ELECTRON MICROSCOPY OF CALCIFIED MICROAGGREGATES OCCURRING WITHIN *CLADOPHORA* SP. MATS

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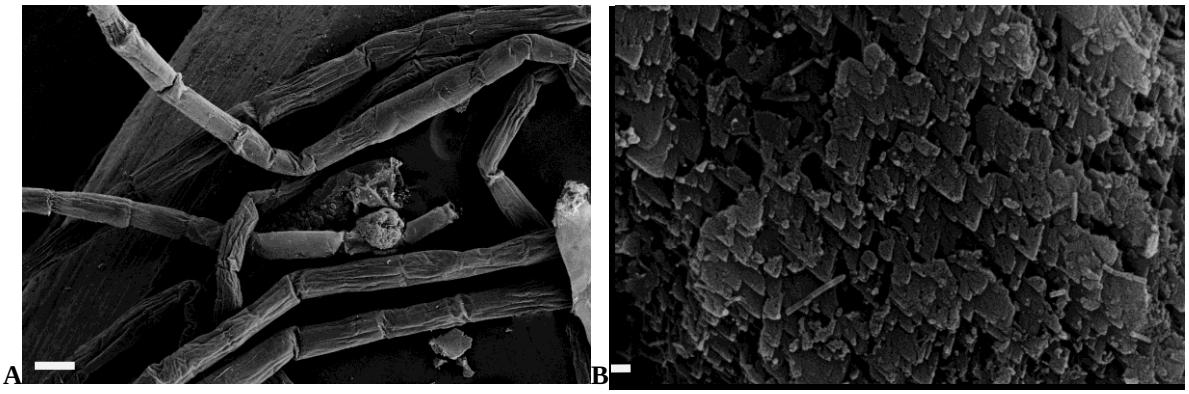
Increased solar exposure, high temperatures and lack of precipitation, conditions common at the end of winter and the early spring in subtropical Andean rivers, favor the production of river algae blooms such as *Cladophora* sp [1]. *Cladophora* is considered the most important of the attached benthic algae as they tend to alter the conditions of their microhabitats by modifying current, blocking sunlight, absorbing dissolved oxygen, collecting detritus and providing refuge for invertebrates [2]. *Cladophora* is also an important substrate for epiphytic growth due to its ability to withstand the shear stress typical in the benthic regions of rivers. In marine systems *Cladophora* has been associated with lithified carbonate structures, where it has been found to form mixed communities with cyanobacteria while, in freshwater streams mats have also been described in association with carbonate layers. During a fifteen year research project in the microhabitat produced by *Cladophora's* mats in the Lules River (Tucumán, Argentina) annual cycles of exceptionally prolonged drought, in conjunction with naturally occurring diffuse organic pollution, generated the unique conditions for the precipitation of lithified carbonate microaggregates (microbialite-like). This work presents an initial characterization of calcified microstructures attached to *Cladophora* sp. mats using scanning electron microscopy coupled to energy-dispersive X-ray spectroscopy (EDS). Samples taken from *Cladophora's* mats of the Lules River were fixed in the field with 4% formaldehyde. The fixed samples were washed three times with phosphate buffer and $CaCl_2$ for 10 min, and fixed with osmium tetroxide (2% v/v) over night. Afterwards, the samples were washed twice with ethanol (30% v/v) for 10 min, dried at a critical point, and sputtered with gold. Specimens were observed under high vacuum using a JEOL 35CF and a Zeiss Supra 55VP (Carl Zeiss NTS GmbH, Germany) scanning electron microscope coupled with an energy dispersive X-ray detector (INCA Oxford, EDS detector). Abundant microaggregates forming around the filaments from *Cladophora* mats were found (Fig. 1A). They varied in both size and weight (mean 2 mm diameter, 0.37 g weight). Electron microscopy allowed a closer analysis of the collected microaggregates; numerous diatomeas and bacteria together with typical crystals of calcite agglutinated surrounding the main filaments of the algae, (Fig. 1B). Small quantities of quartz and albite were also recorded. The mineral presence was confirmed by X-ray diffractometry and also EDS (Fig. 2). We hypothesize that the calcificated microaggregates observed around the filaments of *Cladophora* sp. are produced by the release of extracellular polysaccharides of some diatomea such as *Gomphonema* sp., as previously noted by Winsborough and Golubic [3]. These microaggregates represent incipient formation of microbialite-like structures which are organosedimentary deposits formed from the interaction between benthic microbial communities and detrital or chemical sediments. As such, they constitute interesting models to further explore microbe-mineral interaction processes in subtropical freshwater habitats.

REFERENCIAS

[1] Fernández, H.R., Reid B., (2012) "Invertebrate distribution on a macroalgae/macrophyte mixed mat in flowing wáter" *Fundam. Appl. Limnol.* 181: 289-299.

[2] Dodds, W.K., Gudder D.A., (1992) "The ecology of Cladophora" *J. Phycol.* 28: 415-427.

[3] Winsborough, B.M., Golubic, S., (1987) "The role of Diatoms in stromatolite growth: two examples from modern freshwater settings" *J. Phycol*. 23: 195-201.



A **B**

Fig.1. A. *Cladophora* filaments displaying the calcified microaggregates. Bar: 100 µm B. Detailed view of the calcite crystals forming the microaggregates Bar: 200 nm.

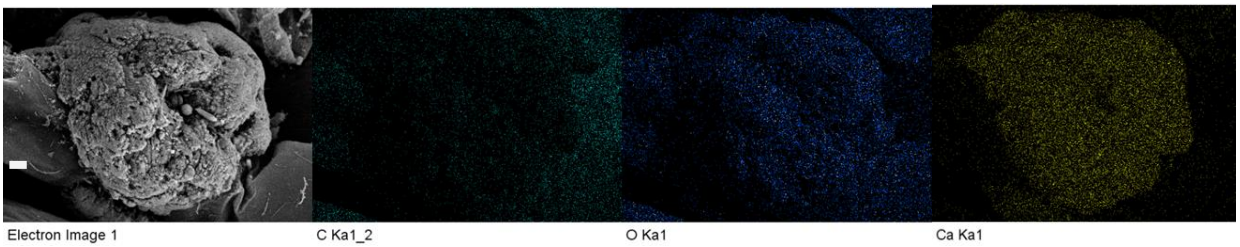


Fig. 2. EDS mapping of one of the microaggregates revealing elemental constitution: mainly C, O and Ca. Bar: 10 µm.

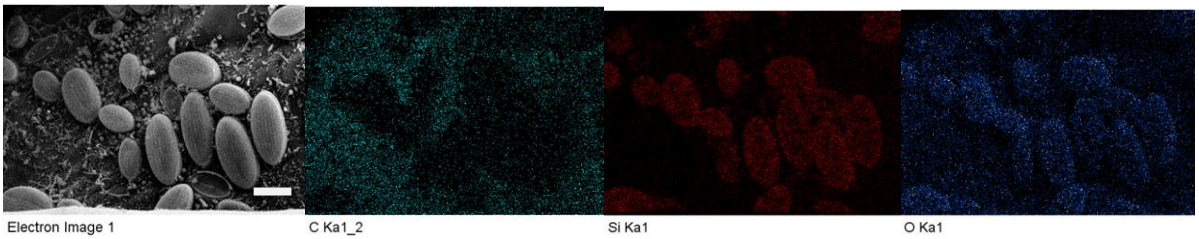


Fig. 3. EDS mapping of the surface of the calcified microaggregates showing diatomea and bacteria on the top. Main elements are C, Si and O. Bar: 10 µm