



	<b>Experiment title:</b> Speciation of engineered Ag-NPs aged in realistic environmental systems	<b>Experiment number:</b> 30-02 1071
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## Report:

### Introduction

Because of their antimicrobial and antifungal properties, silver nanoparticles (Ag-NPs) have numerous uses and are likely to enter in aquatic environments, potentially causing adverse effects not only on humans but also on aquatic ecosystems. Indeed, the oxidative dissolution of Ag-NPs in oxic waters produces Ag ions leading to cell death. Other possible mechanisms of toxicity are oxidative stress generated by the formation of reactive oxygen species (ROS) at the surface of the Ag-NPs and “particle specific” effects from Ag-NPs. Unfortunately, most of the toxicity studies published in the literature do not mimic the complexity of natural environments. Because of the ability of Ag-NPs to transform in aqueous environments (changes in aggregation state, oxidation state, precipitation of secondary phases, sorption of (in)organic species), it is important to assess the toxicity of the Ag-NPs in environmentally relevant conditions. As a consequence, characterization of Ag-NPs biotransformation under realistic environmental conditions is essential to understand and predict their fate and impact in natural aquatic systems.

In this context we assessed the environmental risk of Ag-NPs in environmentally relevant conditions at different levels of complexity. To mimic realistic aquatic environments, indoor aquatic microcosms, with artificial sediment, were used (0.8x0.2x0.6 m, ICEINT, France). A higher degree of complexity was achieved with outdoor mesocosms simulating a large-scale freshwater emergent wetland (3.66x1.22x0.8 m, CEINT, USA). The distribution, transformation, and bioaccumulation of Ag-NPs were studied in the microcosms and mesocosms during 1- and 12-month experiments, respectively. For all experiments we worked with environmentally relevant concentrations of Ag-NPs (<1 mg/L).

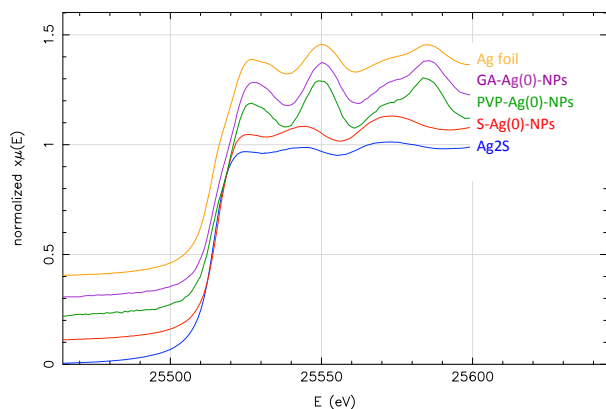
### Experimental details

By combining different analytical approaches (e.g.  $\mu$ XRF, XRD, ICP-MS, DLS) we identified the preferential zones of NPs accumulation in sediments and organisms from the microcosms and the mesocosms. This XAS experiment at the Ag K-edge consisted in determining Ag atomic environments in the different compartments following a single injection or several chronic injections of AgNPs. We evaluated the transformation of different Ag-NPs: surface modified AgNPs with PVP (PVP-AgNPs), or gum arabic (GA-AgNPs), and an altered AgNPs that has been reacted with sulfide prior to exposure (altered-AgNPs).

Ag K-edge XANES measurements on samples and reference compounds were performed at 5 to 10 K (helium cryostat), to avoid any possible dehydration or oxidation of samples. They were carried out on

beamline BM30B/FAME with a Si(220) crystal monochromator operating at 6 GeV and 90 mA. The XAS spectra were all recorded in fluorescence mode, using a thirty-element solid-state germanium detector (Canberra, CT, USA). Surficial sediments and organisms (gammars, freshwater molluscs) were freeze-dried before XAS analysis. Surficial sediments were size fractionated and the most Ag concentrated fractions (<20  $\mu\text{m}$ ) were analyzed.

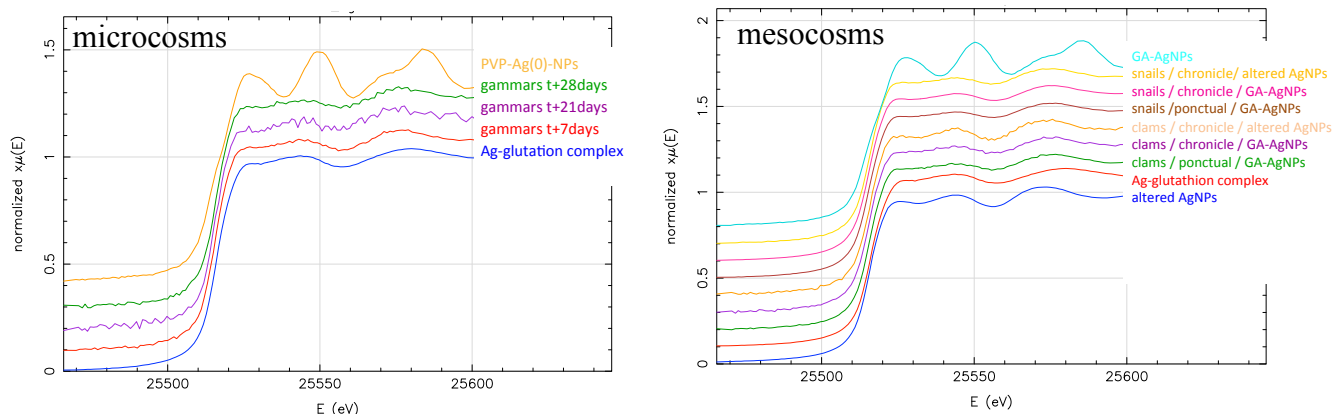
## Results



**Figure 1.** Ag K-edge XANES spectra of Ag-NPs introduced in microcosms and mesocosms and the reference compounds used for comparison.

Ag K-edge XANES analyses on organisms exposed to NPs were performed to determine the transformation in natural aquatic environments. Both PVP-AgNPs and GA-AgNPs are initially composed by Ag(0). This is confirmed by their XANES spectra characterized by the 3 absorption peaks at 25527 eV, 25550 eV and 25585 eV, which match with the spectrum of the silver metallic foil used as a reference (Fig. 1). The spectra for the altered-AgNPs (S-Ag(0)-NPs on Figure 1) was found to be very similar to the Ag<sub>2</sub>S reference compounds. LCF of XANES spectrum indicated that the totality of Ag(0) was sulfidized during the alteration of this AgNPs. Indeed, raw and altered AgNPs presented different Ag speciation before exposition to the organisms in microcosms or mesocosms.

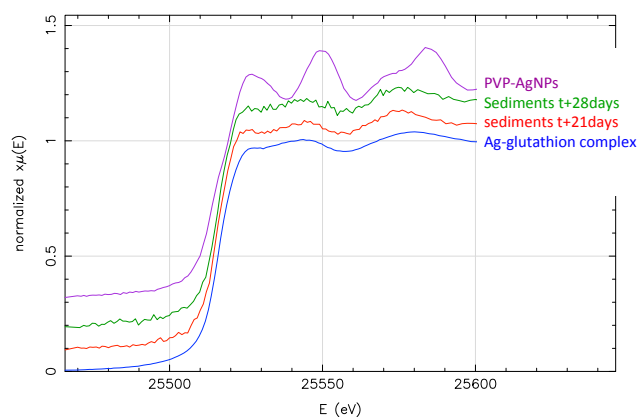
After uptake of Ag-NPs by organisms Ag was found bound to S whatever the kind of NPs introduced (GA-AgNPs vs PVP-AgNPs vs altered-AgNPs) or conditions of exposition (microcosms vs mesocosms and punctual vs chronic) (Fig. 2). The LCF indicated that 100±10% of Ag taken up by organisms was sulfidized and this transformation occurred less than 1 week after exposure (Table 1). These results also revealed that altered Ag-NPs did not transform during exposure and that Ag<sub>2</sub>S would be the final state of AgNPs in natural aquatic environments.



**Figure 2.** Ag K-edge XANES spectra after uptake by organisms exposed to NPs compared to initial AgNPs and organic Ag-S reference spectra. A/. Ag uptake by gammaris exposed to PVP-AgNPs in microcosms. B/. Ag uptake by snails and clams exposed to GA-AgNPs or altered-AgNPs in mesocosms.

XAS analysis performed on the fine fractions of surficial sediments in microcosms revealed XANES spectra with shape and intensities of white line similar to Ag-S signature. LCF of XANES spectra indicated that the Ag was mainly associated to S atoms ( $\geq 87 \pm 10\%$ , Table 1). Ag speciation was similar for Ag present in surficial sediments and Ag taken up by organisms.

XANES analyses were performed on all samples initially envisaged. EXAFS spectra were also recorded to 10  $\text{\AA}^{-1}$  and treatments are in progress. Those results, combined with oxidative stress data will allow us to evaluate the fate and impacts of AgNPs within aquatic ecosystems.



**Figure 3.** Ag K-edge XANES spectra in fine fraction of the surficial sediments of microcosms, compared to initial AgNPs and organic Ag-S reference spectra.

**Table 1.** Speciation of Ag taken up by organisms exposed to AgNPs (gammar, snails and clams) determined from Ag K-edge XANES experimental spectra and their linear combination fitting (LCF) with XANES spectra of reference materials ( $AgNO_3$ ,  $Ag_2S$ , Ag-glutathion complex, introduced NPs).

PVP-AgNPs	Organisms			Sediments	
	Gammar			<20 $\mu m$	
	Chronicle			Chronicle	
	7 days	21 days	28 days	21 days	28 days
Ag-O	13%	8%		8%	13%
Ag-S	87%	92%	100%	92%	87%
R factor	0.00042	0.00013	0.00014	0.00024	0.00025
GA-AgNPs	Snails		Clams		
	125 days		125 days		
	Chronicle	Ponctual	Chronicle	Ponctual	
	Ag-O	6%	6%	6%	
Ag-S	94%	94%	94%	91%	
R factor	0.00006	0.00007	0.00012	0.00011	
altered-AgNPs	Snails		Clams		
	125 days		125 days		
	Chronicle	Ponctual	Chronicle	Ponctual	
	Ag-O		-	6%	
Ag-S	100%		94%		
R factor	0.00010	-	0,00006	-	