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Comparative study on microphytobenthic pigments of muddy and sandy intertidal sediments of the Tagus estuary

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Abstract

The abundance and distribution of microphytobenthic pigments determined by HPLC (chlorophylls and carotenoids) were compared between muddy and sandy sediments of the Tagus estuary (Portugal). In the two types of sediment, with similar periods of illuminated emersion, chlorophyll *a* concentrations on a per area basis (mg m^{-2}) were comparable (down to 2 mm). Pigment analysis also revealed similar microphytobenthic communities in terms of algal classes. Diatoms were the dominant microalgae, but cyanophytes, euglenophytes and phanerogam debris were also present. For both muddy and sandy sediments, microphytobenthic biomass showed a high level of variability both within and between two consecutive years. Microphytobenthos was highly stratified in the mud, with most of the chlorophyll *a* occurring in the top 500 μm. In the sand, relatively constant concentrations were found throughout the sediment profile down to 3 mm. This is probably related to deeper light penetration in sandy sediment and/or increased physical mixing caused by invertebrate activity or overlying currents, leading to the burial of an important fraction of the microphytobenthic cells. Differences observed in the intensity of sediment coloration of muddy and sandy sediments might have resulted from the different vertical distribution of benthic biomass.

Keywords: microphytobenthos; pigments; HPLC; chlorophylls; carotenoids; Tagus estuary

1. Introduction

Benthic microalgae of intertidal sediments show a high degree of spatial and temporal heterogeneity, and factors such as resuspension, nutrient and light availability, grazing, and desiccation have been suggested to control microphytobenthic biomass (see review by Underwood and Kromkamp, 1999). A major factor determining both the abundance and composition of microphytobenthic communities is the nature of the substratum. Some studies report higher biomass in muddy sediments (Riaux-Gobin et al., 1987; Riaux-Gobin and Bourgoin, 2002; Perkins et al., 2003), while others show higher chlorophyll *a* levels associated with sandier substrates (Cahoon et al., 1999; Cahoon and Safi, 2002).

Information regarding the distribution and abundance of benthic microalgae can be derived from methods like spectral reflectance (e.g. Paterson et al., 1998), chlorophyll *a* fluorescence (e.g. Serôdio et al., 2001) or high performance liquid chromatography (e.g. Brotas and Plante-Cuny, 1998). HPLC is a separative method that allows not only the determination of chlorophyll *a*, a reliable index of benthic microalgae biomass, but also sheds light over a complex pool of pigments and their degradation forms that occur in intertidal sediments (Cariou-Le Gall and Blanchard, 1995; Brotas and Plante-Cuny, 2003). The pigment composition determined by HPLC can be used for taxonomic purposes, since several pigments or pigment combinations are found only in certain algal classes (Riaux-Gobin et al., 1987; Klein and Riaux-Gobin, 1991; Brotas and Plante-Cuny, 1998). Furthermore, the presence of chlorophyll *a* degradation products has been related to grazing processes (Bianchi et al., 1988; Buffan-Dubau et al., 1996; Cartaxana et al., 2003).

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The primary goal of this work was to compare the abundance and distribution of microphytobenthic pigments (chlorophylls and carotenoids) determined by HPLC in muddy and sandy sediments of the Tagus estuary (Portugal). Information derived from the optical properties of the sediment surface is increasingly being used in remote sensing studies (e.g. Méléder et al., 2003; Murphy et al., 2005) to quantify chlorophyll *a* in intertidal and subtidal benthic habitats. Furthermore, the surface coloration of the sediment is a general indicator of the nature of the surface assemblages (Paterson et al., 1998). From the observation of sediment coloration, it is hypothesised that muddy sediments, with similar periods of illuminated emersion to that of sandy sediments, are more favourable for benthic cells and are distinct in both the abundance and the composition of the microphytobenthos. The distribution of the microphytobenthic pigments with depth was also compared between the two types of sediment.

2. Material and methods

2.1. Study site

The Tagus estuary (38°44′N, 09°08′W) is a shallow mesotidal estuary covering an area of 320 km^2. Tidal amplitude ranges from 1 to 4 m, and the intertidal area comprise 20–40% of the estuary in neap and spring tides, respectively. Sediments are typically sandier in zones where currents are more active and muddier in enclosed areas.

2.2. Sampling

From September 2002 to July 2004, sediment samples were collected at low tide during spring tides on two different intertidal sites. Both sites were exposed during each low tide for about 2.5–3 h (tidal height ±1 m), but had different sediment characteristics (Table 1). At each site, three different subplots separated by approximately 5 m were chosen for replicate sampling.

Sediment samples were collected using contact corers (Honeywill et al., 2002). The contact corer device, a small strip of metal plate with a cavity at the bottom side, was used to freeze the top 2 mm of the sediment. It was gently placed on the sediment surface so that the metal dish made contact. Liquid nitrogen (LN) was added on top, after which the sediment was allowed to freeze for up to 2 min (the freezing time was assessed for each site and varied according to factors such as weather conditions and sediment water content). Any sediment deeper than 2 mm was removed using an artist's palette knife leaving a flat disk of sediment. The sediment disc was wrapped in foil and stored in LN. In the laboratory, samples were weighed, transferred to −80 °C, then freeze-dried and re-weighed. Additional samples were taken for particle size, organic matter, and interstitial water salinity determination.

Another sampling technique employed a device called the cryolander (Wiltshire et al., 1997). The cryolander was gently placed on the sediment surface and a small amount of LN poured slowly onto the cotton wool in it. The LN vaporised and froze the immediate sediment surface without distortion even on a micrometer scale (Wiltshire et al., 1997). After the surface had frozen, the LN was poured onto it evenly through the cryolander mesh. The cryolanding process varied with environmental and site conditions and took up to 5 min. When a disk of sediment around the cryolander was frozen, the cryolander was lifted from the sediment surface, and a plunger used to remove the sediment disk. The disk was placed face down on a foil (so that the surface was always identifiable), wrapped, and stored in LN. In the laboratory, samples were transferred to −80 °C. The cryolander sediment blocks were cut into slices using a freezing microtome (Leiz Wetzlar Kryomat 1703). The sediment was sectioned into depth intervals of 0–180, 180–360, 360–540, 540–720, 720–1080, 1080–1500, 1500–1980, 1980–3000, and 3000–3480 μm. The sectioned sediment was removed from the microtome blade with a small piece of filter material (pre-weighed), then placed in a 1 ml Eppendorf (pre-weighed). The samples were freeze-dried and re-weighed prior to analysis.

Table 1
General characteristics (average ± standard deviation, $n = 36$) of muddy and sandy intertidal sediments of the Tagus estuary. Significant differences between sites are indicated: ***$p < 0.001$

	Sandy	Muddy
Water content (%)	27.8 ± 3.2	70.5 ± 3.0***
Organic matter (%)	1.3 ± 0.4	9.0 ± 0.5***
Sediment size fractions (%)		***
>1000 μm	5.4 ± 1.9	0.2 ± 0.2
1000–500 μm	31.2 ± 2.7	0.1 ± 0.06
500–250 μm	43.3 ± 3.6	0.8 ± 0.4
250–125 μm	10.4 ± 1.3	1.4 ± 0.8
125–63 μm	1.2 ± 0.4	0.9 ± 0.3
<63 μm	8.5 ± 3.3	96.7 ± 1.6
Wet bulk density (g cm^{-3})	2.04 ± 0.40	1.34 ± 0.17***
Salinity	21.8 ± 8.0	21.6 ± 10

2.3. HPLC analysis

Approximately 0.2 g of freeze-dried sediment from the contact cores were extracted for 15 min in 95% cold buffered methanol (2% ammonium acetate) with 30 s sonication (Cartaxana and Brotas, 2003). After filtration (0.2 μm Whatman membrane filters) extracts were immediately injected in a Shimadzu HPLC with a photodiode array (SPD-M10AVP) and a fluorescence detector (RF-10AXL). Chromatographic separation was carried out using a C18 column for reverse phase chromatography (Supelcosil; 25 cm long; 4.6 mm in diameter; 5 μm particles) and a 35 min elution programme. The solvent gradient followed Kraay et al. (1992) with a flow rate of 0.6 mL min^{-1} and an injection volume of 100 μL. Sediment samples from the cryolanders were extracted in acetone (48 h, dark, −20 °C) and analysed on a Waters system. Samples were injected in between two plugs of Milli-Q water (20 μl Milli-Q, 60 μl sample, 20 μl Milli-Q).