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Determining sediment quality for regulatory proposes using fish chronic bioassays

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Abstract

Sediment quality assessments for regulatory purposes (i.e. dredged material disposal) are characterized by linking chemical and acute ecotoxicological data. The design of chronic bioassays that incorporate more sensible endpoints than acute tests is discussed to address sediment quality for environmental quality assessment and regulatory proposes. The chronic tests use juveniles of commercial species of fish *Sparus aurata* and *Solea senegalensis*, to assess sediment toxicity in samples collected along different littoral areas in the North and the South of Spain. The organisms were exposed during 60 days and sublethal endpoints were selected including biomarkers of exposure to metals (metallothioneins — MTs) and to organic contaminants (ethoxyresorufin-*O*-deethylase activity — EROD activity) and biomarkers of effect (histopathology in different tissues, gill and liver). A Multivariate Analysis Approach was conducted in order to associate these biological responses with sediment metal concentration from the ports and with chemical residues in biological tissues exposed to sediments under laboratory conditions.

Keywords: EROD activity; Metallothioneins; Histopathology; Ecotoxicology; Dredged material; Contamination

1. Introduction

It is well known that human activities developed in the last decades have led to the contamination of the coastal zone. Although contaminated sediments are only one component of the ecosystem, they are probably the major source of stress to the organism presents in aquatic ecosystem (Harding, 1992). Frequently, the Spanish Coast suffers dredging operations which means removing and relocating sediments from marine ecosystems, what involves an environmental impact that seldom can damage the organism's health. Interest in the effects of environmental stressors on health and disease in fish and other marine organisms has increased in recent years, and in particular, histological and cellular alterations have been observed in marine fish from polluted coastal waters and estuaries (Stein et al., 1992; DelValls, 2003) besides and the toxic effects of the heavy metals that have accumulated in aquatic ecosystems (Fingerman et al., 1998).

In order to determine the quality of marine sediments, the physical and chemical properties are usually quantified, including contaminants, nutrients and grain size determination; however, ecotoxicological effects on the organisms should be taken into account by carrying out the suitable toxicity tests (Marín-Guirao et al., 2005). Sediment toxicity bioassays are instruments of increasing importance for scientists to test the toxicity and bioavailability of chemical compounds in sediments to organisms (SETAC, 1993; Marín-Guirao et al., 2005); this can be used to characterize different disposal options of the dredged material in order to reduce the environmental impact (Martín-Díaz et al., 2004b).

Acute toxicity test seems to be a good tool to assess the effects of sediment contamination (Riba et al., 2002; Mariño-Balsa et al., 2003), however, sublethal bioassays should be performed in order to determine chronic biological effects that are not provided by the acute toxicity tests. Taking this into account, the toxicity tests that involve sublethal measurements such as

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biomarkers of exposure and effect have shown to be more sensitive and provide more information about the adverse effects associated with chemicals bound to sediments (Martín-Díaz et al., 2004c).

Two species of fish with different habitats of life and feeding habits were selected to carry out this study: *Solea senegalensis* and *Sparus aurata*. These fishes were chosen because they were common and commercial species in coast Spain, and particularly in the area of study. Moreover two biomarkers of exposure (methallothioneins—MTs and ethoxyresorufin-*O*-deethylase activity—EROD activity) and one biomarker of effect (histopathology in gills and liver) were chosen in order to determine chronic toxicity. The aim of this study is to assess the ecotoxicity of dredged material coming from different Spanish ports by linking chemical data with the sublethal responses measured in the fish exposed to those sediments.

2. Material and methods

2.1. Approach

The present study was carried out by using sediment samples collected in ports located IN the North and in the South of Spain (Fig. 1): Bilbao (B) which is affected by an important maritime traffic, Huelva (H1, H2 and H3) affected by metallic contamination and Cádiz (BC1, which is considered an area without any contamination (DelValls et al., 1998) and BC2, port of Cádiz). Clean sediment from the Bay of Cádiz (BC1) was used as negative control reference and toxic mud from an accidental mining spill in Spain (Aznalcóllar, April 1998) was used as positive control of the toxicity (Riba et al., 2003a).

Surface sediment samples from each of the six stations were collected with a 0.025-m^2 Van Veen grab. Sediment samples were placed in a cooler and transported to the laboratory. The contents of the cooler were homogenized with a Teflon ® spoon and sieved through a 0.5 mm mesh in order to remove any associated macrofauna and other means interferences. After that, sediment samples were maintained in the cooler at 4 °C in the dark until their use in sediment toxicity tests. Testing occurred within two weeks of collection. The samples were subsampled for physical characterization and chemical quantification. Detailed descriptions of analysis carried out to obtain the set of data for sediment chemistry are reported by Riba et al. (2004a) and Casado-Martínez et al. (2006).

For sediment grain size analysis, an aliquot of wet sediment was analyzed with a laser particle-size Fristch analyser (DelValls et al., 1998). The remaining sediment was dried at 60 °C before chemical analysis. Dried sediments were gently homogenized. Organic carbon content was determined using the method of Gaudette et al. (1974) with El Rayis (1985) modification. Metal were determined following methods reported by Riba et al. (2004a) and Casado-Martínez et al. (2006). The analytical procedures were checked using reference material (MESS-1 NRC and CRM 277 BCR) and showed a 90–110 range. Results are expressed as mg kg^{-1} dry sediment. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were analyzed by using gas chromatography equipped with an electron capture detector (ECD) (U.S. Environmental Protection Agency SW-846 Method 8270) (Riba et al., 2002). Quality control was carried out using NRC-CNRC HS-6 sediment reference material and allows agreement with certified values higher than 90%.

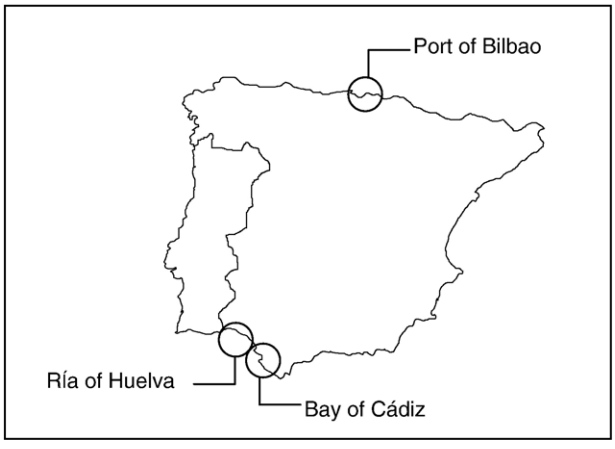

Fig. 1. Map of Spain showing the general areas sampled. Two of the three studied sites are located in the South of Spain, the Bay of Cádiz (BC#) and the Ría of Huelva (H#). The other studied area is located in the North of Spain in the Port of Bilbao (B).

Table 1
Summarized results of chemical contaminants measured in different sediment samples along the Atlantic coast of Spain

Contaminant	Bay of Cádiz 1	Bay of Cádiz 2	Huelva 1	Huelva 2	Huelva 3	Bilbao	Toxic Mud
As	3.42	30.7	839	531	273	67.3	408
Cd	0.92	1.32	4.35	2.50	1.32	2.00	5.40
Cr	0.10	14.9	32.9	24.1	8.13	18.3	3.28
Cu	6.98	202	1938	1497	772	102	210
Hg	0.05	1.98	2.38	1.99	1.20	0.74	5606
Ni	0.06	20.1	34.6	7.10	128	26.4	8.50
Pb	2.28	86.9	383	385	217	147	790
Zn	21.3	378	2458	1857	1176	476	2181
[PCBs]	n.d.	0.11	n.d.	n.d.	n.d.	0.12	n.d.
[PAHs]	0.08	n.d.	n.d.	0.03	0.13	15.7	n.d.

Values of heavy metal (mg kg^{-1}), polychlorinated biphenyls (PCBs, μg kg^{-1}) and polycyclic aromatic hydrocarbons (PAHs, μg kg^{-1}) are calculated at each station. Not detected is expressed by n.d.

2.2. Toxicity test

Toxicity tests were conducted using juveniles of the fish *S. senegalensis* and *S. aurata* obtained from an aquaculture farm and transported to the laboratory. Individuals were exposed to the different sediments during 60 days. Each sediment was tested per triplicate in 25 L glass tanks. Tests were carried out in whole sediment using a 1:4 v/v sediment water relation and with constant aeration. Organisms, with a weight averaged 4±1 g, were acclimatized in the laboratory along 15 days before the beginning of the experiment. Twelve individuals were placed in every tank, fed with artificial food two times per day and water replacement was performed daily using a peristaltic pump during the exposure period. The physicochemical parameters, pH (8.0–8.5), temperature (19±2 °C), dissolved oxygen (80% saturation) and salinity (32±2), were measured and controlled during both sediment toxicity tests. Natural photoperiod was maintained.

At the beginning of the test, a baseline group of 10 randomly chosen individuals from each species were measured, weighed, anaesthetized, and processed for pathological responses to be used as the initial cellular control. At the end of the experiment organisms from each station were anaesthetized and processed to determine histopathological damages (biomarker of effect), MTs induction and EROD activity (biomarkers of exposure).

2.3. Histological analysis

Organisms from the toxicity tests were analyzed to determine the histopathological damages in different target tissues (liver and gills). Fish were removed from the tanks after 60 days of exposure period. They were anaesthetized with 0.1% 2-phenoxyethanol 99% during 5–10 min. Gills and liver tissues of the fish were obtained by dissection and then fixed in phosphate buffered 10% formaldehyde (pH 7.2) for 24 h. After dehydration in graded concentrations of ethanol, the samples were embedded in paraffin wax. Histological sections of 6 to 8 μm thickness were stained with Haematoxylin–Eosin and Haematoxylin–VOF (Gutiérrez, 1967). Sections were reviewed by light microscopy Leitz Laborlux S and photographed (Sony DKC-CM30).

Table 2
Summarized of average semi-quantitative lesions detected in microscopic abnormalities of individuals of juveniles of the fish *S. aurata* collected in the Bay of Cádiz (BC#), the Ría of Huelva (H#), Bilbao (B) and toxic mud (TM) the day 60 of exposure

Histopathology	BC1	BC2	H1	H2	H3	B	TM
Gills							
Hypertrophy/Hyperplasy	−	−	+/−	+/+	+/+	+/+	+
Fusion of secondary lamellae	−	−	−	+	+	+/+	+
Shortening of secondary lamellae	−	+/−	+/−	+	+/+	+/+	+/+
Edematous areas in distal portion of lamellae	+/−	+/−	+/−	+	+/+	+/+	+/+
Necrosis and lost of cells epithelial	+/−	+/−	+	+	+/+	+/+	+/+
IGG	0.17	0.30	0.43	1.70	1.43	1.80	1.90
Liver							
Hepatocytes and exocrine pancreas alteration	−	+/−	+/−	+	+/−	+	+
Vacuolization of hepatocytes	−	+/−	−	+/−	−	+/−	+/+
Necrosis and decrease of the zymogen granules	−	+	+	+	+/−	+/−	+
IGL	0	0.67	0.61	1.05	0.33	0.72	2.11

Incidence of lesions: (−) 0 fish, (+/−) 1 fish, (+) 2 fishes, (+/+) 3 fishes, (+) 4 fishes, (+/++) 5 fishes, (++) 6 fishes.

2.4. Biochemical analysis

Fish were sampled for biochemical analysis and, after dissection, liver was kept at −80 °C prior to the homogenization. The samples were homogenized following the procedure developed by Lafontaine et al. (2000). The methodologies used to determine MTs concentration and EROD activity were detailed described by Martín-Díaz (2004), Martín-Díaz et al. (2004a,c, 2005).

Samples obtained to determine metallothionein (MT) content were centrifuged at 28,000 g for 40 min. The supernatant was added to 0.9 mL of NaCl (0.9%), heated to 95 °C for 4 min, and centrifuged at 10,000 g for 15 min at 4 °C. Supernatant was stored at −80 °C prior to MT concentration determinations by Anodic Stripping Voltammetry (Olafson and Olsson, 1987) using purified rabbit metallothionein (Sigma-Aldrich). Total protein determination was determined by the Bradford method (1976). MT concentrations were expressed as μg mg^{-1} total protein.

Mixed function oxidase activity was measured using the adapted EROD assay (Gagn and Blaise, 1993). Briefly, 50 μL of supernatant (homogenate 10,000 g for 30 min), 10 μM 7-ethoxyresorufin and 10 mM reduced NADPH in 100 mM KH_2PO_4 buffer (pH 7.4). The reaction was started by the addiction of NADPH, being allowed to proceed for 60 min at 30 °C, and stopped by the addition of 100 μL of 0.1M NaOH. The 7-hydroxyresorufin was determined fluorometrically using 520 nm (excitation) and 590 (emission) filters. 7-hydroxyresorufin concentration in the samples was achieved through a standard calibration curve developed with concentrations of 7-hydroxyresorufin. Results were expressed as pmol mg^{-1} min^{-1} total protein.

2.5. Statistical analysis

Histopathological lesions at the end of the bioassay were selected as endpoints. General indexes of histological lesion were calculated for each tissue (lesion index in gills [IGG] and lesion index in liver [IGL]) as an average value of the fish damage semi-quantified as previously reported by DelValls et al. (1998), Riba et al. (2004c,d). The semi-quantification was performed by ranking the frequency of lesions measured in a total number of 6 individuals, so we represent the number of organisms that show prevalence in any of the detected lesions using the next expressions and associated number of individuals:− (0 individuals), +/− (1 individual), + (2 individuals), +/+ (3 individuals), ++ (4 individuals), ++/++ (5 individuals) and finally the maximum is associated with the presence of a disease in the total number of individuals, ++ (6 individuals). Therefore, this value was obtained from the original semi-quantitative assessment of the histological lesions for each organism in each replicate.

The data obtained for metallothioneins, EROD activity and general indexes of lesions from each fish species exposed to different sediments were compared using ANOVA in order to determine significant differences ($p<0.05$; $p<0.01$) among stations. The concentration of metals, PCBs, PAHs, MTs induction, EROD activity and the general index of lesions in the tissues of the different individuals were analyzed by factor analysis, using principal components analysis as the extraction procedure, which is a multivariable statistical technique (MAA) to explore variable distributions (Riba et al., 2003b, 2004b). MAA was used to correlate concentration of contaminants and sublethal effects measured in individual of fish.

3. Results

3.1. Sediment contamination

In Table 1 are shown the chemical concentrations of metals and organic contaminants (PAHs and PCBs) presented in the different sediments collected in the Spanish ports. Among all stations, the negative control (BC1) showed the lowest values of the chemical concentrations of metals, PCBs and PAHs. In general, it is observed that the concentrations of metals in the area of Huelva (H1 > H2 > H3) were higher than those measured in Bilbao and Bay of Cádiz. Besides, the concentrations of PAHs were notably higher in Bilbao than in the other stations. On the other hand, the toxic mud (TM) used as positive

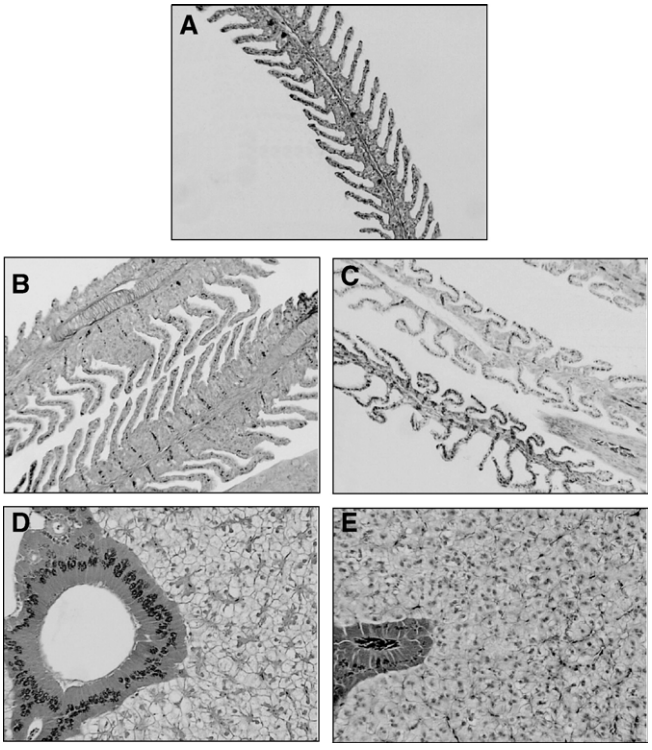

Fig. 2. Histophatological alterations in *S. aurata* after 60 days of exposure to sediments and controls collected along the Atlantic coast of Spain. These alterations were used to semi-quantify lesions. A) Control gills (BC1) (H/VOF x10). B) Fish gills exposed to TM (H/VOF x10). Note the hyperplasy, shortening and fusion of secondary lamellae. C) Fish gills exposed to H2 (H/E x10). Note edematous areas in distal portion of lamellae and lost of cells epithelial. D) Fish liver exposed to BC1 (control) (H/VOF x10). E) Fish liver exposed to H1 (H/VOF x10). Note the vacuolization of hepatic parenchyma and decrease of the zymogen granules.

control showed very high values for Cd, Hg, Pb and Zn in comparison to the other sample sites.

3.2. Biomarker of effect

Histological alterations (gills and liver) were measured at the end of the experiment (60 days). Summarized results of the semi-quantitative evaluation of the frequency of the lesions measured are shown in Table 2. A general index of damage for each analyzed tissue (index of gill damages — IGG and index of liver — IGL) was established to allow comparison of responses among treatments. IGG derived from disease hypertrophy, hyperplasy, fusion of secondary lamellae, shortening of secondary lamellae, edematous areas in distal portion of lamellae and necrosis and lost of cells epithelial, and IGL derived from disease hepatocytes and exocrine pancreas alteration, vacuolization of hepatocytes and necrosis and decrease of the zymogen granules. Histopathological alterations detected in different tissues of fish exposed to toxic mud dilutions and environmental sediment samples are shown in Fig. 2.

It can be observed that in all tissues the histopathological damage for *S. aurata* is lower in individuals exposed to sediments for the Bay of Cádiz (BC1) and the highest damage was observed in tissues of organisms exposed to toxic mud. Gill seem to be the most damaged tissues showing different lesions in specimens treated with TM, H2, H3 and B sediment, which were characterized by hypertrophy and/or hyperplasy, fusion and shortening of secondary lamellae, edematous areas in distal portions of lamellae and necrosis and lost of cells epithelial.

All histological samples of liver showed evidence of necrosis and decrease of the zymogen granules, and hepatocytes and/or exocrine pancreas alteration, except in the negative control sediment. Higher damage was observed in liver of organism exposed to H2 sediments as well as in the toxic mud. The damages identified in both gill and liver analyzed always were present in organisms exposed to stations of H2, B and TM.

Histological evaluations of gill and liver tissues revealed significant differences ($p \leq 0.05$) between the negative control of toxicity and the H2 and TM. Results show the general index of damages in gills (IGG) was significantly different ($p \leq 0.05$) in stations from Huelva (H2 and H3), Bilbao, and toxic mud when compared to negative control (BC1), whereas the index of liver damages was significantly different ($p \leq 0.05$) only in organisms exposed to H2 and TM. The other station located in Cádiz was not significantly different to control sediments in the frequency of histopathological lesion observed. It was observed, with increasing the concentration of metal exposure it was observed an increase of histopathological lesions.

Table 3
Sorted rotated factor loadings (pattern) of 15 variables for the three principal factors resulting from the multivariate analysis of results obtained from the bioassays with juveniles of *S. aurata* and *S. senegalensis*

	Factor 1	Factor 2	Factor 3
% Variance	39	27	15
As	–	0.97	–
Cd	0.89	–	–
Cr	–	0.88	–
Cu	–	0.94	–
Hg	0.90	–	–
Ni	–	–	–
Pb	0.96	–	–
Zn	0.70	0.66	–
[PCBs]	–	–	0.87
[PAHs]	–	–	0.83
EROD (ρmol mg^{-1} min^{-1}) *S. aurata*	–	–	0.78
MT (μg mg^{-1}) *S. aurata*	–	0.98	–
MT (μg mg^{-1}) *S. senegalensis*	0.60	0.41	–
IGG	0.64	–	–
IGL	0.96	–	–

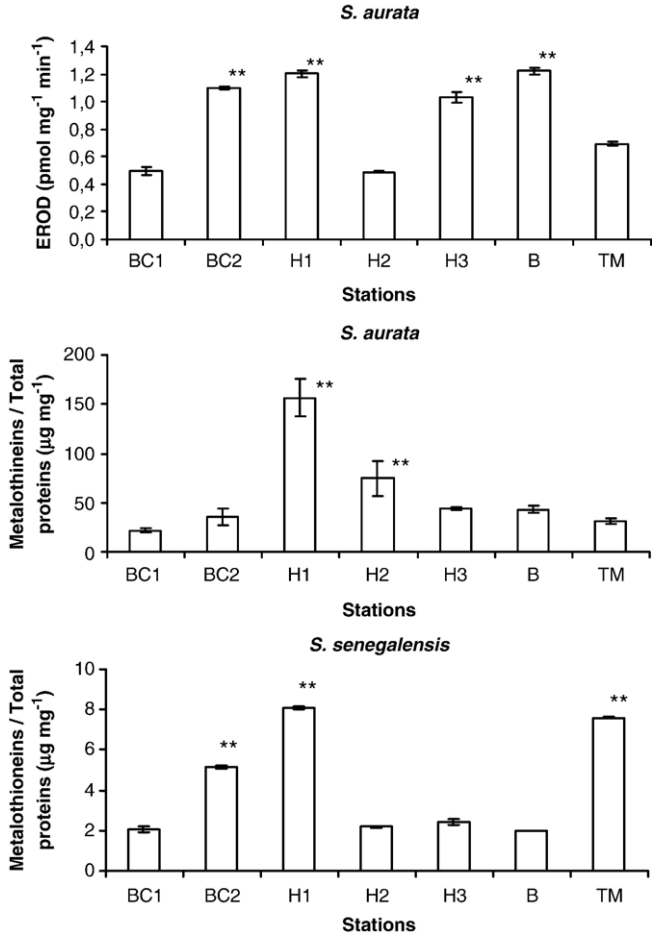

Fig. 3. Summarized results of EROD activity in ρmol mg^{-1}min^{-1} of protein and metallothionein concentration in μg mg^{-1} of protein in liver of *S. aurata* and *S. senegalensis* collected at 60 and 42 days of the experiment respectively. Asterisks indicate significant differences among the biomarker induction in the stations and the control sediment (**$p<0.01$) and the absence of asterisk means no significant difference.

3.3. Biomarker of exposure

The results of EROD activity and MT concentration in liver of *S. aurata* and *S. senegalensis* are summarized in Fig. 3. The major induction of MTs and EROD activity in *S. aurata* was observed in the station with more concentration of metals and organic respectively. Thus, in the course of the bioassay the MT induction for H1, which is the station with the greatest amount of metals in their sediments, is always higher than the MT induction for H2 and H3 decreasing with concentration metals decrease. Significant differences ($p<0.01$) in MT concentrations were observed among individuals of *S. aurata* exposed to H1 and H2 in comparison to negative control (BC1). However, for organisms of *S. senegalensis* these differences were significant in BC2, H1 and TM ($p<0.01$). No significant differences were observed in any of the two species for metallothionein induction among organisms exposed to BC1, H3 and B.

For the stations B, H3, BC1 and H2, the induction of EROD activity in liver increases with the presence of PAHs in the sediment samples (B > H3 > BC1 > H2). Moreover, stations BC2, H1 and TM where PAHs were not detected, present a variable induction of EROD activity. EROD activity in liver of fish exposed to sediments from station BC2, H1, H3 and B were significantly different ($p < 0.01$) from control treatment.

3.4. Links between chemicals and tissue lesions

In order to correlate the physicochemical data with the sublethal responses measured in the organisms exposed to sediments, the MAA was performed using the set of data obtained for the 7 cases defined by the different sampling sites (BC1, BC2, H1, H2, H3, B and TM). In total we applied the MAA on 15 variables (10 chemical concentrations–As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, PCBs, PAHs–and 5 toxicity data—IGG and IGL in *S.aurata*, EROD activity in *S.aurata* and metallothioneins induction in both species) for the 7 cases. The application of MAA indicates that the original variables can be grouped in three new factors. These factors explain 80.5% of the variance in the original data set. The criteria selected to interpret a variable associated with a particular factor was a loading of 0.4 or higher (Table 3). This approximates Comrey's (1973) cut-off of a 0.55 or higher for a good association between an original variable and a factor, and also takes into account the discontinuities in the magnitudes of the loadings of the original variables. Each factor is described according to the dominant group of variables.

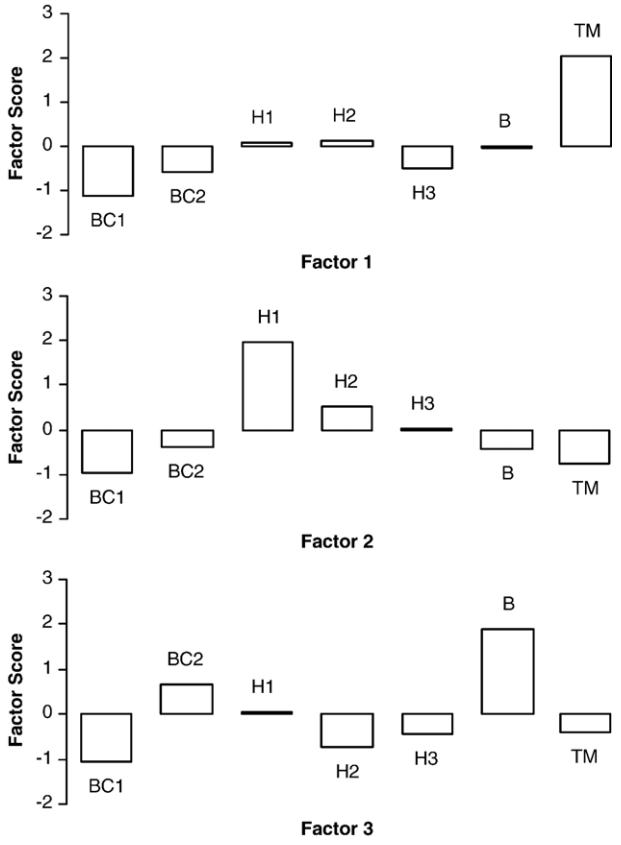

Fig. 4. Estimated factor scores for the three factors in each of the seven cases. The factor scores quantify the prevalence of every factor for each station and is used to establish the description of each factor. Factor 1: toxicity associated with the presence of metals in the sediments, combining the metals and all the indexes of lesions. Factor 2: effect due to the exposure to trace metals with the MTs induction in both species. Factor 3: toxicity of organic contaminants and combines the concentration of PAHs and PCBs in sediment and the EROD activity in liver.

The first principal factor, #1 accounts for 39% of the variance. This factor explains the toxicity associated with the presence of metals in the sediments, combining the metals (Cd, Zn — with lower loading than in #2, Pb and Hg) and all the indexes of lesions (gill, IGG; and liver, IGL). The MT in *S. senegalensis* also is included in this factor with lower loading than in #2. The second factor, #2 accounts for 27% of the variance. This factor relates the effect due to the exposure to trace metals (As, Cr, Cu and Zn) with the MTs induction in both species, *S. aurata* and *S. senegalensis*. The third factor, #3 accounts for 15% of the variance. This factor explains the toxicity of organic contaminants and combines the concentration of PAHs and PCBs in sediment and the EROD activity in liver.

In order to confirm these factor descriptions and to establish the relationship between components to define the environmental degradation at each of the studied stations, we propose a representation of estimated factor scores from each case (stations) to the centroid of all cases for the original data (Fig. 4). Factor 1, which could be defined as the toxic response of the fish to the metals Cd, Hg, Pb and Zn bound to sediment, has prevalence in the sediments of Huelva and the positive control (H1, H2 and TM). The values obtained in the analysis corresponding to factor 2, explains the induction of MTs due to the high concentration of the metals As, Cr, Cu and Zn in individuals exposed to sediment from the area of Huelva, and specially H1 with the value 1.99 (H2 = 0.52; H3 = 0.04). Finally, the factor 3 score explains the induction of EROD in individuals exposed to sediment from B and BC2 with high concentration of PAHs and PCBs, whereas the induction of this biomarker in the organisms exposed to sediments from station H1 could be related to the presence of any contaminant which has not been determined in the present study.

4. Discussions and conclusions

Results show the combined effects of chemical content in sediment and biological effects associated with histopathological lesions, MTs induction and EROD activity. The multivariate analysis approach (MAA), that previously has been shown as a useful tool to link sediment chemistry and biological effects with different proposes (DelValls and Chapman, 1998; DelValls et al., 2002), was used in this study. It grouped different metal with effect and sampling points. The highest difference observed was in sediment from toxic mud, which has a high concentration of metals associated with histopathological damage in gill and liver.

In our study, histopathological lesions seem to be mainly related to the metals Cd, Hg, Pb and Zn bound to sediments. The lesions observed in this study are similar to those previously reported in similar organisms when were affected by sediment contamination caused by metals and organic compounds (González de Canales et al., 1996 and Ortiz et al., 1999 in *Fundulus heteroclitus*; DelValls et al., 1998 in *S. aurata*; Riba et al., 2004c,d in *S. senegalensis*; Acosta and Lodeiros, 2004 in *Tivela mactroides*; Narváez et al., 2005 in *Perna viridis*), such as the hypertrophia, fusion of secondary lamellae, necrotic cells among others observed for *S. aurata* used in this study. Different kinds of contaminants produce different effects in several tissues organisms. In general, gill is the tissue that more affected by different contaminants probably because gill fish is a

multifunctional organ sensitive to chemicals in water, although in sediments collected in stations BC2, H1 and TM show pathologies in liver. Au (2004) reported that histopathological lesions in liver are not specific to pollutants, nevertheless there are numerous reports of histo-cytopathological changes in liver of fish exposed to a wide range of organic compounds and heavy metals (Hinton and Lauren, 1990; Hinton, 1994; Braunbeck, 1998). Besides, gill histopathological changes are, in general, non-specific to pollutant exposure (Au, 2004), although Wood (2001) reported structural changes in fish gills in response to toxicants exposure (organochlorines, petroleum compounds, heavy metals, etc).

On the other hand, the biomarkers of exposure selected (Metalothioneins and EROD activity) have shown that their are mainly specific to pollutant exposure (MTs to metals and EROD activity to organic contaminants) as it has been shown in previous studies (Whyte et al., 2000). Edwards and White (1999) observed high levels of EROD activity in the sites exposed to oil constituents in comparison with the control sites. Moreover, Lafontaine et al. (2000) have reported increases in EROD activities in many species of invertebrates after exposure to organic trace pollutants. This concur with our results; significant EROD induction has been measured in several sampling points, characterized by high concentrations of PAHs and PCBs. The role of MTs sequestering metals is well established as a protection function (Stegeman et al., 1992). Other investigators reported an increased the metallothionein concentrations in body burden with increasing cadmium exposure in crustaceans (Olafson et al., 1979; Olafson and Olsson, 1987; Pedersen et al., 1994). The present study demonstrates the significant relationship between MTs induction and the concentration of metals in sediment.

The toxicity tests used in this study identify the biological responses related to the sediment contamination and have permitted the establishment of differences in the toxic effects measured between stations and controls, validating the use of these species (*S. aurata* and *S. senegalensis*) in sediment assessment. The advantages of these sediment bioassays have been listed by several authors (DelValls and Conradi, 2000; Chapman et al., 2002). Bioassays showed results sensitive enough to determine the hazardousness associated with these dredged materials and, thus, its feasibility to be used as a complementary tool to classical chemical guidelines to the management of dredged material disposal options in Spain. Besides, the results explain the importance of using the chronic bioassays as a useful tool to determine the quality of the sediments and complete the limitations that offer the data of acute bioassays.

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