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Evaluation of Heavy Metal Sediment Toxicity in Littoral Ecosystems Using Juveniles of the Fish *Sparus aurata*

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The toxicity of sediments from two littoral ecosystems of the Gulf of Cadiz was tested using juveniles of the fish *Sparus aurata* (seabream). Concentrations of total carbon and nitrogen, organic carbon, 14 heavy metals (Fe, Mn, Cu, Zn, Pb, Cd, Ag, Hg, As, Sn, V, Ni, Co, Cr), and the surfactant linear alkyl benzenesulfonate (LAS) in the sediments were measured. Chemical analysis was performed in the stations to determine the degree and nature of contamination. Four different endpoints were selected in the toxicity test: survival, superficial alteration, hematocrit analysis, and histological damage. After 14 days, survival, superficial alteration, and hematocrit analysis did not reveal effects of the different sediments tested. The histological and cellular damage revealed a more sensitive response to measured chemicals in sediments and they were found to be a powerful tool to evaluate sediment toxicity effects. Semiquantitative evaluation of the histological damage demonstrated correlation with sediment concentrations of some of the heavy metals (Cr, Cd, Pb, Ag, Cu) and the surfactant (LAS). Data derived from chemical concentrations and toxicity tests were assembled by multivariate statistical techniques (principal components analysis) to identify the ranges of chemical concentrations associated with an adverse effect. The results obtained, as suggested by site-specific sediment quality values, were the following: $Cr \geq 90.2$; $Cd \geq 1.24$; $Pb \geq 52.5$; $Ag \geq 0.68$; $Cu \geq 71.2$; $LAS \geq 8.7\ mg\ kg^{-1}$ of dry sediment. These results are mainly in concordance with studies performed in other areas of the world and therefore support wide application of the method.

INTRODUCTION

Contaminated sediments have been recognized as a significant environmental hazard, since they have the potential to form associations with several classes of anthropogenic pollutants. Contamination represents a possible source of stress for the benthic environment. To measure the stress, toxicological methods have been developed to monitor effects of sediment-associated pollutants on benthic organisms, populations, and communities (Luoma and Ho, 1992). Although contaminated sediments are only one component of the ecosystem, in some aquatic ecosystems, they are probably the major source of stress to the ecosystem health (Harding, 1992). The release of pollutants from contaminated sediments into the interstitial waters and overlying water column represents another environmental hazard posed by contaminated sediments (Larson, 1985).

Several methods developed for water column testing have been adapted for sediment assessments. These kinds of tests normally are used with elutriate or pore water exposures, and they provide nonlethal responses such as development and fertilization success (Williams *et al*., 1986; Long *et al*., 1990; DelValls *et al*., 1996). To examine the biological effects associated with sediment-bound chemicals in two shallow littoral ecosystems from the Gulf of Cádiz, juvenile *Sparus aurata* (seabream) were chosen. This species was selected because it is a common species, easily adapts to laboratory conditions, and has considerable economic importance in the Gulf of Cádiz and other areas in the world (Barnabi and Billard 1984), with intense growth and moderate to extensive aquaculture. 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 (Malins *et al*., 1984; Stein *et al*., 1992). An understanding of the cause-and-effect relationship between xenobiotics and fish diseases remains unclear. The first objective of this study was to determine if the contaminated sediments studied cause water column toxicity. For this purpose, different endpoints in a sediment toxicity test using juvenile *S. aurata* with the concentrations of 14 heavy metals and the surfactant linear alkyl benzenesulfonate in the same sediment sample are compared. The second objective is to identify the ranges in heavy metal and surfactant concentrations associated with adverse effects by means of a multivariate analysis [principal components analysis (PCA) and factor analysis].

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MATERIALS AND METHODS

Approach

The present study was carried out at seven stations in two shallow littoral ecosystems in the Gulf of Cadiz in the southwest of Spain (Fig. 1). Approximately half of each area ($20\,km^2$) has a water depth of less than 3 m and is almost stagnant. For this reason, contamination of the water column is determined largely by the process of chemical transfer across the water–sediment interface. Five sampling stations were selected in the Bay of Cádiz, which is $41.2\,km^2$ and dedicated largely to extensive and intensive marine

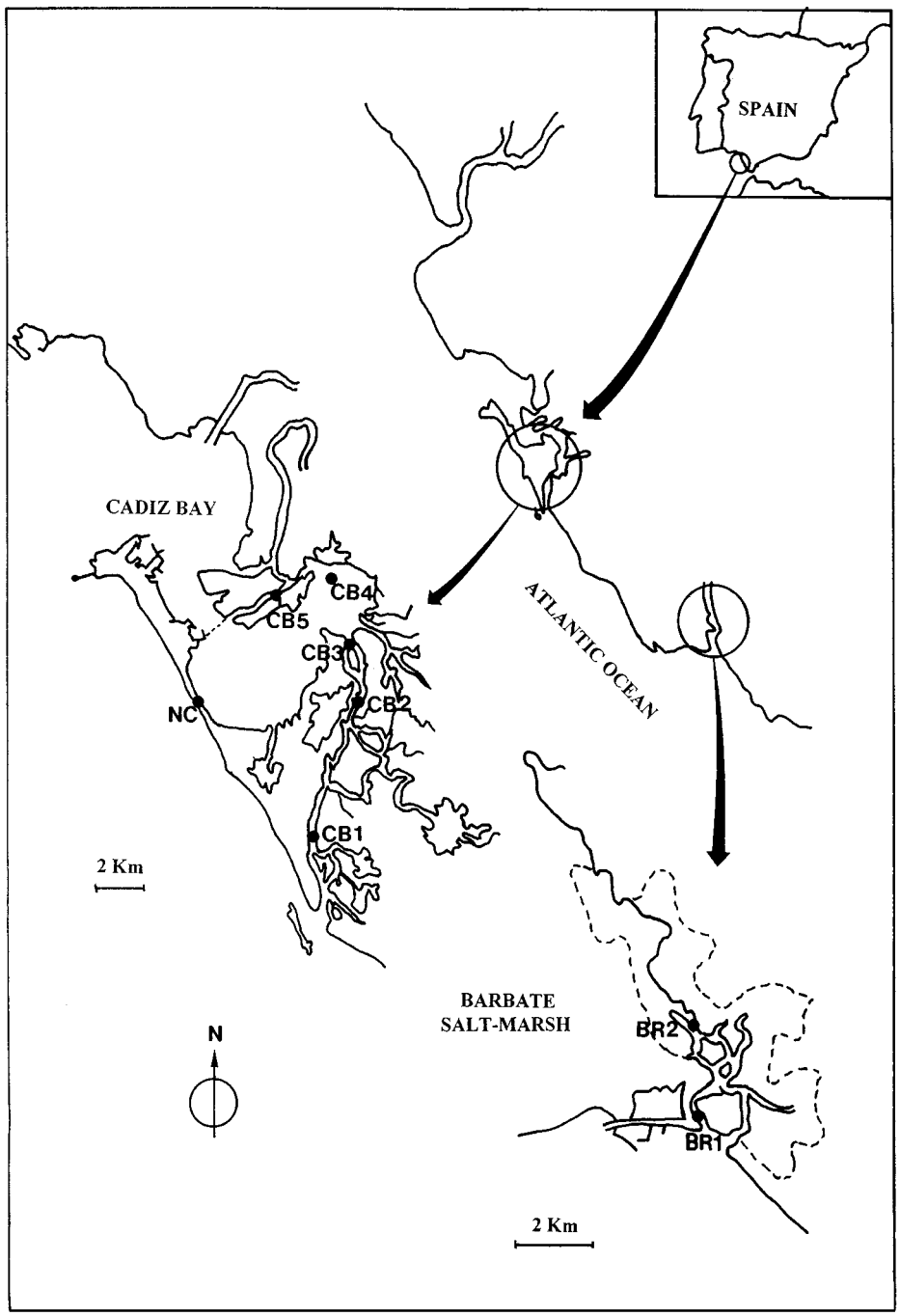

FIG. 1. Map of littoral ecosystems selected in the Gulf of Cadiz indicating locations of seven sampling stations and negative control (NC). The stations were grouped into two areas: the Bay of Cadiz (CB #) and the second Barbate river (BR #).

aquaculture. This semiclosed zone receives a large input of contaminants from the effluents of a population of approximately 600,000 inhabitants. In the last few decades, industrialization, especially the marine industry and car and aircraft component manufacturing, has increased. In addition, the good climatic conditions have caused an increase in recreational activities on the coast. The other two selected stations correspond to salt ponds from the Barbate River. This is a zone with a relative absence of contamination, receiving little urban effluent from a population of approximately 20,000 inhabitants and occasional fluvial effluents from farms draining into it. Therefore, stations were chosen on the best available information to represent presumably low, moderate, and high levels of chemical contamination (Gomez-Parra *et al*., 1984; Establier *et al*., 1985; Blasco *et al*., 1996; González-Mazo *et al*., 1997). Stations were called, in order of decreasing potential anthropogenic influences, CB2, CB3, CB5, CB4, and CB1 in the Bay of Cádiz and BR2 and BR1 in the Barbate River (Fig. 1). Clean sediment from Cádiz beach (CN, Fig. 1) was used as a reference together with samples of oceanic water (DelValls *et al*., 1996, 1997a).

Sample Collection

Sediment samples from each of the seven stations and the control were collected with a 0.025-m^2 Van Veen grab. Only grabs that had achieved adequate penetration (two-thirds of total volume) to collect the superficial 5 cm of sediment and that exhibited no evidence of leakage or surface disturbance were retained for this study. Sediment samples were placed in a cooler until sufficient sediment was collected from a particular station (at least 20 liters). The contents of the coolers were homogenized with a Teflon spoon until no color or textural differences could be detected. Then, the coolers, chilled with ice, were transported to the laboratory. Samples arrived at the laboratory 6–7 h after collection. The sediments were subsampled for chemical quantification and toxicological characterization (1.5-liter aliquots). Afterward, sediment samples were maintained in a walk-in cooler at 4°C in the dark until processing and analysis (toxicity test was carried out the next day after the final sample collection). Sediment was filtered (0.5 mm) prior to the toxicity test. Oceanic water was collected (36°21′N 6°34′W) using Niskin bottles, placed on ice and transported to the laboratory, and then filtered (0.45 μm) to be used as a clean reference control. Prior to sample collection, all beakers for the collection and storage of sediment samples were thoroughly washed with acid (10% HNO_3) and then rinsed in double-distilled (Milli-Q) water before each use.

Chemical Analyses

Sediment aliquots from each station were dried in an oven (60°C) prior to chemical analysis and then gently homogenized. Total organic carbon (TOC) content was determined using the method of Gaudette *et al*. (1974) with the E1 Rayis (1985) modification. Elemental analysis was carried out with a Carlo Erba CHN (Model 1106). Surfactants (LAS) were measured using the procedure described by González-Mazo *et al*., (1997). For trace metal analysis, the sediments were digested according to the protocol of Loring and Rantala (1992). Fe, Mn, Zn, and Cu concentrations in the extracts were determined by flame atomic absorption spectrophotometry with a Perkin–Elmer 2100. Hg and As concentrations were determined with a Perkin–Elmer MHS-FIAS coupled to a Perkin–Elmer 4100 ZL spectrophotometer. The other trace metals were measured by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer, 4100 ZL). Results are expressed as milligrams per kilogram of dry sediment. The analytical procedure was checked using reference material (MESS-1 NRC and CRM 277 BCR).

Sediment Bioassays

Toxicity tests were conducted on juvenile *S. aurata* obtained from an aquaculture farm and transported to their laboratory. The fish were acclimated to test conditions for 1 week. A baseline group of 10 randomly chosen individuals were weighed to provide data for feeding calculations and later weight comparisons. At the end of the acclimation period, the specimen size varied from 3.2 to 4.9 cm total length and weight averaged 3 ± 1 g. At the beginning of the experiment, another baseline group of 10 randomly chosen individuals were measured, weighed, necropsied, and processed to be used as the initial cellular control. Two negative controls [clean seawater (SW) and clean sediment (CN)] and sediment from the seven stations (CB1, CB2, CB3, CB4, CB5, BR1, BR2) were tested in duplicate using 35-liter glass tanks containing 20 individuals each; 6 liters of control and test sediments and 24 liters of clean seawater were placed in each tank. Fish were fed artificial food three times per day. Aeration was provided to maintain adequate oxygen concentrations (80% saturation). Temperature in a photoperiod room (12 h light and 12 h dark with a luminosity of 1000–1500 lx) was constant at 19 ± 1°C. Everyday, 33% of the water column was renewed with clean seawater during the 14 days of exposure.

Data Calculation and Statistical Analysis

When the water was renewed, the survival rate for all tanks was determined. During the test survival was evaluated, and at the end of the toxicity tests, superficial alterations (erosion), hematocrit, histological damage, as well as survival, were evaluated. The exposure was completed on Day 14, when all surviving fish were removed from the tanks. Survival was evaluated in each tank. Then, a sample

of 10 fish was taken from each tank for processing. The fish were measured, weighed, and examined externally. Blood samples were taken to determine hematocrits. The fish were quickly killed. Samples of gill, liver, and intestine were fixed in 0.1 M formaldehyde–phosphate buffer, pH 7.2, and embedded in paraffin. Sections 5–7 μm thick were stained with hematoxylin–eosin or hemaetoxylin–VOF (Guitérrez, 1990).

Water in all the tanks was monitored regularly for salinity, temperature, pH, and dissolved oxygen.

Significant differences between test sediments were determined by ANOVA and by Scheffe multiple comparison tests ($P < 0.001$).

The contamination and toxicity data were analyzed using PCA. PCA was performed on the correlation matrix; the variables were autoscaled (standardized) to be treated with equal importance (DelValls *et al.*, 1997a,b; DelValls and Chapman, 1997). All analyses were performed using the BMDP statistical software package (Frane *et al.*, 1985).

The quality assurance/quality control (QA/QC) program followed the recommendations of Chapman (1988) and ASTM (1991).

RESULTS

Sediment Contamination

Sediment samples had relatively similar texture, being dominated by the clay fraction (DelValls *et al.*, 1997b). At station BR1, the sand proportion (higher than 20 μm) was 7%, whereas the rest was 1%. Levels of organic matter in the sediments were similar and in the normal range for shallow littoral ecosystems [1–3% by dry weight (Dermott and Munawar, 1993)]. A few differences in the concentrations of the major elements (Fe and Mn) were observed between stations (Table 1). Of all the stations, BR1 revealed the lowest values for both metals. At station CB2, the concentration of LAS was notably higher than at the other stations. Cd and Cr concentrations at stations CB5, CB3, and CB2 were, in general, higher than those observed at other stations. Exceptionally high values for Hg concentration at station CB3 and, particularly, for Cr concentration at station CB5 were noted.

Sediment Bioassay

Salinity, temperature, and pH were similar in all tanks (Table 2). Oxygen concentration was near saturation in all toxicity test duplicates. Mortality was observed only at station CB2 ($12.5 \pm 2.5\%$). Hematocrits of fish from the sediment test and control tanks were not significantly different. Only some fish collected from CB2 (four individuals) and CB5 (two individuals) toxicity test tanks exhibited slight fin and dermis lesions. None of those signs were detected in individuals from the other test or control sediments. Differences with respect to body weight and general appearance were not significant. On the other hand, evaluation of the histology of gills, intestine, and liver revealed clear differences between control individuals (Fig. 2) and

TABLE 1
Concentrations of Organic Carbon and Nitrogen, Total Carbon, 14 Heavy Metals, and Tensioactive LAS in Sediment Samples Exposed to Juvenile *Sparus aurata*[a]

Contaminant	CB1	CB2	CB3	CB4	CB5	BR1	BR2
Organic carbon	1.49	3.01	2.71	3.03	2.76	0.62	1.56
Total carbon	3.06	3.73	4.19	4.91	3.89	5.21	2.01
Total nitrogen	0.10	0.23	0.19	0.20	0.21	0.01	0.11
Fe	29,650	36,170	34,970	27,440	28,530	1,350	37,370
Mn	329	201	324	396	293	79	255
Zn	107	185	188	149	128	19	125
Cu	49.4	92.4	71.2	48.8	43.9	13.2	45.3
Pb	25.7	64.7	50.3	51.2	52.5	6.9	26.7
Cd	0.52	0.93	0.87	0.84	1.24	0.94	0.39
Cr	64.1	90.2	67.9	60.3	164.9	39.7	86.2
Ag	0.38	1.59	1.01	0.60	0.64	0.68	0.5
Hg	0.14	0.32	1.02	0.47	0.45	0.27	0.10
V	75.3	125.4	100.4	85.3	75.3	3.1	134.2
Ni	25.5	43.8	34.3	30.5	26.3	1.2	43.6
Co	6.97	8.56	8.48	8.39	6.95	0.40	11.33
As	8.70	7.42	10.88	11.31	9.18	5.12	7.89
Sn	23.4	12.4	14.1	8.1	15.1	1.1	7.4
LAS	1.9	26.7	8.7	1.2	2.3	1.7	2.5

[a] All concentrations are expressed in mg kg^{-1} except those of organic carbon, total nitrogen, and total carbon, which are expressed in percentage of dry sediment.

TABLE 2

Summarized Results of Water Quality Parameters (Salinity, Water Temperature, pH) Measured in the Toxicity Test and Control Tanks for *Sparus aurata* Sediment Toxicity Testing

Parameter	CN	SW	CB1	CB2	CB3	CB4	CB5	BR1	BR2
Salinity	34.3	34.5	34.1	33.9	34.0	34.1	33.9	34.0	34.0
T (°C)	19.0	19.0	19.1	18.9	18.8	18.9	19.0	18.8	19.1
pH	8.01	8.07	7.91	8.05	8.02	7.83	7.91	8.03	8.10

those from the sediment test tanks (Figs. 3–5). Tissue damaged was classified into three different groups: (a) weak alteration (Fig. 3); (b) medium alteration (Fig. 4); (c) strong alteration (Fig. 5). Semiquantitative evaluation of damage was done for each tissue, followed by determination of an average value (from each duplicate). Both values were then combined as a single measure to provide an overall average value for each station (Table 3). Histological alterations varied depending on the sediments to which the specimens were exposed and the tissue analyzed. Thus, individuals exposed to CB2 and CB5 sediments exhibited the strongest alterations, being strong in the gills and medium in the intestine and liver. Specimens exposed to CB3 sediments demonstrated medium histological alterations of the gills and weak damage to the intestine and liver. Juvenile *S. aurata* in contact with the remaining sediments exhibited weak alteration of the gills, whereas no histological damage was detected in intestine or liver. No alterations were measured in the two controls. The average value of disease caused by the sediments from the group of stations comprising CB2, CB3, and CB5 was significantly higher than that for the group comprising CB1, BR1, BR2, and CB4 and both negative toxicity controls (SW and CN). Within the group of CB2, CB3, and CB5, sediment at station CB3 can be considered moderately toxic and sediments at CB5 and CB2, highly toxic. In other words, toxic chemicals in the sediment of the latter two stations may have been becoming bioavailable and, as a result, stressing the system. Lesions of the gills were more severe than in the other tissues, principally because the gills having easier and more direct interaction with chemicals adsorbed by the sediment and later dissolved in the water column.

Links between Chemicals and Tissue Lesions

For a better understanding of the relationship between chemicals and damage, possible correlations between chemical concentrations measured in the sediments and the prevalence of the tissue lesions assessed in fish exposed to those sediments were investigated. A multivariate analysis (PCA) was applied to the sediment concentration data of 14 heavy metals, LAS, TOC, the relation C/N, and the

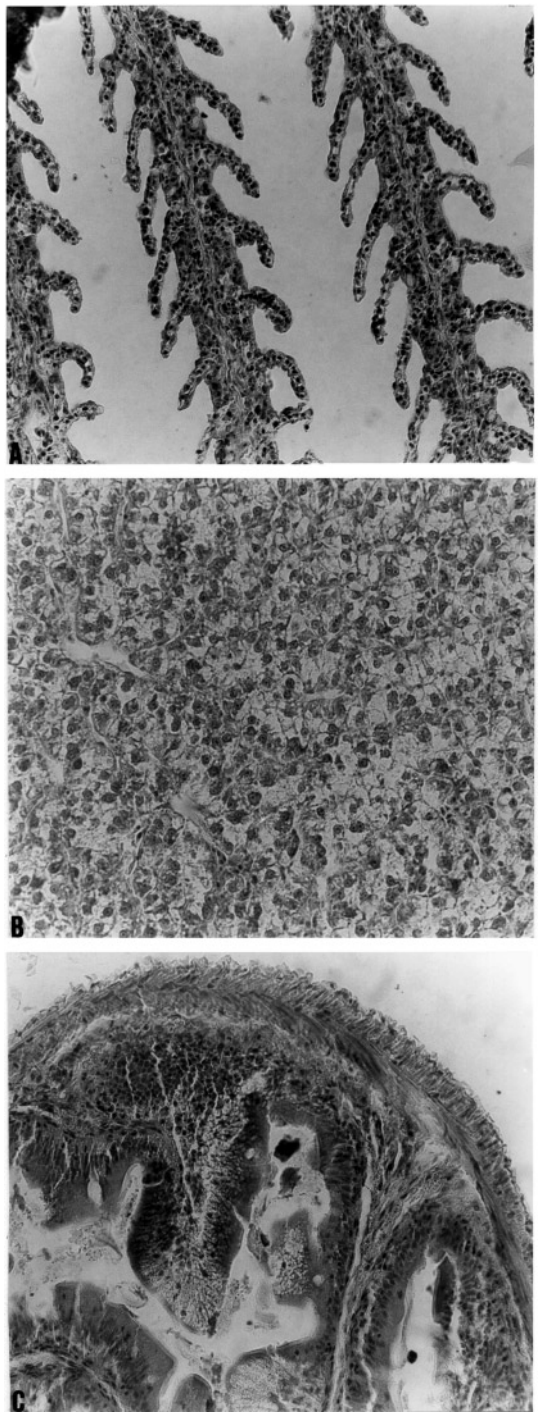

FIG. 2. Histological sections of gills, liver, and intestine of juvenile *Sparus aurata* specimens from control stations (SW and NC). Hematoxylin–VOF. (A) Normal histological section of juvenile *S. aurata* illustrating a gill lamella on a gill filament with squamous epithelium and interlamellar and mucous cells. The vacuolar system (capillary lumen and erytrocytes) is visible. Original magnification, 100 ×. (B) Normal histological section of liver with normal hepatocytes with granular cytoplasm, nuclei, and nucleoli. The vacuolar system is evident. Original magnification, 250 ×. (C) Histological section of intestine indicating different portions of the mucosa with goblet and epithelial cells, as well as lipid vacuoles. Original magnification, 250 ×.

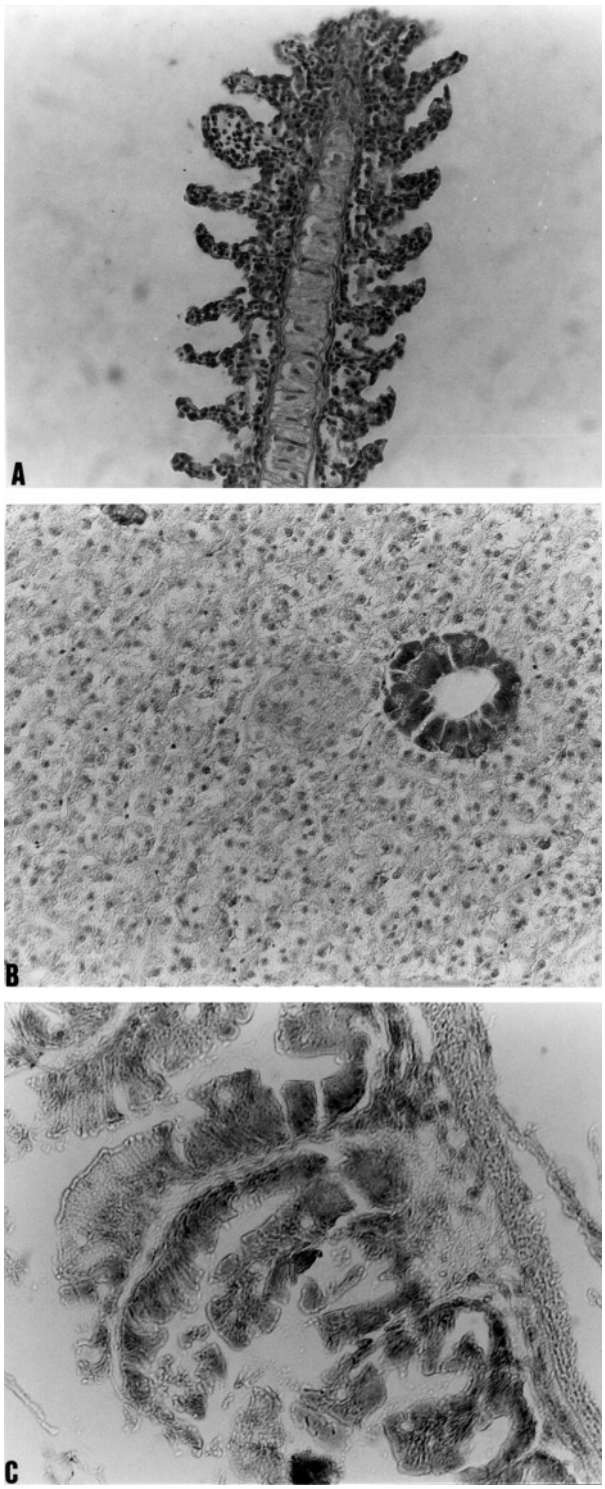

FIG. 3. Histological sections of different tissues of *Sparus aurata* specimens. Evaluated histological alterations are weak and indicated as + in Table 3. Hematoxylin–eosin. (A) Histological section of a gill lamella demonstrating a slight early hypertrophy of epithelial cells covering secondary gill lamellae. Original magnification, 250×. (B) Normal aspect of the intrahepatic pancreas and weak disorganization in the liver. Original magnification, 250×. (C) Desquamation of epithelial cells of the mucosa. Original magnification, 250×.

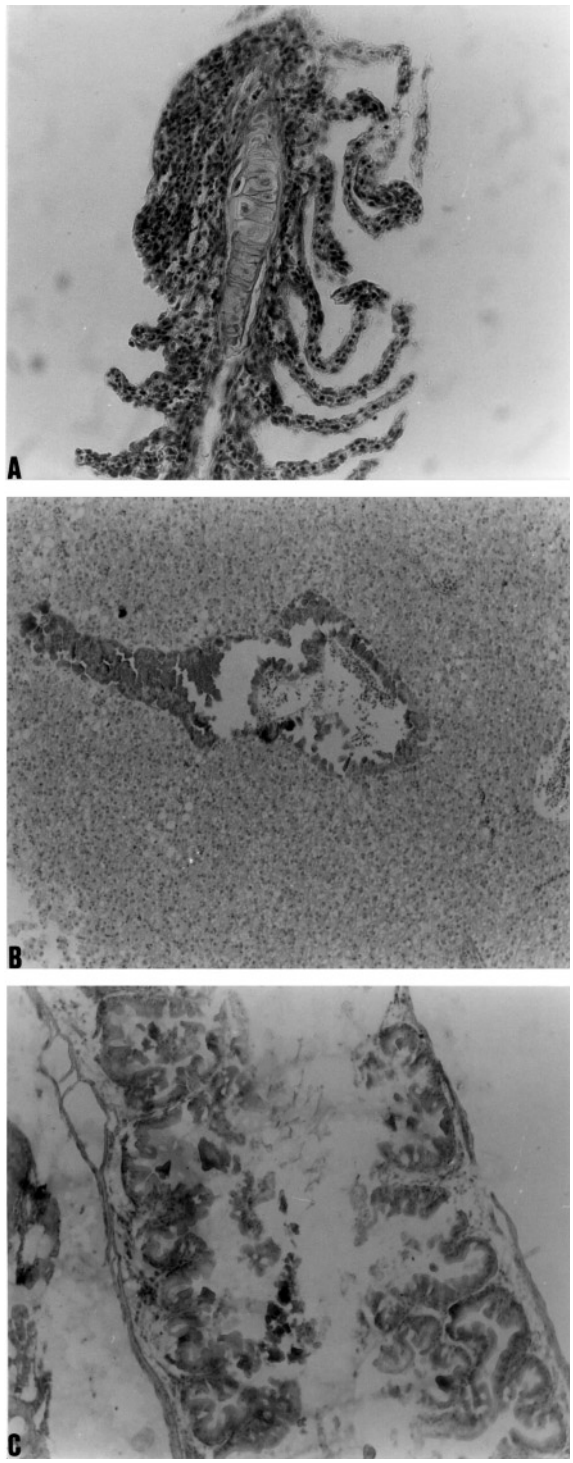

FIG. 4. Histological sections of different tissues of juvenile *Sparus aurata*. The moderate histological alterations are indicated as ++ in Table 3. Hematoxylin–eosin. (A) Lamellar hypertrophy and hyperplasia in the epithelium of gill lamellae and important alterations at the base of secondary lamellae. Original magnification, 250×. (B) Necrosis in the intrahepatic pancreas and strong vacuolization in the hepatocytes. Original magnification, 100×. (C) Disorganization and necrosis of the intestinal mucosa epithelium with general cellular disorganization and partial leakage of cellular integrity and necrosis of epithelial cells. Original magnification, 250×.

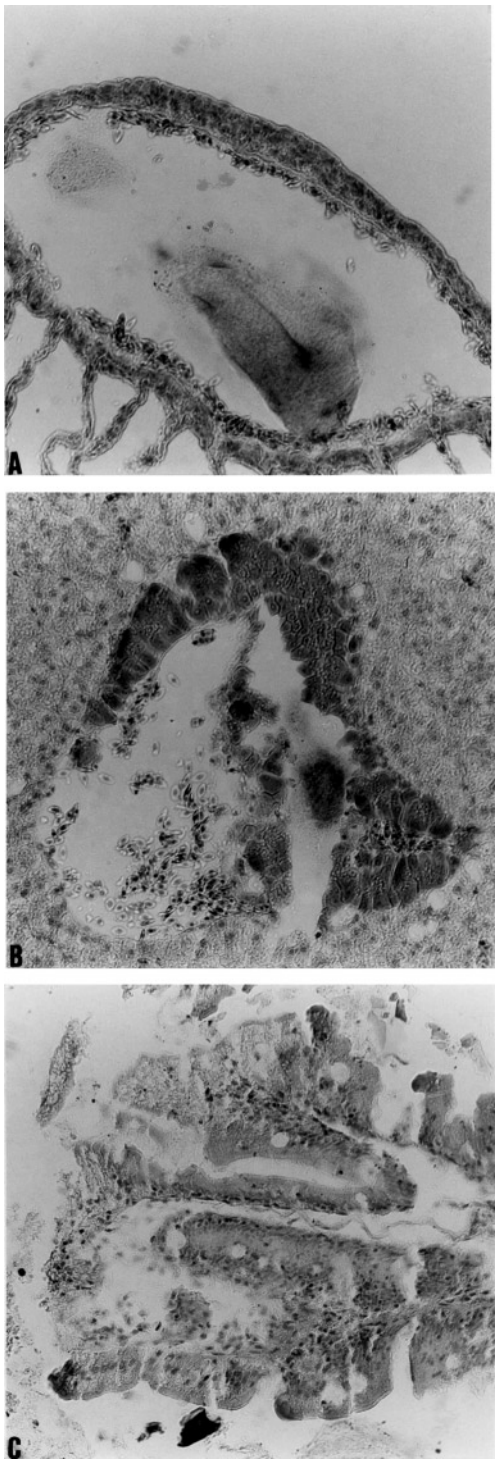

FIG. 5. Histological sections of different tissues of *Sparus aurata* specimens. Histological alterations are strong and are indicated as + + + in Table 3. Hematoxylin–eosin. (A) Intense dilation of gill lamellae and thrombosed lamellar telangiectasis. Original magnification, 250 ×. (B) Pancreatic and hepatic necrosis with evident signs of pycnosis and Karyolysis with partial or total disappearance of zymogen granules. Original magnification, 250 ×. (C) Intense disorganization of the intestinal mucosa epithelium with strong necrosis of the absorptive (enterocytes) and mucous or goblet cells. Original magnification, 250 ×.

TABLE 3
Semiquantitative Results of the Histological Damage Detected in a Sediment Toxicity Test of Juvenile *Sparus aurata*[a]

Station	Gill	Liver	Intestine	Average
NC	−	−	−	0.00
SW	−	−	−	0.00
CB1	+	−	−	0.33
CB2	+ + +	+ +	+ +	2.33
CB3	+ +	+	+	1.33
CB4	+	−	−	0.33
CB5	+ + +	+ +	+ +	2.33
BR1	+	−	−	0.33
BR2	+	−	−	0.33

[a] The number of plus symbols is proportional to the assessed damage. The average is calculated by assigning the value 1 to each plus symbol.

biological effects on *S. aurata* assessed as histological alterations in three different tissues (gills, intestine, and liver) from the seven sampling stations. The application of PCA to the variables described above indicates that those variables can be described by four new variables or principal components (Table 4). These explain 92.0% of the variance in the original data set. The criterion selected to interpret a variable associated with a particular factor was a loading of

TABLE 4
Sorted Rotated Factor Loadings (Pattern) of 20 Variables on the Four Principal Components[a]

Variable	No. 1 (56.18%)[b]	No. 2 (22.52%)	No. 3 (11.86%)	No. 4 (9.44%)
V	0.944	—	—	—
Co	0.941	—	—	—
Ni	0.926	—	—	—
Fe	0.921	—	—	—
C/N	− 0.818	—	—	—
Zn	0.642	—	0.444	0.571
Cr	—	0.887	—	—
Gill effect	—	0.885	0.431	—
Intestine effect	—	0.885	0.431	—
Liver effect	—	0.830	—	—
Cd	− 0.580	0.741	—	—
Pb	0.403	0.565	0.429	0.531
Ag	—	—	0.940	—
LAS	—	—	0.877	—
Cu	0.596	—	0.666	—
As	—	—	—	0.926
Mn	0.464	—	—	0.808
TOC	—	0.497	—	0.651
Hg	—	—	0.406	0.613
Sn	0.405	—	—	0.401

[a] Only loadings greater than 0.4 are provided. Components are numbered consecutively from left to right in order of decreasing variance explained.

[b] Percentage variance in parentheses.

0.4 or higher; this approximates Comrey's (1973) cutoff of 0.55 for a good association between an original variable and a component, and also takes into account discontinuities in the magnitudes of the loadings of the original variables. Each component is described according to the dominant group of variables.

Component 1: Sedimentary matrix and organic matter. The first factor accounts for 56.18% of variance; this combines the concentrations of V, Co, Ni, Fe, Zn, Pb, Cu, Mn, and Sn with positive values and C/N and Cd with negative values. This factor could represent the sedimentary matrix and includes organic matter, Fe, and Mn. The oxides of manganese and iron and organic matter have been emphasized as important scavengers of heavy metals (Arakel and Hongjun, 1991).

Components 2 and 3: Chemical concentrations associated with biological adverse effects in Sparus aurata. The second and third factors account for 22.52 and 11.86%, respectively. The second factor includes the chemical concentrations of Cr, Cd, Pb, TOC, and the adverse effects on the gills, intestine, and liver, whereas the third factor includes the chemical concentrations of Zn, Pb, Ag, LAS, Cu, Hg, and adverse effects on the gills and intestine. Both factors reveal the relationship between some chemical concentrations in sediments and the adverse biological effects observed in tissues of *S. aurata*. Nevertheless, the differences between the factor loadings for components 2 and 3 could be a consequence of the different bioavailability of some heavy metals and, consequently, their capacity to exert a toxic effect. In this sense, those heavy metals associated with component 2 appear correlated with alterations in three tissues, whereas the contaminants associated with component 3 are associated with lesions of only two of the three tissues evaluated. Based on the variance explained for each component (No. 2, 22.52; No. 3, 11.86), and because the chemicals associated with component 2 are correlated with alterations in three tissues, whereas those associated with component 3 are associated only with gill and intestine lesions, it was possible to classify those chemical concentrations associated with component 2 as more stressing to *S. aurata* than those associated with component 3 (Table 4).

Component 4: Heavy metals associated with nautical activities. Finally, the fourth factor accounts for 9.44% of the variance and includes Zn, Pb, As, Mn, TOC, Hg, and Sn. This factor represents the input of those heavy metals in sediments as a consequence of contamination processes associated with nautical activities and the use of antifouling paints. The heavy metals included in this component have been related to this kind of contamination by several authors (i.e., Bryan and Langston, 1992; Claisse and Alzieu, 1993; Blasco *et al*., 1996). Since this component is not associated with damage to any of the tissues evaluated, it demonstrates the contamination phenomena that are not exhibiting toxicity effects.

After analysis of the data obtained in this study, it is proposed that the principal measured chemical concentrations in sediments stressing juvenile *S. aurata* are, from component 2, the heavy metals chromium, cadmium, and lead, and, from component 3, the heavy metals silver and copper and the surfactant LAS. The remaining chemical concentrations [TOC (component 2) and Zn, Pb, and Hg (Component 3)] associated with both adverse-biological effect components are not included in the above proposition because they have more loading relative to component 4, which is not an adverse biological effect components.

To confirm these component descriptions and to establish the suggested site-specific values of sediment quality (SQVs) in the two littoral ecosystems studied in the present paper, representation of the estimated factor scores (and their mahalanobis distances: χ^2 values) from each case (station) to the centroid of all cases for the original data is proposed (Fig. 6). The prevalence of principal components is used for each of the cases studied. When components 2 and 3, which are factors demonstrating relationships between groups of chemicals and adverse effects, are both zero or below, the maximum concentrations of toxic chemicals at any of those stations represent maximum chemical concentrations not associated with adverse effects. These are considered to be chemical concentrations below which biological effects are low or minimal and are termed "no or minimal adverse biological effects." In contrast, to establish the minimal concentrations above which biological effects are always high, those minimal concentrations at stations where components 2 and 3 are higher were selected and named as "major adverse biological effects." Also, an intermediate range of chemical concentrations representing an "area of uncertainty," or a breakpoint between the high and low concentrations, is indicated. The suggested site-specific SQVs for six different chemicals (Cr, Cd, Pb, Ag, LAS, Cu) according to those three concepts (based on the biological effect on *S. aurata*) are listed in Table 5. The rest of the chemicals found in the different sediments of this study occur at concentrations below the biological effect detected in the juvenile fish.

DISCUSSION

Chemical concentrations were similar to those measured in previous studies performed in both ecosystems (Gomez-Parra *et al*., 1984; Establier *et al*., 1985; González-Mazo *et al*., 1997). Silver and mercury concentrations in CB2 and CB3 sediments were near the values analyzed in sediments from contaminated estuaries and littoral sites in Great Britain (Bryan and Langston, 1992). On the other hand, Ni, Co, As, and Sn were present at concentrations considered normal for littoral ecosystems (Alsenoy *et al*., 1993).

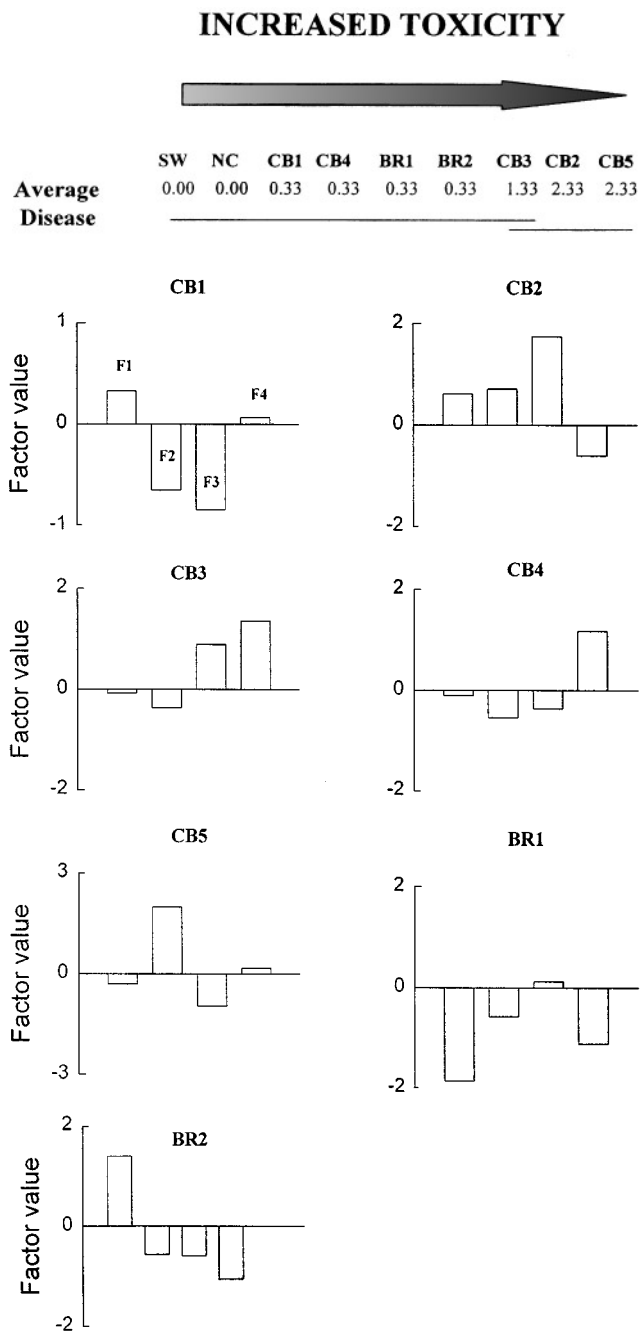

FIG. 6. Estimated factor scores from each of the seven cases (five stations at the Bay of Cádiz—CB1, CB2, CB3, CB4, and CB5—and two stations at the salt pond of the Barbate River—BR1 and BR2) to the centroid of all cases for the original data.

Determining SQVs is a difficult task because of such factors as the partitioning of sediment contaminants between dissolved (i.e., interstitial waters) and particulate-bound fractions, which may render them more or less available to the organisms in the ecosystem. Because of these complexities, comparative evaluation of broad-scale data sets encompassing complex interactions based on sediment toxicological data provides a promising alternative method for developing site-specific SQVs, compared with theoretical or univariate studies. Although the present study uses a limited data set including those considered as usual contaminants in the area studied: [heavy metals (Gómez-Parra *et al*., 1984) and the surfactant (LAS) (González-Mazo *et al*., 1997)], the proposed method provides a *snapshot* in evaluating the sediment quality of the ecosystems studied, resulting in a conservative estimate based on interactions between complex chemical mixtures that may, individually or in combination, be responsible for the observed effects on *S. aurata*. However, these site-specific SQVs should be used cautiously because they are based on evaluation of limited sites, measurement of heavy metals and surfactant (LAS), and biological effects measured for one species. With more sites, more chemical measurements and a battery of sediment toxicity tests (in which the described test would be included) will provide better use of these SQVs. Nevertheless, the objective in this study was to provide site-specific SQVs for the area studied by means of chemical and biological data obtained by simultaneous analysis (synoptic sampling) and use of a multivariate approach to link both data sets. In this sense, the authors note that instead of using large data sets from samples collected and/or analyses performed at different times (coincident), they used this small data set based on samples collected and analyses carried out at once (synoptically), as recommended by different authors when integrative assessment is performed (Chapman, 1995, 1996; Chapman *et al*., 1991b; Green *et al*., 1993).

The complete applicability of the specific SQV derived remains to be determined. However, data sets similar to those used in this study are presently available for North American sites (i.e., Chapman *et al*., 1987, 1991a; Long *et al*., 1995; Clements and Kniffey, 1994; DelValls and Chapman, 1997). To illustrate and confirm the specific SQVs proposed in Table 5, the authors used a summarized comparison of

TABLE 5

Site-Specific Sediment Quality Values (SQVs) for Chromium, Cadmium, Lead, Silver, Linear, Alkyl Benzenesulfonate (LAS), and Copper Proposed in This Study[a]

	Guideline description		
Contaminant	No or minimal adverse biological effects	Major adverse biological effects	Area of uncertainty
Cr	< 86.2	> 90.2	> 86.2, < 90.2
Cd	< 0.94	> 1.24	> 0.94, < 1.24
Pb	< 51.2	> 52.5	> 51.2, < 52.5
Ag	< 0.64	> 0.68	> 0.64, < 0.68
LAS	< 2.5	> 8.7	> 2.5, < 8.7
Cu	< 49.4	> 71.2	> 49.4, < 71.2

[a] All concentrations are expressed as $mg\,kg^{-1}$ dry sediment.

the most recent sediment management guidelines proposed by the U.S. Environmental Protection Agency using site-specific sediment bioassays Army Corps of Engineers (1977), the Ontario Ministry of the Environment using the screening level concentration (SLC) approach (Persaud *et al*., 1989), the National Oceanic and Atmospheric Administration (NOAA) from a compilation of the results of acute and chronic bioassays in several aquatic species (Long *et al*., 1995), and the Washington State Department of Ecology derived by Puget Sound using a combination of the apparent effects threshold (AET) and equilibrium partitioning (EqP) methodologies (WADOE, 1991). Although many of those values are considered site specific and are not intended for use as regulatory guidelines, they provide useful benchmarks for assessing the potential toxicity of contaminated sediments. The similarities reported between data obtained in this study (Table 5) and data proposed by the various agencies (Table 6) suggest that the site-specific SQVs from the Gulf of Cádiz could converge on appropriate SQVs which are supported by substantial synoptic and available biological effect data and could support wide application of both the method and the calculated values.

TABLE 6

Summary of Benchmark Sediment Quality Guidelines (mg kg^{-1} dry sediment) Proposed to Evaluate Potential Sediment Toxicity for Pb, Ag, Cr, Cd, and Cu, by Different North American Agencies and Government Bodies for Developing Sediment Quality Guidelines

	Sediment quality guideline		
	Not polluted	Moderately polluted	Highly polluted
Pb			
A[a]	< 40	40–60	> 60
B	< 23	31	> 250
C	< 35	35–110	> 110
D	—[b]	—[b]	> 450
Ag			
C	1.0	1.0–2.2	> 2.2
Cr			
A	< 25	25–75	> 75
B	< 22	31	> 111
C	< 80.0	145.0	> 145.0
Cu			
A	< 25	25–50	> 50
B	< 15	15–114	> 114
C	< 70	70–390	> 390
D	—[b]	—[b]	> 390
Cd			
A	—[b]	—[b]	> 6
B	< 0.6	0.6–10.0	> 9
C	< 5	5–9	> 10
D	—[b]	—[b]	> 5.1

[a] (A) U.S. EPA (Army Corps of Engineers, 1977); (B) Ontario Ministry of Environment (Persaud *et al*., 1989); (C) U.S. NOAA (Long *et al*., 1995); (D) Washington State Department of Ecology (1991). Surfactant LAS guidelines were not available.

[b] Guideline not available.

CONCLUSIONS

This study presents the results and interpretation of a chemical analysis and biological assessment of several sediment samples, which are intended to provide a *snapshot* of the quality of sediments collected in two littoral ecosystems in the Gulf of Cádiz. Within the context of this study a number of specific conclusions can be derived regarding the general sediment quality of the two ecosystems studied and the use of the proposed sediment toxicity test. These conclusions are summarized:

a. The results presented above clearly demonstrate that histological damage to juvenile *S. aurata* is a useful tool to determine toxic effects associated with the chemicals bound in sediments from the area studied. The damage observed was greatest in the gills. The other three endpoints – survival, superficial alteration, and hematocrit – did not indicate differences related to tested sediments. The introduction of a semiquantitative index (average damage, Table 3) allowed classification of the toxicity of sediment at the sampling stations.

b. Application of PCA demonstrated that the variance of the results could be described by four factors, which explain 92.0% of the variance in the original data set. Also, site-specific SQVs were obtained for the chemicals associated with adverse biological effects, and their similarity to the results for other ecosystems suggests the wide applicability of these guidelines.

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