EUROPEAN SYNCHROTRON RADIATION FACILITY
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Experiment Report Form

	Experiment title: **Iron uptake and binding by phytoplankton – a key parameter in climate global change**	**Experiment number**: 20170410
Beamline: FAME-UHD	**Date of experiment**: from: 07 November 2017 to: 14 November 2017	**Date of report**: 15/02/2018
Shifts: 18	**Local contact(s)**: Olivier Proux	*Received at ESRF:*

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Scientific background and objectives:

Marine phytoplankton species play an important role in the biological carbon pump but iron (Fe) is a limiting nutrient for their growth. It is unknown how the ocean acidification resulting from the global rising CO_2 will affect Fe availability, but no prediction is possible without taking account the mechanisms of uptake and sequestration of Fe by phytoplankton. Our objective was to characterize the Fe speciation in two plankton species, the diatom *Phaeodactylum tricornutum* and the calcifying coccolithophore *Emiliana huxleyi*, particularly the Fe status in the cell wall, which is likely to play an important role in Fe binding. For that, Fe K-edge High Energy Resolution Fluorescence Detected (HERFD)-XAS using the Crystal Analyzer Spectrometer (CAS) was performed at the new FAME UHD beamline.

Experimental:

Diatoms *P. tricornutum* and coccolithophores *E. huxleyi* were grown in synthetic seawater and exposed to 1 µM ferric citrate at pH 7.9. Cells were harvested, rapidly washed and centriguged. Some cells were treated with a mixture of chemical extractants (EDTA and DFOB) to remove Fe bound to the cell walls, resulting in washed cells. Organic cell walls were extracted from cells by sonification and digitonine. Bulk plankton cells, washed plankton cells, and plankton cell walls were then prepared as bulk frozen pressed pellet in liquid nitrogen. They were kept at low temperature until measurements.

As the CAS set-up was used for the first time to speciate Fe, Fe model compounds including mineral and organic forms with various oxidation states and coordinations needed to be prepared and analyzed as solid pellets or aqueous solutions.

Samples were positioned in the He cryostat operating at 12K. Fe K-edge HERFD-XANES spectra were collected by selecting the Fe $K\alpha_1$ fluorescence line using 3 Ge(440) crystals while the diffracted intensity was collected with a one SDD detector. The beamline operates with a monochromator Si(220).

Results and conclusions of the study:

First, results attested the higher sensitivity of HERFD-XANES in comparison to standard fluorescence measurements particularly at the pre-edge level (Fig. 1) as shown by Fe model compounds with Fe(II) in a tetrahedral coordination (chromite) and an octahedral coordination (siderite, fayalite) and with Fe(III) in a tetrahedral coordination (FeIII-phosphate) and octahedral coordination (hematite, goethite). The edge and the pre-edge features of spectra in the HERFD mode are particularly sensitive to the oxidation state of iron and coordination number (Fig. 2). At the first glance, spectra collected on *Phaeodactylum* and *Emiliana* show an edge typical of an oxidated state of Fe. They also show a shoulder after the white line (arrow if Fig. 3), similarly to FeIII-phosphate and phytate spectra. A closer inspection of the edge and pre-edge features indicates variations of Fe depending on the treatment (washing and cell extraction) and plankton species (Fig. 4). After washing, the spectra are shifted to lower energy values for both *Phaeodactylum* and *Emiliana*, suggesting that the proportion of ferrous iron remaining in the cells is higher. We hypothesize that a part of Fe could be associated to the remaining mineral exoskeleton, calcium carbonate for *Emiliana* and silica for *Phaeodactylum*. Spectra for the cell walls for both algae are similar, suggesting a similar binding at the cell surface, where Fe is likely present as a ferric form.

Data treatment is in progress to deconvolute pre-edge features (Wilke et al., 2001) and interpret the spectra by a linear combination fitting approach.

Finally, we tried to collect the XANES spectra of the culture medium containing 1 µM Fe (< 100 ppb Fe). Although only 3 to 4 fluorescence counts per second could be detected, a spectrum could be obtained with the CAS set-up, paving the way for the investigation of highly diluted systems.

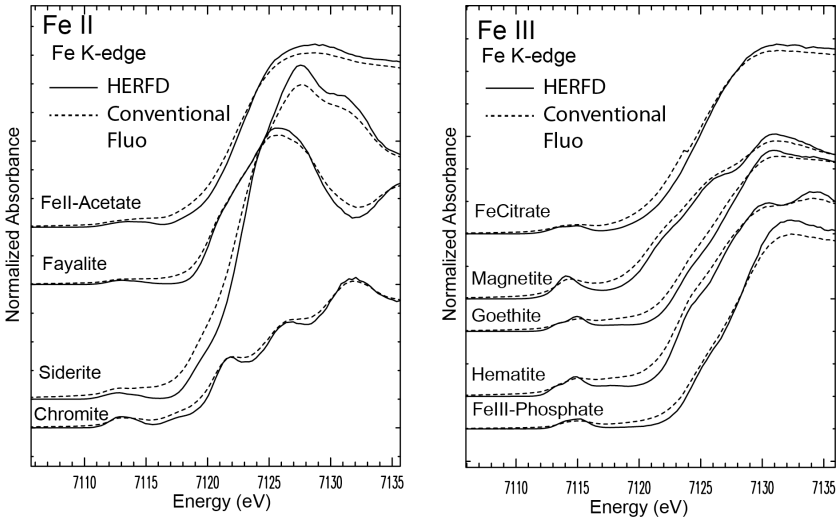

Fig. 1 : Fe K-edge XANES spectra of some Fe(II) and Fe(III) model compounds collected in HERFD mode and conventional fluorescence mode.

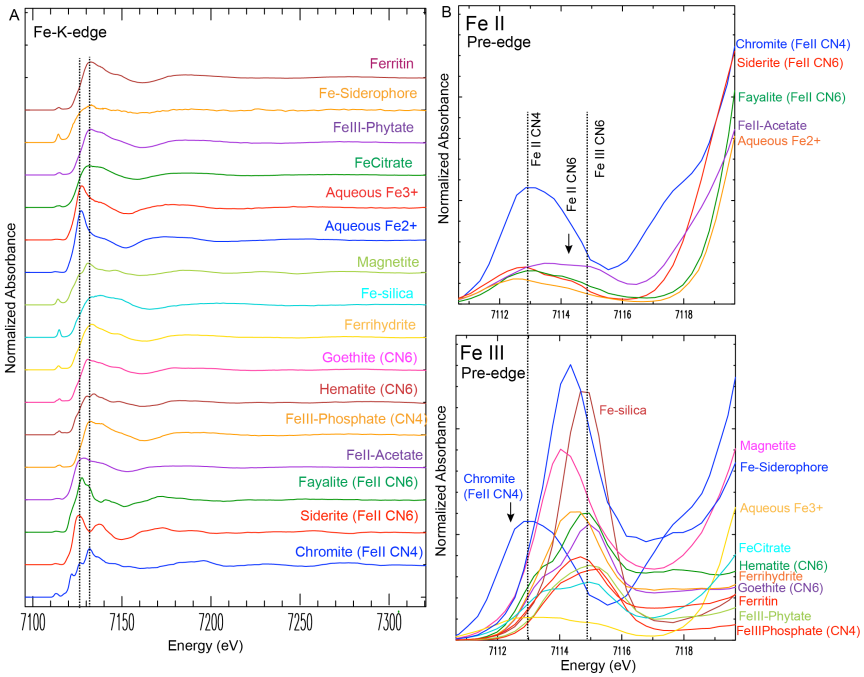

Fig. 2 : Fe K-edge HERFD-XANES spectra of some Fe(II) and Fe(III) model compounds (A) and zoom on the pre-edge features (B).

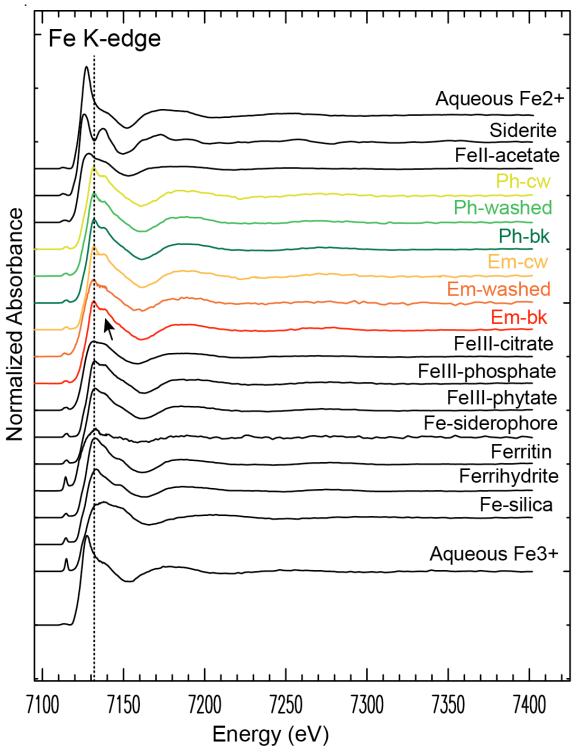

Fig. 3 : Fe K-edge HERFD-XANES spectra of Phaeodactylum (Ph) and Emiliana (Em), as bulk, washed or cell wall samples, compared to Fe model compounds.

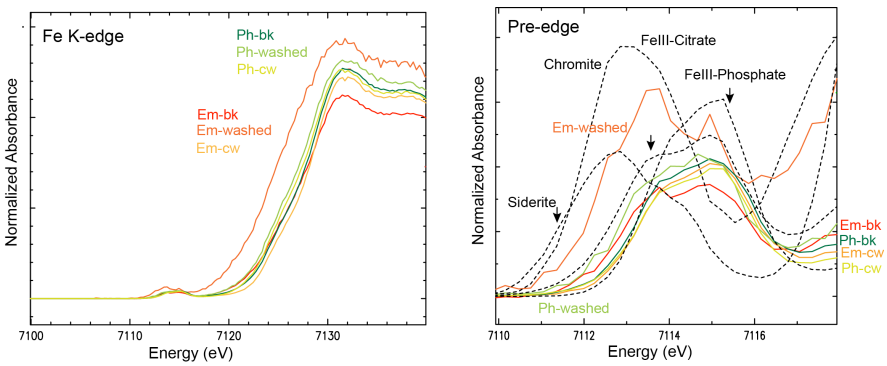

Fig. 4 : Zoom on the edge and pre-edge of Fe K-edge HERFD-XANES spectra of Phaeodactylum (Ph) and Emiliana (Em), as bulk, washed or cell wall samples, compared to Fe model compounds.

Justification and comments about the use of beam time:

The new beamline worked very well. Beamtime was dedicated to the measurements of Fe model compounds (~ 15) and some were diluted. We also investigated the two algae grown at pH 7.9 and various treatments (bulk, washed and cell walls). The alignment of the CAS is time consuming and 2 shifts were used for this step. The use of the cryostat is also time consuming (2 hours are necessary to change sample holders).
The aim of our ANR project is to determine the Fe sequestration by plankton species and the effect of an acidification. We started our work by investigating one pH and we now need to determine the effect of a lower pH on the Fe binding. It is also important to determine if the mineral skeletons sequester iron. We thus applied for additional beamtime to accomplish this study before the ESRF shutdown at the end of 2018.

Publication(s):

Data treatment is still in progress (pre-edge deconvolution and linear combination fitting approach). We need additional data on the effect of an acidification for a publication.

References :

Wilke M. et al. 2001. Oxidation state and coordination of Fe in minerals: An Fe K-edge spectroscopic study. *Am. Mineralogist* 86: 714-730.