UNDERSTANDING THE RISE OF BIOMINERALIZATION THROUGH THE USE OF X-RAY TECHNIQUES: PHOSPHATE DETECTION ON FOSSILS. F. Rodrigues[1], M.L.A.F. Pacheco[2] and D. Galante[3] [1]Institute of Chemistry, University of Sao Paulo (Av. Prof. Lineu Prestes, 748, Butantã, CEP 05508-000, São Paulo, SP, Brazil, farod@iq.usp.br) , [2] Department of Biology, Universidade Federal de São Carlos – Campus Sorocaba, (Rodovia João Leme dos Santos (SP-264), Km 110, Bairro do Itinga - Sorocaba - São Paulo – Brasil CEP 18052-780, forancelli@ufscar.br), [3] Brazilian Sincrotron Light Laboratory, Centro Nacional de Pesquisa em Energia e Materiais (Av. Giuseppe Máximo Scolfaro, 10.000, Bairro Gurá, Campinas, SP, Brazil, CEP 13083-100, douglas.galante@lnls.br)

Introduction:

The rise of hard parts: evolutionary consequences.

We know very little about the early life on Earth, including the Ediacaria biota (580 – 542 Myr), because it was composed mainly by soft-tissue organisms. This fact had implications on their preservation on the fossil record, which was very poor.

At the evolutionary moment known as an apparent "explosion" of life in Cambrian, it was observed a rapid rise on the preserved richness, and the appearance of ecological relations, such as between predator and prey in fossil record. Concomitant to these changes, it was observed the appearance of hard parts, first on rigid organic exoskeletons and then shells, and later inner skeletons. One of the first hard parts were probably the result of biomineralization, which is the formation of minerals due to biological intervention. Associated to organic compounds, such as fibrous proteins like chitin, they formed different organomineral parts, mainly made of calcium phosphate or carbonate, which conveyed resistance and strength to the body, ensuring the survival of new species in a very competitive world.

There are still many controversies around this important evolutionary innovation, and the use of new analytical techniques is contributing to unveil the mechanisms and details of the appearance of the first biological hard parts.

The detection of Phosphate.

In this specific work, we concentrated on the detection of phosphorous, present in association to many biomolecules on the form of calcium phosphate - $Ca_3(PO_4)_2$ – as in many current crustaceans. On the form of nanocrystals (~ 20nm), associated to proteins, such as chitin, it forms a very rigid carapace [1]. Detecting phosphorous by a non-destructive technique is not easy, as it is a light element. Atomic spectroscopic methods normally require the destruction of the material and large quantities of it. Non-destructive techniques, such as micro FT-IR or Raman, or SEM-EDS, are not sensitive enough to the small amounts of phosphorous that last on the fossils, after hundreds of millions of years exposed to weathering, geological and geochemical alteration.

To overcome these difficulties, our group has used X-ray fluorescence spectroscopy, performing the experiments in ultra-high vacuum (to avoid air absorption) at the SXS beamline of the Brazilian Synchrotron Light Laboratory (LNLS), and adapting an X-Ray Absorption Near Edge Spectroscopy (XANES) method to allow the simultaneous quantification and characterization of the phosphorous [2].

Results and discussion: In order to do absolute quantification of P, we constructed calibration curves mixing $Ca_3(PO_4)_2$ in different substrates to compare with the fossils. The difference between post and pre-edge baselines was taken to be proportional to the elemental concentration of P [2], as seen in figure 1, and so the total amount of phosphorous was measured in different substrates and samples as by the Δ.

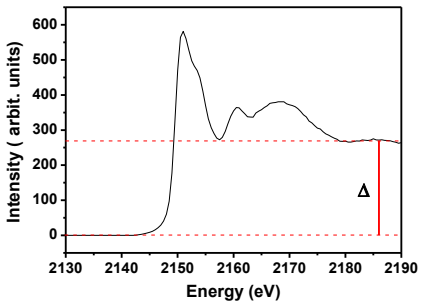

Fig 1: P K XANES spectrum of calcium phosphate and the methodology for P quantification

Conclusions: We demonstrated that the X-ray fluorescence technique, performed in vacuum and using synchrotron light sources, is a reliable way of detecting very low concentrations of phosphorous on geological and paleobiological samples, helping to elucidate the biomineralization process. Having the advantage of being very sensitive (easily better than 10ppm, if necessary), non-destructive and with a potential of doing space-resolved measurements (down to 10nm) if a sufficiently brilliant monochromatic X-ray source is available, such as it will be with Sirius, the next Brazilian synchrotron light source.

[1] Boßelmann, F.; Romano, P.; Fabritius, H.; Raabe, D.; Epple, M. *Thermochim. Acta* **2007**, *463*, p. 65.

[2] Kruse, J.; Leinweber, P. *Journal of Plant Nutrition and Soil Science* **2008**, *171*, p. 613.