# New insights into arsenic toxicity in the model plant *Ceratophyllum demersum* (I): by As µ-XRF

*S. Mishra[1], M. Alfeld[2], U. Boesenberg[2], E. Andresen[1], H. Küpper[1]*

*[1] Universität Konstanz, Universitätsstraße 10, D-78464 Konstanz, Germany*

*[2] DESY, Notkestr. 85, D-22607 Hamburg, Germany*

Heavy metal(loid) uptake in plants is an important research area not only in the sense of basic research, but also because of its impact on human nutrition and its application for the phytoremediation of contaminated soils. Our research mainly deals with mechanisms of active uptake of metal(loid)s, mechanisms of metal(loid) toxicity as well as detoxification. One of the most interesting topics in this area of research is the localisation of the metals&metalloids (e.g. As, Cd, Cu, Cr, Ni, Zn) inside the plant (e.g. [1], [2]).

Among the methods for analyzing metal distribution in tissues, micro X-ray Fluorescence (µXRF) in tomography mode has the advantages of detecting multiple elements simultaneously in contrast to fluorescent dyes, allowing for higher spatial resolution than LA-ICP-MS, and offering far better sensitivity compared to EDX. In earlier parts of our studies [2] the DORIS beamline L has been attractive for these measurements because it allows measurements of all elements of interest for the current questions at a spatial resolution (5 µm according to Sparrow's definition of resolution) that is sufficient for distinguishing between all relevant tissues in our model plant *Ceratophyllum demersum*. Further, with the help of a cryostream the samples can be kept frozen during the measurement, which is absolutely essential for avoiding beam damage and artefacts of element re-distribution that would inevitably occur if a tissue piece would be measured in non-frozen state. Beamline P06 at PETRA allowed for a first measuring session of microscopic analysis of As speciation [2], which showed that all what was possible at beamline L can be done faster and with higher resolution at P06.

The plants were grown in the lab of H. Küpper, where various biochemical and biophysical analyses were carried out [3]. There we also prepared the samples by excising the tissue pieces, mounting them in glass capillaries, attaching them to sample holders, and shock-freezing the whole assembly in supercooled isopentane. In order to optimize sample throughput, we designed improved sample holders that eliminate the necessity to re-center the stage after changing the sample during the beamtime, which led to more efficient use of the beamtime. During the experiments, the sample was cooled using the cryostream from Oxford Instruments. This arrangement of the cryostream proved decisive to prevent icing at the sample, and also allows the Maia detector. The µXRF-experiments were carried out with a Si 111 Double Crystal Monochromator. Focussing was achieved with a KB mirror system. For detection, a Maia detector was used to enable ultrafast data acquisition with dwell times down to 1ms per pixel. The resulting sinograms were tomographically reconstructed using the maximum likelihood expectation maximization (“MLEM”) algorithm. Finally, a multielement-standard measured in the identical geometry was used to relate fluorescence intensities with µmolar concentrations, and to correct for matrix effects. A histogram analysis will be used to correct for the background level as in our previous work [2].

## Results

In the first phase we analyzed the distribution of non-hyperaccumulated As in the metal-sensitive model plant *Ceratophyllum demersum* at tissue-level resolution (about 5 µm, [2]). At 1 µM As in the nutrient solution, As was mainly accumulated in the epidermis. Upon increasing As in the nutrient solution, total As increased in the vein and mesophyll but not in the epidermis of young leaves, as depicted by µ-XRF tomograms of As [3]. Thus, at lethal concentrations (5 µM As), arsenic spread into the mesophyll, causing additional toxicity. Therefore, As toxicity was correlated

with a change in As distribution pattern and As species (revealed by µ-XANES), rather than general increase in many tissues [2].

The new more detailed analysis using the microprobe at P06 combined with the Maia detector and a cryostream allowed to resolve the distribution of As accumulation inside single cells (Fig. 1). As the most important preliminary result (the data analysis is still ongoing), these measurements revealed the As predominantly accumulates in a single large organelle towards the side of the cells. Since only the nucleus has this combination of number, size and position, it is clear that As accumulation mainly occurs in the nucleus. This is an important result because one mode of As toxicity, where *in vivo* relevance is still not clear, is the replacement of P by As in the DNA. The data of our measurements now indicate that this is occurring at physiologically relevant concentrations. This interpretation is also in agreement with µ-XANES measurements on the same samples [2] where it was found that a large proportion of the As was surrounded by oxygen ligands as it is the case for P in DNA.

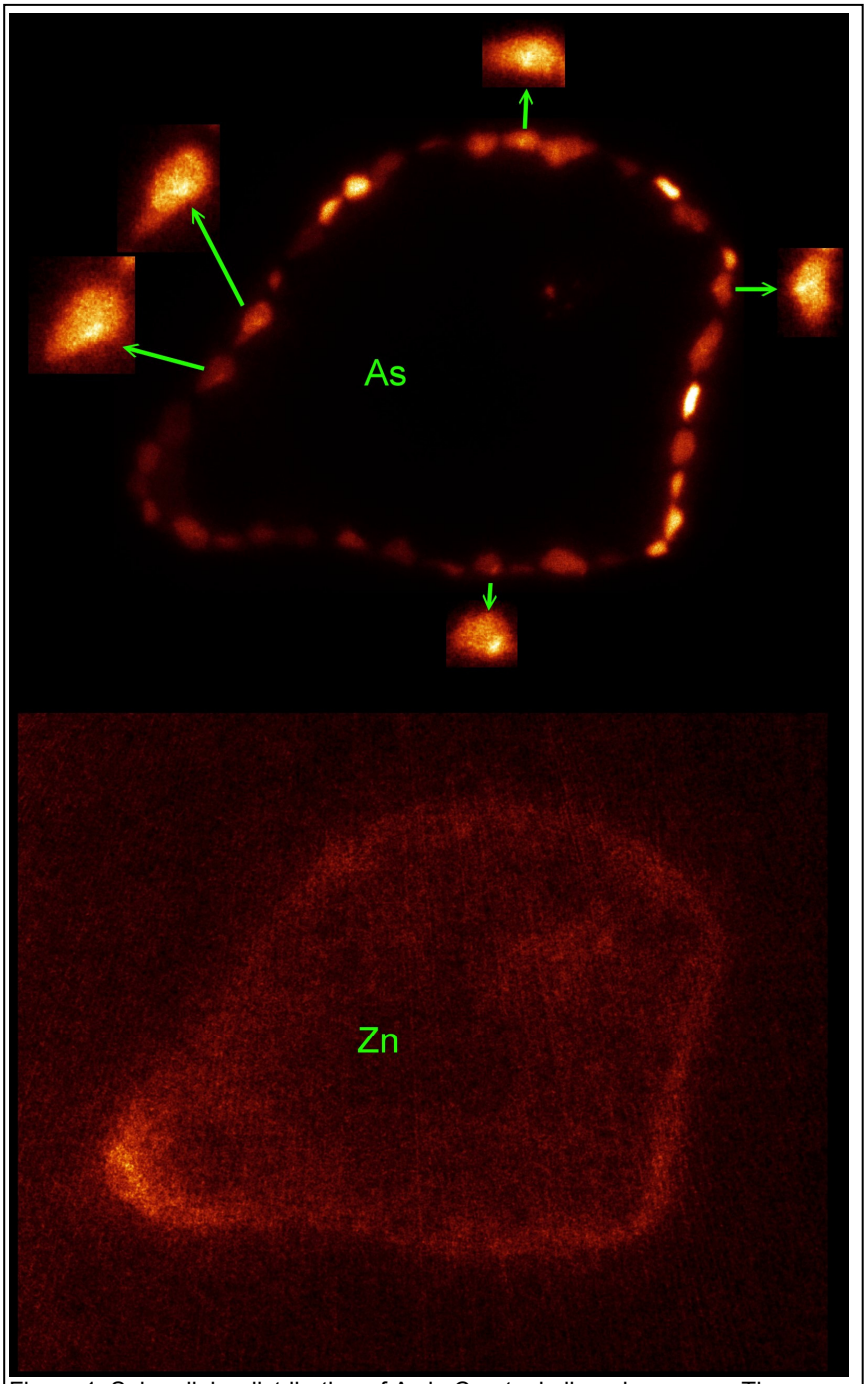


Figure 1: Sub-cellular distribution of As in Ceratophyllum demersum. The map is a preliminary one since the full quantification as published for the low-resolution data [1] is in progress.

**References**

[1] H. Küpper, P.M.H. Kroneck, Heavy metal uptake by plants and cyanobacteria. In: Metal Ions in Biological Systems, Band 44, Kapitel 5. (Eds: Sigel A, Sigel H, Sigel RKO). Marcel Dekker, Inc., New York; pp. 97-142 (2005)

[2] S. Mishra, G. Wellenreuther, J. Mattusch, H-J. Stärk, H. Küpper. Speciation and distribution of arsenic in the non-hyperaccumulator macrophyte Ceratophyllum demersum L. Plant Physiology 163, 1396-1408 (2013)

[3] S. Mishra, H.-J. Stärk, H. Küpper. A different sequence of events than previously reported leads to arsenic-induced damage in Ceratophyllum demersum L. Metallomics 6, 444-454 (2014)