Supplementary Information

Effect of CeO₂ nanomaterial surface functional groups on tissue and subcellular distribution in tomato (*Solanum lycopersicum*)

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The goal of the synchrotron-based micro X-ray Fluorescence experiments (μ -XRF) was to evaluate the effect of surface charge/coating on localization of nano-Ce in roots and leaves of tomato plants. Roots of tomato plants exposed to nano-Ce having neutral, negative or positive surface charge were harvested and prepared as thin sections by routine electron microscopy (EM) processing methods: fixation in 4% paraformaldehyde + 1% glutaraldehyde mixture followed by dehydration series in methanol and then resin infiltration series in LR white resin. Root cross-sections approximately 1 mm from the apex were cut to 1 micron thickness using an ultramicrotome and mounted onto silicon dioxide backing material for μ -XRF; serial sections were prepared on glass microscope slides for light microscopy and stained with toluidine blue dye to define cellular boundaries. Routine EM processing is assumed to cause redistribution of mobile elements (e.g., aqueous K+ ion) in cells and tissues; however, it is unlikely to alter the localization of discrete or agglomerated particles such as CeO₂.

Ultra-high spatial resolution (circa 15 nm) μ -XRF images of root cells were acquired at the Hard X-ray Nanoprobe (HXN) beamline at the National Synchrotron Light Source II (NSLS-II), and μ -XRF images of entire root sections were acquired at GSECARS Beamline 13-ID-E (The University of Chicago) at the Advanced Photon Source (APS). Additionally, fresh tomato leaves from the third position on the apical stem were harvested and mounted onto Kapton tape and select regions were imaged at the Sub-micron Resolution X-ray (SRX) beamline at the National Synchrotron Light Source II (NSLS-II).

For µ-XRF experiments, the incident beam energy was fixed at 12 keV. At the HXN beamline, samples were oriented 15° to the incident beam and scanned on-the-fly in the transverse direction using nanopositioning stages with laser interferometer feedbacks. HXN is an undulator-based, scanning X-ray microscope using a new class of X-ray nanofocusing optics-known as multilayer Laue lenses (MLLs)to enable imaging experiments at a resolution of 10 nm, with an ultimate goal of about 1 nm¹. Images were typically collected for a 1 to 10 µm² area using a pixel size of 10 nm (fine resolution) to 100 nm (course) and a transit time of 50 to 100 ms. At the GSECARS 13-ID-E beamline, samples were oriented 45° to the incident beam and scanned on-the-fly in the transverse direction. Beamline 13-ID-E is an undulator-based hard X-ray microprobe instrument which utilizes dynamically-figured, grazing incidence silicon mirrors placed in Kirkpatrick Baez geometry for X-ray focusing.² Images were collected for areas up to 350 μ m2 using a pixel size of 1 μ m and a transit time of 100 ms. At the SRX beamline, samples were oriented 45° to the incident beam and sub-millimeter regions of tomato leaf were scanned with a step size of 5 to 20 microns using a dwell time of 1 second. Full X-ray fluorescence spectra for each pixel were acquired using Quantum Detectors Xspress3 Electronics with a Hitachi Vortex ME3 silicon drift diode (SDD) detector (HXN and SRX) or a Hitachi Vortex ME4 SDD detector (GSECARS) sitting at 90° to the incident beam and in the plane of the storage ring. The fluorescence signal was normalized to the changes in intensity of the X-ray beam (I0). Data processing for HXN and SRX was performed with NSLS-II/PyXRF software (https://github.com/NSLS-II/PyXRF) and with U. Chicago CARS/LARCH software (https://github.com/xraypy/xraylarch).

 Chu, Y.S., H. Yan, E. Nazaretski, S. Kalbfleisch, X. Huang, K. Lauer and N. Bouet. 2015. Hard Xray nanoprobe facility at the National Synchrotron Light Source II. SPIE (doi: 10.1117/2.1201508.006068) 2. Lanzirotti, A., M. Newville, L. Manoukian, and K. Lange. 2016. High-speed, coupled microbeam XRD/XRF/XAFS mapping at GSECARS: APS Beamline 13-ID-E. The Clay Minerals Society Workshop Lectures Series, Vol. 21, Chapter 5, 53-64.



Figure.S1. Signs of chlorosis in leaves (treatment level mg/L). A. DEX and CM, B. DEAE, C. Control.







Figure. S2. Uptake of ionic cerium from $CeCl_3$ (0.5, 1.5, 5, 15 mg/L Ce). A, dry masses of shoots and roots. B, C, tissue concentration of Ce.





Figure. S3. Cerium concentration in the leaves is much lower than in the stem. DEAE(+), DEX(N), CM(-).





Figure S4. Additional XRF coarse tiling over a large area were used to locate specific regions of interest for high-resolution scans. A, DEAE, B, DEX (aside is a corresponding light microscope image of the part of the section scanned by XRF), C, CM. Scale bars are 10 μ m in A and C and 5 10 μ m in B.

(Figures on next three pages)









Normalized Ce Lα Intensity

Figure S5. Fit of XANES spectra from the epithelial regions of the cross sections A) DEAE, B) DEX and C) CM.

