Available online at www.sciencedirect.com
ScienceDirect
Environmental Pollution 146 (2007) 233–240
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Comparing sediment quality in Spanish littoral areas affected by acute (*Prestige*, 2002) and chronic (Bay of Algeciras) oil spills

C. Morales-Caselles [a,c], J. Kalman [a,c], I. Riba [a,c], T.A. DelValls [b,c,*]

[a] *Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Avda. República Saharaui s/n, Puerto Real 11510, Cádiz, Spain*
[b] *UNESCO UNITWIN/UNICOP, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro s/n, Puerto Real 11510, Cádiz, Spain*
[c] *Unidad Asociada de Calidad Ambiental y Patología (CSIC & UCA), Avda. República Saharaui s/n, Puerto Real 11510, Cádiz, Spain*

Received 7 December 2005; received in revised form 6 April 2006; accepted 12 April 2006

Littoral sediments affected by low or moderated but continuous oil spills are more polluted than those affected by accidental oil spills such as the Prestige.

Abstract

The quality of sediments collected from two areas of the Spanish coast affected by different sources of contaminants has been compared in this study. The areas studied are the coast of Galicia affected by the oil spill from the tanker *Prestige* (November 2002) and the Gulf of Cádiz which suffers continuous inputs of contaminants from industries located in the area and from oil spills. Contamination by several chemicals (metals, PCBs and PAHs) that bind to sediments was analyzed, and two toxicity tests (Microtox® and amphipod 10-day bioassay) were conducted. PAHs were identified as the compounds responsible for the toxic effects. Results show differences between an acute impact related to the sinking of the tanker *Prestige* and the chronic impact associated with continuous oil spills associated with the maritime and industrial activities in the Bay of Algeciras, this being the most polluted part of the two coastal areas studied in this work.

Keywords: Amphipods; Microtox®; PAHs; Toxicity; Contamination

1. Introduction

Sediments are an important part of the ecosystem and play a key role in the distribution of contaminants in the aquatic environment; the study of the quality of sediments provides information about the ecosystem health. Human activities in coastal areas usually involve an input of contaminants to the natural environment that becomes evident in the decreased quality of coastal sediments. Many authors agree that sediment quality is best determined by integrating the information obtained from measures of chemicals concentration and from specific tests to determine sediment toxicity (DelValls and Conradi, 2000; Chapman et al., 2002). The biological effects can be established based on laboratory tests that determine toxic responses. Sediment bioassays are usually relatively simple tests that evaluate the responses of the tested organism to contaminated sediments under controlled conditions (Riba et al., 2004a).

In the present study, we have selected two different tests in order to determine sediment toxicity: the Microtox® test and an amphipod acute bioassay. Use of the commercial bioassay Microtox® has increased in recent years since it detects the ''hot spots'' of field contamination in the screening procedure (Mowat and Bundy, 2001; Stronkhorst et al., 2003; Van Beelen, 2003); Microtox® has also been used before to assess the impact of oil spills and oil contaminated

* Corresponding author. UNESCO UNITWIN/UNICOP, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro s/n, Puerto Real 11510, Cádiz, Spain. Tel.: +34 956 016794; fax: +34 956 016040.
E-mail address: angel.valls@uca.es (T.A. DelValls).

0269-7491/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2006.04.042

sediments (Brohon et al., 2001; Kenneth et al., 2003; Pelletier et al., 2004). The other acute bioassay was carried out with the amphipod *Corophium volutator* which is an important test organism for the ecotoxicological quality assessment of marine and estuarine sediment samples (Peters and Ahlf, 2005).

The bioassay with *C. volutator* is integrated in test batteries for dredged material management (Peters et al., 2002; Stronkhorst et al., 2003) and it is required for compliance with certain International Standardization Organisation quality standards (ISO, 2003). This kind of bioassay has been used in previous studies for the assessment of spills (Grant and Briggs, 2002; Briggs et al., 2003).

In this study a comparison is made between the quality of sediments sampled on two lengths of Spanish coast affected by different sources of contaminants. In the coast of Galicia, the study addresses the acute impact provoked by the oil spill resulting from the break-up and sinking of the tanker *Prestige* (November 2002), whereas in the Gulf of Cádiz the sediments studied have suffered a chronic impact lasting several decades, caused by the input of oil and other contaminants from the various industries located in the area and from accidental spills and deliberate discharges from commercial shipping activities.

The main objectives of this study are: (1) to characterise the contamination by PAHs in the selected areas of study on the Galician Coast and in the Gulf of Cádiz; (2) to establish the sediment toxicity caused by the presence of contaminants in the sediment samples; (3) to compare the sediment quality of the various areas studied by linking contamination and ecotoxicological data.

2. Materials and methods

2.1. Approach

Fig. 1 shows the seven sediment sampling stations located in the National park of the Atlantic Islands that were selected in the area of Galicia, three stations in the island of Ons (D07, D09 and D18) and four stations in the Cíes archipelago (D60, D66, D79 and FIG). In the Gulf of Cádiz seven stations were selected in the area of the Bay of Algeciras: three stations in the mouth of the river Guadarranque (GR1, which is near an oil-fired electricity generating plant, GR3′ and GR4, both near chemical processing plants), one station in the mouth of the river Palmones (P4) and three stations in the Bay (AL1 and AL2, both located in the port and near the city of Algeciras, and AL5, near a chemical plant). All these sediments have suffered repeated impacts by moderate or small oil spills caused by maritime traffic and bunkering activities in the area during recent decades. Clean sediment from the Bay of Cádiz was used as the negative control (Ca1). An artificial sample (TM) was made by mixing a toxic mud from an accidental mining spill in Spain (Aznalcóllar, April 1998) with the same clean sediment and used as positive toxicity control (Riba et al., 2003).

Sediments were collected with a 0.025 m^2 Van Veen grab and transferred to the cooler. When sufficient sediment had been collected from a particular station, the cooler was transported to the laboratory. The contents of the cooler were homogenized with a Teflon spoon until no colour or textural differences could be detected. The samples were subsampled for physical characterization and chemical quantification. After that, sediment samples were maintained in the cooler at 4 °C in the dark until they were used for sediment toxicity testing, but no longer than 2 weeks.

2.2. Chemical analysis

Sediment aliquots for chemical analysis were dried at room temperature and then gently homogenized. Total organic carbon (TOC) concentration and sediment grain size (fines: % of dry sediment < 63 μm) were studied in order to determine the geochemical matrix characteristics. Organic carbon content was determined using the method of Gaudette et al. (1974) with the El Rayis (1985) modification. For sediment grain size, an aliquot of wet sediment was analyzed using a Fristch laser particle sizer (model Analysette 22) following the method reported by DelValls and Chapman (1998).

For trace metal analysis, the sediments were digested as described by Loring and Rantala (1992). Zn and Cu concentrations in the extracts were determined using a Perkin–Elmer 2100 flame atomic absorption spectrophotometer. The other trace metals were measured by graphite furnace atomic absorption spectrophotometry (Perkin–Elmer 4100 ZL). Concentrations of Hg were determined using a Perkin–Elmer MHS-FIAS coupled with a Perkin–Elmer 4100 ZL spectrophotometer. Results are expressed as $mg\,kg^{-1}$ dry sediment. The analytical procedures were checked using reference material (MESS-1 NRC and CRM 277 BCR) and showed agreement with the certified values of more than 90%.

The analyses of PAHs and PCBs were carried out according to USEPA SW-846 Method 827C78082. Briefly, following recommendations by Riba et al. (2002), dried samples were Soxhlet extracted with *n*-hexane for 18 h, and the extracts were isolated by column chromatography on Florisil–alumino-silica. PCBs and PAHs were eluted and their fractions were dried in a rotating evaporator and re-dissolved in isooctane. Aromatic fractions were analyzed on a Hewlett–Packard (HP) 5890 Series II gas chromatographer coupled with an HP 5970 mass spectrometer. Chromatographic resolution was achieved with a 30 m × 0.250 mm DB-5 capillary column, which has a 0.25 μm film thickness, with helium as carrier gas. The 16 priority PAHs considered by the US Environmental Protection Agency were analyzed by GC–MS using selected ion monitoring (SIM). Quality control was carried out using NRC-CNRC HS-6 sediment reference material. Analysis of PCBs as AROCLOR 1242 and AROCLOR 1260 was performed using the same instrument with an electron capture detector (GC/ECD) and a 30 m × 0.25 mm MDN-5S capillary column. Quantification was performed by the external standard technique by comparison of peak areas in the sample with those obtained by injecting a standard mixture of AROCLOR 1242 and 1260. Quality control was carried out with NRC-CNRC HS-1 sediment reference material. For both set of organic chemicals, PAHs and AROCLOR, the analytical procedure showed agreement with the certified values of more than 90%.

2.3. Microtox® bioassay

The commercial Microtox® test is a bioassay that uses the bioluminescence of the bacteria *Vibrio fischeri* as an indicator of the quality of the sample (liquid or solid phase) exposed; the bioluminescence of the bacteria is related to its metabolism therefore, a diminution of the sediment quality will be reflected in the decrease of the quantity of light emitted. In the Microtox® toxicity test, the measures of the IC50 (which is the concentration of dry sediment that provokes a 50% inhibition of the light emitted by the bacteria) provide the information about the sediment toxicity. The bioassay of bioluminescence inhibition with the bacteria *V. fischeri* was conducted on solid phase with the commercial Microtox® apparatus (model 500) by following the protocols for the solid phase test (SPT) according to the standard operating procedure (AZUR Environmental, 1998). Briefly, 7 g (±0.01 g) of sediment were tested as suspensions prepared with 35 mL of commercial Microtox® Solid Phase Test Diluent and diluted to a series of nine concentrations in the cuvettes. The reconstituted bacteria were added to the dilutions which were incubated for a period of 20 min at 15 °C in a waterbath. Next the dilutions were filtered through the filter columns and 500 mL of content of each cuvette of the bath were transferred to its corresponding cuvette in the apparatus and bioluminescence was measured in the "read well". The modification of the basic solid phase test (BSPT) reported by Campisi et al. (2005) was carried out and an average value of the IC50 from using both protocols was obtained for each sample.

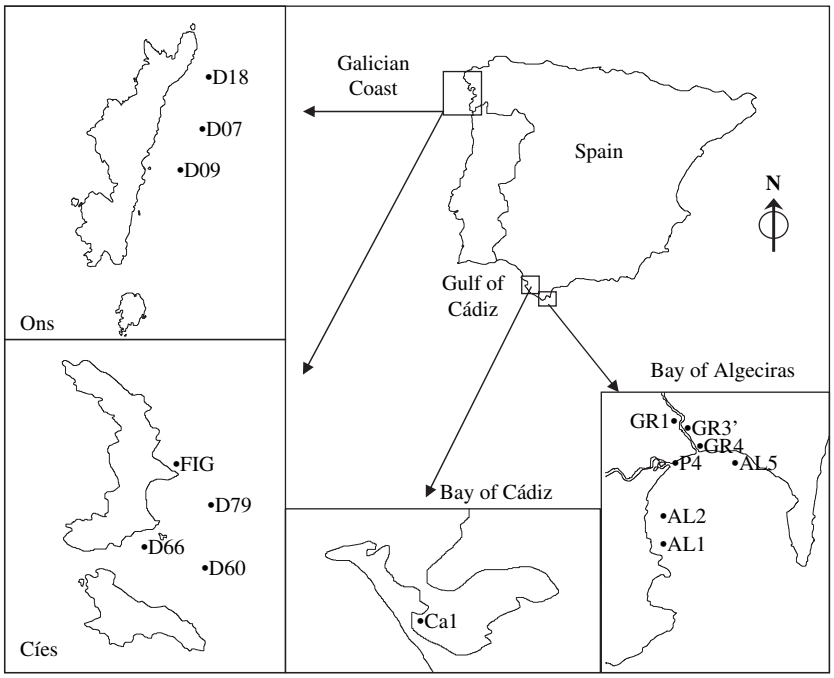

Fig. 1. Map of the coastal area of Galicia, the Bay of Algeciras and the Bay of Cádiz showing the general areas sampled and locations of the sampling stations. D(#) refers to the stations located in Galicia and AL(#), P(#), and GR(#) to those in the Bay of Algeciras. Ca1 was the station selected as negative control in the Bay of Cádiz.

2.4. Amphipod bioassay

Individuals of *C. volutator* were obtained from the field in a clean area located in the coast of Galicia (Morales-Caselles, 2005) by sieving mud through a 1 mm mesh; when the organisms were isolated they were placed in 11 L capacity aquariums with clean seawater and sieved sediment (collected in the same area as the organisms) and were maintained in the laboratory under controlled conditions for acclimation until the start of the test. Aeration was provided and natural photoperiod was selected. During acclimation (1 month) the organisms were fed twice a week with food for invertebrates (''Marine Invertebrate Diet'' which is a mixture of amino acids and organic particles) and water was replaced.

The toxicity test was conducted in replicate (5) by exposing individuals of the amphipods *C. volutator* to bulk sediment using the percentage of survival after 10 days of exposure as the end point (ASTM, 1993). Mortality is measured after the time of exposure and the results obtained have been correlated positively with changes in benthic communities (Long et al., 2001).

Approximately, 250 g of sieved (1 mm) sediment was placed in 2 L glass containers and then about 750 mL of clean seawater were added. Aeration was provided after the sediment had settled down. The individuals of *Corophium* were sieved and separated from sediment of the acclimatising tanks and were placed in each replicate container (20 individuals per container). The containers were covered in order to avoid water evaporation (a hole was made in the lid to provide aeration), and maintained at 19 °C during the 10 days of exposure. After that time, the contents from the different stations with the various replicates were sieved and the organisms' survival rate was recorded.

2.5. Statistical analysis

ANOVA was performed in order to determine significant differences ($p < 0.05$) in amphipod survival among the toxicity results obtained for the control site and the other sampling sites. Also, contamination and toxicity data were linked by factor analysis, using principal components analysis (PCA) as the extraction procedure; this is a multivariate statistical technique for exploring variable distributions (Riba et al., 2003). The original data set used in the analysis included two acute toxicity responses (amphipod survival and the IC50 measured in the Microtox® bioassay), the sediment concentration of different contaminants (PAHs, PCBs, Cd, Cu, Ni, Co, V, Pb, Zn, Hg), and the geochemical matrix characteristics (including total organic carbon and grain size distributions). The objective of PCA is to derive a reduced number of new variables as linear combinations of the original variables. This provides a description of the structure of the data with the minimum loss of information.

3. Results

3.1. Chemical analysis

Summarized results of chemical analyses in the sediments used for both bioassays are shown in Table 1. In general, results do not show a prevailing tendency in the concentration of metals among sediments from the different areas, although the toxic mud presents the highest concentration of metals. On the other hand, organic contaminants (PAHs and PCBs) seem to be at higher levels in sediments collected in the Bay of Algeciras than in the area of Galicia. The negative control (Ca1) shows the lowest levels of metals, and no organic contamination was found.

3.2. Microtox® bioassay

The highest inhibition of bioluminescence, which corresponds to an IC50 < 600 mg L^{-1} dry weight, is shown in the samples collected at the Bay of Algeciras stations AL2 (69 mg L^{-1}), AL1 (208 mg L^{-1}), GR3′ (235 mg L^{-1}), GR4 (249 mg L^{-1}) and GR1 (522 mg L^{-1}), and in the sediments from the coast of Galicia D60 (358 mg L^{-1}), D79 (364 mg L^{-1}), D18 (390 mg L^{-1}) and D66 (486 mg L^{-1}).

Table 1
Average values of total organic carbon (%dry weight), fines (% of dry sediment < 63 μm) and the concentration of contaminants (metals (mg kg^{-1} dry weight); PAHs and PCBs (μg kg^{-1} dry weight)) in sediment samples (negative control: Ca1; positive control: TM; Algeciras Bay: GR1, GR3′, GR4, P4, AL1, AL2, AL5; Galicia: D07, D09, D18, D60, D66, D79 and FIG.) Not detected is expressed by n.d.

	TOC	Fines	Zn	Cd	Pb	Cu	Ni	Co	V	Hg	PAH	PCBs
Ca1	1.07	1.04	21.3	0.92	2.31	6.98	0.06	3.40	80.0	n.d.	n.d.	n.d.
GR1	3.12	75.1	44.8	0.61	9.10	12.6	6.01	n.d.	6.69	n.d.	546	0.86
GR3′	2.15	89.2	138	0.17	22.0	5.01	74.7	12.8	26.1	1.04	2961	22.0
GR4	3.19	39.5	35.3	0.10	6.21	3.67	13.1	5.59	n.d.	0.25	802	1.75
P4	2.09	34	50.4	0.62	5.64	11.3	24.7	1.11	85.0	0.11	21.4	4.64
AL1	2.35	92.3	137	0.16	32.0	30.5	50.9	n.d.	60.8	1.11	1383	0.46
AL2	3.22	90.8	54.0	0.11	9.81	7.59	15.1	1.69	4.82	0.81	1376	0.65
AL5	1.22	3.2	23.0	0.14	7.12	10.8	52.6	5.09	2.19	0.26	1218	0.33
D07	3.79	45.6	85.3	n.d.	23.1	251	1.04	n.d.	81.2	0.08	465	n.d.
D09	4.61	59.9	107	n.d.	28.3	160	11.7	n.d.	116	0.07	240	n.d.
D18	2.42	12.9	55.5	n.d.	14.2	20.8	3.44	2.00	54.0	0.04	480	n.d.
D60	3.56	60.9	101	n.d.	31.0	70.9	16.2	n.d.	125	0.12	702	n.d.
D66	0.37	11.3	14.0	n.d.	4.10	16.2	4.60	0.30	n.d.	0.06	384	n.d.
D79	3.58	70.2	114	n.d.	29.1	150	4.44	n.d.	13.7	0.09	273	n.d.
FIG	2.10	2.12	76.2	n.d.	26	18.5	11.8	0.50	n.d.	0.04	390	n.d.
TM	1.00	10.1	2181	5.40	789	210	8.5	n.d.	n.d.	5.61	n.d.	n.d.

The positive control of toxicity TM shows a 50% inhibition of bioluminescence at very low concentration (142 mg L^{-1}). The sediment obtained from the Bay of Cádiz and used as the clean reference (station Ca1), showed the highest value of IC50 (6013 mg L^{-1}) and confirms its validity as the negative toxicity control. Microtox® results are shown in Fig. 2.

3.3. Amphipod bioassay

Mean mortality results after the 10 days amphipod toxicity test are shown in Fig. 3. The highest mortality measurements (100%) were associated with sediments found in the Bay of Algeciras at station GR3′ and with the positive control of toxicity TM, while lowest mortality (3.3%) was associated with sediments from the reference station Ca1. The mortality measured in the sediments from GR3′ (100%), TM (100%), GR4 (75%), D18 (45%), D66 (60%), P4 (33.3%) and AL5 (35%) was significantly different $*p < 0.05$ from the negative control Ca1. The other stations did not present significant differences in the mortality results compared to the control station (Ca1).

3.4. Statistical analysis

To link the set of data obtained, the original variables from chemical concentration and toxicity responses were analyzed by factor analysis, using principal components analysis (PCA) as the extraction procedure; this is a multivariate statistical technique (MAA) for exploring distributions of the variables (chemical concentration, $n = 14$; toxicity data, $n = 2$).

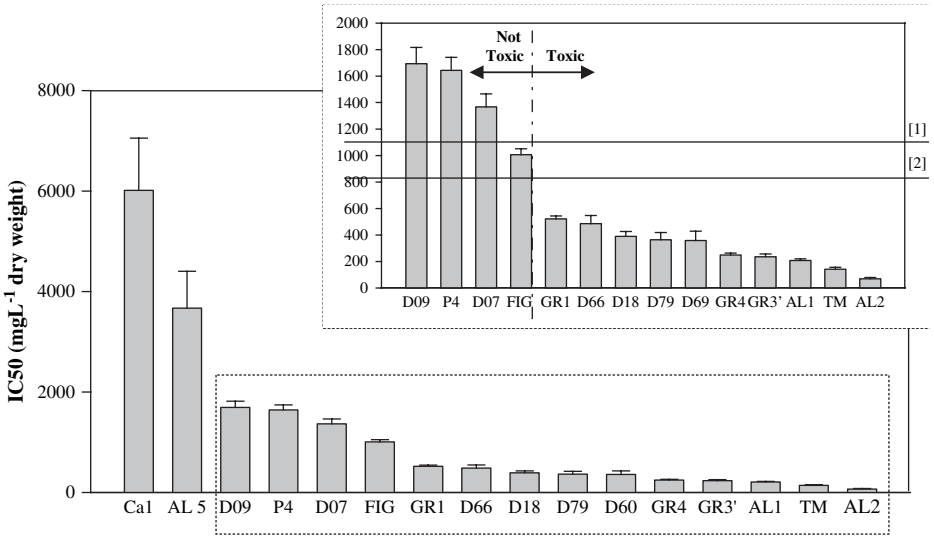

Fig. 2. IC50 results obtained from the application of the Microtox® test to sediment samples from the various stations. A zoom is provided for IC50 < 1800 mg L^{-1} (dry weight). Lines displayed show the limits below which the sediment sample is considered toxic by the Canadian Standards (1000 mg L^{-1} dry weight) and by the proposed Spanish Standards (Casado-Martínez et al., in press), 750 mg L^{-1} (dry weight).

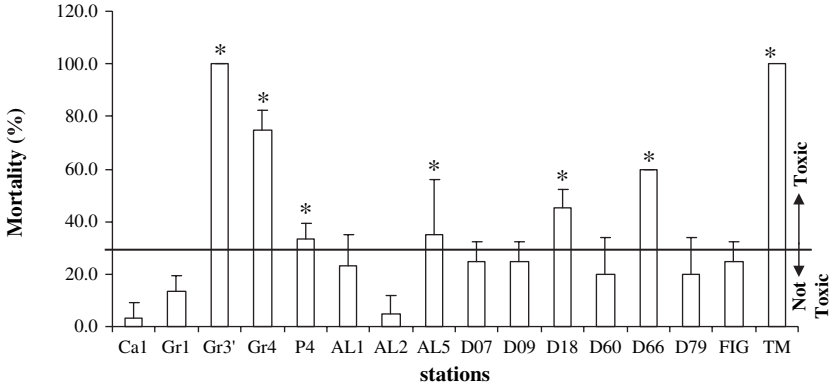

Fig. 3. Mortality results after 10 days of exposing *C. volutator* to the sediment samples. Asterisks indicate significant differences between the amphipod mortality rate in the treatments and the negative control ($*p < 005$). The line displayed shows the limits below which the sediment samples are considered toxic by the proposed Spanish Standards (DelValls et al., 2004; Casado-Martínez et al., in press). Those samples where the mortality rate of the amphipods is 20% higher than the mortality recorded in the negative control (Ca1) and show significantly different ($*p < 005$) results compared to those obtained in Ca1, are considered as toxic.

The factor analysis was performed on the correlation matrix; the variables were autoscaled (standardized) so as to be treated with equal importance (Riba et al., 2004a). The application of MAA to the original 14 variables indicates that they can be grouped in three new factors. These factors explain 78.3% of the total variance in the original data set. In the present study, we decided to interpret a group of variables as those associated with a particular component where the loading was 0.30 or higher (Table 2). This approximates to Comreys' cut-off of 0.55 (Comreys, 1973) for a good association between an original variable and a factor, and also takes into account discontinuities in the magnitudes of loadings approximating the original variables.

The first principal factor, #1 is predominant and accounts for 35.0% of the variance; it shows the toxicity to the bacteria and the amphipods associated with the presence of trace metals in sediments (Zn, Cd, Pb, Cu and Hg). The second factor, #2 accounts for 28.8% of the variance; it explains the amphipods and bacteria toxicity associated with the chemical concentrations of the metals Co and Ni, the organic contaminants PAHs and PCBs. The third factor, #3 accounts for 14.5% of the variance; it shows the relationship between the grain size and the total organic carbon in the sediments with the presence of Cu and V, but toxicity does not contribute to this factor.

Table 2
Sorted rotated factor loadings (pattern) of 14 variables for the three principal factors resulting from the multivariate analysis of results obtained from the chemical analysis and the acute toxicity tests (Microtox® and *C. volutator* bioassay)

%Variance	Factor 1	Factor 2	Factor 3
	35.01	28.76	14.54
TOC	–	–	0.86
Fines	–	0.40	0.84
Zn	0.98	–	–
Cd	0.93	–	–
Pb	0.98	–	–
Cu	0.51	–	0.42
Ni	–	0.86	–
Co	–	0.88	–
V	–	–	0.41
Hg	0.98	–	–
PAH	–	0.94	–
PCBs	–	0.88	–
Microtox®	0.43	0.30	–
Corophium	0.59	0.52	–

The influence of the three factors at the 16 stations is reflected by the Factor score at these stations and is shown in Fig. 4. The definition of Factor 1, with positive loading, is the acute toxicity of the organisms to metals bound to sediment, it is mainly prevalent in the positive control TM (3.65) followed by GR3′ (0.02) and AL2 (0.01) with low prevalence. Factor 2 is defined as the lethal toxicity of the amphipods related to the concentration of metals Co and Ni and to the organic compounds, mainly PAHs, bound to sediments; this factor shows significant prevalence in the stations from the Bay of Algeciras: GR4 (0.49), AL1 (0.49), AL2 (0.47), AL5 (0.61) and mainly in GR3′ (3.28). The definition of Factor 3, with negative loading, does not include information about the toxicity of the contaminants, but indicates the association of geochemical features of the sediment, described by the relationship between total organic carbon and grain size.

4. Discussion

The chemical data obtained in the analyses show how the levels of PAHs are, in general, higher at the stations in the Bay of Algeciras than in the stations selected on the coast of Galicia. These chemical data can be compared to international sediment quality guidelines (SQGs) that specify the levels of chemical contaminants associated with biological effects (DelValls et al., 2004). In this regard, the samples collected at GR3′, AL1, AL2 and AL5 exceed the SQGs for PAHs defined by Dutch agencies (Tweede Kamer, vergaderjaar, 1994–1995); this implies that the sediments from these locations could be considered slightly or moderately polluted

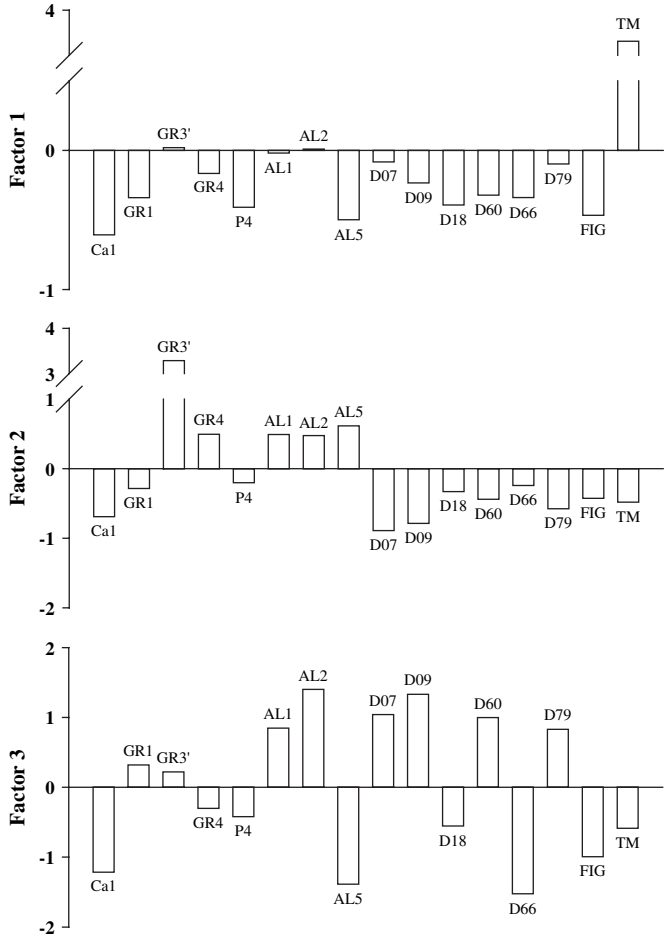

Fig. 4. Estimated factor scores for the three factors in each of the 16 cases. The factor scores quantify the prevalence of each factor for every station and is used to establish the definition of each factor.

according to the Dutch SQGs for PAHs. Following the recommendations described by MacDonald et al. (1996), the sediments from GR4 and D60 would also be considered as slightly polluted by this contaminant and adverse effects could be frequent. There are some stations in the Galician islands (D07, D09, D18, D60, D79 and FIG) where sediment exceeds the SQGs defined for Cu by international agencies and previous studies (CEDEX, 1994; Tweede Kamer, vergaderjaar, 1994–1995; MacDonald et al., 1996; NOAA, 1999). Although contamination by copper was observed in the uppermost layer in the *Prestige* shipwreck area of the Northeast Atlantic Ocean (Prego and Cobelo-García, 2004; Cobelo-García et al., 2004), this Cu contamination should not be related to the shipwreck, because levels of Cu in the fuel oil carried by the *Prestige* were relatively low ($3.39\ mg\ kg^{-1}$) and previous studies have shown that there are other sources of this metal in the area (Carballeira et al., 1997). Also, there are some stations in the Bay of Algeciras (GR3′, AL1 and AL5) where sediment exceeds some international SQGs (CEDEX, 1994; Tweede Kamer, vergaderjaar, 1994–1995; MacDonald et al., 1996; NOAA, 1999) defined for Ni and for Hg (GR3′, GR4, AL1, AL2 and AL5). The positive control TM exceeds almost all the SQGs defined by international agencies and other authors (CEDEX, 1994; Tweede Kamer, vergaderjaar, 1994–1995; MacDonald et al., 1996; NOAA, 1999) for the metals Zn, Cd, Pb, Hg and Cu.

In Fig. 2 the Microtox® results are shown. The lines represent the values below which sediment toxicity is assumed by different international agencies (Casado-Martínez et al., in press). The Canadian Standards (Environment Canada, 2002) considers the limit to be $1000\ mg\ L^{-1}$ (dry weight) while in the Spanish Standards (DelValls et al., 2004; Casado-Martínez et al., in press) this limit is associated with a concentration of $750\ mg\ L^{-1}$ (dry weight). In this case both guidelines agree and the sediments from GR1, D66, D18, D79, D69, GR4, GR3′, AL1, TM and AL2 would all be considered as toxic.

The line displayed in the graph for the *Corophium* mortality (Fig. 3) shows the limit above which sediments are considered toxic by the US Environmental Protection Agency (USEPA, 1994) and the Spanish Standards (DelValls et al., 2004; Casado-Martínez et al., in press). These agencies establish that a sediment sample can be considered toxic when the mortality rate recorded from the treatment is 20% higher than the mortality measured in the negative control sediment; it also shows significantly different ($*p < 0.05$) mortality results compared with those obtained in the negative control. In this case all the samples that are significantly different ($*p < 0.05$) from Ca1 would be considered toxic by these agencies because they also have a mortality rate higher than 20% compared to the control sediment (10%).

From the MMA performed to link together the chemical and ecotoxicological data, we have obtained three factors that account for all the variables and have a different influence for each sampling site. Factor 1 accounts for the toxicity responses of the two bioassays due to the metals that bind to sediments. This factor is seen with most prevalence in the positive control TM which presents the highest levels of metals in the study, a low IC50 and a 100% mortality of *Corophium* after 10 days of exposure.

Regarding the toxicity due to the presence of organic contaminants in the sediments, Factor 2 is mainly prevalent at the stations in the Bay of Algeciras: GR3′, GR4, AL1, AL2 and AL5. This factor also includes toxicity due to the metals Ni and Co and the toxicity is determined by the mortality of the amphipods and the low IC50 measured with the bacteria bioassay. The content of PCBs in the sediments studied does not exceed the SQGs and the highest concentration of PCBs is found in sediments from GR3′ whereas in the Galician sediments, it was not found at all. Thus, the PAHs can be considered as the main organic contaminant producing the toxicity measured in the study. The metals Ni and Co have been previously reported associated with PAHs in the oil spills occurring in the area of Algeciras (CSIC, 2005) caused both by the industrial plants located in the area and by maritime activities, and in other areas affected by oil spills (Massoud et al., 1998). However, both of these metals (Co and Ni) could originate from any of the various local activities, not only accidental oil spills. The toxicity results from Microtox® have a low loading in this factor, maybe because this is not the best test for

organic compounds, due to the insoluble nature of most of the oil compounds (Simon et al., 2004). However, it is important to highlight that the Microtox® is a screening bioassay and it is suggested that the biotests alone may not be representative in certain cases of the full impact of a given pollutant on an ecosystem (Brohon et al., 2001). On the other hand, previous studies have shown that the toxicity of sediments to *Corophium* is closely correlated with their hydrocarbon content (Grant and Briggs, 2002).

In general, results obtained in the MAA suggest that the sediments from the Bay of Algeciras and the Galician islands are contaminated with PAHs and that toxic responses due to these compounds occur in both places. However, toxicity is mainly seen in sediments from the Bay of Algeciras, and toxic sediments show a higher frequency in the stations located in this area (GR1, GR3′, GR4, P4, AL1, AL2 and AL5) than in those sampled on the coast of Galicia (D07, D09, D18, D60, D66, D79 and FIG). This could be associated either with the higher quantity of PAHs or with the presence of a more complex mixture of contaminants that has not been analyzed in this study, in sediments from the Bay of Algeciras rather than in those from Galicia. Also, based on the results obtained in this study, the concentration of PAHs bound to sediments in the Bay of Algeciras could be more bioavailable for the organisms studied and would produce more toxicity than that found in Galicia. However, this greater bioavailability of PAHs bound to sediments in the Bay of Algeciras is only a hypothesis that needs to be taken into account in later work.

Sediment quality guidelines (benchmarks) for PAHs can be determined with the information obtained in this study by means of MAA using the Factor scores. The highest concentrations of PAHs measured at those stations where the value of the Factor 2 score is 0 or below 0, define the concentration of PAHs ''not associated with the toxic effect'' measured in the study. It is determined at station D60 with a value of 702 $\mu g\,kg^{-1}$ (dry weight) (V1). The lowest concentration of PAHs measured at the station where the value of Factor 2 score is positive defines the concentration of PAHs ''associated with the toxic effect measured in this study''. It is determined at station GR4 with a value of 802 $\mu g\,kg^{-1}$ (dry weight) (V2).

In general, our results have shown that the highest pollution measured in the Gulf of Cádiz was determined in sediments sampled from the Bay of Algeciras and especially at station GR3′, related to chronic contamination, while moderate contamination and low toxicity was determined on the coast of Galicia in the Cíes archipelago. Previous studies (Riba et al., 2004b) agree that moderate but chronic inputs of contaminants can produce more pollution in coastal sediments than higher but acute environmental impacts.

5. Conclusions

From the results obtained in the various analyses performed in this study, we can conclude that PAHs are the main contaminant at the sites studied on the coast of Galicia and in the Bay of Algeciras, while there is no such contamination in the sediments from the station located in the Bay of Cádiz; the concentrations of PAHs in sediments from the Bay of Algeciras are, in general, higher than in sediments from the coast of Galicia. Toxicity is also higher in the Bay of Algeciras than in the Galician islands, but no toxicity was detected in the sediment from the Bay of Cádiz station. Finally, it has been shown that sediments from the Bay of Algeciras are chronically polluted by PAHs (V1: 702 $\mu g\,kg^{-1}$; V2: 802 $\mu g\,kg^{-1}$) while those of the coast of Galicia can only be considered as moderately or not polluted. In the Bay of Cádiz no environmental degradation was measured.

To sum up, with the present study we have shown that sediments found in the Bay of Algeciras, affected by chronic oil spills, are more environmentally degradated (polluted) than those found in the coast of Galicia, which was mainly affected by the *Prestige* accidental oil spill.

Acknowledgements

The work described was partly supported by a Grant funded by the Ministry of Education and Science VEM2003-20563 and by CIS funded by the Ministry of Environment. Carmen Morales-Caselles thanks the Ministry of Education and Science for funding her research fellowship (FPU). We are grateful for the support and help of the members of the CIS. Special thanks are given to Mercedes Conradi, Augusto César and Carmen Casado-Martínez.

References

American Society for Testing and Materials, 1993. Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with the Marine and Estuarine Amphipods. ASTM, Philadelphia, pp. 1367–1392.

AZUR Environmental, 1998. Microtox M500 Manual. AZUR Environmental, Carlsbad, CA, USA.

Briggs, A.D., Greenwood, N., Grant, A., 2003. Can turbidity caused by *Corophium volutator* (Pallas) activity be used to assess sediment toxicity rapidly? Mar. Environ. Res. 55, 181–192.

Brohon, B., Delolme, C., Gourdon, R., 2001. Complementarity of bioassays and microbial activity measurements for the evaluation of hydrocarbon-contaminated soils quality. Soil Biol. Biochem. 33, 883–891.

Campisi, T., Abbondanzi, F., Casado-Martínez, C., DelValls, T.A., Guerra, R., Iacondini, A., 2005. Effect of sediment turbidity and color on light output measurement for Microtox Basic Solid-Phase Test. Chemosphere 60, 9–15.

Carballeira, A., Carral, E., Puente, X.M., Villares, R., 1997. Estado de conservación de la costa de Galicia. Nutrientes y metales pesados en sedimentos y organismos intermareales. Universidad de Santiago de Compostela, 105 pp.

Casado-Martínez, M.C., Campisi, T., Díaz, A., Lo Re, R., Obispo, R., Postma, J.F., Riba, I., Sneekes, A.C., Buceta, J.L., DelValls, T.A., 2006. Interlaboratory assessment of marine bioassays to evaluate the environmental quality of coastal sediments in Spain. II. Bioluminescence inhibition test for rapid sediment toxicity assessment. Cienc. Mar. 32 (1B), 129–138.

CEDEX, 1994. Recomendaciones para la gestión del material de dragado en los puertos españoles. Centro de Estudios y Experimentación de Obras Públicas, Puertos del Estado, Madrid, Spain, 45 pp.

Chapman, P.M., Ho, K.T., Munns, W.R., Solomon, K., Weistein, M.P., 2002. Issues in sediment toxicity and ecological risk assessment. Mar. Pollut. Bull. 44, 271–278.

Cobelo-García, A., Labandeira, A., Prego, R., 2004. Two opposite cases of metal accumulation in ria sediments: Ferrol and Corme-Laxe (Galicia, NW Iberian Peninsula). Cienc. Mar. 31 (4), 653–659.

Comreys, A.L., 1973. A First Course in Factor Analysis. Academic New York, NY, USA.

CSIC, 2005. Informe del CSIC sobre la calidad ambiental del Campo de Gibraltar. Centro Superior de Investigaciones Científicas, Algeciras, Spain.

DelValls, T.A., Chapman, P.M., 1998. Site-specific sediment quality values for the Gulf of Cádiz (Spain) and San Francisco Bay (U.S.A.), using the sediment quality triad and multivariate analysis. Cienc. Mar. 24, 313–336.

DelValls, T.A., Conradi, M., 2000. Advances in marine ecotoxicology: laboratory tests versus field assessments data on sediment quality studies. Cienc. Mar. 26 (1), 39–64.

DelValls, T.A., Andres, A., Belzunce, M.J., Buceta, J.L., Casado-Martinez, M.C., Castro, R., Riba, I., Viguri, J.R., Blasco, J., 2004. Chemical and ecotoxicological guidelines for managing disposal of dredged material. Trends Anal. Chem. 23 (10–11), 819–828.

El Rayis, O.A., 1985. Re-assessment of the titration method for the determination of organic carbon in recent sediments. Rapp. Comm. Int. Mer. Medit. 29, 45–47.

Environment Canada, April 2002. Biological Test Method: Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test. Environmental Protection Service, Ottawa, ON. Report EPS 1/RM/42.

Gaudette, H.E., Flight, W.R., Torner, L., Folger, D.W., 1974. An inexpensive titration method for the determination of organic carbon in recent sediments. J. Sediment. Petrol. 44, 249–253.

Grant, A., Briggs, A.O., 2002. Toxicity of sediments from around a North Sea oil platform: are metals or hydrocarbons responsible for ecological impacts? Mar. Environ. Res. 53 (1), 95–116.

International Organization for Standardization (ISO), 2003. ISO DIS 16712 Water Quality – Determination of Acute Toxicity of Marine or Estuarine Sediment to Amphipods (Draft 2003). ISO, 18 pp.

Kenneth, L., Wohlgeschaffen, G., Tremblay, G.H., Johnsosn, T., Sergy, G.A., Prince, R.C., Guénette, C.C., Owens, E.H., 2003. Toxicity evaluation with the Microtox® test to assess the impact of in situ oiled shoreline treatment options: natural attenuation and sediment relocation. Spill. Sci. Technol. Bull. 8 (3), 273–284.

Long, E.R., Hong, C.B., Severn, C.G., 2001. Relationships between acute sediment toxicity in laboratory tests and abundance and diversity of benthic infauna in marine sediments: a review. Environ. Toxicol. Chem. 20, 46–60.

Loring, D.H., Rantala, R.T.T., 1992. Methods for the geochemical analyses of marine sediments and suspended particulate matter. Earth-Sci. Rev. 32, 235–283.

MacDonald, D.D., Carr, R.S., Calder, F.D., Long, E.R., Ingersoll, C.G., 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. Ecotoxicology 5, 253.

Massoud, M.S., Al-Abdali, F., Al-Ghadban, A.N., 1998. The status of oil pollution in the Arabian Gulf by the end of 1993. Environ. Int. 24, 11–22.

Morales-Caselles, C., 2005. Evaluación de la contaminación y sus efectos en sedimentos del Parque Nacional de las Islas Atlánticas (Galicia) asociados con el vertido del petrolero Prestige. Master thesis, Universidad de Cádiz, pp. 1–121.

Mowat, F.S., Bundy, K.J., 2001. Correlation of field-measured toxicity with chemical concentration and pollutant availability. Environ. Int. 27, 479–489.

NOAA, 1999. Sediment Quality Guidelines Developed for the National Status and Trend Programs. NOAA.

Pelletier, E., Delille, D., Delille, B., 2004. Crude oil bioremediation in sub-Antarctic intertidal sediments: chemistry and toxicity of oiled residues. Mar. Environ. Res. 57, 311–327.

Peters, C., Becker, S., Noack, U., Pfitzner, S., Bülow, W., Barz, K., Ahlf, W., Berghahn, R., 2002. A marine bioassay test set to assess marine water and sediment quality – its need, the approach and first results. Ecotoxicology 11, 379–383.

Peters, C., Ahlf, W., 2005. Reproduction of the estuarine and marine amphipod *Corophium volutator* (Pallas) in laboratory for toxicity testing. Chemosphere 59, 525–536.

Prego, R., Cobelo-García, A., 2004. Cadmium, copper and lead contamination of the seawater column on the *Prestige* shipwreck (NE Atlantic Ocean). Anal. Chim. Acta. 524, 23–26.

Riba, I., Forja, J.M., DelValls, T.A., Guerra, R., Iacondini, A., 2002. Determining sediment toxicity associated with the Aznalcollar mining spill (SW, Spain) using a bacterial bioassay. In: First International Conference on Remediation of Contaminated Sediments. Batelle Press.

Riba, I., Zitko, V., Forja, J.M., DelValls, T.A., 2003. Deriving sediment quality guidelines in the Guadalquivir estuary associated with the Aznalcóllar mining spill: a comparison of different approaches. Cienc. Mar. 29 (3).

Riba, I., Conradi, M., Forja, J.M., DelValls, T.A., 2004a. Sediment quality in the Guadalquivir estuary: lethal effects associated with the Aznalcóllar mining spill. Mar. Pollut. Bull. 48, 144–152.

Riba, I., Forja, J.M., Gómez-Parra, A., DelValls, T.A., 2004b. Sediment quality in littoral regions of the Gulf of Cádiz: a triad approach to address the influence of mining activities. Environ. Pollut. 132, 341–353.

Simon, M.A., Bonner, J.S., Page, C.A., Townsend, R.T., Mueller, D.C., Fuller, C.B., Autenrietha, R.L., 2004. Evaluation of two commercial bioaugmentation products for enhanced removal of petroleum from a wetland. Ecol. Eng. 22, 263–277.

Stronkhorst, J., Schipper, C., Brils, J., Dubbeldam, M., Postman, J., Van De Hoeven, N., 2003. Using marine bioassays to classify the toxicity of Dutch Harbor sediments. Environ. Toxicol. Chem. 22, 1535–1547.

Tweede Kamer, vergaderjaar, 1994–1995, Evaluatienota Water, Dutch standards for contaminated sediments, 21 250, 2728.

USEPA, 1994. Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods. United States Environmental Protection Agency (USEPA). EPA/600/R-94/025.

Van Beelen, P., 2003. A review on the application of microbial toxicity tests for deriving sediment quality guidelines. Chemosphere 53, 795–808.