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SEDIMENT QUALITY IN THE ATLANTIC COAST OF SPAIN

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Abstract—Sediments from the Atlantic coast of Spain have been studied to evaluate environmental quality by using an integrated approach including chemical and toxicological data. Sediment samples were collected in four littoral ecosystems located in Spain, Bay of Cádiz, Guadalquivir River estuary, Ría of Huelva, and Ría of Coruña. To characterize the sediments, organic carbon, granulometric content, total sulfide, eight trace metals (Hg, Cd, Pb, Cu, Zn, As, Ni, and Cr), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) were measured. The toxicity of sediments was assessed with the amphipod *Ampelisca brevicornis*, the clam *Ruditapes philippinarum*, juveniles of the fish *Solea senegalensis*, populations of the estuarine rotifer *Brachionus plicatilis*, and populations of the bacterium *Vibrio fischeri* (Microtox®). The results obtained show that in general, stations located in the Ría of Huelva were associated with heavy metal contamination and with the highest toxicity. Only chronic toxicity tests were capable of identifying the effects associated with PCB concentrations. The sediment quality guidelines calculated by means of a multivariate analysis approach for contaminants not associated with biological effects (mg/kg) are Hg, 0.54; Cd, 0.51; Pb, 260; Cu, 209; Zn, 513; As, 27.4; and total PCBs, 0.05.

Keywords—Toxicity tests Contamination Sediment quality guidelines Multivariate analysis

INTRODUCTION

Sediments are significant in studies of pollution in aquatic environments and are known to transport various contaminants. Sediments also serve as a sink or source to the water column of some contaminants [1]. For a better assessment of the pollution process in the marine coastal environment, several authors have proposed determinations based on chemical measurements together with laboratory toxicity tests [2].

The complex nature of the sediment matrix and complex mixtures of numerous potential toxicants make it difficult to identify the components that cause biological effects. Some authors have pointed out that rigorous numerical analysis of relationships between sediment toxicity and contamination may identify significant associations that could determine the cause of toxicity [3]. Sediment toxicity tests can be performed with relatively simple bioassays designed to minimize the effects of naturally occurring properties of sediments and providing a rapid and integrated measure of the toxicological significance of contaminants bound to sediment [4]. In this sense, sediment toxicity tests provide information on the toxicity of contaminated sediments by measuring their effect on tested organisms. Furthermore, a battery of bioassays should be used to cover the different routes of exposure (whole sediment and sediment elutriate) and to ensure that the no-effect level in one test also will discriminate at several trophic levels [5].

In this paper, whole-sediment toxicity tests were applied by using four organisms and six endpoints: amphipod survival, clam mortality and reburial, fish and clam histopathological lesions, and inhibition of bioluminescence of the bacterium *Vibrio fischeri* (Microtox®, Carlsbad, CA, USA). Sediment elutriate toxicity tests were applied by using declines in rotifer populations and inhibition of bioluminescence of *V. fischeri*. The present article combines sediment contamination with sediment toxicity tests by using multivariate analysis to derive quantitative guidelines for the chemical contaminants measured (sediment quality guidelines [SQGs]) associated with effect. The objectives of this study were to establish sediment quality of samples collected in different areas affected by different sources of contamination by using an integrated method that applies sediment chemistry and toxicity assessment and to derive site-specific quality values for the ecosystems studied, defined as ranges of chemical concentrations (SQGs) associated with adverse effects.

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MATERIALS AND METHODS

Approach

The present study was developed by using 11 sediment samples collected in different littoral areas along the Atlantic coast of Spain (Fig. 1). Eight of the samples were located in the South of Spain: three from the Bay of Cádiz (BC1, BC2, and BC3), two from the Guadalquivir River estuary (GL and GR), and three from in the Ría of Huelva (H1, H2, and H3); and three located in the North of Spain from the Ría of Coruña (CO1, CO2, and CO3). The sampling sites represent different sources and origin of contamination. The Bay of Cádiz has low contamination, the Guadalquivir River estuary is affected by a mining spill (April 1998), the Ría of Huelva is affected by mining and industrial activities, and the Ría of Coruña is affected by heavy navigation activities. 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 [6].

Sediment samples were collected with a 0.025-m^2 Van Veen grab and transferred to a cooler. When sufficient sediment (about 40 L) had been collected from a particular station, the

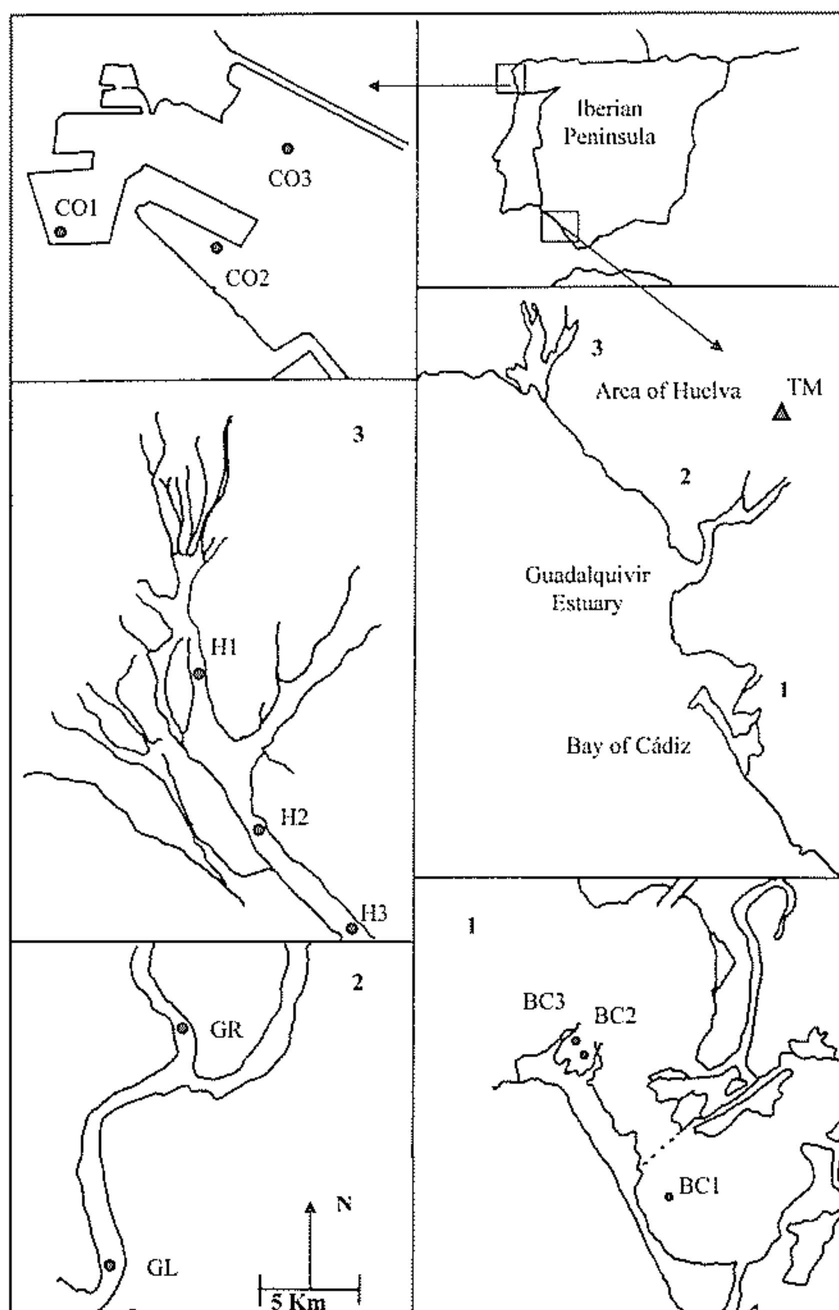

Fig. 1. Map of the Atlantic coast of Spain showing the general areas sampled and locations of the sampling stations. Three of the four studied sites are located in the South of Spain, the Bay of Cádiz (BC1, BC2, and BC3), the Guadalquivir River estuary (GL and GR), and the area of Huelva (H1, H2, and H3). The other studied area is located in the North of Spain in the area of Coruña (CO1, CO2, and CO3). The location of the toxic mud (TM) also is shown.

cooler was transported to the laboratory. The contents of the cooler were homogenized with a Teflon® spoon until no color or textural differences could be detected. The sediments were subsampled for physical characterization and chemical quantification (1.5-L aliquots). After that, sediment samples were maintained in the cooler at 4°C in the dark until their use in sediment toxicity tests and not longer than two weeks to avoid interferences in the toxicity results associated with the storage period [2,6].

Chemical analyses

For sediment grain size analysis, an aliquot of wet sediment was analyzed with a laser particle-size Fristch analyzer (model Analysette 22, Laval lab, Lavel, PQ, Canada) by following the method reported by DelValls et al. [7]. The remaining sediment was dried at 60°C before chemical analysis. Dried sediments were gently homogenized. Organic carbon content was determined by using the method reported by El Rayis [8]. For trace metal analysis, the sediments were digested as described by Loring and Rantala [9]. The concentrations of the heavy metals Cd, Ni, Cr, and Pb were determined with a Perkin-Elmer 4100 ZL graphite furnace atomic absorption spectrophotometer (Norwalk, CT, USA), and the concentrations of Cu and Zn were determined with a Perkin-Elmer 2100 flame atomic absorption spectrophotometer. Results are expressed as mg/kg dry sediment. Mercury was determined by cold vapor tech-

Table 1. Summarized description of sediment bioassays performed in whole sediment and in test elutriates collected on the Atlantic coast of Spain. The ET50 is the median effective time in the clam bioassays, TL50 is the median lethal time of 50% of the population of rotifers, and EC50 is the median effective concentration for the Microtox test with *Vibrio fischeri*

Sediment toxicity organism test	Temperature (°)	Duration	Endpoint
Ampelisca brevicornis (adult amphipod)	20	10 d	Survival (%)
Ruditapes philippinarum (juvenile bivalve)	20	48 h 10 d	Clam reburial ET50 (h), Survival, Histopathology
Solea senegalensis (juvenile flatfish)	20	30 d	Histopathology
Brachionus plicatilis (rotifer population)	25	7 d	Population decline TL50 (h)
Vibrio fischeri (bacteria population)	15	15–30 min	Luminescence decrease, EC50 (mg/L)

nique and As by hydride generation: in both cases a flow-injection hydride analysis system coupled with a Perkin-Elmer 4100 ZL graphite furnace atomic absorption spectrophotometer was employed. Total sulfides were determined by potentiometric titration (Metrohm, 670, Berchem, Belgium) by using a sulfur-specific electrode (F1212S, Radiometer, Westlake, OH, USA) and a reference electrode (Metrohm, 6.0726.100). Briefly, 1 to 5 g of sediment was buffered to a pH of 11.5 with 15 ml of a solution containing 0.2 mM $Na_2PO_4{\cdot}2H_2O$, 0.45 mM $NaNO_3$, and 0.1 mM NaOH. Treatments were performed in a nitrogen atmosphere adapted by Ortega [10]. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were analyzed by using a gas chromatography equipped with an electron capture detector (ECD) (U.S. Environmental Protection Agency method 8080) by following methods reported by Riba et al. [11]. All the analytical procedures were checked with reference materials (Marine Sediment References Material for Trace Metals–1, National Research Council (NRC), Certified Reference Material, 277 BCR, and Conceil National de Reserches Canada, 277 BCR, for heavy metals; and NRC_CNRC HS-1 for organic compounds) and allow agreement with certified values higher than 90%.

Temperature, salinity, and dissolved oxygen were measured during toxicity testing every day. Salinity was determined by an induction salinometer (Beckman RS-10, Franklin Lakes, NJ, USA). The pH (seawater scale) was measured with a potentiometric analyzer (Metrohm, 670) with a glass combination electrode (Metrohm, 6.0210.100). Oxygen concentration was measured by following the Winkler method as reported by Grasshoff et al. [12].

Toxicity tests

Five separate sediment toxicity tests were used to measure sediment toxicity in the selected sampling stations. Tests were chosen to cover a wide range of toxic responses and are described for each species (Table 1). Whole-sediment toxicity tests were conducted with the amphipod *Ampelisca brevicornis* [13], the estuarine clam *Ruditapes philippinarum*, and the juveniles of the fish *Solea senegalensis*. Sediment elutriate toxicity tests were conducted with populations of the estuarine rotifer *Brachionus plicatilis* [14] and the bacterium *V. fischeri* [15]. Both bacterial assays were performed on the solid phase and elutriates by following standard protocols [15].

In each bioassay, the temperature (20 ± 1°C), pH (7.8–8.2), salinity (33.8 ± 0.2), and dissolved oxygen (>5 mg/L, 60% saturation) were measured and controlled every day. Rotifer and Microtox tests had different temperatures, at 25 ± 1°C and 15 ± 1°C.

Amphipod assay

Individuals of the amphipod *A. brevicornis* were obtained from the clean reference area of the Bay of Cádiz. The estuarine amphipods (20 amphipods per tank) were exposed to bulk sediment with percent of survival after 10 d of exposure as the endpoint [13]. After this exposure period, the sediments were sieved and surviving amphipods were removed and counted. The sediment toxicity test was performed with 2-L glass beakers containing a 5-cm layer of test sediment (about 200 ml) overlaid with filtered clean seawater (~ 800 ml). The test was conducted in triplicate.

Clam assay

Clams (*R. philippinarum*) were obtained from an aquaculture farm and maintained in our laboratory under controlled conditions during a one-month acclimation period and before conducting the test. During this period, clams were fed with a mixture of microalgae (*Tetraselmis chuii*, *Isocrhysis galbana*, and *Chaetoceros gracilis*). The organisms were maintained in aerated tanks of about 20 L: the water was replaced (80%, v/v) every 3 d. After the acclimation (one month) the test was developed in whole sediment (2 L per duplicate) with 5-cm-deep sediment and 8 L of overlaying water, as reported by DelValls et al. [16] in 15-L tanks. Briefly, 40 previously acclimated organisms were added to the vessels and exposed for 10 d. Three different endpoints were selected to assess the toxic effects: the rate of burial of the clams during the first 48 h of exposure period (median effective time [ET50]); the percentage of mortality; and the histological lesions in digestive gland, gills, and gut tissues at the end of the experiment (10 d). General indexes of lesions (clam lesion index in digestive gland [CLID], clam lesion index in gills [CLIG], and clam lesion index in gut [CLIGU]) were calculated for each tissue as an average value of the clam damage semiquantified as previously reported by DelValls et al. [17]. Briefly, the frequency of the histological lesions for each organism in each replicate was used to derive an arithmetic average value semiquantifying from 0 (absence of lesion in all the organisms) to 3 (always present in all the organisms).

Fish assay

About 3 L (about 5 cm in depth) of previously wet-sieved (1-mm) aliquots of sediment per duplicate were placed in

aquaria of about 20 L total capacity and filled with clean seawater (12 L). Individual fish (14.21 ± 1.07 cm total length and 3.25 ± 1.50 g wt) were obtained from the aquaculture facilities at the Faculty of Marine and Environmental Sciences before the test. Randomly selected fish of both sexes were placed in the toxicity test and control aquariums by replicate (six fish per replicate). Fish were fed with artificial food (Gemma 0.9–1.6 mm, Trout, Oslo, Norway) two to three times per day. Water was replaced every 2 or 3 d by using a peristaltic pump. Histopathological lesions and survival at the end of the bioassay were selected as endpoints. General indexes of lesions were calculated for gills (fish lesion index in gills [FLIG]), gut (fish lesion index in gut [FLIGU]), and liver (fish lesion index in liver [FLIL]) as an average value of the fish damage semiquantified as previously reported by DelValls et al. [17]. Briefly, the frequency of the histological lesions for each organism in each replicate was used to derive an arithmetic average value semiquantifying from 0 (absence of lesion in all the organisms) to 3 (always present in all the organisms).

Rotifer assay

This test was conducted on sediment elutriates obtained by following methods recommended by the Society of Environmental Toxicology and Chemistry [18]. Briefly, sediment aliquots were maintained in rotation for 6 h with clean seawater at a sediment to water proportion of 1:4 (v/v), and then they were left for 24 h for the particles to settle. The water was centrifuged (1,800 *g* for 2 h) and the supernatant liquid was removed to obtain elutriates used in the tests. Bioassays were conducted in the next 48 h. The sediment toxicity test with *B. plicatilis* was carried out for 7 d under controlled conditions (Table 1). The rotifer inocula came from cultures in exponential growth phase, were fed on the microalga *Nannochloropsis gaditana*, and were exposed to a constant temperature of 25°C and continuous light (1,500–2,000 lux). To initiate tests, rotifers were sieved through a mesh of 63-μm pore size and transferred to a 2-L clean seawater flask without microalgal food and maintained for 48 h. Initial rotifer concentration was about 200 individuals per milliliter for each triplicate of test media (50 ml). The medium was not renewed and the rotifers were maintained unfed and exposed to a constant temperature of 25°C and continuous light during the test (7 d). Subsamples (0.5 ml) of each rotifer test (50 ml) were taken about every 8 h for 7 d and counted by stereoscopic microscopy (×50). A first count was done of dead rotifers (individual showing no internal or external movement for 10 s) and a second count was done of the total number of rotifers that previously had been fixed with 3% buffered formalin. The survival along the time of exposure was selected as the endpoint in this bioassay and the time required to kill 50% of the initial population (LT50) was derived, as reported by DelValls et al. [14,19].

Microtox testing

The bioassay of bioluminescence inhibition with the bacterium *V. fischeri* was conducted on solid and liquid phases with the commercial Microtox apparatus (model 500) by following the user's guide and standard protocols for both bioassays [15]. For the Microtox elutriate bioassay, sediments were centrifuged at 1,800 *g* for 10 min to clear the solution, then the supernatant liquid was removed for testing.

Data calculation and statistical analysis

The resulting parameters calculated from the sediment test and control replicates, percent of amphipod survival, clam reburial rates (ET50), and mortality (%), rotifer decline rates (LT50), Microtox effective sample percent (median effective concentration [EC50]), and general indexes of lesions for clams and fish were compared by analysis of variance and Scheffe's *F* tests to identify significant differences in sensitivity among test sediments and controls ($p < 0.01$). The parameters ET50 (h) and LT50 (h) were calculated by linear regression of log time. The toxic parameter for the Microtox test was obtained from standard software (EC50 in mg/L). The parameters ET50 and LT50 were calculated with a probit modified from the classic methodology by following the method reported by DelValls et al. [14,16].

Contamination and toxicity data were analyzed by factor analysis, by using principal components analysis as the extraction procedure, which is a multivariate statistical technique to explore variable distributions. The original data set used in the analysis included 13 chemical concentrations and 11 toxicity endpoints. Factor analysis was performed on the correlation matrix, that is, the variables were autoscaled (standardized) so as to be treated with equal importance. All analyses were performed by using the principal component analysis option of the FACTOR procedure, followed by the basic setup for factor analysis procedure (P4M) from the BMDP statistical software package [20]. Toxicity data on LT50 for the decline in the rotifer population and on the EC50 for the decrease in bacterial luminescence were transformed as the inverse to show an increase with biological damage ($LT50^{-1}$ and $EC50^{-1}$, respectively). The survival percentage of amphipod and clam were expressed as percent of mortality.

RESULTS

Sediment contamination

Results of heavy metal contamination, PCBs, PAHs, and parameters analyzed as organic carbon, grain size, and total sulfide are summarized in Table 2. In general, sediment samples had relatively similar texture and ranged between 60 and 90% of percentage of fines, except BC1 where the percentage of fines is approximately 2%. Levels of organic carbon in sediments were similar among stations and ranged between 0.7 and 4% except for sediments collected in the Ría of Coruña, which ranged between 5 and 7%. Total sulfides ranged between 0.2 and 7.6 μmol/g, except for station H1 (10.7 μmol/g) and station CO2 (12.6 μmol/g).

Of all the stations, BC1 showed the lowest values of chemical concentrations of heavy metals, PCBs, and PAHs, which could be related to the lowest values in this station of conventional parameters such as organic carbon content and fine sediment fractions. In general, the concentrations of heavy metals in the area of Huelva (H2 > H1 > H3) were higher than those measured in the samples from the Guadalquivir River estuary, in the Bay of Cádiz, and in the Ría of Coruña, although mercury showed the highest value in sediments from CO1. This station was contaminated in PCBs and their concentrations were higher than at CO2 and CO3. The highest concentrations of PAHs was measured in sediments from station CO2, which was considered to be contaminated with PAHs, as well as station CO1; intermediates values of PAHs were measured in stations CO3 and H2.

Amphipod survival

Summarized values of amphipod survival as well as other toxic responses for the different bioassays are shown in Figure

Table 2. Summarized results of chemical contaminants measured in different sediment samples along the Atlantic coast of Spain. Values of organic carbon (OC, % dry wt), granulometric content (% fines), total sulfide (TS, μmol/g dry wt), heavy metals (mg/g), polychlorinated biphenyls (PCBs, μg/kg), and polycyclic aromatic hydrocarbons (PAHs [mg Kg⁻¹]) are calculated at each station[a]

	Station										
	BC1	BC2	BC3	GL	GR	H1	H2	H3	CO1	CO2	CO3
% OC	0.71	2.54	2.27	1.81	2.51	3.95	3.03	2.59	5.97	7.53	5.07
% Fines	1.71	90.4	90.8	80	91	89.3	84.8	58.0	49.7	84.3	74.8
TS	0.21	5.14	4.54	2.89	1.81	10.65	3.01	1.22	7.59	12.63	4.81
Heavy metals											
Hg	ND	0.24	0.22	0.14	0.11	2.32	3.48	1.47	6.41	0.467	0.537
Cd	0.03	0.14	0.13	0.19	0.34	1.76	5.82	0.96	0.96	0.51	0.25
Pb	4.59	41.3	36.0	25.0	35.4	423	433	270	260	82.4	54.1
Cu	1.34	48.7	47.3	30.6	30.0	2012	2438	979	209	53.1	35.3
Zn	6.28	152	140	94.3	152	2551	2695	1310	513	191	135
As	1.85	7.33	5.77	5.24	9.36	343	527	213	27.43	22.5	13.6
Ni	1.70	26.4	28.9	23.9	31.3	33.1	37.2	21.4	19.9	20.0	19.2
Cr	3.47	58.6	56.8	40.2	50.1	64.8	73.5	43.5	28.7	31.4	33.4
PCBs											
PCB-28	ND	ND	ND	ND	ND	ND	0.4	ND	0.6	1.1	0.4
PCB-52	ND	ND	ND	ND	ND	ND	0.1	ND	3.4	0.9	0.3
PCB-101	ND	1.7	0.8	ND	ND	0.3	0.9	ND	32.2	5.6	2.5
PCB-118	ND	1.1	0.7	0.1	0.1	0.3	0.6	0.1	22.3	2.8	1.7
PCB-138	ND	3.9	1.9	0.2	0.1	0.6	1.8	0.3	49.1	11.1	7.9
PCB-153	ND	6.4	3.0	0.3	0.2	1.0	3.0	0.5	77.7	18.3	13.0
PCB-180	ND	7.4	3.1	0.2	0.2	1.0	3.2	0.6	69.1	19.0	14.6
PAHs											
Acenaphthene	ND	ND	ND	ND	ND	ND	ND	ND	0.09	0.06	0.02
Acenaphthylene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anthracene	ND	ND	ND	ND	ND	ND	0.02	ND	0.19	0.11	0.05
Benzo[*a*]anthracene	ND	0.02	0.02	ND	ND	ND	0.11	ND	0.53	0.55	0.26
Benzo[*a*]pyrene	ND	0.03	0.02	ND	ND	ND	0.11	ND	0.76	0.89	0.41
Benzo[*b*]fluoranthene	ND	0.03	0.02	ND	ND	ND	0.10	ND	0.67	0.75	0.36
Benzo[*ghi*]perylene	ND	0.02	0.02	ND	ND	ND	0.06	ND	0.44	0.63	0.30
Benzo[*k*]fluoranthene	ND	0.02	ND	ND	ND	ND	0.06	ND	0.36	0.38	0.17
Chrysene	ND	0.02	0.02	ND	ND	ND	0.11	ND	0.47	0.54	0.26
Dibenzo[*a,h*]anthracene	ND	ND	ND	ND	ND	ND	0.02	ND	0.16	0.17	0.09
Phenanthrene	ND	0.03	0.02	ND	ND	ND	0.14	ND	0.75	0.42	0.17
Fluoranthene	ND	0.05	0.03	ND	ND	ND	0.25	0.02	1.09	0.80	0.35
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND	0.11	0.06	0.03
Indene[1,2,3-*cd*]pyrene	ND	0.03	0.02	ND	ND	ND	0.07	ND	0.52	0.66	0.35
Naphthalene	ND	ND	ND	ND	ND	ND	ND	ND	0.09	0.04	ND
Pyrene	ND	0.04	0.03	ND	ND	ND	0.20	ND	1.12	1.01	0.35

[a] ND = not detected.

2. Mean survival from the amphipod test (10 d of exposure time) ranged between 97.5% at BC1 and 12.5% at H2. Stations GL, BC2, CO3, GR, and BC3 were not significantly different ($p < 0.05$) from the negative control (BC1). All other sediments were toxic ($p < 0.05$). The concentration of total ammonia in the interstitial water was monitored at the beginning and at the end of the test and ranged between 9 and 25 mg/L (pH ranged between 7.5 and 7.9) at all the stations. These concentrations are considered without influence on the toxic results produced by the chemicals.

Clam mortality and reburial

The results of the sediment toxicity test for the ET50 for reburial are shown in Figure 3. The ET50 was faster for the control station (BC1) compared to the rest of the stations, with the highest value that was significantly different from this station measured in H2 ($p < 0.05$; Fig. 2). In general, ET50 values were faster at stations located in the Bay of Cádiz (BC1, BC2, and BC3), GL, CO2, and CO3; slowest at H2; and intermediate at GR, H1, H3, and CO1. Examination of the data from this bioassay endpoint indicates that station H2 was significantly toxic ($p < 0.05$; Fig. 2). The rest of the stations were considered to be nontoxic when using this behavioral endpoint, based on the similarities in the toxic responses compared to the control.

No clam mortalities occurred in the control sediment (BC1) over the 10-d exposure period (Fig. 2). In general, examination of clam mortality data indicates that stations from the Ría of Huelva were significantly toxic ($p < 0.05$; Fig. 2), and sediments at station H2 were considered to be the most toxic for the clams. The rest of the stations were not toxic compared to the control ($p < 0.05$). The concentration of total ammonia in the interstitial water was monitored at the beginning and at the end of the bioassay, and was less than 20 mg/L (pH ranged between 7.5 and 7.9) in all the sediments and controls.

Rotifer population decline

Mean values of the LT50 (h) were calculated for each toxicity test station and the highest value was observed at BC1 (Fig. 2). The LT50 values were fastest at stations H2 and CO1, which were significantly different from the control ($p < 0.05$); slowest at BC1, BC2, and CO3; and intermediate at stations H3, H1, BC3, CO2, GL, and GR. Sediment elutriates at stations

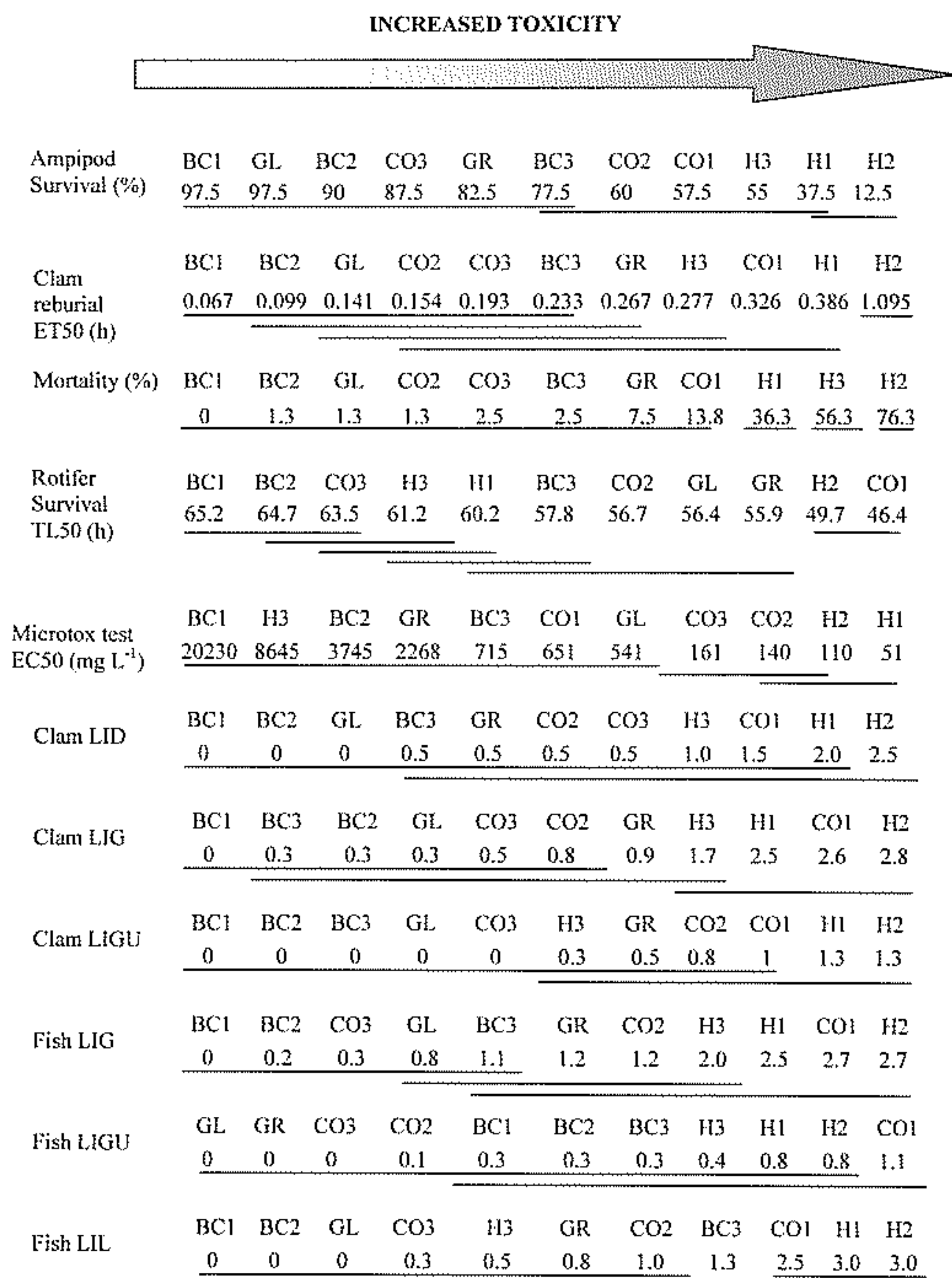

Fig. 2. Summary of the toxicity tests results (mean values). Treatments (stations, see Fig 1) not underlined by the same line are significantly different at $p < 0.05$ (Scheffe's F tests). ET50 = median effective time; LT50 = lethal time of 50% of the population; EC50 = median effective concentration; LID = lesion index in digestive gland; LIG = lesion index in gills; LIGU = lesion index in gut; LIL = lesion index in liver. See Figure 1 for study site locations.

H2 and CO1 were considered to be toxic ($p < 0.05$) for *B. plicatilis*.

Microtox bioassay

Only results obtained by applying the solid phase standard protocol are shown because the test carried out on elutriates produced values not significantly different for all the treatments compared to the controls. The sensitivity of the Microtox test conducted on elutriates of sediments determines that this test as a not valid tool to assess toxicity in the samples studied. Mean values of the quantity of sample needed to produce an EC50 (mg/L) for the decrease of the bioluminescence by the bacteria population were calculated for each treatment. Sediments from the Bay of Cádiz and Guadalquivir River estuary were not toxic, whereas sediments from CO2 were not significantly different from those from H2 and H1 ($p < 0.05$), with the lowest values of EC50 measured in H1. The concentration of sulfides was monitored during this bioassay and in that with rotifers. The rotifer bioassay has been shown not to be affected by sulfide concentrations, although the Microtox test is highly affected. In this sense, some of the results obtained in this test could be explained by sulfide levels found

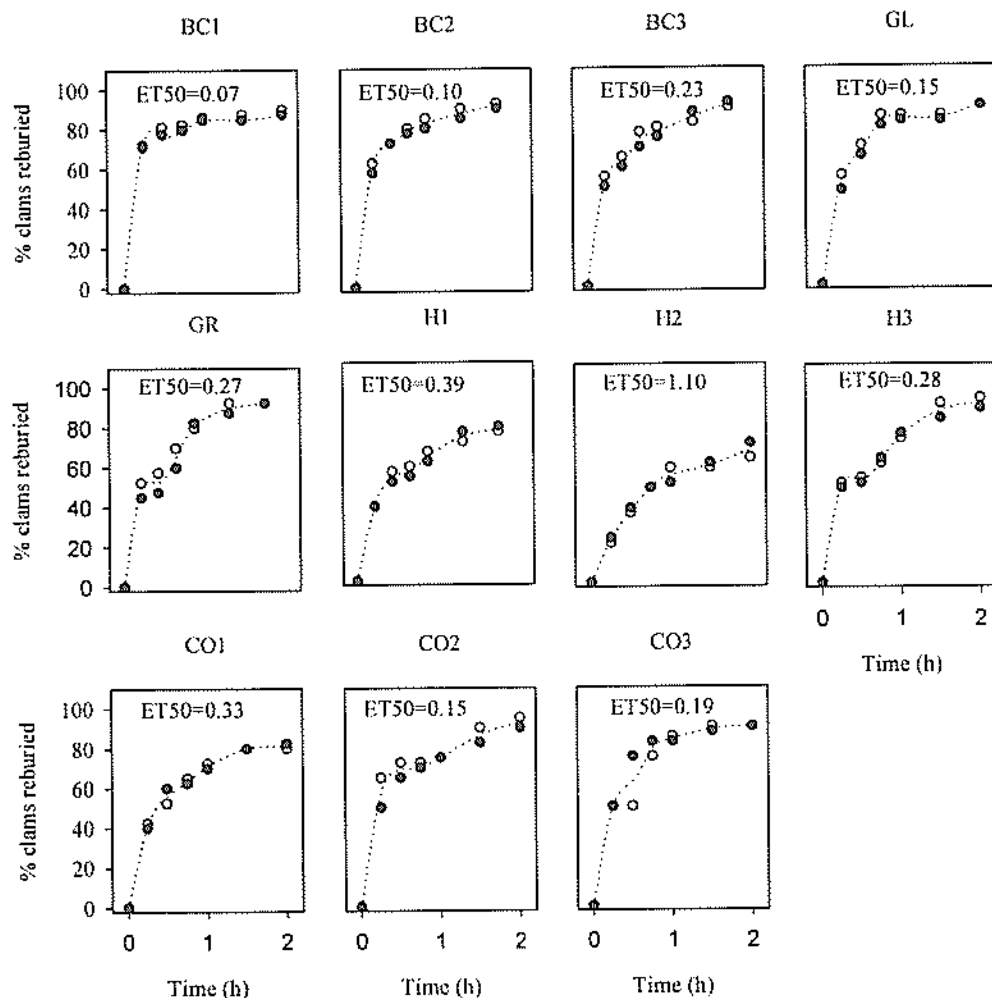

Fig. 3. Sediment toxicity results for the clam reburial test at each sampled location (BC1, BC2, BC3, GL, GR, H1, H2, H3, CO1, CO2, and CO3; see Fig. 1). The percentage of clams buried for each replicate is shown during the first 2 h of a total of 10 d of exposure. The median effective time (ET50) values, calculated as the time (h) required for the 50% of the population to reburied, are calculated from the plots and presented in the figures. See Figure 1 for study site locations.

in elutriates. On the other hand, in the Microtox test on the solid phase, the correction on grain size was used by following the method reported by Stronkhorst [21].

Fish and clam histopathological lesions

No fish mortality was measured in any treatments or at controls; 100% mortality was measured in the positive control (toxic mud). Histopathological alterations were evaluated semiquantitatively by ranking the severity of lesions (grades 1 [+], 2 [++], and 3 [+++]) as described in detail by previous studies carried out in the area [22]. A general index of damage for each analyzed tissue (FLIG, FLIGU, and FLIL for fish; and CLID, CLIG, and CLIGU for clams) was established to permit comparison of toxic responses between treatments and organisms (Tables 3 and 4). An arithmetic average value was obtained from the original semiquantitative assessment of the lesions for each tissue and organism (Tables 3 and 4). To derive these indexes of lesions, we used histomorphological alterations detected by microscopic observation of different tissues. Figure 4 shows examples of some of the lesions (Fig. 4b, d, and f) and controls (Fig. 4a, c, and e) measured in this study. The lesions observed in this figure are similar to those reported previously in similar organisms when affected by sediment contamination [23], such as the lamellae alteration (Fig. 4b), tubular alteration (Fig. 4b), and gill epithelial rupture observed for both kinds of organisms used in this study (Fig. 4). The damages identified in all the tissues analyzed almost always were present in animals exposed to sediments from stations H2 and CO1. The lesions detected in the tissues of fish and clams exposed to sediments collected in the Guadalquivir River estuary (Tables 3 and 4) are lower than those measured in the area of Huelva and Coruña and similar to those observed in organisms exposed to sediments from the Bay of Cádiz, although higher lesion indexes were found in animals exposed to sediments from station GR than from station GL. Fish exposed to sediments from the Ría of Huelva and the Ría of Coruña showed several types of damage, mainly associated with liver and gills.

DISCUSSION

In summary, sediments from stations located in the Ría of Huelva showed the highest toxicity to all the organisms included in the battery used in this study. The acute bioassays (amphipod, Microtox, and clam mortality) clearly detected the toxicity associated with the contaminants bound to sediments from the Ría of Huelva; however, the bioassays did not show a clear response to contaminants bound to sediments from the Ría of Coruña, except the amphipod test. The chronic bioas-

Table 3. Summarized semiquantitative results of lesions detected in microscopic abnormalities of individuals of the clam *Ruditapes philippinarum* exposed to sediments collected along the Spanish Atlantic coast. The common lesions are tubular alteration (AT) in the digestive gland, ciliar alteration (AC), connective alteration (CA) and lamellae alteration (AL) in gills, alteration in the intestinal epithelium (EI), and glandular alteration of the digestive ducts (TG) in gut. General lesion indexes in digestive gland (CLID), gills (CLIG), and gut (CLIGU) are calculated for the damage in each tissue and for each replicate. Lesions scores are (−) absent lesion, (+) frequent, (++) very frequent, and (+++) always present

		Station										
Tissue	Lesion	BC1	BC2	BC3	GL	GR	H1	H2	H3	CO1	CO2	CO3
Digestive gland	AT	−	−	+	−	−	++	+++	+	++	+	+
		−	−	−	−	+	++	++	+	+	−	−
	CLID	0	0	1	0	0	2	3	1	2	1	1
		0	0	0	0	1	2	2	1	1	0	0
Gills	AC	−	+	−	−	−	++	++	++	++	+	−
		−	−	+	+	+	+++	+++	+	+++	+	+
	CA	−	+	−	−	+	+++	+++	++	+++	−	−
		−	−	−	+	+	++	+++	+	++	+	+
	AL	−	−	+	−	+	+++	+++	++	+++	+	+
		−	−	−	−	+	++	+++	++	+++	+	−
	CLIG	0	0.6	0.3	0	0.7	2.6	2.6	2	2.6	0.6	0.3
		0	0	0.3	0.6	1	2.3	3	1.3	2.6	1	0.6
Gut	EI	−	−	−	−	+	++	+	+	+	+	−
		−	−	−	−	+	+	++	−	−	−	−
	TG	−	−	−	−	−	+	++	−	++	+	−
		−	−	−	−	−	+	−	−	+	+	−
	CLIGU	0	0	0	0	0.5	1.5	1.5	0.5	1.5	1	0
		0	0	0	0	0.5	1	1	0	0.5	0.5	0

says (rotifer) and those using sublethal endpoints (histopathological lesions) confirmed the toxicity detected by the amphipods in the area of Coruña. To better understand these differences in the toxic responses and, furthermore to derive SQGs for the areas studied, we have applied a multivariate analysis approach [16,24].

The results obtained after factor analysis to link the chemical and biological variables are shown in Table 5. The application of factor analysis to the chemical and toxicological data obtained in this study in the littoral samples (11 cases) indicates that original variables can be represented by five new variables, or principal factors. These new factors explain 96.12% of the variance in the original data set. In the present study, we selected to interpret a variable or group of variables as those associated with a particular factor where loadings were 0.3 or greater (Table 5), corresponding to an associated ex-

Table 4. Summarized semiquantitative results of lesions detected in microscopic abnormalities of individuals of the fish *Solea senegalensis* exposed to sediments collected along the Spanish Atlantic coast. The alterations detected are epithelial decamation (DE), lamellae alteration (AL), and alteration in cartilaginous tissue (ACR) in the gills; alterations in the mucosa (M), submucosa (SM), muscular (MS), and serosa (S) in the gut; and vacuolization in the hepatocytes (HR) and in the vascular system (AS) in the liver. General lesion indexes in gills (FLIG), gut (FLIGU), and liver (FLIL) are calculated for the damage in each tissue and for each replicate. Lesion scores are (−) absent, (+) frequent, (++) very frequent, and (+++) always present

		Station										
Tissue	Lesion	BC1	BC2	BC3	GL	GR	H1	H2	H3	CO1	CO2	CO3
Gills	DE	−	−	+	−	+	++	++	+	++	+	−
		−	−	−	+	+	++	+++	−	+++	+	+
	AL	−	+	+	+	+	+++	+++	+++	+++	++	+
		−	−	+	+	++	+++	+++	+++	+++	++	−
	ACR	−	−	+++	+	+	++	++	+++	++	+	−
		−	−	+	+	+	+++	+++	++	+++	−	−
FLIG		0	0.3	1.6	0.6	1	2.3	2.3	2.3	2.3	1.3	0.3
		0	0	0.6	1	1.3	2.6	3	1.6	3	1	0.3
Gut	M	−	−	−	−	−	+	+	+	+	+	−
		+	+	+	−	−	++	++	−	++	−	−
	SM	−	−	−	−	−	−	−	−	+	−	−
		+	+	+	−	−	+	+	+	++	−	−
	MS	−	−	−	−	−	−	−	−	+	−	−
		−	−	−	−	−	+	+	+	−	−	−
	S	−	−	−	−	−	−	−	−	+	−	−
		−	−	−	−	−	+	+	−	+	−	−
	FLIGU	0	0	0	0	0	0.25	0.25	0.25	1	0.25	0
		0.5	0.5	0.5	0	0	1.25	1.25	0.5	1.25	0	0
Liver	HV	−	−	+	−	+	+++	+++	−	+++	++	+
		−	−	+	−	+	+++	+++	+	++	+	−
	AS	−	−	+	−	+	+++	+++	+	++	+	−
		−	−	++	−	−	+++	+++	−	+++	−	−
	FLIL	0	0	1	0	1	3	3	0.5	2.5	1.5	0.5
		0	0	1.5	0	0.5	3	3	0.5	2.5	0.5	0

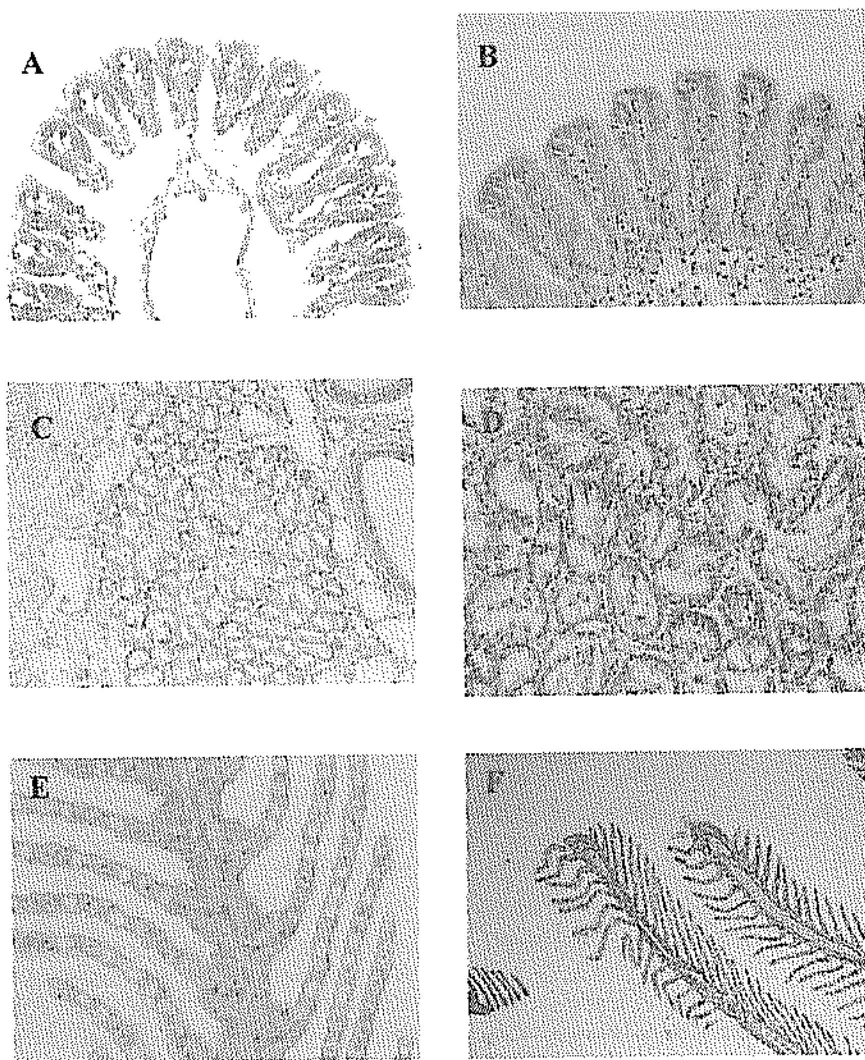

Fig. 4. Example of alterations caused by exposure of clams (*Ruditapes philippinarum*) and fish (*Solea senegalensis*) to sediments and controls collected along the Atlantic coast of Spain. These species were used to semiquantify the lesions shown in Tables 3 and 4. (A) Clam gill exposed to control (×20); (B) clam gill exposed to CO1 (×30); (C) clam digestive gland exposed to control (×30); (D) clam digestive gland exposed to H1 (×40); (E) fish gills exposed to control (×40); (F) Fish gills exposed to H2 (×10).

plained variance of over 65%. This approximates a cutoff of 0.55 for a good association between an original variable and a factor, and also takes into account discontinuities in the magnitudes of loadings approximately of these original variables [25]. Each factor is described according to the dominant group of variables. The first principal factor (factor 1) is predominant and accounts for 58.00% of the variance; this factor combines the chemical concentrations of all the metals, although Hg, Cr, and Ni group with other factors with higher loadings (factor 2, Cr, and Ni; factor 5, Hg) and all the biological responses, except that obtained in the chronic assay using the population of rotifers (LT50). This factor can be associated with the toxic responses associated with heavy metals and use of solid phase tests with both lethal and sublethal endpoints. Factor 2 accounts for 18.56% of the variance and combines the organic carbon and sulfide content of sediments, the concentration of organic contaminants (PCBs and PAHs, although they have higher loadings in factor 5), and two biological responses (CLI-GU and EC50), but these two endpoints have lower loadings than in the two other factors in which they grouped that have higher loadings (factor 1 and factor 5). Factor 2 represents the association of the organic matter and sulfides in sediments and the concentration of organic contaminants not associated with biological response. Factor 3 accounts for 10.45% of the variance and is a combination of sulfides and two metals (Ni and Cr) with the absence of biological effect. Factor 4 accounts for 6.12% of the variance and groups the biological response of the commercial test Microtox and the concentration of sulfides. The rotifer population decline endpoint (LT50) also is included but with negative loading, although this endpoint has a lower loading than in factor 5. This could be related to an effect on the test with *V. fischeri* associated with the concentration of sulfides in sediments. Finally, factor 5 accounts for the lowest variance (2.99%) and associates the sublethal endpoints measured as lesions in clam and fish tissues and the response measured in the chronic bioassay with the population of rotifers in sediment elutriates, with the concentration of Hg (higher than in other factors) and Pb (lower than in that loading in factor 1) and the concentrations of PCBs (higher than in factor 2) and PAHs (lower than in factor 2). This factor suggests that the biological effects determined by sublethal endpoints and chronic bioassays could be related to the concentrations of Hg mobilized from the sediment (rotifer assay) and to the sublethal effects provoked by the PCB concentrations.

Table 5. Sorted rotated factor loadings (pattern) of 24 variables on the five principal factors. The loading matrix has been rearranged so that the columns appear in decreasing order of variance explained by factors. Only loadings greater than 0.3 are shown in the table. Factors are numbered consecutively from left to right in order of decreasing variance explained. Toxicity endpoints ET50, LT50, and EC50 are from Table 1; chemical variables are from Table 2; and histopathological lesions are obtained from Tables 3 and 4

% Variance	Factor 1 58.00	Factor 2 18.56	Factor 3 10.45	Factor 4 6.12	Factor 5 2.99
% OC	—	0.945	—	—	—
% Fines	—	—	0.953	—	—
TS (μmol/g)	—	0.880	—	0.364	—
Hg (mg/kg)	0.445	—	—	—	0.857
Cd	0.905	—	—	—	—
Pb	0.891	—	—	—	0.338
Cu	0.964	—	—	—	—
Zn	0.948	—	—	—	—
As	0.978	—	—	—	—
Ni	0.404	—	0.906	—	—
Cr	0.457	—	0.859	—	—
Σ7PCBs (μg/kg)	—	0.418	—	—	0.855
Total PAHs (mg/kg)	—	0.826	—	—	0.454
Morty_A[a]	0.892	—	—	—	—
Morty_C[a]	0.910	—	—	—	—
ET50 (h)	0.857	—	—	—	—
CLID	0.874	—	—	—	0.392
CLIG	0.772	—	—	—	0.569
CLIGU	0.724	0.406	—	—	0.414
FLIG	0.691	—	—	—	0.602
FLIGU	0.550	—	—	—	0.759
FLIL	0.707	—	—	—	0.508
TL50 (h^{-1})	—	—	—	−0.370	0.794
EC50 (mg/L)	0.627	0.449	—	0.503	—

[a] Morty_A = mortality of amphipods; Morty_C = mortality of clams.

However, based on the low variance explained in this factor, it should be taken with caution. The feasibility of acute bioassays to identify the toxicity associated with compounds such as PCBs is being discussed. In this sense, other bioassays such as DR-CALUX [21] are recommend to establish the effects associated with these kinds of contaminants. The use of histopathology or bioaccumulation assays also is recommended [26].

To confirm these factor descriptions and to establish the SQGs for the Atlantic coasts of Spain, we propose a representation of estimated factor scores from each case (11 stations) to the centroid of all cases for the original data (Fig. 5). Factor 1 scores were negative in all the cases except for the stations located in the Ría of Huelva. On the other hand, the positive scores of factor 5 that were only measured at station CO1 and station H2 confirms the description of this factor related to the sublethal and chronic effects associated with PCB and Hg concentrations, respectively.

For the chemicals grouped with their highest loadings in factor 1 (Cd, Pb, Cu, Zn, and As) and in factor 5 (Hg and PCBs) we developed SQGs by following the procedures reported by DelValls and Chapman [24] and the factor scores shown in Figure 5 for the studied stations. In this approach, we used the prevalence (factor scores) of factors for each of the cases studied to make the three operative definitions based on the data obtained from the toxicity tests. It should be emphasized that this approach is based on comparing those chemicals that group under the same factor as the toxicity parameters (in this study, under factors 1 and 5). The assumption is that these chemicals presumably are correlated in a cause-and-effect manner. In this sense, when the factor score from factors 1 and 5, which suggest a correlation between the above chemicals and biological adverse effects, is 0 or below with respect to all stations (Fig. 5), the maximum concentrations of toxic chemicals at any of those stations represent the maximum chemical concentrations that are not associated with adverse effects. These are considered to be concentrations below which biological effects are low or minimal and here are indicated as not polluted. In contrast, to establish the minimal concentrations above which biological effects are always high, those minimal concentrations at stations where factor scores from factors 1 and 5 were higher than 0 were selected and described here as highly polluted. Also, an intermediate range of chemical concentrations representing an area of uncertainty, or a break point between the high and low concentrations, is shown and described as moderately polluted.

To facilitate the understanding of the mentioned process to derive SQGs, we have described the calculation for Hg (Table 6). The metal is included in the factors 1 and 5, so is correlated to biological effect (Table 5). Also, these factors are positive in stations H1, H2, H3, and CO1, and are negative in the other stations. To derive the guideline being considered not polluted (Table 6; for Hg = 0.54), we should find the higher concentration of Hg measured in the sediments from the stations with negative values for factor scores 1 and 5 (Fig. 5) from Table 2. This value is that measured in sediments from station CO3 (0.54). To derive the guideline being considered highly polluted, we should find the lower concentration of the chemical from the stations with positive values for factor scores 1 and 5 (Fig. 5) from Table 2. This value is that measured in sediments from station H3 (1.47). The moderately polluted value is considered as the uncertainty area between these two calculated values. A similar procedure can be conducted to determine the rest of SQG values shown in Table 6 for metals

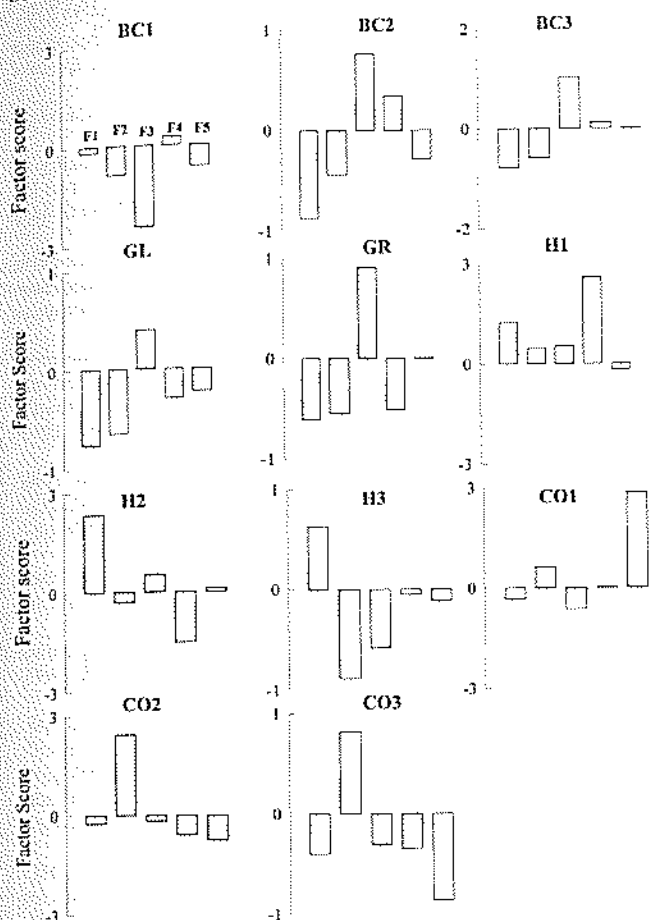

Fig. 5. Estimated factor scores from the 11 cases evaluated to the centroid of all cases for the original data. The factor scores quantify the prevalence of every factor for each station and it is used to calculate the sediment quality guidelines. See Figure 1 for study site locations.

Table 6. Summary of benchmark sediment quality guidelines (SQGs: mg/kg dry wt) proposed to evaluate sediment quality in different areas from the Atlantic coast of Spain for the heavy metals Hg, Cd, Pb, Cu, Zn, and As and the total of seven polychlorinated biphenyls (Σ7PCBs) associated with toxic effects and by using different approaches: A = this study; B = SQGs from Riba et al. [27]; C = SQGs from DelValls and Chapman [24]; D = SQGs from Long et al. [28]

	Sediment quality guideline		
Chemical study	Highly polluted	Not polluted	Area of uncertainty
Hg A	>1.47	≤0.54	0.54–1.47
B	—	—	—
C	>0.57	≤0.37	0.37–0.57
D	>1.30	≤0.15	0.15–1.3
Cd A	>0.96	≤0.51	0.51–0.96
B	>0.90	≤0.80	0.80–0.90
C	—	—	—
D	>5.0	≤4.1	4.1–5.0
Pb A	>270	≤260	260–270
B	>87	≤66	66–87
C	>115	≤49	49–115
D	>110	≤35	35–110
Cu A	>979	≤209	979–209
B	>53	≤45	45–53
C	>98	≤68	68–98
D	>70	≤90	70–90
Zn A	>1,310	≤513	513–1,310
B	>273	≤158	158–273
C	>225	≤156	156–225
D	>270	≤120	120–270
As A	>213	≤27.4	27.4–213
B	—	—	—
C	>64	≤58	58–64
D	>85	≤33	33–85
Σ7PCBs A	>0.254	≤0.054	0.254–0.054
B	—	—	—
C	>0.180	≤0.057	0.057–0.180
D	—	—	—

Cd, Pb, Cu, Zn, and As, by using the prevalence of factor score 1 and for the Σ7PCBs by using the prevalence of factor score 5. All of these values are shown in Table 6.

CONCLUSION

Determining SQGs is a difficult task and comparative evaluation of broad-scale data sets encompassing complex interactions based on sediment toxicological data provides a promising alternative method for developing site-specific SQGs. Furthermore, this method of deriving guideline concentrations provides an estimation based on interactions between complex chemical mixtures that may, individually or in combination, be responsible for the observed effects. The SQGs obtained in this study were compared to SQGs previously derived by different authors [24,27] in the area and SQGs proposed in areas of North America [28]. Highest values of SQGs were obtained in this study for heavy metals, which were strongly influenced by the results obtained in the heavily impacted area of Huelva, except for Cd and As. The SQGs derived for Hg and PCBs were similar to those previously reported in other littoral areas of the Gulf of Cádiz and in North America. The guidelines derived here could converge on appropriate SQGs for the Atlantic coasts of Spain, although further integrated studies should be carried out to be widely applied as part of a tier testing scheme to risk management in the system studied.

This study presents the results of a combined chemical and biological assessment of sediment quality in different areas located on the Atlantic coasts of Spain. Within the context of this study some conclusions are obtained and summarized below.

The battery of sediment toxicity tests used in this study to identify the biological responses related to the contamination detected was useful and permitted establishment of differences in the toxic effects measured between stations and controls. The inclusion in the battery of sublethal endpoints measured as histological lesions in clams and fish identified the effects caused by organic compounds in the absence of lethal and acute effects such as for Σ7PCBs. Additionally, the use of chronic bioassays conducted on sediment elutriates by using the decline in the population of rotifers identified chronic responses associated with the concentrations of Hg mobilized from sediments at station CO1.

The multivariate statistical method (factor analysis) used in this study provides a deeper insight into the structure of complex and diverse data. For example, this multivariate tool revealed groupings of varying degrees of correlation between chemical concentrations in sediment and biological effects. Moreover, the relationships between Cd, Pb, Cu, Zn, and As concentrations in sediment with the acute (lethal) effects, and Hg and Σ7PCBs concentrations in sediments with the chronic and sublethal effects with different organisms are strong enough to permit the establishment of SQGs.

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