ESRF

ESRF	**Experiment title:** Zn-based root (nano)fertilizers; unravelling the bioavailable chemical species, their route of root uptake and translocation to design more efficient formulations.	**Experiment number**: ES1315
Beamline: ID21	**Date of experiment**: from:22/06/2023 to:26/06/2023	**Date of report**: 13/09/2023
Shifts: 12	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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Report:

The goal of our experiment was to study the fate of Zn nanoparticles (Zn-NPs) after root exposure in pepper plants. Here, ZnO nanoparticles (NPs) and surface modified ZnO NPs with $Zn_3(PO_4)_2$ with opposite charges (+14.60 ± 0.42 mV and - 18.10±0.61 mV respectively) were used to assess the impact of NPs surface chemistry and properties for Zn root uptake, transport, and plant distribution. Pepper plants roots were exposed to bare ZnO NPs, surface modified ZnO NPs with $Zn_3(PO_4)_2$ (ZnO_Phosp NPs), a Zn Salt for control. The roots of the pepper plants were exposed to ZnO NPs, ZnO_Phosp NPs and Zn salt, by injecting 270 µL on the sand where the plants were grown, next to the root. Both NPs suspensions and Zn salt solution 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 1 week after exposure into: main root and stem, to assess the translocation of Zn-species upon root uptake. All samples were embedded in OCT resin and flash frozen in liquid nitrogen. Samples were vertically cross-sectioned (20 µm thick) by Hiram Castillo-Michel using the microtome at ID-21 and mounted in the cross-sectioning holder of the beamline. Cross-sections were analyzed using the cryogenic setup of ID21 with at Zn K-edge (9.8 keV).
Elemental µ-XRF on plants that were exposed to ZnO NPs had a different Zn distribution both on exposed roots and stem, when compared to plants that were exposed to ZnO_Phosp NPs (Fig. 1). Plants exposed to ZnO NPs appeared to retain more Zn in both the root and stem epidermis (Fig. 1A and E), while for ZnO_Phosp NPs, Zn is more evenly distributed in epidermis, cortex, and the root vasculature (Fig. 1B and F). Preliminary analysis of the µ-XANES spectra performed in both NPs treatments indicate that no NP were found. Despite the differences observed in cellular distribution between NPs, both present similar Zn speciation to the Zn salt, indicating that Zn is bound to similar organic ligands. Our results suggest that either the Zn-phosphate shell on

the ZnO NPs or its negative charge might have had influence on the root uptake of Zn, and therefore distributed differently than for the non-coated and positively charged ZnO NPs. Since this experiment was performed 1 week after exposure, and even though we did not find evidence of NPs presence, the uptake of NPs in earlier stages might have occurred, which could also explain differences observed in cellular distribution.

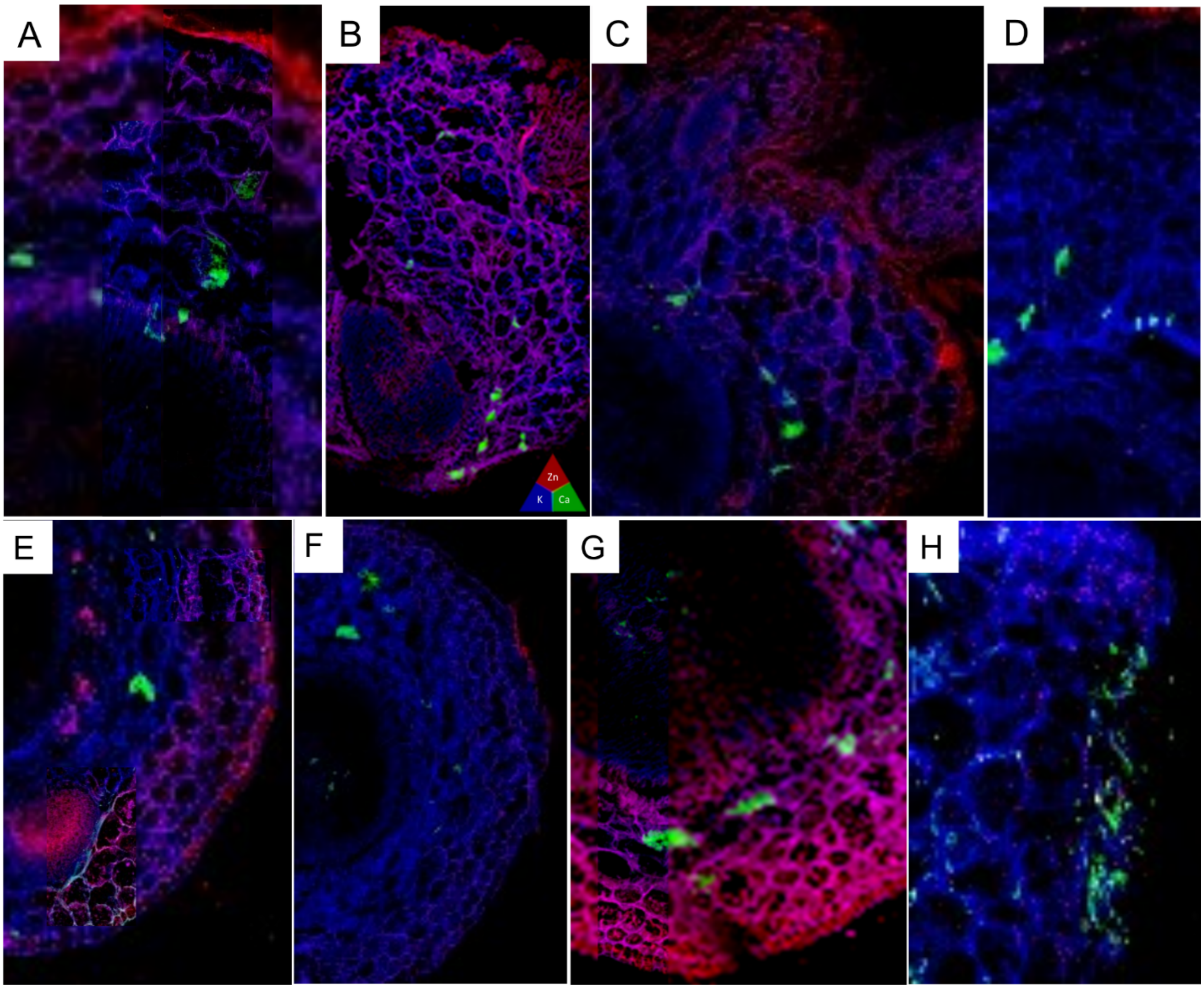

Fig. 1 – Elemental µ-XRF (8µm x 8µm resolution) on the main root and stem of pepper plant exposed to ZnO NPs (A and E), ZnO_Phosp NPs (B and F), Zn Salt (C and G) and a non-exposed control (D and H)