Micro-X-Ray Fluorescence (2D/CT) and Laboratory Absorption Microtomography Reveal Tissue Specific Metal Distribution in Daphnia magna

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In the field of environmental toxicology, the study of the effects of the presence of transition metals such as Co, Cu, Ni and Zn on the health of pelagic and benthic invertebrates is an important research topic. The freshwater crustacean Daphnia magna is a frequently used model organism to investigate the mechanisms of toxicity of the metals mentioned above [1]. Historically, it has often been difficult to link bioaccumulation to toxic effects in daphnids because total organism digestion cannot distinguish the accumulation in critical tissues from whole body accumulation. However, in a previous Hasylab Annual Report article, we showed that the in-situ analysis of the sample volume of interest with minimal influence of the X-ray microbeam allows the investigation of the accumulation of metals within specific organs with microscopic resolution and offers an elegant solution to the previously stated problem [2].

Here, we illustrate a quantitative comparison between two differently exposed Daphnia samples. In order to make comparison possible, both images were normalised to a) measuring time 2) doris Current and 3) dead time. An estimation of the surface concentration was made using the elemental yields of a Bovine Liver Standard (NIST SRM 1577) measured under the same experimental conditions (see Fig. 1). Next to the operational elemental yields, Fig. 1 also shows the measured absolute detection limits for biological samples.

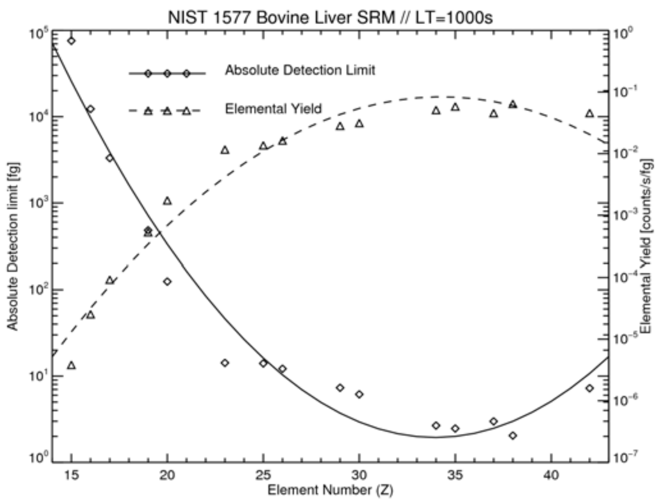


Fig. 1: Typical absolute detection limits (LT=1000s) for NIST 1577 Bovine Liver SRM using the experimental set-up at the German synchrotron facility DESY - HASYLAB Beamline L. The same experimental set-up was also used for the measurements on Daphnia. The beam size was estimated to be ~ 15 μm FWHM. Absolute detection limits for the target elements are in the femtogram range. Corresponding relative detection limits are in the 0.01-0.1 ppm range

The measured elemental distributions (See Fig. 2) have been combined with results from complementary X-ray imaging methods, namely X-ray absorption microtomography [3]. X-ray absorption microtomography offers the possibility to facilitate the interpretation and aid the quantification of 2D/3D elemental imaging results by providing a full 3D absorption model, an accurate frame of reference, for the very same sample which was analyzed by SR micro-XRF techniques. Absorption microtomography provides a complementary dataset with density information, which not only reveals the internal structure/morphology of the sample examined, but also aids the quantitative analysis of the measured 2D/3D elemental distributions. The potential of these combined micro-imaging techniques is illustrated below: by coupling information obtained by synchrotron radiation micro-XRF and absorption microtomography, it is possible to unravel the tissue-specific 2D/3D distribution of metals in-situ within delicate organic samples in an essentially non-destructive manner, as illustrated by Fig. 3

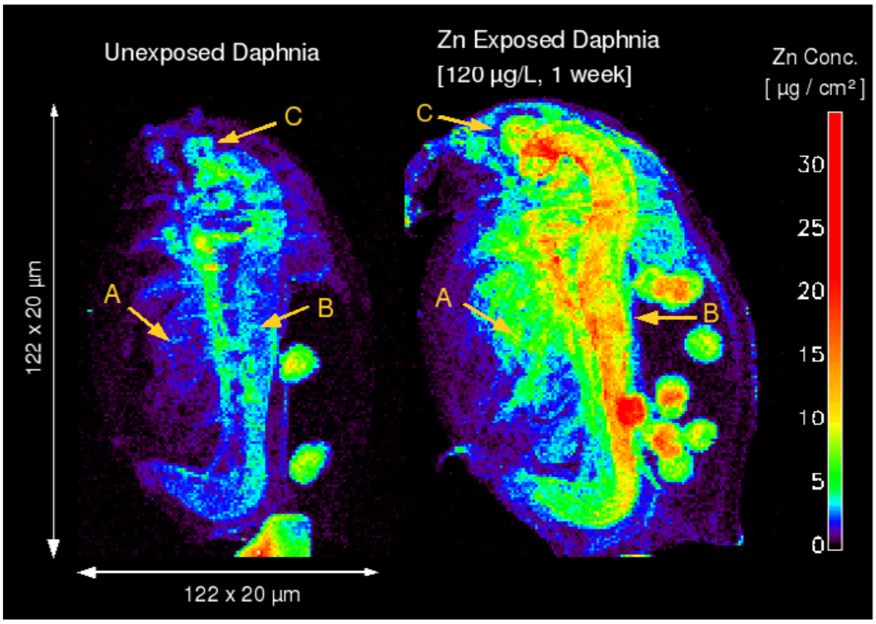
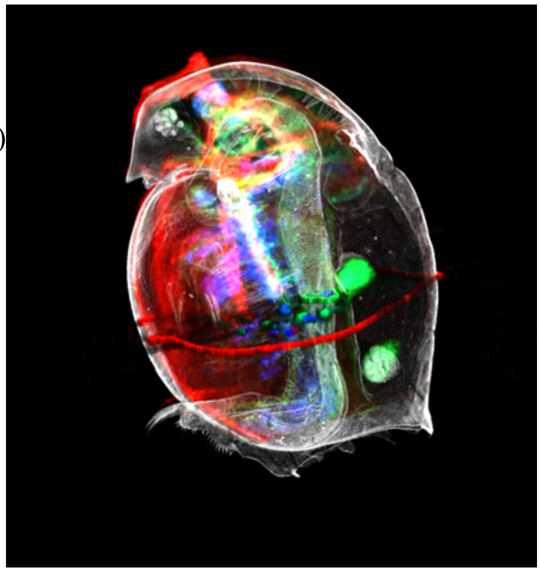


Fig 2: Micro-XRF dynamic scans on 2 differently exposed *Daphnia* samples. The left sample is an unexposed sample, the right sample was exposed to 120 µg/L Zn during one week. The accumulation of the Zn in the different tissues is clearly visible. Both images were normalized and the element contents quantified in a first order approximation, using the elemental yields obtained by measuring NIST SRM 1577 Bovine liver [Fig.1]. A color bar, which gives the areal Zn concentration in µg/cm² for both samples, is given on the right. A distinct enrichment of Zn can be observed in (A) the gill-like osmoregulatory tissue and in the digestive system: (B) the gut and (C) the digestive gland. The accumulation in gills may suggest that zinc accumulation may interfere with osmoregulation (as in fish). The accumulation in the digestive system may suggest that nutrient assimilation from food is a possible Zn toxicity target. It is presently unclear whether elevated levels of Zn in the gut region are located in the gut or in the gut epithelium. More precise analysis will be required to elucidate this matter.

Fig. 3: 3D rendered image of the unexposed *Daphnia Magna* species shown in Fig. 2 (left side) with VGStudioMax. The white dataset gives a full 3D absorption reconstruction of the daphnid (3 µm resolution) obtained via the UGCT micro/nano CT set-up at Ghent University. Two RGB composed micro-XRF datasets obtained at Hasylab, Beamline L are also incorporated in the image: a micro-XRF 2D dynamic scan (length: 175 x 20 µm, height: 122 x 20 µm) and a micro-XRF computed tomography scan (length: 165 x 20 µm, height: 165 x 20 µm) through the gill tissue, eggs and gut. The colors red, green and blue are the scaled Ca, Zn and Fe intensities, proportional to the elemental concentration. The very distinct accumulation of Ca in the exoskeleton, Fe in the gill-like tissue and Zn in the gut and eggs can be clearly observed.



References:

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[2] Micro X-Ray Fluorescence Computed Tomography and Radiography on Daphnia magna, B. De Samber, G. Silversmit, R. Evens, K. De Schamphelaere, C. Janssen, L. Balcaen, F. Vanhaecke, I. Szaloki, G. Falkenberg, L. Vinze, Hasylab Annual Report 2006, 1, 1087-1088 (2006)

[3] Octopus, Scanner independant CT reconstruction software for fan beam, cone beam and parallel beam geometry, www.xraylab.com

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