	Experiment title: Mobilisation of Ni and toxic elements in the human respiratory system	**Experiment number**: LS-2112
Beamline: ID22	**Date of experiment**: from: 5 September 2001 to: 8 September 2001	**Date of report**: 08-08-2003
Shifts: 3	**Local contact(s)**: Alexandre Simionovici and Sylvain Bohic	*Received at ESRF:*

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Report:

Objective of the experiment:

1. To explore micro-XRF capabilities to study elemental deposits in cryosections of few micrometers thick of lung tissue and lung associated lymphoid tissue;
2. To obtain semi-quantitative and quantitative data for toxic metals such as, Cr, Ti, and Pb;
3. To explore the feasibility of applying micro-XANES to assess chemical species of interest in deposits.

Achievements:

1. Mapping enable to identify deposit areas and to visualise distributions for Cr, Ni, Pb among other metals such as Fe, Cu and Zn;
2. Point analysis over regions of interest and scans over selected areas (deposit areas and specific tissue structures) using monochromatic beam mode, enabled to obtain quantitative data;

3. Develop a methodology to carry out quantitative analysis by combining both micro-XRF results obtained at ESRF and Rutherford Backscattering Spectrometry (RBS) data obtained at the Nuclear Microprobe set-up installed at ITN, Sacavém;
   - Areal mass density and/or thickness provided by RBS was used to normalise micro-XRF data. Therefore, the sections irradiated at the micro-XRF set-up were scanned at similar areas with the nuclear micropobe. RBS and PIXE (Particle Induced X-ray Emission) spectral data were collected simultaneously and an average areal mass density for different areas (tissue, various types of deposits, surrounding deposit areas) were obtained. These average values were then used to normalise micro-XRF spectral data.
   - The methodology used to normalise micro-XRF data proved to be quite satisfactory. Differences in concentration values of Ca, Cr, and Fe, obtained for micro-XRF normalised data and micro-PIXE data were of about 10% at deposit regions for both lung and lymphoid tissues. Major departures (larger than 40%) were obtained for the elemental concentrations of tissue free of deposits, mainly due to high uncertainty on micro-PIXE determination derived from poor counting statistics.
4. Identify sources of uncertainties in concentration calculation that can thus, be corrected in future experiments, such as, eliminate backing foil to support samples.
5. A contribution to the Proceedings of the 7th International Conference on X-ray Microscopy (X-ray Microscopy 2002) was prepared and published in Journal de Physique IV, 2003 [1].
   Results obtained were also presented at two conferences as posters and published as abstracts [2,3].

Difficulties and Drawbacks:

1. Orientation of samples and localisation of regions of interest were the major difficulties encountered during the experiment, mainly due to the poor contrast offered by the dry sections.
2. Concentration levels of toxic elements in deposits were often below the detection limit of micro-XANES, thus this technique was not applied.

Methodology improvements that can be carried out for future experiments

Improve sample orientation prior to SXRF experiment. Indicators for regions of interest can be obtained by verifying using different optical microscopy approaches to parallel sections.

Self-supporting sections of 10 to 15 micrometer thick (fresh material) can be produced, without decreasing lateral resolution, what will eliminate the contribution from backing foil on distribution maps and quantification procedures.

In addition, becoming acquainted with the micro-XRF experimental facility, and its capabilities, concerning mapping, resolution and excellent limit of detection for many elements of biological relevance (in biomedical and toxicity studies) will for sure enable us to better delineate new experiments and to better decide on advantageous applications. Also, the combination of various microscopy techniques, such as, optical, and based on synchrotron radiation, electron and accelerated particles, beams, enabling to overlap morphological and elemental distribution information, is of the utmost relevance in biomedical studies.

References of published work associated to ESRF experiment LS-2112

[1] T. PINHEIRO, L.C. ALVES, F. ARAÚJO, A. BARREIROS, S. BOHIC, A. SIMIONOVICI, Imaging and quantification of trace metals in biomedical samples using Synchrotron Induced X-ray Fluorescence and Nuclear Microprobe techniques, Journal de Physique IV, 2003;104:321-324

[2] T. PINHEIRO, L.C. ALVES, F. ARAÚJO, A. BARREIROS, P. MONTEIRO, A. BUGALHO DE ALMEIDA, S. BOHIC, A. SIMIONOVICI, Retention of Inhaled Particles in the Human Respiratory System and in the associated lymphoid tissue, *TEMA11, 11th International Symposium on Trace Elements in Man and Animals*, Berkeley, USA, June 2002, poster.

[3] T. PINHEIRO, L.C. ALVES, F. ARAÚJO, A. BARREIROS, S. BOHIC, A. SIMIONOVICI, Analysis of trace elements in cell inclusions using microprobe techniques: Nuclear Microprobe and Synchrotron Radiation X-ray Fluorescence Microprobe, *ICNMTA2002, 8th International Conference on Nuclear Microprobe Technology & Applications,* Takasaki, Japan, September 2002, poster.