ESRF	**Experiment title: Topographic and quantitative analysis of selected elements in human central nervous system tissue and single neurons.**	**Experiment number**: MD-32
Beamline: ID 22	**Date of experiment**: from: 5 Mar 2003 to: 10 Mar 2003	**Date of report**: 11 Feb 2004
Shifts: 15	**Local contact(s)**: Sylvain Bohic, Alexandre Simionovici	*Received at ESRF:*

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Report:

The synchrotron microbeam-X ray fluorescence (μ–SXRF) was applied for topographic and quantitative analysis of selected elements in central nervous system (CNS) tissue. The main goal of the studies is the investigation of the role of trace metals, in processes leading to degeneration and atrophy of nerve cells in Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS). Since, trace elements may participate in biochemical processes of CNS, elemental analysis of nervous tissue may significantly gain existing information on the pathogenesis of PD and ALS.

For the experiment, samples were taken during the autopsy of patients deceased with PD, ALS and from patient died due to non-neurological conditions. Three areas of CNS i.e. substantia nigra (SN) of brain, brain cortex (BC), and thoracic spinal cord were sampled. The fresh specimens were quickly frozen and cut into sections of 20 micrometers thickness in a cryomicrotome. From each section one slice was taken for routine histopathological investigation and the contiguous one for μ–SXRF analysis. The slices designed for elemental analysis were mounted immediately onto AP1 foil and freeze-dried. Before X-ray analysis, the samples were examined by optical microscopy. From each sample two areas were selected for scanning. In the first one, representative of the gray matter, neurons' perikarial parts were located. The other one, representative of white matter, was without nerve cell bodies. Moreover, preliminary elemental analysis was performed inside single neurons of SN. The μ–SXRF analysis was carried out for SN and BC of PD and the control group samples, as well for spinal cord sections representing ALS and the control group.

A 14? keV monochromatic excitation was used. The applied Kirkpatrick Beaz optics enabled obtaining an intense monochromatic photon beam. focused to a final beam spot dimension of 4 μm x 1.4 μm (horizontal (H) x vertical (V)) and flux at the sample of about $1*10^{11}$ photons/s. In case of big map, typical areas selected for scanning were 500 x 500 μm^2 and were mapped by

steps of 10 μm (H) by 5 μm (V). For single neurons mapping, the scanning areas were 60 x 60 μm^2. In this case more precision of scanning, i.e. by steps 3 μm (H) by 1 μm (V) was applied. Time of measurement was equal to 3 s per point. The 2D maps of elemental distribution in the tissue slices were obtained after normalization of the counts number to the incident photon flux. Measurements of XRF thin film calibration standards and NIST standard reference materials (SRM 1833 and SRM 1832) were performed for spectrometer calibration. For each calibration standard the measurement was performed in 9 points. The acquisition time was equal to 10 s per pixel.

The promising results, obtained during LS-2111 experiment [1,2,3] from medical point of view required verification by investigation of a statistically reliable number of samples relevant for selected pathology. In the performed experiment MD-32, the next samples representing PD, ALS and control group were analysed. The elements such P, S, Cl, K, Ca, Fe, Cu, Zn, Se, and Br were identified in CNS tissue. The results showed that significantly higher intensities of selected elements in μ–SXRF images reflect position of the neurons in SN and spinal cord tissue slices. Such results were not observed for the BC samples where the distribution of elements was quite uniform. The Figure 1 shows distribution of Cl, Cu, Zn, and Se in comparison with the scanned area of the SN. Topographic analysis of neuron pericarial parts showed significant differences in accumulation of elements inside the cells. Moreover, it was noticed that some elements such Cl reveal different distribution between PD and control samples. The selected results from single neurons analysis were presented on Figure 2. Topographic elemental analysis of areas representing white matter was in good agreement with the LS-2111 results. In this part of CNS, the distribution of elements was quite uniform that reflect a more “monotonous” histological structure of this area.

The calibration data obtained from measurements of thin film calibration standards were used for calculation of a mass per unit area of elements in tissue samples. The comparison studies indicated that SN neurons of the PD samples show significantly higher values of mass per unit area for Ca, Fe, Zn in comparison with all control cases. The white matter of PD sample reveals higher level of Ca, Fe, Cu, Zn and Br than the control cases whereas the BC analysis showed increased accumulation of Ca, Zn, and Br in PD case. The quantitative analysis of spinal cord sections show that in ALS case accumulation of Zn in neurons is lower than in the control whereas Br content in white matter is increased with respect to the control.

Most of present results are in good agreement with the results obtained in the previous experiment. However, some differences and new observation were noticed after investigation of a larger number of samples. The elemental analysis of single nerve cells helped to localize more precisely the abnormal accumulation of elements inside nerve cell bodies. The obtained results would be helpful to gain some information on the trace metal function in central nervous system tissue with respect to neurological disorders.

References:

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3. Lankosz M, Szczerbowska-Boruchowska M, Ostachowicz J, Adamek D, Krygowska-Wajs A, Tomik B, Bohic S, Simionovici A. Topographic and quantitative elemental analysis of human central nervous system tissue. *ESRF Highlights 2002.*

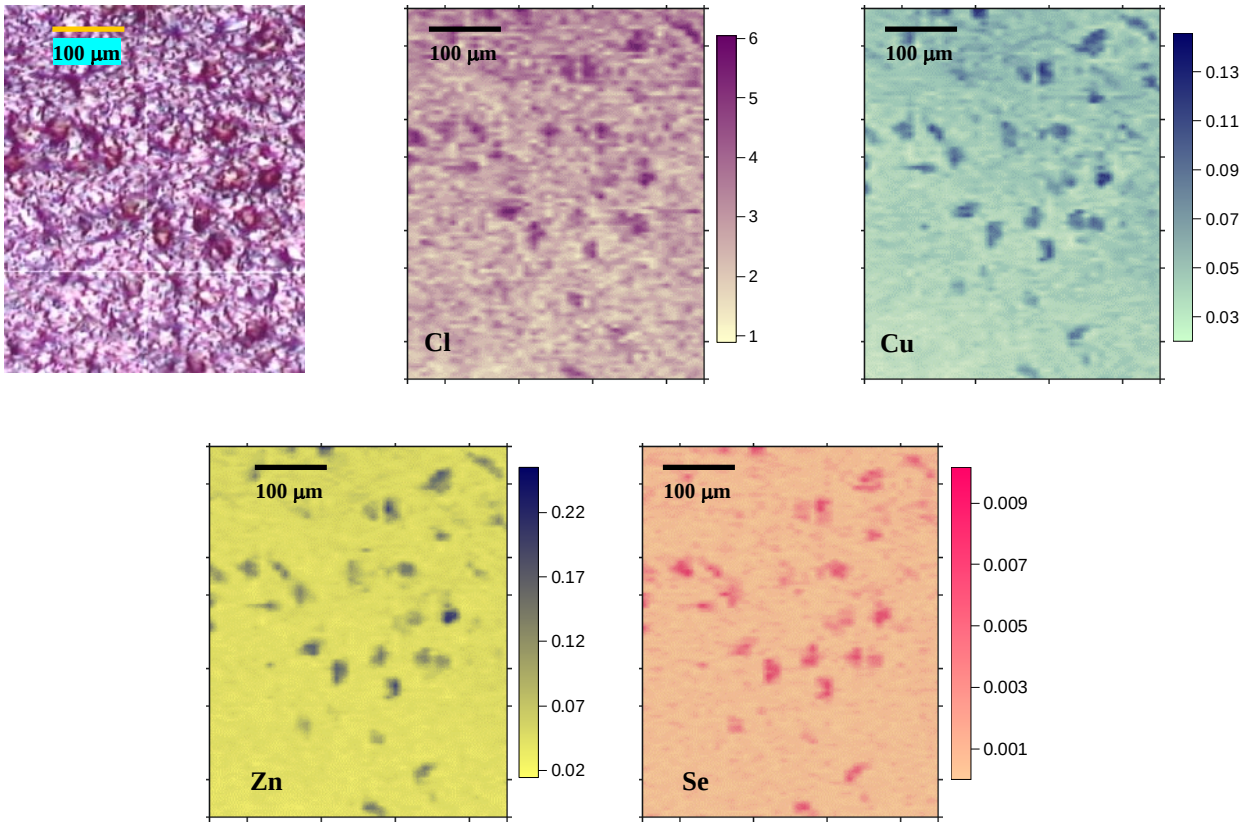

Figure 1. Distribution of selected elements in substantia nigra tissue (the control case) in comparison with scanned area of tissue. The values on the scales represent mass per unit area of element in[µg/cm^2].

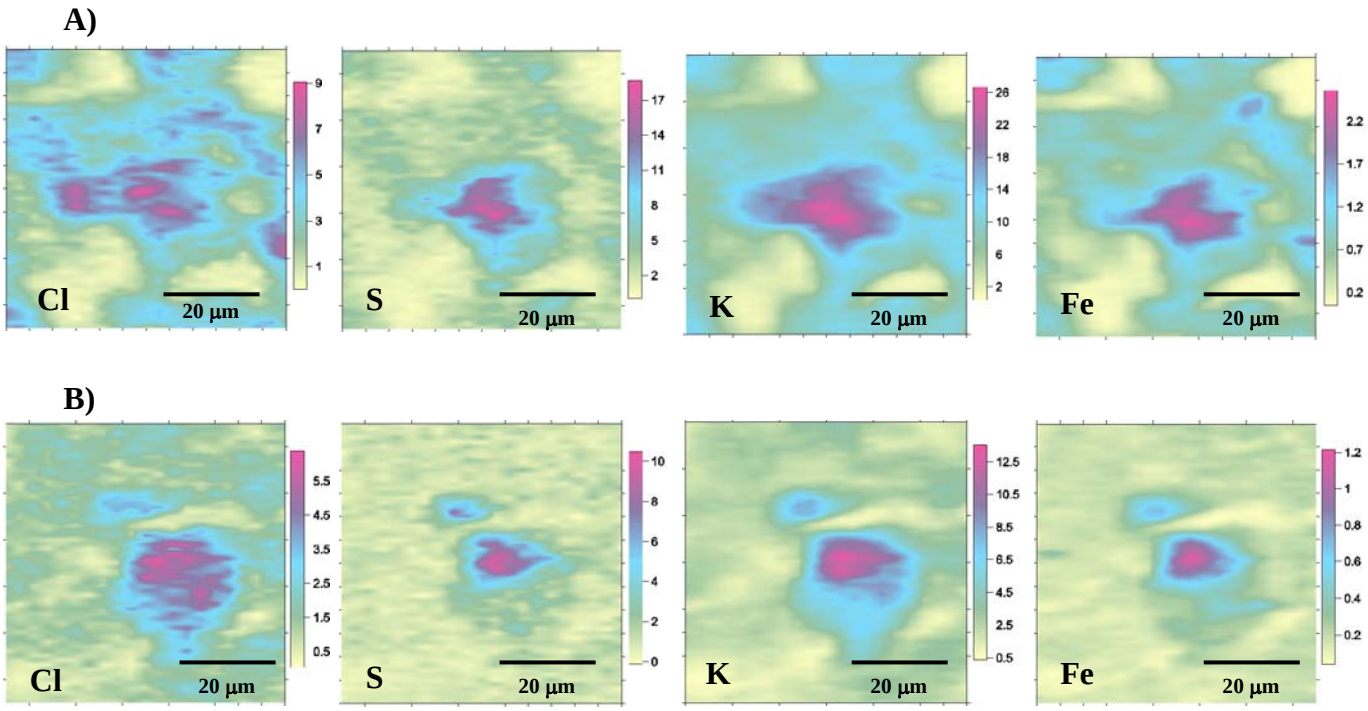

Figure 2. Distribution of elemental masses per unit area (in [µg/cm^2]) for: (A) Parkinson's diseased and (B) the control nerve cells.