

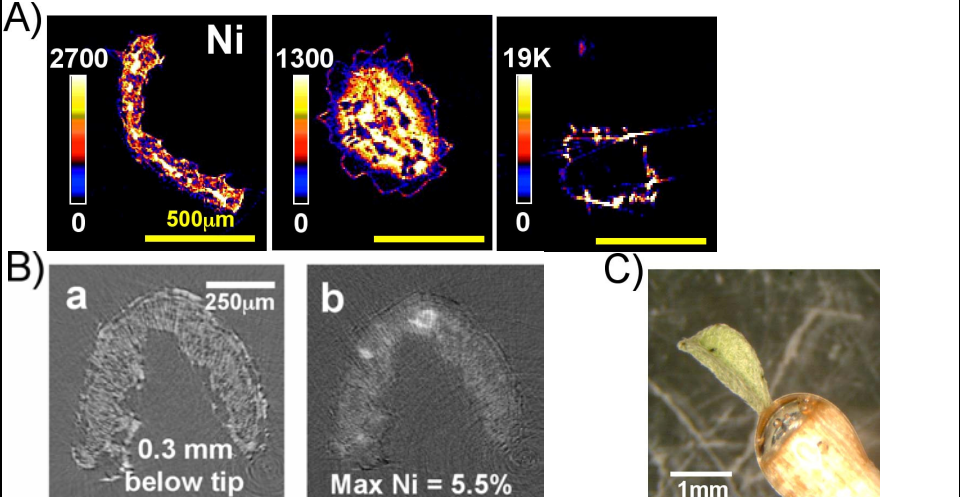


HANDBOOK OF METHODS USED IN RHIZOSPHERE RESEARCH

2.1. Elemental Analysis of Plants and Fungi

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<i>Parameter</i>	Quantitative Elemental Compartmentalization in Plant Tissues
<i>Plant species</i>	Any plant tissue with elemental concentrations exceeding $\sim 100 \mu\text{g g}^{-1}$ D.W.
<i>System</i>	Plant samples grown in a glass house in Ni refinery enriched soils
<i>Method</i>	Synchrotron based X-ray fluorescence and absorption edge computed microtomography (F-CMT and AE-CMT)
<i>Method description</i>	<p><i>Fluorescence CMT</i> Specimen (i.e., leaf, root) is attached to a wooden shaft using epoxy resin (Fig.1C) and then mounted on a rotation-translation stage. XRF intensities are then collected as the specimen is translated through the X-ray beam, rotating by a small angle, repeating the translation-XRF collection, and continuing this process to encompass a total rotation of 180 degrees. Typical datasets are collected with 5 μm translation steps over 1.5 mm, 3° angular steps and dwell times of 1 second resulting in 2-D “sinograms” (intensity on a “position-angle” plot) for each element (up to 13 at once) with total collection times of ~ 5 hours. 2-D element images are then computationally reconstructed using FFT-based Gridrec software (Dowd et. al., 1999), which is controlled by the IDL programming language (Research Systems Inc.) yielding images of the cross-sectional internal metal distributions. See McNear et. al. (2005) for details on elemental quantification.</p> <p><i>Absorption Edge CMT</i> Fresh leaves are removed just prior to analysis and mounted directly to the translation-rotation stage by placing the proximal portion of the leaf into a bead of modeling clay. A synchrotron bending magnet source is used to provide a wide fan X-ray beam for flood field applications. The transmitted X-rays are converted to visible light with a single crystal YAG scintillator and the scintillator is imaged with a microscope objective (10X) projected onto a 1300 x 1030 pixel fast charge-coupled device (CCD) X-ray area detector resulting in a field of view of 2.15 x 1.7 mm and an optical resolution of 3.3 μm. Typical 3D datasets are collected with 0.5 degree steps over 180 degrees and dwell times of 10 seconds. One tomogram is collected with the X-ray beam energy below the absorption edge energy (e.g., 8300 eV for Ni or ~ 30 eV below the Ni K absorption edge) and a second tomogram is acquired above the absorption edge. To enhance absorption contrast, the above-edge energy is selected to coincide with the XANES white line for the element in the plant tissue. This energy is determined empirically by viewing the absorption radiograph in real time as the monochromator energy is scanned manually until the energy of maximum absorption is found. Typical image acquisition times with these settings are 45 min. to 1.5 hours. The two sinograms (above edge and below edge) are subtracted, the difference matrix reconstructed using the Gridrec-based software (Dowd et. al., 1999) and the resulting images stacked and viewed sequentially resulting in a movie depicting the metal distribution from the top (distal) to the bottom (proximal) end of the leaf.</p>

<p><i>Do's, don'ts, potential limitations, untested possibilities</i></p>	<p><i>Limitations:</i></p> <ul style="list-style-type: none"> Freezing and partial drying is required for F-CMT to minimize movement (which compromises reconstruction of the tomogram) due to dehydration from the microfocused X-ray beam. Field of view is limited (F-CMT = 1 mm² ; AE-CMT = 4 mm²) which restricts the size of the object that can be examined. This can be overcome by rolling or folding the material to fit the requisite dimensions. Limited to analyzing elements with atomic numbers greater than Sulfur. Concentration must be greater than 100 mg kg⁻¹ D.W. for F-CMT and 5000 mg kg⁻¹ W.W. for AE-CMT. <p><i>Possibilities</i></p> <ul style="list-style-type: none"> still exist for exploring alternative methods for sample preparation that would prevent X-ray beam damage and allow for the analysis of hydrated tissues via F-CMT (e.g., glycerol solvation, paraffin embedding etc.). Coupling F-CMT with AE-CMT provides highly resolved elemental microdistributions as well as confirmation of the ubiquity of the distributions throughout an entire leaf or selected sections of stems and/or roots.
<p><i>References</i></p>	<p><i>Experimental details:</i> McNear, D.; Peltier, E; Everhart, J.; Chaney, R.L.; Sutton, S.; Newville, M.; Rivers, M.; Sparks, D.L. 2005. The application of fluorescence and absorption edge computed microtomography to image metal compartmentalization in <i>Alyssum murale</i>. Environ. Sci. Technol. 39:2210-2218</p> <p><i>Application to As sequestration:</i> Keon-Blute, N.; Brabander, D. J.; Hemond, H. F.; Sutton, S. R.; Newville, M.; Rivers, M. 2004. Arsenic sequestration by ferric iron plaque on cattail roots. Environ. Sci. Technol. 38: 6074-6077.</p> <p><i>Software description:</i> Dowd, B. A.; Campbell, G. H.; Marr, R. B.; Nagarkar, V. V.; Tipnis, S. V.; Axe, L.; Siddons, D. P. 1999. Developments in synchrotron X-ray computed microtomography at the National Synchrotron Light Source. Proceedings of SPIE, Developments in X-Ray Tomography II 3772: 224-236</p>
<p><i>Links</i></p>	<p>http://cars9.uchicago.edu/gsecars/GSEmain.html</p>
<p><i>Additional information</i></p>	 <p>Figure 1: A) Ni concentrations (ppm dry weight) and micro-distributions in a leaf, stem and root acquired by F-CMT; B) captured frames from AE-CMT movie showing the Ni conc. (% wet weight) and distribution (brighter regions) 0.3 mm below the tip of an <i>Alyssum murale</i> "Kotodesh" leaf; C) leaf mounted in epoxy resin on the end of wooden shaft for analysis via F-CMT</p>