


# A SIMPLE AND LOW-COST METHOD FOR ANALYZING MICROPHYTOBENTHOS IN MARINE SEDIMENTS

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## 1 Introduction

The term phytobenthos refers to photosynthetic organisms inhabiting intertidal and subtidal mudflats as well as other substrates. These organisms consist primarily of algae, although seagrasses form meadows in some subtidal and intertidal zones. The group also includes both photosynthetic and non-photosynthetic components, such as benthic algae, dinoflagellates, cyanobacteria, and other microorganisms. Periphytic algae are typically microscopic, unicellular, or filamentous, and are a major component of microphytobenthos (20–200 µm). These benthic communities are the primary producers and main photosynthetic components of the ecosystem (Macintyre et al. 1996; Wilkinson, 2001; Law, 2011; Haro, 2025).

Benthic diatoms are the most dominant organisms, and two groups can be distinguished according to Vos et al. (1988): epipsammic diatoms (immotile diatoms that live semi-permanently attached to sand grains) and epipellic diatoms (motile diatoms that move freely through the sediment). Microphytobenthos (MPB) also contribute to primary production and sediment stability, and serve as a food source. Moreover, due to their sensitivity to environmental factors, MPB attributes can be used as indicators of aquatic ecosystem conditions (Colijn & De Jonge, 1984; Stal, 2010; Barinova et al., 2019).

Most studies do not taxonomically identify microalgae, but instead assess chlorophyll and pigment biomass. These parameters are generally estimated using spectrophotometry, fluorimetry, and high-performance liquid chromatography (HPLC), which involve higher costs (Brotas & Plante-Cuny, 2003; Brotas et al., 2005; Fonseca et al., 2013; Rezende, 2013; Matos, 2023). Some methods described in the literature require complex protocols and are more costly. Although relatively simple, one such method involves centrifugation and costly reagents (Blanchard, 1988; Vermeulen et al. 2012). To simplify the procedure, the present study proposes a low-cost method for microphytobenthos analysis in marine sediments.

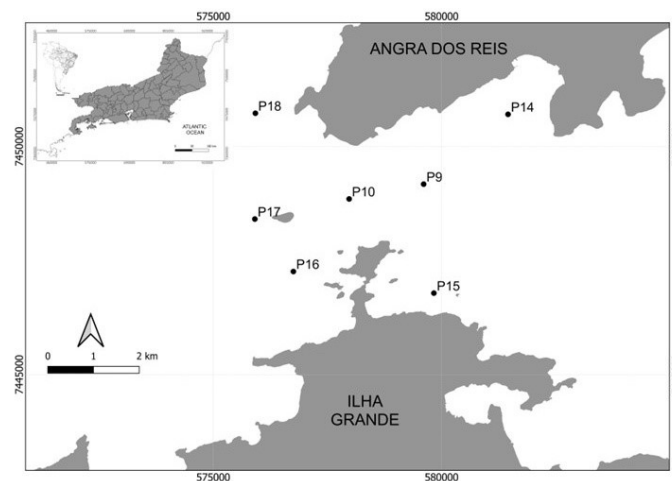
This study is part of an environmental monitoring program designed to ensure compliance with licensing regulations in Baía da Ilha Grande, a bay located on Brazil's southeastern coast, in the State of Rio de Janeiro (Figure 1). The study region is a biodiversity hotspot with high environmental vulnerability due to its proximity to

## ABSTRACT

This study presents a simple and efficient method for analyzing microphytobenthos in marine sediments. Sampling was conducted at seven sites in Baía da Ilha Grande, Rio de Janeiro State, Brazil. A flotation method using a sucrose solution was employed, in which organisms recovered from the supernatant and subsequently analyzed. A total of thirteen taxa were identified, predominantly benthic diatoms, along with silicoflagellates, Cyanobacteria, and Foraminifera. The proposed method eliminates the need for Ludox, centrifugation, or other costly equipment, offering a cost-effective and accessible for similar studies and environmental monitoring.

**Keywords:** Benthic diatom. Flotation method. Environmental assessment. Microphytobenthos.

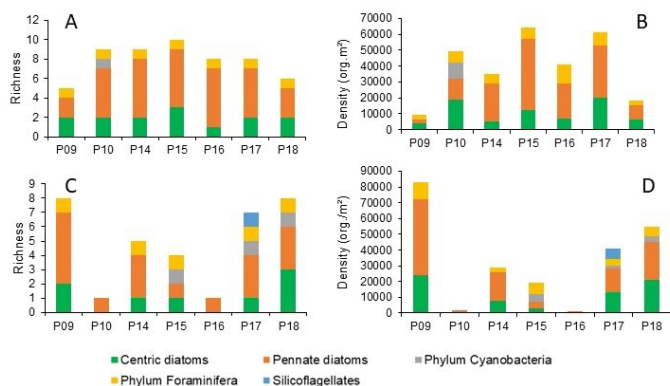
a petroleum terminal and a nuclear power plant (Creed et al. 2007). Baía da Ilha Grande is characterized by three distinct sedimentary groups: very fine sands in the western portion and continental shelf, medium to coarse sands in the eastern portion, and pelites in the central channel and sheltered areas (Mahiques & Furtado, 1989).



**Figure 1.** Map of the seven sampling sites (P#) for the analysis of microphytobenthos communities, Baía da Ilha Grande, Rio de Janeiro, Brazil.

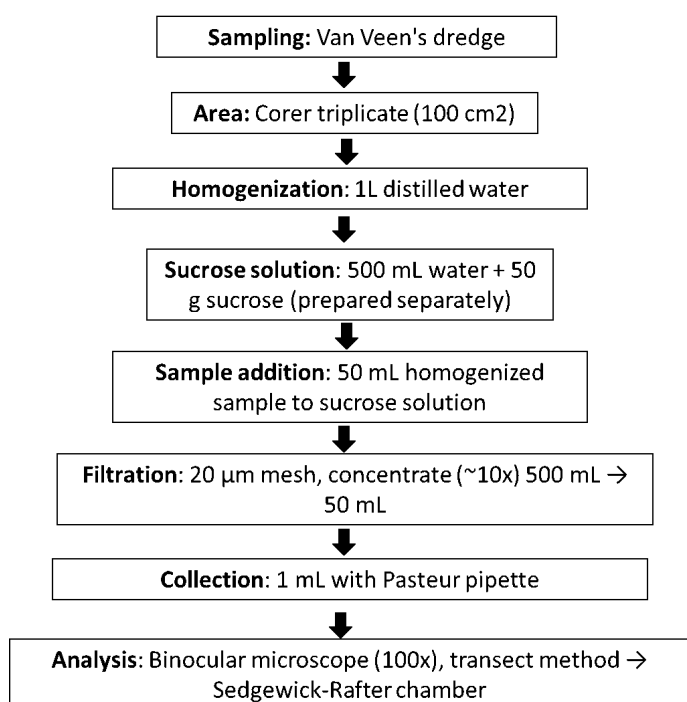
Seasonal sampling (March and August 2024) was conducted at seven sampling sites. Sediment was collected using a 500 cm<sup>2</sup> Van Veen grab and immediately fixed with formalin 2%. Three core replicates (0.01 cm<sup>2</sup> each) were taken at each site. These sediment aliquots were diluted in distilled water to a final volume of 1.0 L. A 50 mL aliquot of the sediment suspension was homogenized with 500 mL of a saturated sucrose solution. The mixture was then filtered through a 20 µm membrane, concentrated to 50 mL, and a 1 mL subsample was transferred to a Sedgewick-Rafter counting chamber. Subsamples were analyzed under a binocular microscope along transects covering the entire chamber. Taxonomic identification was performed using identification guides and specialized literature (Hasle et al. 1996).

A total of thirteen taxa were identified at the genus and major group levels, including centric and six pennate diatoms, silicoflagellates, Cyanobacteria, and Foraminifera, with only one taxon per group (Figure 2). The highest richness (13 taxa) and density (80,000 org./m<sup>2</sup>) were observed during the dry season (August 2024). Protozoa, Foraminifera, and the centric diatom *Thalassiosira* were the most frequent in both periods (>70%).



**Figure 2.** Richness and density (org./m<sup>2</sup>) of microphytobenthos communities at seven sampling sites (P#) during the wet (A, B) and dry (C, D) seasons (March and August 2024) in Baía da Ilha Grande, Rio de Janeiro State, Brazil.

This study presents a simple and low-cost method for conducting microphytobenthos inventories (Figure 3). This method, known worldwide as sucrose flotation, is primarily used for separating meiofauna from sediments (Jenkins, 1964). Due to changes in solution density, organisms become lighter and remain suspended in the supernatant. Compared to the method proposed by Blanchard (1988), our approach is faster and more cost-effective, as it does not require centrifugation. Although Ludox is a standardized reagent for separating microalgae in sediments, it is more expensive than sucrose and distilled water (Table 1). In addition, methods that analyze living organisms and require acclimatization and incubation further increase costs (Eaton, 1966). The simplicity of the present protocol makes it suitable for bioassessment studies involving large sample sizes. However, further studies are needed to evaluate its applicability for biomass determination and primary production.



**Figure 3.** Protocol for Microphytobenthos Analysis Using an Adapted Flotation Method with Density Modification.

**Table 1.** Advantages and disadvantages of the proposed method for separating microphytobenthos from marine sediments.

Parameter	Ludox and centrifugation method	Sucrose flotation method (proposed)
Density	Ideal for density gradient MPB separation	Sucrose is less toxic and lower cost
Need for centrifugation	Yes, required for MPB separation	No, enabling a simpler process
Toxicity	Toxic to some cell types	Sucrose is biocompatible
Cellular viability	Centrifugation may cause cell damage	Minimal mechanical and osmotic stress
Cost	High, due to Ludox and centrifugation	Reduced, sucrose is low-cost
Reproducibility	Standardized and well-documented	Requires further validation

Testing across varied sediment grain sizes and geographic locations is recommended. Given the low taxonomic resolution, further research using electron microscopy is recommended to refine the identification of benthic diatoms and other groups. The use of sucrose as a density-altering medium may be a viable alternative to Ludox, particularly for studies requiring higher cell viability. Eliminating centrifugation simplifies the procedure and facilitates its application in laboratories without access to specialized equipment. However, it is crucial to confirm whether the density of the sucrose solution is sufficient to effectively separate microalgal cells from the sediment without compromising procedural selectivity.

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