ESRF	**Experiment title:** High resolution XANES mapping of phosphorus in calcareous biominerals: implications for the use of calcareous proxies in environmental studies	**Experiment number**: LS 2371
Beamline: ID 21	**Date of experiment**: from: 24 oct 2014 (8:00) to: 27 oct 2014 (8:00)	**Date of report**:
Shifts: 9	**Local contact(s)**: Murielle SALOME	*Received at ESRF:*

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Report:

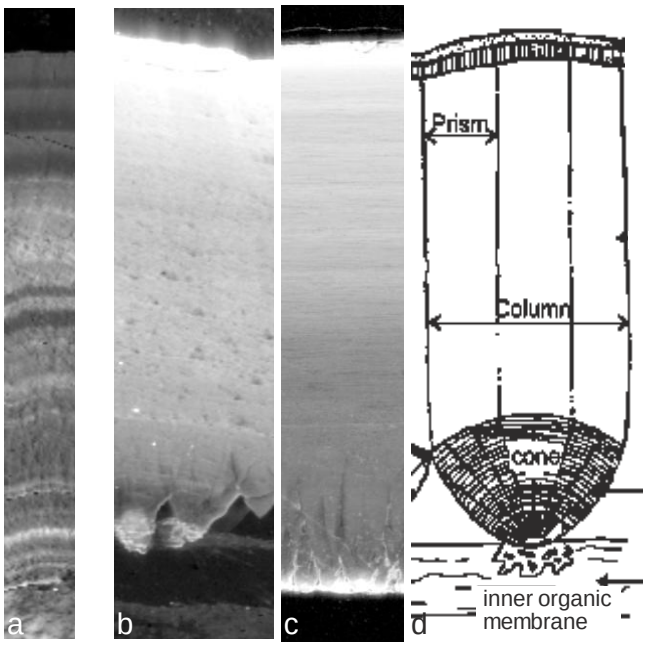
Aims of the experiment: $CaCO_3$ biominerals contain minor elements such as P, but their role is not well established: is P linked to the mineral lattice or a part of the shell organic matrix? As P is also considered to be an environmental proxy and a marker for diet and/or pollution, understanding the speciation of P in calcareous biominerals (eggshells, gastroliths, molluscs) is a key-question for environmental reconstructions, fisheries, poultry farming and fundamentals about biocrystallization mechanisms.

Previous data: the role of P in $CaCO_3$ biomineralization has been repeatedly shown, as well as its potential role as an "environmental monitor data". Nevertheless, data on P in calcareous skeletons are very rare. Quantitative analyses done using electron microprobes (WDS) and chemical maps have shown that, despite low contents (500 ppm), well spatially resolved maps can be obtained.

Results: First, reference spectra have been acquired on several standards. Geological apatite, dibasic Ca phosphate for minerals, phytic acid and glucose 6P as organic phosphate in sugars, and caseine for a phopshate in a protein. XANES spectra of these 3 categories of PO4 differ. Native P was not tested.

Then, XANES spectra have been acquired on the organic matrices extracted from the biominerals. All of them are calcareous: gastroliths from Arthropods, avian eggshells, mollusks. The organic matrices were obtained after decalcification and centrifugation, and were separated in insoluble and soluble organic matrices. All the organic matrices contain organic phosphate. A more detailed examination is now necessary to precize the link between the phosphate group and the sugars and/or the proteins of the matrices because of the similarities of the standard spectra obtained.

A third step of the experiment was to obtain distribution maps to reveal possible gradients or/and heterogeneities related to the microstructural organization of the samples.



Phosphate maps of a gastrolith (a) showing a banding pattern, and two eggshells (b, c) showing the high phosphate content of the inner mammillary layer (white), and a regular gradient form the inner part towards the outer surface, as well as growth lines.

Here three examples are illustrated. Maps were performed at 2.15 keV. Each sample shows a good correlation between the structure and the distribution map of phosphate groups. The micrometer scale resolution at ID1 allows to see the growth lines in all studied samples.

Additionally, some XANES spectra have been acquired in situ in selected zones. The complex profiles of the spectra suggest the potential existence of weak quantities of mineral phosphate in addition to phosphate groups of organic origin. Thus, more detailed analyses of the spectra are now necessary to unravel the composition and complexation of phosphate groups.

Conclusion: These results confirm the presence of P in the form of phosphate groups in calcareous biominerals. Moreover, they confirm that the organic components are not only composed of C, H, O and N. Not only sulphur and magnesium exist, but phosphorus also. The studied examples belong to only three major taxa (invertebrates: crustacea arthropods, mollusks; vertebrate: avian eggsgells), and they represent permanent (mollusks) or temporary structures used as reservoirs (gastrolith, eggshell). They are composed of calcite, aragonite or amorphous calcium carbonate. Nevertheless, all samples possess phosphate groups, probably associated to both the mineral and the organic phases.