Marine Pollution Bulletin 62 (2011) 1538–1549

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Validation of *Arenicola marina* in field toxicity bioassays using benthic cages: Biomarkers as tools for assessing sediment quality

Julia Ramos-Gómez [a,*], Marta Martins [b], Joana Raimundo [b], Carlos Vale [b], M. Laura Martín-Díaz [a,c], T. Ángel DelValls [a]

[a] UNITWIN/UNESCO/WiCoP, Departamento de Química Física, Universidad de Cádiz, Facultad de Ciencias del Mar y Ambientales, Campus Universitario de Puerto Real, 11510 Puerto Real, Cádiz, Spain
[b] INRB/IPIMAR, Instituto Nacional dos Recursos Biológicos, Av. Brasília, 1449-006 Lisboa, Portugal
[c] Centro Andaluz de Ciencia y Tecnología Marinas (CACYTMAR), Universidad de Cádiz, Campus Universitario de Puerto Real, 11510 Puerto Real, Cádiz, Spain

ARTICLE INFO

Keywords:
Dredge material
Sediment quality
Caged organisms
Biochemical biomarkers
Polychaetes
Multivariate analysis

ABSTRACT

Sediment toxicity assessments using caged organisms present advantages over using laboratory and native community studies. The use of caged *Arenicola marina* in sediment toxicity assessments was evaluated. Lugworms were exposed *in situ* to sediments from coastal and port areas in Spain for seven days, and the activities of the biotransformation enzymes ethoxyresorufin O-deethylase, dibenzylfluorescein dealkylase and glutathione S-transferase, the activities of the antioxidant enzymes glutathione reductase and glutathione peroxidase and lipid peroxidation were then analyzed as biomarkers. Biomarker results and sediment physicochemical data were integrated. Cádiz Bay (SW Spain) sediments presented metal contamination that was not linked to a biochemical response. In LPGC Port (SW Spain), Pb contamination exhibited a moderate toxic potential, while PAHs, and presumably pharmaceuticals, provoked biochemical responses that efficiently prevented lipid peroxidation. In Santander Bay (N Spain), exposure to PAHs and, presumably, pharmaceuticals induced biomarker responses, but lipid peroxidation occurred nevertheless. These results indicated that caged *A. marina* were effective for the assessment of sediment quality and that the selected biomarkers were sufficiently sensitive to identify chemical exposure and toxicity.

1. Introduction

Coastal and estuarine areas are impacted by complex mixtures of man-made contaminants that affect marine wildlife and degrade marine ecosystems. The use of bioassays is fundamental for assessing contaminant-related toxicity. Field toxicity tests using caged organisms present numerous advantages over chemical criteria alone, laboratory toxicity testing and indigenous community surveys (Burton et al., 2005). They allow more realistic exposure conditions, in contrast to laboratory tests. Compared with studies of native populations, the use of caged animals allows the exposure history of the organisms to be known (Oikari, 2006) because all tested individuals have the same origin and because the exposure duration is known. Native organisms may have developed adaptations to environmental stress, so measures based on toxic responses may underestimate pollution. Furthermore, areas subjected to periodic dredging cannot support permanent native communities (Martín-Díaz et al., 2004).

Biochemical measures used as biomarkers can offer more sensitive information about toxic chemical impacts on organism health and allow early signs of biological responses to xenobiotics to be detected (Sun and Zhou, 2008). The ecological relevance of biochemical responses lies in their relationship with harmful effects observed at higher levels of organization such as histological- (Morales-Caselles et al., 2007), physiological- (Moreira et al., 2006), behavioral- (Vieira et al., 2009), population- (Tlili et al., 2010) and, potentially, ecosystem-level (Moreira et al., 2006; Vieira et al., 2009) effects. Hence, measuring biomarkers allows contaminant-related effects occurring at low levels of organization to prevent further irreversible damage at higher levels of organization.

Arenicola marina is an ecologically-relevant species (Alyakrinskaya, 2003; Flatch and Beukema, 1994; Volkenborn, 2005). Alterations of individual health and subsequent population perturbations of *A. marina* may be reflected in the ecosystem. The species has been found to be sensitive to sediments at a biochemical level (Morales-Caselles et al., 2009) and to accumulate contaminants (Casado-Martínez et al., 2009; Morales-Caselles et al., 2008). Contaminants may be transferred through the food web and thus represent a risk to the ecosystem and to human health.

* Corresponding author. Tel.: +34 956 016 423; fax: +34 956 016 040.
E-mail address: julia.ramosgomez@uca.es (J. Ramos-Gómez).

0025-326X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2011.03.045

The objectives of the present study were as follows:

(1) To determine the suitability of a battery of biomarkers analyzed in the polychaete *A. marina* after exposure to contaminated sediments under field conditions as tool for the assessment of sediment and dredged material quality. The battery of biomarkers consisted of exposure biomarkers (activities of phase I biotransformation enzymes ethoxyresorufin O-deethylase (EROD) and dibenzylfluorescein dealkylase (DBF); phase II biotransformation enzyme glutathione S-transferase (GST); antioxidant enzymes glutathione peroxidase (GPX) and glutathione reductase (GR)) and lipid peroxidation (LPO) as biomarker of effect (van der Oost et al., 2003).

(2) To evaluate the quality of sediment and dredged material from different Spanish coastal and port areas using an integrated approach based on a multivariate analysis. These areas were Cádiz Bay (SW Spain), Las Palmas de Gran Canaria (LPGC) Port (SW Spain) and Santander Bay (N Spain). Two lines of evidence were integrated: (a) sediment physicochemical characteristics and (b) the responses of exposure and effect biomarkers. Multivariate analysis is a statistical technique that allows significant relationships between chemicals bound to the sediments and biological responses to be elucidated. Distinguishing xenobiotics that are significantly associated with biological responses contributes to the evaluation of contaminant bioavailability and toxicity and facilitates the prevention of ecosystem degradation by identifying potentially harmful substances. Analyses of the contaminant body burdens of lugworms were also conducted as a support tool to better interpret the obtained results.

(3) To design a cage prototype for the polychaete species used in field studies of whole sediments and dredge material toxicity and to optimize the technique so as to broaden the scope of application of these species. This would strengthen caging methodologies as caging techniques have not been rigorously standardized at a national or international level thus far and are not accepted as a routine practice (Oikari, 2006).

2. Materials and methods

2.1. Brief description of the study sites

The locations of the study sites are shown in Fig. 1. The study sites were situated along different regions of the Spanish Coast, namely, Cádiz Bay, LPGC Port and Santander Bay. These areas are characterized by chronic contamination from major urban areas and industrial, mining and port activities. Different stations were selected in each zone to assess sediment quality. A control site (CA1) was established at Cádiz Bay together with CA2 and CA3. Two sites were selected in LPGC Port, namely, C2 and C3. Finally, stations S4 and S5 were set in Santander Bay.

2.2. Sediment sampling and physicochemical characterization

2.2.1. Sediment collection and preparation

Surface (5–20 cm) sediment samples were collected at the study sites for sediment characterization. A 0.025 m^2 Van Veen grab was used. The samples were simultaneously homogenized and sieved through a 1 mm mesh, and subsequently subsampled to assess the following different parameters: percentage of fines and organic carbon; concentrations of Cd, Pb, Cu, Ni, Co and Hg; and concentrations of 15 polycyclic aromatic hydrocarbons (PAHs, consisting of low molecular weight PAHs with three aromatic rings, including acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene; moderate molecular weight PAHs with four aromatic rings, including fluoranthene, pyrene, benzo(a)anthracene and chrysene; and high molecular weight PAHs with five and six aromatic rings, including benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)-pyrene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene and indene).

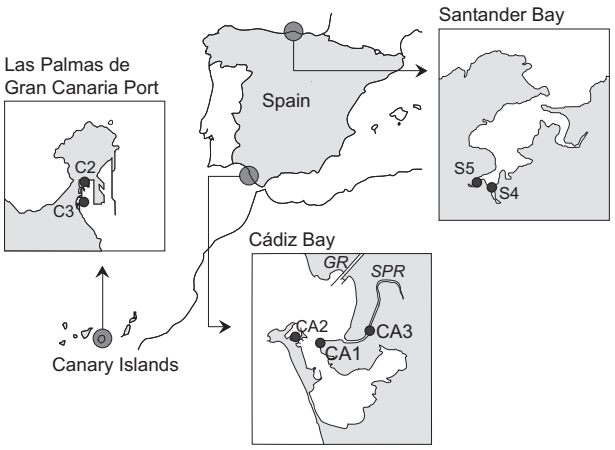

Fig. 1. Location of the study sites at Cádiz Bay (CA1, CA2 and CA3; CA1 is a control station), Las Palmas de Gran Canaria Port (C2 and C3) and Santander Bay (S4 and S5). *GR* and *SPR* designate Guadalete River and San Pedro River, respectively, which are both located in Cádiz Bay.

2.2.2. Percentage of organic carbon

The percentage of organic carbon was analyzed following the adaptation of the Walkley–Black titration method proposed by Gaudette et al. (1974) together with the El Rayis modification (1985). After 1 N $K_2CR_2O_7$ and concentrated H_2SO_4 were added to the dried and ground sediment, the mixture was gently shaken and stored at 100 °C for 30 min. The solution was then diluted with MilliQ water and 85% H_3PO_4. Finally, NaF and the indicator diphenylamine were added, and the final solution was titrated with 0.5 N $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$. A blank with no sediment was run with each batch of samples as a control.

2.2.3. Fine fraction percentage

The percentage of fine sediments was evaluated following the NLT 104/91 norm (CEDEX, 1991). Each sediment sample was sieved using a 2 mm mesh (ASTM 10). The sieved sediment was introduced into 125 ml of 4% sodium hexametaphosphate for 18 h. Then MilliQ water was added, and the mixture was stirred for one minute, washed using a 0.075 mm sifter (ASTM 200) and dried at 110 °C. The dried sample was sieved using <2 mm ASTM sifters.

2.2.4. Metal concentrations in sediments

The metals selected for analysis were Cd, Cu, Pb, Zn, Co, Ni and Hg. The sediment metal concentrations were measured through differential pulse anodic (Cd, Cu, Pb and Zn) and cathodic (Ni and Co) stripping voltammetry as described in the Metrohm Application Bulletin No. 147 (1991). Before that, the sediment samples were digested with 2 N HNO_3 in a microwave (400 W, 15 min, twice) and the organic matter was removed using a C-18 column. Hg was determined utilizing the analyzer AMA254, for which no sample pre-treatment was needed. Metal concentrations were expressed as mg kg^{-1}dried sediment (d.w.).

2.2.5. PAH concentration in sediments

PAHs were measured using the method developed by González-Piñuela et al. (2006). Sediment samples were subjected