ESRF	**Experiment title: Topographic and Quantitative Microanalysis of Selected Elements in Tissue of Human Central Nervous System**	**Experiment number**: LS - 2111
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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 work is the investigation of the role of elements, mainly metals, in processes leading to degeneration and atrophy of nerve cells in cases of two neurodegenerative disorders i.e. Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS). Since, the pathogenesis of PD and ALS is still not known the mechanisms leading to neurodegeneration are investigated.

For the experiment, the samples were taken during the autopsy from patients deceased with PD, ALS and from patient died due to non-neurological conditions. Two areas of CNS i.e. substantia nigra (SN) of brain and thoracic spinal cord were sampled. The specimens were 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 other 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, representing gray matter, neurons' perikarial parts were located. The other one, representing white matter, was without nerve cell bodies. The μ–SXRF analysis was carried out for SN of PD, ALS and the control group samples as well for spinal cord sections representing ALS and the control.

A 17 keV monochromatic and polychromatic excitation were used. In monochromatic mode, the beam was focused with Compound Refractive Lenses (CRL) for a final beam spot dimension of 10 μm x 2 μm (horizontal (H) x vertical (V)) and flux at the sample of about $1*10^{10}$ photons/s. With polychromatic PINK beam mode, additional appliance of pinhole gave the final beam dimension of 5 μm x 2 μm (H x V), and a flux about $1.5*10^{11}$ photons/s. The spectra obtained from tissue sample, for both cases of excitation, revealed about 10 times higher intensities of excited characteristic x-rays of elements for PINK beam mode then for monochromatic beam in the same time of measurement. Finally, the main part of analysis was

performed with application of the PINK beam mode. Typical areas selected for scanning were 500 x 500 μm^2 and were mapped by steps of 10 μm (H) by 5 μm (V). Moreover, during the experiment, analysis of SN single neurons was performed. For this purpose, areas of 100 x 100 μm^2 containing neurons' perikarial parts were scanned with more precision i.e. by steps 5 μm (H) by 2 μm (V). 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. Due to the detector saturation, in this case, the primary beam size was reduced from 0.6 x 0.6 mm^2 (aperture applied during measurements of tissue samples) to 0.2 x 0.2 mm^2. For each calibration standard the measurement was performed in 9 points. The acquisition time was equal to 10 s per pixel. The other parameters were unchanged.

The elements such us P, S, Cl, K, Ca, Fe, Cu, Zn, Se, Br, Rb and Sr were identified in CNS tissue. Two-dimensional maps of elemental distribution were compared with the histopathological sections precisely corresponding to the slices prepared to the μ–SXRF analysis. The results showed that significantly higher intensities of selected elements in μ–SXRF images reflect position of the neurons in tissue slice. Particularly, in control group, neurons of substantia nigra revealed higher accumulation of S, Cl, K, Ca, Fe, Zn, Se and Rb then a surrounding area. However, level of P and Cu is comparable in both areas. The Figure 1 shows distribution of Cu, Zn and Se in comparison with optical image of scanned area of the control tissue. Unlike the control, correlation between increased content of element and neuron position was not observed either for Ca in PD and ALS or for Cl in ALS. Moreover, strong accumulation of Cu was noticed in neurons for PD case and is presented on the Figure 2. As for the spinal cord investigation, the elemental maps obtained from the control case show a correlation between neuron bodies positions and increased content of S, Cl, K, Ca, Zn, Br and Rb. Such correlation is not observed for Fe and Cu whereas accumulation of P is even decreased inside neuron perikarial part. The topographic results obtained for ALS tissue slice of spinal cord are in good agreement with these observed for control group excluding S. Accumulation of this element inside neuron bodies is comparable with surrounding area. The elemental analysis of single neurons of SN showed additionally differences of distribution of selected elements inside body of nerve cell. As example, comparison between localization of Zn and Se inside the neuron is shown in Figure 3.

Topographic elemental analysis of areas representing white matter showed that in this part of the brain distribution of elements is quite uniform that reflect 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 S, Ca, Fe, Cu, Zn, Se and Br in comparison with control case. For the same part of the brain increased content of Cl, Ca, Zn, and Br was noticed for the ALS case. The white matter of PD sample reveals higher level of Ca, Fe, Cu, Zn and Br then the control case whereas increased values of mass per unit area of Ca, Fe, Zn, Br and Sr were observed for ALS case. The quantitative analysis of spinal cord sections show that in ALS case accumulation of Zn in neurons is lower then in the control whereas Br content in white matter is increased with respect to the control.

The present study indicated that high resolution μ–SXRF technique is sensitive for elemental mapping in thin nervous tissue sections at the single cell level. The μ–SXRF technique enabled localization of abnormalities of elemental accumulation for cases of PD and ALS at the single cell level that was not possible using x-ray fluorescence bulk analysis methods. Our results are in good agreement with existing hypotheses of neurodegenerative role of trace elements based on in vitro investigations or experiments with animals. The obtained results would be helpful to gain some information on the elemental function in central nervous system tissue with respect to neurological disorders.

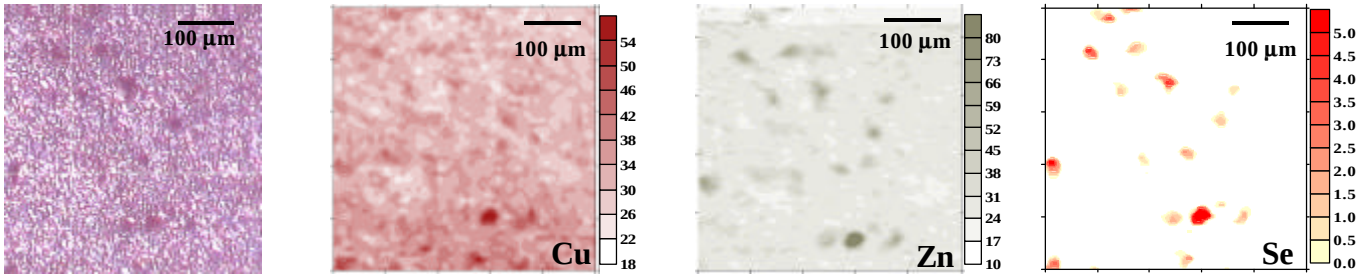

Figure 1. The microscopic view of substantia nigra tissue of the control case in comparison with distribution of selected elements obtained using µ–SXRF technique. The neurons are seen on the optical image as the dark points. The values on the scales represent intensities in relative units.

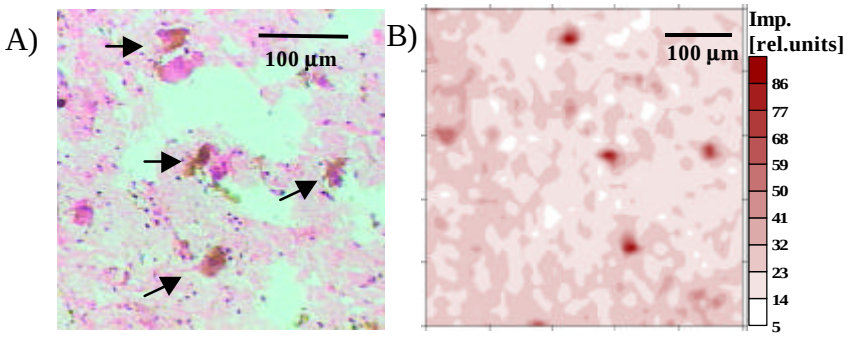

Figure2. Substantia nigra section of PD case. A) the microscopic view of the histopathological slice; B) the map of copper distribution obtained using µ–SRIXE technique. The arrows show neurons localization.

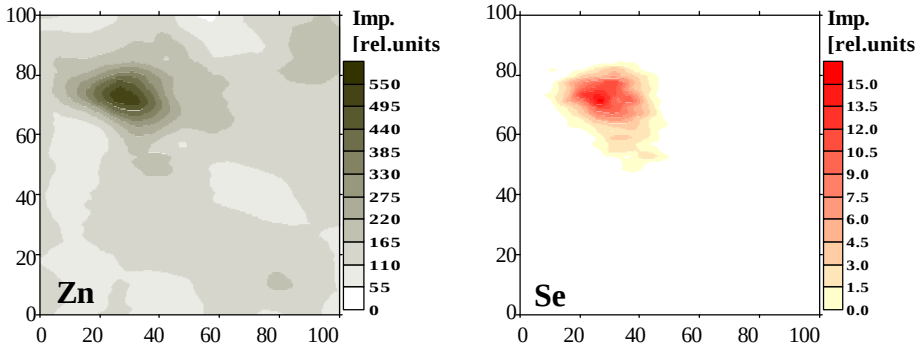

Figure 3. Distribution of Zn and Se inside a single neuron of substantia nigra.