| | Experiment title: Zn-based foliar (nano)fertilizers; unravelling the bioavailable chemical species, their route of foliar uptake and translocation to design more efficient formulations | Experiment number: ES1168 |
|---|---|---|
| **Beamline**: ID21 | **Date of experiment**: from: 21/06/2022 to: 25/06/2022 | **Date of report**: 13/09/2022 |
| **Shifts:** 12 | **Local contact(s)**: Hiram Castillo-Michel | *Received at ESRF:* |


**Names and affiliations of applicants** (* indicates experimentalists):

- Sandra Rodrigues*, DAO/University of Aveiro
- Astrid Avellan*, CNRS/GET
- Camille Larue*, CNRS/LEFE
- Sónia Rodrigues*, DAO/Unversity of Aveiro


**Report:**

The goal of our experiment was to study the fate of Zn nanoparticles (Zn-NPs) after their foliar deposition in pepper plants.We aimed to investigate which Zn-species were bioavailable 2 hours and 1 week after foliar exposure, help unravel Zn-NPs-leaf interaction, paths of uptake and translocation from leaves to stem. Pepper plants were foliarly exposed to two types of NPs: bare ZnO NPs and surface modified ZnO NPs with $Zn_3(PO_4)_2$ (ZnO_Phosp NPs). The $Zn_3(PO_4)_2$ surface modification was meant to study if a less soluble coating would enhance Zn bioavailability through surface charge modification and/or change the ionic release on the leaf.

Pepper plants were foliarly exposed to ZnO NPs and ZnO_Phosp NPs, by drop depositing 270 µL on the 6$^{th}$ and 7$^{th}$ leaf. Both NPs suspenstions were prepared at 150 mg Zn/L, for a total of 40.5 µg of Zn applied per plant.

The exposed pepper plants were sampled 2 hours ans 1 week after foliar exposure into: 7$^{th}$ exposed leaf and stem, to assess the translocation of Zn-species upon foliar uptake. The 7$^{th}$ exposed leaf was sampled, a segment of the stem was cut (with the node of the 7$^{th}$ leaf), samples were embedded in OCT resin and flash frozen in liquid nitrogen. Samples were verticaly cross-sectioned (20 µm thick) using the microtome at ID-21 and mounted in the cross-sectioning holder of the beamline. Cross-sections were analyzed using the cryogenic set-up of ID21 with an incident beam of 9.8 keV.

Plants that were exposed  2h to to ZnO NPs (Figure 1 – A-1and A-2) or ZnO_Ph (data not shown), had Zn accumulation in both the upper and lower epidermis of the exposed leaf, while only the adaxial  (top) part of the leaf was exposed. For both types of NPs, XANES spectra of ZnO were only found on the upper epidermis (Figure 2) of the exposed leaf and we did not find evidences of ZnO NPs in the lower epidermis. This indicates a rapid uptake and translocation all over the leaf. It remains unclear if the ZnO dissolved before or after its leaf uptake.

Plants that were exposed over 1 week also presented Zn accumulation in both the upper and lower epidermis of the exposed leaf (exemple for ZnO NP: Figure 1 – B-1, B-2 and C), but in addition we could visualize some Zn around the vasculature (ZnO NPs: Figure 1 – C). No evidence of ZnO NP persistence was found after a week of exposure.

Zn translocation was further assessed by cross-sectioning the stem of pepper plants near the node of the 7$^{th}$ exposed leaf (Figure 1 – E-1 and E-2 for ZnO NPs). We observed that on ZnO NPs exposed pepper plants, there was high Zn accumulation in the stem epidermis and in the collechyma (right bellow the epidermis) (Figure 1 – E-1), up to the vasculature (Figure 1 – E-2). On plants that were exposed to ZnO_Phosp NPs, Zn was only observed on the stem epidermis and in the parenchyma. No evidence of ZnO persistence in the stem was found on the XANES spectra acquired.

This experiment demonstrated

- A possible plant response to Zn storage in the epidermis as a depuration strategy that would be an interresting topic to investigate further.
- A difference of Zn distribution in the stem between the 2 type of NPs. This could be due to a different Zn rate of uptake and translocation inside the plant and makes sense regarding our ICP-MS results where Zn accumulated more in the stem of ZnO_Phosp NPs exposed plants. This could also mean that Zn is accumulating elsewhere in the stem of ZnO_Phosp NPs exposed plants. Our sp-ICPMS results also showed that there were ZnO particles for both types of NPs exposed plants in the stem.

These results will be of great use to interprete our bulk ICP-MS and sp-ICPMS results investigating foliar uptake of Zn from various Zn sources and its *in planta* translocation in various pepper plant organs (exposed leaves, remaining upper and lower leaves, stem, roots and pepper fruit), results that will be published all together. The article is currently about to be submitted.

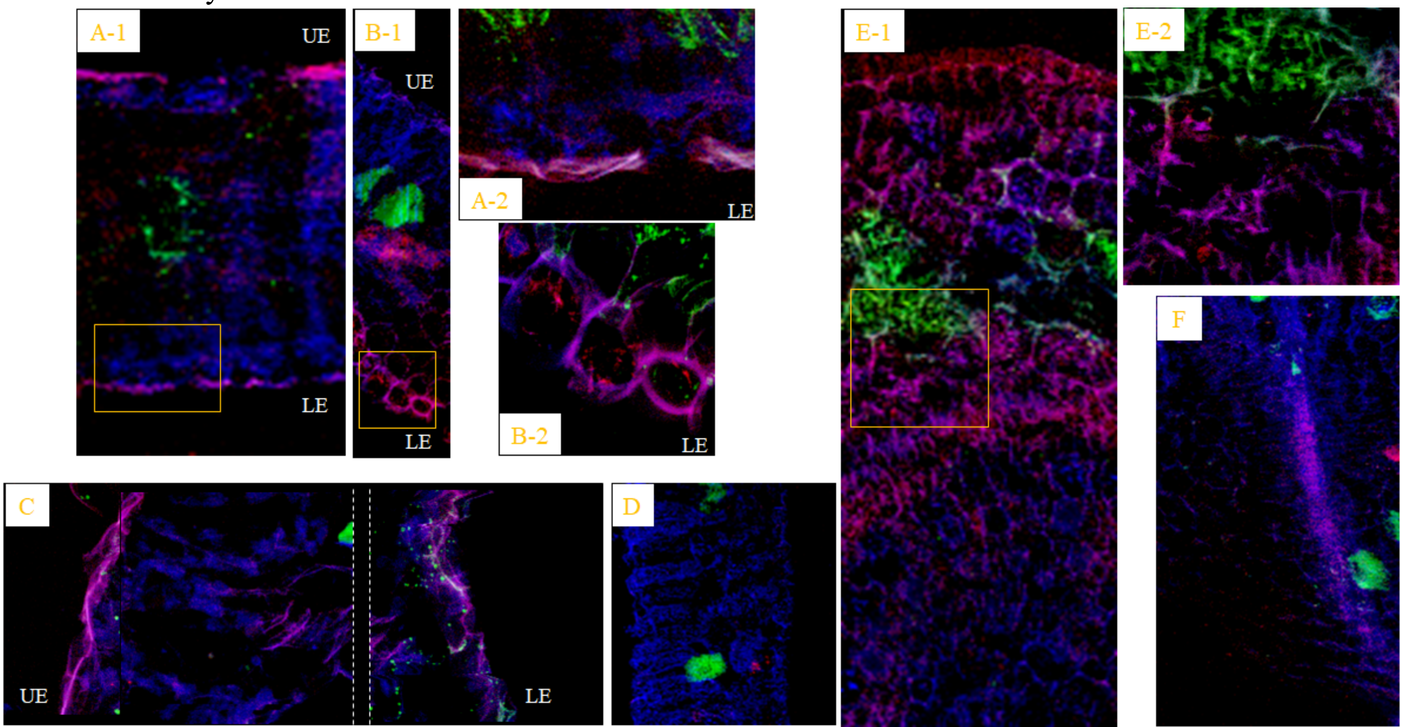


**Figure 1** – Elemental µ-XRF on the Pepper plant 7$^{th}$ **leaf exposed to ZnO NPs**: 2hours after exposure (2µm x 2µm resolution A-1 and 0.5µm x 0.5µm of the highlighted yellow area in A-2); 1 week after exposure (2µm x 2µm resolution B-1 / C and 0.5µm x 0.5µm of the highlighted yellow area in B-2). Elemental µ-XRF on the Pepper plant **stem near 7$^{th}$ leaf node exposed to ZnO NPs**: 1 week after exposure (2µm x 2µm resolution E-1 and 0.5µm x 0.5µm of the highlighted yellow area in E-2). Elemental µ-XRF on the Pepper plant 7$^{th}$ **leaf** and **stem near 7$^{th}$ leaf node exposed to DIW**: 1 week after exposure (2µm x 2µm resolution D and F respectively). Zn is represented in red, K in blue and Ca in green. Upper epidermis is identified as UE and lower epidermis as LE.

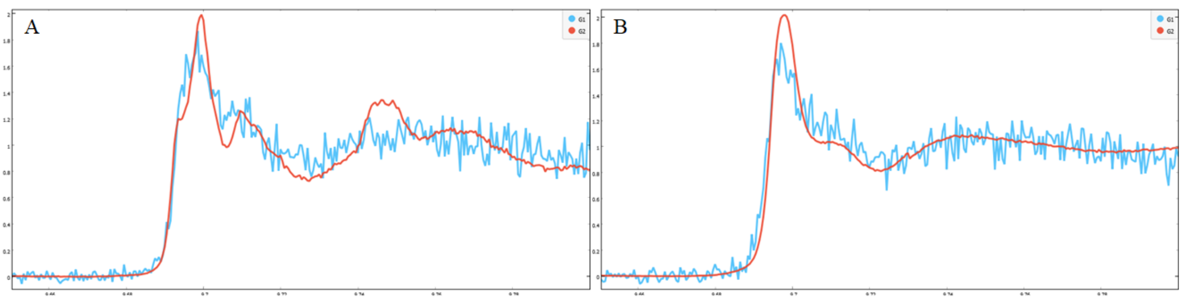


**Figure 2** – XANES specta performed on 2 hours ZnO NPs exposed leaves: A – ZnO NPs (red) VS Upper epidermis ZnO NP (blue) and B – Zn-Citrate (red) VS Mesophyll Zn-Citrate (blue).