Experimental Report – proposal # 20171561

The experiment has been performed on BM30B In February 2018 – 15th-20th

The proposed study aimed at understanding how Ag speciation affect a soil ecosystem, in particular E.Fetida and microorganisms. Ag speciation in two different experiments has been assessed

Experiment 1 : Eisenia fetida earthworms were exposed to a range of concentrations of Ag (AgNPs or AgNO3) in an artificial OECD soil. The main aims were to identify the location and form of accumulation of Ag in the exposed earthworms

The speciation of Ag at the different stages of the experiment has therefore been measured: (i) in the soils and (ii) in the earthworms tissues after 4 weeks exposure.

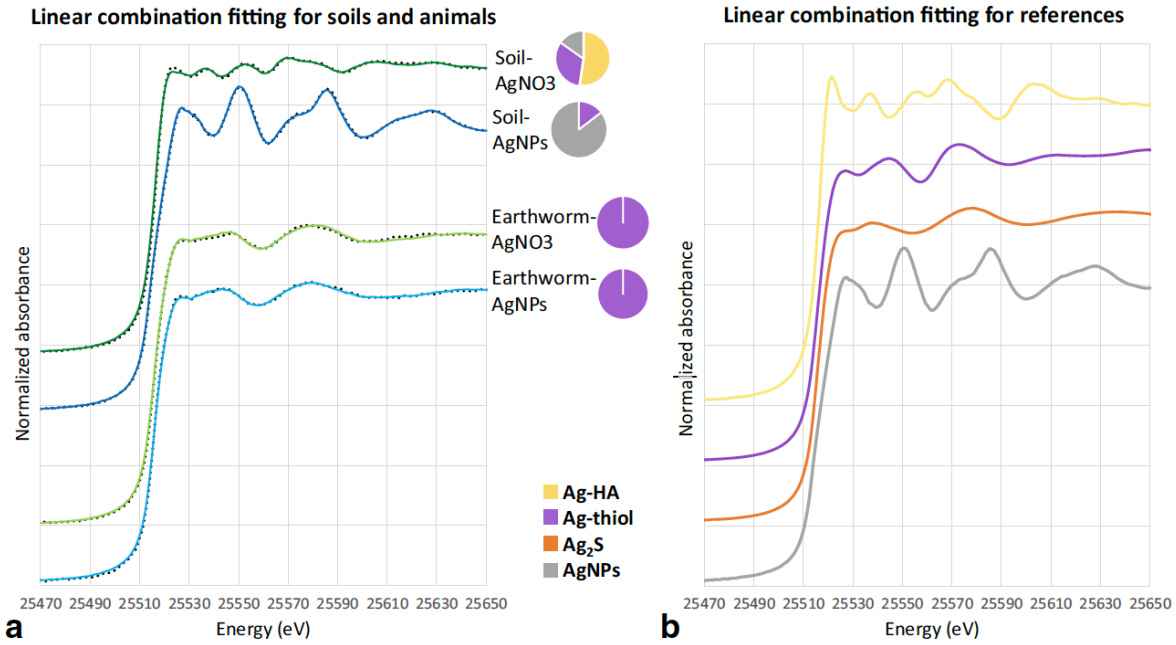

Figure 1. Linear combination fitting (LCF) of the XANES spectra of the samples collected at different time points (dotted lines) and experimental spectra (solid lines) of soil and earthworms after 4 weeks of exposure to AgNPs and AgNO3. The curves for the samples are colored as follows: dark green, soil spiked with AgNO3; dark blue, soil spiked with AgNPs; light green, earthworms exposed to AgNO3; light blue, earthworms exposed to AgNPs. b XANES spectra of the model compounds used for LCF. Ag-HA (in yellow) was used as a proxy for Ag complexed to natural organic matter (humic acids). AgNPs (in grey) corresponds to the initial NM300K AgNPs used for the experiment. Ag-thiol (in purple) was used as a proxy for Ag bound to an organic thiol. Ag2S (in orange) corresponds to silver sulfide (acanthite mineral)

Ag speciation in OECD soil after 4 weeks of incubation depended on the initial form (NPs or ionic). Ag initially spiked as AgNPs remained mainly metallic, but approximately 15% became complexed with natural organic thiols (Fig. 5). Ag initially spiked as AgNO3 was linked with humic acid (52%) and organic thiols (33%), and approximately 15% was in

metallic form. Regardless of the exposure scenario (AgNPs or AgNO3), the speciation of Ag accumulated in earthworms was similar and consisted of Ag bound to thiols (Fig. 5).

Experiment 2. An agricultural soil with Eisenia fetida earthworms was exposed to four different treatments either via a direct or indirect exposure via sewage sludge (i) soil spiked with AgNPs (with or without slugde) (ii) soil spiked with $AgNO_3$ (with or without sludge) (iii) control soil spiked with the dispersant used to solubilize AgNPs (with or without sludge) (iv) control soil without any additive (with or without sludge).

The speciation of Ag at the different stages of the experiment has therefore been measured: (i) in the sludge after anaerobic digestion; (ii) in the soils and (iii) in the earthworms tissues after 5 weeks exposure.

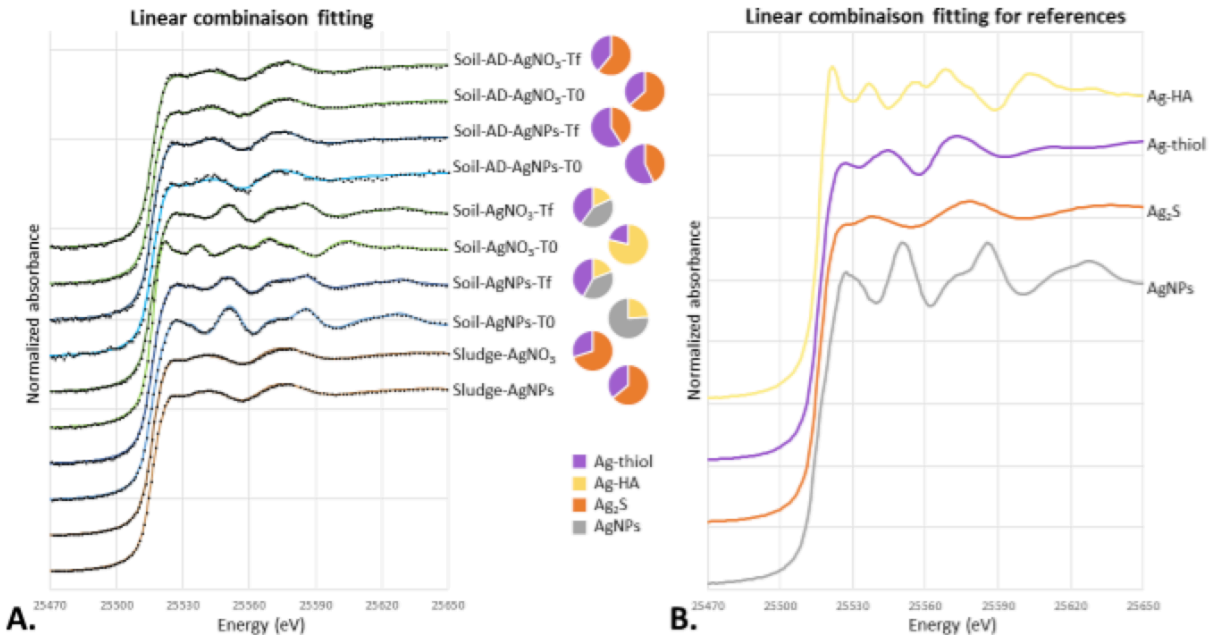

Figure 2: A. Speciation of silver in mixtures: linear combination fitting of μ-XANES spectra obtained taken different points of samples. Samples are in green, blue and brown. B. Speciation of Ag references. Ag-HA (in yellow) corresponds to Ag complexed with humic acids. AgNPs (in grey) corresponds to the linear combination fitting obtained with a sample of NM300K AgNPs used for the experiment. Ag-thiol (in purple) corresponds to Ag linked to an organic thiol. Ag_2S (in orange) corresponds to silver sulfide.

AgNPs and $AgNO_3$ in soil

Initially, the speciation of Ag (around 2h after spiking), varied depending on its initial form. The Ag initially from AgNPs was mainly under its pristine form (76% metallic Ag) with a fraction of Ag bound to organic matter (24%). This latter fraction is probably a fraction of soluble silver that has quickly dissolved and has been complexed by the organic matter present in the soil. The Ag initially from $AgNO_3$ was quickly complexed with organic matter with a fraction of thiol groups (21% Ag-GSH). More importantly, after 5 weeks of experiment, the speciation no longer depends on the initial Ag speciation. In both conditions AgNPs and $AgNO_3$, about 40% is under the metallic form, 40% is complexed to an organic thiol and 20% is characteristic of Ag bound to humic acids.

AD-AgNPs and AD-$AgNO_3$ (soil + sludge)
When spiked in sludge prior to the soil, Ag was transformed into Ag_2S (30-33%) and Ag-thiocarbamate complexes (67-70%) in the sludge whatever the initial state of the silver (ionic or NPs). These two species remained the main ones after spiking the sludge into the soil but the proportion of them changed, from a few tens of minutes after mixing. The quantity of Ag-thiocarbamate complexes decreased a little for $AgNO_3$ and more for AgNPs. The proportion of Ag_2S increased accordingly. The speciation of Ag did not changed after 5 weeks of incubation.

Speciation of Ag bioaccumulated in earthworms
Speciation of silver bioaccumulated in earthworms is similar regardless the initial form of Ag when exposed to AgNPs or $AgNO_3$. The XANES spectra are 93% identical to the Ag-GSH model compound which is illustrative of Ag atoms bound to a thiol group (fig. 7). The remaining 7% correspond to Ag-humic acids. The content of bioaccumulated Ag in earthworms exposed to AD-AgNPs and AD-$AgNO_3$ microcosms was too weak to be analysed by X-ray Absorption Spectroscopy.

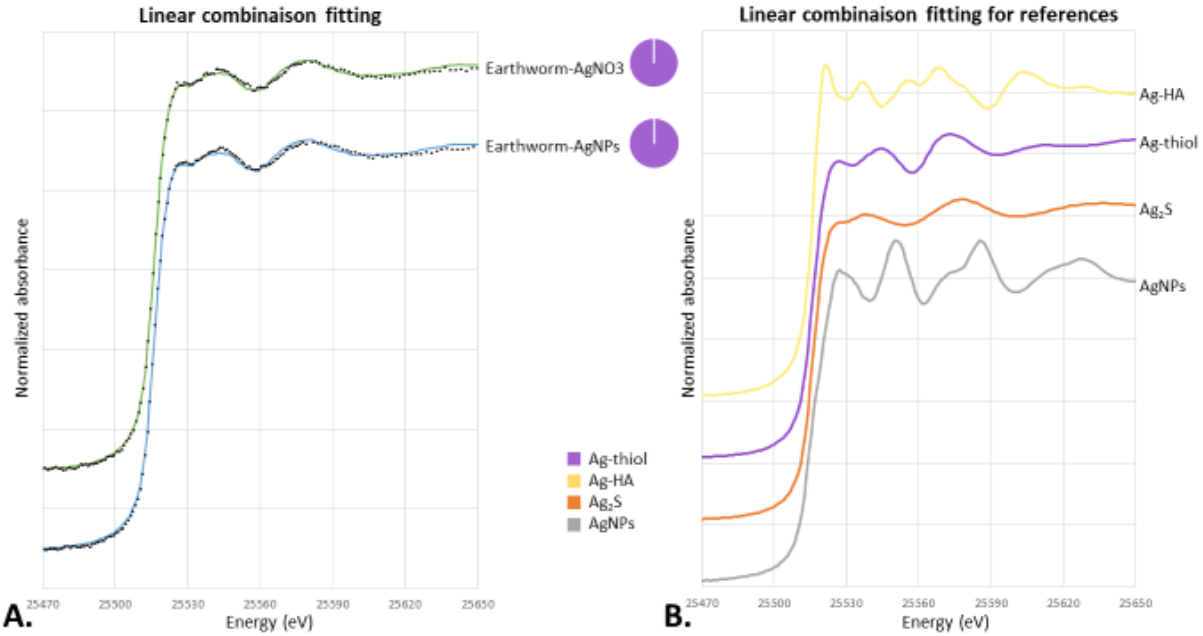

Figure 3: A. Speciation of silver in earthworms: linear combination fitting of µ-XANES spectra obtained taken different points of samples. Samples are in green and blue. B. Speciation of Ag references. Ag-HA (in yellow) corresponds to Ag complexed with humic acids. AgNPs (in grey) corresponds to the linear combination fitting obtained with a sample of NM300K AgNPs used for the experiment. Ag-thiol (in purple) corresponds to Ag linked to an organic thiol. Ag_2S (in orange) corresponds to silver sulfide.

Experiment 1 has led to a publication in ESPR:
Courtois, P.; Rorat, A.; Lemiere, S.; Levard, C.; Chaurand, P.; Grobelak, A.; Lors, C.; Vandenbulcke, F., Accumulation, speciation and localization of silver nanoparticles in the earthworm Eisenia fetida. Environmental Science and Pollution Research 2020. doi: 10.1007/s11356-020-08548-z

Experiment 2 will lead to a second publication.