



## **Development of an Optimal Methodology for the Extraction of Microphytobenthic Chlorophyll**

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*Received January 13, 2009; Accepted February 23, 2009*

**Abstract:** Benthic microalgae are important primary producers in intertidal shallow systems. Their biomass can be estimated by the assessment of chlorophyll *a* concentration. A rapid and reliable method of measuring chlorophyll *a* is by spectrophotometer. There is however, no standard protocol for the analysis of benthic chlorophyll *a*. Although the most common solvent generally used is 90% acetone, some authors showed better results with methanol and ethanol. Some pre-treatments, such as the addition of fine inert granules or ultrasound bath, have also been suggested as factors that improve the extraction efficiency. Sediment samples were collected from two sites, muddy and sandy, located within Ria Formosa (Portugal). The aim of this work was to test the effectiveness of different pre-treatments in the extraction and to develop an optimal method for chlorophyll *a* extraction and analysis. Pre-treating samples did not yield any significant differences in chlorophyll *a* extracted. Treating sediments with acetone was found to yield higher concentrations of chlorophyll *a*, both for muddy and sandy sediments. Acetone was therefore found to be the best solvent for both sediment types, with 90% being the best strength for sandy and 80% the best for muddy sediments. These differences may be related to differences in the structure of the algal communities. Six hours of extraction was found to be sufficient, since after a six hour period the extraction efficiency did not improve.

**Keywords:** *chlorophyll a; extraction efficiency; microphytobenthos; spectrophotometry; Ria Formosa.*

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