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Quantitative analysis of human remains from 18th–19th centuries using X-ray fluorescence techniques: The mysterious high content of mercury in hair

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ABSTRACT

In this work, we report the unusual concentration of mercury in the hair of an individual buried in the 18th to mid-19th centuries and the comparison with the elemental composition of other remains from the same individual. Two energy dispersive X-ray fluorescence (EDXRF) setups, one with tri-axial geometry and the second one with micro-beam capabilities and a vacuum system, for light elements detection, have been used. Quantitative evaluation of the obtained spectra were made by fundamental parameters and winAXIL program by compare mode method. The levels of Hg in the hair of buried samples presented a concentration over 5% (w/w), a significantly lower presence of this element in the cranium, and no Hg in the remaining organs. Furthermore, there was no evidence of Hg in the burial soil, which has been also analyzed. From this result, we could conclude that the possibility of *post-mortem* contamination from the burial surroundings is very unlikely. The obtained results are indicative of the apparent use of a mercury-based compound for medical purposes, most likely lice infestation.

1. Introduction

The analysis of human remains is a reliable source of information regarding the dietary habits, environmental surroundings and exposure to toxic elements of a particular population [1]. Bones, on the one hand, are considered good lifelong monitors for trace elements contamination [2], while hair is believed to provide clues regarding the timeline of the exposure to a given element [3]. Teeth can also be a valuable source to the knowledge of the habits of former civilizations considering that the diffusion pattern of some elements can give information both for archaeological purposes and diagenesis processes affecting the apatite ante-mortem elemental content [4]. However, post-mortem alterations of buried remains is always a problematic factor for archaeological and forensic studies. The extent of these alterations depends on direct environmental conditions such as groundwater, soil pH, redox potential and temperature [2]. Moreover, direct contamination of elements present in the burial soil must be taken into consideration.

X-ray fluorescence methods have proven to be suitable tools for studying the elemental composition of such samples. Carvalho et al. used total reflection X-ray fluorescence (TXRF) to study the elemental distribution and post-mortem intake of trace elements in bone from the middle ages [5] and synchrotron-based micro X-ray fluorescence (Sy-μ-XRF) to evaluate the diffusion of elements such as Ba and Pb in teeth from the same Era [4]. Piga et al. [6] used both a benchtop and a portable energy dispersive XRF equipment to study the bone fragments belonging to King Peter III of Aragon and Queen Blanche of Anjou and discovered the presence of elements related with the mummification processes.

Regarding hair analysis, and because the analysis are mainly performed directly on the hair samples, micro beam is paramount and Sy-XRF is the most used technique [7] sometimes complemented with TXRF [8]. In a more illustrious case study, hair samples of Napoleon I, collected through a course of 16 years, were analyzed using SXRF. The presence of toxic elements was compared in the different hair samples as well as longitudinally through each hair sample [9].

The choice of XRF for these studies relies in the non-destructive nature of the technique, allowing for the analysis without damaging the sample. Moreover, it is a multi-elemental technique, were all element are evaluated simultaneously. With the two setups used in this work combine the improved detection limits of a tri-axial

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geometry with the micro-beam and vacuum capabilities of the M4 Tornado setup.

The Ermida of Espirito Santo, in Almada (Portugal) was built in the 15th century and according to the records of Santa Maria do Castelo parish, it was used as burial site between 1755 until it was closed in the mid-19th century. In 2011, during some archaeological excavations, the remains of 83 individuals were recovered [10]. In this work, we report the elemental analysis of the remains of one specific individual, mainly hair and bones, focusing on the presence of unusual high amounts of mercury in the hair.

One can be exposed to mercury from breathing in contaminated air, from swallowing or eating contaminated water or food, or from having skin contact with it [11]. Not all forms of mercury can easily enter in our body, even when in contact with it. The most dangerous forms, from the toxicological point of view, are the metallic form, the divalent inorganic forms and methylmercury compounds [12]. Classical mercury poisoning is characterized by a triad of signs, namely tremors, erythrism and gingivitis [12]. Some inorganic mercury compounds do not generally vaporize at room temperature like elemental mercury does. If inhaled or swallowed, they are not expected to enter in human body as easily as inhaled metallic mercury vapor [13]. Furthermore, inorganic mercury accumulates mostly in the kidneys and does not reach the brain as easily as metallic mercury [14]. Nevertheless, a small amount of the inorganic mercury can be changed by the organism into metallic mercury and leave in the breath as a mercury vapor [13].

Two different EDXRF setups were used in order to take advantage of their special features: a setup with tri-axial geometry in order to take advantage of the lower detection limits for low Z matrices [15], and a benchtop equipment allowing micro beam as well as performing analysis under vacuum fundamental for the detection of lighter elements, such as phosphorus in bones and teeth [16].

2. Materials and methods

2.1. Specimen description

During archaeological excavations, the remains of 83 individuals were found in the Ermida of Espirito Santo (Almada). In this work we will report the analysis of bones, hair and tooth of one particular individual, because of the unusual amount of Hg found in the remains. This was an adult female, approximately 50 years old, buried in a shroud, without coffin, and about 70 cm deep. The analyzed samples were: a strand of hair, a molar tooth and bones belonging to the cranium, ribs and hand phalange. In order to evaluate possible contaminations from the burial surroundings, we also analyzed the soil.

2.2. Energy dispersive X-ray fluorescence spectrometers

The spectrometer with tri-axial geometry used in this work is a commercial X-ray tube (Philips, PW 1140; 100 kV, 80 mA), equipped with a molybdenum (Mo) secondary target water cooled. The X-ray tube, the secondary target and the sample are in a tri-axial geometry. With this geometry it is possible to obtain a nearly monochromatic source, with Kα and Kβ lines of Mo, energies 17.44 and 19.60 keV, respectively. With this arrangement, we decrease the background, taking advantage of the effect of the polarization of the incident X-ray beam from the tube and the nearly monochromatic radiation. Both the X-ray beam emitted by the secondary target and by the sample are collimated throughout two silver apertures, in order to reduce the scattered radiation and improve the detection limits. The characteristic radiation emitted by the elements is detected by a nitrogen-cooled Si(Li) detector by Oxford Instruments (England) with energy resolution of 138 eV at 5.9 keV [15]. The operating conditions of this system were 50 kV and 20 mA with acquisition times of 1000 s. The quantitative evaluation for hair and bone samples was performed using Fundamental Parameter method, which determines the relative concentration of elements present in the sample making use of fundamental atomic parameters such as cross-sections for absorption and X-ray production, transition intensities, fluorescence yields, etc [17]. The suitability of the used method for the different matrices was recognized using appropriate standard reference materials (SRM): Bone Ash SRM NIST-1400 and Bovine Bone 05-02 (Department of Health, State of New York, USA) for bone samples; Methylmercury spiked human hair IAEA-085 (International Atomic Energy Agency) and DC73347-China National Analysis Center for iron and steel- for hair. Tables 1 and 2 present the Detection Limits (DL) and accuracy results obtained for these standard reference materials. Detection Limits were determined according to Silveira et al. [16].

In this work we also used a commercial spectrometer, the M4 Tornado by Bruker (Germany). 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 background (100 μm Al/ 50 μm Ti/ 25 μm Cu). Detection of 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. This equipment was used to evaluate the presence of low-Z elements in all samples as well as performing mappings of cross sections of the bones and tooth samples. Spectra deconvolution and fitting were performed using WinAXIL software package (Camberra, Belgium) and quantification was performed through compare mode method [18]. 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.

Two SRM were used for the quantification of the burial soil: IAEA – soil 7 and International Soil-Analytical Exchange – ISE- 954.

Table 1 presents the Detection Limits obtained for Bone Ash SRM NIST-1400 and Bovine Bone 05-02 and IAEA – soil 7 (International Atomic Energy Agency) with the M4 Tornado. The accuracy of the proposed method for quantification of soil samples is presented in Table 2.

2.3. Sample preparation

Bone samples to be analyzed in the tri-axial setup were ground into a powder and pressed into pellets (2 cm diameter; 1 cm thickness) as described in Guimarães et al. [19]. Hair was analyzed in a strand as recommended by Török [20] in order to minimize heterogeneity effects. Sample hair was not washed according to the recommendation rules, taking into account that these samples are the only hair samples of the whole estate and are valuable for the museum. They were analyzed without any sample preparation.

Tooth samples were cut in longitudinal cross-sections using a microtome with diamond saw. The elemental mappings were performed directly on the specimen.

3. Results and discussion

The most significant result obtained in this study was the remarkable concentration of Hg in the hair samples, up to 6% (w/w) Fig. 1.

The quantifications obtained for the remaining elements are presented in Table 3. Regarding this amount of Hg it would be