ESRF	**Experiment title:** First direct characterization of ferruginous bodies from lung tissue combining micro-XRF, micro-XRD and micro-XAS techniques	**Experiment number**: MD-550
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Report:

In this experiment we investigated the nature of Asbestos Bodies (AB) in lung tissues of two subjects subjected to prolonged occupational exposure to asbestos. Elemental distribution maps were acquired at 14.4keV on AB deposited on porous membranes after the removal of the organic tissue by digestion with sodium hypochlirite. Bovine liver and SRM1832-1833 from NIST where used as calibrants for accurate elemental quantification.

Elemental quantification indicated that Fe is by far the most concentrated element (about 200x the other elements detected) and it is highly enriched on the *AB* with respect to the surrounding organic tissue (~20% *vs.* less than 0.1%, Table 2). The Fe levels found on the *AB* are twice higher than that measured on the ferritin standard (~11%), but still in line with the Fe concentration reported for ferritin in the literature (10–30%wt, [1]. The average Fe concentration detected in the lung tissue (~0.06%) is of the same order of that measured in the bovine liver standard (~0.02%). Similar amounts of Ca (0.3–0.4%) were detected in *AB*. Calcium occurring in the *AB* could arise from $CaPO_4$, which could take part in the biomineralization process, as already proposed by Pooley [2]. High levels of Cl (~5%), originating from NaCl crystals formed during the digestion procedure and not fully removed by washing the samples with deionized water, were also detected.
Copper, Zn, and As in the 0.1–0.8% range were also detected in the *AB*. The presence of significant amount of Ba found in the *AB* and, at lower concentration, in the lung tissue, was totally unexpected. Barium was absent in control samples (pristine porous membrane and porous member filtered with pure NaClO), excluding the possibility of external contamination.
A representative fluorescence map acquired at 14.4 keV on an *AB* is shown in Figure 1a. Only elements that were found in higher concentrations on the *AB* with respect to the background (cellulose membrane plus residual organic tissue) are shown. The XRF map reveals that the spatial distribution of Fe, Ba, Cu, Zn, and As mimics the morphology of the *AB*, indicating either that those elements take part in the biomineralization process, or that they are efficiently adsorbed by the *AB* during its formation. From the figure it can also be seen that elements such as Br and Cl, originating from the original organic tissue or from sodium hypochlorite used to digest the tissue, are uniformly distributed on the background, and are not associated with the *AB*. The distribution of the above discussed elements was the same in all the *AB* examined. In particular, Cu, Zn, and Ba appear to be distributed in higher concentrations in the inner part of the *AB*, were the concentration of Fe is highest.
Elemental quantification has rarely been attempted on *AB* due to the difficulty of isolating a sufficient amount of material. In addition, due to the high dilution level of important trace elements, elemental quantification has always been performed using ICP-MS, which is a bulk technique. Another disadvantage of ICP-MS is that it requires incineration of the organic tissue and the complete dissolution of the *AB* in strong

acids, which can severely alter the chemistry of the samples and destroys any spatial information. Although not sensitive and reliable as ICP-MS, elemental quantification by micro X-ray fluorescence can be performed with little sample pre-treatments, which allowed studying the elemental concentration of single *AB* avoiding chemical treatments and retaining spatial information, thus revealing details essential to understand the bio-mineralization process leading to the formation of the *AB*. Although this is the first work attempting to determine the quantitative elemental composition of single *AB*, due to technical limitations discussed in the paper, the results obtained must be considered as a rough estimation. Elemental quantification confirmed that Fe is by far the most concentrated element in the *AB*, and the Fe levels detected (~20%) are compatible with the presence of high Fe-loaded ferritin or hemosiderin. Moreover, with respect to previous works exploiting synchrotron radiation for this subject [4] the presence and distribution of elements heavier than Fe (Cu, Zn, As, and Br) is reported for the first time. The fortuitous presence of Ba in the studied samples and its distribution (together with that of Cu, Zn, and As) suggested a speculative compositional and growth model for the *AB*, which supports the presence of hemosiderin in the inner part of the Fe-coating, while the outer part would be mainly composed by ferritin. The results were presented at an international conference [and a publication is in preparation.

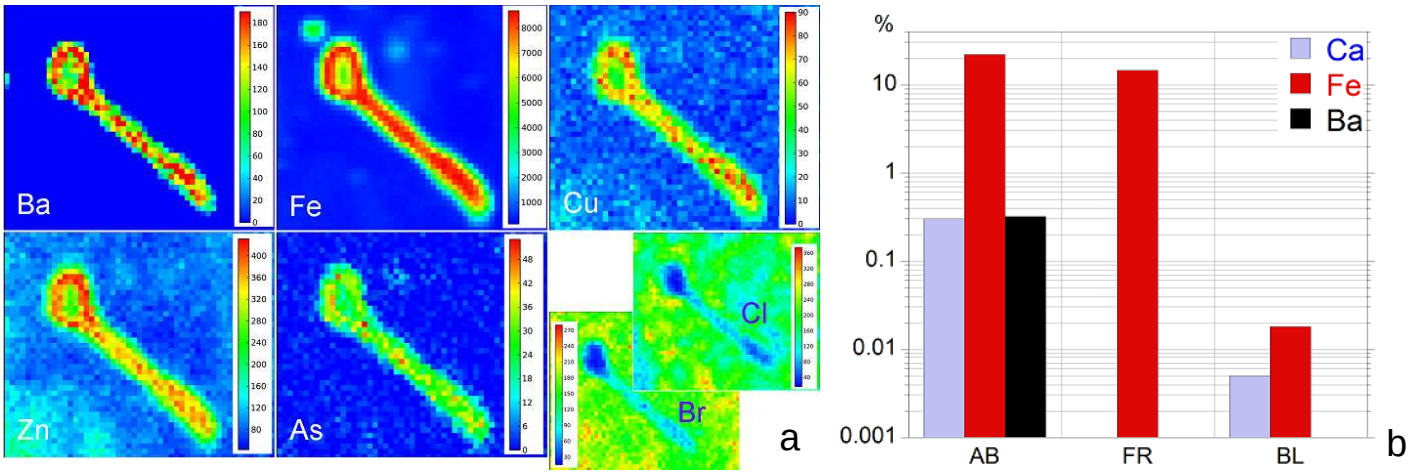

Figure 1. (a) µXRF spatial distribution maps of selected elements acquired at ID18F at 14.4 keV on an *AB* belonging to case A. The maps clearly reveal elements not associated with the *AB*, as is the case of Br and Cl, which are uniformly distributed in the background, but absent on the *AB*. The size of the map is 130 x 120 µm^2 and the pixel size (resolution) is 2.5 x 2.0 µm^2. The scale bars indicate the intensity of the fluorescence signals and are informative of the relative elemental concentrations. (b) Concentration of Ca, Fe, and Ba in *AB*, and in the ferritin (FR) and bovine liver (BL) standards.

	AB	[a]*FR*	[b]*BL*
K	nd	0.004±0.001	0.073±0.008
Ca	**0.30±0.04**	**nd**	**0.005±0.003**
Mn	nd	nd	0.001±0.001
Fe	**21.9±0.3**	**14.6±1.2**	**0.018±0.002**
Cu	0.15±0.02	nd	0.021±0.002
Zn	0.78±0.01	0.017±0.001	0.017±0.001
As	0.10±0.02	0.013±0.003	nd
Br	0.85±0.04	0.080±0.002	0.002±0.001
Ba	**0.32±0.05**	**nd**	**nd**

Table 1. Quantification of elements from K to Br and Ba, performed on *AB*, and on the ferritin (*FR*) and bovine liver (*BL*) standards at incident photon energy of 14.4 keV. The errors represent the standard deviations on several measurements (up to 10). Concentrations values below 0.001% (10ppm) were neglected. The elements appearing in bold style are reported in the histogram shown in Figure 1b for easier comparison.

[a] Horse-spleen ferritin (F7879, Sigma-Aldrich); [b] Bovine liver (SRM1577b, NIST). "nd" stands for not detected or under the detection limit.

References

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