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The application of a weight of evidence approach to compare the quality of coastal sediments affected by acute (*Prestige* 2002) and chronic (Bay of Algeciras) oil spills

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Chronic inputs due to the continuous entrance of contaminants result in much more harm to coastal ecosystems than major but precise environmental impacts.

Abstract

To evaluate sediment quality in different areas affected by oil spills, a weight of evidence approach was employed by including a complete set of parameters as part of four lines of evidence: sediment contamination, biological effects (including biomarkers) and bioaccumulation under laboratory conditions, toxicity in field conditions and benthic community structure. The methodology was applied to sediments from the Bay of Algeciras (S Spain) chronically impacted by different spills, and the Galician Coast (NW Spain) acutely impacted by an oil spill (*Prestige* 2002). Results obtained have elucidated the sources and fates of pollutants and the type of risk involved for the ecosystem. Factorial analysis revealed that the main factors were those containing toxicity, chemistry and benthic community variables indicating degradation in Algeciras. It has been demonstrated that the impact associated with chronic event of contamination by oil spills are significantly more dangerous and polluted than those related to acute effects.

Keywords: Sediment contamination; Sediment toxicity; Sediment Quality Triad; Bioaccumulation; Sublethal; Benthic alteration

1. Introduction

Nowadays, human activities in coastal areas involve a high pressure and a source of different contaminants to the natural environment that becomes evident in the decreased quality of coastal sediments. Sediments act as a trap of contaminants and may become sufficiently polluted to disrupt natural biological communities (Adams et al., 1992; Tolun et al., 2001). Substances introduced into the environment may be more or less bioavailable to organisms depending on their chemical form, modifying factors in the environment, the environmental compartment they occupy, and the reactions (behavioural and physiological) of exposed biota (Chapman et al., 2003; Chapman, 2007). The biological effects can be established based on laboratory tests that determine toxic responses; besides, field data on the communities living in the sediments allow to establish whether there is observable pollution-induced degradation effect in the biota (Chapman et al., 1991).

Integrated studies use different lines of evidence (LOEs) which address different questions about the presence of contaminants, their bioavailability and their adverse biological effects (Riba et al., 2004) in a weight of evidence (WOE) framework. In the present study a WOE following four

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LOEs has been applied to compare the sediment quality of two areas of the Spanish Coast affected by oil spills: the Bay of Algeciras (S Spain) and the Galician Coast (NW Spain). The four LOEs include the determination of sediment contamination, sediment toxicity under laboratory conditions, biological effects under field conditions, and benthic alteration. These lines comprise different approaches to the study and complement each other as part of a WOE investigation. Integration of the obtained information allows correlating the data and discriminating the causes and effects that determine the sediment quality in each of the study sites.

The Bay of Algeciras has suffered a chronic impact lasting several decades, caused by the input of oil and other contaminants from the various industries located in the area and from accidental spills and deliberate discharges from commercial shipping activities (Morales-Caselles et al., 2007), whereas the Galician Coast was impacted by the sinking of the tanker *Prestige* (2002), which spilt about 63,000 tonnes of heavy fuel oil (Mariño-Balsa et al., 2003; Blanco et al., 2006; Fernández et al., 2006). In addition a third area located in the Bay of Cádiz (SW Spain) and widely characterized by different ecotoxicological studies was selected as the reference site (DelValls and Chapman, 1998; Riba et al., 2004; Martín-Díaz et al., 2005; Morales-Caselles et al., 2007).

The aim of this study are: (a) to determine the feasibility of using the selected parameters as part of four LOEs to assess sediments contaminated by different types of oil spills; (b) to establish the environmental degradation in the studied areas; and (c) to elucidate what is more harmful to the environment: acute or chronic impacts associated with oil spills.

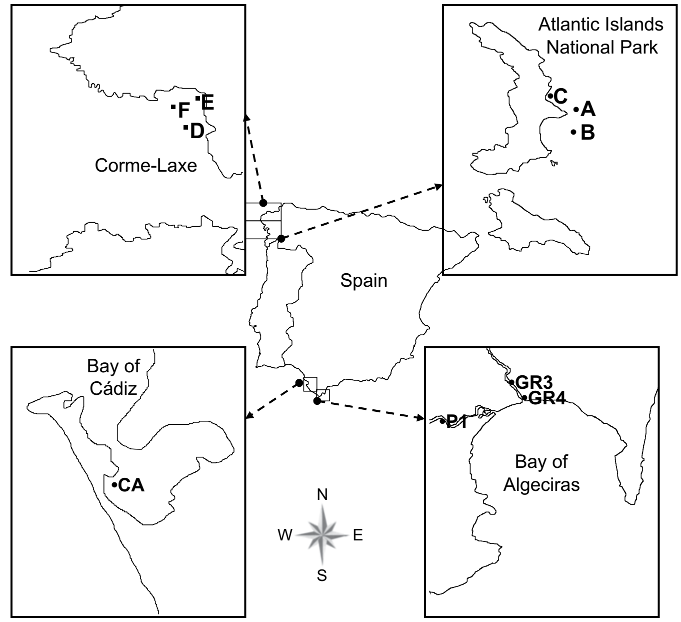

Fig. 1. Map of the coastal area of Galicia, the Bay of Algeciras and the Bay of Cádiz showing the general areas sampled and locations of the sampling stations. A, B and C are the stations located in the Cies Island in the Atlantic Islands National Park (Galician Coast); D, E and F are the sites from the Bay of Corme-Laxe (Galician Coast); GR3, GR4 and P1 are located in the Bay of Algeciras whereas the reference station CA is placed in the Bay of Cádiz.

2. Methodology

2.1. Approach

Fig. 1 shows the six sediment sampling stations located in the area of Galicia (NW Spain), three stations in the Atlantic Islands National Park (A, B and C) and three stations in the Bay of Corme-Laxe (D, E and F). Both areas were importantly affected by the *Prestige* oil spill and are considered of high ecological importance. In the Gulf of Cádiz (S Spain) three stations were selected in the area of the Bay of Algeciras (GR3, GR4, P1) which is highly industrialized area where a large number of petrochemical activities take place that comprise several accidental oil spills; besides this, a reference site was chosen in a clean area in the Bay of Cádiz (CA) (Riba et al., 2003).

2.2. The WOE components

A weight-of-evidence approach (WOE) was conducted in the sites selected which includes four lines of evidence (LOEs) incorporating the following analysis (Fig. 2).

(a) Sediment contamination: Includes the concentration of total PAHs (acenaphthalene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*g,h,i*]perylene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene, fenanthrene, fluoranthene, fluorene, indene[*1,2,3,cd*]pyrene, naphthalene, and pyrene) and trace metals (Zn, Pb, Cu, Ni, Co and V). Sediment characterization by organic carbon and percentage of fines is also included in this section (methodologies described in Morales-Caselles et al., 2006).

(b) Sediment toxicity under laboratory conditions: Including the bacteria assay Microtox® (Morales-Caselles et al., 2007), the amphipod mortality test with *Corophium volutator* (Morales-Caselles et al., 2007) and the polychaeta mortality and bioaccumulation assay (Casado-Martínez et al., 2006) with *Arenicola marina*; sublethal assays were also conducted based on biomarkers by using two invertebrate species, the crab *Carcinus maenas* and the clam *Ruditappes philippinarum*, and a suite of biomarkers measured after 28 days of exposure: ethoxyresorufin *O*-deethylase (EROD), phase I detoxification enzyme implicated in monooxygenation reactions of dioxins and PAHs; glutathione-*S*-transferase (GST) phase II detoxification enzyme but also implicated in oxidative stress events; glutathione peroxidase (GPX) and glutathione reductase (GR), antioxidant enzymes (Martín-Díaz et al., 2007); ferric reducing ability of plasma (FRAP) assay as a measure of antioxidant capacity (Benzie and Strain, 1996); and the vitellogenin variation in crabs (Martín-Díaz, 2004).

(c) Field bioassays were carried out to determine the *in situ* effects. These toxicity tests were performed using field deployments in cages of the crab *Carcinus maenas* and the clam *Ruditappes philippinarum*. The same suite of biomarkers described above and used under laboratory conditions was employed to determine sublethal effects in the organisms exposed during a 28-day period (Martín-Díaz et al., 2007).

(d) "*in situ* alteration": Benthic alteration was selected and determined by measuring parameters *in situ* based in taxonomic identifications and community descriptive statistics (abundance–biomass analysis, species richness, diversity, dominance and proportions of the major taxonomic groups).

2.3. Data integration

The integration of the data obtained from the four LOEs was performed through a multivariate analysis approach based on linking all the variables obtained which determines (a) the environmental degradation of the studied ecosystems (Riba et al., 2004) and (b) a representation using pie charts by an ANOVA approach and by means of the determination of different factors (Riba et al., 2004; Morales-Caselles et al., in press). The multivariate analysis was performed using principal components analysis (PCA) in order to derive a reduced number of new variables (factors) as linear combinations of the original variables. This provides a description of the structure of the data with the minimum loss of information (Riba et al., 2003). The correlations between the

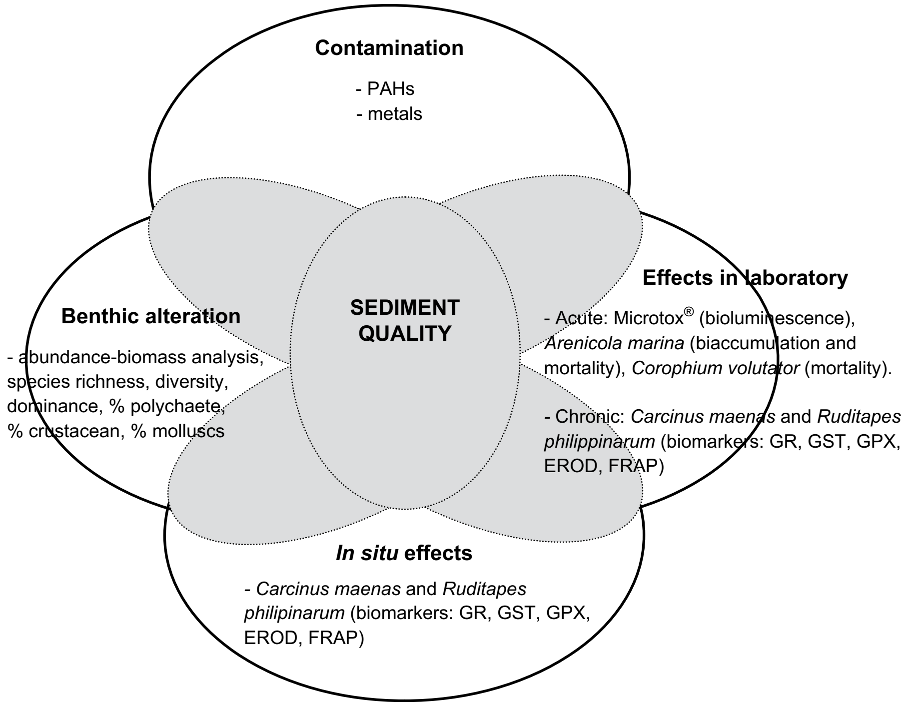

Fig. 2. Summarized description of the four lines of evidence selected in the weight of evidence approach using the schematic representation modification of the classical triad.

original variables are the factor loadings whereas the factor scores estimate the actual values of individual cases (each study site) for the factors. the pie charts were obtained by conducting an ANOVA and Tukey tests which identified significant differences ($p < 0.05$; $p < 0.01$) in sensitivity among stations and the reference station for each of the factor scores obtained from the PCA (Morales-Caselles et al., in press).

3. Results

Table 1 shows the summarized results of the different parameters analyzed in the study. In general, no organic contamination was observed in the reference site whereas the highest levels of PAHs were detected in stations from the Bay of Algeciras. The concentration of metals in sediments varies among the sites and the organic carbon and fines contents are higher in sediments collected in Algeciras. No general pattern was observed in biological parameters between the sampling sites except for site GR3 which presented the highest mortality in the acute bioassays and also a remarkable benthic alteration. To elucidate the matrix of data the PCA was performed to link the variables included in the four LOEs (contamination, effects under laboratory conditions, *in situ* effects and benthic alteration) applied to determine the sediment quality of the two areas affected by oil spills, we have obtained five new factors that account for all the variables and have a different influence for each sampling site (Table 2). These factors explain 82.7% of the variance in the original data set, and negative loadings are considered as important as positive values. The predominant factor, Factor #1, accounts for 34.5% of the variance and shows the relationship between the concentration of Pb, Ni and PAHs in sediment, the percentage of organic carbon and fines, the lethal toxicity determined by the amphipod bioassay, the bioaccumulation of PAHs in *A. marina*, the induction of GPX (crabs), EROD (crabs and clams), vitellogenin variation (crab) and FRAP (clam) activity under laboratory conditions, and the induction of EROD activity (clams) after field exposures; moreover, all the variables related to benthic alteration (abundance, species richness, diversity, dominance and proportions of molluscs, polychaetes and crustaceans) are gathered in Factor #1. Taking this into account, this factor represents environmental degradation by PAHs, Pb and Ni.

Factor #2 (18.0%) combines, with negative loading, the presence of Hg in the sediments with a set of biomarkers (GPX and GST activity in crab and GR induction in clams under laboratory and field conditions, GPX, GR and GST in crabs after field exposures, GR and FRAP activity in clams under laboratory conditions and inverse relation with GST in clam in laboratory studies and FRAP in crabs under field deployments) and the variables of benthic alteration described by species richness and percentage of crustaceans. This factor can be explained as pollution produced by the presence of Hg in the sediments.

The third factor, Factor #3, accounts for a 14.0% of the variance and links, with negative loading, the toxicity detected by the *Arenicola* assay, with the induction of some biomarkers mainly under field conditions (GPX and FRAP in clam in field and laboratory exposures; GST induction in clam and EROD activity in clam and crabs under field conditions; opposite link with GPX activity in crabs from the laboratory experiments). This factor is related to an unknown stressor which is producing a general stress to the exposed organisms but not a benthic alteration, nor pollution or degradation in the benthic environment.

Table 1
Summarized results of physicochemical analysis (mg kg^{-1} for metals, μg kg^{-1} for PAHs, percentage of organic carbon (o.c.) and fines in sediment), the acute toxicity tests (Corophium and Arenicola: % mortality; Microtox: IC_{50}; bioaccumulation of PAHs: μg kg^{-1}), biomarker responses under field and laboratory conditions (glutathione peroxidase activity GPX: nmol min (mg protein)$^{-1}$, glutathione transferase GST activity nmol min (mg protein)$^{-1}$, glutathione reductase GR activity nmol min (mg protein)$^{-1}$, ferric reducing ability of plasma FRAP activity μM mg min^{-1}, EROD activity pmol mg min^{-1} and vitellogenin variation ng 100 ml^{-1}); and the alteration parameters for sediments from the Galician Coast: AINP (A, B, C) and Corme-Laxe (D, E, F), the Gulf of Cádiz—Bay of Algeciras (GR3, GR4, P1) and the Bay of Cádiz (CA)

		A	B	C	D	E	F	GR3	GR4	P1	CA
Physicochemical analyses	Zn	377	91	164	25	19.9	271	138	35.3	56.7	21.3
	Pb	1.5	0.9	0.85	3.7	7.3	5.9	21.6	6.21	12.3	2.28
	Cu	5.2	1.4	1.4	0.7	0.43	4.2	5.01	3.67	75.2	6.98
	Ni	13.3	2.4	4.5	1.7	1.5	5.7	74.7	13.1	13.3	0.06
	Hg	0.7	0.8	0.6	2	2.1	3.4	1.04	0.25	0.65	n.d.
	PAH	108	67	n.d.	38	52	323	2961	802	641	n.d.
	o.c.	0.28	0.26	0.30	0.31	0.37	0.65	2.15	3.19	3.86	1.07
	Fines	4.32	2.81	2.76	3.79	5.50	5.95	69.35	59.33	35.44	2.5
Toxicity tests	Corophium	23	20	17	10	17	20	100	75	20	0
	Arenicola	28	28	22	39	17	17	30	17	46	0
	Microtox	5631	9422	1801	3977	21041	4398	235	249	1642	6013
	Bioaccumulation PAH	2927	2573	2666	2616	3912	3285	5158.9	4809.1	4097.0	2421.0
Biomarkers (laboratory)	GPX-crab-lab	11.6	9.7	8.2	19.3	19.5	15.9	18.8	9.1	12.4	6.3
	GPX-clam-lab	2.1	2.9	4.5	6.1	3.1	4.2	3.8	2.7	2.5	5.1
	GR-crab-lab	1.1	0.7	0.9	0.9	0.6	1.5	1.1	0.5	1.0	0.9
	GR-clam-lab	2.1	1.6	2.3	3.4	11.7	4.0	2.8	1.4	1.5	2.7
	GST-crab-lab	140	218	407	430	684	1071	294.6	203.2	377.7	611.2
	GST-clam-lab	1293	839	1624	1199	910	848	901.1	1634.5	1117.7	1542.1
	EROD-crab-lab	0.1	0.1	0.1	0.0	0.1	0.1	0.3	0.3	0.0	0.0
	EROD-clam-lab	0.3	0.3	0.4	0.4	0.4	0.2	1.2	0.7	0.8	0.1
	FRAP-crab-lab	3.9	2.1	2.6	2.9	2.9	1.6	2.1	n.a.	6.1	1.7
	FRAP-clam-lab	10.6	7.8	4.0	13.7	12.1	6.4	15.4	3.6	8.7	9.5
	VIT-crab-lab	0.0936	0.0380	0.0879	0.1130	0.0889	0.1440	0.3074	0.2332	0.4301	0.0755
Biomarkers (field)	GPX-crab-field	17.8	23.1	15.9	41.4	193.1	125.7	4.8	6.5	4.0	6.3
	GPX-clam-field	10.5	3.6	4.0	25.5	3.2	7.0	2.9	2.8	3.6	5.1
	GR-crab-field	0.7	1.4	1.4	9.9	9.5	23.4	0.8	1.5	0.5	0.9
	GR-clam-field	2.9	1.3	3.8	9.7	14.7	8.0	1.9	1.1	0.8	2.7
	GST-crab-field	1098	1564	690	1489	7523	6073	443.4	1352.7	592.7	611.2
	GST-clam-field	2061	372	1199	3366	131	1558	997.8	1031.6	876.5	1542.1
	EROD-crab-field	0.1	3.0	0.0	8.5	0.4	0.5	0.0	0.0	0.0	0.0
	EROD-clam-field	0.2	0.1	0.1	0.6	0.1	0.1	0.3	0.3	0.3	0.1
	FRAP-crab-field	2.7	n.a.	n.a.	2.4	n.a.	n.a.	1.9	3.5	4.2	1.7
	FRAP-clam-field	10.4	3.1	2.6	23.6	2.0	6.6	2.4	7.7	4.1	9.5
	VIT-crab-field	0.0049	0.0099	0.0879	0.0047	0.0426	0.0098	0.0189	0.1652	0.0303	0.0721
Benthic alterations	Species No.	28.5	33.9	42.4	28.6	32.1	48.2	0.67	4.67	4.67	14
	Specific richness	5.1	5	4.3	3	3	2.9	0	1.21	1.25	2.57
	Diversity	15.3	28.4	39.1	30	40.1	15.4	0	1.29	1.24	1.64
	Dominance	0.50	0.10	0.06	0.15	0.19	0.20	0	0.72	0.68	0.66
	% Mollusca	15.3	28.4	39.1	30.0	40.1	15.4	0.0	34.4	25.4	78.5
	% Polychaete	20.0	21.5	21.7	20.0	22.2	23.1	100.0	45.3	64.4	12.7
	% Crustacea	37.0	41.0	39.1	50.0	51.4	61.5	0.0	20.3	10.2	8.8

n.d., not detected; n.a., not available.

Table 2
Sorted rotated factor loadings for the five principal factors obtained after applying the principal components analysis to the original data set of 41 parameters included in the weight of evidence approach

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
	34.5	*18.0*	*14.0*	*9.0*	*7.1*
Zn	–	–	–	–	0.78
Pb	0.90	–	–	0.35	–
Cu	–	–	–	0.93	–
Ni	0.95	–	–	–	–
Hg	–	−0.81	–	–	0.43
PAH	0.98	–	–	–	–
o.c.	0.45	–	–	0.67	−0.38
Fines	0.86	–	–	–	–
Corophium	0.92	–	–	–	–
Arenicola	–	–	−0.35	0.70	0.43
Microtox	–	–	–	−0.30	−0.47
Bioaccumulation PAHs	0.83	–	–	–	–
GPX-crab-lab	0.47	−0.46	0.68	–	–
GPX-clam-lab	–	–	−0.57	−0.31	–
GR-crab-lab	–	–	–	–	0.64
GR-clam-lab	–	−0.87	–	–	–
GST-crab-lab	–	−0.72	–	–	–
GST-clam-lab	–	0.50	–	–	−0.49
EROD-crab-lab	0.85	–	–	–	–
EROD-clam-lab	0.88	–	–	0.38	–
FRAP-crab-lab	–	–	–	0.77	0.39
FRAP-clam-lab	0.45	–	−0.45	–	–
VTG-crab-lab	0.55	–	–	0.77	–
GPX-crab-field	–	−0.96	–	–	–
GPX-clam-field	–	–	−0.94	–	–
GR-crab-field	–	−0.76	–	–	–
GR-clam-field	–	−0.88	–	–	–
GST-crab-field	–	−0.94	–	–	–
GST-clam-field	–	–	−0.73	–	–
EROD-crab-field	–	–	−0.89	–	–
EROD-clam-field	0.30	–	−0.86	0.34	–
FRAP-crab-field	–	0.47	–	0.65	–
FRAP-clam-field	–	–	−0.93	–	–
VTG-crab-field	–	–	–	–	−0.80
Species No.	0.96	–	–	–	–
Specific richness	0.52	0.42	–	0.36	−0.54
Diversity	0.93	–	–	–	–
Dominance	0.93	–	–	–	–
% Mollusc	0.55	–	–	–	0.70
% Polychaeta	0.89	–	–	0.38	–
% Crustacea	0.53	0.60	–	–	−0.35

Factor #4 (9.0%) is a combination of the concentration of Pb and Cu in the sediments, with the percentage of organic carbon, toxicity in the *Arenicola* toxicity test, *in situ* alteration of the polychaete population and the variation of some biomarkers (EROD in clams and FRAP in crabs under laboratory an field conditions, Vitellogenin variation in crabs and GPX activity in clams under laboratory conditions). In general, Factor #4 can be related to a contamination by Cu and Pb that can be considered a potential risk to the environment but not associated with pollution.

Factor #5 represents 7.1% of the variance and groups the metals Zn and Hg bound to sediment with toxic responses in the *Arenicola* toxicity test, the antioxidant activity determined by the induction of GR and FRAP in crabs in laboratory exposures and the alteration of the mollusc population. Other variables present opposite behaviour such as the GST in clams and vitellogenin variation in crabs, specific richness and the percentage of crustaceans. In this sense, this factor could be explained as contamination by Zn and Hg which is producing some stress in the environment but not pollution.

In order to establish the meaning of each factor in the area of study, the factor scores have been represented for every single station (Fig. 3). The factor scores indicate the importance of every of the factors obtained (described above) in each of the study sites. Factor #1 related to the environmental degradation by PAHs, Pb and Ni presents a positive loading mainly in station GR3 (2.7) followed by site GR4 (0.6) from the Bay of Algeciras. The second factor which explains with negative loading the stress produced by the presence of Hg in the sediments shows prevalence in the study sites located in the Bay of Corme-Laxe E (−2.2) > F (−1.4) > D (−0.3). The unknown stressor described by Factor #3 with negative loading has only prevalence in the station D (−2.8) from Corme-Laxe. In the case of Factor #4 related to a contamination by Cu that could be considered a potential risk, the stations affected are D (0.2) and E (0.1) located in the area of Corme-Laxe and mainly station P1 (2.8) located in the Bay of Algeciras. Finally, the prevalence of Factor #5, which explains the stress caused by the contamination by Zn and Hg, is detected in the stations located in the Atlantic Islands National Park (AINP) A (1.3), B (0.6) and C (0.2), site F (1.1) in Corme-Laxe and site GR3 (0.6) in the Bay of Algeciras.

Fig. 4 shows the significant differences between the stations and the reference site for each of the five studied factors. Significant differences ($p < 0.01$) from the reference site (CA) were observed for Factor #1 and #2 in the stations from Corme-Laxe (D, E, F) and the Bay of Algeciras (GR3, GR4 and P1). Factor #3 was significantly different for B, C ($p < 0.05$) in Cies, D ($p < 0.01$), E ($p < 0.01$) in Corme-Laxe and P1 ($p < 0.05$) in Algeciras. On the other hand Factors #4 and #5 were significantly different ($p < 0.01$ except for F#4 in C which presented $p < 0.05$) to the reference for all the studied stations except for Factor #5 in P1 which did not present these differences. According to the Cluster analysis (Fig. 5) the stations were grouped in a way similar to that in their real location in the field.

4. Discussion

In the present study the integration of four LOEs as part of a WOE approach to assess oil-contaminated sediments is proposed. The different lines employed include a set of 41 variables related to contamination, toxicity and bioaccumulation under laboratory conditions, sediment toxicity measured under field conditions and benthic alteration analysing the macrobenthic structure parameters. The use of sublethal bioassays

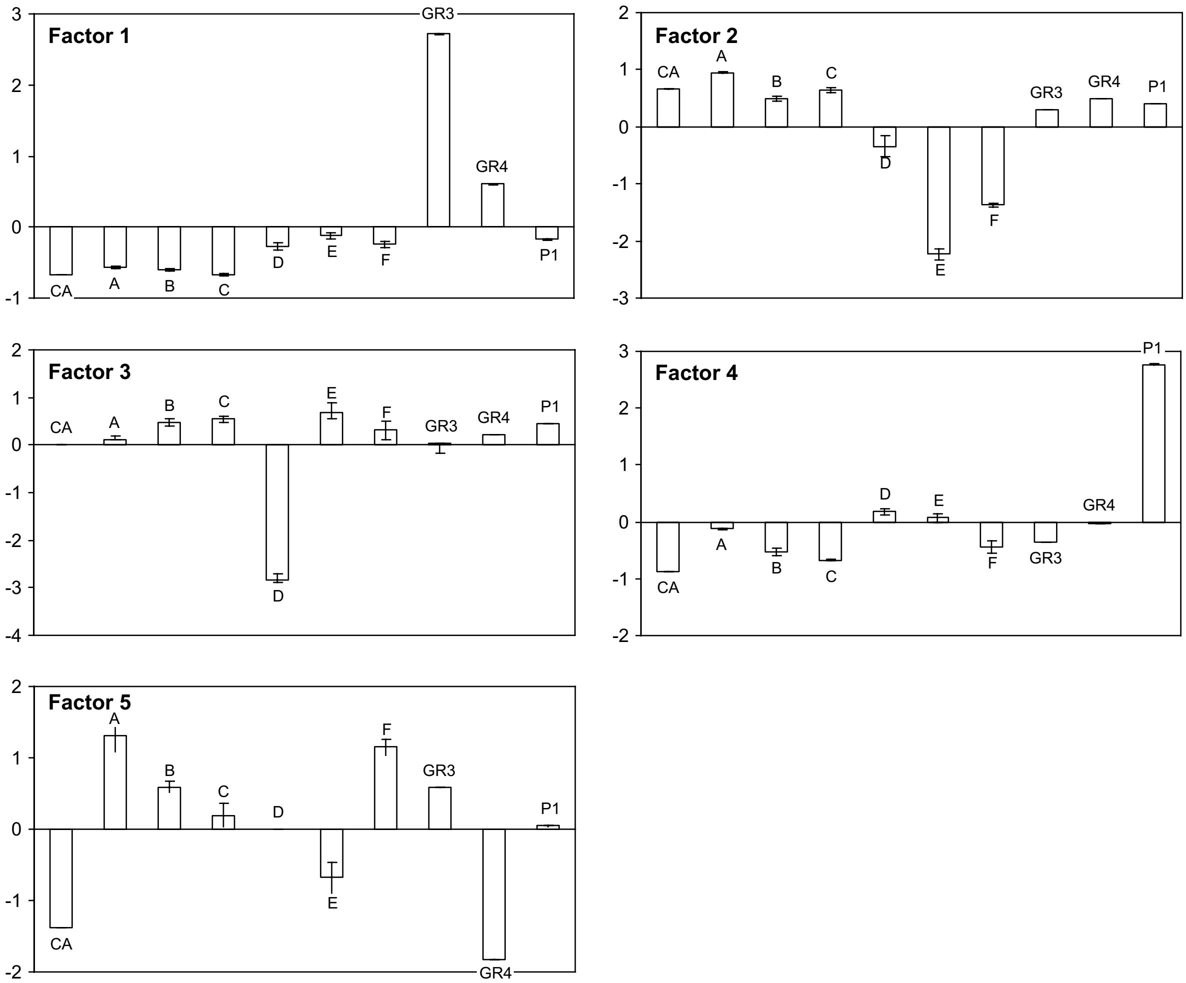

Fig. 3. Estimated factor scores for the three factors in each of the 10 cases. The factor scores quantify the prevalence of each factor for every station.

validated both in laboratory and field exposures by using biomarkers contributes to a better understanding of the toxic processes of the contaminants and supplies the lack of information often shown by acute toxicity tests performed alone. The application of this methodology to sediments affected by oil spills in different manners has allowed determination of the environmental quality of the impacted areas as well as differentiating the most probable causes of the environmental degradation.

The multivariate analyses have demonstrated the suitable use of the site CA as reference station. Results have shown that the Galician coast which was affected by the oil spill from the tanker *Prestige* in 2002 does not present an environmental degradation due to hydrocarbons when compared with the Bay of Algeciras 4 years after the spill; however, significant differences ($p < 0.01$) were detected with the reference station regarding sediment pollution due to fuel oil in the Bay of Corme-Laxe. On the other hand the study sites located in the Cies Island in the AINP present absence of pollution due to fuel oil leakage from the tanker *Prestige*, although an environmental risk caused by metallic contamination of Cu, Zn and Hg is present in the area. Previous studies have demonstrated sources of metals coming from anthropogenic sources located in the area close to the AINP (Carballeira et al., 1997; Pérez-López et al., 2003). The Bay of Corme-Laxe also presents environmental stress due to Cu, Zn and Hg deriving from anthropogenic sources which could include different kind of spills arising from land and maritime traffic. Even though no signals of alteration of the benthic community have been detected in the area, a non-quantified stressor has been determined as potentially toxic. The stress was mainly detected under field exposure, suggesting that the stressor(s) could come from the water; a possible cause could be related to the presence of industrial culture of caged mussels particularly close to station D, which implies a source of organic matter to the water column affecting the exposed organisms. On the other hand, the results observed in the study sites from the Bay of Algeciras have shown an important environmental degradation in the Guadarranque River due to chronic contamination by a mixture of contaminants that include mainly Pb, Ni and PAHs, all of them representative of hydrocarbon contamination in the ecosystem and previously reported by other studies (CSIC, 2003, 2005). Acute and sublethal toxicological responses besides an important alteration on the biota were associated with this kind of pollution. The presence of petrochemical industries, the high maritime

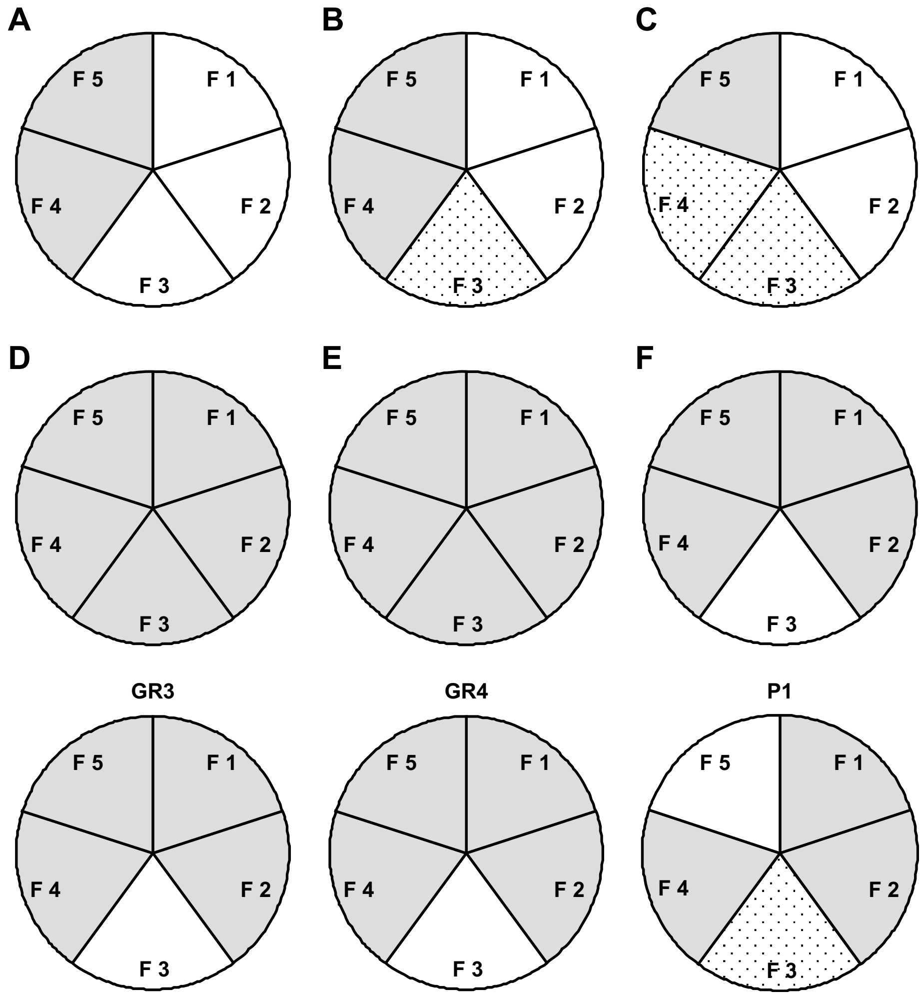

Fig. 4. Pie charts which represent the significant differences of the factors score in every study site related to the reference site CA (dotted: $p < 0.01$; slightly dotted: $p < 0.05$; not dotted: no significant differences, $p > 0.05$).

traffic and the oil bunkering activities of ships are the main factors which involve a threat to the marine ecosystem of the Bay of Algeciras in addition to the human risk represented by the collection of goods for consumption in the zone. Other contaminants such as Cu, Zn and Hg are producing stress to the biota in stations P1 and GR3; on the contrary, the site GR4, which is located in the mouth of the river, does not present this environmental pressure by metals, suggesting that the pollution by these contaminants might come from direct spills to the rivers from the industries located in the area. Despite the tide regime of the area which implies an important water renewal, the degradation of the ecosystem in the mouth of the River Palmones and Guadarranque is a fact. Taking this into account, it is possible that there are other types of contaminants in the area which may be contributing to the environmental impact, but not measured in this study (Antón, 2007). The cluster analysis has confirmed the disparity of the stations, from the Bay of Algeciras > Corme-Laxe > AINP > reference station.

Regarding the results obtained, the recovery of the Galician coast affected by the *Prestige* oil spill is significantly notable (Morales-Caselles et al., in press), although other sources of contaminants should be taken into consideration due to the potential risk involved. On the other hand, the chronic pollution in the Bay of Algeciras which is not only composed by hydrocarbon spills but with the existence of a complex mixture of

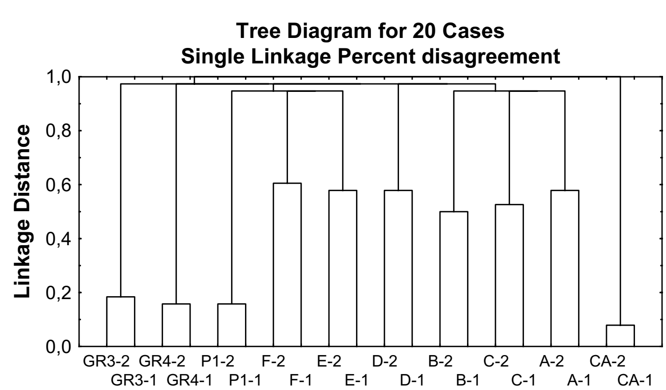

Fig. 5. Tree diagram classification of the 10 stations (in duplicate) based in Cluster analysis (CA: reference station; A, B and C: AINP; D, E and F: Corme-Laxe; GR3, GR4; P1: Bay of Algeciras). The tree clustering method uses the dissimilarities or distances between objects when forming the clusters. In this case, the distance between sites is computed as percentage of disagreement.

contaminant inputs, is producing considerable additional damage to the ecosystem. In this sense, chronic inputs due to the continuous entrance of contaminants result in much more harm to coastal ecosystems than major but precise environmental impacts, as confirmed in previous studies (Riba et al., 2004).

5. Conclusions

In the present study we have successfully integrated four LOEs in a WOE approach to assess sediments affected by oil spills and different sources of contaminants. The use of physicochemical characterization, biological responses under laboratory and field conditions, and *in situ* alteration of the biota as part of a WOE approach is considered a suitable tool to carry out sediment quality studies. This methodology, based on the evaluation of a complete set of parameters under an integrated framework, goes further than the classical studies by studying the real status of the environment and including early warning signals of risk. The combination of field and laboratory analysis supposes an added value to the assessment whereas the complete methodology employed has elucidated the contaminants' sources and fates, in addition to their implication as an environmental risk.

The existence of a wide group of sources in the Bay of Algeciras, including urban and industrial activities in addition to the maritime traffic which involves accidental spills, makes it difficult to elucidate the main cause of the environmental health decrease. Results obtained indicate that the high environmental degradation present in the Bay of Algeciras is mainly due to continuous oil spills. On the other hand, 4 years after the *Prestige* oil spill, a general recovery of the sediments affected in the Atlantic Islands National Park and an improvement in the environmental quality in the Bay of Corme-Laxe was observed. Other inputs of contaminants not related to oil spills were also detected in these areas; at present these sources of stress are not producing damage to the biota although they constitute an environmental risk that should not be ignored.

To sum up, the environmental capacity of recovery after a major oil spill episode such as the *Prestige* has been demonstrated, whereas littoral sediments affected by low or moderate but continuous oil spills have resulted in more degradation. This conclusion should lead to reflection on our perception and major concerns of environmental pollution.

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