High throughput Laser Ablation ICP-MS bioimaging of silver distribution in animal organisms and plant tissue after exposure to silver sufide nanoparticles

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Supplemental information

METHODS

1. Nanoparticles characterization

For the single-species tests with woodlice, the polyvinylpyrrolidone (PVP) coated sulfidised silver nanoparticles colloids (Ag_2S NPs) used in the study were provided by Applied Nanoparticles (Barcelona, Spain).

Size distribution by Transmission Electron Microscopy (TEM)

Images of particles were acquired using a JEOL1010 transmission electron microscope (JEOL, Japan) working at 80keV (Figure S1A). For sample preparation, formvar-coated and carbon stabilized 200-mesh copper grids (Ted-pella Inc., USA) were dipped in aliquots of Ag_2S stock solution with 1:10 dilution in miliQ water and left to dry for at least 12h. ImageJ software (NIH, USA) was used to calculate particles' mean size and size distribution. Analysis of altogether 1238 NPs was done (Figure S1B).

UV-visible spectroscopy

UV-Visible absorption spectra of the three NPs colloids were performed using an Agilent Cary 60 UV-Vis Spectrophotometer setting spectra measuring limits between 300 and 800nm (Figure S1C). Spectra for the Ag_2S NPs colloids were measured at a 1:5 dilution in miliQ water.

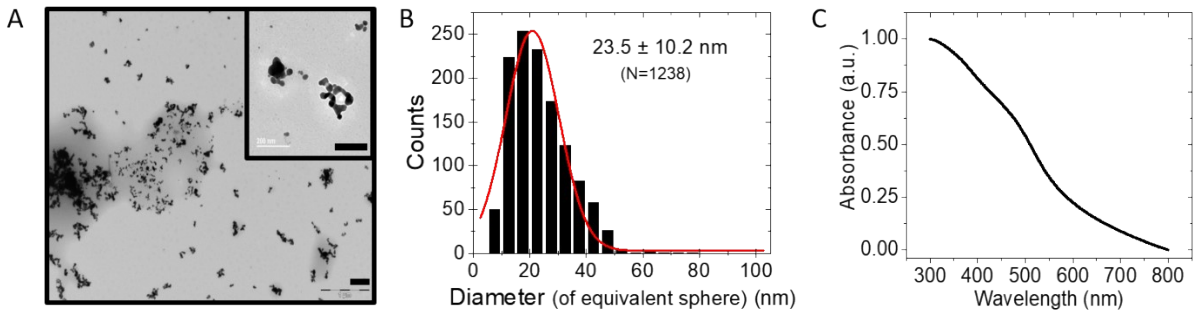

Figure S1: Characterization data from the Ag_2S NPs colloids used in this study. A) shows a low magnification TEM image with a higher magnification top-right inset; scale bar equalling 200 nm; B) shows their corresponding size distribution by analysis of the TEM images (23.5 ± 10.2 nm; 1238 NPs analysed); C) shows their normalized UV-vis spectra in colloidal dispersion in milli-Q water.

Dynamic Light Scattering and Zeta potential measurements

The hydrodynamic diameter and the surface charge of the NMs colloids were measured by Dynamic Light Scattering (DLS) and Zeta Potential (ζ-Potential) on a Malvern Zetasizer Nano

ZS90 which incorporates a Zeta potential analyser (Malvern Instruments Ltd, Worcestershire, UK). In order to be within the technical experimental limits, samples had to be diluted to 1:5 dilution in milliQ water. DLS mean size measured by intensity was 308.1 ± 28.2 nm and by number 81.6 ± 34.2 nm (standard deviation of the mean within 3 replicates was 0.267). ζ-Potential mean value was -32.4 ± 0.6 mV.

2. The main anatomical digestive structures of the organisms used in the study

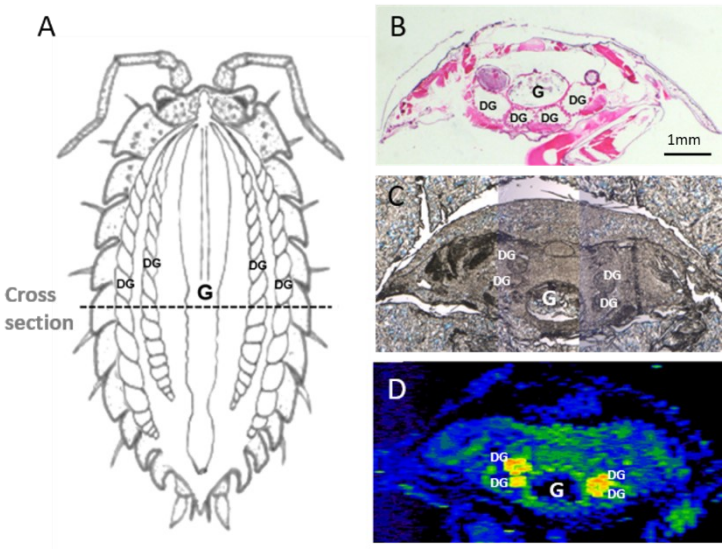

Figure S2: Scheme of woodlice *Porcellio scaber*. (A) Part of the digestive system showing the four blind-ended tubes of the digestive glands (DG), and the gut (G) with one layer of epithelial cells often protected by a cuticle;(B) Transverse section (8 µm) of *P. scaber* showing the internal organs of the body, stained with hematoxylin and eosin;(C) the unstained cross section embedded without stainingas prepared for LA-ICP-MS measurement;(D) the corresponding elemental map of Cu.

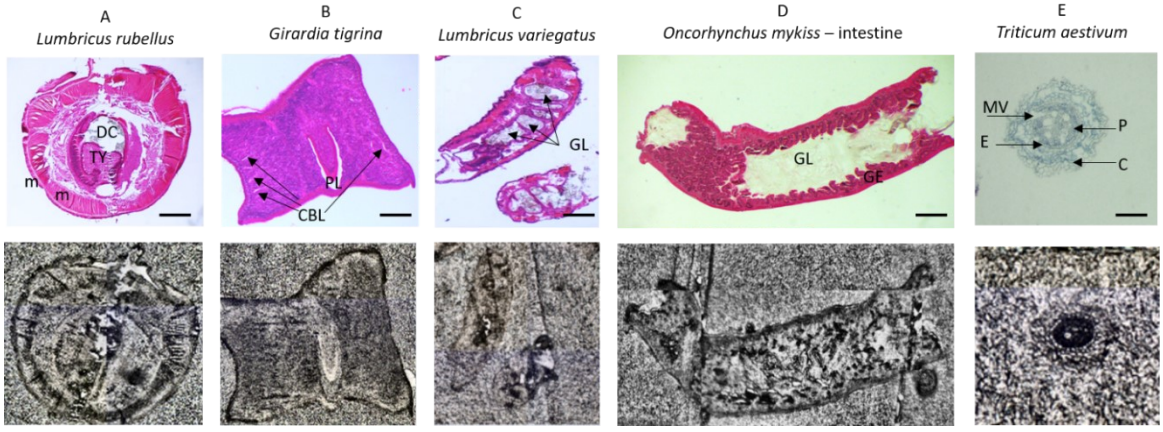

Figure S3: Histological sections (8 µm) of organisms exposed in terrestrial (A, E) and aquatic (B, C, D) mesocosm experiment. Hematoxylin and eosin stained sections are represented in the first row. Major biological structures on micrographs are labelled on earthworm cross section (A): DC - digestive cavity,TY – typhlosole; planarian cross section (B): PL - pharynx lumen, CBL - cavity bowel lumen; blackworm longitudinal section (C): GL - gut lumen; part of rainbow trout intestine longitudinal section (D): GL – gut lumen, GE: gut epithelium; wheat upper root cross section (E): C – cortex, MV - metaxyleme vessel, E – endodermis, P- phloem Under micrographs the images taken before LA-ICP-MS measurements are represented for each organism. The black scale bar line on images of the tissue of all organisms represented in the first row represents 1000 µm.

3. LA-ICP-MS analyses

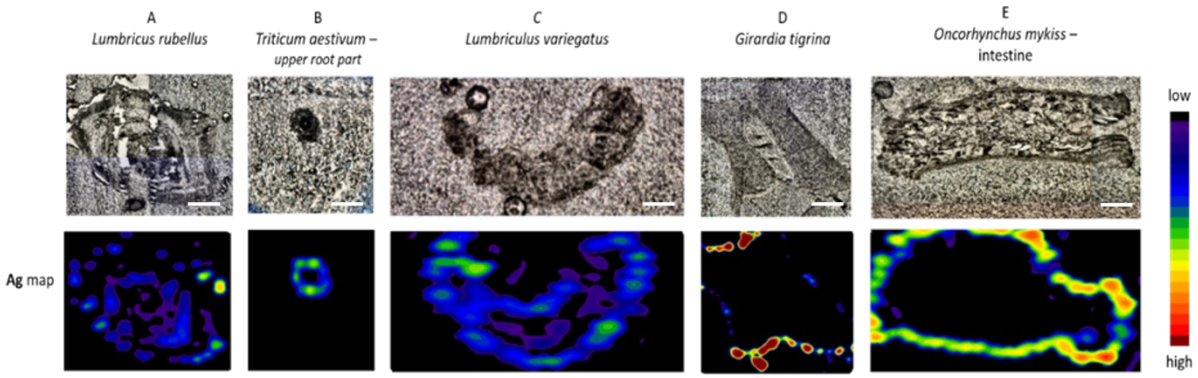

Figure S4: Elemental maps of Ag measured in control samples of terrestrial (A, B) and aquatic (C-E) organisms used in the study, which were identified as background values. The colour bar represents low to high element counts per µm of measured spot. The white scale bar line on images in the first row represents 1000 µm.

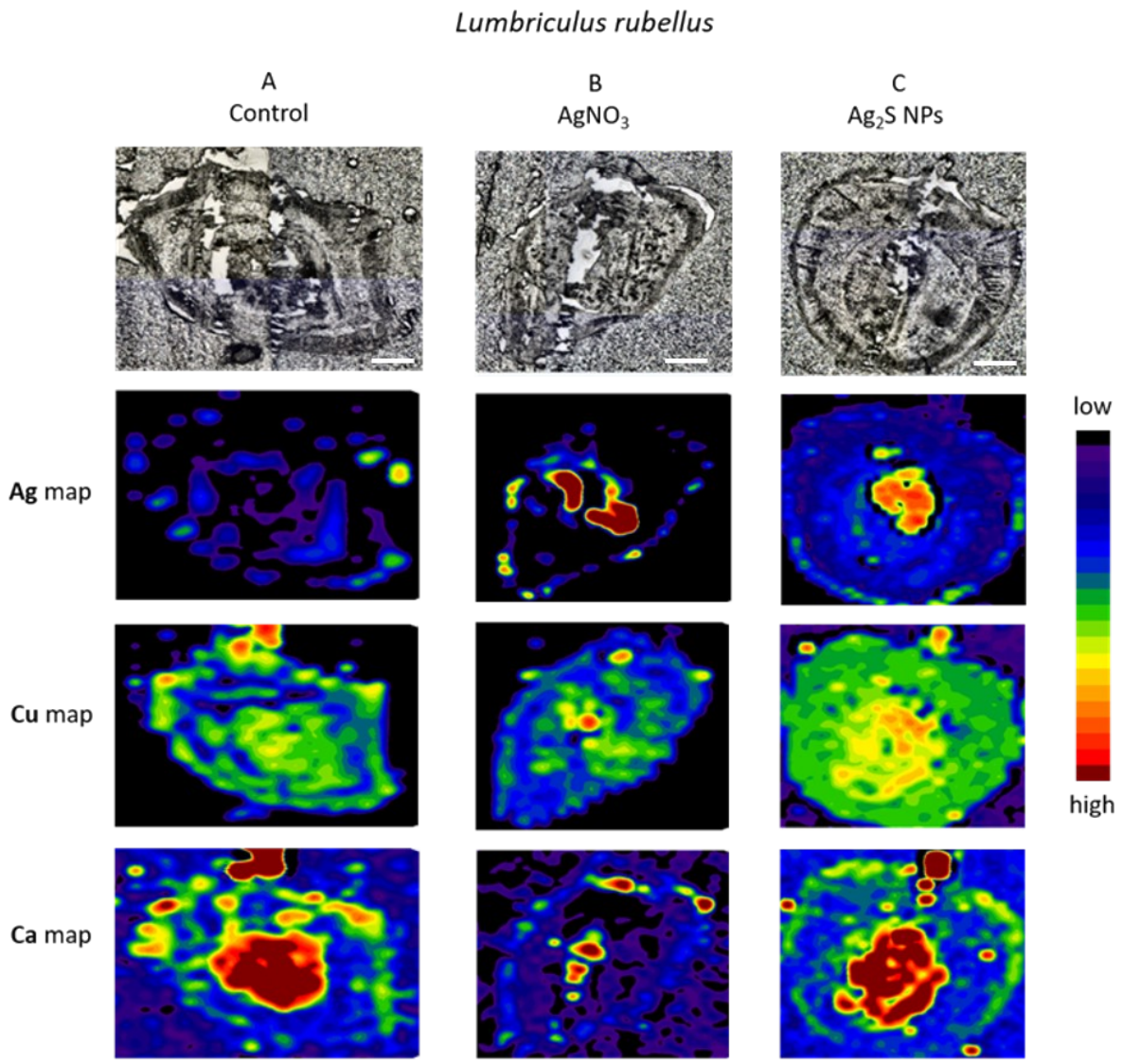

Figure S5: Silver (Ag), copper (Cu), and calcium (Ca) elemental maps of cross section of earthworm *Lumbricus rubellus* as unexposed controls (column A) compared to earthworms exposed to $AgNO_3$ (column B) or Ag_2S NPs (column C), with a final concentration of 10 mg Ag/kg soil. The Cu and Ca maps are used as a reference to identify known biological structures. The colour bar represents low to high element counts per µm of measured spot. The white scale bar line on images in the first row represents 1000 µm.