ESRF	**Experiment title: Analysis of lanthanide localizations at physiological and sublethally toxic concentrations in the green alga *Desmodesmus quadricauda***	**Experiment number**: LS-2847
Beamline: ID-16A	**Date of experiment**: from: 10 Nov 2018 to: 16 Nov 2018	**Date of report**: 18 February 2020
Shifts: 15	**Local contact(s)**: Dr. Peter Cloetens	*Received at ESRF:*

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Report:

Metal uptake by algae has many applications that range from sewage treatment to precious metal recovery. Further, many aspects of metal uptake and metabolism are common in algal, animal and even bacterial cells, thus microalgae can serve as a useful model for other organisms. The aim of the experiments at ESRF was to reveal the localization of lanthanides in organelles of the cells, in order to understand better in which way they interact with cellular metabolism.

For sample preparation, a dense suspension of *D. quadricauda* coenobia was filled into 0.3 mm diameter polyimide capillaries, which were mounted on sample holder and shock-frozen in supercooled isopentane. Subsequently, the samples were stored in liquid nitrogen until they were measured in the cryochamber of beamline ID16A. This technique efficiently preserved sample integrity, as shown also by the phase contrast images of taken at this beamtime (Fig. 1) - as suggested by the referees, we combined the µXRF tomography with a second technique (here: phase contrast tomography) in order to obtain structual information.

For the µXRF measurement, the beam was focussed at 17 keV, and single-slice tomograms were measured by scanning lines across the sample at various rotation angles. X-ray fluorescence was detected with 2 single-element silicon drift detectors (SDD's) coupled to Xpress2 readout electronics. XRF spectra were deconvoluted and background subtracted. Tomograms were reconstructed using the filtered backprojection (FBP) and Maximum-Likelihood Expectation-Maximization (MLEM) algorithms, where also the quantification was performed by our beamline contact Dr. Peter Cloetens. The MLEM reconstructions and full quantification with tomographic standards are not complete yet, but we now submit this report of "work in progress" not to block our next beamtime application by this delay. Further image processing (smoothing, contrast, colour scales) was performed in ImageJ.

The beamtime became a success, yielding data on La distribution in the cells, which are now part of a larger study that is about to be submitted for publication. At sublethal La concentrations (100 nM), La was accumulated inside the cells, often in small spots (biominerals?) (Fig. 1). This means that under these conditions most of the La is in direct contact with the metabolism of the cells, which can explain the

surprisingly high toxicity that was observed in our experiments. The combination of these µXRF results with metalloproteomics analysis by HPLC-ICPMS showed that at this concentration La had not only a physiologically important localisation, but was specifically bound to one approx. 20kDa soluble protein. Current work focusses on the identification of this protein, with the subsequent aim of its biochemical and physiological characterisation. At a lethal La concentration (1000nM), however, essential elements like potassium and zinc became released through leaky membranes, and La bound more to the cell wall (Fig. 1). In this scenario, effects of La are less specific because the cell wall is physiologically not directly active but only acts as a mechanical support, as an ion binding matrix and to some extent as a barrier (filter).

Although the beamtime was altogether a success, we would like to note that some optimisations of the beamline would allow for even better results in the future. The most important would be the implementation of faster detector readout electronics, as the slow readout capabilities of the existing hardware forced us to reduce the count rate, which in turn restricted the number of pixels and thus the resolution of the µXRF tomograms. Additionally, we suffered from some software instability that caused crashes of scans and that was caused by interference from an automated backup.

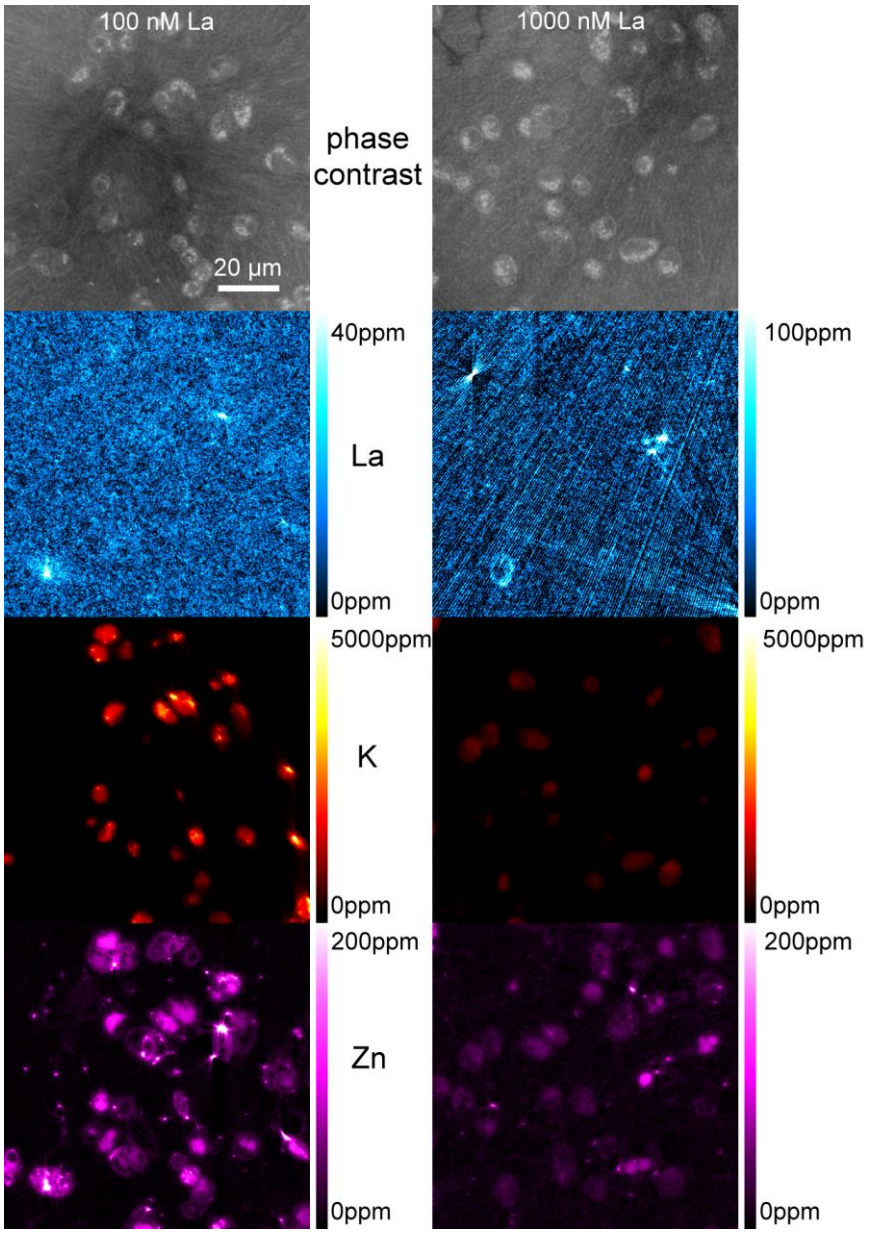

Fig. 1. Phase contrast tomograms and µXRF maps of concentrations of key elements in *Desmodesmus quadricauda* coenobia treated with 100 nM or 1000 nM lanthanum.