

# **Analysis of compartmentation and binding of metals in plants at physiological and toxic concentrations analysed in frozen-hydrated samples by $\mu$ XRF tomography and related techniques**

Hendrik Küpper<sup>a,b</sup>

<sup>a</sup>*Biology Center of the Czech Academy of Sciences, Institute of Plant Molecular Biology,*

*Department of Biophysics & Biochemistry of Plants & University of South Bohemia,*

*Department of Experimental Plant Biology; České Budějovice, Czech Republic, <sup>b</sup>(former address) University of Konstanz, Department of Biology, Konstanz, Germany*

*Author Email: hendrik.kuepper@umbr.cas.cz*

Various transition metals are essential nutrients, but toxic at elevated concentrations. Many mechanisms of metal uptake, transport, binding and sequestration are shared between animals (incl. humans), fungi, plants and even bacteria, others are specific for one group. Analyzing metal distribution (compartmentation) and speciation is a crucial step in revealing mechanisms of metal uptake, transport sequestration, deficiency, toxicity and detoxification. The least artifact-prone techniques for this task all belong to the field of X-ray spectroscopy, including EDX, PIXE, XRF and XAS. For minimizing artifacts, element re-distribution and ligand exchanges inside the measured tissues have to be prevented. The most reliable method for reaching this aim is measurement of shock-frozen, hydrated tissues. Ideally, these samples are analyzed as bulk tissues, replacing physical thin sectioning by recording  $\mu$ XRF tomograms [1-3]. In the ideal case the same samples can be analyzed by  $\mu$ XAS as well, allowing a direct correlation between element distribution and local differences in speciation [1]. Depending on the beamline, resolution of details even on the subcellular level is possible in such samples. In this talk, this ideal case will be compared to related techniques of measurement, in particular SEM-EDX [4-7] and XAS on powdered frozen-hydrated samples [8-12]. Alternative sample preparation techniques (e.g. conventional freezing in liquid nitrogen, freeze-drying and freeze-substitution) will be discussed as well. Differences between the techniques, especially in terms of artifact risks, will be highlighted.

## **References**

- [1] Mishra S, Wellenreuther G, Mattusch J, Stärk H-J, Küpper H (2013) *Plant Physiology* 163, 1396-1408
- [2] Andresen E, Mattusch J, Wellenreuther G, Thomas G, Abad UA, Küpper H (2013) *Metallomics*, 5, 1377-1386
- [3] Thomas G, Stärk H-J, Wellenreuther G, Dickinson BC, Küpper H (2013) *Aquatic toxicology* 140-141, 27-36
- [4] Küpper H, Zhao F, McGrath SP (1999) *Plant Physiology* 119, 305-311
- [5] Küpper H, Lombi E, Zhao FJ, McGrath SP (2000c) *Planta* 212, 75-84
- [6] Küpper H, Lombi E, Zhao FJ, Wieshammer G, McGrath SP (2001) *Journal of Experimental Botany* 52(365), 2291-2300
- [7] Carr HP, Lombi E, Küpper H, McGrath SP, Wong MH (2003) *Agronomie* 23(8), 705-710
- [8] Küpper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH (2004) *Plant Physiology* 134(2), 748-757
- [9] Küpper H, Mijovilovich A, Götz B, Küpper FC, Wolfram Meyer-Klaucke W (2009) *Plant Physiology* 151, 702-714
- [10] Mijovilovich A, Leitenmaier B, Meyer-Klaucke W, Kroneck PMH, Götz B, Küpper H (2009) *Plant Physiology* 151, 715-731
- [11] Trampczynska A, Küpper H, Meyer-Klaucke W, Schmidt H, Clemens S (2010) *Metallomics* 2, 57-66
- [12] Leitenmaier B, Witt A, Witzke A, Stemke A, Meyer-Klaucke W, Kroneck PMH, Küpper H (2011) *Biochimica et Biophysica Acta (section Biomembranes)* 1808, 2591-2599