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The effects of mercury contamination on the structure and functioning of the macrobenthic assemblages of the Ria de Aveiro (Portugal)

Patrícia Alexandra Nunes Matos

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Miguel Ângelo Carmo Pardal (Universidade de Coimbra) e da Doutora Patrícia Cardoso Teixeira

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A todos,

Muito obrigada!

Resumo

Os estuários estão entre os ambientes naturais mais afectados pela elevada pressão urbana e industrial, que aumentam o risco de contaminação por poluentes, como o mercúrio.

Altos níveis de contaminação de mercúrio no ambiente podem afetar os ecossistemas e os seres humanos, especialmente através do consumo de peixe e bivalves. A Ria de Aveiro tem, recebido nas últimas cinco décadas, uma descarga contínua de um efluente rico em mercúrio proveniente de uma fábrica de produção de cloro e soda. Isto levou à contaminação generalizada dos sedimentos, água e organismos criando um gradiente bem definido de mercúrio no sistema.

As comunidades macrobentónicas têm um papel crucial nos ecossistemas aquáticos como consumidores primários e secundários. Devido à sua forte interação com os sedimentos, que são um excelente repositório de mercúrio, existe uma grande preocupação sobre os seus efeitos nas comunidades bentónicas. Assim sendo, os principais objetivos deste trabalho foram: compreender como as comunidades macrobentónicas foram afetadas pela contaminação por mercúrio, especialmente através da análise de sua estrutura (densidade / biomassa), diversidade e produtividade.

Podemos observar que no local mais contaminado, a comunidade macrobentónica apresentou baixas biomassas e baixa diversidade. Este local foi caracterizado por uma comunidade empobrecida constituída principalmente por

um grande número de pequenas poliquetas oportunistas (como por exemplo *Alkmaria romijni*) e outras espécies que se tornaram tolerantes à contaminação por mercúrio como por exemplo, *Cyathura carinata*.

Por outro lado, o local menos contaminado apresentou maior diversidade, tanto em termos do número de espécies como em termos de heterogeneidade e equitabilidade. Este local é caracterizado por espécies com taxas de crescimento mais lento e tempo médio de vida superiores, como por exemplo *Nephtys hombergii* e *Scrobicularia plana*.

Concluindo, a contaminação por mercúrio demonstrou ter claras implicações negativas sobre a estrutura e funcionamento das comunidades macrobentónicas e, consequentemente, pode afetar os níveis tróficos superiores que dependem dessas espécies.

Abstract

Estuaries are among the most affected natural environments due to the high urban and industrial pressure, which greatly increase the risk of contamination by pollutants like mercury.

High mercury contamination levels in the environment might affect ecosystems and the humans especially through fish and shellfish consumption. The Ria de Aveiro has, for the past five decades, received a continuous discharge of a mercury-rich effluent from a chlor-alkali factory. This led to widespread sediments, water and biota contamination, creating a well-defined anthropogenic mercury gradient in the system.

The macrobenthic communities have a crucial role in the aquatic ecosystems as primary and secondary consumers. Due to their strong interaction with the sediments, which are an excellent repository of mercury, there is a great concern about its effects on those communities. Therefore, the main goals of this work were to understand how macrobenthic communities were affected by mercury contamination, especially by analysing their structure (density/biomass), species diversity and productivity.

We could observe that in the most contaminated area, the macrobenthic community was characterized by low biomasses and low species diversity. This site was characterized by an impoverished community constituted mainly by a great number of small opportunistic polychaetes (e.g. *Alkmaria romijni*) and

other species that became tolerant to mercury contamination like, *Cyathura carinata*.

On the other hand, the lowest contaminated site presented the highest species diversity, both in terms of number of taxa and also in terms of heterogeneity and evenness. This site is characterized by species with lower growth rates and higher life spans, like for example *Nephtys hombergii* and *Scrobicularia plana*.

Concluding, mercury contamination demonstrated to have clear negative implications on the structure and functioning of the macrobenthic communities and consequently may affect the higher trophic levels that depend on these species.

Contents

1. Introduction

1.1. Estuaries – Ecological Importance and Deterioration

Estuaries are places where rivers and streams flow into the ocean, mixing their freshwater with the seawater. These places are known for having high nutrient loads, shallow depths, tidal mixing and extremely high primary productivity (McLusky & Elliott, 2005) supporting a wide variety of birds, fish, and other wildlife. Most species with economic interest, such as fishes and bivalves, depend on estuarine systems for survival at least in one stage of their lifecycle (Kennish et al., 2002). On the other hand, estuaries are also among the most affected natural environments due to the high urban and industrial pressure, which greatly increase the risk of contamination by a variety of toxic compounds such as heavy metals (Zonta et al., 2007; Covelli et al., 2011) and organometallic (Berto et al., 2007; Covelli et al., 2011). In addition, the destruction of fringing wetlands and the loss or alteration of estuarine habitats usually degrades biotic communities. High population densities of microbes, plankton, benthic flora and fauna, and nekton characterize estuaries, however, these organisms tend to be highly vulnerable to human activities in coastal watersheds and adjoining embayments (Kennish et al., 2002).

1.2. Mercury Contamination

Mercury (Hg) is one of the most hazardous contaminants that can be present in aquatic environments. This element is widely considered to be one of the most important environmental pollutants by the European Water Framework Directive (WFD) (Pereira et al., 2009).

Mercury is considered a pollutant in terms of environmental damage and a threat to human health. Its high mobility, environmental persistence and lipophilicity, explains the importance of mercury environmental studies, as it is a toxic element to all living organisms (Boening, 2000; Coelho et al., 2007).

Environmental Hg levels affecting the aquatic ecosystems have increased substantially during the industrial age (Mason et al., 1994; Taylor et al., 2012). It is estimated that 80% of the contamination of mercury in the biosphere is due to anthropogenic sources (Mason et al., 1994). The majority of the inorganic mercury arrives to the aquatic systems via anthropogenic discharges, primarily related to chlor-alkali plants (e.g. Alonso et al., 2000; Ram et al., 2003; Bloom et al., 2004; Pereira et al., 2009) and mining activities (e.g. Donkor et al., 2006) or through diffuse sources (e.g. Rothenberg et al., 2008).

Sediments are considered to be the primordial depository of mercury (Balcom et al., 2004; Taylor et al., 2012) and despite of the reduction of anthropogenic emissions of mercury, in the last decades, resulting from the application of restriction rules, the mercury that remains in the sediments has been a concerning problem (Válega et al., 2008).

A large fraction of this Hg may be subjected to methylation by anaerobic bacteria (Gilmour et al., 1992; Benoit et al., 2003 Taylor et al., 2012). The rate of methylation in estuaries is highly accelerated due to the large input of inorganic Hg from anthropogenic sources (Taylor et al., 2012).

The organic form that results from the methylation, called methylmercury (MeHg), can have a toxic effect on the neurological, cardiovascular, and reproductive systems of biota (Wolfe et al., 1998: Taylor et al., 2012). This form of mercury is known to be extremely toxic even at low concentrations (Covelli et al., 2012).

In its organic form, mercury is bioaccumulated in biota (e.g. shellfish with age (Coelho et al., 2006) and biomagnified in aquatic food webs (Fitzgerald et al., 2007; Covelli et al., 2011). So, in aquatic environments, upper trophic level organisms are exposed to MeHg almost exclusively through their diet (Hightower and Moore, 2003). The dominance of this exposure pathway reflects the tendency for MeHg to biomagnify across successive trophic levels, resulting in MeHg concentrations that are potentially toxic to top-level consumers, including humans (US EPA, 2001,Taylor et al., 2012).

1.3. Case study- Ria de Aveiro

The Ria de Aveiro has, for the past five decades, received continuous discharges of a mercury-rich effluent from a chlor-alkali factory. This led to widespread sediments, water and biota contamination, creating a well-defined anthropogenic mercury gradient in the system (Coelho et al., 2007). The discharges resulted in an accumulation of about 33 t of mercury in the lagoon, much of which (about 27 t) is known to be sediment-associated in the Laranjo bay (Pereira et al., 2009). In the last decade, the mercury discharge diminished considerably, being nowadays within the regulatory levels (e.g. 50 μg l^{-1}, the limit value for discharges from chlor-alkali electrolysis industry, in accordance within Directive 82/176/EEC 1982)(Pereira et al., 2009).

Concerns now exist about the future of environmental health of the Ria and attempts have been made to comprehend the processes affecting the historical accumulation of mercury as well as its impacts on the ecosystem functioning (Pereira et al., 2009). Nevertheless, the induced changes by mercury loading on the structure and dynamics of macrobenthic communities have never been addressed before.

1.4. Macrobenthic community

Macrobenthic communities, in Ria de Aveiro are affected by mercury because of its direct contact with the contaminated sediments (Pereira et al., 2009). Previous studies demonstrated a great degree of bioaccumulation in the Laranjo Bay (e.g. *Scrobicularia plana* by Coelho et al., 2006). In this bay have been registered higher levels of mercury in the macrobenthic organisms than in the rest of the Ria de Aveiro (Pereira et al., 2009). The monitoring of contaminated aquatic environments has focused on the collection of potential contaminants, ecotoxiological tests or mesocosms experiments (Grubaugh and Wallace 1995). However, new monitoring approaches have emerged based on biological assessment techniques, including secondary production (Dolbeth et al., 2012).

Secondary production represents an interesting proxy to the functional responses of macrobenthic community subjected to various environmental stresses. Species productivity provides precise information about the state of contaminated sites allowing the comparison of the production between habitats along a gradient of contamination. These approaches have been applied to a wide range of contaminants (Dolbeth et al., 2012).

Given the huge economic and ecological importance of some of the macrobenthic fauna, the aim of the present work is to assess the impact of mercury loading on the macrobenthic community structure and functioning. Generally, it is intended to characterize the macrobenthic community along the mercury gradient and specifically to analyse its structure (density/biomass), species diversity, and productivity comparing with a reference site.

2. Materials and methods

2.1. Study area

Ria de Aveiro supports a population of 250.000 inhabitants in the watershed area, and its main municipality (Aveiro city) is located 15 km south of an industrial complex located in Estarreja including a chlor-alkali plant that has discharged mercury into the Ria from the 1950s until the mid 1990s (Pereira et al., 2009).

Ria de Aveiro is a shallow (mean depth of 1 m), well-mixed mesotidal coastal lagoon on the northwest coast of Portugal (40°38’N, 08°45’W) connected to the sea by a single channel (1.3 km long, 350 m wide and 20 m deep). The system is 45 km long (NNE-SSW direction) with a maximum width of 10 km, and covers an area of approximately 83 km^2 of wetland at high tide and 66 km^2 in low tide (Abrantes et al., 2006; Dias and Fernandes, 2006).

The topography of Ria de Aveiro consists of channels with several branches, islands and mudflats. The Mira and Ílhavo channels run to the south and are narrow and shallow. The S. Jacinto–Ovar channel lies to the north and is wide and deep in its southern part but changes northwards, forming secondary narrow and shallow channels and bays (the Murtosa channel and the Laranjo bay) (Dias et al., 2000).

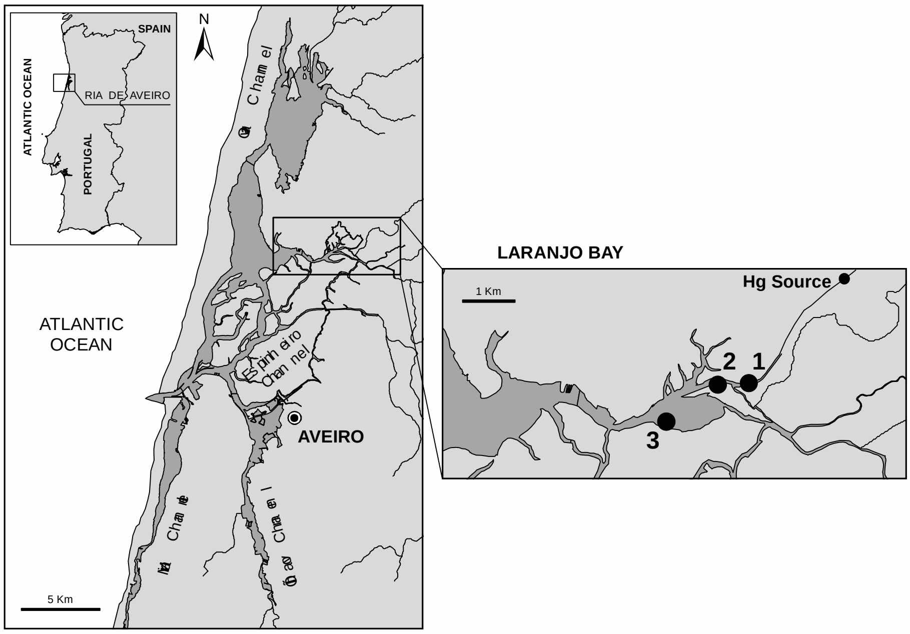

Figure 1- The sampling locations in the Ria de Aveiro (Laranjo Basin).

The macrobenthic communities were monitored monthly during a year, between September 2010 and September 2011 at three distinct sites in Laranjo Bay. The sites were selected according to the well-documented mercury contamination gradient (Fig. 1): Station1 is considered to be at the point source in the estuary), Station 2 (located 600 m away from the point source) and Station 3 (located 3000 m away from the point source and considered as reference site).

2.2. Field and laboratory procedures

At each site and sampling occasion were randomly collected 8-10 sediment cores (141 cm^2 surface area) to a depth of 20 cm. The samples were collected during low tide and washed in estuarine water through a 500 μm mesh. The remaining material was preserved in 4% buffered formalin. Temperature, oxygen, pH and salinity were measured *in situ* in the intertidal pools and sediment samples were collected to quantify the organic matter, and total mercury content. At the same time were collected in the intertidal pools, water samples for determination of chlorophyll *a*, total dissolved mercury and SPM (suspended particulate mercury). Organisms were separated and transferred to 80% ethanol, identified to the lowest possible taxon and counted. Biomass was calculated as ash free dry weight (AFDW) (oven dried at 60°C for 72h and combustion 450ºC for 8h). The same procedure was used to quantify the organic matter content of the sediment.

2.2.1. Mercury quantifications

2.2.1.1. Sediments

Total mercury content of the sediments was analysed by atomic absorption spectrometry with gold amalgamation, using a leco AMA-254 (Advanced Mercury Analyser) (Costley et al., 2000). The analytical quality control of the total mercury determination was performed using Certified Reference Materials (CRMs), MESS-3 (for less contaminated sediments) and PACS-2 (for high contaminated sediments). The results were corrected according to the daily recovery percentage of the CRM analyses. The values obtained for the whole CRM analysis ranged from 96.8 to 103.3% (at 0.05 significance level). Analyses of CRMs were always performed in triplicate and coefficient of variation was less than 10%.

2.2.1.2. Water

Water samples were filtered with 0.45 µm pore size Millipore filters and acidified with concentrated HNO_3 "mercury free" to pH < 2 and maintained in a room at 4ºC.

Total mercury analysis in water samples was performed by cold vapour atomic fluorescence spectroscopy (CV-AFS), on a PSA cold vapour generator, model 10.003, associated with a Merlin PSA detector, model 10.023, and using SnCl2 as reducing agent. This analytical methodology is highly sensitive, allowing the measurement of 1 ng L^{-1} of mercury.

2.2.1.3. SPM (Suspended Particulate Mercury)

Filters (from the previous process of water filtration) were oven-dried at 60º C and digested with HNO_3 4 mol. L^{-1} for determination of the total mercury concentration in the SPM fraction. (as described in Coelho et al., 2007).

2.3. Data analysis

2.3.1. Environmental parameters

One-way ANOVAs were carried out for the environmental parameters in order to detect differences between sampling stations. All data were previously checked for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using the Levene's test (Zar, 1996). Data not meeting these criteria were transformed appropriately (Zar, 1996) and checked again for normality and homocedasticity.

2.3.2. Macrofaunal diversity

The diversity of the macrobenthic communities in the three areas was assessed as species richness (simple count of number of species recognised) by the Shannon-Wiener (log base 2) and Pielou's evenness measures (Krebs, 1999).

2.3.3. Secondary production

Secondary production of the macrobenthic communities was calculated based in different methodologies, according to the species. For *Hydrobia ulvae* (Gastropoda), production was estimated based on the increment summation method after definition of cohorts through size-frequency distribution analysis of successive sampling dates (after Ferreira et al., 2007; Cardoso et al., 2008, Grilo et al., 2009 and Dolbeth et al., 2011).

Length-frequency data of *Hydrobia ulvae* (Gastropoda) individuals from successive sampling dates, at each station, were analysed using FISAT II software (FAO-ICLARM STOCK assessment Tools), as explained in detail by Gayanillo et al. (2005). Annual net production estimates (P) based on cohort recognition, were estimated as described in Dauvin (1986).

Total values of P for the population are expressed as:

$$P = \sum_{n=1}^{N} Pcn$$

Where P_{cn} is the growth production (biomass assimilated by a constant number of individuals in a certain period of time) of a cohort n. The annual mean population biomass ($\overline{B}$) was calculated as follows:

$$\overline{B} = (1/T) \sum_{n=1}^{N} (\overline{B}_n t)$$

Where T is the period of study (one year); N the number of successive cohorts in the period T; B_n the mean biomass of cohort n; t the duration of the cohort n.

When cohorts are not recognized, size-based methods may be applied using the instantaneous growth method (= mass growth method), as long as the maximum size, the life span and the form of the growth curve are known (Benke et al., 1993, Dolbeth et al., 2012). Concerning *Scrobicularia plana* it was impossible to detect cohorts. Consequently, it was used the Mass Specific Growth Rate Method (MSGRM) (Benke et al., 1993; Brey, 2001). This method requires population size-structure with data that express changes in size-structure densities over the whole population cycle (Dolbeth et al., 2012). For this calculation, was used the worksheet provided in Brey 2001 http://www.thomas-brey.de/science/virtualhandbook/navlog/index.html).

Brey´s (2001) method, version 4.04 (worksheet for model computation provided in Brey 2001, http://www.thomas-brey.de/science/virtualhandbook/navlog/index.html) was used as an alternative empirical method for secondary production estimation (after Dolbeth et al., 2005) for other abundant species - *Carcinus maenas*, *Crangon crangon* (Decapoda), *Alkmaria romijni*, *Capitella* *capitata*, *Hediste diversicolor*, *Streblospio shrubsolii*, *Nephtys hombergii*, *Pygospio elegans*, *Tharyx sp*., *Glycera tridactyla*, *Polydora ligni*, *Polydora ciliata*, *Pseudopolydora paucibranchiata* (Polychaeta), *Tubificoides sp*., *Oligocheta sp*. (Oligochaeta), *Cerastoderma edule*, *Scrobicularia plana* (Bivalvia), *Cyathura carinata*, *Lekanesphaera levii* (Isopoda), *Corophium multisetosum*, *Melita palmata* (Amphipoda), *Diptera larvae* (Insecta).

Mean biomass and $P/\overline{B}$ ratios (annual production divided by the annual mean biomass) were also computed for the species.

2.3.4. Multivariate analysis of the macrofaunal assemblages

It was used permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) to test hypotheses about differences in macrobenthic assemblages between sampling sites along the Hg gradient. To visualize multivariate patterns revealed by PERMANOVA, we used canonical analysis of principal coordinates (CAP, Anderson & Willis, 2003). In our study we used a specific case of CAP (canonical correlation), since we were interested in seeing how well the biotic data differentiated the samples along a quantitative Hg gradient. Previous studies have demonstrated the existence of a clear Hg gradient in the Laranjo basin (Coelho et al., 2007; Nunes et al., 2008). Based on this, first it was constructed a PCA with Hg concentrations (sediment, water and SPM) and selected the single variable with the scores for samples along PC1. This information was used to run the CAP and relate the pollution gradient to the biotic matrix. The abiotic data was previously log-transformed while the biotic matrix was square-root transformed for posterior calculation of Bray-Curtis distances (Anderson et al., 2008).

All multivariate analyses were done using the PERMANOVA+ for PRIMER software (Anderson et al., 2008).

3. Results

3.1. Environmental variables and Mercury distribution among sites

The values for the environmental parameters obtained in the Laranjo Bay are shown in Figure 2.

The temperature and salinity values in the intertidal water pools, showed a seasonal variation: low values during the winter and high values in the summer (Fig. 2A, B). No significant differences were obtained for these parameters between the three sampling sites (1-way ANOVA, Temperature: F_2 =0.14, $P > 0.05$; Salinity: F_2 =0.18, $P > 0.05$).

Dissolved oxygen (DO) was always relatively high (Fig. 2C) and generally presented higher values during the winter and lower values during the summer. This environmental parameter did not show significant difference between sampling sites (1-way ANOVA, F_2 =0.22, $P>0.05$). The pH showed a similar pattern during winter and summer months (Fig. 2D), having no significant difference between the three sites (1-way ANOVA, F_2 =1.27, $P>0.05$).

The organic matter content (OM) had a low variation between winter and summer (Fig. 2E). At st1 was found a greater percentage of OM throughout the year than in the other sites, however, this difference was not significant (1-way ANOVA, F_2 =2.57, $P>0.05$). Concentration of chloropyll *a* showed a seasonal variation, the highest concentrations were found in the summer (Fig. 2F). Chlorophyll *a*, as well as the other environmental parameters mentioned above, did not reveal a significant difference between sampling sites (1-way ANOVA, F_2 =1.35, P>0.05).

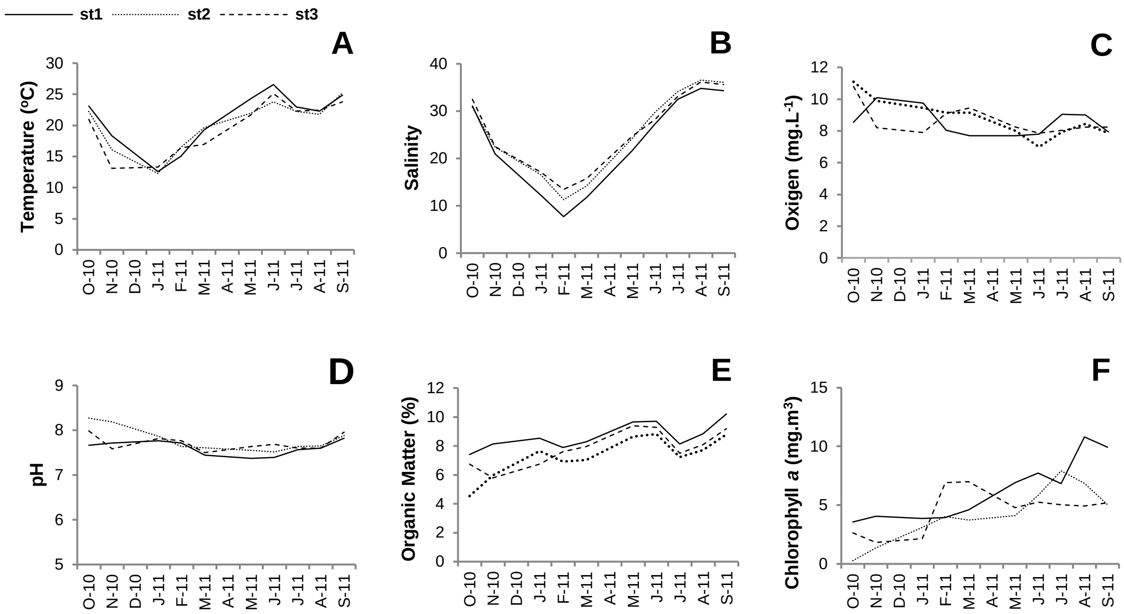

Figure 2 -Environmental parameters: Temperature (A), salinity (B), dissolved oxygen (C), pH (D), Organic matter (E) and chlorophyll *a* (F).

Regarding the mercury concentrations in the sediments it was clearly observed a spatial gradient (Fig. 3A). The highest levels were found in the most upstream area (st1). The remaining locations denoted significantly lower values. The site 2 presented the intermediate values and site 3 the lowest ones. The total mercury in sediments presented significant differences between the three sampling sites (1-way ANOVA, F_2 =93.38, P<0.05).

Dissolved mercury, in the intertidal water pools, showed a spatial gradient with higher values at stations 1 and 2 and lower at station 3. Also, it was possible to observe a seasonal variation (Fig. 3B), with slightly higher values during autumn/winter and lower values during summer. However, no significant differences were observed between the three sites (1-way ANOVA, F_2 = 1.10, P>0.05).

The SPM concentrations also revealed a spatial gradient, with higher values at stations 1 and 2 and lower values at station 3. Seasonally, the SPM values at sites 1 and 2 were higher in summer than in the winter, the opposite occurred at site 3 (Fig. 3C). The SPM showed significant differences between the three sampling sites (1-way ANOVA, F_2 =25.88, P<0.05).

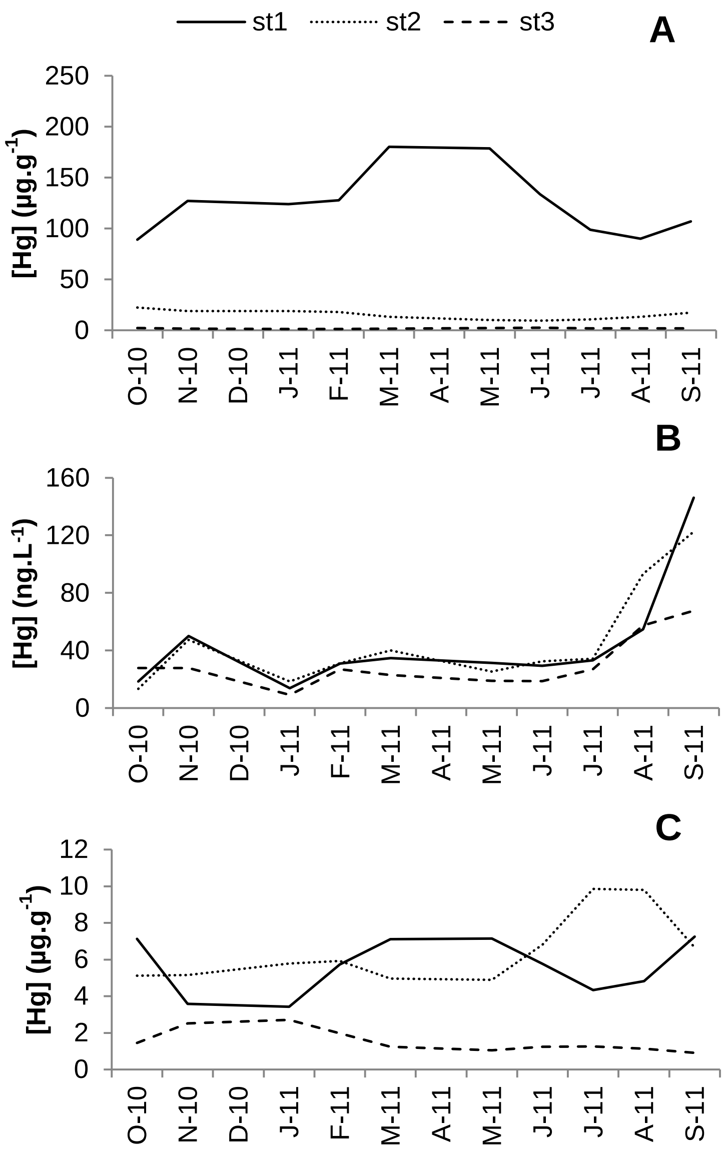

Figure 3 - Total mercury concentrations in sediments (A), dissolved in intertidal water pools (B) and SPM (C).

3.2. Macrobenthic community structure

The organisms collected in the sampling campaigns resulted in 33 macrobenthic taxa, in which six of them represented 98% of the mean total density (71904 ind.m^{-2}). These taxa included *Alkmaria romijni* (polychaeta, 52%), *Hydrobia Ulvae* (gastropoda, 17%), *Hediste diversicolor* (polychaeta, 16%), *Streblospio shrubsolii* (polychaeta, 8%), *Capitella capitata* (polychaeta, 4%) and *Scrobicularia plana* (bivalvia, 1%).

In the Figure 4 is represented the variation in density and biomass of the total community at each sampling station. In terms of density (Fig. 4A), site 1 exhibited the highest abundance during autumn and early winter while during spring/summer the highest values were observed at station 2 (mean values). The lowest values were observed at st1 during the summer.

Concerning the biomass pattern, it is important to highlight that at site 1, biomass was always lower than in the other sites. The site 2 presented the highest biomass values (Fig. 4B).

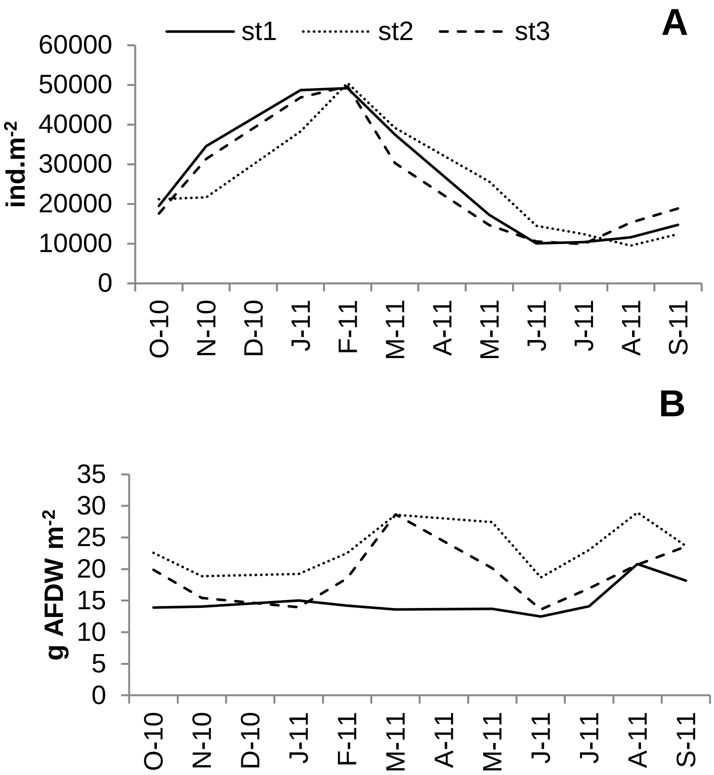

Figure 4 – Variation for the macrobenthic community by density (A) and biomass (B).

Variations in diversity measures (S, J´, H´) at each sampling station are shown in Figure 5.

The sites 1 and 2, closer to the effluent discharge, presented lower species richness than the site 3 (Fig. 5A). Also, the highest values occurred during the winter at all sampling sites.

Concerning heterogeneity and evenness (Fig. 5B, C) they showed similar patterns, characterized by lower values at site 1 than at the other sites. Site 3, generally, presented the highest values.

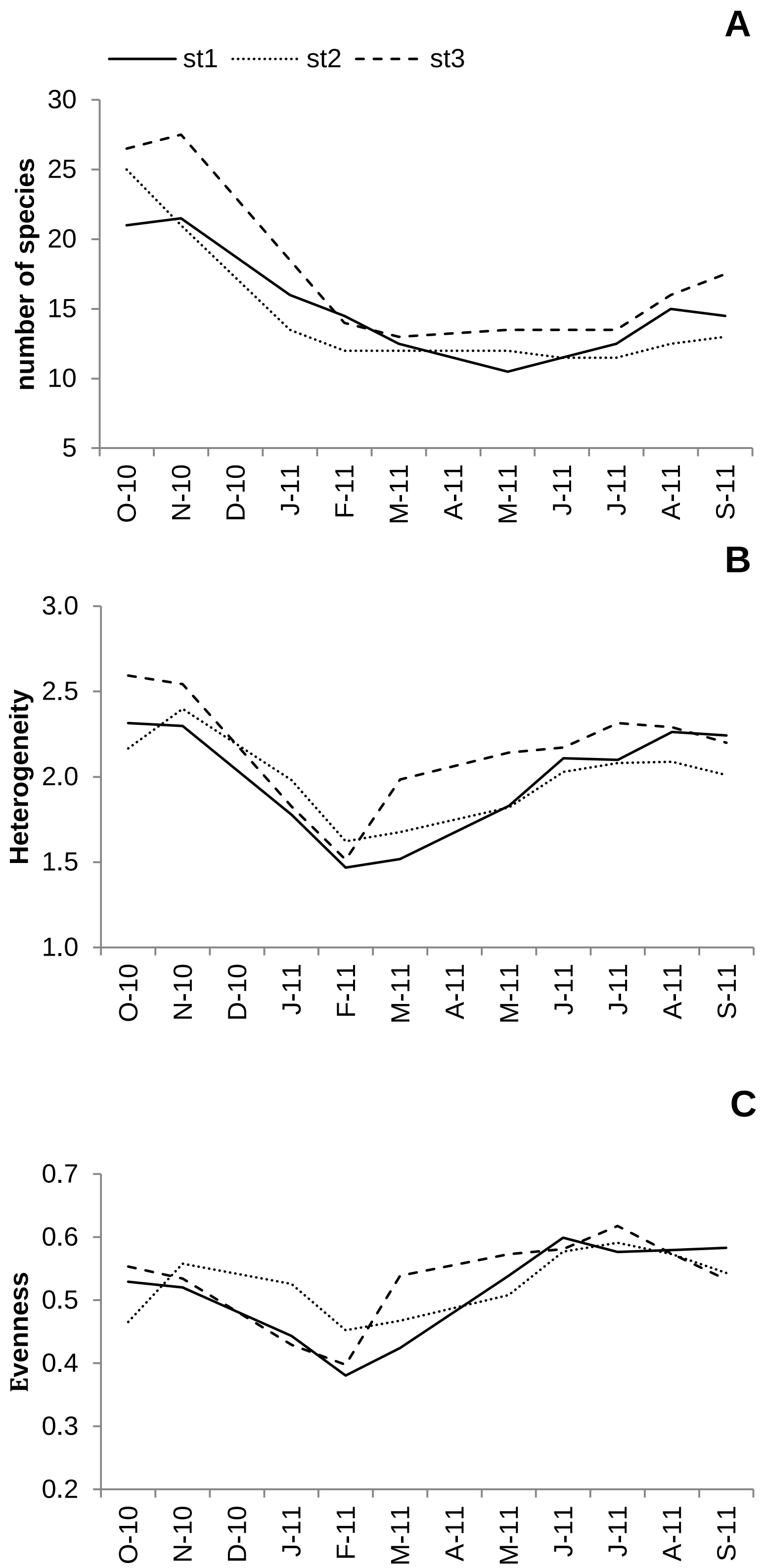

Figure 5 - Variations of biodiversity indices in the three sampling stations over the study period: number of taxa (A), Shannon-Wiener index (B) and evenness (C).

The taxonomic composition of the studied macrobenthic community included: polychaetes (86% of total density), gastropods (10% of total density), bivalves (2% of total density), crustaceans (1% of total density) and 3 miscellaneous taxa (1% of total density).

In the macrobenthic community there are species that exhibited different behaviour in relation to the mercury gradient. Some species were found to be less tolerant to mercury contamination, such as the isopod *Lekanesphaera levii* that was only found at site 3 while the polychaete *Tharyx sp.* was never found at the site 1. The bivalve *Scrobicularia plana* showed a well-defined density gradient with higher densities in the less contaminated site (st3) and lower in the most contaminated one (st1) (Fig. 6A). Moreover, this species had greater biomasses in the less contaminated sites (ST2 and ST3) than at site 1 (Fig. 6B). The polychaete *Pseudopolydora paucibranchiata* revealed higher values of biomass and density in the least contaminated site (ST3) in opposition to the most contaminated (st1) (Fig. 6C, D). This species showed a greater abundance in the winter. On the other hand, the polychaete *Alkmaria romijni* and the isopod *Cyathura carinata* showed an opposite behaviour than the two species above. In the case of *Alkmaria romijni* a higher density of this species was found in the most contaminated site (st1) (Fig. 6E). The same is true for biomass though in this case the values represented a clear gradient with the highest values at site 1 and the lowest ones at site 3 (Fig. 6F). *Cyathura carinata* presented higher density and biomass values in the most contaminated site (st1) (Fig. 6G, H). The highest density values for *Cyathura carinata* were found in the summer.

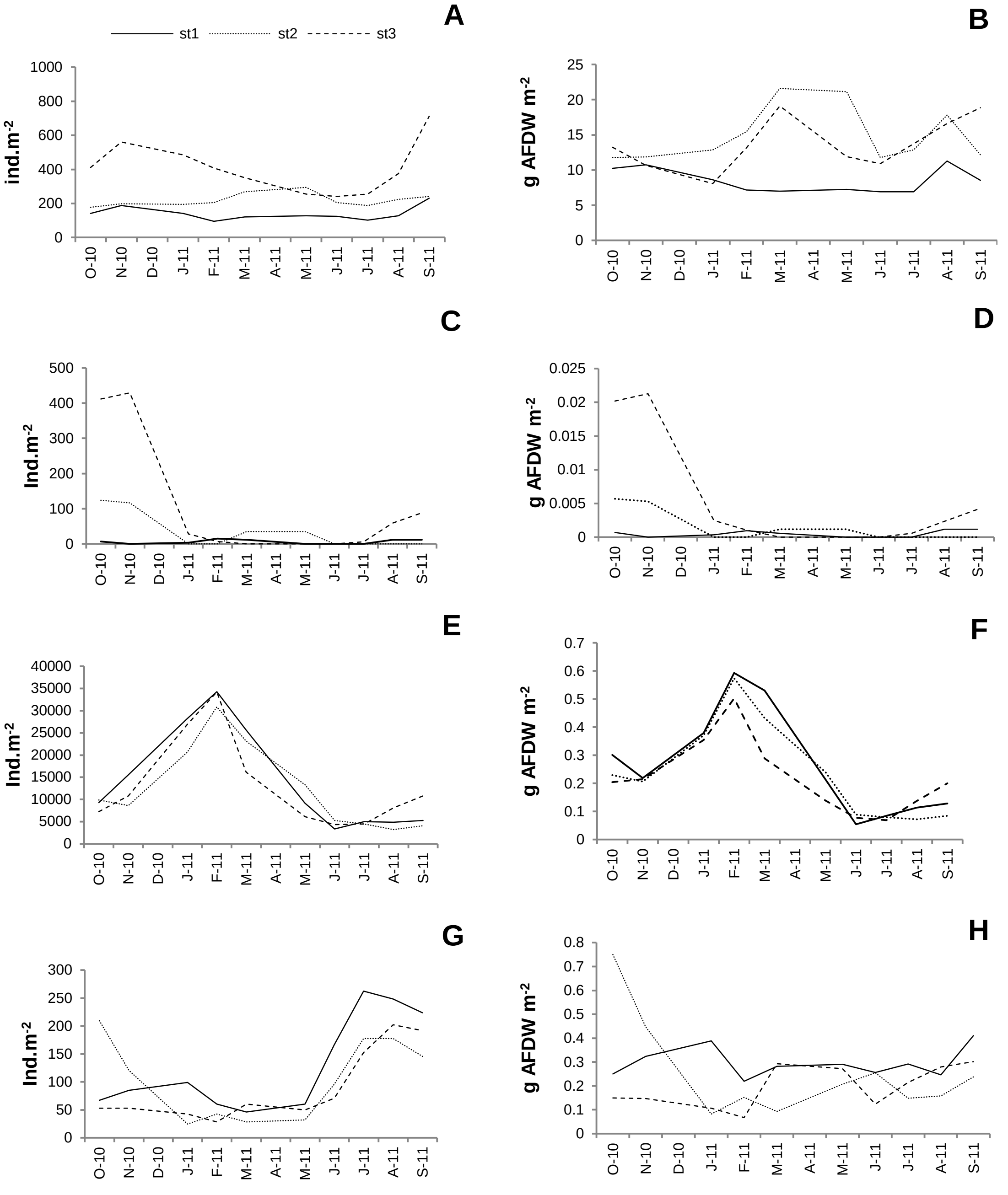

Figure 6 – Values of Density (left) and Biomass (right) for: *Scrobicularia plana* (A and B), *Pseudopolydora paucibranchiata* (C and D), *Alkmaria romijni* (E and F) and *Cyathura carinata* (G and H).

3.3. Secondary production

The Table 1 is a list of the secondary macrobenthic production for each sampling site in Laranjo Bay. The annual macrobenthic community productions for each sampling site showed that the intermediate site (st2) had the highest value. The least mercury-contaminated site (st3) had the lowest value for annual production.

The polychaetes constituted the taxonomic group that represented the greatest contribution for the annual production presenting at site 1 the highest value while the lowest was observed at site 2. The bivalves in the most contaminated site (st1) showed a considerably lower value for the secondary production in comparison to the other sites. Two smaller groups, Isopoda and Decapoda, showed an interesting behaviour regarding productivity. Both annual productions demonstrated that they responded to the gradient of mercury contamination. Isopoda showed the highest value of production at site 1 and the lowest value at site 3. Decapoda (*Carcinus maenas* and *Crangon crangon*) had an opposite behaviour (the highest value was recorded at site 3 and the lowest value at site 1).

The species that mostly contributed to the annual production for all sampling sites was *Hediste diversicolor*. This species had a higher production at site 1 (more contaminated) and a lower production at site 2.

The species that mostly contributed to the higher productivity at site 1 were: *Hediste diversicolor*, *Alkmaria romijni*, *Hydrobia ulvae* and *Scrobicularia plana*. In site 2 were: *Hediste diversicolor*, *Scrobicularia plana* and *Hydrobia ulvae*.

On the other hand, for site 3, the species that most contributed to the secondary production were: *Hediste diversicolor* and *Scrobicularia plana*. Specifically, the polychaete *Pseudopolydora paucibranchiata* showed a perfect gradient with highest production values at site 3 and the lowest ones at site1. The small polychaetes *Alkmaria romijni*, *Capitella capitata* and *Streblospio shrubsolii* attained higher production levels at the most contaminated site. The sampling site with the lowest mercury value showed higher production for the larger polychaetes such as *Glycera tridactyla* and *Nephtys hombergii*.

Table 1 - Species discriminating for each study area with indication of mean production (g AFDW m^{-2} y^{-1}), $P/\overline{B}$ values for each specie and sum of the species production to whole community.

		st1		st2		st3	
Taxa	Species	P	$P/\overline{B}$	P	$P/\overline{B}$	P	$P/\overline{B}$
Nemertinea	*Tetrastemma sp.*	0.042	11.148	0.047	10.304	0.055	6.743
	Nemertinea sp.	0.004	6.674	0.002	5.793	0.006	6.043
Oligochaeta	*Oligochaeta sp.*	0.062	8.432	0.012	8.032	0.066	9.747
	Tubificoides sp.	0.001	8.677	0.005	9.341	0.003	8.899
Polychaeta	*Alkmaria romijni*	3.789	11.259	3.093	13.564	2.781	13.082
	Capitella capitata	0.357	12.802	0.181	12.561	0.309	12.135
	Hediste diversicolor	16.775	4.206	13.370	3.806	14.254	3.978
	Nephtys hombergii	0.002	8.677	0.003	3.524	0.011	5.256
	Pygospio elegans	0.013	13.960	0.005	9.468	0.016	8.791
	Tharyx sp.			0.002	8.147	0.090	8.990
	Glycera tridactyla	0.043	3.425	0.033	3.951	0.080	2.518
	Streblospio shrubsolii	0.551	15.059	0.342	14.469	0.460	13.452
	Polydora ligni	0.013	9.592	0.015	8.965	0.012	9.019
	Polydora ciliata	0.004	9.388	0.010	9.292	0.003	7.997
	Pseudopolydora paucibranchiata	0.005	9.207	0.013	10.581	0.047	9.375
Bivalvia	*Cerastoderma edule*	0.008	3.997	0.598	0.929	0.052	1.783
	Scrobicularia plana	4.846	0.802	8.570	0.737	8.137	0.875
Gastropoda	*Hydrobia ulvae*	4.340	3.665	11.600	3.066	2.700	3.303
Isopoda	*Cyathura carinata*	1.012	3.255	0.828	3.045	0.608	3.008
	Lekanesphaera levii					0.001	8.723
Amphipoda	*Corophium multisetosum*	0.006	6.563	0.009	5.098	0.003	6.677
	Melita palmata	0.002	14.764	0.011	12.093	0.004	12.395
Decapoda	*Carcinus maenas*	0.052	4.019	0.087	3.299	0.129	2.677
	Crangon crangon	0.024	4.616	0.062	3.798	1.045	5.058
Insecta	*Diptera (larvae)*	0.193	5.886	0.090	5.189	0.106	5.273
	Whole community production	**32.142**		**38.991**		**30.977**	

3.4. Multivariate analyses (PERMANOVA and CAP)

PERMANOVA analysis showed significant differences in macrobenthic assemblages between the 3 sampling sites along the mercury gradient (Table 2).

These results were confirmed by the CAP analysis, in which the macrobenthic assemblages followed the Hg gradient. The squared canonical correlation was quite high (δ_1^2= 0.78), suggesting that this is a well-adjusted model (Fig. 7).

Table 2 - Summary of results of permutational multivariate analysis of variance (PERMANOVA) and post-hoc pairwise comparisons of macrobenthic assemblages at 3 sampling stations.

Source of variation	df	SS	MS	F	P
Station (St)	2	3004.1	1502.1	3.4878	0.0002

Pairwise post-hoc comparisons of stations

Groups	t	P
st1,st2	1.5737	0.0213
st1,st3	1.8593	0.0071
st2,st3	2.1191	0.0041

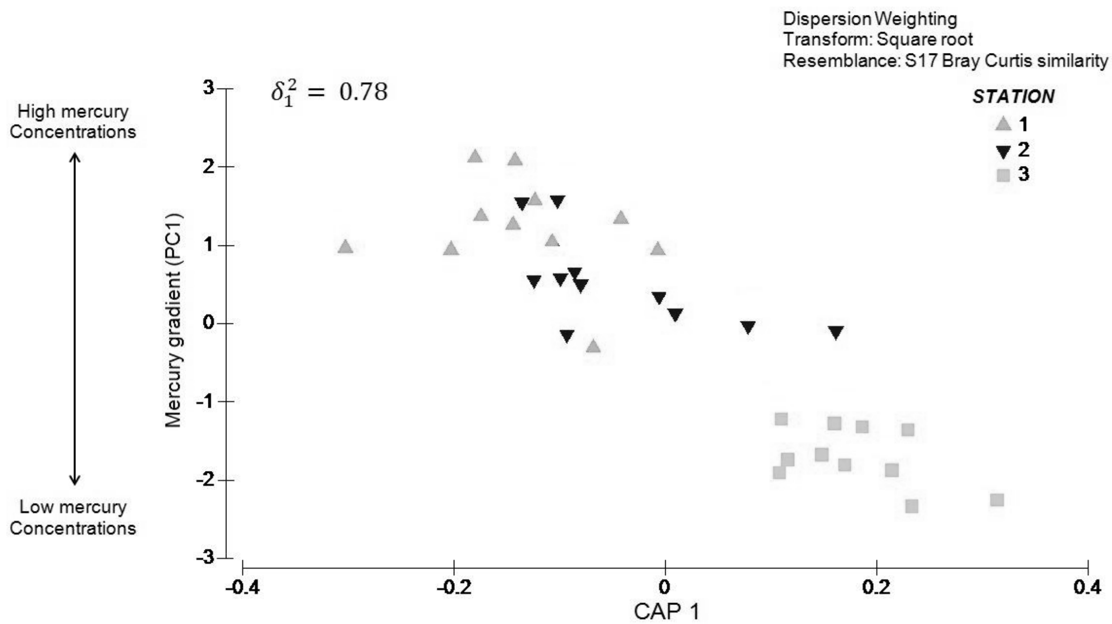

Figure 7 - CAP analysis relating biota from sites in the Laranjo Bay to the mercury gradient.

4. Discussion

4.1. Environmental variables and mercury

The temperature variation registered in the intertidal water pools demonstrated a climatic seasonality. In the winter, the temperature increased towards the estuary mouth, the opposite occurring in the summer. Salinity also showed a seasonal variation possibly related to the higher precipitation in the winter and consequent freshwater flow to the Laranjo Bay resulting in a salinity decrease. The highest salinity values were recorded in the summer possibly due to the freshwater flow decrease and consequent seawater penetration in the estuary (Lillebø et al., 2005).

The values for dissolved oxygen were similar to previous studies (Ramalhosa et al., 2002; Lopes et al., 2003). The pH showed a similar range of values all over the year. Chlorophyll *a* concentration showed a seasonal variation accompanying the seasonal variations in solar radiation and consequent primary production.

The organic matter content (OM) demonstrated a low seasonal variation. The values for OM and mercury were the highest at site 1, possibly due to their strong association (Ramalhosa et al., 2006; Pereira et al., 2009).

All previously analyzed environmental variables had no significant differences between the three sites.

The analysis of mercury content (sediments and water) showed a spatial gradient with higher values at stations 1 and 2 and minimum values at station 3 in accordance with previous studies (Nunes et al., 2008; Pereira et al., 2009). The sediments revealed to have the highest values for mercury concentration throughout the study, especially at station 1, suggesting that mercury is strongly associated with sediment (Pereira et al., 2009). The values for Ecotoxicological Assessment Criteria (EACs) for mercury in sediments and water were measured by the OSPAR Commission in 2004 revealing that these values ranged from 0.05 mg Kg^{-1} to 0.5 mg Kg^{-1} for the sediments and from 5 ng L^{-1} to 50 ng L^{-1} for the water (OSPAR, 2004).

In this study, the concentrations of total mercury in sediments were all above the EACs thresholds, despite at station 3 the Hg concentrations were close to the acceptable values. However, at stations 1 and 2, the levels of mercury were much higher than the upper-EAC, contributing to long-term biological effects, such as mortality (OSPAR, 2004). In terms of dissolved mercury, the values obtained for stations 1 and 2 were close to the upper limit during most of the study period and in the late summer overcame those values. On the other hand, at station 3 were generally lower than the upper EACs threshold.

The total mercury concentrations in sediments and SPM revealed significant differences between the three sampling sites. These results suggested that mercury was the only environmental variable affecting the macrobenthic communities in the three sampling sites of the Laranjo Basin.

4.2. Macrobenthic community structure

Pearson & Rosenberg (1978) mentioned that unidirectional stress caused by a particular environmental disturbance would result in changes of fauna composition along a stress gradient. A species-specific factor that may determine the presence of macrobenthic organisms in highly contaminated sediments is the tolerance. Some previous studies have already revealed the tolerance of macrobenthic species to polluted areas (Dauvin, 2008; Nunes et al., 2008). This suggests that the tolerance to contaminated sites may be acquired at genetic, cellular or biochemical levels. In addition to the occurrence of tolerant species, tolerant genotypes can appear in polluted areas resulting in populations that have a greater tolerance to contaminants (Weis et al., 2004). Substitution of sensitive species by pollution tolerant species may be occurring in contaminated sites (Millward & Klerks, 2002).

The station 1 is the most contaminated sampling site presenting low biomass values, species richness, heterogeneity and evenness. The low species richness at sites 1 and 2 may occur because it is affected by pollution (Ponti et al., 2011). These results are in accordance with other study performed in the lower Douro estuary indicating that the sampling site with the highest levels of heavy metal contamination had the lowest diversity (Mucha et al., 2005).

Site 1 registered high abundance levels during the autumn/winter mainly due to the presence of two species: *Alkmaria romijni* and *Hediste diversicolor*. The most abundant of these species, *Alkmaria romijni*, is a highly opportunistic species. These species, at Ria de Aveiro prosper in organically enriched sediments (Rodrigues et al., 2011). Other studies showed that organic matter is associated to mercury in sediments, suggesting that *Alkmaria romijni* and *Hediste diversicolor* may be a mercury tolerant species-specific (Nunes et al., 2008). Other study at Ria de Aveiro revealed that *Hediste diversicolor* is the least responsive species for mercury when compared to *Scrobicularia plana* and *Carcinus maenas* (Coelho et al., 2007). The *Hediste diversicolor* can survive in sediments containing high levels of copper (Dauvin, 2008), therefore this species seems to be tolerant to a large range of pollutants.

In the opposite, *Lekanesphaera levii* (Isopoda) demonstrated sensibility to mercury contamination thriving in the less contaminated site. This can be supported by previous studies that concluded that Crustacea are sensible to heavy metal pollution (Dauvin 2008). However, *Cyathura carinata* (Crustacea) presented its highest density and biomass values at station 1, suggesting that, like *Hediste diversicolor,* this species is tolerant to mercury contamination (Nunes et al., 2008).

The polychaete *Tharyx sp*. revealed to be sensitive to higher mercury concentrations, as it was not found at station 1. Also, the bivalve *Scrobicularia plana* recorded the lowest values of density and biomass at the most contaminated site, which means that is very sensitive to pollutants.

Site 2, characterized by intermediate mercury concentrations presented the highest biomass values all over the study, which will be reflected in secondary production. This station showed an intermediate pattern in terms of heterogeneity and evenness and as station 1, it showed low species-richness.

The highest values of species-richness were found at station 3. In terms of heterogeneity and evenness, the values at this station were generally higher than at the other stations. This may be due to the lower stress caused by the mercury contamination at this station. In the Douro estuary study the station that had the lowest metals concentration showed a relatively high diversity on the macrobenthic community in terms of number of species (Mucha et al., 2004).

At site 3 it was possible to observe species with higher growth rates and life spans. *Scrobicularia plana* is a good example since it presented a well-defined density gradient with higher densities and biomasses in the less contaminated site. This is in accordance with previous studies (Nunes et al., 2008). This species seems to be sensitive to mercury contamination.

The polychaete *Pseudopolydora paucibranchiata* had a curious behaviour in this study, demonstrating a sensibility to mercury concentration. This species showed higher values of biomass and density at the least contaminated site. In a study in the Oslo Fjord the abundance of *Pseudopolydora paucibranchiata* was significantly reduced as the copper-concentrations increased (Olsgard, 1999) showing that this species is sensitive to metals contamination.

4.3. Secondary production

The species that most contributed to the annual production for all sampling sites was *Hediste diversicolor*. The bivalves in the most contaminated sites showed a considerable lower value of secondary production in comparison to site 3. *Scrobicularia plana* was the species that most contributed for this. Other study at the Laranjo Bay indicated that *Hediste diversicolor* is less responsive to mercury compared to *Scrobicularia plana* (Nunes et al., 2008).

Annual macrobenthic community production for each sampling site showed that the intermediate site (st2) had the highest value. This result can be supported by the intermediate disturbance hypothesis (IDH). This hypothesis predicts that species diversity will be highest on the intermediate levels of disturbance. Joseph Connell (1975, 1978) proposed that the disturbance is the prevalent feature of nature that significantly influences the diversity of a community. Therefore, he proposed that high diversity is a consequence of continually changing conditions, like provoked by a contamination, and not the result of a competitive accommodation at equilibrium. In fact, station 2 showed relatively high values on the diversity parameters (heterogeneity and evenness) and also on secondary production levels, which can be related to the IDH. At this station, *Scrobicularia plana* contributed with 22% of total annual production and *Hydrobia ulvae* contributed with 30% of total annual production. These two species, in this station, presented the highest values of production (especially *Hydrobia ulvae*) and the lowest values of $P/\overline{B}$ ratio in comparison to the other sites, suggesting that these two populations have a higher stability at station 2 (Dolbeth et al., 2007).

Two smaller groups, Isopoda and Decapoda, showed an interesting behaviour regarding productivity. Both annual productions demonstrated that they responded to the gradient of mercury contamination. Isopoda showed the highest production value at site 1 and the lowest value at site 3. This resulted essentially because *Cyathura carinata* has already been reported as tolerant species to several disturbances (Ferreira et al., 2004). Decapoda (*Carcinus maenas* and *Crangon crangon*) had an opposite behaviour in relation to Isopoda (the highest value was recorded at site 3 and the lowest value at site 1). This occurred probably because *Crangon crangon* has a proven vulnerability to metal pollution (Dauvin et al., 2008) and *Carcinus maenas* is sensitive to mercury contamination (Depledge, 2004; Coelho et al., 2008).

The polychaetes constituted the taxonomic group that most contributed for the annual production, presenting at site 1 the highest value while the lowest was observed at site 2. The substantial annual secondary production registered at all sites may have occurred because polychaetes have an extremely variable sensitivity to different heavy metal concentrations (Dauvin 2008).

The small polychaetes *Alkmaria romijni*, *Capitella capitata* and *Streblospio shrubsolii* attained higher production levels at the most contaminated site. They have a small body size, short life span and a clearly opportunistic behaviour at heavy metal contaminated sites (Dauvin, 2008). The less contaminated site showed higher production for the largest polychaetes such as *Glycera tridactyla* and *Nephtys hombergii*. These species of polychaetes are not opportunistic species like the smaller ones and present a longer life span.

The polychaete *Pseudopolydora paucibranchiata* showed a perfect gradient with highest production values at site 3 and the lowest ones at site1. Despite being an opportunistic species, it is sensitive to the mercury contamination.

4.4. Final considerations

In this study we could conclude that the structure and functioning of the macrobenthic communities were negatively affected by the mercury contamination.

The most contaminated site was characterized by low biomasses and low species diversity. This site was characterized by an impoverished community constituted mainly by a great number of small opportunistic polychaetes (e.g. *Alkmaria romijni*) and other species that are tolerant to mercury contamination like, *Cyathura carinata*. On the other hand, the lowest contaminated site presented the highest species diversity, both in terms of number of taxa and also in terms of heterogeneity and evenness. This site is characterized by species with lower growth rates and higher life spans, like for example *Nephtys hombergii* and *Scrobicularia plana*.

Macrobenthic communities are an important component of the estuarine ecosystem since they are crucial to system dynamics reflecting the anthropogenic impacts like mercury contamination. The results from this study constitute a great concern, since mercury contamination demonstrated to have clear negative implications on the structure and functioning of the macrobenthic communities and consequently may affect the higher trophic levels that depend on these species.

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