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Effects of sediment contamination on physiological and biochemical responses of the polychaete *Diopatra neapolitana*, an exploited natural resource

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ABSTRACT

The present study reports metal and arsenic contamination in sediments, as well as element accumulation and partitioning in the polychaete *Diopatra neapolitana* in the Ria de Aveiro lagoon (Portugal). The polychaetes biochemical performance and tissue regenerative capacity were also evaluated. The concentration of elements in sediments showed an increase of contamination among areas (areas A–G), but higher bioaccumulation was observed in organisms from a less contaminated area (area C, BAF > 1). This study evidenced that individuals with higher elements bioaccumulation presented higher LPO and lower GSH/GSSG and also exhibited lower capacity for body regeneration. Polychaetes biotransformation capacity as well as antioxidant defense mechanisms were not sufficiently efficient to withstand the excess of ROS leading to increased LPO when organisms presented higher bioaccumulation levels. Additionally, an increase of methalotionines was also observed in individuals with higher bioaccumulation of metals and As, suggesting an induction of detoxification processes.

1. Introduction

Sediments constitute a major reservoir of persistent contaminants storage in aquatic environments and, therefore, a potential source to interstitial waters and inhabiting organisms, namely benthic species (Chapman et al., 1998; Simpson and Batley, 2007). Macrobenthic organisms are key components of coastal ecosystems, as they play an important role in detritus decomposition, nutrient cycling, and energy flow to higher trophic levels and communities (Levin et al., 2001; Serrano et al., 2003). Additionally, benthic macroinvertebrate species can accumulate large amounts of contaminants, as they live in close contact with the sediment for long periods and present feeding strategies that involve ingestion of sediment particles, resulting in maximal exposure to contaminants both in the sediment and pore water (Dean, 2008). These characteristics can enable macrobenthic invertebrates to provide a time-integrated indication of environmental contamination (accumulation and impacts) and offer the possibility to be used as bioindicators of pollution in coastal areas (Langston et al., 2012). Among all benthic taxa, polychaetes are frequently the most abundant taxonomic group in estuarine ecosystems, and are key elements in estuarine and coastal food webs, constituting an important food source for many commercial fish and crustaceans, enabling them to become transfer vectors of contaminants to higher trophic levels (Lewis and Watson, 2012; Serrano et al., 2003).

Physiological endpoints such as feeding activity, growth, reproduction and survival rate, have been used to measure responses at the individual level in polychaetes which can be correlated with pollutant exposure (Méndez et al., 2013). Chandler and Scott (1991) demonstrated that Endosulfan (insecticide and acaricide) strongly inhibits larval colonization and early juvenile growth of the polychaete *Streblospio benedicti*. A significant depression of feeding activity (from 30 to 70%) was observed in the polychaete *Hediste diversicolor* after exposure to sediments essentially polluted with metals (Moreira et al., 2006a). Gomes et al. (2014) reported that growth and egestion rate of the polychaete *Capitella teleta* were severely impaired when exposed to Barcelona harbour sediment (metal and PCB contamination). Nusetti et al. (2005) and Reish et al. (1989) demonstrated that the regenerative ability of polychaete (*Eurythoe complanata*) was severely affected in organisms exposed to organic and inorganic contaminants. Recently the study of the regenerative capacity of the posterior end of the polychaete *Diopatra neapolitana* was also used to assess the effect of emerging contaminants such as pharmaceuticals (Freitas et al., 2015a; Pires et al., 2016a).

Besides traits at the individual level, organisms responses can be measured at the sub-cellular level. These responses can offer sensitive information about toxic impacts on organism health and allow to observe early signs of biological response to xenobiotics (Sun and Zhou, 2007). Therefore, measuring biomarkers at the sub-cellular level allow to detect contaminant-related effects occurring at low levels of

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organization to prevent further irreversible injury at higher levels of organization (Ramos-Gómez et al., 2011a). Several studies proved that biochemical alterations, namely associated with oxidative stress, are of prime relevance to assess the impacts of inorganic contamination in several polychaete species, both under field (Carregosa et al., 2014; Freitas et al., 2012 in *D. neapolitana*, Ramos-Gómez et al., 2011a, 2011b, in *Arenicola marina*; Durou et al., 2007; Gomes et al., 2013; Moreira et al., 2006a; Solé et al., 2009, in *H. diversicolor*) and laboratorial (Nusetti et al., 2005 in *E. complanata;* Catalano et al., 2012 in *H. diversicolor*; Geracitano et al., 2004; Ventura-Lima et al., 2011 in *Laeonereis acuta*) conditions.

Among the most commonly used bioindicator polychaete species, the tubiculous onuphid polychaete *Diopatra neapolitana* presents a wide geographical distribution, and has been reported in several intertidal and shallow subtidal habitats (Cunha et al., 2005; Dagli et al., 2005; Wethey et al., 2011). Additionally, *D. neapolitana* specimens play an important ecological role, since their tubes stabilize the sediment, increasing its structural complexity and therefore its biodiversity, by providing refuge from disturbance and predation (Bailey-Brock, 1984) while facilitating the settlement and the attachment of some algal species (Thomsen and McGlathery, 2005). This species is also economically important, since it is usually collected by bait diggers to be used as fresh fish bait, constituting an important source of household income (Cunha et al., 2005; Dagli et al., 2005; Berke et al., 2010). *D. neapolitana* has been shown to be a good bioindicator of inorganic contamination (Freitas et al., 2012), organic enrichment (Carregosa et al., 2014), pharmaceuticals (Freitas et al., 2015b; Pires et al., 2016a, 2016b) and water acidification, temperature shifts and salinity fluctuations (Freitas et al., 2015c, 2016a; Pires et al., 2015).

There are few studies comparing the impact of elements contamination in physiological and biochemical responses of polychaetes (Coppola et al., 2016; Reish et al., 1989), and none, to our knowledge, with environmental metal and As contamination. Thus, given its ecological and economical importance, the present study aimed to characterize metal and As bioaccumulation and cellular partitioning, as well as the physiological and biochemical responses of *D. neapolitana* collected in areas characterized by different metals and As contamination levels. For this, polychaetes regenerative ability and biochemical responses (indicators of cellular damage, the activity of antioxidant, biotransformation enzymes and Metallothioneins) were evaluated in organisms collected along the Ria de Aveiro.

2. Materials and methods

2.1. Description of the study area and field sampling

Diopatra neapolitana individuals were collected in seven intertidal areas situated along the Ria de Aveiro (Fig. 1), a shallow estuarine ecosystem located on the northwest Atlantic coast of Portugal (40°38′ N, 8°45′ W). This ecosystem has an area of approximately 47 km^2 (Fig. 1) and comprises a complex system of bays, channels and extensive intertidal sand and mud flats (Dias et al., 2000). The Ria de Aveiro lagoon has been subjected to anthropogenic pressure through waste loadings, some of which with high metal and As content (Coelho et al., 2006; Freitas et al., 2012; Nunes et al., 2008; Velez et al., 2015a, 2015b). For this reason, 7 different areas (named A to G) characterized by different metals and As contamination levels were selected for sampling, where area A was the least contaminated, and then considered as the reference area.

Organisms were collected during low tide in middle October 2014. At each area, twelve individuals were collected for regeneration experiments and eight organisms for biochemical analysis and elements quantification (chromium (Cr), nickel (Ni), copper (Cu), lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As)). Organisms that were regenerating in the field were discarded and not used in this study. Also, at each sampling area three sediment replicates were collected for sediment grain size analysis, total organic matter (TOM) content determination and for elements (Cr, Ni, Cu, Pb, Cd, Hg and As) quantification.

The environmental variables pH, salinity and temperature were measured with specific probes at each sampling area.

After sampling, specimens for biochemical analysis and elements quantification were transported in ice-cold plastic containers to the laboratory, where they were frozen. Organisms for the regeneration experiment were transported to the laboratory in plastic containers.

2.2. Laboratory analysis

2.2.1. Environmental parameters

Sediment grain-size was analyzed by wet and dry sieving following Quintino et al. (1989). The sediment was classified according to the median value (P50), following the Wentworth scale (Doeglas, 1968): very fine sand (median between 0.063 and 0.125 mm); fine sand (0.125–0.250 mm); medium sand (0.250–0.500 mm) or coarse sand (0.500–1 mm). Additionally, the silt and clay fraction was determined by wet sieving through a 0.063 mm mesh screen and classified with “clean“, “silty,” or “very silty” according to fraction ranges (0–5%, 5–25%, and 25–50%, respectively) of the total sediment, dry weight (Doeglas, 1968; Larsonneur, 1977). Sediments with >50% fines were classified as mud.

The total organic matter content was determined by weight loss on ignition at 450 °C, during 5 h (Byers et al., 1978) of 1 g sediment sample after an initial drying at 60 °C for 24 h.

2.2.2. Metals and As quantification

Cr, Ni, Cu, Pb, Cd, Hg, and As concentrations were quantified in organisms (soluble and insoluble fractions) and sediments, following the methodology described by Freitas et al. (2012). The soluble fraction can be defined as the element's concentration in its free form or bound to proteins present in the cytosol, while the insoluble fraction can be defined as the unavailable element concentration, precipitated in insoluble metal-rich granules, and cellular debris (Wallace et al., 2003; Wallace and Luoma, 2003).

Metal and As quantification was done by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry), in a certified laboratory at the University of Aveiro. Concerning quality controls, the calibration of the apparatus was made with successive dilutions of multi-element standard ICP 71A from IV (Inorganic Venture, Christiansburg, VA, USA). The fitness of the calibration curve was checked with CRM NIST 1643e. The whole procedure was verified with standard certificated reference materials (CRM): MESS-3 (for sediments) and TORT-2 (for polychaetes), both from NRCC (National Research Council of Canada). The values obtained for all the CRM analysis ranged from 90% to 110% of the concentration defined for these materials. All samples below this accuracy level were rejected and the analysis repeated. Polychaetes and sediment elements determinations were performed in three replicates. The concentration of elements in polychaetes was expressed in mg/kg dry weight (DW) and the percentage of soluble and insoluble fractions were calculated.

The concentration of elements in sediments was express in mg/kg DW, since the sediments were previously dried during 48 h at 25 °C.

To access the ability of the polychaetes to accumulate elements, the Biota-Sediment Accumulation Factor (BSAF) was determined according to Cheng et al. (2013), dividing the total concentration of an element in the soft tissue of an organism (dry weight) by the concentration of the that element in the sediment (dry weight).

2.2.3. Physiological parameters - regenerative capacity

In the laboratory, individuals were pushed out from their tubes and washed with seawater. Then, organisms were anaesthetized with a solution of 4% $MgCl_2 \cdot 6H_2O$, and the width of the 10^{th} chaetiger was measured for each individual under a stereomicroscope (Leica KL200 LED),

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