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Comparative Toxicity of Contaminated Sediment from a Mining Spill Using Two Amphipods Species: *Corophium volutator* (Pallas, 1776) and *Ampelisca brevicornis* (A. Costa, 1853)

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Over the last several decades the effects of chemicals on living systems have been intensively studied experimentally by measuring responses in organisms following exposure to the contaminants. Biological tests can be used to inform decisions regarding the environmental management of chemicals or materials contaminated with hazardous substances. One of the widely used sediment toxicity tests is that evolving the exposure of individuals of amphipods to aquatic sediments (Long et al., 2001). *Corophium volutator* is the recommended species to carry out this kind of sediment toxicity test by several regulatory agencies in Europe (CEFAS, 1997; RIKZ, 1999). Furthermore the use of this specie is specially recommended during a tier testing assessment of sediment quality previously to the final decision on the management of dredged material in ports and harbours in Europe (Nendza, 2002; DelValls et al., 2001). However the use of the *Corophium volutator* test is limited to assess the sediment toxicity in samples collected in the ecosystems from the South of Europe. The limitation is based on the low density and frequency of this specie found in the ecosystems located in areas of the South of Europe. On the other hand, the amphipod specie *Ampelisca brevicornis* has a wide distribution in coastal ecosystems from these littoral areas (Hastings, 1981; DelValls, 1994). Different regulatory agencies in North America have recommended the use of the taxa *Ampelisca sp.* to assess sediment toxicity and in tier testing assessments on the management of dredged material (US EPA, 1994).

After the Aznalcóllar mining spill (April, 1998) different aquatic ecosystem (Guadiamar and Guadalquivir rivers, Doñana National Parks) were impacted and their sediments polluted by complex mixtures of heavy metals. The concentrations of five metals Zn, Cd, Pb, Cu and As were used as tracers of the extension of the impact provoked by the spill. Samples of contaminated sediments located in the area of the Guadalquivir estuary were assessed under an integrated point of view and their toxicity demonstrated (Riba et al., 2002). The aims of this work are to assess the sensitivity of both amphipod species to the contamination associated with the material from the accidental spill and to establish the usefulness of the toxic response measured by a new specie such as *Ampelisca brevicornis* tested versus the standard test organism *Corophium volutator* using contaminated sediments enriched by complex mixtures of heavy metals from an accidental spill.

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MATERIALS AND METHODS

The present study was developed using three different sediment dilutions of contaminated sediment from the Aznalcóllar mining spill using reference clean sediment from the Bay of Cádiz (0.3%, 1.8%, 7.9% -dry weigh of contaminated sediment-). Clean sediment was collected from a pristine area located in the Bay of Cádiz (DelValls et al.,1996, 1997) and sieved through a 0.6-mm mesh into a tank in order to remove any associated macrofauna and larger sediment particles. Contaminated sediment was collected 40-45Km far from the source of the spill the day after the accident and was sieved through a 0.6-mm mesh too. These sediments were dried and homogenized at room temperature not higher than 40°C prior to chemical analysis. Aliquots for toxicological analysis were stored wet in dark till processing.

All individuals of the specie *Ampelisca brevicornis* used in these experiments were collected from the sediment located in the intermareal zone of the Bay of Cádiz, by sieving the sediment through a 0.6-mm mesh. They were then held and transported to the laboratory in 10L glass beakers with filtered seawater and constant aeration. The crustacean amphipod *Corophium volutator* was collected by sieving mud through a 1-mm mesh at Oesterput in the Ooesterschelde estuary located in Netherland. The animals were shipped to Spain and received in less than 48 hours. Individuals of both species were maintained in acclimation during one month prior to their use in the bioassay under the next conditions: glass aquarium of 20 L, filtered sea water (renewal every three days), dissolved oxygen concentration higher than 6 mg L^{-1}, pH and temperature ranged between 8.0-8.2 and 20-21°C respectively, artificial fed (Advanced Invertebrate Formula, Marine Enterprise International) was provided each water replacement day.

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).

For trace metal analysis, sediment samples of clean sediment, contaminated sediment and their dilutions were totally digested as described by Loring and Rantala (1992). The concentrations of heavy metals Zn and Cu in the extracts were determined with a Perkin-Elmer 2100 Flame Atomic Absorption Spectrophotometer. The trace metals Cd and Pb were measured by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer, 4100 ZL) and As in the extracts was determined by means of Perkin-Elmer MHS-FIAS coupled with a Perkin-Elmer 4100 ZL spectrophotometry. 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 El Rayis (1985) modification based on a acidification of the sediment sample.

The PAHs and PCBs analysis were carried out according to U.S.EPA SW-846 Method 827C78082 and were measured as described by Riba et al. (2002). For

PAHs quality control was carried out using NRC-CNRC HS-6 sediment reference material and NRC-CNRC HS-1 sediment reference material for PCBs expressed as AROCLOR 1242 and 1260. For both sets of organic chemicals PAHs and AROCLOR the analytical procedure allow agreement with the certified values higher than 90%.

Results of theoretical (C_{th}) and measured concentrations (C_r) of heavy metals (Zn, Cd, Pb, Cu and As) are shown for each of the contaminated sediment dilution tested in sediment bioassay. The relative error (C_r-C_{th}/C_{th}) is expressed as percentage and using an absolute value. Organic contaminants were not measured in the dilution treatments but theoretical.

The toxicity tests were performed exposing individuals of the amphipods *Ampelisca brevicornis* and *Corophium volutator* to bulk sediment using the percentage of survival after ten days of exposure as the endpoint (ASTM, 1993). Sediment toxicity tests were performed using 2 litter glass beakers containing a 5 cm layer of test sediment (about 200 ml) overlaid with filtered clean seawater (about 800 ml). Both tests were carried out in whole sediment using a 1:4 v/v sediment water relation. The experimental set-up was maintained under constant aeration for 24h before any *Ampelica* and *Corophium* were added to each replicate. Aeration was provided with a Pasteur pipette without disturbing the sediment surface. The temperature (20ºC±1ºC), pH (7.8-8.2), salinity (33.8±0.2) and dissolved oxygen (>5 mg L^{-1}, 60% saturation) were measured (temperature: platinum resistance thermometer –Hart model 1502-; pH: ion analyzer – Radiometer ION85- using combined glass electrodes –GK2410G-, salinity: induction salinometer -Beckman RS-10-, and oxygen: oxygen electrode –WTW CellOx 325-) and controlled every day in each replicate and bioassay.

Twenty individuals of *Ampelisca brevicornis* were selected from the acclimation beakers and placed into each replicate (5 replicates per dilution of contaminated sediment and negative control of toxicity). All *Ampelisca* selected were adults (3-5 mm) and with the sexes in equal proportion. Twenty adult of *Corophium volutator* (average 5-7 mm) were arbitrarily selected from the acclimation beakers and placed into each of five replicates with different dilutions of contaminated sediment.

No food was supplied during the course of the experiments and after ten days of exposure period, sediment samples were taken for chemical analysis and were sieved for counting the surviving amphipods.

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

The mortality of the *Ampelisca brevicornis* and *Corophim volutator* measured after ten days of exposure time was used to derive a toxic parameter (LC50) associated with the contaminated sediment. Three different dilution of contaminated sediment with control sediment were used (0.3%, 1.8% and 7.9%)

to define the concentration (percentage of contaminated sediment) that provokes the mortality of the 50% of the population exposed (LC50 -% of contaminated sediment, dry weigh-). It was calculated by linear regressions of log toxicant dilution of contaminated sediment on declining probit values.

To identify the concentration of heavy metal consistent with the toxicity associated with the contaminated sediment from Aznalcóllar mining spill and to compare the lethal effects measured using two different amphipods we have derived sediment quality values (SQVs) for five metals (Zn, Cd, Pb, Cu and As). These SQVs for both species of amphipods were calculated using the LC50 values and the concentration of metals Zn, Cd, Pb, Cu and As measured in the contaminated sediment (table 1). These SQVs are defined as the concentrations of Zn, Cd, Pb, Cu and As associated with the mortality of 50% of the total populations of amphipods after 10 days of exposure.

RESULTS AND DISCUSSION

Summarized results of chemical analysis in the treatments used in the two bioassays are shown in table 1.

Table 1. Organic Carbon (O.C. -%-), grain size composition (F.S. -% fine sand-), total PAHs, total PCBs and metal concentration (mg Kg^{-1}-dry sediment-) measured in the treatments for the *Ampelisca brevicornis* and *Corophium volutator* bioassays.

	Clean sediment	Contaminated sediment	0.3%	1.8%	7.9%
O.C	1.4	0.4	1.397	1.382	1.321
F.S.	90	92	90.01	90.04	90.16
PAHs	0.30	n.d.	0.001	0.005	0.024
A 1242	0.45	n.d.	0.001	0.008	0.036
A 1260	0.09	n.d.	n.d.	0.002	0.007
[Zn] C_r	41.6	21618	97.80	398.8	1600
C_{Th}	--	--	106.4	430.0	1746
ε	--	--	8.050	7.260	8.370
[Cd] C_r	0.1	45.66	0.220	0.551	3.026
C_{Th}	--	--	0.200	0.900	3.600
ε	--	--	10.00	38.78	15.94
[Pb] C_r	71.90	7873	80.02	250.23	623.9
C_{Th}	--	--	95.30	212.3	688.2
ε	--	--	15.84	17.90	9.328
[Cu] C_r	9.5	2033	15.96	54.23	180.2
C_{Th}	--	--	15.53	45.89	169.4
ε	--	--	2.769	18.17	6.425
[As] C_r	11.20	4088	22.10	80.12	301.0
C_{Th}	--	--	23.43	84.58	333.2
ε	--	--	5.676	5.273	9.689

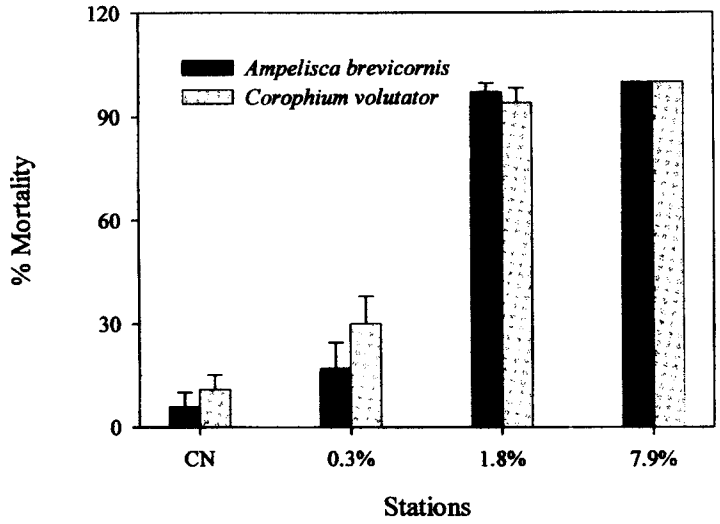

Figure 1. Average and standard deviations of the mortality calculated from five replicates for each of the sediment toxicity test using species of amphipods *Ampelisca brevicornis* and *Corophium volutator* after ten days of exposure. CN refers to clean sediment from the Bay of Cádiz.

Organic contamination associated with concentration of PAHs and PCBs are not detected in any of the contaminated sediment dilutions because concentrations of PCBs and PAHs in the positive and in the negative control of toxicity are not significant.

To establish the differences between the concentrations of heavy metal measured in the contaminated sediment dilution (C_r) and the theoretical concentration (C_{Th}) calculated from the concentrations measured at the negative control and at the contaminated sediment used in the sediment dilutions an error coefficient (ε) was derived. Summarized results are shown in Table 1.

The heavy metal concentration increases with the contaminated sediment dilution being highest at 7.9%. 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 2.8% (Cu measured at 0.3% of contaminated sediment dilution) and 18.2% (Cu measured at 1.8% of contaminated sediment dilution), except for the heavy metal Cd at contaminated sediment dilution of 1.8%. Those heavy metals (Cd and Cu) with low concentrations in the negative control showed higher errors than those with high concentrations (Zn, Pb, As).

In the figure 1 it is shown the summarized results of the amphipod *Ampelisca brevicornis* and *Corophium volutator* exposed to the contaminated sediment dilution 0.3%, 1.8% and 7.9% and to the negative control. Overall mean survival

in all the clean reference sediments from the Bay of Cádiz (CN) was higher than 94% for both species.

The mortality of amphipods increases with the contaminated sediment dilution. *Corophium* survival at dilution of contaminated sediment 0.3% ranged between 60-80% during the ten days of exposure and *Ampelisca* survival ranged between 75-95% at the same dilution. The mortality of both species at dilution of 1.8% was about 90%. Besides, the mortality for *Ampelisca* and *Corophium* at dilution of 7.9% was 100%.

It has been calculated the lethal concentration of contaminated sediment (LC50-% dry weight-). The obtained results are presented in table 2. The lethal concentration of contaminated sediment (LC50 -% dry weight-) associated with the toxic responses measured in *Ampelisca brevicornis* bioassay (0.68±0.06) is similar to that obtained in *Corophium volutator* bioassay (0.57±0.03).

Table 2. Mean values of LC50 (% -dry weight-) calculated for the *Ampelisca brevicornis* and *Corophium volutator* bioassays. Sediment quality values for Zn, Cd, Pb, Cu and As (mg Kg^{-1} -dry weight-) are obtained from the $LC50_s$ and calculated for the *Ampelisca brevicornis* and *Corophium volutator* used in the sediment toxicity tests. *not-detected

	Ampelisca brevicornis	*Corophium volutator*	SQVs -Triad-	SQVs -sublethal-
LC50	0.676±0.06	0.567±0.03	---*	---*
Zn	146.1±13.2	122.5±7.0	156-225	---*
Cd	0.309±0.03	0.259±0.02	---*	0.94-1.24
Pb	53.22±4.81	44.61±2.56	49-115	51.2-52.5
Cu	13.52±1.22	11.33±0.65	68-98	49.4-71.2
As	27.63±2.50	23.16±1.33	58-64	---*

These results show that both species of amphipods have the same lethal responses to the complex mixtures of heavy metals from the contaminated sediment associated with the Aznalcóllar mining spill although *Ampelisca* show values slightly higher than those derived using *Corophium*.

Summarized results of sediment quality guidelines (SQVs) calculated from the LC50 values and the heavy metal concentrations measured in the contaminated sediment and in the negative control are shown in table 2. It is not observed differences between the concentrations of heavy metals derived using LC50 for each species. The SQVs are compared to those using different integrative

assessments previously reported in the area. The SQVs-Triad- were derived by DelValls and Chapman (1998) using the Sediment Quality Triad in the Gulf of Cádiz and in the Bay of San Francisco by means of a multivariate analysis approach. The SQVs -sublethal- were derived by DelValls et al (1998), using sediment toxicity tests (endpoint: subletal, histopathology) in samples from the Bay of Cádiz and the same approach using multivariate analysis. Two different values are provided in table 2. The low value is considered a no observed effect concentration (NOEC) whereas the high value is calculated as the lowest observed effect concentration (LOEC) of heavy metals. The SQVs obtained in this study are in the same range than those previously reported. In general for both species, SQVs derived in this study are similar to the lower values of the SQVs previously obtained in the area.

From the obtained results it can be shown that the specie of amphipod *Ampelisca brevicornis* can be used to establish the sediment toxicity associated with sediment samples in South of Europe. The similarities (linear correlation r^2 = 0.96) in the toxic responses measured by the new specie *Ampelisca brevicornis* compared to the standardized *Corophium volutator* determine the potential of the *Ampelisca* to be included in the battery of sediment toxicity tests to characterize dredged material in Spanish Ports.

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