Investigation of Copper Accumulation in the marine bivalve mollusc *Mytilus edulis* with Zone plate optics

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The marine bivalve mollusc *Mytilus edulis* is a model organism used to investigate the effect of (toxic) metals on ecosystem health. Larvae of *Mytilus edilus* (48h old, 100 µm height) were exposed to Cu containing medium in different concentrations (control solution, 5µg/L Cu and 10µg/L Cu). The samples were subsequently fixed in graded water-acetone solutions and treated with 1,1,1,1-hexamethyldisilazane (HMDS). Finally, the dried specimens were glued onto a carbon tip before undergoing elemental analysis as shown in Fig. 1.

X-ray fluorescence analysis on *Mytilus edulis* was performed in the nanoprobe hutch of the P06 Hard X-ray Micro/Nanoprobe. A Si(111) monochromator was used for generating a 10 keV X-ray beam. As focussing optics, Ir-HSQ zone doubled Fresnel zone plates (FZPs) were deployed (h ~ 600 nm, d_n=25nm, 150 µm diameter, 30.3 mm focal length @ 10 keV, 7.7% efficiency @ 6.2 keV). By means of ptychographic beam reconstruction, a beamsize of 21 x 24 nm^2 was deduced. Other optical elements included: 1) slit system for beam confinement before FZPs (2) central beamstop (Ta) and (3) pinhole optic (50 µm diameter). A silicon drift detector (SII NanoTechnology Inc. USA) was used for energy dispersive photon detection. Measurement on NIST SRM 1577C (bovine liver) provided relative detection limits of 10 ppm (300ms scanning time) for the most sensitive elements as shown in Fig. 2.

Fig. 3 shows a comparison between the elemental distributions of a control/unexposed organism (0µg/L Cu exposure) and an exposed organism (10µg/L Cu exposure). Overview scans of both organisms were performed with 1µm step size to reveal the overall elemental distribution. In the overview scan of the control organism, a homogeneous Ca distribution is observed, probably corresponding to the presence of the bivalve shell. However, the Zn elemental distribution is inhomogeneous and is likely to correspond with the structure of the larvae inside the bivalve shell. *Mytilus edulis* exposed to 10µg/L Cu shows the presence of specific Cu and S 'hot spots' (as indicated with red rectangles). Detailed scans of these areas (100nm step size for the S containing area and 200nm step size for the Cu containing area) revealed the specific submicroscopic structure of the hot spots.

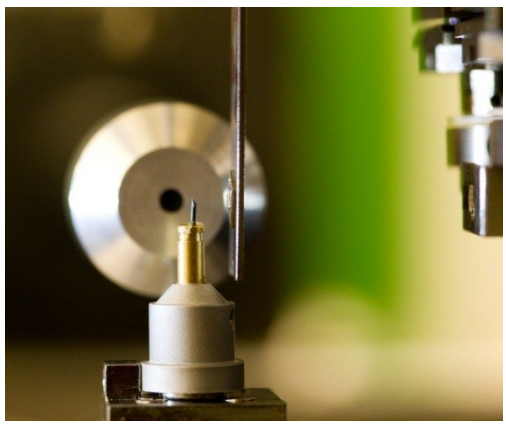

Fig. 1: Experimental setup at sample position

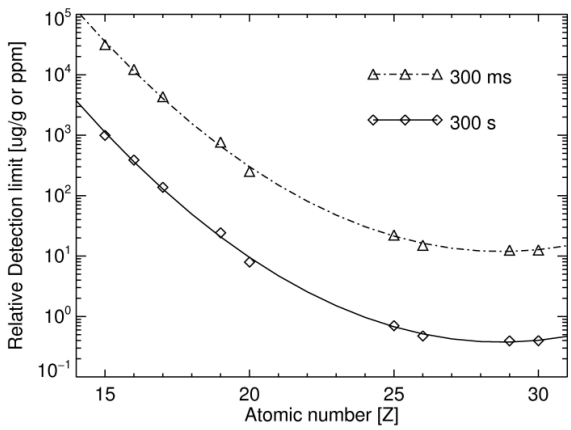

Fig. 2: Relative detection limits (RT=300ms/300s)

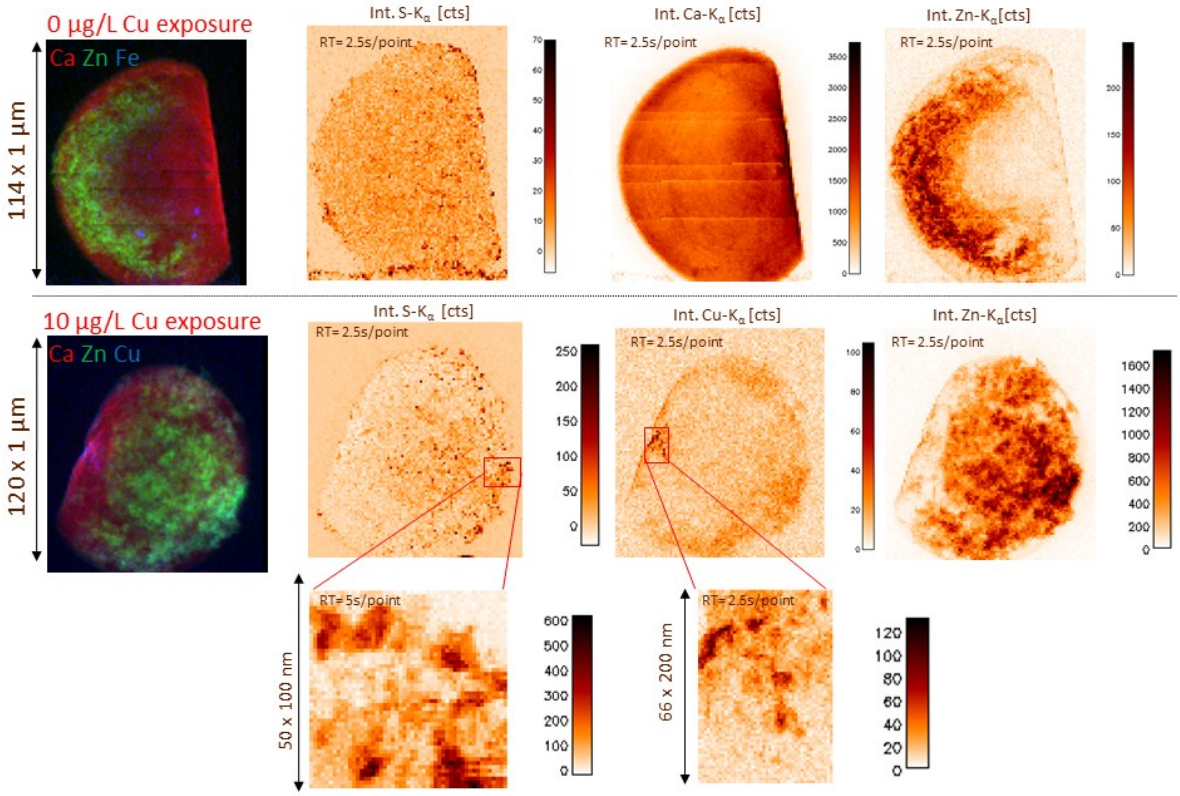

Fig. 3: Elemental distributions of S, Ca, Fe, Cu and Zn within Mytilus edulis

References

[1] C.G. Schroer, G. Falkenberg, C. David, Photon Science 2011, 'Hard X-ray nanoprobe at PETRAIII', pp. 100-101 (DESY, 2011)