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THE ROLE OF BIOMARKERS TO ASSESS OIL-CONTAMINATED SEDIMENT QUALITY USING TOXICITY TESTS WITH CLAMS AND CRABS

CARMEN MORALES-CASELLES,*†‡ MARÍA LAURA MARTÍN-DÍAZ,†‡ INMACULADA RIBA,†‡
CARMEN SARASQUETE,† and TOMÁS ÁNGEL DELVALLS†‡
†Unidad Asociada de Calidad Ambiental y Patología (Centro Superior Investivaciones Científicas-Universidad de Cádiz), Avda. República Saharaui, Puerto Real 11510, Cádiz, Spain
‡United Nations Educational, Scientific and Cultural Organization/University Twinning/Wise Coastal Practices for Sustainable Human Development, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro, Puerto Real 11510, Cádiz, Spain

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Abstract—A 28-d bioassay was conducted with two invertebrate species with different feeding habits, the clam *Ruditapes philippinarum* and the shore crab *Carcinus maenas*. The purpose of the present study was to assess the quality of sediments affected by oil spills in different areas of the Spanish coast. The organisms were exposed to environmental samples of oil-contaminated sediments during four weeks and, after the experiment, a suite of biomarkers of exposure was measured: The phase one detoxification system was assessed by ethoxyresorufin-*O*-deethylase (EROD) activity; glutathione-*S*-transferase (GST) is a phase-two detoxification enzyme but also is implicated in oxidative stress events; glutathione peroxidase (GPX), glutathione reductase (GR), and the ferric reducing ability of plasma (FRAP) assay were analyzed to determine the antioxidant activity of the tissues. The biomarker results were correlated with the chemical compounds bound to sediments (polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], Zn, Cd, Pb, Cu, Ni, Co, V) and a principal component analysis was carried out with the purpose of linking all the variables and to detect those contaminated sediments potentially harmful to the biota. Results showed induction of biomarkers in both invertebrate species and significant differences ($p < 0.05$; $p < 0.01$) were established among sediments affected by different spills. The use of the selected biomarkers together with the sediment chemical analysis assesses the bioavailability of contaminants and has proven to be a suitable tool to monitor the environmental quality of sediments affected by oil spills.

Keywords—Polycyclic aromatic hydrocarbons Toxicity Bioassay Oil spill

INTRODUCTION

The presence of persistent pollutants related to oil spills such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and toxic metals (Cd, Pb, Zn, Cu, Ni, Co, V, etc.) in different compartments of the marine environment has become a major threat to the health of marine ecosystems due to accumulation of their residues in the tissues of marine organisms [1]. Biomarkers have been shown to be useful tools in characterizing the health status of animals from affected areas, where complex mixtures of pollutants are usually present [2–4]. Biomarkers present the inherent capacity to detect early biological effects within the organism and to monitor the temporal progression (or regression) of the disturbance of various levels of biological organization [5]. Under controlled conditions in the laboratory, it is relatively straightforward to standardize biomarker assays and to regulate the chemical exposures that organisms receive, so that cause-effect and, indeed, exposure-relationships, can be established [6].

The fluctuation of different biomarkers in response to different toxicants provides a pattern of results that can give clues as to the type of pollutant that is causing the observed effect [6]. Biomarkers previously have been used to assess oil spill episodes [5,7–9]. In the present study a suite of biomarkers was chosen in order to investigate biological responses of organisms exposed to oil-contaminated sediments from the Galician coast (northwest Spain), acutely affected by a fuel spill (*Prestige*, 2002), and the Bay of Algeciras (south Spain), chronically affected by different spills. Ethoxyresorufin-*O*-deethylase (EROD) was selected as the phase-one detoxification enzyme implicated in monooxygenation reactions of dioxins and PAHs. Glutathione-*S*-transferase (GST) is a phase-two detoxification enzyme but also is implicated in oxidative stress events; glutathione peroxidase (GPX) and glutathione reductase (GR) were chosen as antioxidant enzymes together with the ferric reducing ability of plasma (FRAP) assay. The combination of biological responses and chemical data of the sediment helps identify the integrated impact of chemical contamination on organisms. Many authors agree that sediment quality is best determined by integrating the information obtained from measures of chemical concentration and from specific tests to determine sediment toxicity [10,11].

The purpose of the present study is to test the suitability of using a set of biomarkers in two invertebrate species, the clam *Ruditapes philippinarum* and the crab *Carcinus maenas*, in order to assess the environmental quality of sediments affected by oil spills. To achieve this objective, the selected biomarkers were linked with the concentration of contaminants in the sediments and the results are discussed.

MATERIALS AND METHODS

Approach

The sediments employed in the present study were collected in two areas of the Spanish coast affected by oil spills. The area of the Galician coast (northwest Spain) suffered the acute

* To whom correspondence may be addressed (carmen.morales@uca.es).
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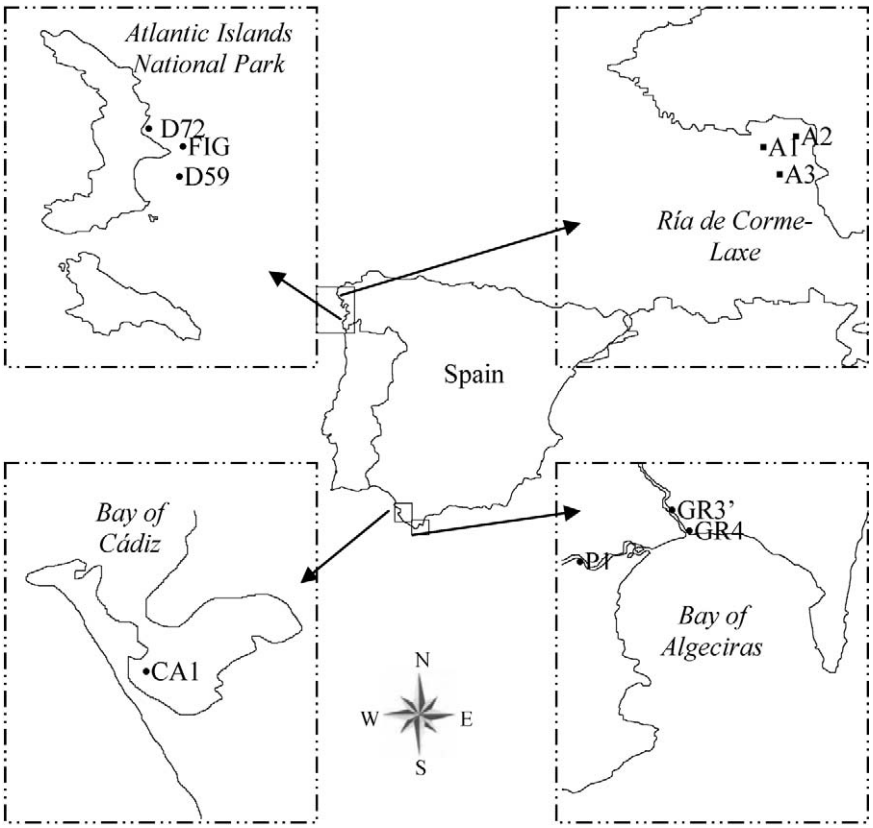

Fig. 1. Map of the coastal area of Galicia, Spain, showing the locations of the sampling stations. FIG, D59, and D72 refer to the stations located in the Cíes Islands in the Atlantic Islands National Park and A1, A2, and A3 to those in the Bay of Corme-Laxe. Stations located in the Bay of Algeciras are GR3′, GR4, and P1. Station Ca1 located in the Bay of Cadiz corresponds to the sediment used as a reference.

impact of the *Prestige* oil spill in 2002 whereas the Bay of Algeciras (south Spain) is affected continuously by minor spills, including oil and other contaminants from industries and discharges from commercial shipping activities [12]. A reference site with no organic pollution was selected in the Bay of Cádiz (south Spain). This site has been validated widely as a reference area [9,12–14]. The 10 selected study sites are shown in Figure 1.

Bioassays

The clam *Ruditapes philippinarum* and the crab *Carcinus maenas* were obtained from an aquaculture farm and were kept under laboratory conditions in tanks with continuous water replacement and acclimated for 10 d. Twenty-five–liter tanks were employed to perform the bioassay with crabs, whereas 11-L aquariums were selected to carry out the experiment with clams. Sediment collected in the study sites was placed in replicate in the tanks: 4 L of sediment was put in the 25-L glass tanks and 2 L of sediment sample was placed in the 11-L aquariums. Clean seawater then was added and, after particle settling, aeration was provided to maintain adequate oxygen concentrations (greater than 80% saturation). The organisms then were transferred to the tanks under controlled laboratory conditions. The temperature was kept at 19 ± 1°C and the natural photoperiod was maintained. The bioassays were performed in duplicate and lasted 28 d; over this time the water in the tanks was replaced and the crabs were fed every week with a mixed diet of mussels or fish, and the clams were fed with an algae preparation.

Biochemical analysis

After 28 d of the exposure period, a survey was carried out and the hepatopancreas (in crabs) and digestive gland (in clams) were extracted and kept at −80°C prior to homogenization. The samples were homogenized according to the procedure developed by Lafontaine et al. [15].

Following homogenization, the samples were centrifuged at 10,000 *g* for 30 min, and the supernatant was used for the biomarker determination. Mixed-function oxygenase activity, which is the first mode of detoxification of many organic pollutants, was measured using the adapted EROD assay [16]. The FRAP assay allows a measure of the antioxidant capacity and was carried out as described by Benzie and Strain [17]. The antioxidant GST activity was determined by monitoring the rate of conjugation of glutathione (GSH) to 1-chloro-2,4-dinitrobenzene at 340 nm [18]. The oxidation of 1 mM nicotinamide adenosine dinucleotide phosphate by GR activity in the presence of 10 mM oxidized glutathione also was monitored at 340 nm [18]. The phase two metabolizing enzyme GPX activity was measured according to McFarland et al. [18]. All the biomarker responses were normalized to the total protein content [19].

Chemical analysis

The analyses of PAHs and PCBs were carried out following the methodologies described in Morales-Caselles et al. [8], according to U.S. Environmental Protection Agency methods. Briefly, following recommendations by Riba et al. [20], dried samples were Soxhlet extracted with *n*-hexane for 18 h, and

the extracts were isolated by column chromatography on Florisil alumino-silica (Sigma-Aldrich, St. Louis, MO, USA). The PCBs and PAHs were eluted and their fractions were dried in a rotating evaporator and redissolved in isooctane. The aromatic fractions were analyzed using a Hewlett-Packard 5890 Series II gas chromatographer coupled with a Hewlett Packard 5970 mass spectrometer (Ramsey, MN, USA). The PAHs were analyzed by gas chromatography coupled with mass spectrometry using selected ion monitoring. Analysis of PCBs such as Aroclor 1242 (Chemical Abstracts Service number 53469-21-9) and Aroclor 1260 (Chemical Abstracts Service number 11096-82-5; Accu Standards, New Haven, CT, USA) was performed using the same instrument with an electron capture detector. For both sets of organic chemicals, PAHs and Aroclor, the analytical procedure showed agreement with the certified values of more than 90%.

A trace metal analysis was carried out as described by Casado-Martínez et al. [21]. Briefly, 2.5 g of sediments (<0.065 mm) were placed in Teflon® containers and were digested in a microwave oven (400 W, 15 min, twice) with 2N HNO_3. The extracts were purified by passing them through a C-18 column and metal analyses were performed by anodic voltamperometry (Zn, Cd, Pb, Ni, Co, and Cu; Metrohm Application Bulletin 147; Metrohm Application Note V-81, Herisau, Switzerland). The cold-vapor technique was used for Hg and was quantified using atomic absorption spectrometry. The analytical procedures were checked using reference material MESS-1 (marine sediment, National Research Council Canada, Ottawa, ON) and certified reference material 277 (estuarine sediment, Bureau Communautaire de Référence, European Union) and showed a recovery greater than 90% of the certified concentration.

Statistical analysis

The biomarker results were analyzed with analysis of variance and Tukey test with the aim of determining significant differences ($p < 0.05$; $p < 0.01$) between the results obtained for the reference site and the other sampling sites (SPSS® 11.5, Chicago, IL, USA). The chemical concentrations in sediments and biomarker responses were correlated with the Pearson analysis ($p < 0.05$) in order to detect relationships between the variables (Statistica 6.0, Statsoft, Tulsa, OK, USA). Finally, a multivariate analysis was carried out with the purpose of linking chemical and biological data; the principal component analysis was used as the extraction procedure to derive a reduced number of new variables (factors) as linear combinations of the original variables (Statistica) [22].

RESULTS

Biomarker responses

The biomarker responses in crabs and clams after 28 d of exposure to the sediment samples are shown in Figure 2. The GPX activity results (Fig. 2A) showed the lowest values in *C. maenas* exposed to the reference sediment. Significant differences ($p < 0.01$) in GPX induction were detected between crabs exposed to the reference sediment and crabs exposed to test sediments collected in A2 and A3 in Corme-Laxe and GR3′ in the Bay of Algeciras. The induction of this biomarker in clams did not present significant differences between treatments. The induction of the antioxidant biomarker GR (Fig. 2B) in crabs exposed to sediment from A1 and clams from the A2 treatment was significantly different ($p < 0.05$ and 0.01, respectively) from the reference site; on the other hand, crabs exposed to sediment from FIG and clams exposed to GR3′ presented significant differences ($p < 0.05$) and lower values than the reference site Ca1. In relation to the phase one detoxification system, clams from all treatments showed significant differences with the reference site in EROD activity (Fig. 2D); these differences were greater ($p < 0.01$) for those clams that had been exposed to the sediments collected in the Bay of Algeciras (GR3′, GR4, and P1). Ethoxyresorufin-*O*-deethylase activity induction in crabs from A1 and A2 in Corme-Laxe, and GR3′ and GR4 in the Bay of Algeciras, also was significantly different from the reference station ($p < 0.05$). The antioxidant activity obtained from the FRAP assay (Fig. 2E) showed significant differences between crabs exposed to A2 ($p < 0.05$), A3 ($p < 0.05$), FIG ($p < 0.05$), and P1 ($p < 0.01$) and the reference site; in the case of clams, GR3′ ($p < 0.01$), GR4 ($p < 0.01$), and D72 ($p < 0.05$) presented significant differences to the reference site; although GR3′ showed higher values than Ca1, GR4 and D72 presented lower values.

Chemical analysis

The results of the chemical analysis are shown in Table 1. The sediments from the reference site did not present organic contamination, whereas the sediments from the Bay of Algeciras (GR3′ > GR4 > P1) chronically affected by different spills and those of Corme-Laxe (A1 > A2 > A3) presented higher concentrations of PAHs in their sediments than sites from the Cíes Islands (59 > FIG >72). No general pattern was observed in the concentration of metals in sediments from the different areas; however sediments from site GR3′ from the Bay of Algeciras presented the highest values of Ni (74.7 mg kg^{-1}), whereas sites A1 and A3 from Corme-Laxe had the highest content of Zn and Pb.

Correlation between variables

A correlation analysis was conducted in order to detect relationships between the presence of contaminants in the sediments and the induction of biomarkers in the invertebrates exposed to the same as biological mechanisms to defend against these compounds. Significant correlations ($p < 0.05$ and $p < 0.01$) were observed between sediment contamination and biological responses in the organisms exposed (Table 2). Similarly, significant associations ($p < 0.05$ and $p < 0.01$) were observed between organic contaminants (PAHs and PCBs) bound to sediments, the metals Ni and Co and the induction of EROD activity in both clams and crabs after the 28-d exposure time. Meanwhile, the antioxidant GR and FRAP activity measured in both species was significantly ($p < 0.01$) correlated with the presence of Zn and Cu in the sediment, respectively. A relationship has been shown between the metals V and Cd ($p < 0.01$), although this association did not present any relationship with the biomarkers studied.

Principal components analysis

Based on correlations between the chemical and biological results, the principal components analysis enables grouping of the 19 original variables into four new factors, which account for 63.3 % of the variance. The purpose of this analysis is to reduce the number of variables with the minimum loss of information in order to simplify the interpretation of the results. In the present study, it was decided to interpret a group of variables as being associated with a particular component

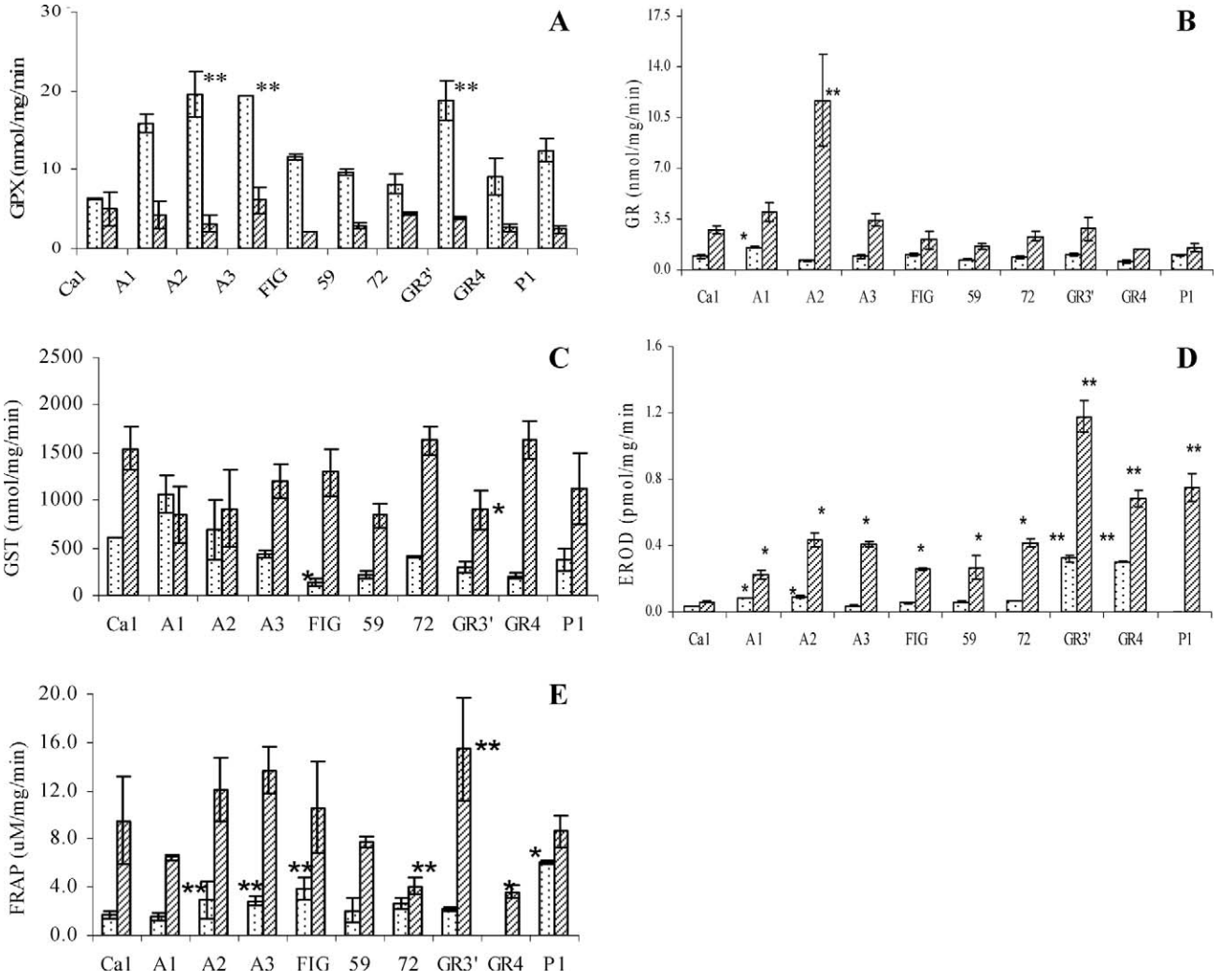

Fig. 2. General health biomarkers for both invertebrate species, the clam *Ruditapes philippinarum* (▨) and the crab *Carcinus maenas* (⬚): (**A**) glutathione peroxidase (GPX) activity (nmol/min/mg protein), (**B**) glutathione reductase (GR) activity (nmol/min/mg prot), (**C**) glutathione transferase (GST) activity (nmol/min/mg protein), (**D**) Ethoxyresorufin-*O*-deethylase (EROD) activity (pmol/mg/min), and (**E**) ferric reducing ability of plasma (FRAP) activity (μM/mg/min). Asterisks (*) indicate significant differences from the reference treatment Ca1 (*$p < 0.05$; **$p < 0.01$). See Figure 1 for location labels on the *x*-axis.

where the loading was 0.30 or higher (Table 3), approximating to Comreys' [23] cut-off of 0.55 for a good association between an original variable and a factor. Factor 1 (31.2 %) links the concentration of PAHs, PCBs, Ni, and Co in sediments with the induction of EROD activity in clams and crabs, the GPX activity induction in crabs, and the FRAP activity in clams after 28 d of exposure. The metals Zn and Pb in sediment are related with GPX and GR activities in both crabs and clams, GST activity in crabs, and FRAP induction in clams as defined by factor 2 (17.3 %). Factor 3 (14.9 %), with negative loading, groups Cu with the induction of GPX and FRAP activities in crabs after 28 d of exposure to the sediments.

After defining the meaning of each factor, the principal component analysis enables identification of the importance

Table 1. Concentration of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls ([PCBs], μg kg^{-1} dry wt) and metals (mg kg^{-1} dry wt) in the sediment samples used in the bioassays. See Figure 1 for location labels. ND = not determined

	Reference	Corme-Laxe			Cíes Island			Bay of Algeciras		
	Ca1	A1	A2	A3	FIG	59	72	GR3′	GR4	P1
PAHs	ND	820	558	537	257	370	239	2,961	802	641
PCBs	ND	2.28	4.29	2.60	ND	6.52	4.76	22.0	1.75	0.84
Zn	21.3	244	31.8	65.7	76.2	43.4	37.5	138	35.3	56.7
Cd	0.92	ND	ND	ND	ND	ND	ND	0.17	0.10	0.12
Pb	2.28	14.3	4.25	44.0	26.6	9.13	6.54	21.6	6.21	12.3
Cu	6.98	19.1	ND	22.1	18.9	ND	31.6	5.01	3.67	75.2
Ni	0.06	7.03	5.61	9.39	12.0	6.88	5.02	74.7	13.1	13.3
Co	3.40	0.67	0.37	1.21	0.52	ND	0.87	12.8	5.59	ND
V	80.0	5.94	2.34	13.4	ND	ND	ND	26.1	ND	6.84

Table 2. Pearson correlation (*$p < 0.05$, **$p < 0.01$) results among chemical compounds bound to sediments (polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], Zn, Cd, Pb, Cu, Ni, Co, V) and biomarkers: Ethoxyresorufin-*O*-deethylase (EROD) activity, glutathione-*S*-transferase (GST) activity, glutathione peroxidase (GPX) activity, glutathione reductase (GR) activity, and ferric reducing ability of plasma (FRAP) activity

	PAHs	PCBs	Zn	Cd	Pb	Cu	Ni	Co	V	GPX Crab	GPX Clam	GR Crab	GR Clam	GST Crab	GST Clam	EROD Crab	EROD Clam	FRAP Crab	FRAP Clam
PAHs		0.905**					0.969**	0.863**								0.754*	0.851**		
PCBs	0.905**						0.914**	0.799**								0.648*	0.727*		
Zn												0.843**							
Cd									0.970**										
Pb																			
Cu																		0.791**	
Ni	0.969**	0.914**						0.882**								0.709*	0.843**		
Co	0.863**	0.799**					0.882**									0.857**	0.725*		
V				0.970**															
GPX–Crab																			
GPX–Clam																			
GR–Crab			0.843**																
GR–Clam																			
GST–Crab																			
GST–Clam																			
EROD–Crab	0.754*	0.648*					0.709*	0.857**									0.692*		
EROD–Clam	0.851**	0.727*					0.843**	0.725*								0.692*			
FRAP–Crab						0.791**													
FRAP–Clam																			

Table 3. Sorted rotated factor loadings of 19 variables for the three principal factors resulting from the multivariate analysis of results obtained from the chemical analysis (polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], Zn, Cd, Pb, Cu, Ni, Co, V) and the biomarker responses in crabs and clams: Ethoxyresorufin-*O*-deethylase (EROD) activity, glutathione-*S*-transferase (GST) activity, glutathione peroxidase (GPX) activity, glutathione reductase (GR) activity, and ferric reducing ability of plasma (FRAP) activity. An em dash represents factor loading less than 0.30

	Factor 1	Factor 2	Factor 3
	31.23%	17.25%	14.85%
PAHs	0.95	—	—
PCBs	0.91	—	—
Zn	—	0.80	—
Cd	—	—	—
Pb	—	0.34	—
Cu	—	—	−0.49
Ni	0.95	—	—
Co	0.91	—	—
V	—	—	—
GPX–Crab	0.48	0.38	−0.30
GPX–Clam	—	0.31	—
GR–Crab	—	0.82	—
GR–Clam	—	0.34	—
GST–Crab	—	0.73	—
GST–Clam	—	—	—
EROD–Crab	0.86	—	—
EROD–Clam	0.85	—	—
FRAP–Crab	—	—	−0.59
FRAP–Clam	0.42	0.44	—

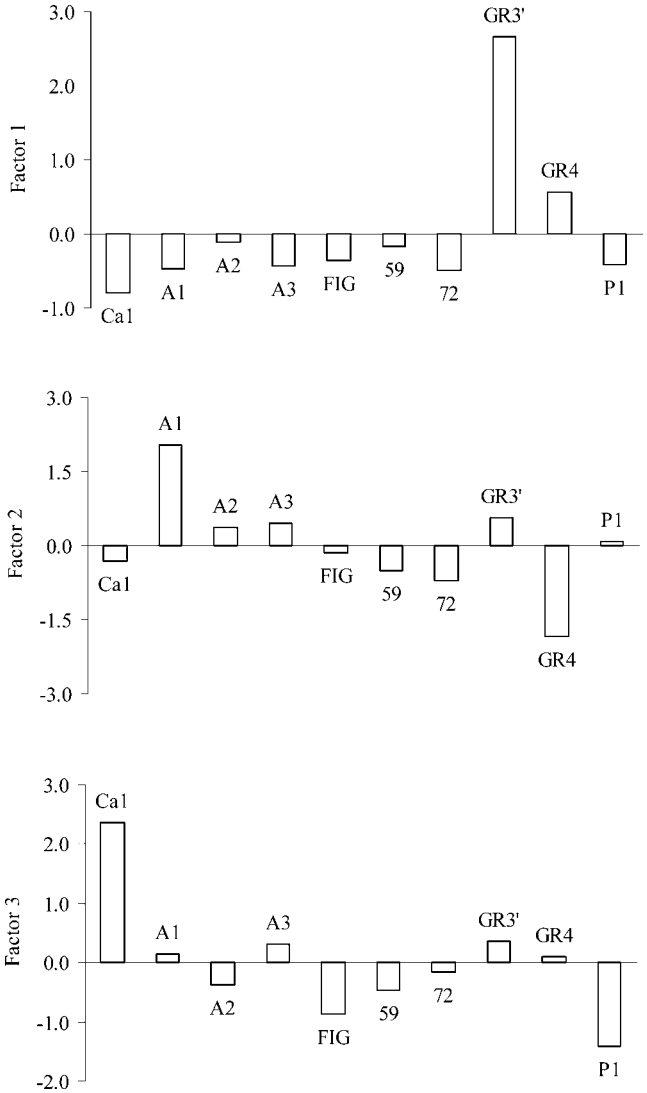

Fig. 3. Factor loadings for the three principal factors resulting from the multivariate analysis of results obtained from the chemical analysis and the suite of biomarkers. See Figure 1 for location labels.

of each factor at each study site by using the factor score. Figure 3 shows the factor score at each of the stations. The influence of factor 1, related with the biomarker response of the organic contaminants (PAHs and PCBs), and the metals Co and Ni in sediments, is prevalent in the stations GR3′ (2.66) and GR4 (0.57) from the Bay of Algeciras; factor 2, which explains the relationship between the content of Zn and Pb in the sediment and the biomarker responses in clams and crabs, presents a positive loading in the stations A1 (2.02), A2 (0.36), and A3 (0.46) from Corme-Laxe and GR3′ (0.58) and P1 (0.07) from the Bay of Algeciras. Finally, factor 3, which with negative loading relates Cu with some biomarker responses (GPX and FRAP induction in crabs), shows a prevalence (negative scores) in the stations from the Cíes Islands FIG (−0.88), 59 (−0.46), 72 (−0.16), and A2 (−0.38) from Corme-Laxe and P1 (−1.40) from the Bay of Algeciras.

DISCUSSION

The present study analyzes the relationship between biomarker responses in organisms exposed to sediments contaminated by oil spills in northwest and south Spain and their chemical content. Two invertebrate species with different feeding habits have been employed in the present study as bioindicator species, the shore crab *Carcinus maenas* and the clam *Ruditapes philippinarum*.

Despite the difficulty of testing complex mixtures of contaminants, the results have shown clear relationships between the different antioxidant enzymes in the tested organisms in the presence of metals. The phase one detoxification system measured by the EROD activity was related to organic contaminants (PAHs and PCBs) and metals bound to complex organic mixtures (Ni and Co). These contaminants, especially PAHs and Ni, often have been linked to oil spills. Antioxidant response (GPX in crabs and FRAP in clams) also was identified for these compounds. Ethoxyresorufin-*O*-deethylase activity often is used as a biomarker of exposure to lipophilic organic contaminants and measures the enzymatic activity of phase one catalyzed by the complex cytochrome CYP1A. The complex cytochrome CYP1A transforms some lipophilic xenobiotics into more water-soluble metabolites, so that they are easier to excrete. Induction of EROD activity has been reported previously in crabs and clams subsequent to organic pollutant exposure [9,15,24]. The highest values of EROD activity were obtained in organisms exposed to sediments from the Bay of Algeciras, and the multivariate analysis linked this induction with organic compounds (PAHs and PCBs). In addition, the induction of antioxidant enzymes measured by the GPX and FRAP analysis was linked with organic contaminants in sediments by factor 1; Cheung et al. [25] demonstrated that some PAHs are potent oxidative stress inducers in the marine mussel, *Perna viridis*, and obtained increasing values of antioxidant parameters, including GPX. Many pollutants (or their metabolites) may elicit toxicity related to oxidative stress. Oxygen toxicity can be a potent oxidant capable of reacting with critical cellular macromolecules, possibly leading to DNA damage and

cell death. Defense systems that tend to inhibit oxyradical formation include antioxidant enzymes such as GR and GPX [9]. It is well known that GPX transforms organohydroperoxide to alcohol and water at the expense of GSH [26]. Thus, GPX activity likely is influenced by the GSH level and GR activity, which regulates the level of GSH [25]. The results obtained in the multivariate analysis demonstrated a relationship of the antioxidant biomarkers GPX and GR in both invertebrate species due to the presence of Zn and Pb in sediments. This means that these metals are producing some stress in the exposed organisms, as is reflected in the antioxidant responses.

The induction of lipid peroxidation by copper is well known in other invertebrates [27], which could explain the connection between Cu and the induction of antioxidant enzymes explained by factor 3 in the multivariate analyses. It is important to note that Cu belongs to a group of metals that are redox-active and are capable of directly generating free radicals [28], which may lead to antioxidant defense responses. Previous studies also reported an induction of GPX activity in *Mytilus galloprovincialis* exposed to copper in controlled conditions or to complex mixtures of metals in field conditions [29]. The induction of FRAP activity in crabs and clams seems to be less relevant than other biomarkers related to oxidative stress, although the correlations observed, especially with metals, suggest the importance of analyzing a group of biomarkers rather than single use.

Glutathione-*S*-transferase activity inducted in crabs has been related to Zn and Pb contamination in the multivariate analysis of factor 2. Glutathione transferases phase two detoxification enzymes that utilize GSH as a substrate in reactions that permit the biotransformation and disposal of exogenous compounds [30]; the induction of GST activity in *Carcinus maenas* previously has been related with metal contamination [9].

The results obtained in the present study showed the activation of different defence systems in both the organisms tested. This mainly was related with an input of chronic fuel oil contamination into the studied sediments, predominantly in the Bay of Algeciras, followed by Corme-Laxe, which was affected by an acute oil impact; these results correspond with histopathological damage observed in the tissue of crabs and clams under laboratory conditions exposed to these sediments (C. Morales-Caselles, personal observations). The Ni and Co sediment content correlates with the organic contaminants, which are usual in the case of hydrocarbon contamination episodes. Zinc and lead contamination also was detected in the area of Corme-Laxe and the Bay of Algeciras, producing stress in the animals exposed. In addition, a source of Cu in Cíes and Algeciras was linked with antioxidant responses in the organisms tested. The presence of metals in these areas suggests that other alternative sources of contaminants should be investigated apart from the *Prestige* oil spill.

Both species employed in the present study, the clam *Ruditapes philippinarum* and the crab *Carcinus maenas*, have responded biochemically to the contamination present in the sediments. All the biomarkers presented higher values in the digestive gland of clams than in the hepatopancreas of crabs, except for GST. The use of two invertebrate species with different feeding habits allows a more complete study of the biological effects of contaminants bound to sediments. According to other authors [31,32], the information provided by each biomarker individually is of limited relevance, because there is a considerable likelihood of misinterpretation; thus, biomarkers are best used as selected batteries of tests rather than individually. In addition, combining chemical analysis with suites of biomarkers addresses the need for more pragmatic environmental assessment techniques linking environmental degradation with its causes [2]. The higher sensitivity of sublethal bioassays compared to acute toxicity tests indicates the advantages of incorporating this approach as part of a more complete and integrated study based on a weight-of-evidence approach, as previously recommended by some authors [8,9,33].

CONCLUSION

In the present study, an evaluation has been carried out of the environmental quality of coastal areas affected by different contaminants. The biomarkers have demonstrated that they are activated depending on the kind and level of contamination and have proven to be a suitable tool to assess oil-contaminated sediments. The clam *Ruditapes philippinarum* and the crab *Carcinus maenas* have demonstrated the importance of using different species of organisms when assessing environmental management and have responded satisfactorily to the contamination present in the sediments, demonstrating the bioavailability of organic and inorganic contaminants related to oil spills. The application of the methodology in two areas affected in different manners by oil spills (acutely, the Galician coast and chronically, the Bay of Algeciras) has shown how biomarkers are activated in diverse ways depending on the source of the pollutants.

The present study has demonstrated the important role that biomarkers play as part of the weight-of-evidence approach, and its use in the assessment of oil spills is strongly recommended.

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