ESRF	**Experiment title:** **Foliar uptake of aged Ag nanoparticles**	**Experiment number**: EC1053
Beamline: ID21	**Date of experiment**: from: 26 Sept 2012 to: 01 Oct 2012	**Date of report**: 21/01/2013
Shifts: 15	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
Camille Larue, Géraldine Sarret, Stijn Smulders

Report:

On this experiment we focussed on the impact of Ag nanoparticles (NPs) on plants and especially the uptake of pristine Ag NPs or Ag NPs coming from aged paints after foliar exposure of lettuce. This experiment was the continuation of experiment EC886 with a special emphasis to (i) confirm the role of stomata for the transfer of NPs into leaves, (ii) identify Ag secondary species in the leaves, (iii) determine wether Ag NPs embedded in a paint matrix could be internalized in lettuce leaves.

The experiment was performed on lettuce (*Lactuca sativa*, cultivar laitue romaine) at the 5-leaf stage. Plants were exposed to (1) pristine Ag NPs, (2) ionic Ag and (3) leachates from aged paints. Leachates were obtained according to the ISO 12457-3:2002 protocol. Briefly, aged paint particles were in contact with ultrapure water (ratio S:L 1:10) for 24h, then the supernatant was filtered at 0.45 µm. Foliar exposure was performed by adding 1 µL per leaf mm² of a suspension of pristine NPs at 1000 ppm or of a solution of 5 ppm $AgNO_3$ or of paint leachate. For paint leachate, application of droplets was performed every day during the whole timecourse of exposure. A root exposure experiment was also performed. Lettuces grown in hydroponics were exposed to 1000 ppm pristine Ag NPs dispersed in a nutrient solution or to paint leachate.

At the end of the 7-day exposure, leaves were thoroughly rinsed with deionised water, embbedded in OCT resin and immediately cut in thin sections (20 µm) on a cryomicrotome. Sections were then placed between two ultralene films and inserted in the cryo sample holder for analysis. Mapping was performed in cryo conditions using a a vibration-free cryo-stage, passively cooled by a liquid nitrogen (LN2) dewar in fluorescence mode using a Silicon Drift Detector. The beam size was 0.3 x 0.7 µm. µXRF maps were recorded at 3.45 keV. µXANES spectra were acquired between 3.33 and 3.45 keV on Ag-rich regions. Ag model compound spectra were recorded to complement our database. µXRF data were processed using PyMCA software to extract elemental maps, and µXANES spectra were analyzed by linear combination fits of standard spectra using Athena software.

Results

Our results demonstate that stomata is one way for Ag internalisation (Figure 1A, B) since some sub-stomatal chambers evidenced an overaccumulation of Ag (Figure 1C). However, NPs probably enter by other pathways since agglomerates of NPs were observed in leaf tissue distant from the stomata.

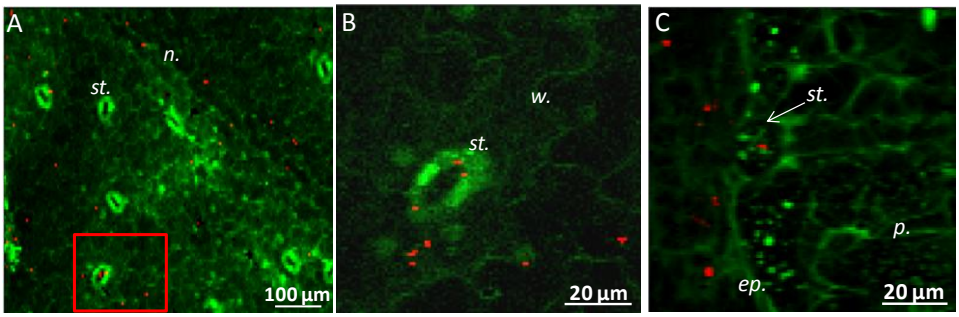

Figure 1. Lettuce foliar exposure to pristine Ag NPs, µXRF distribution maps of P in green and Ag in red. A. Map of leaf surface (3 µm x 3µm). B. Zoom of the red inset in A (1 µm x 1 µm). C. Map of leaf cross section containing a stomata (1 µm x 1 µm). (*st. stomata, n. nerve, w. cell wall, ep. epidermis, p. parenchyma*).

Ag NPs present either on the leaf surface or internalised in the parenchyma underwent partial or total dissolution phenomena. µXANES spectra prove the occurence of Ag^+ species, often bound to glutathione (GSH). In some cases Ag NPs were totally converted into Ag-GSH (Figure 2). There was no particular relationships between the localization of Ag in the leaf tissue and its speciation.

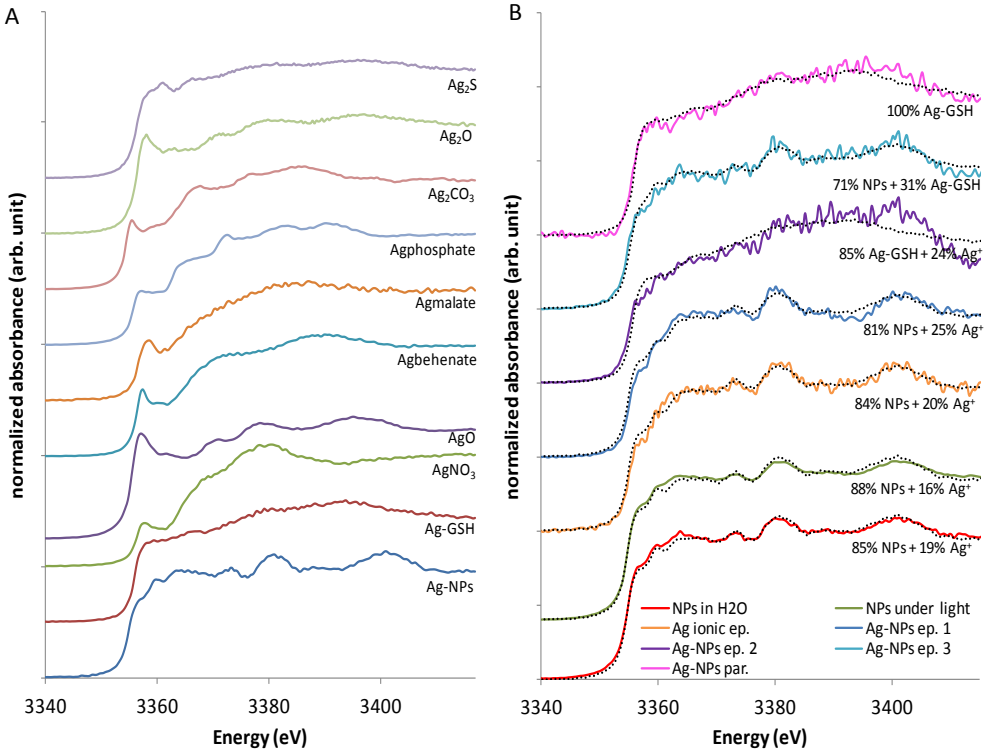

Figure 2. A. µXANES spectra of Ag reference compounds. B. µXANES spectra and linear combination fits (dotted lines) of aged Ag NPs (in H_2O and under light) and Ag-rich spots in leaf tissue exposed to pristine Ag NPs and to ionic Ag. Ag^+ stands for free Ag^+ or Ag^+ bound to various compounds. *(ep. epidermis, p. parenchyma)*

ICP-MS analysis demonstrates that paint leachate contained 25 µg Ag/L. In our exposure conditions, Ag was not detectable on/in lettuce leaves.

This beamtime was also used to investigate the potential uptake of Ag NPs after root exposure in hydroponic conditions. After exposure to 1000 ppm pristine NPs, Ag was detectable on root epidermis but also inside root, up to xylem vessels (Figure 3). Ag was subsequently detected in stem vascular bundles suggesting that these NPs can be transferred to leaves together with the sap (results not shown). Ag was not detectable in roots after exposure to paint leachate.

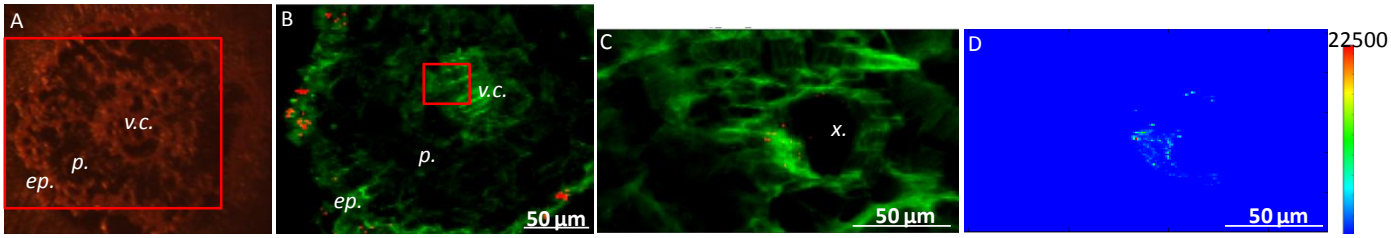

Figure 3. Root exposure, root cross sections. A. Optical microscope picture of the analyzed area (red square). B. µXRF distribution map of P in green and Ag in red. C. Map of the xylem vessels (red inset in B, green:P, red: Ag). D. µXRF distribution map of Ag in temperature color (*ep. epidermis, p. Parenchyma, v.c. vascular cylinder, x. xylem)*

Scientific production related to this experiment

Results obtained during this beamtime were exposed during oral presentations at the Goldschmidt conference (June 24-29, 2012. Montreal, Canada) and at the 5th International IMBG meeting (Institut des Métaux en Biologie de Grenoble) on metal homeostasis (September 17-21, 2012. Autrans, France). They were also presented in a poster at the NanoSafe conference (November 13-15, 2012. Grenoble, France).

Two articles are in preparation.