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Macroinvertebrates and fishes as biomonitors of heavy metal concentration in the Seixal Bay (Tagus estuary): Which species perform better?

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A B S T R A C T

The present study aims to evaluate the utilization of several benthic macroinvertebrate and fish species as bioindicators of heavy metal contamination in a relatively enclosed estuarine area, the Seixal Bay (Tagus estuary, Portugal). 266 specimens of 16 taxa were captured during four sampling campaigns and the concentration of lead, chromium, nickel, copper, zinc, cobalt and cadmium were quantified in fish muscle and whole soft body of the invertebrates, as well as in suspended particulate matter in the water column and sediments. Larger predator fishes with higher mobility presented low levels of contamination, probably due to their feedings sites being located outside the bay in less contaminated areas. The species of the Gobiidae family, *Pomatoschistus microps*, *Pomatoschistus minutus* and *Gobius niger* presented higher values of non-metabolic elements (Pb, Ni and Cd) while *Scrobicularia plana*, *Nereis diversicolor*, *Carcinus maenas* and *Palaemon serratus* were more associated to the accumulation of Cu, Cr and Zn. *Nephtys hombergii* and *Crangon crangon*, showed intermediate concentrations of all metals. These results points out to a future possible utilization of *S. plana*, *N. diversicolor*, *P. serratus* and *C. maenas* and of *Pomatoschistus* species as bioindicators of heavy metal contamination in ecological quality monitoring programs.

1. Introduction

Aquatic environments are highly impacted by human activities, either by its use for resources exploitation, transportation, recreational proposes or as dumping sites. In the particular case of an estuary, the system naturally drains the surrounding areas, during tidal flooding, for example, introducing in the aquatic environment large amounts of harmful substances (Shinn et al., 2009). Many countries began to develop monitoring systems for ecological impact assessment, control and surveillance of these endangered water bodies. For the implementation of the Water Framework Directive (Directive 2000/60/EC of the European Commission) all the European Union member states should develop a program for monitoring transitional water bodies in order to maintain the good water quality standards, including low levels of toxic substances.

The presence of toxic elements, like heavy metals, can or not be a threat to the exposed organisms, as they may not be up taken by the organisms, or if up taken the organisms may be able to tolerate and/or excrete them (Fernandes et al., 2008). On the other hand when affected, at sub-letal levels, the inhabitant aquatic organisms are useful indicators of the presence of heavy metals (Arain et al., 2008; Válega et al., 2008). Therefore, the analysis of invertebrates and fish tissues can be very helpful to understand not only the sources of metal contamination but also the environmental risks to the trophic chain and in some extreme cases to the humans as top consumers. However, further investigation to know which species could function as indicators is still needed.

With the assessment of the heavy metals concentration in invertebrates and fishes tissues from the Seixal Bay and in the respective abiotic compartments, this study aims to understand which species perform better as indicators of heavy metal contamination. For this, several organisms inhabitant on this relatively enclosed bay, belonging to different trophic levels and with different levels of spatial fidelity were chosen for the evaluation of their metal contamination and their performance as bioindicators.

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2. Materials and methods

2.1. Study area

The Tagus estuary is one of the largest estuaries of the Europe and it is located in the most populated area of Portugal. After 1950, more than 15 large dams were built in the Tagus water basin, affecting the river discharge regime (Costa et al., 2001). Apart from harbouring, a major Portuguese seaport, the Tagus estuary receives effluents from agricultural, industrial, and urban sources. It is adversely affected mainly by the inflow of effluents from about 2.5 million Greater Lisbon inhabitants (Chainho et al., 2010), together with contamination from several chemical, petrochemical, metallurgic, shipbuilding and cement manufacture industries, and agriculture fertilizers and pesticides, although in some of these cases there have been a progressive reduction in pollutant loads (Duarte et al., 2008) This system has been contaminated mainly by two industrial areas located in both margins, the Vila Franca de Xira-Alverca and the Barreiro-Seixal industrial regions, being the study area located in this last region (Bettencourt et al., 1999; Vale, 1990; Cottè-Krief et al., 2000; Canário et al., 2005).

With a total surface of 4.2 km^2, the Seixal Bay is a relatively small water body located in the left margin of the lower Tagus estuary (Fig. 1). Water connection to the remaining estuarine system is made through a 100 m wide single opening. Depth may exceed 6 m in the main channel but about 95% of the bay is composed by intertidal areas, including an important saltmarsh in the NW region (Corroios saltmarsh). Average salinity ranges between 20 and 25 during most of the year (Costa, 1999) but can reach less than 10 and more than 30 in winter and summer, respectively (unpublished data). In the Tagus estuary water temperature usually varies between 8 °C and 22 °C (Costa, 1999), but thermal amplitude is expected to be higher in the Seixal bay due to its shallow characteristics. The human pressure around the bay is very high, with several urban areas close to the water body. Until 2009 only 49% of the domestic effluents in the Seixal Municipality were treated. Among the net of urban areas a few number of familiar farms and small factories (mainly for tannery, fertilizers and pesticides production) are still operating, as well as two aquaculture units. In the past the bay harboured an important set of economic activities connected with the estuary, like grinding in tide mills, industrial fish processing and naval construction and repairing, but nowadays only some shipyards are still in business (unpublished data, source Seixal Municipality). Considering the described human pressures, a certain degree of contamination should be expected in the water, sediments and organisms of the Seixal Bay, including contamination by heavy metals.

2.2. Sampling procedure

The present work focused on the most abundant species of benthic invertebrates and fishes present in the Seixal Bay, since these are the animal groups that should be monitored in the scope of the WFD implementation in transitional waters. However, some of the most abundant benthic invertebrates in the area had to be discarded from the study because of their small size, which impaired the collection of enough organisms to obtain the necessary quantity of tissues for metal concentration determination. Therefore, a total of 16 species were analysed: the bivalve *Scobicularia plana* (da Costa, 1778), the polychaets *Nereis diversicolor* (O.F. Müller, 1776) and *Nephtys hombergii* Savigny in Lamarck, 1818, the shrimps *Crangon crangon* (Linnaeus, 1758) and *Palaemon serratus* (Pennant, 1777), the crab *Carcinus maenas* (Linnaeus, 1758), and several fish species, namely the seabass *Dicentrarchus labrax* (Linnaeus, 1758), the Senegal seabream *Diplodus bellottii* (Steindachner, 1882), the white seabream *Diplodus sargus* (Linnaeus, 1758), the common two-banded seabream *Diplodus vulgaris* (E. Geoffroy Saint-Hilaire, 1817), the black goby *Gobius niger* (Linnaeus, 1758), the common goby *Pomatoschistus microps* (Krøyer, 1838), the sand goby *Pomatoschistus minutus* (Pallas, 1770), the golden grey mullet *Liza aurata* (Risso, 1810), the common sole *Solea solea* (Linnaeus, 1758) and the Lusitanian toadfish *Halobatrachus didactylus* (Bloch & Schneider, 1801).

Between April 2009 and March 2010, 4 sampling campaigns were seasonally conducted all over the Seixal Bay to get an overall picture of the contamination in the area, independently of the season or specific location (Fig. 1). Captures of fishes and decapod crustaceans were conducted with a beam-trawl (width ≈ 1.5 m; mesh size of 5 mm) while other invertebrates were collected using a modified van Veen LMG grab (0.05 m^2) and sieved through a 0.5 mm mesh. The dredges were always made in triplicate at each site and fish and decapod crustaceans sampling areas were swept once in each season. Previously to sieving the dredged sediments a sub-sample from each dredge was collected for sediment metal content. During the same sampling periods an in the same stations, surface water samples were collected and stored in refrigerated bottles for further suspended particulate matter (SPM) filtration and metal analysis.

2.3. Sample preparation and heavy metal analysis

For the evaluation of the heavy metal content in the several analysed matrices, a group of seven elements (Zn, Pb, Ni, Co, Cr, Cu and Cd) was chosen to be analysed since they are the most abundant and have the higher ecological significance for the Tagus estuary area (Vale et al., 2008). Harvested tissues for metal analysis consisted in muscle of fishes and whole body (except shells) for the macroinvertebrates. Samples were stored at −80 °C and previously freeze-dried during 48–72 h. After this process, samples were reduced to powder using liquid nitrogen and a mortar and pestle. The acid digestion procedure for liquefaction of the samples consisted in digesting 100 mg of processed sample with 2 mL of HNO_3:$HClO_3$ (7:1) mixture during 3 h in a Teflon reactor at 110 °C (França et al., 2005). After digestion and cooling down, the resulting solution was filtered using Whatman n° 42 filters.

Since the group of animal species, above described, comprised both benthic macroinvertebrates, and decapod crustaceans and fishes associated with different levels of the water column, also the sediments and SPM heavy metal concentrations were analysed. This allowed a comparison between the bottom sediments and the suspended matter heavy metal contents and their influence on the species metal content. For the determination of SPM in the water column, 500 mL of water sample was filtered through pre-weighted acid-washed filters. After filtration, samples were dried at 60 °C until constant weight was achieved. The determination of particulate metals in the water column was carried out by acid digestion as above described for the animal tissues. For heavy metal content determination in sediments, samples were freeze-dried for 48 h and subsequently digested with the same protocol as above described. This allowed a more accurate comparison between the heavy metal concentrations in the several sample types.

Heavy metal concentrations were determined by Flame Atomic Absorption Spectrometry (FAAS, SpectraAA 50, VARIAN). Blank filters were also made using the same digestion and analysis procedure and their metal content used to correct the SPM filters metal concentrations. All concentration values were above the detection limit of the FAAS. In order to validate the accuracy of the digestion procedure, reference material CRM 145 and CRM 146 (sediment and SPM) and TORT-2 (animals) was simultaneous analysed. For all metals investigated, obtained values were consistently within the ranges of certified values ($p < 0.05$).