EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experiment Report Form

	Experiment title: Sub-cellular imaging of Cu and Zn in plants by nano X-ray fluorescence	**Experiment number**: EC 897
Beamline:	**Date of experiment**: from: Sept 20 -23 and Nov 9-12	**Date of report**: August 20, 2012
Shifts:	**Local contact(s)**: Rémi Tucoulou	*Received at ESRF:*

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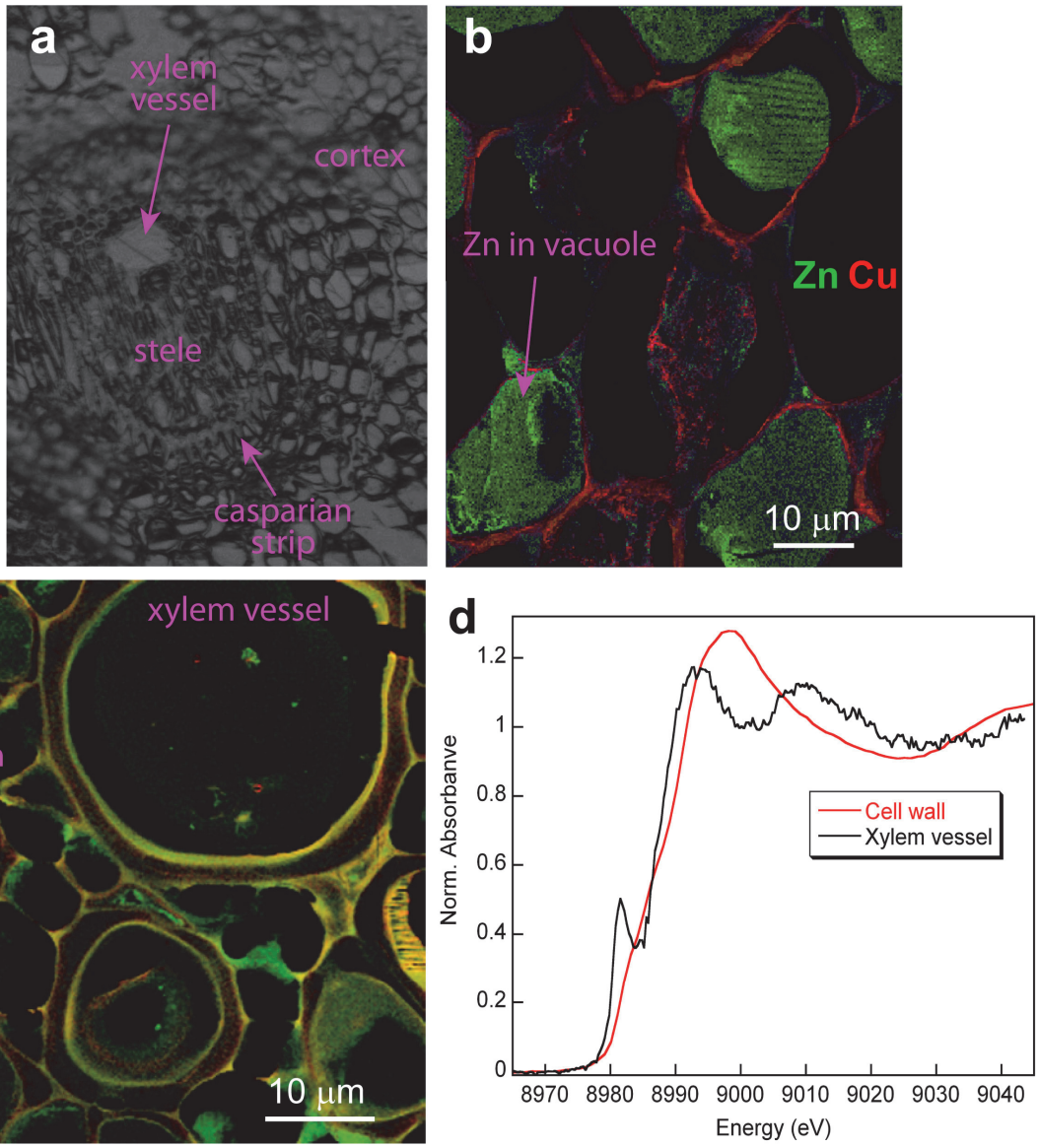
Report:

The allocated beamtime has been split in two sessions of three days to optimize our measurements. The distribution of Cu and Zn in the roots of two plants, one monocotyledon, *Phalaris arundinacea*, and one dicotyledon, *Thlaspi arvensis*, have been imaged at several scanning resolutions down to 180 x 180 nm using micro- and nano-SXRF, and the chemical form of Cu in the two plants interrogated using micro-XANES. The fresh samples were cryofixed at high pressure, then cryosubstituted to preserve the fine structures, and finally sectioned in slices of two micrometers in thickness. As an example of the results, we show below, (1) an optical image of a prepartion taken in transmission with the beamline camera (a), (2) two high-resolution nano-SXRF maps (b,c), and (3) one micro-XANES spectrum (d).

The optical image (a) is from a root of *P. arundinacea*. It shows that the vacuolar content and cytoplasm were preserved in several regions of the preparation. We focused our analyses on these areas and examined in total four microtomic slices for each plant. Fluorescence maps collected in the cortex region showed that Cu is associated mainly with the cell walls and present also in the cytoplasm and on inner-cell membranes (such as vacuoles) (b). Zinc, instead, is predominantly stored inside the vacuoles, and to a lesser extent also present on cell walls. These observations gives an inkling into the metal homeostasis in the plants. Zn, as we know it, is preferentially complexed to small organic acids, such as

malate, and its sequestration in the vacuoles can be explained by the high content of endogenous carboxylic acids in this organelle. In contrast, the presence of Cu in the cytosol can be explained by its inferred complexation to amino acids and peptides. The compartmentation of Cu and Zn described in the cortex is also observed in the stele (c). The xyleme vessels, which translocate metals from roots to shoots, contain Cu and Zn "grains". Importantly, the grains were never multimetallic, suggesting that Cu and Zn are transported to the leaves by different molecules. Because Cu has a strong affinity for amine and amid groups, it has been proposed to be transported by free amino acids, as reported for nickel in other plants. The micro-XANES spectrum shown in (d), characterized by a deep diagnostic pre-peak, provides the first direct evidence that Cu is indeed strongly chelated to one or several N-containing residues, such as histidine and methionine. More complete analysis of the data is now under course.



Metallomics

RSC Publishing

PAPER

Thlaspi arvense binds Cu(II) as a bis-(L-histidinato) complex on root cell walls in an urban ecosystem†

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Root cell walls accumulate metal cations both during acquisition from the environment and removal from the protoplast to avoid toxicity, but molecular forms of the metals under field conditions remain elusive. We have identified how copper is bound to cell walls of intact roots of native *Thlaspi arvense* by combining synchrotron X-ray fluorescence and absorption techniques (XANES and EXAFS) at the nano-, micro-, and bulk scales. The plants grew naturally in sediment in a stormwater runoff basin at copper concentrations typical of urban ecosystems. About 90% of acquired copper is bound *in vivo* to cell walls as a unique five-coordinate Cu(II)–bis(L-histidinato) complex with one L-histidine behaving as a tridentate ligand (histamine-like chelate) and the other as a bidentate ligand (glycine-like chelate). Tridentate binding of Cu(II) would provide thermodynamic stability to protect cells against copper toxicity, and bidentate binding may enable kinetic lability along the cell wall through protein–protein docking with the non-bonded imidazole group of histidine residues. EXAFS spectra are provided as ESI† to facilitate further identification of Cu–histidine and distinction of Cu–N from Cu–O bonds in biomolecules.

Introduction

The multiple roles of copper in biochemical reactions result from the redox-sensitivity of the Cu^{2+}/Cu^{+} couple, which has elevated standard electrode potentials when complexed in proteins that facilitate electron transfer in cellular processes.[1] Imbalances in cellular copper are implicated in multiple diseases that strike eukaryotic organisms. In plants, copper is essential for photosynthesis, mitochondrial respiration, lignin synthesis, root growth, ethylene sensing, and reactive oxygen metabolism.[2,3] Typically, the concentration of nutrient copper falls between 5 and 30 mg kg^{-1} dry weight (DW) in vegetative tissues, regardless of the copper concentration of the soil in which the plant grows.[4] When in excess, copper can catalyze the production of hydroxyl radicals, with subsequent damage to macromolecules involved in the production (chloroplasts) and storage (mitochondria) of energy, to protein synthesis, and to biomembranes through the peroxidation of unsaturated fatty acids.[5–9] To maintain nutrient requirements, while simultaneously protecting photosynthetic and reproductive tissues from excess amounts, vascular plants have evolved physiological mechanisms to regulate the concentration of copper in root cells and its transport in vascular bundles (*i.e.*, xylem sap) from the stele.[10–13]

Knowledge of the location and molecular forms of copper in the root is sparse, especially at non-toxic concentrations. This information is critical for developing approaches to preserve copper homeostasis in plants as environmental conditions change, and for applying phytotechnology to remediate contaminated soil and water.[14,15] Results of previous electron microscopy studies show that copper is dominantly contained on cell walls as are other metals.[16–19] Copper may enter the cell wall through intercellular spaces during the uptake of water from the environment, and as a result of trace metal removal from the protoplast during the sequestration process for detoxifying excess copper. Inside cells, copper typically is not sequestered in root cortical vacuoles, as is commonly the case for zinc and cadmium detoxification.[20–22] Sequestration of copper in vacuoles has been observed only in two Cu-tolerant plants, *Armeria maritima* sp. halleri grown in the wild on Zn-polluted soil with a root content

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† Electronic supplementary information (ESI) available: File S1: Results and figures. File S2: Cu K-edge EXAFS spectra of the cell wall and model compounds. See DOI: 10.1039/c3mt00215b

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