



## Can benthic foraminifera be used as bio-indicators of pollution in areas with a wide range of physicochemical variability?



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### ABSTRACT

The Ria de Aveiro, a lagoon located in the NW coast of Portugal, presents a wide range of changes to the natural hydrodynamical and physicochemical conditions induced for instance by works of port engineering and pollution. In order to evaluate the response of living benthic foraminifera to the fluctuations in physicochemical parameters and pollution (metals and TOC), eight sediment samples were collected from canals and salt pans within the Aveiro City, in four different sampling events. During the sampling events, salinity showed the most significant fluctuations among the physicochemical parameters with the maximum range of variation at Troncalhada and Santiago salt pans. Species such as *Haynesina germanica*, *Trochammina inflata* and *Entzia macrescens* were found inhabiting these hypersaline environments with the widest fluctuations of physicochemical parameters. In contrast, *Ammonia tepida* dominated zones with high concentrations of metals and organic matter and in lower salinity waters. Parameters related to benthic foraminiferal assemblages (i.e., diversity and evenness) were found to significantly decline in stations polluted by metals and characterized by higher TOC content. Foraminiferal density reduced significantly in locations with a wide range of physicochemical temporal variability. This work shows that, even under extreme conditions caused by highly variable physicochemical parameters, benthic foraminiferal assemblages might be used as valuable bioindicators of environmental stress.

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### 1. Introduction

Coastal environments such as lagoons, estuaries and bays are transitional areas that commonly display high biodiversity and

serve as nursery and refuge for numerous species (Kennish and Paerl, 2010). These areas are also densely populated and influenced by high anthropic pressure (Kennish, 1992) that induces adverse effects on the living organisms and in the ecosystem thereof (Castro et al., 2006). Assessment of the environmental quality in coastal environments can not only be achieved by analyzing the abiotic factors (i.e., metal concentrations, presence of persistent organic pollutants, and amount of organic matter) but also through the evaluation of organisms living therein (e.g., Borja et al., 2009; Hamza-Chaffai, 2014).

Since the beginning of the 1960's, benthic foraminifera have been widely used as environmental bioindicators in coastal areas

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(Alve, 1995; Yanko et al., 1998; Dimiza et al., 2016a, b). Changes to the abundance and diversity of benthic foraminifera as well as to the composition of the assemblages are commonly used to assess environmental quality in coastal areas (Frontalini and Coccioni, 2011). Among the different types of pollutants, metals are widely reported to negatively impact foraminiferal assemblages (Frontalini and Coccioni, 2008; Martins et al., 2013, 2015a, b).

In the light of previous researches on application of benthic foraminifera as bioindicators, this work intends to investigate if foraminifera are suitable to be used as bioindicators in a polluted area with a wide range of physicochemical variability. The Aveiro City canals (Portugal) were selected for this study since they undergo temporal variability of physicochemical parameters and are considerably polluted (Martins et al., 2010). Additionally, two salt pans characterized by low pollution but the highest seasonal variability of physicochemical parameters were also considered in this work. Several researches, aiming to assess the response of foraminiferal assemblages to pollution in punctual sampling events, have been performed in this coastal ecosystem (Martins et al., 2010, 2011, 2013, 2015a, b). However, there is as yet very limited information available on the response of benthic foraminifera to the variability of temporal and spatial physicochemical parameters and pollution in this ecosystem as well as in most transitional and coastal environments.

### 1.1. Study area

The study was carried out in the Aveiro city canals and salt pans, which are part of the lagoon system commonly known as Ria de Aveiro, located in the coastal center-west zone of Portugal (40°38'N, 08°45'W) (Fig. 1A). From an ecological point of view, the Ria de Aveiro is characterized by rich biodiversity (França et al., 2012) and represents a Special Protected Area (Sumares and Fidélis, 2013). Eleven cities, of which Aveiro is the most important one, are located around this lagoon (Fig. 1A and B). This city, crossed by canals connected with the main body of Ria de Aveiro, hosted several industries, mostly ceramics, glass and metallurgy, between the 18th and mid-20th centuries. In the past, urban and industrial sewages were directly discharged into the Aveiro city canals, resulting in a high degree of pollution and environmental degradation (Martins et al., 2010). During the second half of the 20th century, most industries shut down or moved to other regions. Domestic effluents and municipal waste are no longer being discharged into Aveiro City canals. However some improper discharges of waste may still be prevalent.

## 2. Materials and methods

Eight points in the Aveiro city canals and the Troncalhada and Santiago salt pans were selected and sampled during four sampling campaigns from 2009 to 2011 (Fig. 1C; Appendix A). The samplings took place in autumn (28.10.2009, sampling event 1, SE1), early winter (07.01.2010; SE2), early spring (22.03.10; SE3) and late winter (02.03.2011; SE4). During the samplings, physicochemical parameters such as temperature and salinity of water, and the pH and Eh in sediments, were measured with a multiparameter probe (Consort C933). Sediment samples for textural, mineralogical, geochemical and foraminiferal analyses were collected by box-corer and only the top first cm of sediment was considered. Samples for textural, mineralogical and geochemical analyses were collected only in the first and fourth SEs. The sediment samples for foraminiferal analysis were preserved and stained with Rose Bengal (2 g per liter of ethanol 90%) to identify the living specimens (Murray, 1991).

### 2.1. Textural, geochemical and mineralogical analyses

Samples were oven dried at about 45 °C. A homogenized portion of the dry sediment sample was used for grain-size analysis. The fine fraction was separated from the coarser one by wet sieving using a 63 µm mesh screen. The dry fraction >63 µm was sieved through a series of sieves of 125 µm, 250 µm, 500 µm, 1000 µm. Percentage of each sediment fraction was determined. The textural parameters were evaluated by Folk and Ward method (1957). Mineralogical and geochemical analyses were carried out on the fine sediment fraction (<63 µm). The mineralogical analysis was performed by X-ray diffraction (XRD) techniques, using the Philips PW1130/90 and X'Pert PW3040/60 and Cu K $\alpha$  radiation. The scans were performed between 2° and 40° 2 $\theta$  (in non-oriented powder). Mineralogical semiquantification was performed according to the methodology described by Martins et al. (2007). In order to identify general changes in the sediments' composition, a mineralogical index, namely coarse minerals resulting from the sum of quartz, K-feldspar and plagioclase (Rocha et al., 1999), was considered. The total organic carbon (TOC) was determined in 2 g of sediment using SC144 LECO equipment. Geochemical analysis of metals was performed on the fine fraction by ICP-MS after complete digestion of sediment with four acids (HClO<sub>4</sub>, HNO<sub>3</sub>, HF, and HCl) in ACME Analytical Laboratories (Canada). The concentrations of Al, As, Cd, Co, Cr, Cu, Ni, Pb and Zn were considered in this work. The pollution load index (PLI), defined by Tomlinson et al. (1980) and adapted by Martins et al. (2011) was used to estimate the general contamination by metal content. Total available concentrations of metals (TAC) were evaluated in SE4, for As, Cd, Co, Cu, Ni, Pb and Zn, from three extracted phases: 1) clay and elements co-precipitated with carbonates, 2) organic matter and 3) amorphous Mn hydroxides. These results were statistically compared with physicochemical, sedimentological and biotic data related to SE4.

### 2.2. Foraminiferal analysis

A total 50 ml of sediment was used for foraminiferal analyses. The sediment was wet sieved using 63 µm and 500 µm sieve mesh screens. The sediment fraction 63–500 µm was retained for foraminiferal analysis and oven dried at 50 °C. Only well-stained foraminiferal specimens were considered living at the time of sampling. Composition of foraminiferal assemblages was based on the identification and count of ideally 300 specimens per sample. Despite the complete sediment analyses, some samples did not yield the minimum number of 100 specimens; such samples were not considered in the statistical analysis and in the calculation of ecological indices. Foraminiferal genera were taxonomically classified in accordance with Loeblich and Tappan (1987). The taxonomic identification, at species level, was based on several publications, notably Martins and Gomes (2004) and Camacho et al. (2015). Ecological indexes such as species richness (S), diversity (H'), and evenness (J') were calculated using the Primer 6 software. Foraminiferal density (FD: number of specimens per gram) was also determined.

### 2.3. Mapping and statistical analysis

Selected parameters were mapped using the Surfer v.10 (Golden Software). Stations are labeled with C1–C8. The position of station C7 was changed in the maps to reduce the size of the figures. The statistical analyses were based on abiotic and biotic data selected after an additive logarithmic normalization. The transformed matrix data was then analyzed through an R-mode Cluster Analysis (CA) aiming to evaluate the relationship between biotic and abiotic

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