ESRF	**Experiment title:** Toxicity of CeO_2 nanoparticles on a freshwater experimental trophic chain: insight into the involved mechanisms through the use of mesocosms	**Experiment number**: EV 160
Beamline: ID21	**Date of experiment**: from: 23/07/2015 to: 29/07/2015	**Date of report**: 25/02/2015
Shifts: 15	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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Report: The goal of our experiment was to investigate the spatial distribution and speciation of Ce in an aquatic trophic chain. The specificity of the experiment was that these animals were exposed in an aquatic mesocosm mimicking a natural environment including mineral water, sediments, primary producers (diatoms), primary consumers (chironomid larvae) and final consumers (pleurodele larvae). Contamination was done by repeated additions of CeO_2 nanoparticles (NPs) to reach a final concentration of 1 mg/L after 4 weeks.

Pleurodele larvae organs were dissected in spleen, liver, external gills, internal gills, intestine and gallblader. Pleurodele organs and individuals (for diatoms and chironomid larvae) were then embedded in OCT resin and sectionned on ID21 cryomicrotome (30 µm). Cross-sections were analyzed using the cryogenic set-up of ID21 with an incident beam of 5.8 keV focused at 1 µm.

Ce was detected as a diffuse signal at the surface of internal gills (*ie.* the part inside the body of the larva and connected to the external gills) and in the intestine associated with S, Ti (Figure 1) and Ba. Ti and Ba were part of the sediment which suggests that Ce had been taken up together with soil particles throughout the food chain or by direct uptake. The presence of S can also indicate the trigerring of detoxification mechanisms in pleurodele larvae. Ce was not detected in the other organs observed because either it did not reach those organs or because it was under detection limits of the technique. However, the most probable hypothesis is that gills played well their role of filter and that the Ce found in the intestine is the result of dietary uptake. Ce was not often detected in the intestine meaning that an efficient depuration process had taken place and that Ce is not redistributed inside the animal body.

It was not possible to map other individuals of the trophic chain in the mesocosm because they had been either eaten or mixed to the sediments. So we investigate the fate of CeO_2-NPs in pleurodele and chironomid larvae and diatoms under monospecific exposure (one species + water + NPs). Results for the pleurodele larave were the same as in the mesocosm conditions. Chironomid larvae were found with a very high Ce content in intestine lumen (Figure 1), also associated with Ti and Ba. Chironomids are filter dwellers; they ingest sediments which explains the presence of Ba and Ti together with Ce which has probably sedimented at the interface between water and sediment. Diatoms are microalgae (10 to 20 µm) having an external skeleton made of Si. For some but not all of the indiviuals we detected a colocalisation between Si and Ce signals (also associated to Ca, S and P). However, the lateral resolution was not high enough to conclude if the detected Ce was adsorbed on the skeleton of diatoms or included in the skeleton or even internalized inside the algae.

The second goal of our experiment was to investigate Ce speciation throughout the food chain. However, in the mesocosm experiment the concentration found in the different organs was too low to obtain good quality XANES spectra.

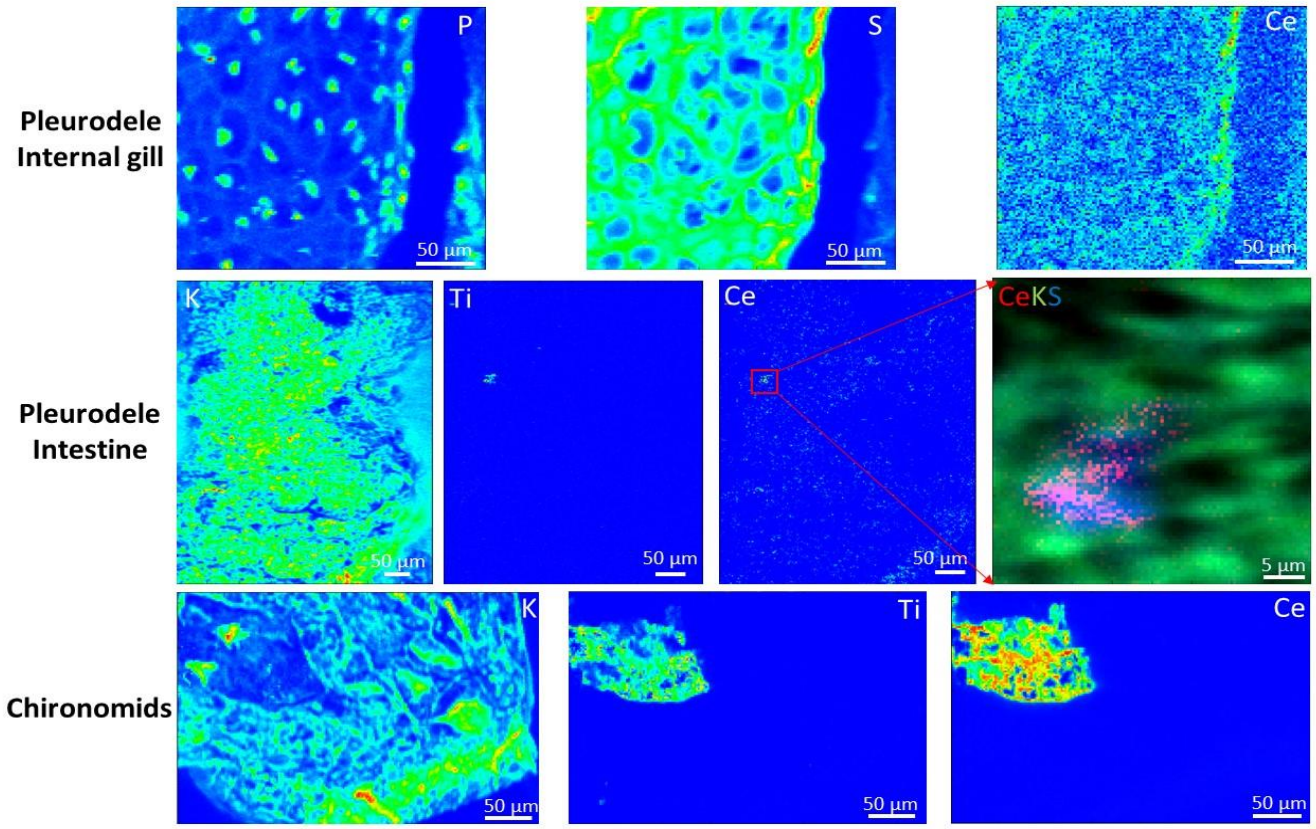

Figure 1. Elemental µXRF maps of pleurodele larvae (mesocosm) and chironomid larvae (monospecific exposure).

XANES acquisitions were thus performed on samples from monospecific exposure (in that case several exposure concentrations were available, up to 10 mg/L). Ce in the NPs is Ce(III) and was also detected in this form in the diatoms and the chironomid larvae under monospecific exposure conditions (Figure 2). However, in the sediments from the mesocosm, Ce was also present under Ce(IV) form (Figure 2, sediment). This form is potentially more toxic than the Ce(III) which could explain the toxicity symptoms noticed on pleurodeles in the mesocosm and not in monospecific conditions.

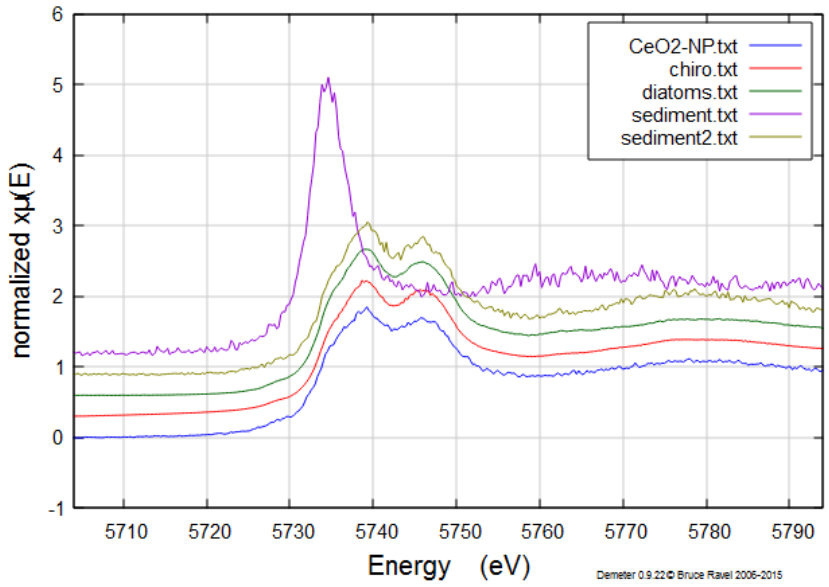

Figure 2. µXANES spectra recorded in situ.

Scientific production related to this experiment

A paper is currently under redaction. It will come in support of a previously published article (Bour et al., 2015: Toxicity of CeO_2 nanoparticles on a freshwater experimental trophic chain: A study in environmentally relevant conditions through the use of mesocosms, Nanotoxicology).