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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia, realizada sob a orientação científica do Professor Doutor João Carlos Marques (Universidade de Coimbra)

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ABSTRACT

The coastal areas have historically played a crucial role in human life. A large proportion of the human population inhabits coastal areas, and human density is expected to increase in the coming years. Consequently, coastal ecosystems are particularly exposed to human pressures, and some of them are among the most disturbed ecosystems of the biosphere. In rocky shores, as well as in other coastal ecosystems, benthic communities show spatially heterogeneous distributions and experience seasonal variations due to both natural and anthropogenic stresses.

The major goal of this study was to assess the existence of a disturbance gradient regarding the spatial distribution of the intertidal macrozoobenthic communities of hard substrata, across the horizontal axis of three rocky platforms, and zones within and across platforms, in Buarcos bay during spring 2009. For this purpose, physcochemical parameters and macroalgae taxa were utilized in the assessment to confirm sampling was performed inside a disturbance gradient, and to compare with results obtained for the macrofauna. The behaviour of ecological indices calculated from macroinvertebrate data were compared with results obtained with the ecological tool MarMAT – Marine Macroalgae Assessment Tool. During the survey, a total of 27930 macroinvertebrate individuals corresponding to 122 different taxa were found, belonging to Phyla Annelida (44), Arthropoda (41), Cnidaria (1), Echinodermata (2), Mollusca (31), Nematoda (1), Nemertea (1) and Sipuncula (1). The species *Mytilus galloprovincialis* (mean density of 14345.4 ind m^{-2}) and *Chthamalus montagui* (mean density of 12870.4 ind m^{-2}) were dominant in the assemblages, accounting for 39.94% and 35.83% of the total individuals, respectively, while the remaining taxa represented individually less than 6%.

The various statistical and ordination tools allowed the verification of a disturbance gradient from St *A*, the most proximate platform from the point source pollution, to St *C*, the furthermost platform. The gradient was also found from *zone a* (upper shore) *to zone c* (lower shore) within the two immediate platforms, and across platforms. Furthermore, St *C* and *zone c*, the outermost sampling areas, were found to show the highest similarities (43.14% and 48.47%, respectively) with *Mytilus galloprovincialis* contributing mostly to these similarities.

The ecological indicators captured the differences in the communities between platforms and zones, and confirmed that disturbance gradient. The indices results were in compliance to the results obtained with the MarMAT, which according to the EQRs indicated the St *A* was the platform with worse ecological condition, whereas St *C* was the platform showing the best ecological condition.

This survey contributed for a better knowledge on the rocky shore intertidal communities, aiming at improving decisions with regard to further management routines.

RESUMO

As áreas costeiras têm desempenhado historicamente um papel crucial na vida humana. Uma grande proporção da população humana habita em áreas costeiras, e espera-se que a sua densidade aumente nos próximos anos. Consequentemente, os ecossistemas costeiros estão particularmente expostos a pressões humanas, e alguns deles estão entre os mais perturbados ecossistemas da biosfera. Nas costas rochosas, e também em outros ecossistemas costeiros, as comunidades bentónicas apresentam distribuições espaciais heterogéneas e experienciam variações sazonais devidas a pressões naturais e antropogénicas.

O principal objectivo deste estudo foi a avaliação da existência de um gradiente de perturbação tendo em conta a distribuição especial de comunidades macrozoobentónicas intertidais de substrato rochoso, ao longo de um eixo horizontal de três plataformas, e de zonas dentro e ao longo das plataformas, na praia de Buarcos durante a Primavera de 2009.

Para tal, parâmetros físico-químicos e taxa de macroalgas foram utilizados na avaliação para confirmar que a amostragem seguiu um gradiente de perturbação, e comparar com os resultados obtidos para a macrofauna. O comportamento de índices ecológicos calculados com os dados dos macroinvertebrados foi comparado com os resultados obtidos com a ferramenta ecológica MarMAT – Marine Macroalgae Assessment Tool. Durante o estudo, um total de 27930 indivíduos de macroinvertebrados foram encontrados correspondendo a 122 taxa diferentes, pertencendo aos Phyla Annelida (44), Arthropoda (41), Cnidaria (1), Echinodermata (2) e Mollusca (31), Nematoda (1), Nemertea (1) e Sipuncula (1). As espécies *Mytilus galloprovincialis* (densidade média de 14345.4 ind m^{-2}) e *Chthamalus montagui* (densidade média de 12870.4 ind m^{-2}) foram dominantes nas comunidades, representando 39.94% e 35.83% do total de indivíduos, respectivamente, enquanto os restantes taxa representaram individualmente menos de 6%.

As várias ferramentas estatísticas e de ordenação permitiram a verificação de um gradiente de perturbação da St *A*, a plataforma mais próxima do foco pontual de poluição, para a St *C,* a plataforma mais distante. O gradiente foi também encontrado da *zona a* (*upper shore*) para a *zona c* (*lower shore*) dentro das duas plataformas mais imediatas, e entre plataformas. Ademais, a St *C* e a *zona c*, as duas áreas de amostragem mais afastadas do foco de poluição, foram as que apresentaram maior similaridade (43.14% e 48.47%, respectivamente) com *Mytilus galloprovincialis* a contribuir maioritariamente para essas similaridades.

Os índices ecológicos capturaram as diferenças nas comunidades entre plataformas e entre zonas, e confirmaram a existência daquele gradiente. Os resultados dos índices estiveram de acordo com os resultados obtidos com a ferramenta MarMAT que, de acordo com os EQRs obtidos, indicou que a St *A* foi a plataforma com pior condição ecológica, enquanto a St *C* foi a plataforma com melhor condição ecológica.

Este estudo contribuiu para um melhor conhecimento das comunidades macrozoobentónicas intertidais de costa rochosa, procurando esclarecer e fundamentar medidas de gestão a implementar em avaliações futuras.

CONTENTS

1.INTRODUCTION

1. INTRODUCTION

The coastal areas have historically played a crucial role in human life. They are considered of great importance in the context of marine ecosystems as they provide valuable resources in terms of biological diversity, contribution to productivity, fisheries and tourism (Salomão & Coutinho, 2007). A large proportion of the human population inhabits coastal areas, and human density is expected to increase in the coming years. Consequently, coastal ecosystems are particularly exposed to human pressures, and some of them are among the most disturbed ecosystems of the biosphere (Martínez-Crego et al., 2010). This already extensive natural habitat is further increased by the plethora of artificial hard structures (offshore platforms, docks, dykes, sea walls) all of which function essentially as artificial rocky shores (Thompson et al., 2002).

Rocky shores are heterogeneous environments representing the transition from a terrestrial to a marine environment. They are important habitats for several fish and marine benthic invertebrates, serving many vital ecological functions including spawning, recruitment, nursery, feeding and refuge (Orth & van Montfrans, 1990; Beck et al., 2001). These areas are the most densely inhabited by macroorganisms and have the greatest diversity of animal and autotroph species (Nybakken, 2000) existing where the effect of waves on the coast is mainly erosive. Rocky shores are variable coastal habitats and, depending on local geology, they may range from steep overhanging cliffs to wide gently shelving platforms, from smooth uniform slopes to highly dissected irregular masses or even extensive boulder beaches. Therefore, rocky shores are rarely smooth slabs of rocks, but instead crossed with cracks, crevices, gullies and pools which provide special habitats with their own set of advantages and problems (Raffaelli & Hawkins, 1999).

The vertical distribution of rocky intertidal benthic communities is characterized by the organisms', or groups of organisms', allocation across horizontal areas (Stephenson & Stephenson, 1949; Lewis, 1964). The shore's vertical variability usually exists in a degree of centimetres or of few metres.

The horizontal spatial variability across the horizontal axis is an issue widely cited in literature (Underwood, 1981; Benedetti-Cecchi & Cinelli, 1997; Underwood & Chapman, 1998a, b; Guichard et al. 2001; Araújo et al., 2005), and it is related to a specific observation level. For the Portuguese coast, Araújo et al. (2005) referred that the large scale (kilometres) of horizontal variability was related with the wave exposure level, while the small scale (metres) variability was related to habitat heterogeneity. The topographic complexity of the substrate is another important physical characteristic particularly in intertidal areas, where mechanical action of waves and desiccation are of major importance (Jacobi & Langevin, 1996). The heterogeneity of substrates may alter the hydrodynamical pattern during high tide and, on the other hand, influence shading and wind intensity during low tide (Guichard et al., 2001; Masi & Zalmon, 2008).

Intertidal rocky communities (fauna and flora) must contend with severe abiotic conditions, such as wave action, desiccation, tidal regime, wind and temperature fluctuations, or even hypersaline conditions in evaporating rockpools, but also biotic conditions such as recruitment or biological interactions (herbivory, predation and competition) (Masi & Zalmon, 2000); conjunctly with the interface between air and water, and also with the action of tides and waves, the result is a vertical emersion gradient (essentially unidirectional) with increasing stress from emersion at higher shore levels. The horizontal gradient associated with exposure to wave action (non-unidirectional) also exists both among microhabitats within shores and among different shores. Furthermore, the degree of exposure to wave action can modify the extent of the vertical gradient. The interaction between these gradients is of prime importance in determining the type of organisms that any area of hard substrata will support. Consequently, clear, and well studied, patterns of zonation of fauna and flora exist on rocky shores (Lewis, 1964; Stephenson & Stephenson, 1972; Hill et al., 1998; Thompson et al., 2002).The alternating flood and exposure to air (during tidal regime) are considered the most important environmental factors in determining the organisms occurring in intertidal areas, and are the reasons why sessile organisms of those areas on any coast are similar, despite striking dissimilarities in climate (Masunari & Dubiaski-Silva, 1998).

Although the organisms are well adapted (morphological, physiological and behaviourally) to tolerate environmental extremes, disturbance by physical and biological factors may reduce the number of organisms in the community to the point at which there is less competition for resources, and hence less competitive exclusion, leading to greater species diversity (Dethier, 1984; Raffaelli & Hawkins, 1999); thus, rocky shores communities are composed by numerous fauna and flora species, and are especially rich in invertebrates belonging to almost all invertebrate phyla.

The combination of the aforesaid factors allows the rocky shores to be dynamic systems subject to seasonal and spatial changes and lead to the development of a characteristic zonation of habitats (Menconi et al., 1999), being often characterized by striking horizontal bands of species or species assemblages. Several models of vertical zonation of organisms on rocky shores have been developed to characterize their distribution. In Portugal, rocky intertidal ecosystems are divided into three major zones (the upper littoral, the mid littoral and the lower littoral) in relation to a gradient of emersion/desiccation, containing distinct organisms (Araújo et al., 2005). Some species occur in more than one and the boundaries can be blurred in places (Lewis, 1964;

Boaventura et al., 2001), as described in general zonation schemes by Stephenson & Stephenson (1949), Lewis (1964), Pérès & Picard (1964) and Seoane-Camba (1969). The upper littoral is permanently exposed and subject to wave splashing, being dominated by incrustant lichens and by the gastropod *Melaraphe neritoides*. The mid littoral, is restrained by intense tidal influence, either being submersed or exposed, usually presenting sessile filter feeders such as *Patella* spp., *Chthamalus* spp. and *Mytilus galloprovincialis* which are the most common organisms on the shore of exposed zones. The lower littoral is permanently submerged, is characterised by the presence of a considerable diversity of turf forming algae and canopy species like *Saccorhiza polyschides* and *Laminaria ochroleuca*, among others (Boaventura et al., 2002; Araújo et al., 2005).

Although natural physical disturbance are a common and often important factor affecting the structure and dynamics of rocky shore communities, there are another major threats to marine and other aquatic habitats as result of increasing human population and coastal development. As consequence, rocky intertidal areas worldwide are subject to considerable and increasing anthropogenic impacts (Schiel & Taylor, 1999) with origin either in land or at sea, more frequently than any other marine system (Schramm, 1991). These habitats have been, and are currently, affected by oil spills, direct harvesting of plants and animals (for food, bait, aquaria, or curiosity), exploratory manipulation of rocks and specimens (Addessi, 1995), introduction of alien species, habitat destruction and hydrology alterations (e.g. though the construction of sea walls, boat ramps, marinas, etc.) and climate change (Suchanek, 1994; O'Hara, 2002). The increased tourist activity translating into higher trampling levels also represents a significant source of impact to rocky shore communities (Murray et al., 1999; Schiel & Taylor, 1999; Milazzo et al., 2002, 2004; Ferreira & Rosso, 2009).

Coastal and estuarine waters are the most nutrient-enriched ecosystems on earth (Nixon et al., 1986; Kelly & Levin, 1986). As global human populations have increased, there has been also an unsustainable increase in the input of nutrients, especially nitrogen and/or phosphorus compounds, to coastal and transitional waters (Maier et al., 2009; Fitch & Crowe, 2010) in some cases to harmful levels. Nutrient pollution defies simple categorization and is difficult to control as it may come from point (wastewater treatment plants, sewer system overflows, septic systems, industrial facilities, and animal feeding operations), nonpoint (many diffuse sources and occurs when rainfall and snowmelt wash pollutants) (McCarthy et al., 2008), and/or atmospheric sources, from near and far.

Rocky shore species are sensitive to both acute impacts, such as oil spills, and chronic impacts, such as recreational activities. Studies of benthic communities show great potential for revealing the cumulative effects of disturbances on marine biota as benthic organisms may integrate the effects of long-term exposure to natural and anthropogenic disturbances (Pinedo et al., 2007). Use of benthic communities in marine pollution assessments are based on the concept that they reflect not only conditions at the time of sampling but also conditions to which the community was previously exposed (Reish, 1987; Gappa et al., 1990). Therefore, benthic organisms can be good indicators of pollution level in a given area (Anger, 1977; Leppakoski, 1979; Young & Young, 1982; Reish, 1986; Gappa et al., 1990), and are useful for impact studies by responding to local disturbances, as they are relatively non-mobile organisms with short generation times, and play an important role in cycling nutrients and inorganic compounds between sediments and water column (Silva et al., 2006). Due to their permanence over seasonal time scales, benthic invertebrates integrate the recent history of disturbance that might not be detected in the water column. Different benthic species exhibit different tolerance to stress, covering the Water Framework Directive (WFD) (EC, 2000) requirement of integrating sensitive species (Goela et al., 2009) in the ecological quality assessment.

The present study intends to aid in future surveys in the scope of the WFD. This is a key directive in the European Union legislation, with several goals such as to prevent water ecosystems deterioration, and to protect and enhance the status of water resources, having as main objective the achievement and maintenance of a good ecological status for all water bodies by 2015, mandatory for all Member states. The WFD provides a challenge in the development of new and accurate methodologies, addressing to the assessment of the Ecological Quality Status (EQS) within European rivers, lakes, groundwater, estuaries and coastal systems (Borja et al., 2004) taking into account biological quality elements (e.g. benthic invertebrates) and supported by physicochemical and hydromorphological quality elements, in order to implement management plans that prevent their further deterioration. Also, and according to the WFD, the resulting ecological status should be expressed as a ecological quality ratio (EQR) between the values of the biological elements observed at a given body of surface water and the values for these elements in a site with no, or very minor, disturbance from human activities (reference conditions) (Ballesteros et al., 2007).

The present study pretends to assess the existence of a disturbance gradient regarding the spatial distribution of the intertidal macrozoobenthic communities of hard substrata, across the horizontal axis of three rocky platforms in Buarcos bay during the spring of 2009. Accordingly, five null hypothesis (H_0) will be tested:

H_{01}: Communities are not different between platforms due to a perturbation influence;

H_{02}: Communities are not different between zones within each platform due to a perturbation influence;

H_{03}: Communities are not different between levels within zones at each platform in order to test if the sampling procedures are adequate;

H_{04}: Communities are not different in zones across platforms due to a perturbation influence;

H_{05}: Communities are not different between levels within zones across platforms in order to test if the sampling procedures are adequate.

Ultimately, the results obtained in the present study will be compared with unpublished results obtained with MarMAT – Marine Macroalgae Assessment Tool for the same period.

2. MATERIAL AND METHODS

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2.1. Study site description

2.1.1. Buarcos Bay characterization

Buarcos Bay is located in the Western Portuguese coast, north of the city of Figueira da Foz (40º09´54´´N; 8º52´11´´W), and falls in the category of Mesotidal Atlantic Exposed Shore defined for Portuguese typologies (Bettencourt et al., 2004). It has a NW-SE general orientation until Cabo-Mondego, with approximately 2.8 km length. The beach is located in a warm temperate coastal system with a mediterranean temperate climate experiencing a clear seasonal pattern of precipitation with higher rainfall periods during winter and dry warm periods during summer (Portuguese Institute of Meteorology) (www.meteo.pt).

Buarcos is a narrow sandy beach, limited landward by urban infrastructures, namely coastline protection adjacent to a seaside avenue. Almost the total longshore extension of the beach is covered by hard rock outcrops, which have an onshore-offshore orientation and average development from 2 m depth above chart datum (CD) to 1 m depth below CD. The beach sediments are mainly medium and coarse sand (D50 = 0.69 mm). The mean tidal range is 2.2 m (Larangeiro & Oliveira, 2003).

2.1.2. Geological characterization

The lithostratigraphic unit of Buarcos beach is formed by the Boa Viagem sandstones, named like that due their location near the Boa Viagem Hill. This unit (over 400 m high) that constitutes the geological substrate of the region, as can be observed in the Geological of Portugal (sheet 19C – Figueira da Foz) (Fig. 1), was formed during the Upper Jurassic or Malm (Low Kimmeridgian to Tithonian; 141 MA to 152 MA), and corresponds to a thick sandstone - clay- series of reddish and yellowish colour with crisscrossed stratification and some limestone, marly limestone or marly beds, where the continental character increases to the top; this series settles over the underlying layers in stratigraphic unconformity (Kullberg et al., 2006).

2.1.3. General Coastal Water Circulation

The Portuguese Current System (PCS) is characterised by a North-South water flow from 46º N to 36º N in latitude, and offshore up to 24º W in longitude. It is a complex system and of difficult spatial definition, due to the interaction between coastal and oceanic currents, bathymetry and water bodies. It encompasses several currents (the Portuguese Current, the Portuguese Coastal Current and the Portuguese Coastal Counter-Current), the PCS is dominated by the North Atlantic Gyre, which is characterised as being a slow circulation region between the North Atlantic Current and the Azores Current (Portuguese Geographic Information System – SNIG) (snig.igeo.pt/). During summer the strong and persistent north/northwesterly winds results in a general circulation pattern dominated by an equatorward flow on the continental shelf and slope (Portuguese Coastal Current). Also during summer the area is protected from the influence of atmospheric synoptic low pressure systems, showing a low energy wave regime (significant wave heights of about 2 m). During the winter, the northerly component of the wind weakens, or even reverses, reversing the surface flow and this way originating a relatively narrow, warmer and saltier poleward current (Portuguese Coastal Counter-Current), flowing along the continental shelf and slope. These conditions are responsible for a highly energetic wave regime with significant wave heights exceeding 5 m during storms (Garcia, 2008).

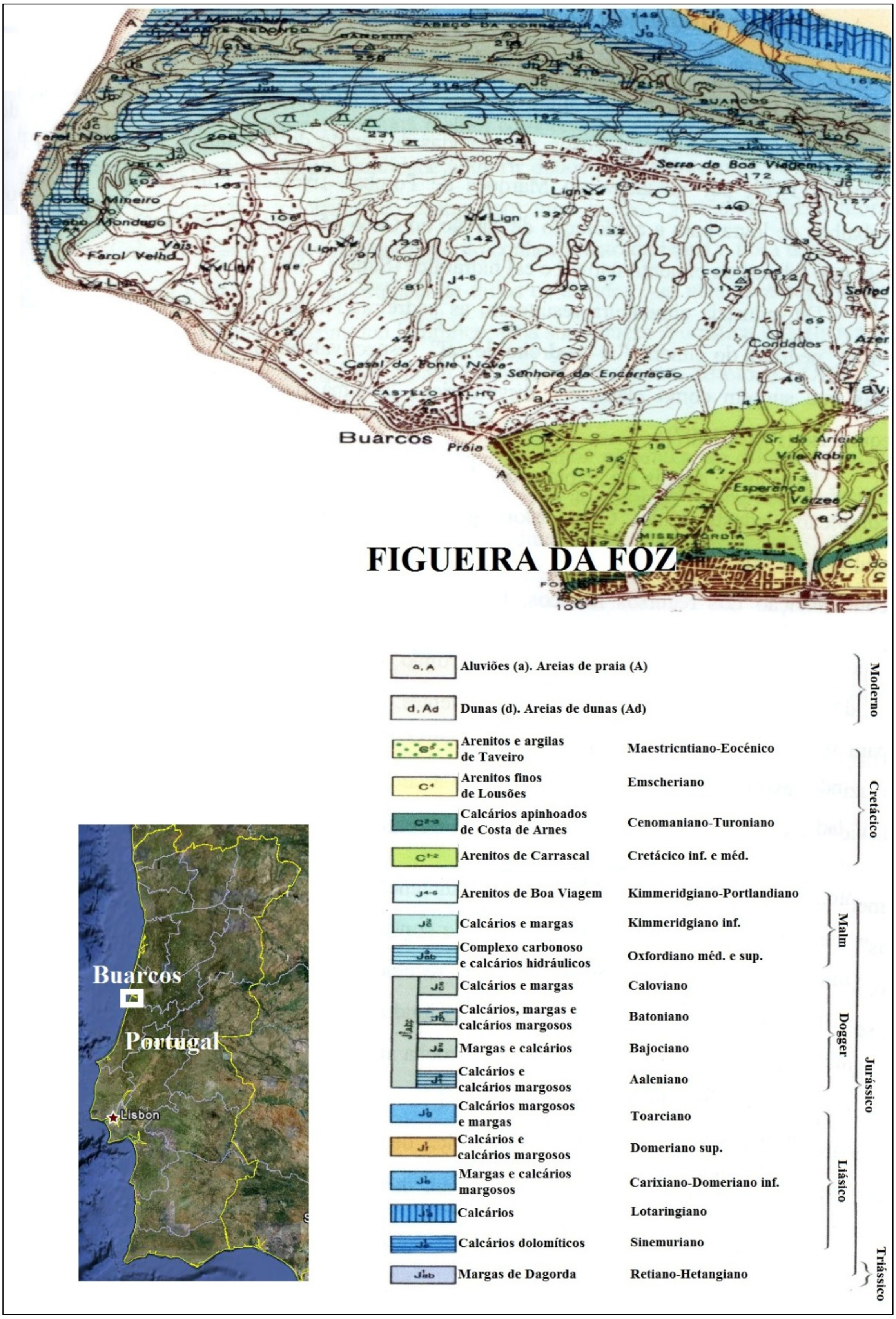

Figure 1 – Partial Geologic Chart of Portugal, sheet 19C – Figueira da Foz.

2.2. Sampling design and laboratorial procedures

2.2.1. Midlittoral benthic macrofauna and macroalgae

On the 12 June 2009, during low tide, three intertidal platforms were sampled near of a waste water discharge point one in front of the point of discharged (Station *A*) and other two located north of this point (Station *B* and Station *C*). Considering the intertidal zonation referred in the previous chapter all these 3 platforms correspond to the mid littoral zone. Concerning the pattern of occurrence of organisms, each platform was subdivided in three horizontally distributed zones – *a* (upper midlittoral, approximately 20m from the beginning of the platform)), *b* (mid midlittoral, approximately 60m)) and *c* (lower midlittoral, approximately 90m)). Each of these zones was subdivided in two levels (1 and 2) – *Stratified sampling*, and three replicates using 12cm x 12cm squares were randomly collected at each level – *Random sampling*. Coordinates for each platform were taken and saved in a GPS device for future sampling at the same sites.

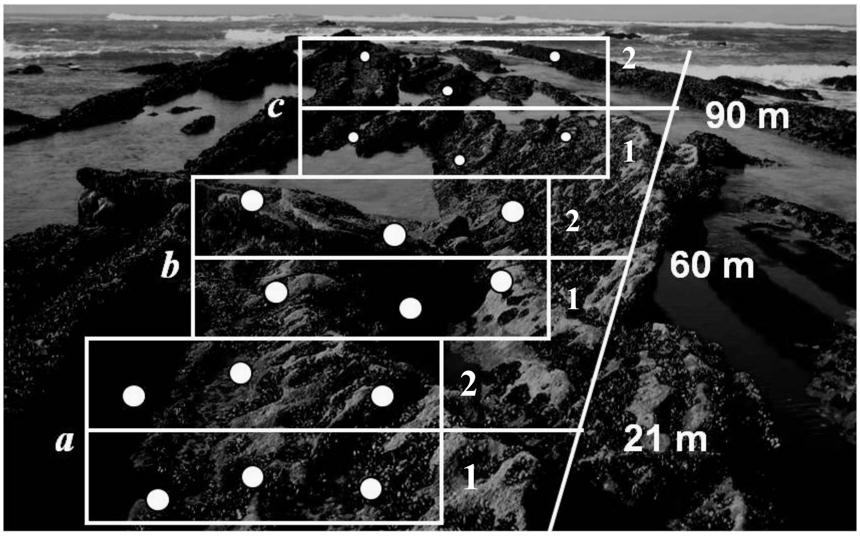

Figure 2 – Sampling schematics of the survey. *Zone a* (upper midlittoral, 21 m), *b* (mid midlittoral, 60 m) and *c* (lower midlittoral, 90 m). 1 and 2 refers to the levels subdividing each zone. White dots represent replicates.

At each replicate site, when in the presence of sessile organisms (e.g. barnacles), photographs were taken before removing the totality of the macrofauna and the associated macroalgae with a chisel.

Each sample was kept in a properly labelled bag, outside and inside with the site designation (Station [*A, B* or *C*]), zone (*a, b* or *c*), level (1or 2), number of the replicate (1, 2 or 3) and sampling date (ex.: StAa1R1, June 2009).

Once in the laboratory, samples were immediately preserved in 4% buffered formalin solution. *A posteriori*, samples were washed through a 1 mm sieve and all faunal organisms were sorted, counted and identified to the lowest possible category, preferentially to species level. Algal individuals were also identified to the lowest possible category, preferentially to species level, and biomass was estimated as dry weight (DW) by drying at 60 ºC, until reaching a constant weight.

2.2.2. Water Physicochemical Parameters

In parallel with biological samples, water samples (3 L) were collected at each platform and at the source of pollution point. Physicochemical parameters [salinity, temperature (ºC) and pH] were measured *in situ* using a Data Sonde Survey 4, the remaining parameters [nutrients, silica and chlorophyll *a*], concentrations were after analysed in the laboratory.

Water samples were immediately filtered using a “Whatman GF/F glass-fibre filter”. Approximately 250 mL of the filtered water were stored frozen at -18 ºC until analysis following standard methods described in Limnologisk Metodik (1992) for ammonium ($N\text{-}NH_4$) and phosphate ($P\text{-}PO_4$) and in Strickland & Parsons (1972) for nitrate ($N\text{-}NO_3$), and nitrite ($N\text{-}NO_2$). The filter was wrapped in aluminium foil and frozen until analysis for Chlorophyll *a* determination following Strickland & Parsons (1972) method.

2.3. Data analysis

2.3.1. Statistical analysis

2.3.1.1. Physical-chemical parameters analysis

A Principal Component Analysis (PCA) on the environmental variables was performed to find patterns in data of high dimension by reducing the number of dimensions, without much loss of information. Prior to the calculation of the environmental parameters resemblance matrix based on the Euclidean distance, nitrites, nitrates and silica were "1/Y" transformed, while salinity, pH and temperature were square-root-transformed. Afterwards, all parameters followed normalisation.

2.3.1.2. Macroalgae data analysis

Macroalgae biomass was converted to dry-weight per unit (g DW m^{-2}). Total macroalgae biomass was square-root transformed and total number of species was not transformed. The Euclidean distance was calculated, followed by normalization.

The statistical significance of variance components were tested using 9999 permutations of residuals under a reduced model, with *a priori* chosen significance level of α=0.05. One-way PERMANOVA was used to test differences between the three study platforms (fixed factor; St *A*, St *B* and St *C*) and a three-way analysis PERMANOVA was performed to examine interactions, that included (1) platforms (fixed factor; St *A*, St *B* and St *C*), (2) zones (fixed factor; *zone a*, *zone b* and *zone c*) and (3) level (fixed factor; 1 and 2). Both tests were performed for total biomass and total number of species. Afterwards, pair-wise analysis was performed in order to infer witch pairs of platforms (one-way PERMANOVA) and terms or interactions (three-way analysis PERMANOVA) were significantly different. When the possible number of permutation was lower than 150, the Monte Carlo-p was considered.

Macroalgae biomass data was and square-root transformed, on Bray Curtis similarity matrix. Principal Coordinate Analysis (PCO) was used as an ordination method to visualize patterns in data. One-way PERMANOVA and a three-way analysis PERMANOVA were performed to test differences between platforms and terms and interactions, followed by pair-wise tests. The statistical significance of variance components were tested using 9999 permutations of residuals under a reduced model, with an *a priori* chosen significance level of $\alpha = 0.05$. The Similarity Percentages-species contributions (SIMPER) analysis was used to determine which macroalgae species contributed most for the similarity within platforms and zones or for the dissimilarity between platforms and zones.

The relationship between environmental variables and the maroalgae was explored by carrying out a Distance-based Linear Models analysis (DistLM) (Anderson, 2005) with “Best” as selection procedure and “BIC” (Bayesian Information Criterion) as selection criterion. Distance based redundancy analysis (dbRDA) was performed in order to visualize the model in the multivariate space of the chosen resemblance matrix.

All analysis were performed using the PRIMER 6 + PERMANOVA© software (software package from Plymouth Marine Laboratory, UK) (Clarke, 2001; Anderson et al., 2008).

2.3.1.2.1. Ecological Quality Ratio: MarMAT (Marine Macroalgae Assessment Tool)

The MarMAT is a multimetric methodology, compliant with the European WFD requirements, based on 'Composition' (Chlorophyta, Phaeophyceae and Rhodophyta) and 'Abundance' (coverage of opportunists) of marine macroalgae (Neto et al., *submitted*). Within the EQR scale (0–1) five ecological quality status classes are defined to establish the final EQS (EC,2000): “Bad” (0-0.19), “Poor” (0.20-0.39), “Moderate” (0-40-0.59), “Good” (0.60-0.79) and “High” (0.80-1).

MarMAT unpublished results will be compared to the behaviour of ecological indices calculated from macroinvertebrate data, in order to assess the ecological condition of the assemblages.

2.3.1.3. Macrofauna data analysis

Abundance data of invertebrates was converted to density (ind. m^{-2}). Total density was fourth-root transformed and total number of species was square-root transformed. The ecological indices i) Margalef richness index (d); ii) Shannon-Wiener diversity index (H’); iii) Pielou evenness index (J’); and iv) Simpson domination index (1-D) results were not transformed. The Euclidean distance was calculated, followed by normalization.

The statistical significance of variance components were tested using 9999 permutations of residuals under a reduced model, with *a priori* chosen significance level of α=0.05. One-way PERMANOVA was used to test differences between the three study platforms (fixed factor; St *A*, St *B* and St *C*) and a three-way analysis PERMANOVA was performed to examine interactions, that included (1) platforms (fixed factor; St *A*, St *B* and St *C*), (2) zones (fixed factor; *zone a*, *zone b* and *zone c*) and (3) level (fixed factor; 1 and 2). Both tests were performed for total density total and total number of species, and for the ecological indices results. Afterwards, pair-wise analysis was performed in order to infer witch pairs of platforms (one-way PERMANOVA) and terms or interactions (three-way analysis PERMANOVA) were significantly different. When the possible number of permutation was lower than 150, the Monte Carlo-p was considered.

Macrofauna density data was fourth-root transformed, on Bray Curtis similarity matrix. Principal Coordinate Analysis (PCO) was used as an ordination method to visualize patterns in data. One-way PERMANOVA and a three-way analysis PERMANOVA were performed to test differences between platforms and terms and interactions, followed by pair-wise tests. The statistical significance of variance components were tested using 9999 permutations of residuals under a reduced model, with an *a priori* chosen significance level of $\alpha = 0.05$. The Similarity Percentages-species contributions (SIMPER) analysis was used to determine which macrofauna species contributed most for the similarity within platforms and zones or for the dissimilarity between platforms and zones.

The relationship between environmental variables and the macrofauna was explored by carrying out a Distance-based Linear Models analysis (DistLM) (Anderson, 2005) with "Best" as selection procedure and "BIC" (Bayesian Information Criterion) as selection criterion. Distance based redundancy analysis (dbRDA) was performed in order to visualize the model in the multivariate space of the chosen resemblance matrix.

All analysis were performed using the PRIMER 6 + PERMANOVA© software (software package from Plymouth Marine Laboratory, UK) (Clarke, 2001; Anderson et al., 2008).

2.3.1.3.1. Ecological Indicators

The diversity of macrobenthic fauna was assessed by different ecological indices: i) Margalef richness index (d) (Margalef, 1968); ii) Shannon-Wiener diversity index (H') (Shannon & Weaver, 1963); iii) Pielou evenness index (J') (Pielou, 1969); and iv) Simpson domination index (1-D) (Simpson, 1949). Indices were calculated as

$$d = \frac{(S-1)}{\ln N}$$

, where S is the number of species and *N* is the total number of individuals. The higher is the index's value, higher is the diversity (e.g. a value of 0 means all individuals belong to the same species).

$$H' = -\sum p_i \log_2 p_i$$

, where *pi* is the proportion of individuals belonging to species *i* in the sample. This can be estimated as *Ni / N*, the reason between the number of individuals of species *i* (*Ni)* and number of total individuals (*N*). The index's unit depends on the utilized logarithm. In this study the log_2 was used, being expressed as bits/individual. It can assume values between 0 and any other positive number, nevertheless numbers above 5 bits/individual are rare (Marques et al., 2009).

$$J' = \frac{H'}{H'max} = \frac{H'}{\log S}$$

, where *H'*max is the maximum diversity possible. This index's values can range between 0 (all individuals belong to the same species) and 1 (all individuals belong to different species).

$$D = \sum_{i=1}^{n} N_i (N_i - 1)$$

, where *Ni* is the number of individuals of species *i* and *N* is the total number of individuals. This index can assume values between 0 and 1, and high values imply a low diversity (e.g. 1 means all individuals belong to the same species). Simpson index was calculated on the 1-D algorithm; hence, the results should be interpreted inversely to Simpson's dominance (D).

Indices were calculated per replicate and a mean value was estimated per zone within each platform.

3. RESULTS

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3.1. Environmental data

At Buarcos beach the Portuguese Coastal Current was not observed during the day and time of sampling (Fig. 3), this could be due to the geomorphological phenomenon of the Hill of Boa Viagem which may have lead to a current turnover from North-South to South-North orientation.

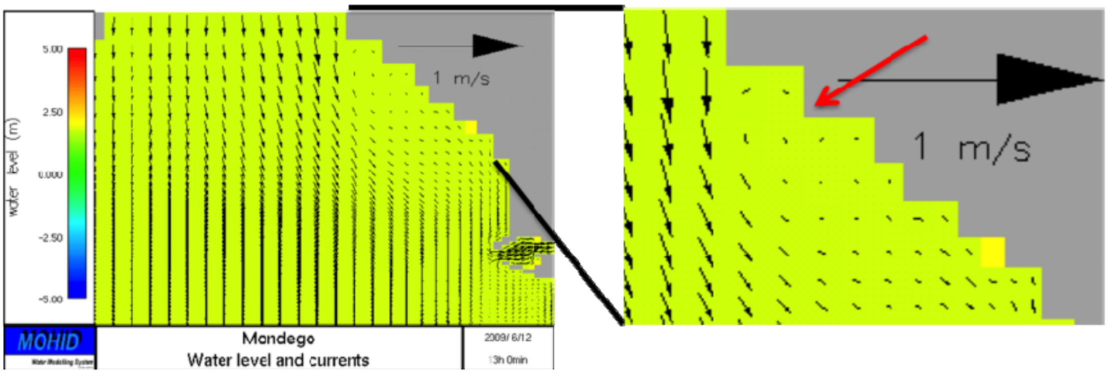

Figure 3 – Current velocity and direction at Buarcos beach during the day (June 12th, 2009) and time (1 pm) of sampling (red arrow represents the point pollution source).

The physical-chemical parameters results are shown in Table I.

Water temperature (Fig. 4) did not vary much, ranging from 21.4 ºC at St *Fonte* (source of pollution) and St *C* sites, to 22.1 ºC at St *A*. Regarding salinity and pH, higher values were registered for St *A* (35.7 and 8.38, respectively), while the lowest values were found for St *Fonte* (0.4 and 7.71, respectively).

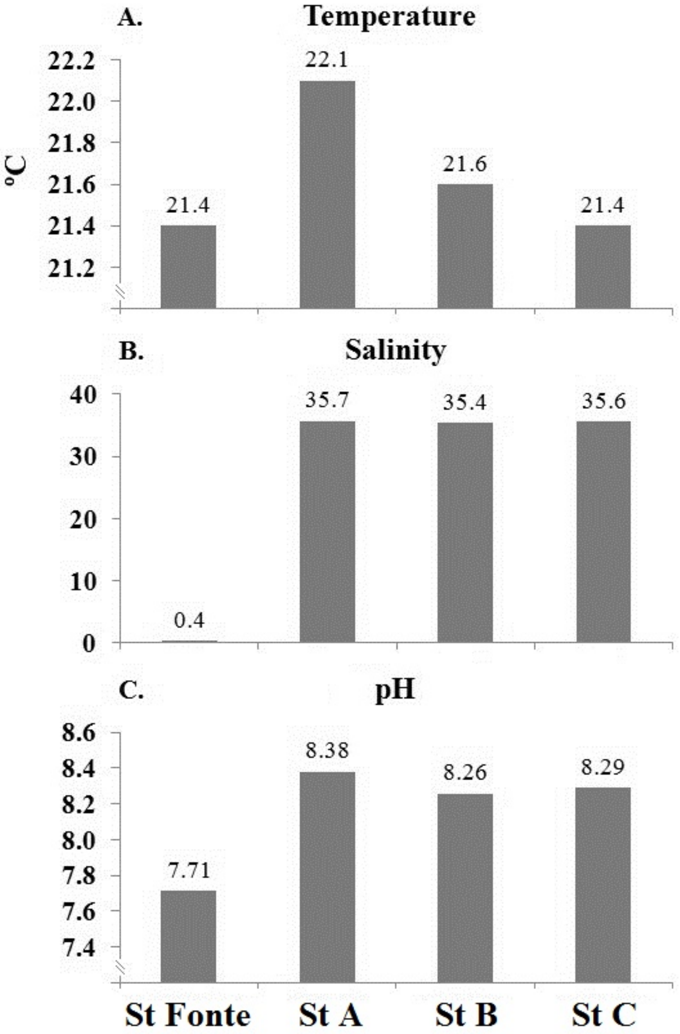

Figure 4 – Values for physical-chemical parameters (A.) Temperature, (B.) Salinity and (C.) pH found at each station (St).

Chlorophyll *a* (Fig. 5) concentration ranged from 0.779 mg m^{-3} at St *C* and 2.17 mg m^{-3} at St *A*. Regarding the nutrients concentration, higher values were always found at St *Fonte* site, with 0.003 mg L^{-1} for nitrites (N-NO_2), 0.580 mg L^{-1} for nitrates (N-NO_3), 0.019 mg L^{-1} for phosphates (P-PO_4), with a similar value for St *B* (0.018 mg L^{-1}), and 0.031 mg L^{-1} for ammonia (N-NH_4). Lower values were found for N-NO_2 at St *C* (0.001 mg L^{-1}), for N-NO_3 at St *A* (0.029 mg L^{-1}), for P-PO_4 at St *A* and St *C* (0.003 mg L^{-1}), and for -NH_4 at St *C* (0.0004 mg L^{-1}). The St *Fonte* site also presented the maximum silica value (2.579 mg L^{-1}), while St *B* registered the lowest (0.034 mg L^{-1}).

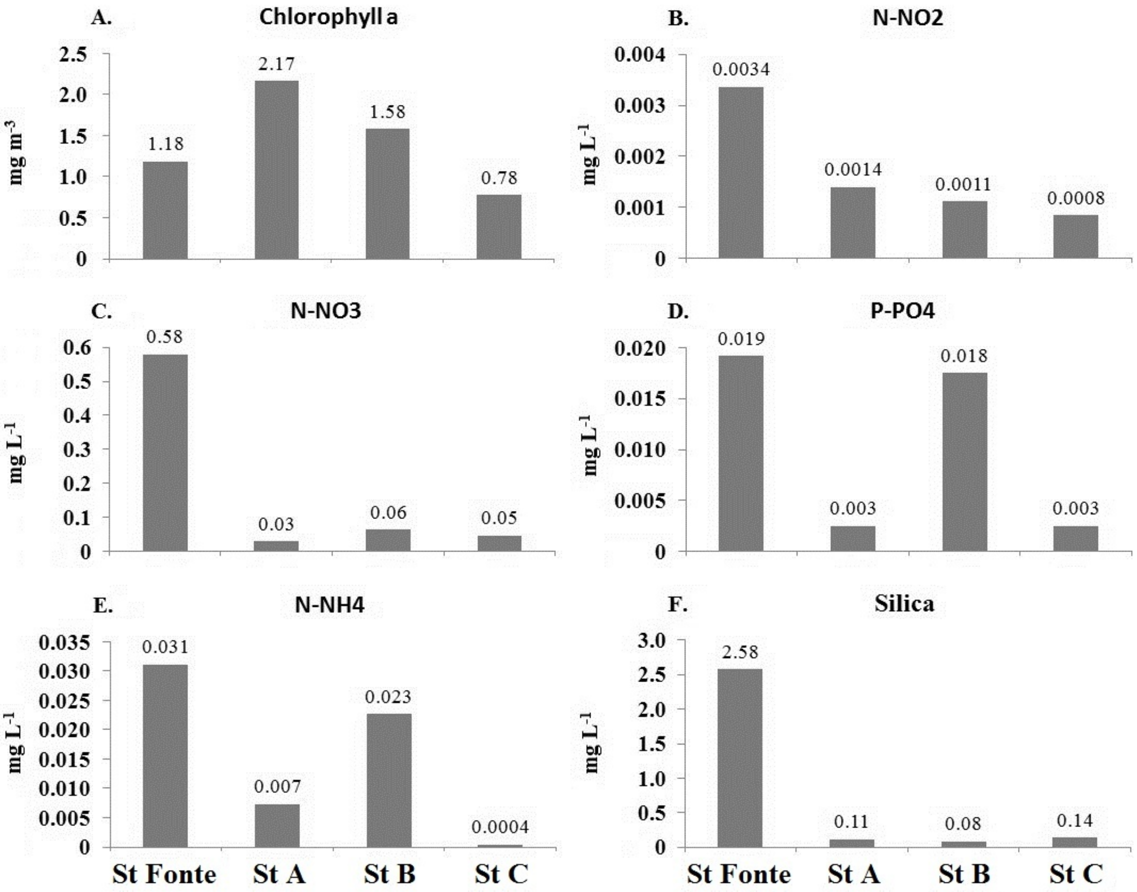

Figure 5 – Values for physical-chemical parameters (A.) Chlorophyll *a*, (B.) N–NO_2; (C.) N–NO_3; (D.) P-PO_4; (E.) N–NH_4; and (F.) Silica found at each station (St).

Table I – Physical-chemical parameters values found for the three platforms and the source of pollution.

	St Fonte	St A	St B	St C
Temperature (ºC)	21,4	22,1	21,6	21,4
Salinity	0,40	35,7	35,4	35,6
pH	7,71	8,38	8,26	8,29
Chlorophyll *a* (mg m^{-3})	1,183	2,168	1,579	0,779
N-NO2 (mg L^{-1})	0,003	0,001	0,001	0,001
N-NO3 (mg L^{-1})	0,580	0,029	0,064	0,047
Phosphate (mg L^{-1})	0,019	0,003	0,018	0,003
N-NH4 (mg L^{-1})	0,031	0,007	0,023	0,0004
Silica (mg L^{-1})	2,579	0,113	0,084	0,143

The Principal Component Analysis (PCA) for physical-chemical environmental factors provided a clear distinction between platforms (Fig. 7). The first two principal components (PC1 and PC2) explained 88.4% of data variability. The first axis (PC1) explained most (65.4%) of this variability, where N-NH_4 and P-PO_4 contribute for the positive component, and chlorophyll *a,* $N\text{-}NO_2$, $N\text{-}NO_3$, pH, salinity, silica and temperature contribute for the negative component of this axis. The second axis (PC2) explained 23.0%, with chlorophyll *a*, $N\text{-}NH_4$, $N\text{-}NO_3$, $P\text{-}PO_4$, silica and temperature contribute for the positive component, and pH, $N\text{-}NO_2$ and salinity contribute for the negative component of this axis.

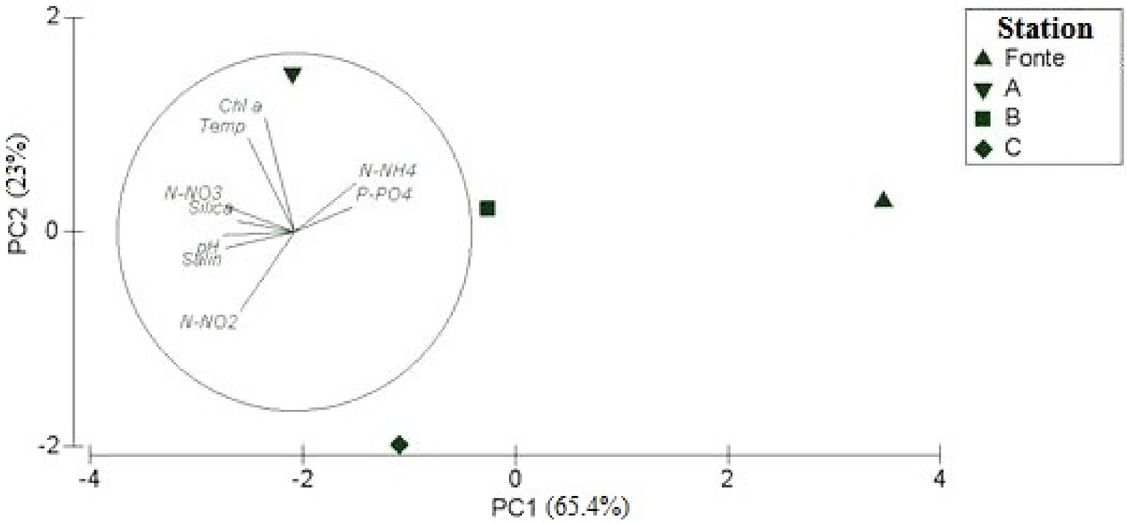

Figure 6 – Two-dimensional Principal Component Analysis (PCA) plot of physicochemical parameters for the three platforms – St *A,* St *B* and St *C*, and the source of pollution – St *Fonte*. (Chl a. Chlorophyll *a*; Salin. Salinity; Temp. Temperature).

3.2. Spatial variation in macroalgae

During the study period 49 different macroalgae taxa were found, belonging to Divisions Chlorophyta (9) and Rhodophyta (37), and to Class Phaeophyceae (3). Table II shows the spatial occurrence for all recorded taxa. The species *Ulva lactuca/rigida* and *Ulva intestinalis/compressa* were dominant, accounting for 50.46% and 15.21% of total biomass (with mean biomass of 58.93 g DW m^{-2} and 17.76 g DW m^{-2}, respectively), while the remaining taxa represented individually less than 7%.

Table II – Macroalgae taxa found in the study, their occurrence (platforms St *A,* St *B* and St *C*; zones *a, b* and *c*; and levels 1 and 2), mean biomasses (MD) (g DW m^{-2}) and related standard deviation (SD), and their proportion of the total biomass (PT) (%). A cross (x) corresponds to presence.

STATION	**A**						**B**						**C**						**MB (g DW m^{-2})**	**SD**	**PT (%)**
ZONE	**a**		**b**		**c**		**a**		**b**		**c**		**a**		**b**		**c**				
LEVEL	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**			
Rhodophyta																					
Acrochaetium spp.									x										0.0001	0.001	0.0001
Aglaothamnion spp.											x						x	x	0.0004	0.002	0.0003
Anotrichium furcellatum																		x	0.0001	0.001	0.0001
Apoglossum ruscifolium/ Hypoglossum hypoglossoides				x			x	x	x	x	x			x	x	x			0.0086	0.054	0.0074
Boergeseniella spp.			x								x						x	x	0.0501	0.360	0.0429
Callithamnion/ Aglaothamnion/ Antithamnion spp.											x								0.0001	0.001	0.0001
Callithamnion tetragonum											x						x	x	0.1870	1.359	0.1601
Callithamnion tetricum					x						x				x		x		4.6813	34.077	4.0082
Caulacanthus ustulatus													x				x		0.0031	0.022	0.0027
Ceramium spp.	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x	0.6140	2.553	0.5257
Chondracanthus acicularis																	x		0.3902	2.841	0.3341
Chondracanthus teedei var. lusitanicus	x						x	x		x	x	x					x	x	3.1540	13.639	2.7004
Chondria coerulescens			x								x								0.0003	0.001	0.0002
Chondrus crispus										x							x		0.0628	0.392	0.0537
Colaconema daviesii			x	x			x	x	x	x				x					0.0010	0.003	0.0009
Corallina elongata	x							x	x	x	x	x						x	5.1490	28.786	4.4085
Corallina officinalis				x															0.0138	0.100	0.0118
Corallina spp.			x				x	x	x	x	x	x			x			x	0.3041	1.159	0.2604
Cryptopleura ramosa											x				x		x		0.0621	0.450	0.0532
Gastroclonium reflexum										x									0.0001	0.001	0.0001
Gelidium pulchellum	x															x			2.2596	16.450	1.9346
Gracilaria gracilis	x		x				x	x			x	x			x	x	x	x	1.2862	6.859	1.1012
Gymnogongrus griffithsiae							x	x	x										0.4176	2.874	0.3575

Table II. (Continued)

Halurus equisetifolius					x			x											0.0016	0.011	0.0013
Herposiphonia secunda	x		x				x		x	x	x				x			x	0.0020	0.003	0.0017
Jania spp.			x																0.0001	0.001	0.0001
Lophosiphonia reptabunda										x									0.0001	0.001	0.0001
Mastocarpus stellatus/ *Petrocelis cruenta*			x							x	x			x				x	0.3200	1.869	0.2740
Osmundea pinnatifida			x	x				x	x	x	x	x		x	x	x	x	x	7.4080	17.570	6.3427
Pleonosporium spp.															x				0.0001	0.001	0.0001
Plocamium cartilagineum												x							0.0815	0.593	0.0698
Polysiphonia spp.			x					x	x		x	x					x	x	0.0238	0.145	0.0204
Porphyra spp.		x			x				x		x	x							4.3415	21.953	3.7171
Pterosiphonia complanata				x							x	x					x	x	0.1247	0.648	0.1068
Pterosiphonia parasitica				x															0.0001	0.001	0.0001
Pterosiphonia pennata			x	x			x		x		x				x			x	0.0009	0.002	0.0008
Rhodothamniella spp.									x										0.0001	0.001	0.0001
Chlorophyta																					
Chaetomorpha spp.							x	x	x							x			0.0005	0.002	0.0004
Cladophora spp.	x		x				x	x	x	x									1.1668	7.641	0.9990
Codium spp.																		x	0.2121	1.544	0.1816
Rhizoclonium riparium/ Ulothricales								x											0.0003	0.001	0.0002
Ulva compressa									x										0.0001	0.001	0.0001
Ulva intestinalis/ *compressa*	x	x	x			x	x	x	x		x	x							17.7612	72.107	15.2071
Ulva intestinalis							x		x	x									1.3607	9.904	1.1650
Ulva lactuca									x	x			x						58.9348	87.852	50.4599
Ulva lactuca/rigida	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	6.2847	26.995	5.3810
Phaeophyceae																					
Dictyota dichotoma									x		x	x							0.1232	0.512	0.1055
Ectocarpales/ *Sphacelaria* spp.			x				x		x			x							0.0007	0.002	0.0006
Stypocaulon scoparium							x												0.0001	0.001	0.0001

The macroalgae mean number of species and mean biomass (g DW m^{-2}) found per zone at each platform are represented on Figure 7.

Zone b of St *C* obtained the highest mean number of species (9.17), whereas *zone b* of St *A* obtained the lowest (0.41). Mean biomass highest value was found for *zone b* of St *A* (227.7 g DW m^{-2}) while the lowest value (0.91 g DW m^{-2}) was found for *zone c* of that platform.

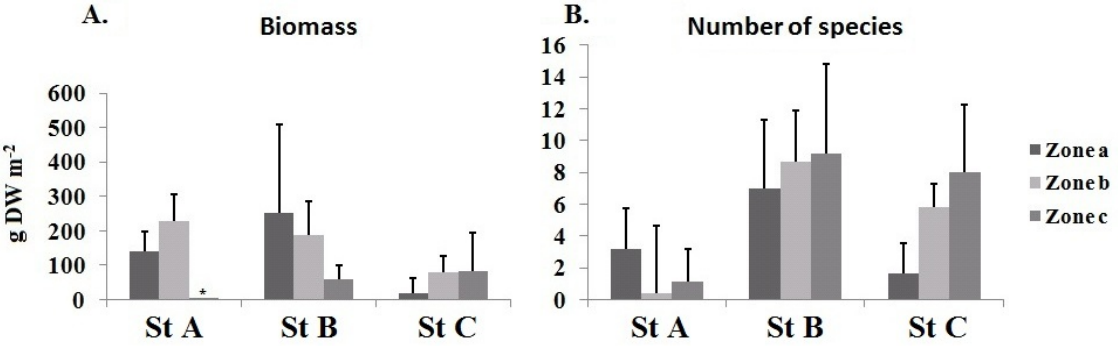

Figure 7 – Macroalgae mean density (A.) and mean number of species (B.) per zone for all platforms. An asterisk (*) means value close to 1.

PERMANOVA revealed statistically significant differences in species number between platforms ($F_{(Pl)2,51}$=6.725; p=0.0024) and also the interaction Platform*Zone ($F_{(Pl*zn)4,36}$=2.7887; p=0.0421). The Pair-wise test on the “Platform” revealed significant differences between the pairs St *A* and St *B* ($t_{A,B}$=3.548, $p(MC)_{A,B}$=0.0015), and between St *B* and St *C* ($t_{B,C}$=2.295, $p(MC)_{B,C}$=0.027). For the term “Platform*Zone” the pair-wise test showed, within “Zone” levels “a” and level “b”, sites St *B* and St *C* (t=2.604, p=0.0401 and t=2.272, p=0.0126, respectively) being significantly different. For levels of factor “Platform” within level “c” the test revealed statistically significant differences between St *A* and St *B* ($t_{A,B}$=3.3045, $p_{A,B}$=0.011), and between St *A* and St *C* ($t_{A,C}$=3.4406, $p_{A,C}$=0.011). Regarding the term “Platform*Zone” within “Platform” levels the analysis showed that within St *A* only the *zone b* and *zone c* were significantly different (t=2.9034, p=0.0269). Within St *B* there were no significant differences

($p>0.05$) between all pairs of zones. For St *C* significant differences were found between *zone a* and *zone* b ($t_{a,b}=5.4554$, $p_{a,b}=0.0014$) and between *zone a* and *zone c* ($t_{a,c}=3.3328$, $p_{a,c}=0.0163$).

Regarding total biomass, significant statistical differences were found between platforms ($F_{(Pl)2,51}=3.3583$, $p=0.0428$), and also the interaction Platform*Zone ($F_{(Pl*Zn)4,36}=5.8024$; $p=0.0008$). The pair-wise test showed only St *B* and St *C* were significantly different ($t=2.7246$, $p=0.0118$). For the term "Platform*Zone" significant differences were found between all zones across platforms: *zone a* was significantly different between St *A* and St *C* ($t_{A,C}=5.1552$, $p_{A,C}=0.0025$); *zone b* was significantly different in St *C* ($t_{A,C}=3.9198$, $p_{A,C}=0.0034$ and $t_{B,C}=3.4751$, $p_{A,C}=0.0079$, respectively); and *zone c* was significantly different in St *A* ($t_{A,B}=4.9469$, $p_{A,B}=0.0038$ and $t_{A,C}=2.7708$, $p_{A,C}=0.0156$, respectively). For the term "Platform*Zone" the analysis showed that within St *A* all zones revealed statistically significant differences ($p<0.05$). Within St *B* only *zone b* and *zone c* were significantly different ($t=4.4633$, $p=0.0019$). Within St *C* on the other hand, *zone b* was different from *zone a* ($t=2.9802$, $p=0.0204$).

Principal Coordinate Analysis (PCO) didn´t show clear differences between the studied platforms and zones (Fig. 8), with the first two principal component axis explaining 52.1% of the samples variability.

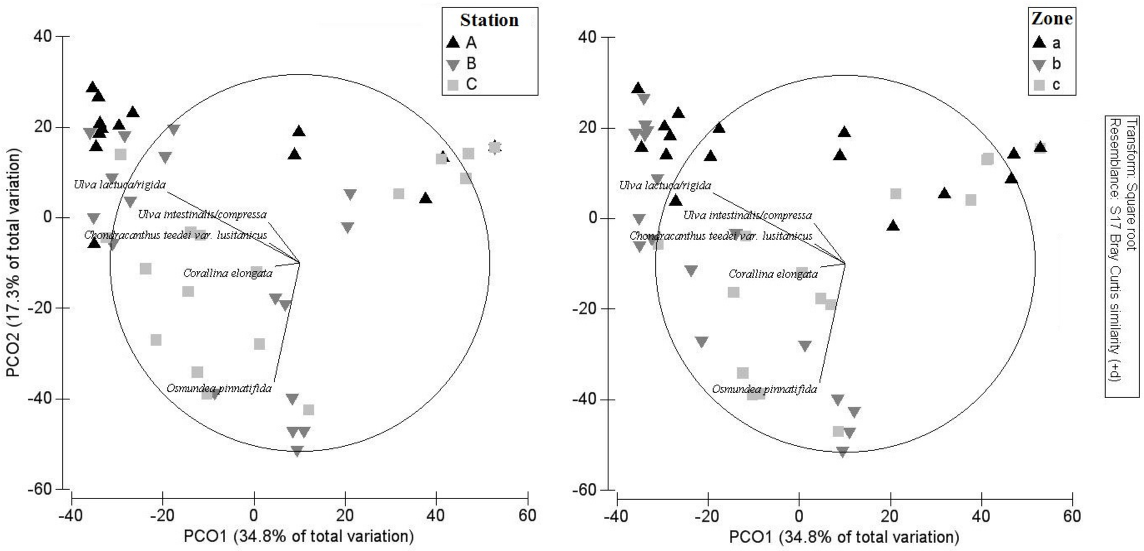

Figure 8 – Principal Coordinate Analysis (PCO) plot based on macroalgae for platforms (A) and zones (B) with the representation of the species that contributed most to groups' similarities (Axis 1 = 34.8%; Axis 2 = 17.3%).

Multivariate analyses (PERMANOVA) for the algal community, revealed statistically significant differences between platforms ($F_{(Pl)2,51}$=2.874; p=0.0022), and also the interaction Platform*Zone ($F_{(Pl*Zn)4,36}$=3.978; p=0.0001). The Pair-wise test for "Platform" revealed significant differences between platforms (p<0.05). The pair-wise test for "Platform*Zone" for "zone" showed statistically significant differences in *zone a* between St *A* and St *C* (t=2.880 p=0.0042). For both zones *b* and *c* significant differences were found between St *A* and St *B* (t=2.1345, p=0.0495 and t=2.6936, p=0.0029, respectively), and between St *A* and St *C* (t=2.8735, p=0.0032 and t=2.7277, p=0.0054, respectively). Regarding the term "Platform*Zone" within "Platform" levels, the analysis showed for St *A* statistically significantly differences between all pairs of zones (p<0.05). Within St *B* differences were found between *zone a* and *zone c* (t=1.4815, p=0.0324). For St *C* significant differences were found between *zone a* and *zone* b (t=2.5628, p=0.0071) and between *zone a* and *zone c* (t=1.9352, p=0.0198).

SIMPER analysis (80% cut-off) showed the similarities within platforms were quite low (from 18.34% for St to 25.10% for St *A*). Five species contributed for these similarities, with *U. lactuca/rigida* contributed the most for St *A*, St *B* and St *C* similarities (86.40%, 48.88% and 60.19% respectively). Dissimilarities between platforms were 82.44% between St *A* and St *B,* 83.53% between St *B* and St *C*, and 85.34% between St *A* and St *C.* The species *U. lactuca/rigida* was the most contributing species for all dissimilarities*,* with 33.19%, 24.20% and 44.80%, respectively (Tale III).

Table III – SIMPER (80% cut-off) similarities (in gray) and dissimilarities (in white), between platforms – St *A, B* and *C* (A). (Ct: contribution (%); AD: average density (ind m^{-2}); "+": higher biomass in the factor on top; "-": higher biomass in the factor on the left).

	St A			**St B**			**St C**		
St A	**25.10%**	**Ct (%)**	**AD (g DW m^{-2})**						
	Ulva lactuca/rigida	86.4	7.4						
St B	**82.44%**			**18.34%**	**Ct (%)**	**AD (g DW m^{-2})**			
	Ulva lactuca/rigida (+)			*Ulva lactuca/rigida*	48.9	5.0			
	Ulva intestinalis/compressa (-)			*Osmundea pinnatifida*	18.2	1.8			
	Osmundea pinnatifida (-)			*Corallina elongata*	6.7	1.7			
	Ulva lactuca (-)			*Ulva intestinalis /compressa*	5.4	2.5			
	Porphyra spp. (-)			*Chondracanthus teedei var. lusitanicus*	4.8	1.5			
	Corallina elongata (-)								
	Chondracanthus teedei var. lusitanicus (-)								
St C	**85.34%**			**83.53%**			**19.53%**	**Ct (%)**	**AD (g DW m^{-2})**
	Ulva lactuca/rigida (+)			*Ulva lactuca/rigida* (+)			*Ulva lactuca /rigida*	60.2	3.2
	Osmundea pinnatifida (-)			*Osmundea pinnatifida* (-)			*Osmundea pinnatifida*	26.8	2.1
	Ulva intestinalis/compressa (+)			*Ulva intestinalis/compressa* (+)					
	Gracilaria gracilis (-)			*Ulva lactuca* (+)					
	Porphyra spp. (+)			*Corallina elongata* (+)					
	Gelidium pulchellum (-)			*Porphyra spp.* (+)					
				Chondracanthus teedei var. lusitanicus (+)					
				Gracilaria gracilis (-)					
				Ulva intestinalis (+)					
				Gelidium pulchellum (-)					
				Dictyota dichotoma (+)					

Regarding the zones, 5 different species contributed for similarities, ranging from 14.55% in *zone c* to 39.80% in *zone b*, being *U. lactuca/rigida* the species with higher percentage of contribution for all zones (59.69%, 76.42% and 55.21% for *zone a*, *zone b* and *zone c* respectively). Dissimilarities were 80.40% between zones *a* and *b,* 82.92% between *zones b and c*, and 91.66% between *zones* a *and* c. The species *U. lactuca/rigida* was the most contributing species for all dissimilarities*,* with 38.04%, 42.29% and 28.78%, respectively (Table. IV).

Table IV – SIMPER (80% cut-off) similarities (in gray) and dissimilarities (in white) between zones – *zone a, b* and *c*. (Ct: contribution (%); AD: average density (ind m^{-2}); "+": higher biomass in the factor on top; "-": higher biomass in the factor on the left).

	Zone a			**Zone b**			**Zone c**		
Zone a	**15.37%**	**Ct (%)**	**AD (g DW m^{-2})**						
	Ulva lactuca/rigida	59.7	4.8						
	Ulva intestinalis/compressa	35.8	4.1						
Zone b	**80.40%**			**39.80%**	**Ct (%)**	**AD (g DW m^{-2})**			
	Ulva lactuca/rigida (-)			*Ulva lactuca /rigida*	76.4	7.4			
	Ulva intestinalis/compressa (+)			*Osmundea pinnatifida*	17.3	2.3			
	Osmundea pinnatifida (-)								
	Ulva lactuca (-)								
	Chondracanthus teedei var. lusitanicus (+)								
	Gelidium pulchellum (-)								
	Gracilaria gracilis (+)								
Zone c	**91.66%**			**82.92%**			**14.55%**	**Ct (%)**	**AD (g DW m^{-2})**
	Ulva lactuca/rigida (+)			*Ulva lactuca/rígida* (+)			*Ulva lactuca /rigida*	55.2	2.1
	Ulva intestinalis/compressa (+)			*Osmundea pinnatifida* (+)			*Osmundea pinnatifida*	21.2	1.8
	Osmundea pinnatifida (-)			*Ulva lactuca* (+)			*Gracilaria gracilis*	5.5	0.4
	Porphyra spp. (-)			*Porphyra spp.* (-)					
	Corallina elongata (+)			*Corallina elongata* (+)					
	Chondracanthus teedei var. lusitanicus (+)			*Gelidium pulchellum* (+)					
	Ulva intestinalis (+)			*Gracilaria gracilis* (+)					
	Mastocarpus stellatus/Petrocelis cruenta (+)								
	Gracilaria gracilis (+)								

DistLM analysis didn´t show a significant relationship between biological and environmental data when considering predictor variables individually, as none of the studied parameters were statistically significant. Nevertheless, N-NO_2 was the best solution (R^2=61%) to explain the total variability of the macroalgae.

The dbRDA (Fig. 9) calculated the variation percentage explained out of the fitted model (100%) and the variation percentage explained out of the total variation (100%). Chlorophyll *a*, N-NH_4, N-NO_3, pH, P-PO_4, silica, and temperature contributed positively in the first axis, while N-NO_2 and salinity contributed negatively. In the second axis chlorophyll *a*, pH, N-NO_3, salinity and temperature had a positive contribution while N-NH_4, N-NO_2, P-PO_4 and silica had a negative contribution.

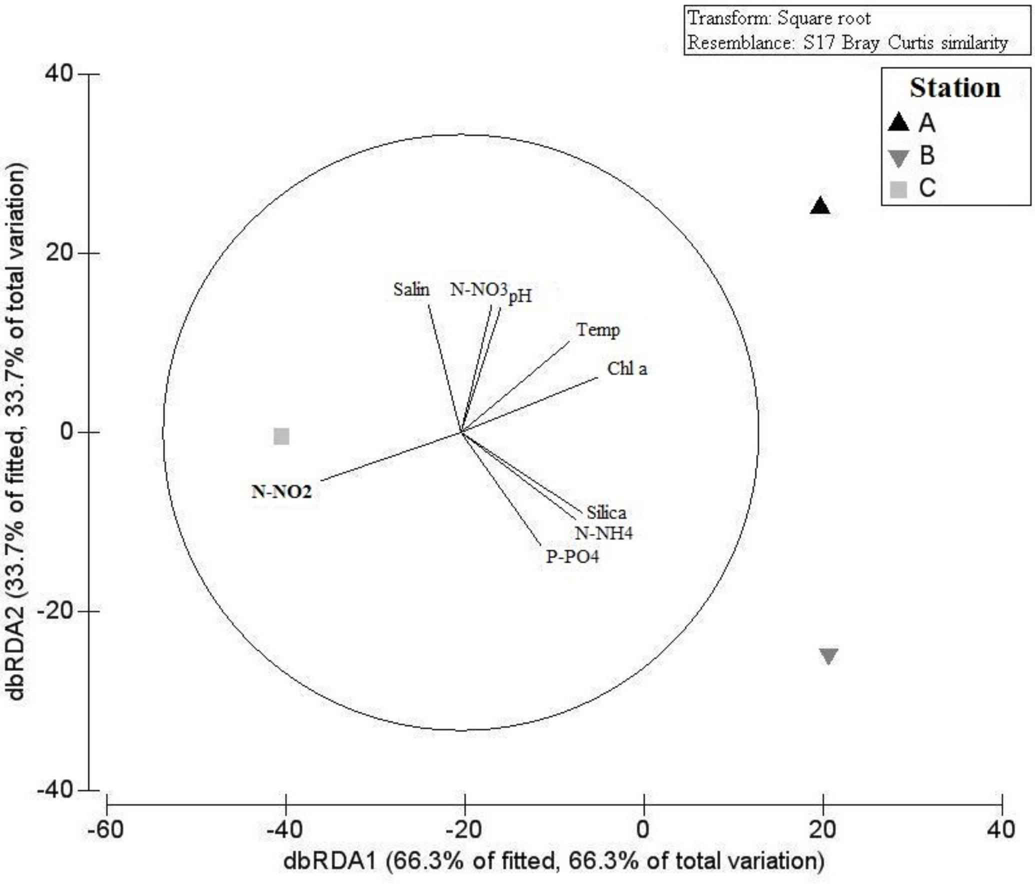

Figure 9 – Two-dimensional Distance based redundancy analysis (dbRDA) plot of all physicochemical parameters for the different station samplings (Axis 1 = 65.1% of fitted model, 65.1% of total variation; Axis 2 = 34.9% of fitted model, 39.9% of total variation). In bold is the best variable solution.

3.2.1. Ecological Quality Status: MarMAT (Marine Macroalgae Assessment Tool)

The MarMAT ecological tool presented distinct results (unpublished data) (Table V), with EQRs found for the sampling stations ranging from 0.47 – Moderate Status – in St *A* to 0.72 – Good Status – in St *C*.

Table V - MarMAT results obtained for the surveyed platforms (stations *A, B* and *C*) in spring 2009 (unpublished data). (EQR: Ecological Quality Ratio; EQS: Ecological Quality Status).

EQR	**0.47**	**0.61**	**0.72**
EQS	Moderate	Good	Good
Site	St A	St B	St C

3.3. Spatial variation in benthic macrofauna assemblages

During the study period, a total of 27930 individuals corresponding to 122 different macrobenthic taxa were found, belonging to Phyla Annelida (44), Arthropoda (41), Cnidaria (1), Echinodermata (2), Mollusca (31), Nematoda (1), Nemertea (1) and Sipuncula (1).

The species *Mytilus galloprovincialis* and *Chthamalus montagui* were dominant, accounting for 39.94% and 35.83% of total individuals (with mean densities of 14345.4 ind m^{-2} and 12870.4 ind m^{-2}, respectively), while the remaining taxa represented individually less than 6%. The taxa *Acanthochitona crinita, Acanthochitona fascicularis,* Actiniaria, *Dynamene bidentata, Eulalia viridis, Gibbula umbilicalis, Idotea pelágica, Lepidochitona cinérea, Lumbrineris impatiens, M. galloprovincialis,* Nemertea, *Sabellaria alveolata,* Syllinae and *Venerupis* sp. occurred in all zones of all platforms (with minor exceptions). Table VI shows the spatial occurrence for all recorded taxa, their total mean densities (ind m^{-2}) and related standard deviation, and their proportion of the total density.

Table VI – Macrobenthic taxa found in the study, their occurrence (platforms St *A,* St *B* and St *C*; zones *a, b* and *c*; and levels 1 and 2), mean densities (MD) (ind m^{-2}) and related standard deviation (SD), and their proportion of the total density (PT) (%). A cross (x) corresponds to presence.

STATION	A						B						C						MD (ind m^{-2})	SD	PT (%)
ZONE	a		b		c		a		b		c		a		b		c				
LEVEL	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2			
Annelida																					
Oligochaeta			x						x		x				x				7.716	29.142	0.021
Polychaeta				x															1.286	9.450	0.004
Aphroditidae					x									x					2.572	13.238	0.007
Aonides oxycephala									x	x	x		x		x		x		19.290	87.858	0.054
Arenicolides ecaudata													x		x		x		9.002	40.574	0.025
Capitella capitata			x		x					x									5.144	22.781	0.014
Cirriformia tentaculata	x					x			x							x		x	7.716	25.832	0.021
Eulalia sp.						x											x		5.144	26.476	0.014
Eulalia viridis			x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	119.599	161.553	0.333
Harmothoe sp.																	x		1.286	9.450	0.004
Laeonereis glauca													x	x					2.572	13.238	0.007
Lepidonotus clava																	x	x	5.144	22.781	0.014
Lumbrineris impatiens	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	237.912	273.354	0.662
Lumbrineris sp.									x						x				2.572	13.238	0.007
Malacoceros ciliatus	x		x	x					x										11.574	44.230	0.032
Nainereis cf. *laevigata*														x		x			1.286	9.450	0.004
Nainereis laevigata																	x		3.858	20.971	0.011
Naineris quadricuspida															x				3.858	28.351	0.011
Neanthes sp.														x					1.286	9.450	0.004
Nereididae		x		x	x			x	x	x	x				x				12.860	30.387	0.036
Orbiniidae			x																1.286	9.450	0.004
Perinereis cultrifera									x		x								6.430	27.871	0.018
Perinereis marionii	x			x	x	x			x				x	x	x		x		20.576	49.803	0.057
Platynereis dumerilii							x	x	x		x	x	x	x	x	x	x	x	84.877	253.336	0.236
Platynereis sp.			x																2.572	18.900	0.007
Pholoe minuta														x	x			x	3.858	16.056	0.011
Phyllodocinae					x				x					x		x	x	x	12.860	35.880	0.036
Phyllodoce sp.					x	x				x							x		7.716	25.832	0.021
Polycirrus sp.		x	x			x			x						x	x			24.434	68.994	0.068
Sabellaria alveolata	x	x		x	x	x	x		x	x	x	x	x	x	x	x	x	x	2025.463	3179.419	5.639
Sabellaria sp.						x													2.572	18.900	0.007
Sabellaria spinulosa																	x		10.288	59.429	0.029
Scolelepis cantabra							x		x						x				6.430	30.964	0.018
Scolelepis sp.															x				2.572	18.900	0.007

Table VI (Continued)

Scolelepis squamata					x														1.286	9.450	0.004
Spio filicornis									x										2.572	18.900	0.007
Spirobranchus lamarcki											x			x	x	x	x	x	11.574	37.555	0.032
Sthenelais boa					x							x							2.572	13.238	0.007
Spionidae													x						1.286	9.450	0.004
Syllidae					x														1.286	9.450	0.004
Syllinae	x	x	x	x	x	x		x	x	x	x	x	x	x	x			x	178.755	298.799	0.498
Syllis amica															x				1.286	9.450	0.004
Syllis garciai																		x	2.572	18.900	0.007
Syllis gracilis														x	x		x		6.430	24.389	0.018
Arthropoda																					
Chelicerata																					
Acarina														x					3.858	28.351	0.011
Araneae														x					1.286	9.450	0.004
Pycnogonida													x						2.572	13.238	0.007
Crustacea																					
Amphipoda									x		x				x				7.716	22.029	0.021
cf. **Aoridae**															x				1.286	9.450	0.004
Apohyale prevostii													x	x					3.858	20.971	0.011
Atylus swammerdami								x				x							2.572	13.238	0.007
Elasmopus rapax											x						x	x	5.144	18.358	0.014
Gammaropsis maculata																		x	1.286	9.450	0.004
Gammaropsis sp.									x										2.572	18.900	0.007
Guernea coalita									x										2.572	18.900	0.007
Hyale perieri														x			x		10.288	39.115	0.029
Hyale sp.									x			x	x	x		x			14.146	49.344	0.039
Hyale stebbingi					x	x			x	x		x	x	x		x			81.019	212.763	0.226
Jassa marmorata												x							1.286	9.450	0.004
Melita palmata								x			x	x							21.862	92.310	0.061
Microdeutopus chelifer						x					x	x			x				14.146	43.462	0.039
Microdeutopus damnoniensis (nomen nudum)															x				1.286	9.450	0.004
Photis longicaudata														x					1.286	9.450	0.004
cf. *Protomedeia fasciata*															x				2.572	18.900	0.007
Tritaeta sp.											x								1.286	9.450	0.004
Decapoda																					
Pachygrapsus marmoratus														x				x	5.144	22.781	0.014
Pilumnus hirtellus																	x		1.286	9.450	0.004
Pirimela denticulata							x	x	x		x	x			x	x	x	x	28.292	64.015	0.079
Isopoda																					
Paragnathia formica															x				3.858	28.351	0.011
Idotea balthica									x										3.858	28.351	0.011
Idotea granulosa		x					x									x			6.430	24.389	0.018
Idotea pelagica		x			x	x			x	x	x	x	x	x	x	x	x	x	605.710	1236.491	1.686
Idotea sp.												x							1.286	9.450	0.004

Table VI (Continued)

Cymodoce truncata															x		x	x	9.002	42.758	0.025
Dynamene sp.		x																	1.286	9.450	0.004
Ischyromene lacazei						x											x	x	21.862	94.261	0.061
Lekanesphaera sp.															x				1.286	9.450	0.004
Tanais dulongii				x					x	x	x			x	x	x		x	79.733	193.810	0.222
Sphaeromatidae															x				1.286	9.450	0.004
Sessilia																					
Chthamalus montagui	x		x		x	x							x	x	x	x			12870.370	32496.111	35.832
Elminius cf. *modestus*	x				x	x							x	x		x			12.860	35.880	0.036
Hexapoda																					
Diptera			x																2.572	18.900	0.007
Chironomidae	x								x		x								5.144	18.358	0.014
Dolichopodidae	x									x				x					11.574	53.537	0.032
Cnidaria																					
Actiniaria	x		x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	113.169	213.849	0.315
Echinodermata																					
Echinoidea						x					x								2.572	13.238	0.007
Holothuroidea														x			x		3.858	20.971	0.011
Mollusca																					
Bivalvia					x					x		x							7.716	34.831	0.021
Hiatella arctica									x		x				x	x	x	x	18.004	48.984	0.050
Irus irus										x									1.286	9.450	0.004
Musculus costulatus				x		x			x	x	x				x	x	x		12.860	30.387	0.036
Mytilus galloprovincialis	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	14345.422	15548.702	39.939
Psammobiidae	x				x	x			x	x	x		x	x			x	x	78.447	200.565	0.218
Tellinoidea											x		x						3.858	16.056	0.011
Veneroidea					x	x	x			x	x	x				x			414.095	1784.058	1.153
Venerupis sp.	x	x	x	x	x	x	x		x	x	x	x	x		x	x			826.903	1944.176	2.302
Gastropoda						x													2.572	18.900	0.007
Buccinum humphreysianum					x						x	x		x					7.716	22.029	0.021
Buccinum sp.					x	x				x	x	x		x	x		x	x	60.442	105.401	0.168
Gibbula umbilicalis	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	986.368	1451.760	2.746
Epitonium pulchellum			x			x		x			x								12.860	67.550	0.036
Melarhaphe neritoides	x											x	x	x					87.449	388.220	0.243
Tectura tessulata					x	x						x	x	x	x	x	x	x	61.728	157.705	0.172
Nucella lapillus									x		x	x		x	x	x	x	x	18.004	36.160	0.050
Urosalpinx cinerea			x						x			x			x		x		9.002	33.171	0.025
Omalogyra atomus							x	x	x	x									7.716	25.832	0.021
Patella depressa			x		x	x	x						x	x	x	x		x	111.883	237.677	0.311
Patella ulyssiponensis			x		x	x					x	x		x	x	x	x	x	131.173	209.278	0.365
Tricolia pullus																	x		1.286	9.450	0.004
Pleurobranchus sp.												x							1.286	9.450	0.004
Odostomia eulimoides		x	x						x	x	x	x			x			x	163.323	455.309	0.455
Rissoa parva								x	x	x		x			x		x		33.436	103.391	0.093
Skeneopsis planorbis				x			x		x	x		x							14.146	52.904	0.039

Table VI (Continued)

Opisthobranchia											x							x	10.288	54.643	0.029
Nudibranchia				x							x	x							3.858	16.056	0.011
Polyplacophora																					
Acanthochitona crinita	x	x	x	x	x		x	x	x	x	x	x		x	x	x	x	x	123.457	155.380	0.344
Acanthochitona fascicularis	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x		83.591	117.123	0.233
Lepidochitona cinerea	x		x	x			x		x		x	x	x		x			x	34.722	83.975	0.097
Nematoda									x	x	x		x	x	x				375.514	2402.126	1.045
Nemertea	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	967.078	2306.167	2.692
Sipuncula																					
Golfingia sp.																	x		2.572	18.900	0.007

The mean number of species and mean density found per zone in each platform are represented on Figure 10. The macroinvertebrates mean number of species highest value was registered in *zone b* in St *C* (24.2 species) and the lowest values were recorded in zone *a* on St *B* (8.0 species) and in *zone a* on St *A* (8.8 species).

Regarding the mean density the highest value was found for *zone a* in St *C* (45109.6 ind m^{-2}), while lower values were found for *zone a* in St *B* (6342.6 ind m^{-2}) and for *zone b* in St A (10520.8 ind m^{-2}).

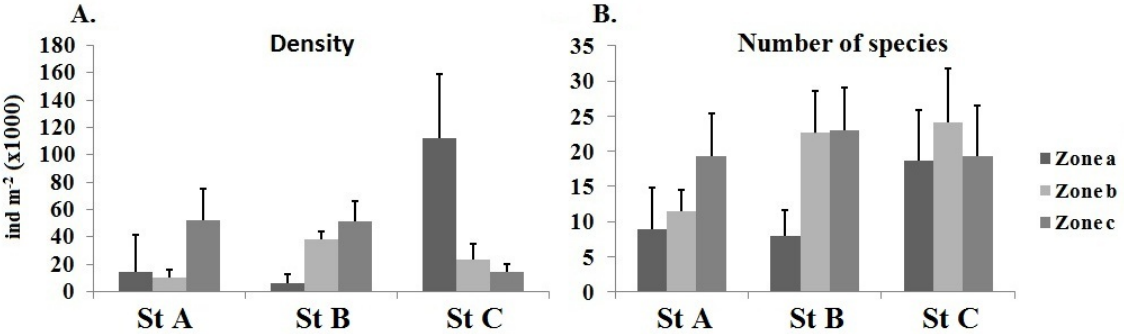

Figure 10 – Macroinvertebrate mean density (A.) and mean number of species (B.) per zone for all platforms.

PERMANOVA revealed statistically significant differences in species number between platforms ($F_{(Pl)2,51}$=4.1335; p=0.0217) and also the interaction Platform*Zone ($F_{(Pl*Zn)4,36}$=3.6364; p=0.0149). The Pair-wise test on the “Platform” revealed significant differences between the pairs St *A* and St *C* (t=3.1836, p=0.0029). For the term “Platform*Zone” the pair-wise test showed, within “Zone” level “a” sites St *A* and St *C* (t=2.3841, p=0.0494) and St *B* and St *C* (t=3.0649, p=0.0136) were significantly different. For levels of factor “Platform” within level “b” the test revealed statistically significant differences between St *A* and St *B* (t=4.3513, p=0.0063), and between St *A* and St *C* (t=5.3183, p=0.0026). Finally within level “c” there were no significant differences (p>0.05) between all pairs of platforms. Regarding the term “Platform*Zone” within “Platform” levels the analysis showed that within St *A* only the *zone a* and *zone c* and *zone b* and *zone c* were significantly different ($t_{a,c}$=2.6031, $p_{a,c}$=0.0349 and $t_{b,c}$=2.5342, $p_{b,c}$=0.0373, respectively). Within St *B* significant differences were found between *zone a* and *zone b* ($t_{a,b}$=4.7124, $p_{a,b}$=0.0034) and between *zone a* and *zone c* ($t_{a,c}$=4.7256, $p_{a,c}$=0.0027). For St *C* there were no significant differences (p>0.05) between all pairs of zones.

Regarding total density, there were no significant differences (p>0.05) between platforms (p>0.05), in contrast statistically significant differences were found in the interaction Platform*Zone ($F_{(Pl*Zn)4,36}$=22.919; p=0.0001). For the term “Platform*Zone” significant differences were found between St *A* and St *C* (t=4.694, p=0.0052 and t=4.2341, p=0.0039, respectively), and between St *B* and St *C* (t=6.4772, p=0.0022 and t=7.1595, p=0.0019, respectively) within *zone a* and *zone c;* significant differences within *zone b* were found between all the pairs of platforms (p<0.05). For the term “Platform*Zone” the analysis showed statistically significant differences within St *A* between *zone a* and *zone c* (t=3.2084, p=0.023) and between *zone b* and *zone c* (t=5.3331, p=0.0023). Within St *B* significant differences were found between all pairs of zones (p<0.05). Within St *C* significant differences were found between *zone a* and *zone b* (t=4.9393, p=0.0034), and between *zone a* and *zone c* (t=6.5381, p=0.002).

Principal Coordinate Analysis (PCO) did not show clear differences between the studied platforms and zones (Fig. 11), with the first two principal component axis explaining 33.4% of the samples variability. Only the platform St *C* was separated from St *A* and St *B*, and was less variable than these two sites. Regarding zones, no separation was clear and *zone c* was the less variable.

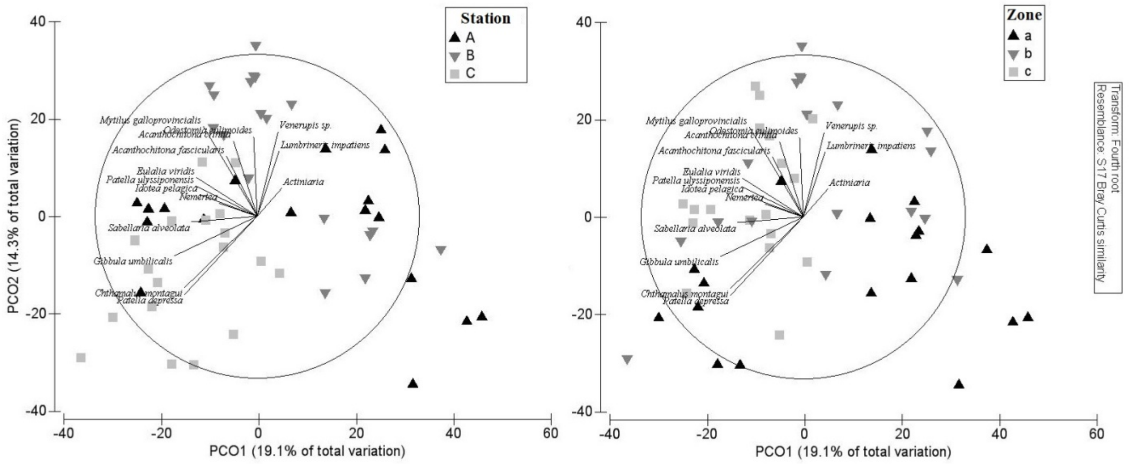

Figure 11 - Principal Coordinate analysis (PCO) plot based on macrofauna density for platforms (A) and zones (B) with the representation of the species that contributed most to groups' similarities (Axis 1 = 19.1%; Axis 2 = 14.3%).

Multivariate analyses (PERMANOVA) for the fauna community (with individual densities) revealed statistically significant differences between platforms ($F_{(Pl)2,51}$=4.527; p=0.0001), and also the interaction Platform*Zone ($F_{(Pl*zn)4,36}$=3.3713; p=0.0001). The Pair-wise test for "Platform" revealed significant differences between all the platforms ($p<0.05$). The pair-wise test for "Platform*Zone" for "zone" showed statistically significant differences in *zone a* between St *A* and St *C* (t=2.1795 p=0.0023), and between St *B* and St *C* (t=2.1189 p=0.0013). For both zones *b* and *c* significant differences were found between all pairs of platforms ($p<0.05$). Regarding the term "Platform*Zone" within "Platform" levels, the analysis showed for St *A* statistically significant differences between *zone a* and *zone c* (t=2.1742, p=0.0044), and between *zone b* and *zone c* (t=2.8264, p=0.002). Within St *B* and St *C* significant differences were found between all pairs of zones ($p<0.05$).

SIMPER analysis (75% cut-off) showed similarities within platforms ranging from 39.88% for St *B* to 43.14% for St *C*. Fifteen species contributed for these similarities, with *M. galloprovincialis* contributing the most for St *A*, St *B* and St *C* similarities (33.72%, 28.95% and 16.94% respectively). Dissimilarities between platforms were 64.00% between St *A* and St *B* with species *Mytilus galloprovincialis* contributing the most (6.37%), 64.21% between St *A* and St *C* with *C. montagui* the most contributing species (8.51%), and 66.98% between St *B* and St C with the species *C. montagui* contributing the most (7.60%) (Table VII).

Table VII – SIMPER (75% cut-off) similarities (in gray) and dissimilarities (in white), between platforms – St *A, B* and *C*. (Ct: contribution (%); AD: average density (ind m^{-2}); "+": higher densities in the factor on top; "-": higher densities in the factor on the left).

	St A			**St B**			**St C**
St A	**40.33 %**	**Ct (%)**	**AD (ind m^{-2})**				
	Mytilus galloprovincialis	33.7	9.3				
	Lumbrineris impatiens	13.2	3.8				
	Gibbula umbilicalis	12.7	4.2				
	Venerupis sp.	7.3	3.6				
	Nemertea	6.4	3.7				
	Sabellaria alveolata	4.5	3.5				
St B	**64.00 %**			**39.88 %**	**Ct (%)**	**AD (ind m^{-2})**	
	Mytilus galloprovincialis (-)			*Mytilus galloprovincialis*	28.9	11.2	
	Venerupis sp.(-)			Nemertea	7.3	3.7	
	Sabellaria alveolata (+)			Actiniaria	5.9	2.7	
	Nemertea (+)			*Venerupis sp.*	5.9	4.0	
	Idotea pelágica (-)			*Gibbula umbilicalis*	5.6	2.8	
	Chthamalus montagui (+)			*Acanthochitona crinita*	4.8	2.5	
	Gibbula umbilicalis (+)						
	Actiniaria (-)						
	Lumbrineris impatiens (+)						
	Odostomia eulimoides (-)						

Table VII (Continued)

Syllinae (-)			
Acanthochitona crinita (-)	*Lumbrineris impatiens*	4.8	2.8
Veneroidea (-)			
Eulalia viridis (-)	*Idotea pelagica*	4.7	3.4
Acanthochitona fascicularis (-)			
Buccinum sp. (-)	*Odostomia eulimoides*	4.3	3.1
Patella ulyssiponensis (-)			
Psammobiidae (-)	*Sabellaria alveolata*	3.2	2.8
Pirimela denticulata (-)			
Dynamene bidentata (+)			
Tanais dulongii (-)			
Lepidochitona cinérea (-)			
Rissoa parva (-)			
Patella depressa (+)			
Platynereis dumerilii (-)			
Nereididae (-)			
Omalogyra atomus (-)			
Hyale stebbingi (-)			
Skeneopsis planorbis (-)			

St C	**64.21 %**	**66.98 %**	**43.14 %**	**Ct (%)**	**AD (ind m^{-2})**
	Chthamalus montagui (-)	*Chthamalus montagui* (-)	*Mytilus galloprovincialis*	16.9	7.9
	Sabellaria alveolata (-)	*Sabellaria alveolata* (-)	*Gibbula umbilicalis*	13.3	6.1
	Mytilus galloprovincialis (+)	*Mytilus galloprovincialis* (+)	*Sabellaria alveolata*	13.2	6.6
	Venerupis sp. (+)	*Gibbula umbilicalis* (-)	*Chthamalus montagui*	9.1	8.1
	Nemertea (-)	*Venerupis sp.* (+)	Nemertea	7.3	3.9
	Idotea pelagica (+)	*Idotea pelágica* (+)	*Patella depressa*	3.8	2.6
	Gibbula umbilicalis (-)	Patella depressa (-)	*Eulalia viridis*	3.6	2.5
	Patella depressa (-)	*Odostomia eulimoides* (+)	*Lumbrineris impatiens*	3.4	2.3
	Patella ulyssiponensis (-)	Nemertea (-)	*Patella ulyssiponensis*	3.3	2.5
	Eulalia viridis (-)	*Patella ulyssiponensis* (-)	*Platynereis dumerilii*	2.9	2.5
	Lumbrineris impatiens (+)	Actiniaria (+)			
	Platynereis dumerilii (-)	*Platynereis dumerilii* (-)			
	Tectura tessulata (-)	Syllinae (+)			
	Syllinae (+)	*Tectura tessulata* (-)			
	Acanthochitona crinita (-)	*Eulalia viridis* (-)			
	Acanthochitona fascicularis (+)	*Lumbrineris impatiens* (+)			
	Actiniaria (-)	*Acanthochitona crinita* (+)			
	Melarhaphe neritoides (-)	Veneroidea (+)			
	Dynamene bidentata (-)	*Acanthochitona fascicularis* (+)			
	Hyale stebbingi (-)	Nematoda (+)			
	Tanais dulongii (-)	*Buccinum sp.* (+)			
	Buccinum sp. (+)	*Hyale stebbingi* (-)			
	Perinereis marionii (+)	*Melarhaphe neritoides* (-)			
	Nucella lapillus (-)	*Dynamene bidentata* (-)			
	Lepidochitona cinerea (-)	Psammobiidae (+)			
	Pirimela denticulata (-)	*Pirimela denticulata* (+)			
	Ischyromene lacazei (-)	*Tanais dulongii* (+)			
	Nematoda (-)	*Nucella lapillus* (-)			

Table VII (Continued)

Phyllodocinae (-)	*Rissoa parva* (+)	
Polycirrus sp. (-)	*Lepidochitona cinerea* (+)	
Psammobiidae (+)	*Hiatella arctica* (-)	
Elminius cf modestus (-)	Phyllodocinae (-)	
Spirobranchus lamarcki (-)	*Ischyromene lacazei* (-)	
Hiatella arctica (-)	*Perinereis marionii* (-)	
	Omalogyra atomus (+)	
	Spirobranchus lamarcki (-)	

Regarding the zones, 14 different species contributed most for similarities, ranging from 29.18% in *zone a* to 48.47% in *zone c*, being *M. galloprovincialis* the taxa with higher percentage of contribution for all zones (41.28%, 20.80% and 23.18% for *zone a*, *zone b* and *zone c*, respectively). Dissimilarities were 57.73% between zones *b* and *c* with species *S. alveoloata* contributing the most (5.06%), 67.45% between *zones* a *and b* with *Chthamalus montagui* the most contributing species (6.73%), and 68.94% between *zones* a *and* c with the species *C. montagui* contributing the most (6.43%) (Table VIII).

Table VIII – SIMPER (75% cut-off) similarities (in gray) and dissimilarities (in white) between zones – *zone a, b* and *c*. (Ct: contribution (%); AD: average density (ind m^{-2}); “+”: higher densities in the factor on top; “-“: higher densities in the factor on the left).

	Zone a			**Zone b**	**Zone c**
Zone a	**29.18%**	**Ct (%)**	**AD (ind m^{-2})**		
	Mytilus galloprovincialis	7.7	9.3		
	Gibbula umbilicalis	3.4	3.8		
	Nemertea	3.3	4.2		
	Chthamalus montagui	6.3	3.6		
	Lumbrineris impatiens	1.7	3.7		
	Actiniaria	1.8	3.5		

Table VIII (Continued)

Zone b	**67.45%**	**43.46%**	**Ct (%)**	**AD (ind m^{-2})**			
	Chthamalus montagui (-)	*Mytilus galloprovincialis*	20.8	9.2			
	Venerupis sp. (+)	*Gibbula umbilicalis*	11.2	4.8			
	Mytilus galloprovincialis (+)	*Lumbrineris impatiens*	9.7	4.0			
	Sabellaria alveolata (+)	*Venerupis sp*	9.4	4.8			
	Gibbula umbilicalis (+)	Nemertea	8.2	4.0			
	Nemertea (+)	*Acanthochitona crinita*	6.6	3.1			
	Lumbrineris impatiens (+)	Syllinae	3.9	2.8			
	Acanthochitona crinita (+)	*Sabellaria alveolata*	3.8	3.7			
	Syllinae (+)	*Acanthochitona fascicularis*	2.9	2.2			
	Actiniaria (-)						
	Idotea pelágica (+)						
	Acanthochitona fascicularis (+)						
	Eulalia viridis (+)						
	Tanais dulongii (+)						
	Odostomia eulimoides (+)						
	Patella depressa (-)						
	Dynamene bidentata (+)						
	Veneroidea (+)						
	Platynereis dumerilii (+)						
	Nematoda (+)						
	Lepidochitona cinerea (+)						
	Melarhaphe neritoides (-)						
	Hyale stebbingi (-)						
	Tectura tessulata (+)						
	Psammobiidae (+)						
	Patella ulyssiponensis (+)						
	Polycirrus sp. (+)						
	Pirimela denticulata (+)						
	Rissoa parva (+)						
	Nereididae (+)						
	Perinereis marionii (+)						
Zone c	**68.94 %**	**57.73%**			**48.47%**	**Ct (%)**	**AD (ind m^{-2})**
	Chthamalus montagui (+)	*Sabellaria alveolata* (-)			*Mytilus galloprovincialis*	23.2	11.6
	Sabellaria alveolata (-)	*Chthamalus montagui* (-)			*Sabellaria alveolata*	13.5	7.0
	Mytilus galloprovincialis (-)	*Mytilus galloprovincialis* (-)			*Gibbula umbilicalis*	9.7	5.0
	Idotea pelágica (-)	*Venerupis sp.* (+)			*Idotea pelagica*	7.6	4.8
	Patella ulyssiponensis (-)	*Idotea pelagica* (-)			*Patella ulyssiponensis*	6.2	3.6
	Nemertea (-)	*Patella ulyssiponensis* (-)			*Lumbrineris impatiens*	5.7	3.2
	Gibbula umbilicalis. (-)	Nemertea (+)					
	Buccinum sp. (-)	*Buccinum sp.* (-)					
	Eulalia viridis (-)	Syllinae (+)					
	Lumbrineris impatiens (-)	*Odostomia eulimoides* (+)					
	Venerupis sp. (-)	*Eulalia viridis* (-)					
	Actiniaria (+)	Veneroidea (+)					

Table VIII (Continued)

Actiniaria (+)	*Acanthochitona crinita* (+)	Nemertea	5.7	4.0
Patella depressa (+)	*Acanthochitona fascicularis* (+)	*Eulalia viridis*	4.2	2.9
Syllinae (-)	*Tanais dulongii* (+)			
Acanthochitona crinita (-)	Actiniaria (+)			
Odostomia eulimoides (-)	*Gibbula umbilicalis* (-)			
Acanthochitona fascicularis (-)	*Platynereis dumerilii* (+)			
Platynereis dumerilii (-)	*Patella depressa* (-)			
Tectura tessulata (-)	Psammobiidae (-)			
Melarhaphe neritoides (+)	*Lumbrineris impatiens* (+)			
Dynamene bidentata (+)	*Tectura tessulata* (-)			
Psammobiidae (-)	*Dynamene bidentata* (+)			
Hyale stebbingi (+)	*Pirimela denticulata* (-)			
Pirimela denticulata (-)	*Lepidochitona cinerea* (+)			
Nucella lapillus (-)	*Nucella lapillus* (-)			
Ischyromene lacazei (-)	*Hyale stebbingi* (+)			
Veneroidea (-)	*Rissoa parva* (+)			
Perinereis marionii (-)	*Hiatella arctica* (-)			
Lepidochitona cinerea (+)	*Ischyromene lacazei* (-)			
Hiatella arctica (-)	*Polycirrus sp.* (+)			
Nematoda (+)	*Perinereis marionii* (-)			
Melita palmata (-)	Nematoda (+)			
	Musculus costulatus (+)			
	Nereididae (+)			
	Microdeutopus chelifer (-)			
	Phyllodocinae (-)			

DistLM analysis did not show any significant relationship between biological and environmental data when considering predictor variables individually, as none of the studied parameters were statistically significant. Nevertheless, silica was the best solution (R^2=59%) to explain the total variability of the macrofauna.

The dbRDA (Fig. 12) calculated the variation percentage explained out of the fitted model (100%) and the variation percentage explained out of the total variation (100%). Salinity contributed positively in the first axis, while $N\text{-}NH_4$, $P\text{-}PO_4$, silica, $N\text{-}NO_2$, $N\text{-}NO_3$, chlorophyll *a*, pH and temperature contributed negatively. In the second axis, $N\text{-}NH_4$, $P\text{-}PO_4$, silica, $N\text{-}NO_2$ had a positive contribution, $N\text{-}NO_3$, chlorophyll *a*, pH, temperature and salinity had a negative contribution.

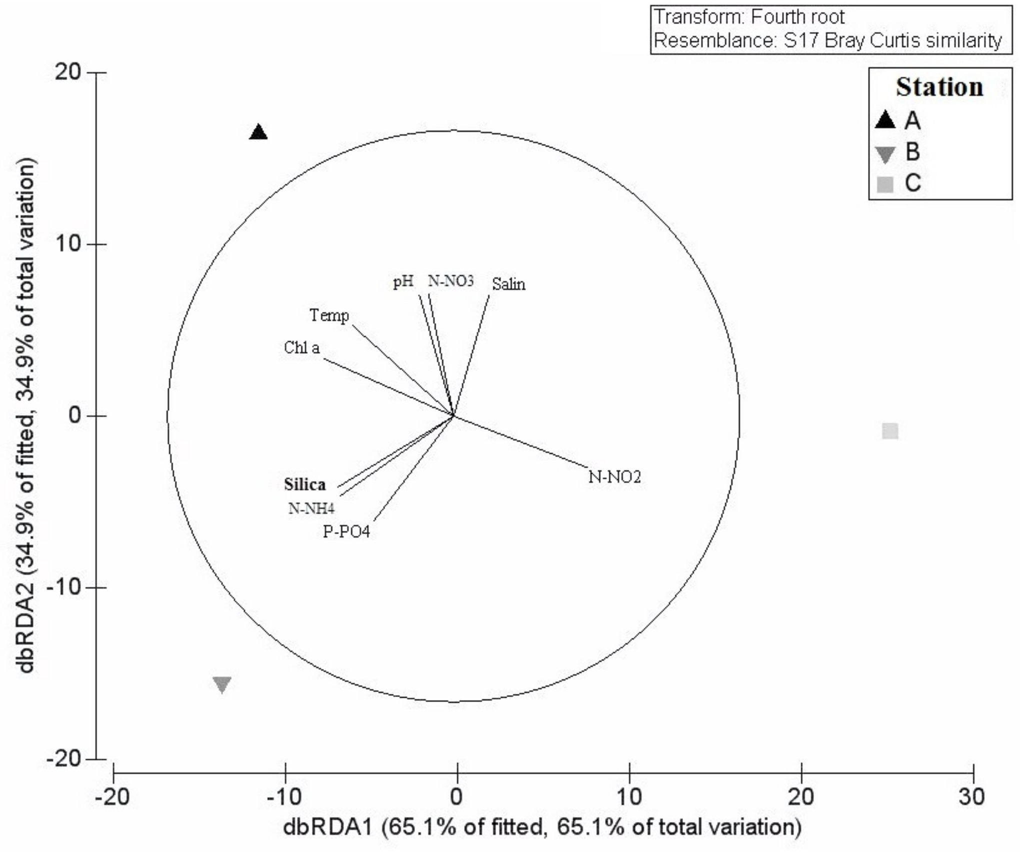

Figure 12 – Two-dimensional Distance based redundancy analysis (dbRDA) plot of all physicochemical parameters for the different station samplings (Axis 1 = 65.1% of fitted model, 65.1% of total variation; Axis 2 = 34.9% of fitted model, 39.9% of total variation). In bold is the best variable solution.

3.3.1 Ecological indicators

Margalef´s index presented higher value in *zone c* (Fig. 13), and also in St *C* (Fig. 14), decreasing towards *zone a,* and St *A*. Values ranged from 1.94 and 2.06 in *zone a* of St *B* and St *A*, respectively, to 4.99 in *zone b* of St *C,* and from 2.92 in St *A* to 4.34 in St C . Shannon-Wiener's index showed a similar pattern, with values ranging from 2.60 and 2.69 for *zone a* in St *B* and St *A*, respectively, to 4.41 *zone b* of St *C,* and from 3.61 in St *A* to 4.12 in St *C*.

Values for Pielou and Simpson indices were always close to 1 for zones within platforms, and for platforms. Pielou index showed the minimum and maximum values in St *C,* for zones *a* (0.941) and *b* (0.976), although values did not vary from St *A* to St

C. Simpson index (1-D) showed a similar pattern to the Margalef and Shannon indexes, with values ranging from 0.75 for *zone a* in St *B* to 0.95 *zone b* of St *C,* and from 0.88 in St B to 0.94 in St *C*.

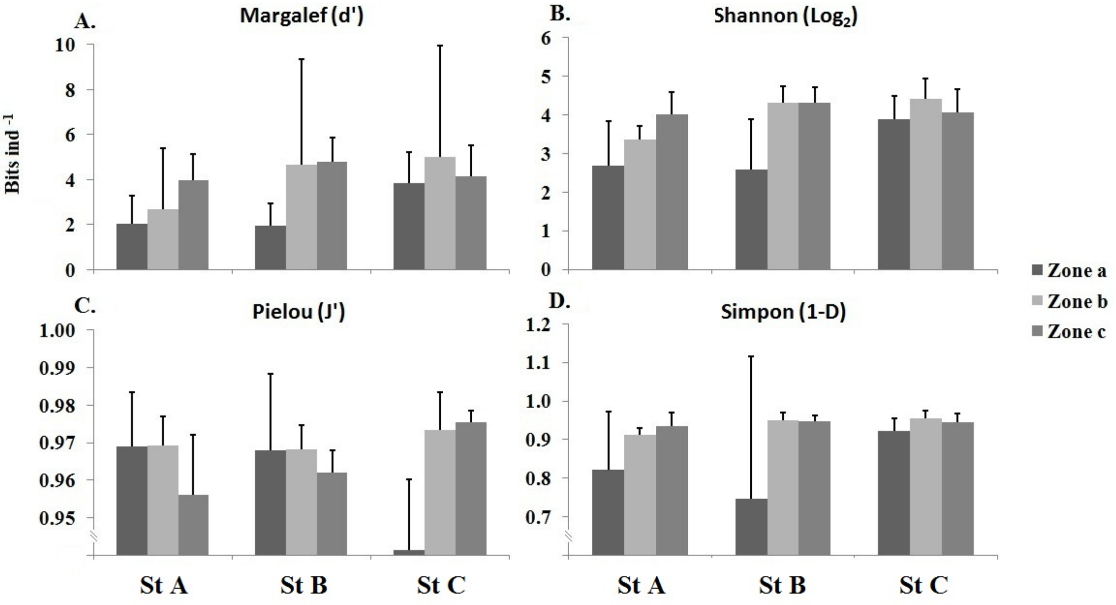

Figure 13 – Variation of Margalef (A.), Shannon-Wiener (B.), Pielou (C.) and Simpson (D.) indices per zone within platform.

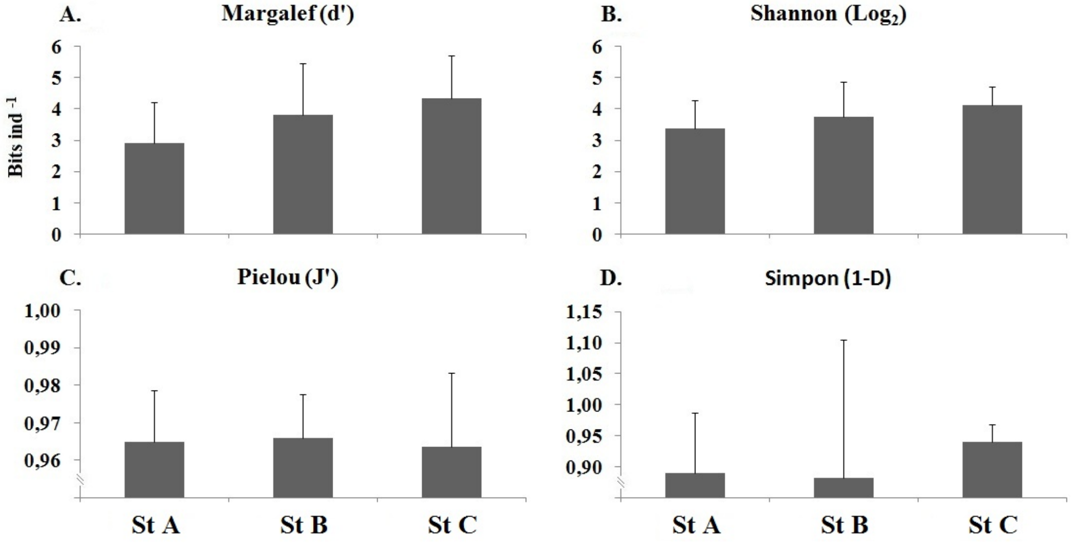

Figure 14 – Variation of Margalef (A.), Shannon-Wiener (B.), Pielou (C.) and Simpson (D.) indices per platform.

PERMANOVA using the macrofauna dataset revealed statistically significant differences in Margalef index between platforms ($F_{(Pl)2,51}$=4.4009; p=0.0198) and also the interaction Platform*Zone ($F_{(Pl*zn)4,36}$=2.9782; p=0.0337). The Pair-wise test on the

“Platform” revealed significant differences between the pairs St *A* and St *C* (t=3.2167, p=0.0034). The pair-wise test for “Platform*Zone” for “zone” showed statistically significant differences in *zone a* between St *B* and St *C* (t=2.6758 p=0.0232). Within “Zone” level “b” the test showed statistically significant differences between the pairs St *A* and St *B* (t=4.1231, p=0.0053) and between St *A* and St *C* (t=5.5166, p=0.001). For levels of factor “Platform” within level “c” there were no significant differences (p>0.05) between all the pair of platforms (p>0.05). Regarding the term “Platform*Zone” within “Platform” levels the analysis showed that within St *A* only the *zone a* and *zone c* were statistically different (t=2.5006, p=0.0394). Within St *B* statistically significant differences were found between *zone a* and *zone* b ($t_{a,b}$=4.8084, $p_{a,b}$=0.0011) and between *zone a* and *zone c* ($t_{a,c}$=4.7212, $p_{a,c}$=0.0014). For St *C* there were no statistically significant differences (p>0.05) between all pairs of zones.

Regarding the Shannon index, using the macrofauna dataset, PERMANOVA revealed statistically significant between platforms ($F_{(Pl)2,51}$=3.1833; p=0.0494), in contrast there were no statistically significant differences in the interaction Platform*Zone (p>0.05). The Pair-wise test on the “Platform” revealed significant differences between the pairs St *A* and St *C* (t=3.2167, p=0.0034).

Regarding Pielou and Simpson indices’, there were no significant differences between platforms (p>0.05) and the interaction Platform*Zone (p>0.05).

4.DISCUSSION

4. DISCUSSION

This study was proposed to assess the existence of a disturbance gradient regarding the spatial distribution of the intertidal macrozoobenthic communities of hard substrata. For this purpose, physcochemical parameters and macroalgae taxa were utilized in the assessment to confirm sampling was performed inside a disturbance gradient, and to compare with results obtained for the macrofauna.

4.1. Environmental data

The physicochemical parameters values utilized in the present study were taken from the sampling moment (spring 2009) and therefore show only a “snapshot” of the environmental conditions. Nevertheless, it is to notice that macrofauna reflects not only conditions at the time of sampling but also conditions to which the community was previously exposed (Reish, 1987; Gappa et al., 1990), thus, it will be assumed the prevailing environmental conditions would not be much different from the ones found for the spring of 2009.

During the survey the physical-chemical parameters (temperature, salinity and pH) varied accordingly to the spring season, as expected.

Regarding the chlorophyll *a* and the nutrients (N-NH4, N-NO3, N-NO2 and P-PO4) values registered, there may have been a possible influence from the source of pollution. Chlorophyll *a* values decreased from *St A* (the most immediate sampling site) to St *C* (the furthermost sampling site), which may have been related to the higher values found for the nutrients at St *Fonte* (source of pollution) (as chlorophyll *a* is used as a *proxy* for primary production). The nutrients and silica showed a pattern probably explained by the current turnover from North-South to South-North orientation during the sampling day (Fig. 3). Moreover, the chlorophyll *a* and nutrients consumption by micro and macroalgae algae may have contributed for the decrease in these parameters concentrations in the water column, and measuring them in the algae directly would be more sensible (Goodsell et al., 2009).

The PCA analysis on the physicochemical parameters revealed a separation of St *Fonte* from all sampling sites (St *A,* St *B* and St *C*) in axis 1, namely due to higher values of phosphates and ammonia and lower values of pH, salinity, silica, nitrates and nitrites. The axis 2 separated St *A* from St *B* and St *C,* with higher values of chlorophyll *a* and temperature registered for that site. These results suggest the existence of the disturbance gradient from the point source of pollution – St *Fonte* across the sampling stations *A, B* and *C*.

4.2. Intertidal macroalgae assessment – MarMAT

The macroalgae are suitable elements for the assessment of communities variation across a disturbance gradient. It is recognized that, due to their capacity to accumulate the disturbance effects, they are biologic quality elements which may be used in the classification of ecological quality status of aquatic systems. The utilization of macroalgae Ecological Quality Ratio (EQS) and Ecological Quality Status (EQS) will allow to proof the existence of a disturbance gradient and to compare with indices results for macrofauna.

The MarMAT ecological tool calculated the EQRs for the sampling stations *A, B* and *C* (unpublished data), and revealed the EQSs of these sites. The results were in agreement with the results obtained with PERMANOVA analysis on the macroalgae dataset, which were supported by SIMPER and dbRDA analysis, and also for the physical-chemical parameters. The EQRs confirmed the presence of the disturbance gradient from the most proximate (St *A*), to the furthermost (St *C*) sampling station to the point source of pollution.

4.3. Intertidal macrofauna assemblages

Man-induced variations from natural trends are not easy to assess. Knowledge about natural temporal variation in the distribution and abundance of communities is necessary for impact-detection studies or ecological observation programmes. The spatial and temporal heterogeneity of rocky shore communities are of great importance for monitoring programmes, regarding the sampling design and frequency (Benedetti-Cecchi et al., 2003; Hartnoll & Hawkins, 1980; Underwood, 2000; Underwood & Chapman, 2003). In this present survey the temporal approach was not able to be undertaken.

It has been referred that in littoral systems the abundance and number of macrofauna species increases from the upper to lower shore levels (Dailey et al, 1993; Davidson, 2004), existing in the lower shore a much more hospitable environment to live in, the habitat is more stable than in higher levels, the temperature is more consistent, less desiccation occurs and the salinity is more constant. This effect diminishes to upper areas, making these much inhospitable to live in. However, variation in abundance of intertidal species according to height on the shore is not only attributable to physiological stresses, but also to biological interactions such as competition (Dayton, 1971; Hawkins & Hartnoll, 1985), grazing (Hawkins & Hartnoll, 1983; Jenkins et al., 1999) and predation (Dayton, 1971; Lubchenco & Menge, 1978).

These may influence the upper and/or lower limits of distribution of individual species similarly on rocky shores (Reichert, 2008).

It has been recognized that most intertidal algae and invertebrates are distributed in extremely patchy patterns at small spatial scales (centimetres to metres) within any height on rocky shores (Aberg & Pavia, 1997; Benedetti-Cecchi, 2001; Chapman, 2002; Fraschetti et al., 2005). Small-scale variation in distribution patterns of species assemblages may be related to small-scale changes in behavioural responses (Underwood & Chapman, 1989; Chapman & Underwood, 1994; Reichert, 2008), recruitment (Chapman & Underwood, 1998; Reichert, 2008), patchy distributions of microhabitats (Underwood & Chapman, 1996; Reichert, 2008) and interactive effects of abiotic and biotic factors (Benedetti-Cecchi et al., 2000b; Reichert, 2008).

In general, the trend mentioned earlier was found in the present study, being the mean total number of species and mean total density higher in *zone c* in all platforms. *Zone a* of station *C* was an exception, with an impressively higher mean density in comparison with the others zones, mainly due to the occurrence of the barnacle *C. montagui* in very large densities. This species reaches a maximum recruitment during spring (sampling date) and summer months (O'Riordan et al. 2004; Jacinto & Cruz, 2008). In addition, the reduced mean total number of macroinvertebrate species and mean total density found in *zone c* of St *C,* in comparison to the ones found for *zone b*, may be related to an undersampling of macrofauna species in that zone.

The univariate (with total number of species, and total densities) and multivariate (individual densities) analysis provided other aspects of the macroinvertebrate community.

When checking for differences between stations, and regarding the total density, the stations were not different from each other. Nevertheless, when considering the individual densities, all stations were different from each other, with dissimilarities above the 63% showing the variability of species from one station to another. Regarding the total number of species, only St *A* and St *C* were different (higher values were found in St *C*) as expected, since St *A* and St *C* are the nearest and furthermost stations from the source of pollution, respectively. This analysis suggets the macroinvertebrate communities are subjected to different disturbance levels, with St *A* being the most disturbed, St C the less disturbed and St *B* at an intermediate level.

When checking for differences between zones within each station, the zonation scheme assessed seems clear regarding the individual densities. At St *B* and St *C* all zones were different from each other, although in St *A* only *zone c* being different. Therefore, in St *A* the disturbance effect is verified. As referred by Pinedo et al. (2007), ephemeral algae such as *Ulva* begin to dominate in highly disturbed environments and near freshwater discharges (Golubic, 1970; Bellan & Bellan-Santini, 1972; Rodriguez-Pietro & Polo, 1996). The proximity of St *A* to the source of pollution enables the opportunistic species *U. lactuca/rigida* and *U. intestinalis/compressa* to increase their biomass in zones *a* and *b* and, thus, competing with *C. montagui* for space (which is usually very abundant in these upper areas) (Benedetti-Cecchi et al., 2000a), translating in much lower densities of that species, as well as other macroinvertebrate species and, consequently, altering the community structure. This shift occurred also in *zone b*. For these two reasons, the *zone c* in St *A* was different from the others also regarding the total density and the total number of species. For the total number of species, the effect of the source of pollution was not so evident. This may be related to the much lower macroalgae biomass in *zone* c, enabling areas available for more macroinvertebrate species to settle.

In St *B* all zones were different regarding total and individual densities, while regarding the number of species only *zone a* was different from the others in this station (for the same reasons mentioned for this zone in St *A*). Here, the effect of disturbance was still verified.

In St *C*, regarding the number of species, no differences were found between zones, most probably explained by a better adaptation of species occurring in this area to the environmental extremes. Regarding the individual species all zones in this station were different, which was the expected result; regarding the total densities only *zone a* was different from zones *b* and *c*, due to large densities of *C. montagui* in *zone a.* This station shows a more structured community, probably with no impact from the source of pollution.

When checking for differences in the communities in zones across stations, *zone b* was the most variable regarding total number of species, total densities and individual densities, which may reveals the existence of a disturbance gradient. *Zone a* of St *C* differed from the other *zone a*, being less disturbed than those, which indicates that the effect there is minor in comparison to the other stations. Moreover, higher macrofauna (namely sessile organisms) densities (St *C*) and macroalgae biomasses (St *A* and St *B*) found in that zone, contributed for this difference. For *zone c* no differences were found in the total number of species, as stated earlier this is a more “stable” zone. This zone differed in St *C* regarding the total densities, which is explained by large densities of *M. galloprovincialis* and *C. montagui* in station *A*, and of *M. galloprovincialis* in station *B*. The great variability of these species densities may be explained by the shift in the community structure occurring in the first two zones of these stations, due to their proximity to the source of pollution, when comparing to St *C*. Regarding the individual densities, *zone* c varied between all stations. This was again caused by a shift in the species composition in the first two zones enhanced by the disturbance, with different species occurring in different density levels.

Finally, when checking for differences between levels within zones at each and across stations, results revealed homogeneity for the levels and, thus, the sampling procedures where adequate.

SIMPER analysis for the macroinvertebrates revealed higher similarities for St *C* and for *zone c*, and higher dissimilarities between St *B* and St *C*, and between zones *a* and *c*. These results support what was stated earlier. St *C*, being further away from the source of pollution, presents a less variable environment regarding nutrients than the other stations, allowing the community to be more constant. In *zone c* the community also tends to be more constant due to less physical and environmental constraints (e.g. higher submersion and lower desiccation) and, thus, less physiologic stress. Furthermore, being St *C* and *zone c* the most distant areas from the source of pollution, the disturbance there is much less intensified resulting in the higher similarities. The dissimilarities are explained by the presence of high, and much variable, densities of *C. montagui* in *zone a* and, in a lesser extent, of *M. galloprovincialis* and *S. alveolata* in *zone c*. The presence of *C. montagui* and *M. galloprovincialis* in those zones is common and has been referred in several studies (e.g. Jones *et al.*, 2000).

The dbRDA revealed a pattern among stations, with the existence of effectively three groups in the community structure of the macrofauna that can be modelled by the environmental variables mentioned initially. Salinity, pH, N-NO_3, Temperature and Chlorophyll *a* separated St *A* (not surprisingly) from St *B* and St *C*. Silica, N-NH_4 and P-PO_4 separated St *B* from St *A* and St *C*. Finally, St *C* is separated from the others due to N-NO_2. For macroalgae, the same groups were formed by the same variables as the macroinvertebrates. Once again, the disturbance gradient from St *A* to St *C* was recognized.

4.4. Ecological indicators

In the present study several ecological indices were utilized to assess the ecological condition of the macrofauna communities. These were: i) Number of species, ii) Margalef richness index (d); iii) Shannon–Wiener diversity index (H'); iv) Simpson domination index (1-D); and v) Pielou evenness index (J').

The indices of Margalef, Shannon-Wiener, Simpson and Pielou revealed a very diverse community, with very high diversity found for each station and each zone within stations. Moreover, the individuals of each species were widely distributed among them. When checking for statistically significant differences between stations, and between zones within and across stations, only the Margalef and Shannon-Wiener indices presented differences. Regarding both indices, differences were found between St *A* and St *C*, as expected, indicating these stations are subjected to different levels of disturbance, being St *A* the most disturbed and St *C* the less disturbed. For the interaction Platform*Zone, only the Margalef index showed significant differences. *Zone a* was different between St *B* and St *C*, following the trend found for the number of species. *Zone b* of St *A* differed from the other stations *zone b*, again following the number of species, since this zone is being more affected by the disturbance in St *A* than in the other stations. For *zone c* no differences were found, showing no signs of disturbance.

Checking for differences in the indices in zones within stations, it was found for St *A* differences between *zone a* and *zone c*. Due to its proximity to the source of pollution, *zone a* of St *A* is the most disturbed site and *zone c* of St *A* is the least disturbed site, occurring the expected disturbance gradient form upper to lower zones in this station. For St *B* it was found *zone a* being different from *zone b* and *zone c*, being *zone a* the most disturbed site in this station. For St *C* no differences were found between zones, which were found to be not disturbed.

The behaviour of the ecological indices are in compliance with the EQRs obtained with the MarMAT ecological tool (for macroalgae) for the same sampling stations and period, showing an improvement of ecological status from St *A* to St *C* and, therefore, the presence of a disturbance gradient from the stations most proximate to the source of pollution to the station most distant from that source. The EQSs translated from the EQRs followed the same trend, although they were not as sensible to detect the disturbance gradient as the EQRs, since some stations (namely St *B* and St *C*) obtained the same final status classification with distinct EQRs.

Other ecological indices based on faunal communities could be used in the assessment of a disturbance gradient, such as the Bellan's one (based on polychaetes), the Bellan–Santini's one (based on amphipods), the BENTIX or the Indicators Species Index (ISI), all which attempt to characterise environmental conditions by analysing the dominance of species indicating some type of pollution in relation to species considered as indicative of an optimal environmental situation, or the Benthic Response Index (BRI) which is based upon the type of species (pollution tolerance) in a sample, although its applicability is complex (Marques e tal, 2009).

5. CONCLUSION

5. CONCLUSION

In the present survey the physical-chemical parameters did not quite show the disturbance gradient conferring different disturbance levels to the sampling stations.

The macroalgae, due to their capability of accumulating disturbance effects were used to reinforce the certainty of the presence of the gradient. The obtained EQSs, and in a more sensible way the EQRs, in comparison with ecological indices applied to macroinvertebrates, allowed the certainty of the existence of that disturbance gradient caused by a point source pollution, from the most proximate sampling station – St *A* to the most distant sampling station – St *C*.

The zonation scheme was helpful to recognize the existence of the disturbance gradient from St *A* to St *C*, and probably from *zone a* to *zone c* in stations *A* and *B*. The different disturbance levels were captured by the indices utilized – Number of species, Margalef, Shannon-Wiener, Pielou and Simpson indices, which were in conformity with the MarMAT ecological tool.

Nevertheless, further assessment should be undertaken, using data from other sampling periods and other ecological indicators (that were not tested due to time-related issues), to improve the results obtained, and to allow a better understanding of rocky shore macrofauna assemblages when in presence of a disturbance gradient.

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