Ecological Indicators xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

The use of *Cerastoderma glaucum* as a sentinel and bioindicator species: Take-home message

Cátia Velez[a,b], Adília Pires[a,b], Leandro Sampaio, Paulo Cardoso[a,b], Anthony Moreira[a,b], Sérgio Leandro[c], Etelvina Figueira[a,b], Amadeu M.V.M. Soares[a,b], Rosa Freitas[a,b,*]

[a] Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[b] CESAM – Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[c] MARE – Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria, 2520-641 Peniche, Portugal

ARTICLE INFO

Article history:
Received 3 June 2015
Received in revised form 18 October 2015
Accepted 18 October 2015
Available online xxx

Keywords:
Bivalves
Cockles
Cellular biomarkers
Element partitioning
Maximum permissible limits
Óbidos lagoon

ABSTRACT

Bivalves are frequently used to assess environmental contamination, and are often considered good sentinel and/or bioindicator species. For that reason the bioaccumulation and toxicity induced by metals and As in the cockle *Cerastoderma glaucum*, collected from areas with different contamination levels along the Óbidos lagoon (Portugal), were used to evaluate the use of this species as sentinel and/or bioindicator. The results showed that areas in the middle of the lagoon presented lower metals and As concentrations, lower total organic matter content and lower percentage of fine particles than areas in the Bom Sucesso arm. In all areas Cr, Pb and Cu were the most abundant elements, while Ni, As, Cd and Hg were less abundant. Results also showed a moderate correlation between total elements concentrations found in *C. glaucum* and in sediment, and thus caution should be taken when considering this species as a good sentinel species. The present study also revealed that, in general, *C. glaucum* from areas in the middle of the lagoon accumulated higher concentrations of metals and As (Biota-Sediment Accumulation Factor >1) than cockles from the most polluted areas located in the Bom Sucesso arm. However, in all areas, the majority of metals (Cu, Cr, Pb) were found in cockles insoluble fraction which may explain low cellular damage and reduced oxidative stress responses observed. Therefore, our results may further alert for caution when identifying *C. glaucum* as a good bioindicator species. Thus, our findings highlight the fact that studies should be cautious when selecting species for environmental monitoring, since good sentinels or bioindicators in highly polluted systems may not act in the same way in low or moderately contaminated areas. Furthermore, our study warns for the misclassification of cockles in different ecosystems.

1. Introduction

Coastal systems have been constantly threatened by pollution, due to the increase of urbanization, agriculture and industrial activities (Green-Ruiz and Páez-Osuna, 2001; Poulos et al., 2000). Considering this, it is well established that sediment act as a sink for a variety of contaminants, such as metals and metalloids (Buruaem et al., 2012; Hoffman et al., 2002), affecting benthic organisms (Dauvin, 2008). Environmental impact assessment studies have relied on monitoring benthic community parameters (e.g. species richness and abundance), measuring the concentrations of selected contaminants in sediments, water and organisms, and assessing induced toxicity in organisms (Box et al., 2007; Calabretta and Oviatt, 2008; Cheggour et al., 2005; Machreki-Ajmi and Hamza-Chaffai, 2006). Benthic communities typically consist of a variety of species that exhibit a wide range of physiological stress tolerances, feeding modes, and trophic interactions, making them good biological indicators to evaluate the impact of environmental contamination (Calabretta and Oviatt, 2008; Cheggour et al., 2005; Moschino et al., 2012). Despite their importance, studies conducted at the community level are time consuming and require a lot of expertise for species identification. For this reason the use of different species as sentinel organisms has become a common practice (e.g. Ferreira et al., 2009; Lima et al., 2008; Moschino et al., 2012). In this particular case the concentration of contaminants is measured in the organisms, and then used to assess environmental contamination. However, measuring the concentration of contaminants in organisms does not give information about impacts on

* Corresponding author at: Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234370782; fax: +351 234372587.
E-mail address: rosafreitas@ua.pt (R. Freitas).

http://dx.doi.org/10.1016/j.ecolind.2015.10.051
1470-160X/

their health status. Therefore, recent studies regarding environmental pollution assessment have relied on the use of bioindicator species, defined as species or group of species that readily reflect the abiotic or biotic state of an environment, revealing the impact of environmental changes on a population, community or ecosystem (Hamza-Chaffai, 2014; Holt and Miller, 2011). In the last years, different biochemical markers have been used in marine species to predict potential detrimental effects induced by different contaminants on organisms (Bergayou et al., 2009; Hamza-Chaffai, 2014; Valavanidis et al., 2006). This approach is suitable for early and sensitive detection of exposure to chemicals, since the measurement of toxicity at the cellular level constitutes the initial interaction between chemicals and biological systems (Monserrat et al., 2007). For this reason, the assessment of biochemical alterations imposed by a contaminant into the aquatic environment has become a common practice. Measuring the same biomarkers in the same species but in different sites gives us information about the pollution status and provides for a better comprehension of organisms responses (Chandurvelan et al., 2015). Recent studies have demonstrated that benthic organisms, namely bivalves, are successfully used as sentinel and/or bioindicator species to monitor coastal areas (Chandurvelan et al., 2015; Hamza-Chaffai, 2014; Karray et al., 2015; Torres et al., 2002). These organisms are chosen due to their ability to accumulate contaminants usually from water and food, reflecting the bio-available fraction (Chandurvelan et al., 2015). In addition, their relative immobility, wide distribution among different aquatic habitats, abundance, persistence, and ease of collection, make them good long-term indicators of environmental contamination (Hamza-Chaffai, 2014). In fact, several studies regarding environmental pollution and biomarkers have focused on bivalves such as the clams *Ruditapes philippinarum* (Adams & Reeve, 1850) (Chora et al., 2009; Moschino et al., 2012), *Chamelea gallina* (Linnaeus, 1758) (Monari et al., 2011), *Ruditapes decussatus* (Linnaeus, 1758) (Bebianno et al., 2004; Chora et al., 2009; Hamza-Chaffai et al., 2003; Smaoui-Damak et al., 2004; Velez et al., 2015a,b), the mussels *Mytella guyanensis* (Lamarck, 1819) (Torres et al., 2002), *Mytilus galloprovincialis* (Lamark, 1819), *Mytilus edulis* (Linnaeus, 1758) (Smaoui-Damak et al., 2004) and *Perna viridis* (Linnaeus, 1758) (Yusof et al., 2004), and the cockle *Cerastoderma edule* (Linnaeus, 1758) (Cheggour et al., 2001; Freitas et al., 2012; Nilin et al., 2012). Few studies are however known using the cockle *Cerastoderma glaucum* (Bruguière, 1789) (e.g. González-Fernández et al., 2015; Ladhar-Chaabouni et al., 2009; Machreki-Ajmi et al., 2008, 2011; Machreki-Ajmi and Hamza-Chaffai, 2006, 2008; Hamza-Chaffai, 2014). This species is a sedentary suspension and deposit feeder (Caspers, 1981) with a wide spatial distribution (WoRMS, 2015), which allows the use of this species as a good candidate as a sentinel (Karray et al., 2015; Szefer et al., 1999) and bioindicator (Machreki-Ajmi and Hamza-Chaffai, 2006; Machreki-Ajmi et al., 2008) species, reflecting environmental pollution levels and impacts. Biochemical alterations induced in this species by contaminants have been focused on a limited number of biomarkers (acetylcholinesterase activity, lipid peroxidation and metallothioneins levels), on few aquatic systems (Tunisian coast and Arcachon Bay, France) and particularly, in low contaminated areas (González-Fernández et al., 2015; Hamza-Chaffai, 2014; Ladhar-Chaabouni et al., 2009; Machreki-Ajmi et al., 2008; Machreki-Ajmi and Hamza-Chaffai, 2006, 2008; Paul-Pont et al., 2010). Nevertheless, due to *C. glaucum* ecological and economic importance (Abdallah et al., 2011; Kandeel et al., 2013), and wide spatial distribution (WoRMS, 2015), it is of prime relevance to evaluate this species performance when under different contamination scenarios.

Thus, the present study aimed to characterize metals and As bioaccumulation and cellular partitioning, and the overall biochemical responses of *C. glaucum* under different environmental conditions. For that purpose, a multi biomarker approach was used, including the measurement of lipid peroxidation (LPO), total protein content, reduced (GSH) and oxidized (GSSG) glutathione content, glutathione *S*-transferases (GSTs), superoxide dismutase (SOD) and catalase (CAT) activities and metallothioneins (MTs). This approach used different biomarkers to reflect the effects of different contaminants in *C. glaucum* collected from different areas along the Óbidos lagoon (Portugal). To ensure the correct identification of the species, often misidentified as *C. edule*, morphological and genetic analyses were performed.

2. Material and methods

2.1. Study area

The Óbidos lagoon is a small and shallow coastal system permanently connected to the sea, located in the Atlantic West Coast of Portugal (Oliveira et al., 2006). This lagoon covers an area of approximately 7 km^2 and with an average depth of approximately 1 m. The Óbidos lagoon is divided in two arms: Barrosa and Bom Sucesso arms. The Barrosa arm receives agriculture and urban effluents from Caldas da Rainha city, resulting in an area with high nutrients availability, previously classified as eutrophic (Carvalho et al., 2011; Pereira et al., 2009a). This arm is mostly contaminated by metals and metalloids, and the major source of these contaminants is related to wastewater discharges in Cal River (Oliveira et al., 2006; Pereira et al., 2008). The Bom Sucesso arm receives a smaller freshwater flow (Vala do Ameal) with better water quality than the Cal River (Carvalho et al., 2011). According to previous studies this lagoon has been classified as a moderately contaminated system (Pereira et al., 2009b,c; Carvalho et al., 2011).

2.2. Sampling procedure

Cerastoderma sp. specimens were collected in 6 different areas (named A to F) of the Óbidos lagoon (Fig. 1), representing different contamination levels and physico-chemical characteristics. In each area three sites were selected and in each site all cockles present in a 11.25 cm^2 rectangle (45 cm × 25 cm) were collected. At each area, three sediment replicates were collected (one per site) for sediment grain size analysis, total organic matter (TOM) content determination, and quantification of elements concentration (chromium (Cr), nickel (Ni), copper (Cu), lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As)).

The environmental variables pH, salinity and temperature were measured with specific probes at each sampling site. After sampling, specimens and sediment were transported on ice (0 °C) to the laboratory.

2.3. Laboratory analysis

2.3.1. Sediment grain size and organic matter content determination

Sediment grain-size was analyzed by wet and dry sieving following the procedure described by Quintino et al. (1989). The median value, P_{50}, was calculated and expressed in phi (ϕ) units, corresponding to the diameter that has half the grains finer and half coarser (dry weight). The median and the percent content of fines were used to classify the sediment, according to the Wentworth scale: very fine sand (median between 3–4ϕ); fine sand (2–3ϕ); medium sand (1–2ϕ); coarse sand (0–1ϕ); very coarse sand (−1 to 0ϕ). The silt and clay fraction was obtained by wet sieving through a 0.063 mm mesh screen and classified as "clean", "silty" or "very silty" according to fine fraction range (0–5%, 5–25% and 25–50%, respectively) of the total sediment, dry weight (Doeglas, 1968). Samples with more than 50% fines content were classified as mud.