

# APPENDIX II

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## LOCALIZATION OF NANOPARTICLES AND NANO-IMAGING

Following exposure of *Mytilus galloprovincialis* to 5 and 50 nm AgENPs, analyzes were performed for localization of AgNPs in different tissues of the animals.

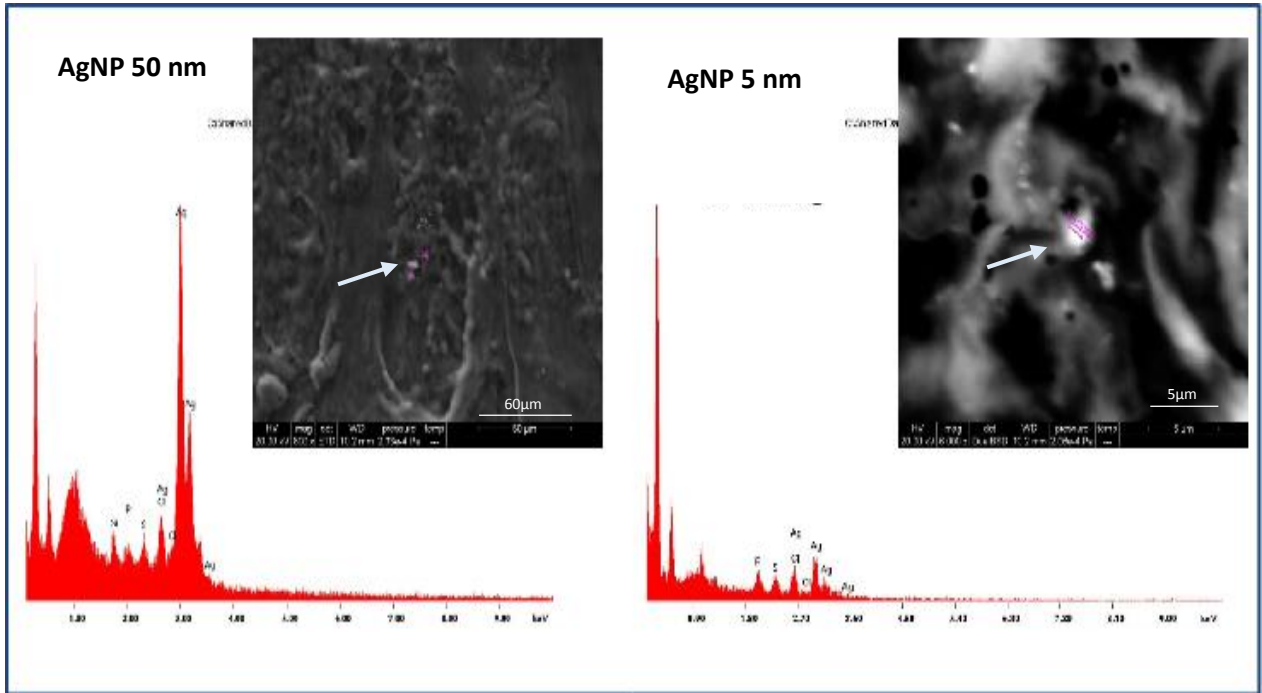
Scanning Electron Microscopy coupled with Energy-Dispersive X-ray spectroscopy elemental analysis (SEM-EDX) was carried out to indentify silver in neutral phormaline buffer fixed gills.

The SEM-EDX is an analytical instrument that uses the emission of X-rays generated by an accelerated electron beam incident on the sample.

The practical applications are mainly addressed to the qualitative characterization of solids and elemental analysis, with the ability to detect even the presence of trace elements. It is also an essential tool for the quantitative characterization of metal alloys and for the determination of the purity of the metals.

The Scanning Electron Microscopy (SEM) enables a morphological analysis of the sample at very high magnification (up to 200000x).

The analysis of fresh gills of *Mytilus* organisms exposed to 1 mg/L of 5nm and 50 nm AgNPs showed the presence of micron sized silver aggregates adhering to the surface of the tissue (Figure 1B). From the elemental analysis it can be also observed that silver is found along with sulfur, phosphorus, chlorine.



***Fig 1B – Silver aggregates on gills of Mytilus exposed to 1 mg/L of AgNPs 5nm and 50nm***