ESRF	Experiment title: **Chemical environment and speciation of adsorbed and incorporated Cd and Zn in living diatoms**	**Experiment number**: SI-853
Beamline: ID 26	**Date of experiment**: from: 30 January 2003 to: 4 February 2003	**Date of report**: 25 February 2004
Shifts: 15	**Local contact(s)**: Dr. Laurent Alvarez, ID 26, ESRF	*Received at ESRF:*

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Report:

Experimental: Adsorption of Zn^{2+} and Cd^{2+} on growing marine and freshwater diatom cells was studied using conventional macroscopic techniques which allowed us to identify the nature and to determine the concentration of the major surface functional groups (carboxyl, amine and silanol) responsible for the amphoteric behavior of cell surfaces and their affinity to trace metals (Pokrovsky et al., 2002; Gélabert et al., 2004a). Local atomic structure of metal ion adsorbed on or incorporated into cells at 25°C and various pH (3 to 8) was characterized by in-situ XAFS spectroscopy. XANES and EXAFS spectra of solutions, live suspensions and freeze-dried cells containing Zn (from 10 to 300 ppm dry weight) were recorded at Zn K-edge (9.66 keV) in fluorescence mode at ID 26 beamline of ESRF. Details of spectra acquisition and treatment procedure were similar to those described by Pokrovsky et al. (2004).

Results on Zn local atomic structure in diatom cultures: Attempts of beam optimization at Cd K-edge (26.7 keV) at the undulator-based ID26 beamline failed. Consequently, all measurements were performed on Zn K-edge. Because of the fragility of organic samples used (aqueous suspensions of cells, freeze-dried biomass) and low concentration of Zn (20-50 ppm for non-contaminated "most interesting" samples with incorporated Zn), long acquisition time required changing the beam position at the sample for each scan to prevent sample degradation and bubbles formation induced by the high X-ray photon flux. Optic microscopic observations of reacted cells revealed that cells remained whole and intact, the structure of frustules and organic coatings being well preserved and the chloropolasts remained colored. One of the important methodological findings of this study is that the XAFS spectra of Zn associated with live and freeze-dried cells are almost identical.

For zinc adsorbed on diatom frustules ($SiO_2 \cdot nH_2O$) or amorphous silica, similar local atomic environment of tetrahedrally coordinated metal with Zn-O distances of 1.94 ± 0.01 Å was revealed. The second neighbor (Si) was detected at 3.56 ± 0.01 Å for diatom frustules. Modeling of diatom cultures spectra shows that both adsorbed and incorporated Zn have an oxidation state of +2 and is surrounded by 4 ± 0.5 oxygens/nitrogens with an average Zn-O distance of 2.00 ± 0.02 Å for adsorbed Zn and 1.97 ± 0.02 Å for Zn incorporated inside the cells. The presence of nitrogen in the first coordination sphere of incorporated Zn allowed some better description of EXAFS spectra. The chemical status of Zn adsorbed on freshwater peryphytic species, *Achnanthidium minutissimum* and *Navicula minima*, and marine planktonic cells (*Skeletonema costatum*) was found to be almost identical: a tetrahedrally coordinated with N/O atoms (Fig. 1). This strongly suggests that the mechanism of Zn interaction with different types of diatoms is essentially the same and no significant change in the zinc environment in diatom cells occurs as a function of level of exposure or of time of exposure. However, Zn incorporated in *Skeletonema c.* during its growth from Zn-bearing solution exhibits a clear presence of two sulfur atoms in the first coordination sphere at a distance of 2.33±0.02 Å and two oxygen/nitrogen atoms at 2.02±0.02 Å. Such an environment, consistent with mixing of cysteine and

hystidine ligands, suggests specific detoxification mechanisms, like the phytochelatin production, which is well documented for marine diatoms (Roberts S.B., Lane T.W., Morel F.M.M. (1997) *J. Phycol.* **33,** 845-850). This mechanism is not identified for the freshwater species (AMIN, NMIN and MVAR). At the same time, the adsorption of Zn on *Skeletonema c.* is unlikely to be coupled with phytochelatin cysteine-bearing ligands or zinc-histidine complexes production (Cox E.H., McLendon G.L., Morel F.M.M. et al. (2000) *Biochemistry* **39,** 12128-12130); rather, similar to freshwater species, fixation of Zn on the outer polysaccharidic matrix prevents its further penetration through the membrane.

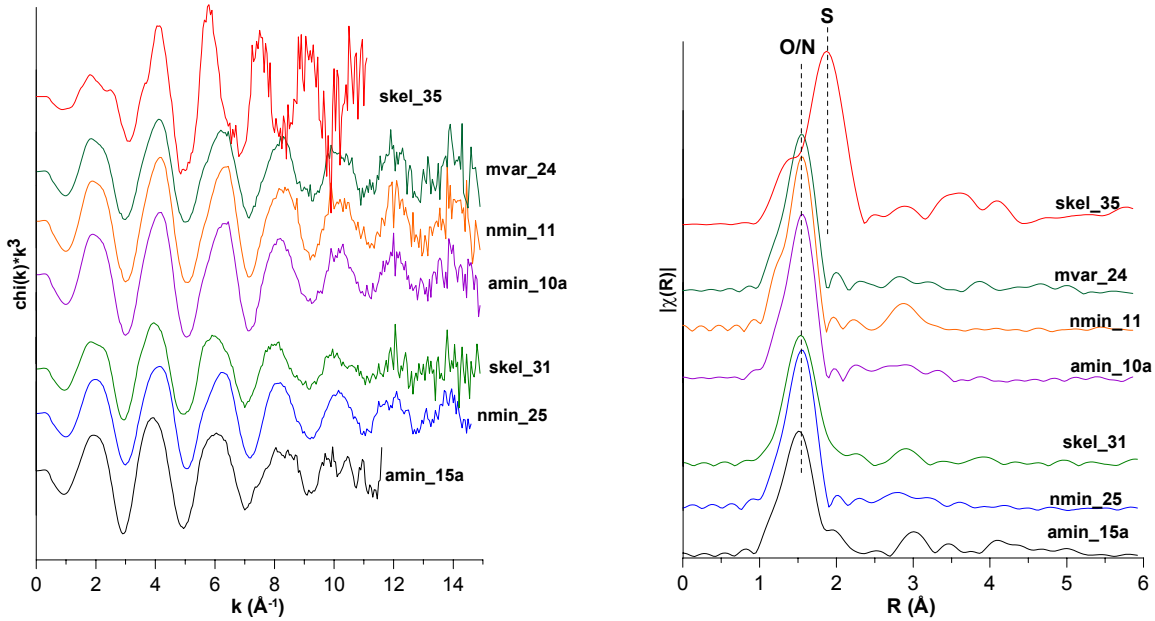

Fig. 1 Normalized k^3-weighted EXAFS spectra of selected Zn samples and their corresponding Fourier Transforms. Skel_35 = *Skeletonema c.* (SC), low Zn incorporated; skel_31 = SC, high Zn, adsorbed; mvar_24 = *Melosira var.,* low Zn, incorporated; amin_15a = *Achnanthidium m.* (AMIN), high Zn, adsorbed; amin_10a = AMIN, low Zn, incorporated; nmin_11 = *Navicula m.* (NMIN), low Zn, adsorbed; nmin_25 = NMIN, high Zn, adsorbed.

Overall these results are consistent with previous microscopic observations (Gelabert et al., 2004a): the diatom cell wall structure can be viewed as a layer of opalline silica (frustule) attached to a protein template from the interior of the cell and covered by a polysaccharide layer bearing negatively charged $>COO^-$ moieties. Metal speciation on the outermost cell walls (short-term adsorption) is controlled by Zn-COO surface complexes while under longer exposure time, Zn binding to N-bearing groups from the cell wall proteins is possible.

Conclusions and perspectives: Our EXAFS observations allowed, for the first time, to infer in the molecular mechanisms of Zn interaction with diatom cultures. They demonstrated that during long-term incorporation into cells and short term adsorption on diatoms surfaces, zinc reduces its coordination number from six (octahedrally coordinated H_2O in bulk solution) to 4 or 5 (oxygen and nitrogen coordinated complexes). Such an important change of metal local environment is likely to produce a significant isotopic fractionation which has been evidenced by measuring $^{66}Zn/^{64}Zn$ isotopic fractionation between diatoms and Zn-bearing solution using a multi-collector ICP-MS (Gélabert et al., 2004b). Further work should be devoted to another environmentally important pollutant, cadmium, which is often considered as biological analogue of Zn for diatoms.

References:

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