EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Nano-XAS arsenic speciation in cellular structures in Sargassum algae	**Experiment number**: EV 445
Beamline: ID16b	**Date of experiment**: from: 06/10/2021 to: 10/10/2021	**Date of report**: 11/09/2022
Shifts: 12	**Local contact(s)**: Remi Tucoulou	*Received at ESRF:*

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Report:

Objectives & expected results in the proposal:

The objective of the present proposal was to study the cells of *Sargassum* algae at ID16b . The aim was to determine the mechanisms in which these algae absorb and accumulate pollutants, especially arsenic. In particular, we aimed to 1) measure the As speciation in the cell compartments of *Sargassum* algae, such as cell walls and cell organelles. 2) study the speciation differences between leaves and stems of algae, 3) determine speciation changes during algae degradation and 4) elucidate the mechanisms of As binding and accumulation in the seaweed. This information was expected to contribute to assess the toxicity and the environmental effects of the As-rich algae, and provide an essential insight to develop a method to handle the contaminated algae.

Experimental results:

Our study had to be adapted from the initial objectives, due to issues with the samples. Fresh algae were collected in the open sea, beaches and inland in Guadeloupe and Martinique during algae blooming season (around the month of august). Subsequently, they were sent to our laboratories in France, with the adequate conditions, in their sea water medium, and with the shortest delay, in order to preserve the sample and ensure that the speciation was not changed during transport. However, problems with the transport company delayed the delivery of the samples, who started to degrade before the arrival to our laboratory, and thus could not be used. The magnitude of the sampling campaigns (which has to be performed during the adequate blooming time, and require the use of boats and needs several people involved) prevented a new sampling campaign to be performed before the start of the experiment at the beginning of October.

Nonetheless, there were samples from previous sampling campaigns that could be used for the experiment. These samples, collected one year in advance, were not anymore fresh, but they had been preserved following several steps for cryofixation, cryo-substitution and resin embedding, which is a method that we usually use for

the sample analysis using NanoSIMS and TEM. Consequently, could not be taken to the beamtime as freeze-dried samples, as planned in the proposal, but as tissue embedded in epoxy resin. The process of resin embedding is known to modify the speciation of elements, including arsenic. Therefore, it was not possible to perform nano-XAS measurements, but our experiment had to focus exclusively in nano-XRF mapping.

However, since the samples had been prepared for TEM imaging as well, they had been treated with osmium tetroxide as it acts as a staining and fixing agent for lipid membranes in electron microscopy. Unfortunately, the fluorescence of As (kα=10.5 keV) overlaps with the one of Os (Lβ2=10.6 keV). Besides, the signal from the added Os (2% solution) was much higher than the one from the As present naturally in the sample (average of 35 ppm). Despite the efforts from the local contact to find a beamline configuration to visualise As, it was not possible to differentiate As from Os. Accordingly, the nano-XRF mapping of other elements were obtained, such as Ca, Fe, Zn, Co, I and Cu, as shown in Figure 1.

The distribution of As in the cell, the original aim, was studied by NanoSIMS. The fact that the same samples were used for both techniques, as well as for TEM, allowed to find the same cell. Therefore, interesting accumulation tendencies could be visualised. Several metals and non-metals show the same tendency of as to accumulate mainly in the cell walls of the cells, with a small amount in the organelles, which could indicate parallel mechanisms of absorption for pollutants, as well as for essential metals.

Potential publications:

The results, in convination with additional data, have been presented as oral communications in two conferences: the "Spectratom 2022", conference, celebrated in Pau, France and the "X Conference of the Spanish Synchrotron User Association (AUSE) and the 5th ALBA User's Meeting" that took place at ALBA synchrotron , Spain.

Additionally, they will be submitted, with additional data from other techniques into the appropriated peer review journal with high visibility in the field (e.g., Environmental Science and Technology, Metallomics...).

Conclusions:

The adquisition of the nano-XRF for several metals and non-metals in cells of sargassum leaves and stems was carried out at ID16b. It was not possible to obtain the mapping of As and neither the speciation of As, which was the main aim of the proposal. Consequently, a further study is needed in order to achive the localised speciation to fulfill the aims of the proposal, i.e. study the As speciation differences between cell organs (wall and organelles) in order to understand the absorption mechanisms and to observe the speciation changes with degradation to assess the fate of the As species.

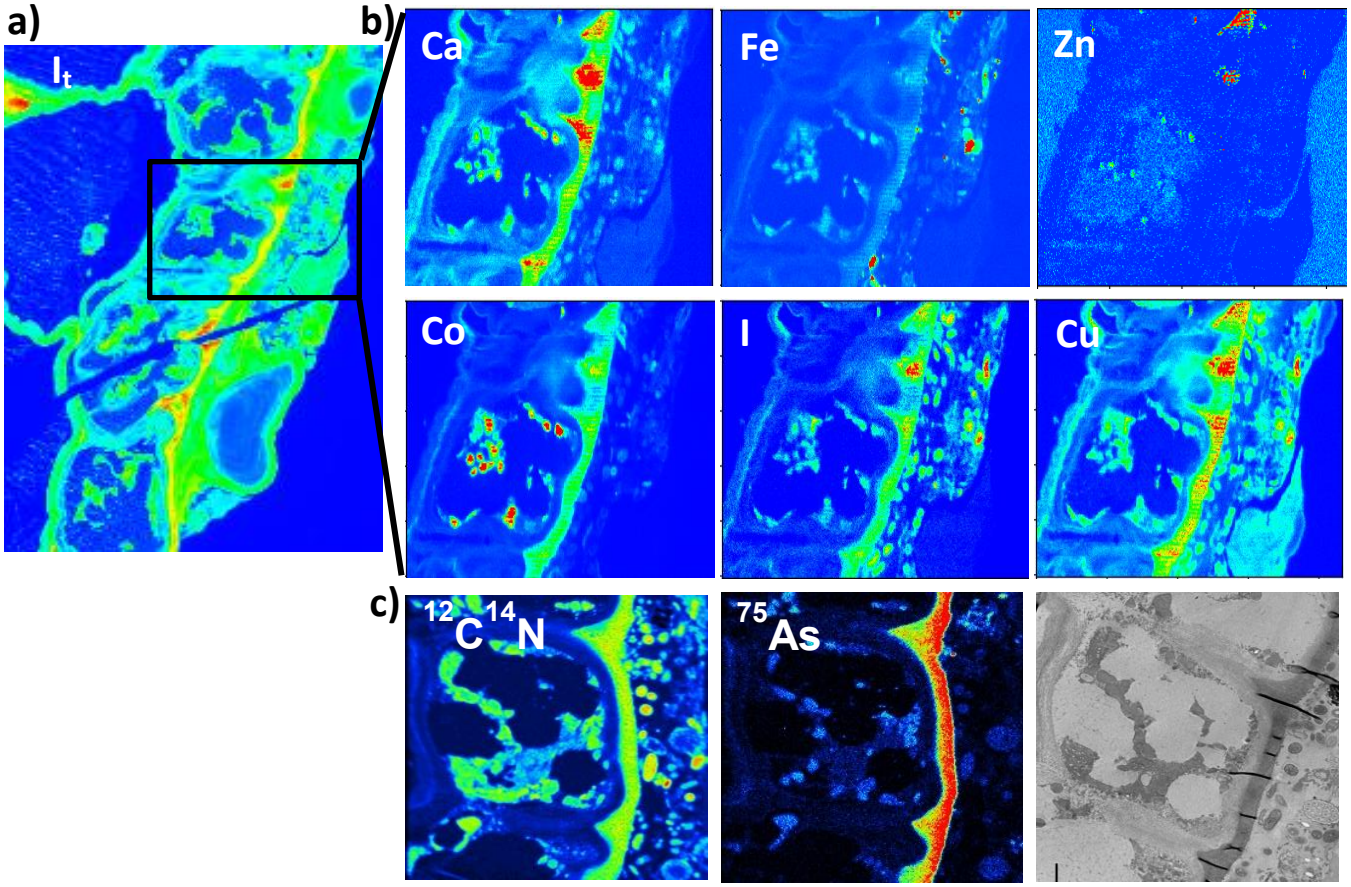

Figure 1: a) and b) nano-XRF images of I_t, Ca, Fe, Zn, Co, I and Cu in a leaf cell of *Sargassum* algae, c) correlative NanoSIMS mapping nitrogen ($^{12}C^{14}N^-$), arsenic ($^{75}As^-$) and TEM image of the same cell.