ESRF	**Experiment title:** **Foliar uptake of nano-sized Ag and TiO_2**	**Experiment number**: Ec886
Beamline: ID21	**Date of experiment**: from: 9 Nov 2011 to: 15 Nov 2011	**Date of report**:
Shifts: 15	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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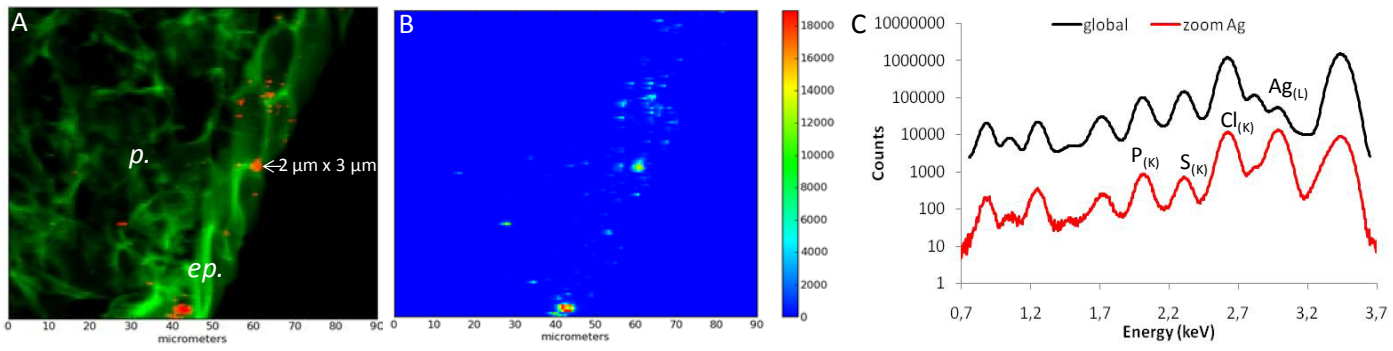
Report:

The aim of this experiment was to clarify the mecanisms of foliar uptake of both Ag and TiO_2 nanoparticles (NPs) in lettuces. To elucidate this mecanisms Ag and Ti distribution were mapped by µXRF and their speciation was determined *in situ* thanks to µXANES. In addition, the impact of NP exposure on the composition/distribution of organic molecules was investigated by µFTIR.

The experiment was performed on lettuces (*Lactuca sativa*, cultivar laitue romaine) at the 5-leaf stage. A droplet of NP suspension (1000 ppm) was deposited on 3 leaves per lettuce once a day during 7 days. At the end of the 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. µXRF data were recorded at 3.45 keV for Ag and 5.1 keV for Ti. µXANES spectra were acquired between 3.33 and 3.45 keV for Ag and from 4.85 to 5.27 keV for Ti. Finally, data were processed using PyMCA software to extract maps from µXRF data and Athena software was used to analyse µXANES spectra.

Internalization inside leaf tissue of NPs was observed for both Ti and Ag (Figure 1). Large agglomerates (few µm) were observed under stomata (Figure 1D) and smaller ones were found throughout leaf parenchyma without preferential localization. A gradient of concentration from the exposed upper epidermis to the lower non-exposed epidermis was observed. These results suggest that stomata act as an entry point for Ti and Ag.



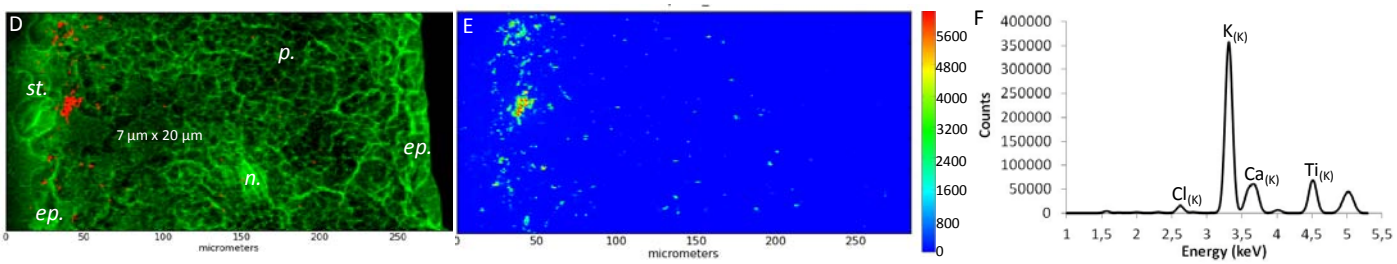


Figure 1: µXRF analysis of lettuce leaves after foliar exposure to Ag (A-B-C) or TiO_2 (D-E-F) NPs. A and D map Cl in green and Ag (A) or Ti (D) in red (ep. epidermis, p. parenchyma, n. nerve, st. stomata). B and E are distribution maps in temperature color of Ag and TiO_2 respectively. C (log scale) and D are fluorescence spectra acquired on the whole surface (black spectra) and on Ag hot spot (red spectra).

The second goal of this experiment was to investigate the speciation of TiO_2 and Ag NPs inside plant tissues. Some Ti K-edge µXANES spectra were well reproduced by anatase, the initial form (anatase) (Figure 2A). However, other spectra showed some discrepancies with the anatase spectrum, and adding a secondary compound (Ti-acetate) improved the fit quality. This might correspond to the presence of an organic coating around the TiO_2 NP. Ag speciation was studied by Ag L_{III}-edge µXANES. We detected both elemental and monovalent forms in the leaves (Figure 2B). This suggests that Ag NPs in plant tissues undergo oxidation and release Ag^+ ions. We have been able to detect these oxidized forms but not to precisely identify them. We would need more reference spectra for that purpose. This identification is of importance since different oxidized forms exert different toxicity, both for plants and their consumers (animals and humans).

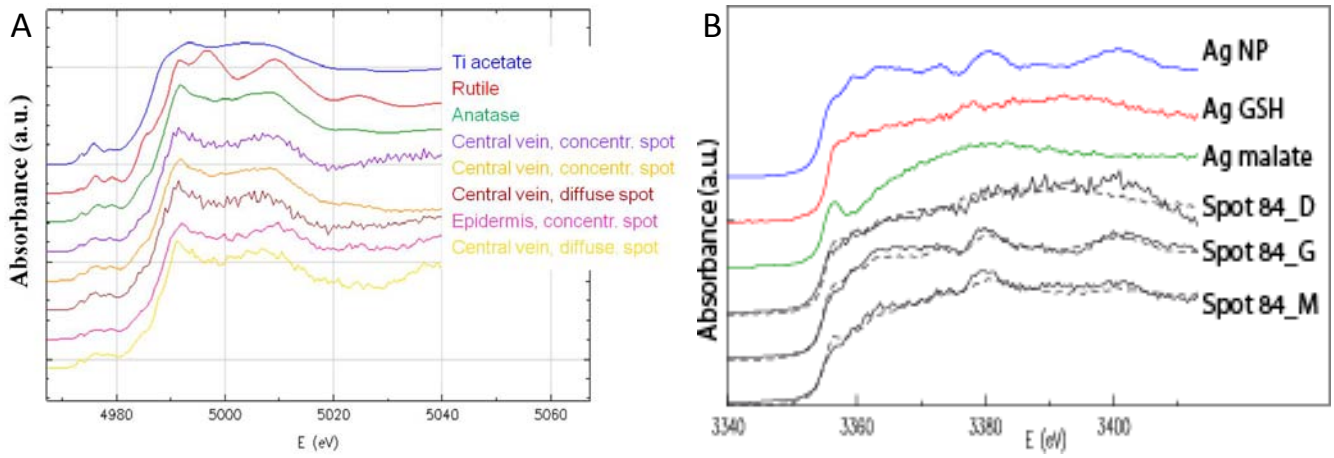


Figure 2: A. µXANES spectra of Ti in leaves and associated reference compounds. B. µXANES spectra of Ag in leaves (spot 84) and associated reference compounds.

Finally, leaf sections were studied by µFTIR in transmission mode. Portions of leaves were embedded in OCT, frozen and sectioned in the cryomicrotome at 15 µm thickness, and thin sections were deposited on BaF2 discs and placed in a dry atmosphere for dehydration.

µFTIR maps were recorded with a 8x8 µm spot size. Maps were analyzed using OMNIC and PyMCA, and spectra were compared to reference compound spectra recorded previously in ATR mode (malate, oxalate, citrate, cell wall , pectin, phosphate, etc...).

The FTIR signal of the OCT was quite strong compared to the signal of the leaf. The spectra recorded at the edge of the leaf (cuticle and epidermal cells) may contain a contribution of the OCT. These spectra showed strong band at 1730 and 1600 cm^{-1} corresponding to C=O and COOH groups. The spectra for the veins showed the same pattern. This likely corresponds to the thickening of the cell walls.

A certain spectral variability was observed for a given condition and within a given region (cuticle, vein or mesophyll). Spectra varied in peak position and peak intensities. This variability made it difficult to find significant differences between different conditions. The only difference, which has to be confirmed, could be a higher ratio for the band at 1730 cm^{-1} to the band at 1600 cm^{-1} for the plant exposed to Ag NPs (Figure 3). The band at 1730 cm^{-1} corresponds to ester and COOH groups, and the one at 1600 cm^{-1} to ketone and COO^- groups.

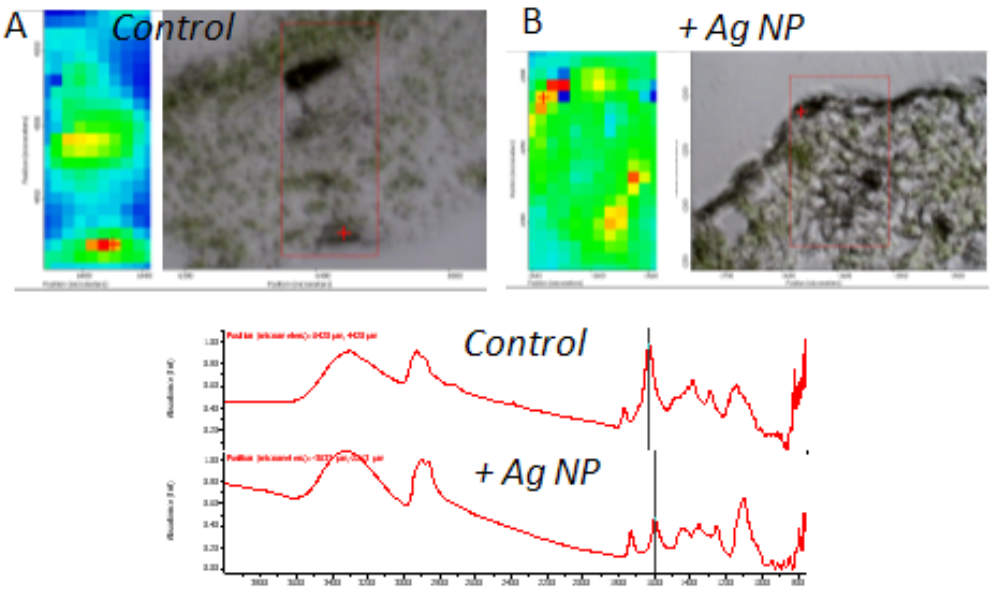


Figure 3: Comparison of µFTIR maps for the peak area of the band at 1600 cm^{-1} for a leaf section of lettuce exposed to Ag NPs (B) and for the control (A) and spectra obtained at the edge of the leaf exposed to the NPs.

Concerning the lipids bands, the ratio of the bands ν(CH): ν_{as}(CH3) (3012:2958) and ν_{as}(CH2): ν_{as}(CH3) (2921:2958) band ratio have been used as markers of the lipid peroxidation in animal tissues (Petibois & Deleris, 2006). In our case, we did not notice significant differences between control and exposed leaves (the variation between different pixels of the same sample was higher than the variation between two conditions).

The transmission mode and 15 µm thickness of the sections were well adapted for this study. The embedding in OCT generated a high background and other types of resins should be tested.

This beamtime makes it possible to improve our knowledge of NP fate in plants upon foliar exposure. Pristine NPs of Ag (40 nm) and TiO_2 (10 nm) are internalized in lettuce leaves upon foliar exposure, likely by the stomata. These NPs are ditributed without preferential localization throughout the parenchyma. TiO_2 NPs keep their crystalline form and seem to be coated with organic molecules. Conversely, Ag NPs undergo oxidation. These results suggest that crops might be a point of entry for NPs throughout the food chain.

Scientific production related to this experiment

Cécillon L, Castillo-Michel H, Larue C, Barthès V, Magnin V, Findling N, Bureau S, Sarret G. Foliar transfer of TiO_2 and Ag nanoparticles in lettuce. NanoImpactNet Conference, February 27 – March 2, 2012. Dublin, Ireland.

Abstract submitted to the Goldschmidt conference (June 24-29, 2012. Montreal, Canada)