ESRF	**Experiment title:** **Tissue-specific metal toxicity studies on Daphnia magna by confocal µ-XANES**	**Experiment number**: 26-01-858
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Report:

Scientific background and aim of the proposed experiments

The freshwater crustacean *Daphnia magna* (better known as the 'water flea', length ~3 mm) is an important model organism to investigate the ecotoxicological effects of exposure to transition elements such as Cu and Zn. Earlier research has provided insights into the possible physiological mechanisms by which metals invoke toxicity at the molecular and physiological level [1-2]. Since metals can only invoke toxic effects in tissues where they actually accumulate, the link between accumulation and toxicity on a tissue-specific level should be established. Tissue-specific accumulation of Zn in *Daphnia magna* has been demonstrated by our group through a combination of synchrotron µ-XRF (conventional and confocal) and laboratory absorption µ-tomography [3-5].

As a further expansion of this earlier tissue-specific study on *Daphnia magna*, we now proposed to identify the chemical state of the accumulated metals inside *Daphnia magna* using µ-XANES and confocal µ-XANES. This knowledge can considerably contribute to the understanding of how metals interact with biomolecules and give more insight into their mechanisms of toxicity. The XMI research group at the Ghent University is involved in the development of µ-XAS at DUBBLE, using a glass polycapillary half lens as focusing element [6]. By mounting a second polycapillary lens in front of the energy dispersive detector, a confocal fluorescence detection is achieved, allowing 3D resolved µ-XANES [7,8]. We have proposed to apply confocal XANES for the tissue-specific speciation of transition metals within *Daphnia magna*. This will allow to identify whether metals accumulated in a given tissue are in a toxicologically active or inactive form.

Experimental method

Daphnia magna samples were prepared according to a HMDS (hexamethyldisilazane) drying method, as used in [3,4]. The experiments focused on 3 different Zn exposure levels of *Daphnia magna*: (i) species exposed to a control Zn concentration in both growth media and algae feed stock, (ii) species exposed to an excess of Zn in their growth medium and (iii) species fed with algae exposed to an excess of Zn.

Zn-K XANES spectra of biological relevant amino acid solutions (ZnAc, ZnGlut, ZnHist and ZnCyst) and of a variety of inorganic Zn compounds were recorded as reference spectra. The algae cells and the growth media for *Daphnia magna* and algae feed stock were also studied at their control and exposure Zn concentration levels.

Results obtained

The data analysis of the XANES spectra is not yet finished, but the preliminary linear combination analysis already indicates the following trends.

Zn-K XANES reference spectra

The measured Zn-K reference XANES spectra are given in Figure 1, left.

Growth media

A shift of the absorption edge towards lower energy values is observed for an increased Zn concentration in the *Daphnia magna* growth medium, pointing towards a larger fraction of Zn-S bonds (possible candidates are ZnS, ZnCyst and/or ZnGlut) see Figure 1, middle.

Algae

The Zn-K XANES spectrum for the algae grown in a medium with higher Zn concentration have a larger edge position, suggesting to have less ZnCyst and/or ZnGlut compared to the non-exposed algae, see Figure 1, right.

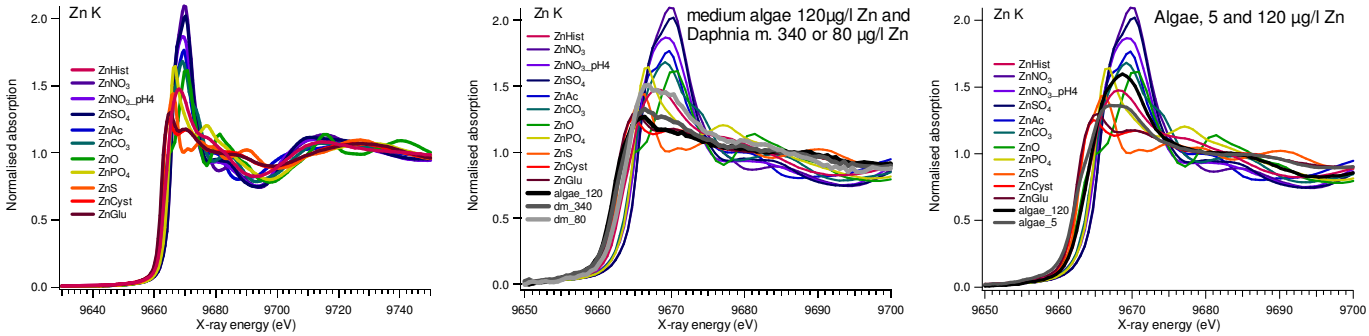

Figure 1: Normalised Zn-K XANES spectra for (left) the reference compounds measured; (middle) Daphnia magna and algae growth media; (right) algae at different Zn level exposure..

Daphnia magna

Due to a cascade of technical problems (failing Vortex fluorescence detectors, issues with the integration of the XIA xmapscan with the EXAFS acquisition software), the confocal detection was not achieved during this beamtime. The tissue-specific analysis could therefore not be performed. We recorded however broad beam spectra on the proposed *Daphnia magna* samples at selected heights corresponding to the digestive gland, osmoregulatory system and eggs. Due to the use of the broad beam, it is not clear which tissues were measured exactly, the spectra however suggest that the head region contains more ZnHist compared to the eggs for example.

References

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