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A WEIGHT OF EVIDENCE APPROACH FOR QUALITY ASSESSMENT OF SEDIMENTS IMPACTED BY AN OIL SPILL: THE ROLE OF A SET OF BIOMARKERS AS A LINE OF EVIDENCE Carmen Morales-Caselles^{1,2,*}, Inmaculada Riba^{1,2}, Carmen Sarasquete¹, T. Ángel DelValls^{1,2}

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24 Abstract

25	In an attempt to incorporate both line of evidence (LOE) and classical weight of
26	evidence (WOE) approaches for the assessment of sediment quality, a set of biomarkers
27	were analyzed in target tissues of two invertebrate species after 28 days of exposure to
28	sediments impacted by oil (derived from the tanker Prestige, 2002). The integration of
29	biomarkers with sediment contamination, acute toxicity and benthic alteration
30	parameters provides an "early warning" tool which not only indicates the environmental
31	quality of an area, but also constitutes an advisory tool for potential ecological risks.
32	The selected biomarkers provide information about the first biological responses due to
33	the presence of contaminants in the environment providing predictable reports about
34	further effects to the ecosystem. The present study demonstrates that the use of a set of
35	biomarkers as part of a WOE approach designed to assess contaminated sediments
36	contributes added value to the classical LOE and allows characterisation of the
37	environmental status of the studied area in a more precise and accurate way.

- 38 Keywords: PAHs, contamination, toxicity, sublethal, WOE, bioassay, clam, crab.

C

1. INTRODUCTION

47	Chemical analyses normally provide the main tool in sediment quality assessment, even
48	though chemical concentrations alone are inadequate for the prediction of biological
49	consequences. Biological effects can be established using laboratory tests that determine
50	toxic responses and field surveys of the communities living within impacted sediments,
51	i.e. observing pollution-induced degradation of the biota (Chapman et al., 1991).
52	Weight of evidence (WOE) investigations determine the possible ecological impacts of
53	chemicals or other stressors based on multiple lines of evidence (LOE) (Chapman,
54	2007). They have been widely used in recent years to assess sediment quality around the
55	world (Chapman, 2000; Borgmann et al., 2001), including different areas within the
56	Iberian Peninsula (DelValls and Chapman, 1998; Caeiro, 2004; Riba et al., 2004;
57	Martín-Díaz, 2004; Morales-Caselles, 2008 a).
58	The sinking of the tanker <i>Prestige</i> (2002) spilt about 63,000 tonnes of heavy fuel oil (a
59	mixture of saturated hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes, with
60	most of the PAHs being of medium to high molecular weight) mainly affecting the
61	Galician Coast, NW Spain. Several investigations have focused on determining the
62	biological and environmental effects of this dramatic episode by following single lines
63	of evidence, such as chemical analyses (CSIC, 2003a; Franco et al., 2006; González et
64	al., 2006), toxicity (Mariño-Balsa et al., 2003; Martínez-Gómez et al., 2006; Marigómez
65	et al., 2006; Morales-Caselles et al., 2006) or benthic alteration (Junoy et al., 2005;
66	Serrano et al., 2006). Recently, authors presented a report (Morales-Caselles et al., 2008
67	a) where a classical WOE approach, the Sediment Quality Triad (SQT), based on three
68	lines of evidence (physicochemical characterization of the sediments, determination of

69 acute toxicity and benthic alteration) was carried out in the Galician Coast. This study 70 suggested a general recovery of the environmental health within the area. However, 71 there were signs (Morales-Caselles et al., 2008 a) that other sources of contaminants 72 apart from the *Prestige* oil spill could be producing some environmental stress. The aim 73 of the present study is to use a biomarker line of evidence (LOE) with the weight of 74 evidence (WOE) approach to further improve the sediment quality assessment of the 75 Galician Coast. Chapman and Hollert (2006) suggested that future applications of the 76 SQT should consider specific LOE in terms of risk assessment, ensuring that both 77 exposure and assessment of biological effects are adequately addressed, as both are of 78 causation and ecological relevance. Thus, the LOE selected in this study constitutes an 79 improvement of the classical WOE laboratory and field studies based on biomarker 80 determinations. Biomarkers can act as an important early warning system by indicating 81 whether environmental pollutants are present at sufficiently high concentrations to cause 82 an effect (Walker et al., 2006). In addition, biomarker responses are specific to certain 83 groups of contaminants, providing information related to possible causation (Galloway 84 et al. 2004). Chemicals such as the Polycyclic Aromatic Hydrocarbons (PAHs) have a 85 very short biological half-life in most species, yet nevertheless, may have long-term effects (Walker et al., 2006). In this sense, some compounds may not produce acute 86 87 toxic effects, however sublethal effects are likely to be observed; carefully selected 88 biomarkers can highlight these sublethal effects, thus providing increased sensitivity 89 and robustness to WOE assessments. Thus, the aim of this research was to examine the 90 feasibility and viability of incorporating a biomarker LOE within the classical WOE 91 approach as applied to the assessment of oil contaminated sediments. This new 92 approach is demonstrated by examining the extent of biological impact and identity of

93 contaminants within sediments influenced by the accidental oil spill of the Pr	restige
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94 (2002) on the Galician coast.

95 2. METHODOLOGY

96 **2.1** Approach

- 97 The study was performed in two areas of the Galician coast (NW Spain) affected by the
- 98 Prestige oil spill in 2002 (Figure 1): Cies Island (A, B, C) within the Atlantic Island
- 99 National Park (AINP) and the Bay of Corme-Laxe (D, E, F). Cies Island acted as a
- 100 natural barrier protecting the rias from entry by the fuel. The Bay of Corme-Laxe is also
- 101 considered a place with high ecological relevance, with a low anthropogenic and
- 102 industrial influence (fishing and farming being the main economic activities).
- 103 The 4 LOE employed within the WOE approach were:
- 104 (a) Sediment contamination; physicochemical characterization of sediments by
- 105 analyzing PAHs (acenaphtalene, acenaphtylene, anthracene, benzo(a)anthracene,
- 106 benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i) perylene, benzo(k)fluoranthene,
- 107 chrysene, dibenzo(a,h) anthracene, fenanthrene, fluoranthene, fluorene, indene (1,2,3,cd
- 108)pyrene, naphthalene, and pyrene) using GC-MS with selected ion monitoring and trace
- 109 metals (Zn, Pb, Cu, Ni and Hg) with anodic stripping voltammetry (Morales-Caselles et
- 110 al., 2006);
- 111 (b) Acute toxicity and bioaccumulation; by performing sediment bioassays such as the
- 112 commercial assay Microtox[®] (Morales-Caselles et al., 2007), the amphipod mortality
- 113 test with *Corophium volutator* (Morales-Caselles et al., 2007), the polychaetes mortality
- 114 assay (Casado-Martínez et al., in press) and bioaccumulation experiments with
- 115 Arenicola marina (Morales-Caselles et al., 2008 b);

116 (c) 'In situ alteration'; benthic alteration was selected and determined by measuring 117 parameters in situ based on taxonomic identification and community descriptive 118 statistics (abundance-biomass analysis, species richness, diversity, dominance and 119 proportions of the major taxonomic groups) (DelValls and Chapman, 1998); 120 (d) Laboratory and field studies based on biomarkers; using two invertebrate species the 121 crab Carcinus maenas and the clam Ruditappes philippinarum, and a set of biomarkers 122 (Morales-Caselles et al., 2008 c): mixed function oxygenase activity, which is the first 123 mode of detoxification in many organic pollutants, was measured using the adapted 124 Ethoxyresorufin-O-deethylase (EROD) assay; the Ferric Reducing Ability of Plasma 125 (FRAP) assay allows a measure of the antioxidant capacity; antioxidant Glutathione-S-126 transferase (GST) activity was determined by monitoring the rate of conjugation of

127 glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm; oxidation of 1

128 mM NADPH by Glutathione Reductase activity (GR) in the presence of 10 mM

129 oxidized glutathione was also monitored at 340 nm; phase II metabolizing enzyme

- 130 Glutathione Peroxidase activity (GPX); all the biomarker responses were normalized
- 131 with the total protein content (n=5).

132 2.2 Data integration

133 The data obtained from the different LOE were integrated through a multivariate 134 analysis approach based on linking all the variables obtained (Riba et al., 2004) and a 135 pie chart representation of comparisons between sites of multivariate factors (Riba et al., 136 2004; Morales-Caselles et al., 2008 a). The multivariate analysis was performed using 137 principal component analysis (PCA) as the extraction procedure, which is a multivariate 138 statistical technique to explore variable distributions (Riba et al., 2003). The original 139 data set used in the analysis included the variables obtained from the 4-LOE and its 140 objective was to derive a reduced number of new variables as linear combinations of the

original variables. This provides a description of correlations between the set of theoriginal variables and their meaning in each study site with a minimum loss of

143 information. For the representation of the pie charts the new factors obtained from the

144 PCA were submitted to ANOVA and Tukey tests which identified significant

145 differences in sensitivity among stations and controls for each factor (Morales-Caselles

146 et al., 2008 a). Every study site has a pie chart divided into the obtained factors which

147 use different colours depending on the level of significant differences in relation to the

148 reference.

149 **3. RESULTS AND DISCUSSION**

150 Table 1 shows the summarized results of the different parameters analyzed. Sediment

151 Hg and Pb content was significantly higher in all Corme-Laxe sites; The highest metal

152 contents were observed at stations A and F. There was no observed general pattern in

153 toxicity and benthic parameters between the sampling sites.

154 Factor analysis revealed that the original variables could be grouped into three principle

155 components (factors) that explained 79 % of the total variance (Table 2). The

156 multivariate analysis is a tool that allows us to interpret large groups of different

157 variables by grouping them using correlations; in addition it indicates the importance of

158 each factor in every single study site.

159 3.1 Factor #1

160 Factor 1 explained 35.2 % of the variance and was influenced by the relationship

161 between different variables related with chemicals, sublethal responses,

162 bioaccumulation and benthic alteration. Sediment PAHs, Pb and Hg contents were

163 positively correlated to the bioaccumulation of PAHs in Arenicola marina (under

164 laboratory conditions), but negatively correlated to their mortality in the acute assays. A

165	set of antioxidant and detoxification biomarkers analyzed in crabs (activity of GPX,
166	GR, GST, EROD and FRAP) and the digestive gland of clams (GR and GST activity)
167	under laboratory conditions were also correlated with Factor #1. EROD and FRAP of
168	organisms exposed to sediments in situ were not significantly correlated with other
169	variables (Table 2). The aforementioned contaminants and the toxicity variables are
170	slightly connected to the benthic alteration explained by alteration of the specific
171	richness and an increase of the polychaete population, while a positive development of
172	crustaceans was detected. Thus, Factor #1 may be interpreted as representing
173	contamination by PAHs, Hg and Pb, which does not produce lethal effects; although
174	PAH bioaccumulation and sub-lethal responses in organisms (under laboratory and field
175	conditions) are associated with a slight alteration of the <i>in situ</i> benthic community.
176	Environmental alterations due to these contaminants have been reported by other
177	authors (DelValls et al., 1998). Factor 1 scores for stations E (1.0) and F (1.4) located in
178	Corme-Laxe (Figure 2) were rather high, suggesting an accumulation of contaminants
179	including fuel oil from the <i>Prestige</i> (Labandeira et al., 2001; Prego et al., 2006).
180	Although initial studies did not detect the presence of Pb and Hg in emulsified samples
181	of the Prestige fuel (with 54–59% water) (CSIC, 2003b), the presence of Pb was later
182	corroborated by other authors (Andrade et al., 2004). Thus, the contamination by PAHs,
183	Hg and Pb observed at Corme-Laxe appears to be caused by the remnants of the
184	Prestige (Morales-Caselles et al., 2008 b).
185	Although no lethal effects were observed, the bioaccumulation of PAHs by A. marina
186	demonstrates the high bioavailability of these compounds. The induction of different
187	biomarkers in the hepatopancreas of crabs and in the digestive glands of clams was also

189 the deployed organisms suffered stress due to the presence of these substances in the

correlated with the presence of these contaminants (PAHs, Hg and Pb), suggesting that

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190 sediments (Morales-Caselles et al., 2008c). The correlation observed among the 191 biomarkers and the different variables defined by Factor #1 was stronger under 192 laboratory conditions, implying that field deployments result in less sensitivity (Astley 193 et al 1999). The flushing action of the open water environment may act to reduce the 194 impacts of sediment contaminants when compared with laboratory incubations.

Although, a good correlation was observed between the biomarkers induced in both invertebrate species, the crab *Carcinus maenas* was more sensitive i.e., showed stronger responses, than the clam *Ruditappes philippinarum* to this kind of pollution. On the other hand some of the variables related to the benthic alteration present in Factor #1 corroborate the effects observed in the sub-lethal experiments.

200 3.2 Factor #2

201 The second factor, Factor #2 (24.5 % of the variance) connects the set of biomarkers 202 measured under field conditions (EROD and FRAP activity in crabs and clams, and 203 GPX and GST in clams), the mortality of *Arenicola* in the acute experiment, no toxicity 204 for amphipods, the alteration in the number of species and the decrease of the 205 Polychaete population. Positive and negative correlations for a few biomarkers were 206 also detected under laboratory conditions and no concordance with the amphipod 207 toxicity test was observed. The relationships between the biological responses identified 208 in Factor #2 are not correlated with any of the chemicals analyzed suggesting that a 209 contaminant or group of compounds that was not analysed, is the cause of the biomarker 210 responses. Taking into account that the acute toxicity observed in *Arenicola* and the rest 211 of the bioassays was relatively low (less than 30% mortality) (Morales-Caselles et al., 212 2008 b), a source of contaminant not related to the sediment is the most likely cause of 213 these effects. Station A (0.1) located on Cies Island and site D (2.0) in Bay of Corme-214 Laxe had the highest positive correlation with Factor #2 (Figure 2). The good

215 correlation observed between the biomarkers in both crabs and clams incubated under 216 field conditions suggests that these locations suffer the stress of non-measured variables 217 which, in the case of site D, could be related to the proximity of aquaculture 218 infrastructures (mussel culture). Other authors (Otero et al., 2006), have described the 219 negative impacts of these mussel aquaculture, including: the discharge of a large 220 volume of bio-deposits containing high concentrations of nutrients; the release of drugs 221 and pesticides into the environment; an increase in sedimentation and accumulation of 222 organic matter and an increase in the concentration of nutrients in sediments and waters 223 (mainly N and P). Negative effects on wild populations of animals have also been 224 observed, ranging from genetic interaction and disease transmission, to changes in the 225 composition of the structure of benthic fauna due to a change from oxic to anoxic 226 conditions (Otero et al., 2006).

227 3.3 Factor #3

The third factor, Factor #3 explained 19.2 % of the variance and was correlated with 228 229 sediments contaminated by the metals Zn, Cu and Ni, and PAHs; these contaminants 230 were correlated with the antioxidant responses under laboratory assays with crabs and 231 clams (GR) and the acute toxicity as determined by the amphipods assay, which was not 232 significant (the toxicity detected was not enough to consider sediment samples as toxic 233 according to this acute assay). Samples where the mortality rate of the amphipods was 234 20 % higher than the mortality recorded in the reference and showed significantly 235 different (*p < 0.05) results compared to those obtained in the reference are considered 236 as toxic (Morales-Caselles et al., 2008 b) The effects on the benthic community were 237 manifested as an alteration in the diversity and percentage of molluscs. This suggests 238 the presence of a stress related to the source of metals (Zn, Cu and Ni) and organic 239 compounds (PAHs), which is different than the source explained by Factor #1. Sites A

240 (1.2) in the AINP and F (1.3) in the Bay of Corme-Laxe had positive Factor #3 values 241 (Figure 2). Previous studies in Bay of Corme-Laxe detected severe contamination of the 242 sediments by Cu (Prego et al., 2006), however a fuel oil origin was unlikely, with the 243 major source of Cu being antifouling paints from the hulls of fishing vessels (Cobelo-244 García and Prego, 2003). Although contamination by Cu and Zn was observed in the 245 uppermost sediment layers of the Prestige shipwreck area (Prego and Cobelo-García, 246 2004; Cobelo-García et al., 2005), this contamination is unlikely not be related to the 247 shipwreck, because levels of Cu in the fuel oil carried by the *Prestige* were relatively low (3.39 mg kg⁻¹) and previous studies have shown that inputs from terrestrial sources 248 249 are probably higher than from the spilled fuel oil (Carballeira et al., 1997; Villares et al., 250 2007). Some of the studied variables demonstrate the stress of these contaminants and 251 the effects on the benthic community; however, the results mostly point to chronic 252 contamination with low bioavailability, and potential, but unconfirmed biological risk. 253 Thus, despite the high trace metal content of sediments from the Galician coast, their 254 reactivity and bioavailability were very low (Otero et al., 2005), which maybe related to 255 the high degree of pyritization found for some of the most toxic trace metals, favouring 256 their release by oxidation of the sulphides that they form, thus making them 257 bioavailable to benthic fauna.

258 **3.4 Significant differences among stations**

Station C, with an absence of PAH contamination and the lowest biological effects, was
considered as the most suitable site to use as a reference station. Factor loadings
obtained in the MAA were submitted to ANOVA and post-hoc examination using the
Tukey test (Figure 3).

Factor # 1 scores of sediments from Cies Island in the AINP were not significantly

different, suggesting that the effects of the *Prestige* oil spill were no longer detectable

265 within the area. However, Factors # 2 and # 3 scores for station A were significantly 266 different (p < 0.01) from the reference station (C), suggesting the influence of non-267 measured stressors from sources other than the *Prestige*. The factor #3 score of station 268 B was also significantly different (p < 0.05) from the reference station, suggesting the 269 presence of some contaminants considered a potential risk within the area, although in 270 general, the environmental quality of the sediments was relatively good. On the other 271 hand, Factor #1scores of sediments from Corme-Laxe D, E and F were significantly 272 different (p < 0.01) from the reference station, suggesting the remaining contaminants 273 from the *Prestige* oil spill are still producing sub-lethal effects to the biota of the bay. In 274 addition, the significant difference (p < 0.01) in Factor #2 score observed at station D 275 suggests other unknown sources of contaminants responsible for biological stress in the 276 study site. In the case of station F a mixture of metals and PAHs from different sources 277 could be considered a potential risk in the area as shown by Factor #3 (p < 0.01). 278 Biomarkers are useful "early warning" indicators of the health status of animals from 279 impacted areas (Galloway et al., 2004; Montserrat et al., in press), including systems 280 impacted by oil (Anderson and Lee 2006), where complex mixtures of pollutants are 281 usually present. The use of a set of biomarkers, as part of a WOE approach designed to 282 assess contaminated sediments, contributed added value to the classical LOE concept 283 and allows for the characterization of the environmental status of the studied area in a 284 more precise and accurate way. In addition, the inclusion of chronic bioassays with two 285 invertebrate species, not only under laboratory conditions but also in field deployments, 286 elucidate different sources of contaminants apart from the sediments permitting a more 287 realistic approach to the original situation of the ecosystem, and the potential ecological 288 risks.

4. CONCLUSIONS

290 Multiple lines of evidence suggest that 4 years after the impact of the *Prestige* oil spill, 291 the fuel is not producing acute toxic effects within the environment (Morales-Caselles et 292 al., 2008 a); however, the use of a biomarker LOE within the WOE approach 293 demonstrated that sub-lethal responses may occur in the area of Corme-Laxe, related to 294 PAHs, Pb and Hg. No effects of oil spill were observed in the study sites located in 295 AINP although contamination by metals, especially Zn, Cu and Ni was observed in 296 some sites on Cíes Island. The inclusion of biomarkers in the SQT indicates that coastal 297 anthropogenic influences (a mixture of pollutants that act as environmental stressors) 298 are evident in both areas and should be considered a potential risk. In the case of 299 Corme-Laxe, a possible impact related to the aquaculture of mussels was detected. 300 Biomarkers demonstrated higher sensitivity when compared to acute toxicity 301 approaches providing a more comprehensive and integrated weight-of-evidence 302 approach (Morales-Caselles et al., 2008 a), that as demonstrated, can be easily incorporated as a fourth line of evidence into the classical SQT. Furthermore, this study 303 304 confirms the suitability of biomarkers as a tool to assess the sources and potential 305 ecological risk of metallic and petrogenic contaminants within sediments.

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446 FIGURE CAPTIONS

- 447 Figure 1. Map of the coastal area of Galicia (NW Spain) showing the sampling sites in
- the Atlantic Island National Park (A, B, C) and the area of Corme-Laxe (D, E and F).
- 449 Figure 2. Estimated factor scores for the three factors in each of the 6 cases. The factor
- 450 scores quantify the prevalence of each factor for every station and are used to establish
- 451 the definition of each factor.
- 452 Figure 3. Pie charts which represent the significant differences of the factors score in
- 453 every study site related to the reference site C (heavy shading: p < 0.01; light shading: p

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454 < 0.05; no shading: no significant differences, p>0.05).

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22 FIGURE CAPTIONS

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24	the Atlantic Island National Park (A, B, C) and the area of Corme-Laxe (D, E and F).
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26	scores quantify the prevalence of each factor for every station and are used to establish
27	the definition of each factor.
28	Figure 3. Pie charts which represent the significant differences in the factors' score in
29	every study site related to the reference site C (high density shading: $p < 0.01$; low
30	density shading: p < 0.05; no shading: no significant differences, p>0.05).
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33	
34	Table 1. Summarized results and SD of chemical analysis (mg kg ⁻¹ for metals, μ g kg ⁻¹
35	for PAHs) the acute toxicity tests (Corophium volutator and Arenicola marina: %
36	mortality; Microtox: IC50; bioaccumulation of PAHs: $\mu g k g^{-1}$), biomarker responses
37	under field and laboratory conditions (glutathione peroxidase activity GPX: nmol min ⁻¹
38	mg prot ⁻¹ , glutathione transferase GST activity nmol min ⁻¹ mg prot ⁻¹ , glutathione
39	reductase GR activity nmol min ⁻¹ mg prot ⁻¹ , ferric reducing ability of plasma FRAP
40	activity $\mu M \text{ mg}^{-1} \min^{-1}$ and EROD activity pmol mg ⁻¹ min ⁻¹) and the alteration
41	parameters for sediments from the Atlantic Islands National Park (AINP) (A, B, C) and
42	Corme-Laxe (D, E, F). n.d.: not detected; n.a.: not available.
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		Α	В	С	D	Е	F
ses	Zn	377±36.06	91 ±1.34	164±11.31	25±1.41	19.9±1.34	271±16.26
alys	Pb	1.5±0.14	0.9±0.00	0.85±0.01	3.7±0.21	7.3±0.07	5.9±0.14
ans	Cu	5.2±0.35	1.4±0.14	1.4±0.21	0.7±0.21	0.43±0.05	4.2±0.21
cal	Ni	13.3±1.91	2.4±0.21	4.5±0.14	1.7±0.14	1.5±0.00	5.7±0.57
emi	Hg	0.7±0.21	0.8 ± 0.07	0.6 ± 0.07	2±0.14	2.1±0.28	3.4±0.28
Che	РАН	108±7.78	67±16.33	n.d.	38±2.47	52±0.71	323±13.44
sts	Corophium	23±1.18	20±1.53	17±0.02	10±1.77	17±1.18	20±3.54
tes	Arenicola	28±1.96	28±1.97	22±1.96	39±0.79	17±0.02	17±0.24
city	Microtox	5631±134	9422±301	1801±101	3977±214	21041±407	4398±282
Toxi	Bioaccumulation PAH	2927±1.48	2573±1.48	2666±1.24	2616±2.40	3911±0.71	3285±0.71
	GPX-crab-lab	11.6±0.59	9.7±0.55	8.2±1.72	19.3±0.00	19.5±2.88	15.9±1.18
ry)	GPX-clam-lab	2.1±0.01	2.9±0.59	4.5±0.04	6.1±2.36	3.1±1.47	4.2±2.42
ato	GR-crab-lab	1.1±0.06	0.7 ± 0.08	0.9±0.12	0.9±0.18	0.6±0.01	1.5 ± 0.04
bor	GR-clam-lab	2.1±0.65	1.6 ± 0.00	2.3±0.36	3.4±0.43	11.7±3.17	3.9±0.03
(lal	GST-crab-lab	139.6±52.3	218.1±44.2	407.3±8.97	429.6±36.1	684.1±422	1070.9±198
ers	GST-clam-lab	1292.8±339	838.6±0.00	1624.5±209	1199.1±174	910.2±566	847.7±293
ırk	EROD-crab-lab	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.04±0.00	0.10 ± 0.00	0.09 ± 0.00
me	EROD-clam-lab	0.26 ± 0.00	0.27 ± 0.00	0.42 ± 0.00	0.41 ± 0.00	0.44 ± 0.00	0.22 ± 0.00
Bic	FRAP-crab-lab	3.85±0.92	2.08±1.01	2.56±0.47	2.86±0.43	2.93±0.56	1.56 ± 0.30
	FRAP-clam-lab	10.58±3.8	7.77±0.5	4.04±0.70	13.72±1.9	12.09±2.6	6.43±0.30
	GPX-crab-field	17.8±0.07	23.1±2.12	15.9±4.10	41.4±2.83	193.1±0.1	125.7±11
	GPX-clam-field	10.5±0.49	3.6±0.14	4.0±0.21	25.5±0.35	3.2±0.49	7.0 ± 2.40
(pl	GR-crab-field	0.7±0.21	1.4±0.92	1.4±0.99	9.9±0.71	9.5±2.83	23.4±1.06
(fie	GR-clam-field	2.9±0.00	1.3 ± 0.00	3.8±0.00	9.7±0.85	14.7±0.07	8.1±1.77
ers	GST-crab-field	1097.8±571	1563.8±224	690.3±10.6	1489.0±219	7523.4±1129	6072.9±0.0
urko	GST-clam-field	2060.8±926	371.6±130	1198.7±45	3365.6±250	130.5±2.5	1557.9±192
) M	EROD-crab-field	0.13±0.00	2.96 ± 0.00	0.02 ± 0.00	8.51±0.00	0.39 ± 0.00	0.48 ± 0.00
Bid	EROD-clam-field	0.15 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.63 ± 0.00	0.07 ± 0.00	0.13 ± 0.00
	FRAP-crab-field	2.72±0.35	n.a.	n.a.	2.42±0.32	n.a.	n.a.
_	FRAP-clam-field	10.39±1.4	3.09±0.53	2.63±0.91	23.58±2.5	2.01±0.65	6.58±1.01
SL	Number species	28.5	33.9	42.4	28.6	32.1	48.2
tion	specific richness	5.1	5	4.3	3	3	2.9
era	Diversity	15.3	28.4	39.1	30	40.1	15.4
alt	Dominance	0.50	0.10	0.06	0.15	0.19	0.20
hic	% Mollusca	15.3	28.4	39.1	30.0	40.1	15.4
enti	% Polychaete	20.0	21.5	21.7	20.0	22.2	23.1
Ã	% Crustacea	37.0	41.0	39.1	50.0	51.4	61.5

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Table 2. Sorted rotated factor loadings of 36 variables for the three principal factors resulting from the multivariate analysis of the single results obtained from the chemical analysis, the acute toxicity tests, the suite of biomarkers and the alteration parameters for the study of the sediments quality in the Galician Coast. Chemicals: loadings are related to the concentration of contaminants in sediments; Acute effects: loadings explain the toxicity detected by the acute assays and the bioaccumulation of PAHs in Arenicola marina. Sublethal effects: loadings are related to the induction of biomarkers. Benthic alteration: loadings are related to alteration of the biota (decrease of number of species, specific richness, diversity, diminuition in the percentage of molluscs and crustacea and increase in the polychaete population. The group of variables selected for the interpretation represented a loading of 0.40 or higher for a good association between an original variable and a factor.

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		Factor 1 35.2	Factor 2 24.5	Factor 3 19.2
	Zn	_	_	0.90
sle	Pb	0.95	—	_
nica	Cu	_	—	0.93
hen	Ni	_	_	0.76
U U	Hg	0.95	_	_
	РАН	0.59	_	0.76
	Corophium	—	-0.52	0.75
ute cts	Arenicola	-0.41	0.87	
Acı effe	Microtox	—	—	
	Bioaccumulation	0.73	-	
	GPX-crab-lab	0.48	-0.77) –
	GPX-clam-lab	—	0.53	_
	GR-crab-lab	0.41	-9	0.78
	GR-clam-lab	0.65		0.49
(pl	GST-crab-lab	0.85		—
fie	GST-clam-lab	-0.53	<u> </u>	_
Ś	EROD-crab-lab	0.62	-0.78	—
ory	EROD-clam-lab		_	-0.89
rat	FRAP-crab-lab	-0.46	—	—
abc	FRAP-clam-lab		0.64	—
s (]	GPX-crab-field	0.88	—	—
ect	GPX-clam-field	V –	0.99	—
eff	GR-crab-field	0.91	—	—
hal	GR-clam-field	0.77	—	-0.46
olet	GST-crab-field	0.89	—	—
Suł	GST-clam-field	_	0.75	_
•1	EROD-crab-field	_	0.88	_
	EROD-clam-field	_	0.98	_
	FRAP-crab-field	—	0.76	—
C .	FRAP-clam-field	_	0.99	_
ų	Number of species	—	0.56	—
ati	specific richness	0.91	—	—
lter	Diversity	—	—	0.97
c al	% Molusca	—	—	0.96
thi	% Polychaeta	0.62	-0.68	—
3en	% Crustacea	-0.96	_	_