	Obtained triaxial setup	Obtained Tornado	Certified	Detection limits triaxial setup	Detection limits Tornado	Obtained triaxial setup	Obtained Tornado	Certified	Detection limits triaxial setup	Detection limits	Obtained triaxial setup	Obtained Tornado	Certified
Ca	27%±5%	30% ± 7%	26%	90	150	37% ± 1%	30% ± 7%	38.28% ± 0.13	100	150	27%±5%	29% ± 7%	26.4%
Zn	75±5	90 ± 20	80	2	30	180 ± 10	160 ± 30	181 ± 3	4	20	80±2	70 ± 10	81
Sr	150±10	170 ± 10	160	2	7	210 ± 20	240 ± 10	249 ± 7	3	7	155±5	150 ± 9	150
Pb	17±2	15 ± 9	16.1 ± 0.3	2	3	11 ± 3	7 ± 3	9.1 ± 0.1	3	3	33±3	30 ± 20	31.5 ± 0.7

Table 2. Accuracy and detection limits in μg g^{-1} obtained for the microanalytical system in SRM IAEA-soil 7 samples (N= 4)

Element	Ca	Zn	Sr	Pb
Certified value	16.3% ± 6.0%	104 ± 3	108 ± 5	60 ± 5
Present work	16% ± 6%	110 ± 40	130 ± 50	50 ± 20
Detection limits	180	50	8	5

Table 3. Concentration values in, μg g^{-1}, for different bones obtained with microanalytical M4-Tornado system in pressed pellets (N=9)

Bones	Ca	Zn	Sr	Pb
Ribs	40% ± 10%	600 ± 100	220 ± 30	600 ± 90
Femur (spongy)	35% ± 10%	1080 ± 200	170 ± 20	280 ± 70
Skull	33% ± 10%	196 ± 34	160 ± 20	220 ± 50
Foot	34% ± 10%	230 ± 40	160 ± 20	260 ± 70
Fibula (comp)	40% ± 10%	260 ± 50	230 ± 50	120 ± 30
Fibula (spongy)	30% ± 10%	80 ± 20	150 ± 30	250 ± 50
Tibia (comp)	30% ± 10%	110 ± 20	170 ± 40	100 ± 25
Tibia (spongy)	39% ± 10%	170 ± 30	190 ± 40	250 ± 60

Table 4. Concentration values in, µg g^{-1}, for different bones obtained with triaxial system in pressed pellets (N=9)

Bones	**Ca**	**Zn**	**Sr**	**Pb**
Ribs	39% ± 5%	500 ± 30	210 ± 10	560 ± 30
Femur (spongy)	36% ± 5%	980 ± 50	200 ± 10	300 ± 20
Skull	32% ± 5%	150 ± 10	170 ± 10	220 ± 20
Foot	32% ± 5%	170 ± 10	180 ± 10	300 ± 20
Fibula (compact)	35% ± 5%	220 ± 20	210 ± 10	190 ± 20
Fibula (spongy)	37% ± 5%	210 ± 10	220 ± 10	250 ± 20
Tibia (compact)	37% ± 5%	110 ± 10	190 ± 10	120 ± 10
Tibia (spongy)	32% ± 5%	140 ± 10	220± 20	260 ± 20

Table 5. Concentration values in, µg g^{-1}, for different tooth region obtained with M4 Tornado for P, Ca, Zn, Sr and Pb in a tooth cross section (N=9)

Tooth					
	P	Ca	Zn	Sr	Pb
Enamel	19% ± 5%	35% ± 8%	110 ± 22	100 ± 6	16 ± 9
Dentine	16% ± 4%	31% ± 7%	140 ± 30	170 ± 10	60 ± 20
Root	16% ± 4%	34% ± 8%	190 ± 30	190 ± 10	130 ± 50
Pulp	3% ± 1%	4% ± 1%	40 ± 8	60 ± 4	140 ± 50