Marine Environmental Research 68 (2009) 82–88

Contents lists available at ScienceDirect

Marine Environmental Research

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

The use of a kinetic biomarker approach for *in situ* monitoring of littoral sediments using the crab *Carcinus maenas*

M. Laura Martín-Díaz [a,b,*], Julián Blasco [a], Diego Sales [c], T. Ángel DelValls [b]

[a] Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Ciencias Marinas de Andalucía (ICMAN), Polígono Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain
[b] Universidad de Cádiz, Facultad de Ciencias del Mar y Ambientales, Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Polígono Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain
[c] Departamento de Ingeniería Química, Tecnología de Alimentos y Tecnologías del Medio Ambiente, Facultad de Ciencias del Mar y Ambientales, Polígono Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain

ARTICLE INFO

Article history:
Received 18 November 2007
Received in revised form 5 February 2009
Accepted 10 April 2009

Keywords:
Metallothionein
Enzymatic activity
Oxidative stress
Carcinus maenas
Ecotoxicology
Dredged material
Invertebrates

ABSTRACT

Caged, transplanted, intermoult, female shore crabs (*Carcinus maenas*) were exposed to sediments from the Port of Cadiz (SW, Spain); the Port of Huelva (SW, Spain), the Port of Pasajes (NE, Spain) and the Port of Bilbao (NW, Spain) for a period of 28 days.

Organisms were sampled on days 0, 7, 14, 21 and 28 allowing examination of different biomarkers to exposure to metals and organic compounds; metallothioneins (MTs), ethoxyresorufin O-deethylase (EROD), glutathione-S-transferase (GST) and glutathione peroxidase (GPX). Sediment samples were also analyzed to determine chemical concentration of metals (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn), PAHs and PCBs.

Metals such as As, Cu and Zn associated with mining activities (Port of Huelva), and contaminants such as Ni, Pb, Hg and PCBs, associated with oil spills and industrial activities (Ports of Cadiz, Pasajes and Bilbao), were found to interfere in detoxification and/or anti-stress oxidative defenses in *C. maenas.* Positive, significant ($p < 0.05$) induction of MTs concentration was associated with increasing sediment As, Cu, Zn and Hg concentrations. EROD activity was associated with PCBs, GST activity with Hg and GPX activity with As, Cu, Ni, Pb and Zn. The use of the parameter AR, which involves the kinetic patterns of the battery of biomarkers tested, was found to be a powerful and sensitive tool for evaluating the bioavailability and adverse effects of contaminants bound to sediments, enabling polluted and nonpolluted sites to be easily distinguished.

1. Introduction

Contaminated sediments are a risk to aquatic life, human health, and wildlife throughout the world. Overwhelming evidence demonstrates that chemicals in sediments are responsible for toxicological and adverse ecological effects (Andersen et al., 1998). Frequently, the chemicals causing these effects are present in the sediment as mixtures of organic, metal, and other types of compounds. The adverse environmental effects associated with contaminated sediments in ports and estuaries have resulted in a number of international treaties and protocols for their environmental management. The OSPAR and Helsinki Conventions (North Sea, North-East Atlantic, Baltic Sea) propose strict guidelines to control the disposal of contaminated sediments. In recent years, remediation and management of contaminated sediments is becoming increasingly important, which has led to an increasing economical and technological burden on management agencies. Thus, there is a clear need for the development of science based tools for the identification of sediments that are impaired, allowing agencies to set priorities and make effective management decisions for dealing with contaminated sediments.

The most widely applied methods for the assessment of sediment toxicity are acute and chronic sediment bioassays. Although a wide range of acute toxicity tests are already recommended, standardized and included in sediment assessment regulatory frameworks, the development and application of chronic toxicity tests including the measurement of sub lethal responses (biomarkers), although widely supported, is still under investigation. The use of biomarkers in the evaluation of the toxicity of dredged material is under continuous development and improvements are still desirable.

An important advantage of biomarkers in assessing the impact of dredged materials is their inherent capability to detect the early

* Corresponding author. Address: Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Ciencias Marinas de Andalucía (ICMAN), Polígono Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain. Tel.: +34 956016423; fax: +34 956016040.
E-mail address: laura.martin@uca.es (M.L. Martín-Díaz).

0141-1136/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2009.04.005

occurrence of various stress conditions within the organism and monitor the temporal progression (or regression) of the disturbance at various levels of biological organization (Regoli et al., 2002). When assessing the exposure to a mixture of contaminants in dredged materials within the aquatic environment, biotransformation and antioxidant enzymes as well as metal-binding proteins such as metallothioneins (MTs) play an important role in identifying contamination by organic and metal pollutants, respectively. However, ecotoxicological measurements obtained in laboratory studies can be difficult to translate into accurate predictions of toxicological effects in the natural environment. Since both overestimation and underestimation of effects may occur, laboratory observations must always be validated with field research. The transplanting and caging of bioindicator organisms provides a combination of the experimental control of laboratory bioassays and the environmental realism of field monitoring (Salazar and Salazar, 1997). Thus, the combination of this methodology with biomarker measurements of suitable indicator species may provide a strong tool for the monitoring of contaminated sediments, allowing the determination of the total external load that is biologically available in the "real world" situation (van der Oost et al., 2003).

Still, studies that use biotransformation and antioxidant enzymes as tools to monitor polluted sites should be performed carefully. Various authors have pointed out the serious risk of misinterpretation associated with using a single "snap-shot" (i.e., a single measurement at the end of exposure) to rationalize oxidizing versus other effects of toxic contaminants in the environment (Bouskill et al., 2006). Thus, a study of a continuous series of biomarker responses over time rather than a single measurement after concrete exposure time should be performed in order to overcome the problem mentioned above.

The aims of this study were to determine the measured biomarker responses within *Carcinus maenas* over a 28-day exposure period to contaminated sediments. This protocol was chosen to: (1) fill the lack that exists from measuring a single time point, (2) reveal temporal variation in oxidative injury related to increasing exposure time, and (3) allow the distinction and classification of sites with different magnitudes (and types) of sediment toxicity. To do this caged, female crabs were exposed *in situ* to sediments with different levels of metals and organic compounds from a number ports within Spain. The responses of a suite of biomarkers, including metallothioneins (MTs), mixed function oxidase (EROD), Glutathione-S-tranferase (GST), and glutathione peroxidase (GPX): antioxidant enzyme were determined during a 28-day exposure period and through a kinetic approach.

2. Materials and methods

2.1. Study site

Eight sampling sites were chosen (Fig. 1) for the assessment of sediment toxicity in Spanish ports. Ca2 and Ca3 (negative toxicity control) were situated in the Port of Cádiz (SW, Spain), which has

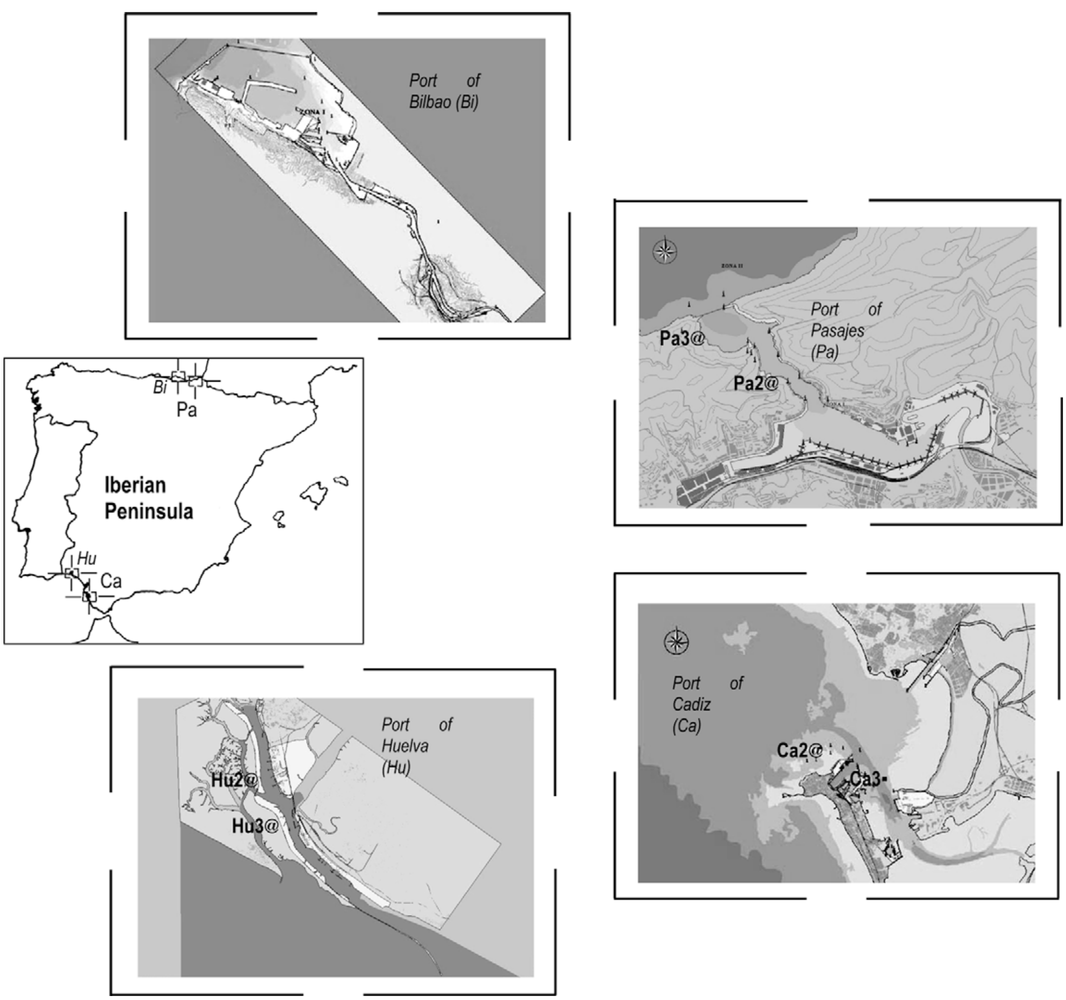

Fig. 1. Localization of sampling points in the Iberian Peninsula.