ID21 Beam Time - exp EC 619 *Local contact : Barbara Fayard*

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Experiment report

Introduction

Silver nanoparticles (AgNPs) are frequently used as antimicrobials. While the mechanism(s) by which AgNPs are toxic are unclear, their increasing use raises the concern that release into the environment could lead to environmental toxicity. We assessed the physicochemical behavior, uptake, toxicity (growth inhibition, embryo mortality), and mechanism of toxicity of AgNPs coated with polyvinylpyrrolidone (PVP), gum arabic or citrate coatings to different organisms: the nematode *Caenorhabditis elegans*, the embryo of *Fundulus heteroclitus* (killifish), and the roots of *Lolium multiflorum*. The aim of this experiment was to correlate the biological responses to the physico-chemical modifications of the AgNPs and their localization within the organisms.

Experimental details

The general frame of the experiment was as follow. The analysis were performed at ambient temperature using a Si(111) monochromator. Entire organisms and cross sections of 55 µm of thickness (cut using cryo-microtome) were analyzed. The reference compounds (AgNPs before any interaction with organisms, Ag_2S and Ag_2O standards) were diluted in cellulose and pressed into thin pellets.

Three sets of samples (prepared at Duke university, USA) were analyzed during this experiment. First, AgNPs were added at a concentration of 40ppm to *L. multiflorum*. Their roots were found to concentrate gum arabic-coated AgNPs, with ~400 ppm silver per unit dry mass. We analyzed the outside of the roots (near the apex area) and the inside at different depth (50 µm, 300 µm, 800 µm, and 4 mm from the apex). Both micro-X-ray maps and micro-XANES at the Ag L_3-edge were performed on these samples.

The second set of samples were fish embryo incubated with citrate-, and gum arabic-coated AgNPs in water (0‰ of salinity) or in saline water (10‰ of instant ocean® salts). The cross sections were analyzed. Micro-X-ray maps were performed on the chorion (outermost membrane around the embryo), and within the embryo (eyes, brain, hart…). Micro-XANES at the Ag L_3-edge were performed on concentrated spots of silver.

The third set of samples corresponds to the nematode *C. elegans*. Two generations of worms incubated with PVP- and citrate-coated AgNPs were analyzed: (i) the adults about 1mm of length (1^{st} generation) and their larvae 200 µm of length (2^{nd} generation). Only micro-X-ray maps were performed to localize silver on these challenging samples. Such potential trans-generational transfers were never studied in the literature.

Main results

Surprisingly, no silver was detected within the 1^{st} generation of the nematodes *C.elegans*. However, in these conditions, it is known from biological assays that the citrate-coated and the PVP-coated nanoparticles induce a strong decrease of growth of the nematodes (*Meyer et al.*

aquatic toxicology 2010). This conclusion is still very qualitative and deserves quantitative verification, but a strong internalization of silver by the nematodes may not be the main cause of the toxicity observed. However, it was less surprising to not detect silver in the 2nd generation of the nematodes. If such trans-generational transfers exist, the concentration of silver within the 2nd generation should be very low.

On the contrary, silver was detected in the eggs of the killifish. For both citrate- and gum arabic-coated AgNPs in pure water and salty media, silver was observed in the chorion (see figure 1 for the citrate-coated AgNPs, left). The XANES spectra of the gum arabic- and the citrate-coated nanoparticles are similar before incubation and evolved similarly after contact with the eggs. In the case of the citrate-coated AgNPs in pure water, silver passed through the membrane and was detected close to the brain (figure 1, right). No difference in the XANES spectra are observed within the chorion or inside the embryo, but this affinity for the surface and this internalization can be responsible for the embryotoxicity observed (Matson et al. 2010).

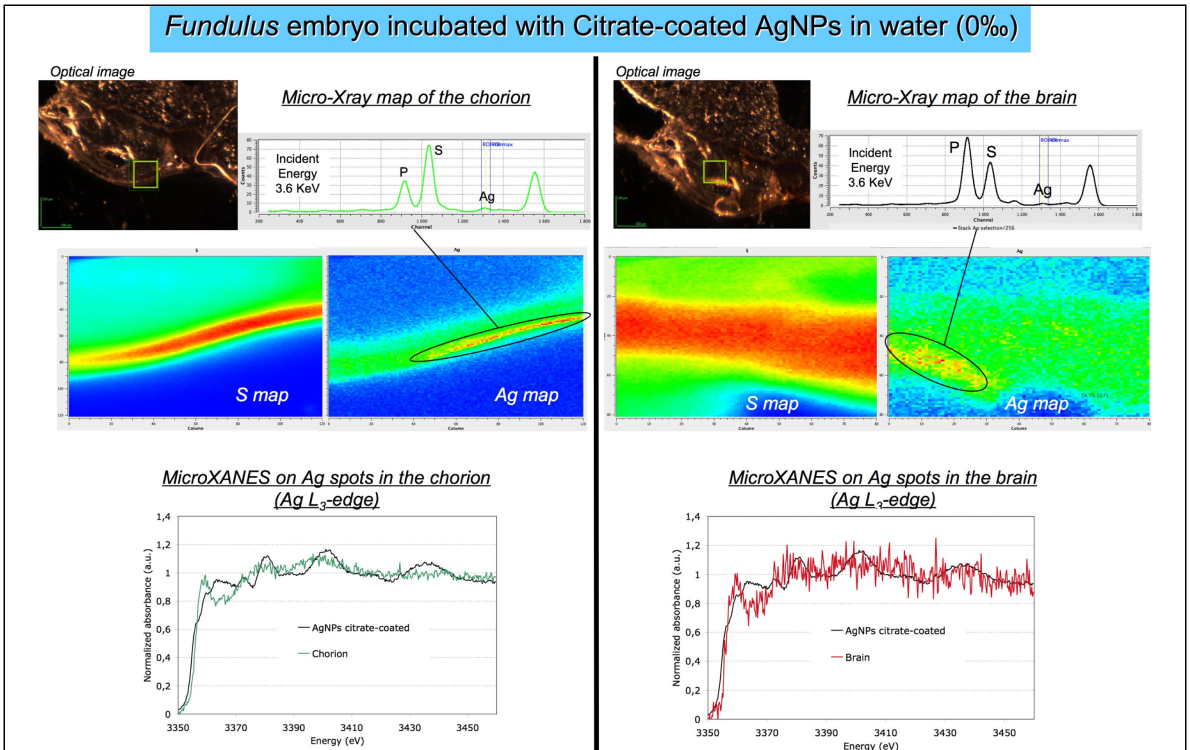

Figure 1: Fish embryo incubated with citrate-coated AgNPs in water. Localization and speciation of silver (left) in the chorion and (right) within the embryo (brain).

Regarding the roots of *L. multiforum*, silver was detected at the surface, but also inside the roots (figure 2) at least from the tip to 4 mm of the apex. The shape of the XANES spectra obtained in the roots are in all cases different from the gum arabic-coated AgNPs before incubation with *L. multiforum*.

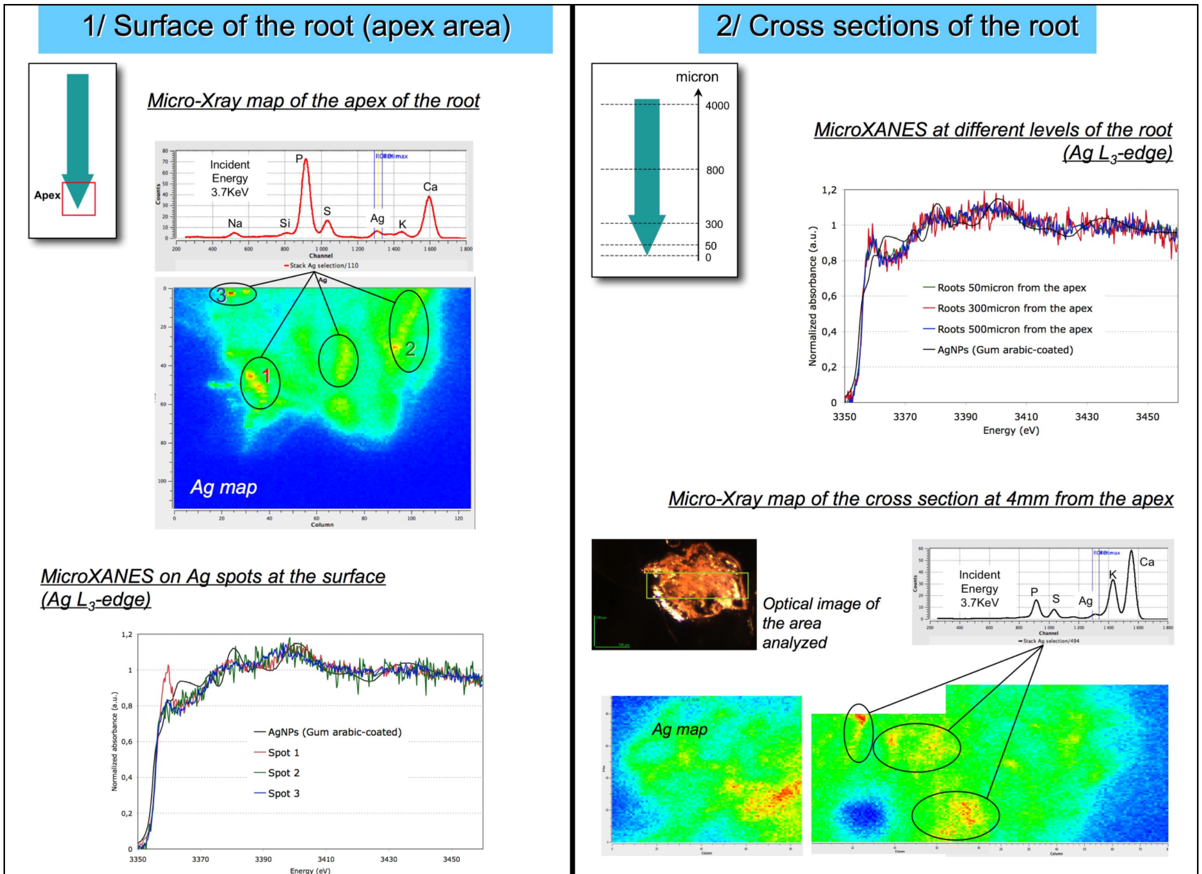

Figure 2: Incubation of gum arabic-coated AgNPs with *L. multiforum.* Localization and speciation of silver (left) at the surface of the apex area of the roots, and (right) within the roots (cross sections).

Experiment performed on the entire roots does not allow us to differentiate silver inside and outside the roots, but two different speciations were observed by XANES (Figure 2, left: XANES spectra of spot 1 is different from spots 2 and 3). One is similar to the XANES spectra obtained within the cross section (Figure 2, right). The exact speciation of silver has still to be determined.

Reference

Meyer, J., C. A. Lord, X. Y. Yang, E. A. Turner, A. R. Badireddy, S. M. Marinakos, A. Chilkoti, M. Wiesner and M. Auffan (2010). "Intracellular uptake and associated toxicity of silver nanoparticles in Caenorhabditis elegans." aquatic toxicology **in press**.

Matson, C. W., (conferencier), M. Auffan , M. R. Wiesner and R. T. Di Giulio (2009 november). "Silver nanoparticle behavior and fish embryotoxicity across a salinity gradient." SETAC **New Orleans, USA**.