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Marine Pollution Bulletin 48 (2004) 144–152
MARINE POLLUTION BULLETIN
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Sediment quality in the Guadalquivir estuary: lethal effects associated with the Aznalcóllar mining spill

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Abstract

Monitoring from 1998 to 2001 has assessed the impact of the Aznalcóllar mining spill on the sediment quality in the Guadalquivir estuary. Chemical analysis has been completed with biological effects measured in different organisms. The toxicity of sediments obtained from dilutions of toxic mud and from environmental stations affected by the accidental spill was tested using the amphipod *Ampelisca brevicornis* and the clam *Scrobicularia plana*.

The results obtained show that amphipods are more sensitive to the accidental spill than the clams. A dilution of clean sediment by more than 1.8% of toxic mud produced 100% mortality of amphipods. In GR2 station is detected toxicity to amphipods but not to clams. The rest of the environmental stations show no toxicity. Toxicity to amphipods in GR2 station decreased along time (from 50% to 60% of mortality in 1998 to 10 to 15% in 2001) and it can be associated with a recovery of the areas impacted by the accidental spill.

Keywords: Sediment contamination; Aznalcóllar mining spill; Lethal effects; Amphipod test; Clam bioassay; Heavy metals

1. Introduction

On 25th April 1998 part of the tailings pond dike of the ''Los Frailes'' zinc mine, situated in Aznalcóllar collapsed, releasing an estimated 4 million cubic meters of acidic water and 2 million cubic meters of toxic mud rich in toxic metals over the next 5 d. The Aznalcóllar accident was one of the worst disasters related to acute pollution ever recorded in Spanish history. It damaged about 4328 ha situated at both shores of the Guadiamar river. It occurred in the vicinity of the Doñana National Parks and affected to Doñana Natural Park (Grimalt and Macpherson, 1999).

Monitoring from 1998 to 2001 has assessed the impact of the Aznalcóllar mining spill (April 1998) on the Guadalquivir estuary determining the transport, fate and enrichment of heavy metals from the accidental spill in water, sediment and organisms from the estuary (Gómez-Parra et al., 2000; Riba et al., 2002a,b,c) as a first step in the integrated assessment of the impact of the spill on the estuary. These results show an enrichment of metals in sediments from some areas of the estuary but they do not determine the biological effects associated with the contamination of heavy metals.

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. Their potential advantages have been listed by several authors (Chapman and Long, 1985; Cairns and Mount, 1990; DelValls and Conradi, 2000; Chapman et al., 2002). One of the classification of the biological effects measured in sediment toxicity tests is related to the type of endpoint used: lethal that determines mortality after the time of exposure selected in the test or sublethal that evaluates other different endpoint that informs about biological effect without killing the organisms (burial rate, histopathology—damage of tissues-, growth, reproduction, etc.).

The species of organisms used in the sediment toxicity tests should provide an appropriate indication of the hazards of chemical stressors in the sediment (Chapman et al., 2002). Amphipods are generally acknowledged as

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0025-326X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0025-326X(03)00391-6

the organism's choice for many sediment toxicity assessments, and amphipod toxicity test results can correlate positively with changes in benthic communities (Swartz et al., 1994; Long et al., 2001). Mollusks are also widely applied to determine the sediment toxicity from different origins of contamination (SETAC, 1993; PIANC, in press). Mollusks, particularly bivalves, have assumed a major role in assessing levels of contaminants world-wide. This is a result of strategic advantages in terms of easy of collection, widespread distribution, relatively sedentary habits, suitable size, and often ecological and economic importance. The use of mollusk species as bioindicators encompasses a number of diverse strategies for accumulation, regulation, and immobilization of contaminants (Langston et al., 1998).

These sediment toxicity tests can be developed using samples collected in different areas of the site to study and/or dilution of these samples with reference sediments by dilution experiments. Both approaches are suitable to be used in the determination of toxicity associated with contaminants in sediments.

The main objective of this study is to determine the biological effects associated with the contamination of heavy metals from the accidental spill on the Guadalquivir estuary by comparing toxic responses measured in dilution of toxic mud with reference sediments to those measured in toxicity tests exposing organisms to sediments collected in different areas of the Guadalquivir estuary. Both sediment toxicity tests are performed using lethal endpoint in two different organisms living in the area: the estuarine amphipod *Ampelisca brevicornis* and the estuarine clam *Scrobicularia plana*.

2. Material and methods

2.1. Approach

The present study was developed using four different environmental stations (GL2, GL6, GR2 and GR4) selected in the Guadalquivir estuary (Fig. 1) to cover the influence of the contamination from the accidental spill. The criteria of selection were to cover as much as possible the heavy metal contamination gradient described in previous studies (Riba et al., 2002a,b,c). Also sediment toxicity tests using sediment dilution of toxic mud with reference clean sediment from the Bay of Cádiz (0.3%, 1.8%, 7.9%, 20% and 32% dry weight of toxic mud) were used to correlates the toxic response produced by the contaminants from the mine with those responses measured in the environmental samples.

Previous studies carried out in the estuary (Gómez-Parra et al., 2000) determines it as vertically homogeneous, showing little variation in salinity. Maximum concentrations of all metals are associated with low salinity areas within the estuary. The estuary show a non-conservative behavior of metals that has been reported in other estuaries and in general it is related to precipitation or adsorption processes on the suspended particle matter (SPM) of both organic and inorganic origin (see e.g., Benoit et al., 1994).

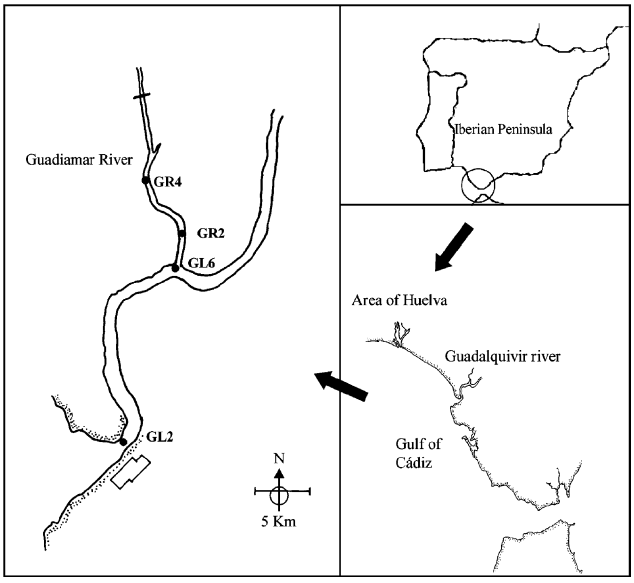

Fig. 1. Map of the Guadalquivir estuary and the Guadiamar river area showing the general areas sampled and locations of the sampling station. GR(#) is selected for the stations located at the Guadiamar river area and GL(#) for Guadalquivir estuary.

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 color or textural differences could be detected. The sediments were sub sampled for chemical quantification (1.5 l aliquots). After that, sediment samples were maintained in the cooler at 4 °C in the dark until they were used for sediment toxicity testing and no longer than two weeks.

2.2. Chemical analysis

For sediment grain size an aliquot of wet sediment was analyzed using a laser particle size Fristch (model Analysette 22) following the method reported by DelValls et al. (1998a).

The sediment samples from the four stations located in the Guadalquivir estuary, the negative and positive control, and the dilutions of toxic mud were dried and homogenized at 60 °C prior to chemical analysis.

For trace metal analysis, the sediments were digested as described by Loring and Rantala (1992). Zn and Cu concentrations in the extracts were determined with a Perkin–Elmer 2100 Flame Atomic Absorption Spectrophotometer. The other trace metals were measured by

graphite furnace atomic absorption spectrophotometry (Perkin–Elmer, 4100 ZL). The analytical procedures were checked using reference material (MESS-1 NRC and CRM 277 BCR) and allow agreement with the certified values higher than 90%.

Organic carbon content was determined using the Gaudette et al. (1974) method, with the El Rayis (1985) modification.

The PAHs and PCBs analysis were carried out according to US EPA SW-846 Method 827C78082. Briefly, following recommendations by Riba et al. (2002a), 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 rotatory evaporator and re-dissolved in isooctane. Aromatic fractions were analyzed on a Hewlett–Pakard (HP) 5890 Series II gas chromatograph coupled with 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 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 were performed with the same instrument with an electron capture detector (GC/ECD) using 30 m × 0.25 mm MDN-5S capillary column. Quantization was performed with 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 allow agreement with the certified values higher than 90%.

2.3. Toxicity tests

Two sediment bioassays were developed to measure sediment toxicity in the environmental stations located in the Guadalquivir estuary (Fig. 1) and in the dilution tests (0.3%, 1.8%, 7.9%, 20% and 32%) using two benthic species widely distributed in the studied areas: the amphipod *A. brevicornis* and the clam *S. plana*. The dilution experiment was performed using clean sediment (NC) collected in a clean area of the Bay of Cádiz (DelValls et al., 1998b) and toxic mud (TM) collected 40–45 km far from the tailing spill point, the day after the accident. Once the clean sediment and the toxic mud are dried, different dilutions were performed on a dry weight base on the same vessels used in the bioassays. The dilutions are referred as percentage of toxic mud. The toxic mud and the clean sediment were mixed by means of polyethylene spoon and homogenized using an aliquot of clean sea water selected to be used during the toxicity tests. The sediments from the environmental stations located in the Guadalquivir estuary were wet used as collected in the field.

The test using the estuarine clam *S. plana* was developed in whole sediment. Clams were provided by an aquaculture farm located in the Bay of Cádiz and with an absence of contamination. Ten clams per tank were used during 96 h of exposure and the mortality during the bioassay was selected as the endpoint. The sediment toxicity test was developed using 15 l vessels containing 2 l of sediment overlaid with filtered clean seawater (about 8 l). An extra sediment toxicity test using this organism was conducted during 10 d of exposure to compare the lethal responses between the amphipod and the clams with the same period of exposure. This test (10 d) was conducted with the same conditions as described above although a water replacement was carried out once during the bioassay (5 d).

Individuals of the estuarine amphipod *A. brevicornis* were collected in the same 'clean' sediments used for the dilution of toxic mud. Twenty amphipods per tank were exposed to bulk sediment with percent of survival after 10 d of exposure as the endpoint (ASTM, 1993). After this exposure period the sediments were sieved and surviving amphipods were removed and counted. Sediment toxicity test was performed using 2 l glasses beakers containing a 5 cm layer of test sediment (about 200 ml) overlaid with filtered clean seawater (about 800 ml). The concentration of total ammonia in the interstitial water was monitored at the beginning and at the end of the bioassays ranged between 9–25 $mg\,l^{-1}$ (pH ranged between 7.5–7.9) in all the stations. These concentrations are considered with absence of influence in the toxic results produced by chemicals.

Both tests were carried out in whole sediment using a 1:4 v/v sediment water relation and with constant aeration. The temperature (20 °C ± 1 °C), pH (7.8–8.2), salinity (33.8 ± 0.2) and dissolved oxygen (>5 $mg\,l^{-1}$, 60% saturation) were measured and controlled every day. The concentration of total ammonia in the interstitial water was monitored at the beginning and at the end of the bioassay being lower than 20 $mg\,l^{-1}$ (pH ranged between 7.5 and 7.9) in all the sediments and controls.

After the acclimation of the organism (1 month) the two different bioassays were developed by replicate.

Adequate quality assurance/quality control (QA/QC) measurements were followed in all aspects of the study, from field sampling through to laboratory and data entry as per ASTM (1991a,b).

2.4. Data calculation and statistical analysis

The mortality of the *A. brevicornis* (10 d) and *S. plana* (96 h and 10 d) measured in the toxic mud dilution

assays was used to derive a toxic parameter (LC_{50}) associated with the toxic mud. The parameter LC_{50} is defined as the lethal concentration of toxic mud (% dry sediment) that provokes the mortality of the 50% of the population exposed. It was calculated by linear regressions of log toxicant dilution of toxic mud on declining probit values using software provided by the US Environmental Protection Agency (EPA) probit-analysis-program (version 1.5).

Another toxic parameter (LT_{50}) was obtained from the clam assay and it was defined as the time required for the 50% of the initial populations to die. The percent of amphipod mortality and the parameter LT_{50} were compared using ANOVA and Scheffe's F tests to identify significant differences between the environmental stations affected and the dilutions prepared with the toxic mud compared to the negative controls ($p < 0.05$).

3. Results and discussion

3.1. Chemical analysis

Summarized results of chemical concentrations of selected contaminants, grain size and percentage of organic carbon in environmental stations and negative and positive control are shown in Table 1. Levels of organic matter and texture are similar among the sediment sample. Organic contamination at environmental stations was low and only related to the concentration of PCBs at stations GL2, GL6 and GR4 being PAHs not detected in any of the environmental stations. There was not detected contamination by PCBs and PAHs in the positive control.

To compare the contamination related to the concentration of heavy metals in the Guadalquivir estuary to that from the mining spill it has been measured the concentration of the metals in each of the toxic mud dilutions. An error coefficient (ε) was derived and defined as

$$\varepsilon = \frac{C_i - C_{\mathrm{Th}}}{C_{\mathrm{Th}}} * 100 \quad (1)$$

being C_i the concentration of the heavy metals measured in each toxic mud dilution and C_{Th} the theoretical concentration of the same metal derived from the concentrations measured at the negative control and the toxic mud used in the sediment dilutions and derived as an arithmetic concentration based on their relative proportions. Summarized results are shown in Table 2. In general, the measured concentrations of heavy metal in the dilutions were in an acceptable agreement with the theoretical concentration for each dilution. The percentage of error (ε) ranged between 0.5% (Fe measured at 1.8% of toxic mud dilution) and 19% (Cu measured at 1.8% of toxic mud dilution), except for the heavy metal Cd at toxic mud dilution of 0.3%, 1.8% and 7.9%. Those heavy metals (Cd and Cu) with low concentrations in the negative control showed higher errors than those with high concentrations (Zn, Fe, Pb and Mn), and associated with the low dilutions of toxic mud.

The concentrations of heavy metals in the sediments collected in the environmental stations were higher at station GR2 than in the rest of the environmental samples. Compared to the highest value measured in other stations the concentrations of heavy metals Zn were 73%; Cd, 63%; Pb, 57%; and Cu 19% higher than those measured in the estuary (GL2, GL6, and GR4). The concentration of all heavy metals in the environmental stations, except Mn, were in range between the heavy metal concentrations measured at dilutions 7.9% and 0.3% of toxic mud being similar to those measured at dilution 1.8%. In this sense, the concentration of heavy metals Fe and Cd were higher in GR2 than those measured at dilution of 1.8% of toxic mud and metals Zn, Cu and Pb have lower concentrations than those

Table 1
Heavy metal concentration (mg kg^{-1} for all metals except Fe as % dry weight)

	Negative control	Positive control	GL2	GL6	GR2	GR4
Zn	41.6	21618	81.9	138	273	158
Fe	0.5	41.6	0.6	2.1	3.2	2.9
Cd	0.1	45.7	0.1	0.3	1.3	0.8
Pb	71.9	7873	66.0	55.1	86.7	44.7
Cu	9.5	2033	15.9	30.6	53.3	44.7
Mn	163	381	186	400	812	510
CO (%)	1.4	0.4	0.7	1.1	1.0	1.1
% Fine sand	90	92	80	85	91	93
PAHs	0.30	n.d.	n.d.	n.d.	n.d.	n.d.
Aroclor 1242	0.45	n.d.	0.73	0.43	n.d.	0.92
Aroclor 1260	0.09	n.d.	0.24	0.22	n.d.	0.12

Organic carbon (%), grain size composition (% fine sand), total PAHs and total PCBs (mg kg^{-1} dry sediment) expressed as Aroclor standards (1242 and 1260) measured in the negative control, positive control and in the environmental stations selected in the Guadalquivir estuary (GL2, GL6, GR2, GR4) for the *A. brevicornis* and *S. plana* bioassays.
n.d: non-detected. (<0.1).

Table 2
Theoretical (C_{Th}) and measured (C_m) concentrations of heavy metals (Zn, Fe, Cd, Pb, Cu and Mn expressed as mg kg^{-1}, except Fe as % dry weight) for each of the toxic mud dilution tests used in the sediment toxicity test using *A. brevicornis* and *S. plana*

		0.3%	1.8%	7.9%	20%	32%
[Zn]	C_m	100	404	1810	4617	6876
	C_{Th}	106	430	1746	4335	6946
	ε	5.79	5.97	3.65	6.49	1.01
[Fe]	C_m	0.54	1.21	3.45	9.42	14.8
	C_{Th}	0.58	1.20	3.71	8.65	13.6
	ε	7.25	0.61	7.05	8.80	9.19
[Cd]	C_m	0.14	0.67	2.56	9.48	15.3
	C_{Th}	0.20	0.90	3.6	9.20	14.70
	ε	30.0	25.6	28.9	3.04	3.81
[Pb]	C_m	88.6	244	653	1853	2976
	C_{Th}	95.3	212	688	1632	2568
	ε	7.06	15.0	5.06	13.5	15.9
[Cu]	C_m	16.2	54.7	180	334	588
	C_{Th}	15.5	45.9	169	412	657
	ε	4.44	19.1	6.30	18.8	8.56
[Mn]	C_m	140	175	180	222	240
	C_{Th}	163	166	180	206	233
	ε	14.2	5.33	0.19	7.63	3.00

The relative error (ε) is calculated as described in the text and expressed as percentage.

measured at 1.8% of toxic mud dilution. These results ($p < 0.05$) agree with those previously reported in the area during the initial assessment of the impact in the estuary (e.g., Gómez-Parra et al., 2000; Riba et al., 2002a,b,c) and that detected an enrichment of heavy metal concentration of Zn and Cd in the areas of the estuary.

3.2. Toxicity tests

In the Fig. 2 are shown the summarized results of amphipod survival for the environmental stations and the toxic mud dilution measured during the 3 years of monitoring of the impact of the spill on the estuary. These results were obtained during the sediment toxicity testing using the amphipod *A. brevicornis* selected as the key toxicity test to determine the impact of the accidental spill on the estuary (Riba et al., 2003).

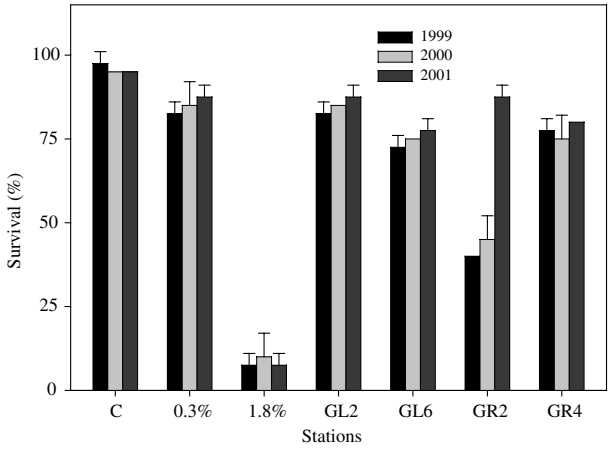

Fig. 2. Percentage of amphipod survival after 10 d of exposure to diluted toxic mud (0.3% and 1.8%) and to sediments collected in the Guadalquivir estuary (GL2, GL6, GR2, and GR4) from 1998 to 2001.

Only the survival measured in the toxic mud dilutions 0.3% and 1.8% were shown because the survival measured at the other high dilutions of toxic mud was 0% of the amphipod exposed. Amphipods survival at dilution of toxic mud 1.8% ranged between 5% and 15% during the 3 years of monitoring and it classifies this dilution as toxic. Differences along time were not detected associated with the toxicity testing of toxic mud dilutions.

Regarding to the results of amphipod survival measured at the environmental stations during the monitoring of the impact of the spill on the estuary it can be observed that during the first 2 years (1999 and 2000) a toxic response (about 60–50% of mortality of amphipod) was detected in the station GR2 located in the Guadiamar River. This toxic response decreased to about 10–15% of mortality in the last monitoring survey of the impact during the year 2001. The rest of the stations located both in the Guadalquivir and Guadiamar river were considered as not toxic (survival higher than 78% during the 3 years) using the amphipod *A. brevicornis*. These results can be associated with the recovery of those impacted areas in the estuary after 3 years of the accidental spill.

The results of mortality of individuals of the clam *S. plana* along time during the exposure of the organ-

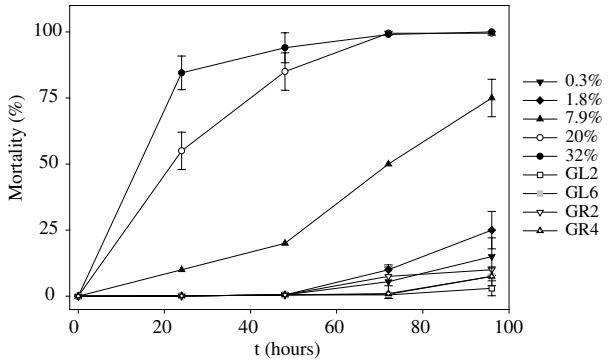

Fig. 3. Mortality of clams along 96 h of exposition to diluted toxic mud (0.3%, 1.8%, 7.9%, 20% and 32%) and sediments from the Guadalquivir estuary (GL2, GL6, Gr2, and GR4).

isms to the toxic mud dilutions and the environmental stations collected during the year 1999, for 96 h of exposure are shown in Fig. 3. Toxic responses for all the stations increase with time being highest at the end of the bioassay. During the first 72 h no mortalities were measured at environmental stations and at toxic mud dilutions of 0.3% and 1.8%. At the end of the bioassay little differences can be associated with the mentioned environmental stations and the mentioned toxic mud dilution.

Higher dilutions of toxic mud than 1.8% provoke toxicity during the first hours. It can be observed that highest dilution of toxic mud (32%) killed most of the individuals of the clam after the first 24 h of exposure. Both toxic mud dilution 32% and 20% are related to a 100% of the mortality of the clams after the 96 h of exposure. Dilution of 7.9% increases toxicity with time and it was measured a mortality of about 75% at the end of the bioassay.

From these results it can be calculated a lethal parameter, defined as the time required for the 50% of the initial population of clams to die (LT_{50}). The results of the LT_{50}s are shown in Table 3. The values of the calculated LT_{50}s for all the environmental stations and for the dilution of toxic mud 0.3% and 1.8% were higher than the exposure time so considered as not toxic. The lowest value of the LT_{50} is calculated for the highest dilution of toxic mud and increases when the dilution decreases.

Both endpoints from the two bioassays performed during the year 1999, survival of amphipods and LT_{50} for clams, were used to ordinate the environmental stations and the toxic mud dilutions based on the toxic responses measured. The results of the ANOVA and the Scheffe's multiple comparison tests ($p < 0.05$) for all the treatments are shown in Fig. 4. For both lethal responses the environmental stations GL2, GL6 and GR4 are not toxic and non-significant differences ($p < 0.05$) were measured compared to the control. The amphipod

Table 3
Average values of the time required to die by the 50% of the clam populations (LT_{50}) for each environmental station and dilution of toxic mud

Station	LT_{50}	Average value
0.3% A	139.409	>96 (136.95)
0.3% B	134.495	
1.8% A	115.024	>96 (112.22)
1.8% B	139.409	
7.9% A	66.904	69.43 ± 3.58
7.9% B	71.961	
20% A	25.229	23.20 ± 2.87
20% B	21.174	
32% A	13.96	10.04 ± 5.54
32% B	6.12	
GL2 A	7018	>96 (6009)
GL2 B	5000	
GL6 A	155.06	>96 (139.47)
GL6 B	123.89	
GR2 A	121.1	>96 (118.8)
GR2 B	116.5	
GR4 A	134.49	>96 (194.78)
GR4 B	255.06	

The values of LT_{50} are expressed as hours. In brackets it is shown the results of the LT_{50} output by the probit method.

mortality shows that sediments collected at the station GR2 can be considered moderately toxic because they are significantly different ($p < 0.05$) compared to the mortality measured in the control. It is also significantly different ($p < 0.05$) compared to the mortality measured at the toxic mud dilution of 1.8% or higher.

From the clam bioassay it can be considered that none of the environmental stations neither the toxic mud dilution 0.3% and 1.8% are toxic because their LT_{50}s are not significantly different ($p < 0.05$) compared to the control. Dilutions of toxic mud of 7.9% or higher are considered toxic because their LT_{50}s are significantly different ($p < 0.05$) compared to those calculated at the control. It is necessary to note that the toxic parameter LT_{50} calculated at dilution of 7.9% was homogenous ($p < 0.05$) to those measured at low dilutions 0.3% and 1.8%.

From these results it can be determined that the amphipod test was more sensitive that the clam toxicokinetic assay to the contamination measured in the estuary and to that associated with the toxic mud from the mining spill.

To corroborate it and to assess the effects associated with the heavy metal contamination from the Aznalcóllar mining spill we have calculated the lethal concentration of toxic mud (LC_{50}—% dry weight) associated with the toxic responses measured at both bioassays. The obtained results are presented in Table 4

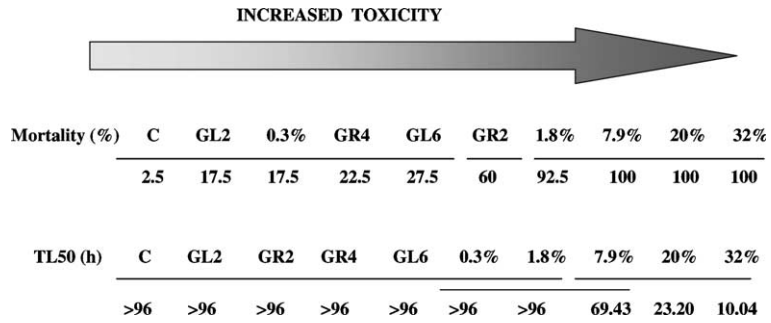

Fig. 4. Summary of the results of the toxicity tests (mean values). Treatments not underlined by the same line are significantly different at $p < 0.05$ (Scheffe's F-tests).

Table 4
Values of LC_{50} (the concentration needed to kill the 50% of the *A. brevicornis* and *S. plana*) expressed as percentage of toxic mud (dry weight) for the two bioassays

Year	Bioassay	LC_{50}	Minimum	Maximum
1999	Amphipods (10 d)	0.68	0.39	1.06
2000	Amphipods (10 d)	0.75	0.45	1.10
2001	Amphipods (10 d)	0.67	0.36	1.05
1999	Clams (96 h)	3.25	1.25	5.75
2003	Clams (10 d)	2.99	1.02	5.58

Two different LC_{50} were calculated for clams at different periods of exposure.

for the different amphipod bioassays conducted during the years 1999 to 2001 and for two different tests using clams using two exposure period of time (10 d and 96 h). The values of LC_{50} derived for the clam test during 1999 and after 4 years (2003) are similar. The values of LC_{50} derived for the mortality of amphipods are lower than those associated with the LT_{50} derived at clam bioassays.

The mortality of the amphipod identifies a toxic response associated with the contaminants bound to sediments located in the station GR2 and not significant ($p < 0.05$) mortality was measured in the other sediments collected in the estuary (GL2, GL6 and GR4). It confirms that the sensitive of the amphipods is higher than the bivalves as previously reported by other studies (Pearson and Rosenberg, 1978) and that the accidental spill provoked an acute and moderated effect on the sediment quality in the estuary that is associated with the toxic responses using amphipods and not using clams.

This toxicity can be related to the high concentration of the heavy metal Cd that was higher than those measured at the dilution of toxic mud of 1.8% and demonstrated as toxic (mortality 90%) to the *A. brevicornis*. Besides the heavy metal Zn although with lower concentrations than those measured at dilution of toxic mud of 1.8% can be contributing to the toxic responses measured in the GR2 station. It is based on the relationship between both metals with a common origin related to the accidental spill, and that was pointed out in previous studies performed in the area (Gómez-Parra et al., 2000; Riba et al., 2002a,b,c,d). To confirm this relationship a multivariate analysis approach is conducted by Riba et al. (2003) linking the lethal responses and the concentration of chemicals obtained in this study with the sublehtal responses described in the mentioned paper.

4. Conclusions

This study presents the results and interpretation of a chemical analysis and a biological assessment of four environmental samples from the Guadalquivr estuary and different dilutions of toxic mud from the Aznalcóllar mining spill (April 1998) with control reference sediment to provide a relationship between the contamination detected in the estuary and the toxicity associated with it. Two different sediment toxicity tests using two indigenous species (amphipod: *A. brevicornis* and clam: *S. plana*) and measuring as endpoint the mortality after 10 d and 96 h of exposure of the amphipods and the clams, respectively, were performed to establish the lethal effects associated with the accidental spill. Within the context of this study a number of specific conclusions can be derived regarding the lethal effects associated with the metals from the accidental spill. These conclusions are summarized as:

(a) In general, the contamination measured in the Guadalquivir estuary is lower than that from the toxic mud (1.8%).
(b) It can be assumed that the sediment toxicity test using the amphipod *A. brevicornis* establishes a better assessment of the adverse effect in the Guadalquivir estuary and associated with the Aznalcóllar mining spill than the sediment toxicity test using the mortality of the clam *S. plana*. The use of the two bioassays are suitable to determine the toxicity associated with

the sediment contamination because these organisms use different exposure routes, amphipods fed through the sediment and clams are deposit-feeder through filtration of the overlain water.

(c) The toxic response measured only by one of the bioassays in one of the environmental stations selected in this study determine that the adverse effects of the accidental spill on the estuary were moderated and located in specific areas of the estuary conferring an acute classification of the impact. Besides the decreases in the toxic responses determined by the amphipod test during the last years of monitoring identify a potential recovery of the system once the Guadiamar river was cleaned up and the spill was stopped.

Acknowledgements

We thank Rosa Vazquez and the personnel at aquaculture plant in the faculty of Marine Science for their help during the acclimation of both species. Thanks are due to Ruchi, Isabel, Carmen, Pablo, Martin and Dr. García-Luque for their help in this study from the sampling to the end of the bioassays. This research was supported by the 'Consejería de Medio Ambiente de la Junta de Andalucía', Convenio PICOVER and partially by grant REN2002-01699 from the Spanish National Plan for Research, Innovation and Development (Ministerio de Ciencia y Tecnología). Inmaculada Riba thanks the 'Consejería de Educación y Ciencia de la Junta de Andalucía' for her Doctoral fellowship (F.P.D.I.).

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