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1 **A WEIGHT OF EVIDENCE APPROACH FOR QUALITY ASSESSMENT OF**
2 **SEDIMENTS IMPACTED BY AN OIL SPILL: THE ROLE OF A SET OF**
3 **BIOMARKERS AS A LINE OF EVIDENCE**

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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.

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46 **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 *Prestige* (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
86 effects (Walker et al., 2006). In this sense, some compounds may not produce acute
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 *Prestige*
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, fenantrene, 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) (DeIvals and Chapman, 1998);
120 (d) Laboratory and field studies based on biomarkers; using two invertebrate species the
121 crab *Carcinus maenas* and the clam *Ruditapes 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

141 original variables. This provides a description of correlations between the set of the
142 original 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 *in situ* 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 *Prestige* (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
188 correlated with the presence of these contaminants (PAHs, Hg and Pb), suggesting that
189 the deployed organisms suffered stress due to the presence of these substances in the

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.

195 Although, a good correlation was observed between the biomarkers induced in both
196 invertebrate species, the crab *Carcinus maenas* was more sensitive i.e., showed stronger
197 responses, than the clam *Ruditapes philippinarum* to this kind of pollution. On the
198 other hand some of the variables related to the benthic alteration present in Factor #1
199 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**

228 The third factor, Factor #3 explained 19.2 % of the variance and was correlated with
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
248 low (3.39 mg kg^{-1}) and previous studies have shown that inputs from terrestrial sources
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**

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

263 Factor # 1 scores of sediments from Cies Island in the AINP were not significantly
264 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 #1 scores 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.

289 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
303 incorporated as a fourth line of evidence into the classical SQT. Furthermore, this study
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**

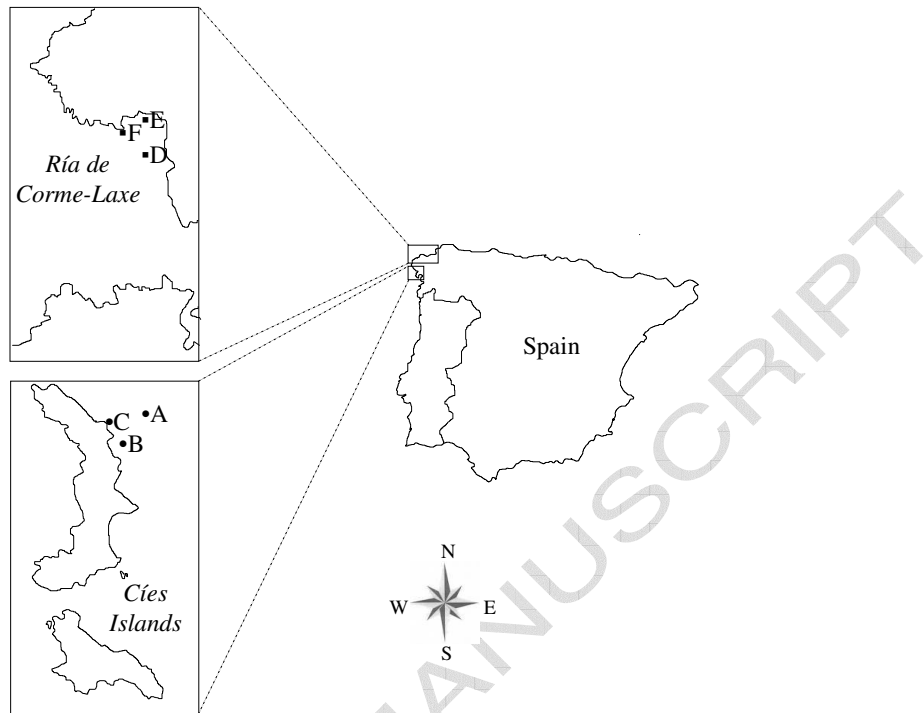
447 Figure 1. Map of the coastal area of Galicia (NW Spain) showing the sampling sites in
448 the Atlantic Island National Park (A, B, C) and the area of Corme-Laxe (D, E and F).

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

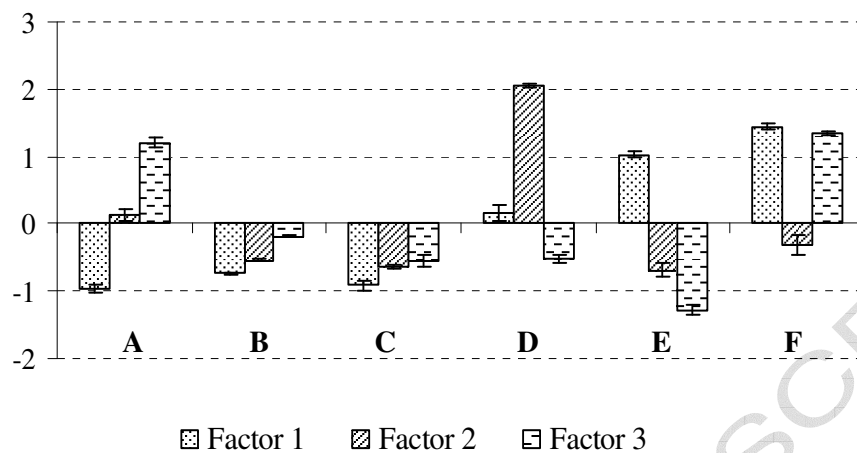
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2 Figure 2.



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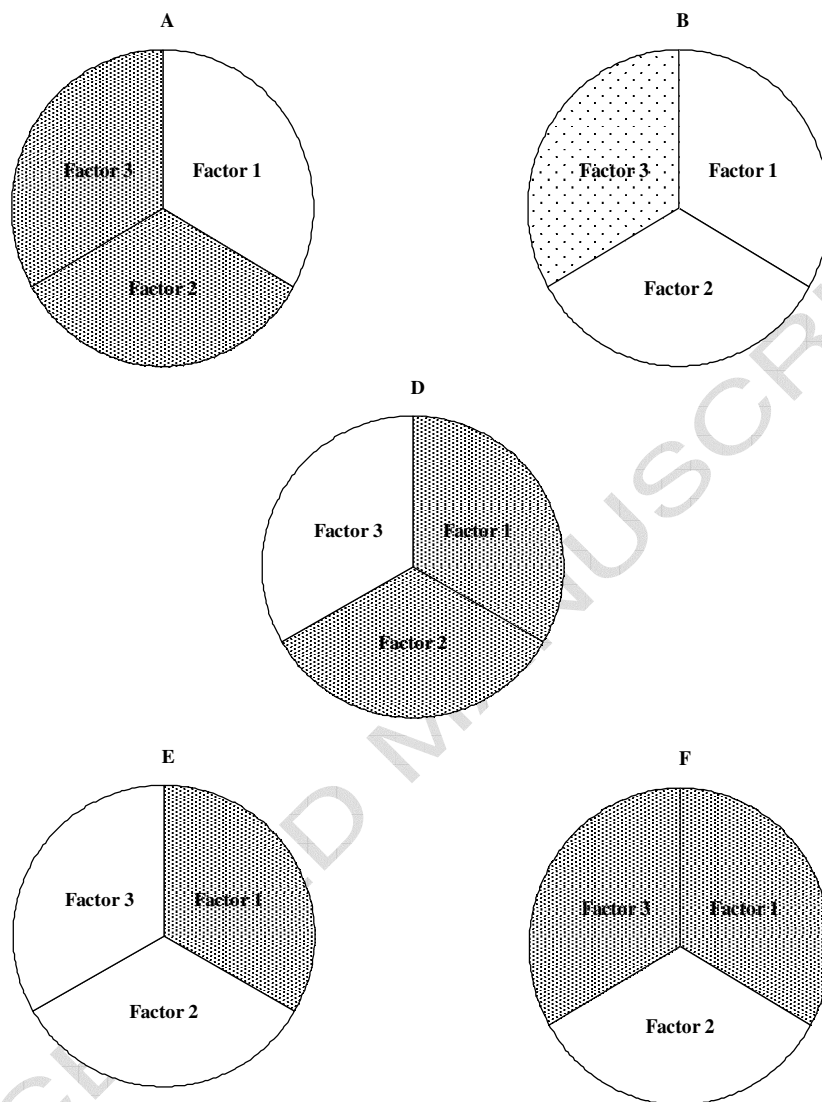
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19 Figure 3.

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22 **FIGURE CAPTIONS**

23 Figure 1. Map of the coastal area of Galicia (NW Spain) showing the sampling sites in
24 the Atlantic Island National Park (A, B, C) and the area of Corme-Laxe (D, E and F).

25 Figure 2. Estimated factor scores for the three factors in each of the 6 cases. The factor
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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34 Table 1. Summarized results and SD of chemical analysis (mg kg^{-1} for metals, $\mu\text{g kg}^{-1}$
35 for PAHs) the acute toxicity tests (*Corophium volutator* and *Arenicola marina*: %
36 mortality; Microtox: IC50; bioaccumulation of PAHs: $\mu\text{g kg}^{-1}$), biomarker responses
37 under field and laboratory conditions (glutathione peroxidase activity GPX: nmol min^{-1}
38 mg prot^{-1} , glutathione transferase GST activity $\text{nmol min}^{-1} \text{mg prot}^{-1}$, glutathione
39 reductase GR activity $\text{nmol min}^{-1} \text{mg prot}^{-1}$, ferric reducing ability of plasma FRAP
40 activity $\mu\text{M mg}^{-1} \text{min}^{-1}$ and EROD activity $\text{pmol mg}^{-1} \text{min}^{-1}$) 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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		A	B	C	D	E	F
Chemical analyses	Zn	377±36.06	91±1.34	164±11.31	25±1.41	19.9±1.34	271±16.26
	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
	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
	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
	Hg	0.7±0.21	0.8±0.07	0.6±0.07	2±0.14	2.1±0.28	3.4±0.28
	PAH	108±7.78	67±16.33	n.d.	38±2.47	52±0.71	323±13.44
Toxicity tests	Corophium	23±1.18	20±1.53	17±0.02	10±1.77	17±1.18	20±3.54
	Arenicola	28±1.96	28±1.97	22±1.96	39±0.79	17±0.02	17±0.24
	Microtox	5631±134	9422±301	1801±101	3977±214	21041±407	4398±282
	Bioaccumulation PAH	2927±1.48	2573±1.48	2666±1.24	2616±2.40	3911±0.71	3285±0.71
Biomarkers (laboratory)	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
	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
	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
	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
	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
	GST-clam-lab	1292.8±339	838.6±0.00	1624.5±209	1199.1±174	910.2±566	847.7±293
	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
	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
	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
Biomarkers (field)	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
	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
	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
	GST-crab-field	1097.8±571	1563.8±224	690.3±10.6	1489.0±219	7523.4±1129	6072.9±0.0
	GST-clam-field	2060.8±926	371.6±130	1198.7±45	3365.6±250	130.5±2.5	1557.9±192
	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
	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
Benthic alterations	Number species	28.5	33.9	42.4	28.6	32.1	48.2
	specific richness	5.1	5	4.3	3	3	2.9
	Diversity	15.3	28.4	39.1	30	40.1	15.4
	Dominance	0.50	0.10	0.06	0.15	0.19	0.20
	% Mollusca	15.3	28.4	39.1	30.0	40.1	15.4
	% Polychaete	20.0	21.5	21.7	20.0	22.2	23.1
	% Crustacea	37.0	41.0	39.1	50.0	51.4	61.5

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

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		Factor 1	Factor 2	Factor 3
		35.2	24.5	19.2
Chemicals	Zn	—	—	0.90
	Pb	0.95	—	—
	Cu	—	—	0.93
	Ni	—	—	0.76
	Hg	0.95	—	—
	PAH	0.59	—	0.76
Acute effects	Corophium	—	-0.52	0.75
	Arenicola	-0.41	0.87	—
	Microtox	—	—	—
	Bioaccumulation	0.73	—	—
Sublethal effects (laboratory & field)	GPX-crab-lab	0.48	-0.77	—
	GPX-clam-lab	—	0.53	—
	GR-crab-lab	0.41	—	0.78
	GR-clam-lab	0.65	—	0.49
	GST-crab-lab	0.85	—	—
	GST-clam-lab	-0.53	—	—
	EROD-crab-lab	0.62	-0.78	—
	EROD-clam-lab	—	—	-0.89
	FRAP-crab-lab	-0.46	—	—
	FRAP-clam-lab	—	0.64	—
	GPX-crab-field	0.88	—	—
	GPX-clam-field	—	0.99	—
	GR-crab-field	0.91	—	—
	GR-clam-field	0.77	—	-0.46
	GST-crab-field	0.89	—	—
	GST-clam-field	—	0.75	—
	EROD-crab-field	—	0.88	—
	EROD-clam-field	—	0.98	—
	FRAP-crab-field	—	0.76	—
	FRAP-clam-field	—	0.99	—
Benthic alteration	Number of species	—	0.56	—
	specific richness	0.91	—	—
	Diversity	—	—	0.97
	% Mollusca	—	—	0.96
	% Polychaeta	0.62	-0.68	—
	% Crustacea	-0.96	—	—

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