SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 23, No. 5, pp. 1100–1107, 2004

Printed in the USA
0730-7268/04 $12.00 + .00

THE INFLUENCE OF pH AND SALINITY ON THE TOXICITY OF HEAVY METALS IN SEDIMENT TO THE ESTUARINE CLAM *RUDITAPES PHILIPPINARUM*

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(*Received* 10 *December* 2002; *Accepted* 14 *October* 2003)

Abstract—An approach is presented for determining the influence of two key variables, pH and salinity (S), on the toxicity of four common heavy metals bound to sediments in estuaries. Two samples of environmental sediment taken from two estuaries in southern Spain (the Huelva estuary and the Guadalquivir River estuary), together with a dilution of toxic mud from the Aznalcóllar (Spain) mining spill (April 1998) were used to determine their toxicity at different values of pH (6.5, 7.5, and 8.5) and salinity (10, 20, and 30) on the estuarine clam *Ruditapes philippinarum*. Two different endpoints, sublethal, indicated by clam reburial (median effective burial time [ET50]), and relative mortality (median lethal concentration [LC50]), were used to quantify the toxicity associated with the heavy metals. Neither salinity nor pH was found to influence the toxic responses measured by the behavioral endpoint (ET50). However, a strong effect on the LC50 related to pH and salinity was detected, with the toxicity of the heavy metals being increased at low values of both variables (pH = 6.5 and S = 10). The mechanism of heavy metals uptake through water may explain this influence of pH and salinity on the lethal toxicity detected. The results show differences in the toxicity of these heavy metals bound to sediments depending on whether the origin of metal contamination is chronic or acute.

Keywords—Sediment toxicity Proton concentration Salinity Estuaries Heavy metals

INTRODUCTION

Estuaries are among the most productive marine ecosystems in the world [1], therefore sediment contamination in estuaries and its biological effects must be properly and fully assessed. The methods devised specifically for assessing these effects in estuarine sediments are few and relatively new [2]. Most of these methods do not take into account the unique and dynamic nature of estuarine ecosystems. Recent papers recommend conducting specific studies to determine the effects of salinity on bioavailability and toxicity of contaminants bound to estuarine sediments.

Trace metals are among the most common contaminants bound to estuarine sediments. The bioavailability and toxicity of these metals to aquatic organisms depend on the physical and chemical forms of the metal [3]. Because estuaries provide an interface between freshwaters and salt waters, estuaries present steep gradients in many physical and chemical variables, including salinity (S), pH, dissolved oxygen, temperature, nutrient content, and the amount and composition of particulate matter. Unlike freshwaters, where pH is the controlling factor, in estuaries salinity is the controlling factor for the partitioning of contaminants between sediments and overlying or interstitial waters; however, both pH and salinity are recognized as the key variables that control the bioavailability and the toxicity of heavy metals bound to sediments.

Few studies have actually measured the effects of pH and salinity changes on the toxicity of metals bound to sediments [4]. In some cases, sediment toxicity has been evaluated by using marine species exposed to interstitial or elutriate waters [5–8] or to sediments whose salinity has been artificially increased [9–12]. In other cases, freshwater species have been exposed to estuarine sediments overlain with freshwater [13]. None of these studies took into account potential differences in contaminant bioavailability and toxicity due to original interstitial salinity. A more reliable method of determining the toxicity and bioavailability of contaminants in estuarine sediments would be to test the sediments with their original characteristics, by using estuarine organisms sensitive to a wide range of estuarine conditions, in particular salinity.

The main objective of this paper is to assess the influence of pH and salinity on the toxicity of heavy metals (Zn, Pb, Cd, and Cu) bound to estuarine sediments by using a true estuarine species [14,15] (the clam *Ruditapes philippinarum*) and simulating the typical values recorded for pH and salinity in most estuaries worldwide (pH ranging from 6 to 8 and salinity ranging from 10 to 35). These effects were evaluated by means of sediment toxicity assays both on environmental estuarine sediments collected from two estuaries in southern Spain and on sediment consisting of dilutions of a toxic mud resulting from an accidental mining waste spill (Aznalcóllar, Spain, April 1998).

MATERIALS AND METHODS

Approach

Two Spanish estuaries affected by heavy metal contamination of widely different origins were selected (Fig. 1). The Huelva estuary has suffered chronic contamination by mining activities continuously since the Roman occupation of the Iberian Peninsula [16]; and the Guadalquivir River estuary recently was affected by a single acute contamination event associated with an accidental mining waste spill [17]. In addition, samples were prepared from different dilutions of toxic mud collected in the proximity of the mine [18] by using clean sediment from the Bay of Cádiz (Spain) and identical assays were performed.

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Presented at the 12th Annual Meeting, SETAC Europe, Vienna, Austria, May 12–16, 2002.

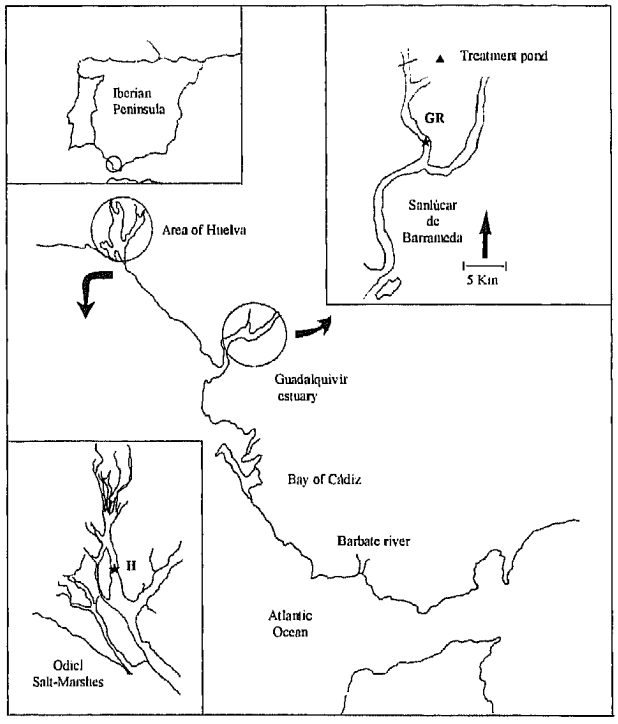

Fig. 1. A schematic map of the studied area in southern Spain showing the locations of the environmental sampling stations selected in the two estuarine ecosystems, the Huelva estuary (H) and the Guadalquivir River estuary (GR).

Sediments were collected with a 0.025-m^2 Van Veen grab and transferred to a 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 subsampled for chemical quantification (1.5-L aliquots). The sediment samples then were kept in the cooler at 4°C in the dark until they were used for sediment toxicity assays, but in no case for longer than two weeks.

Toxicity test

Clams (*R. philippinarum*; shell length about 1 cm, average weight about 0.35 g) were obtained from an aquaculture farm (AMALTHEA, S.L., Cádiz, Spain) and kept in the laboratory for one month before being acclimated to the salinity and pH values selected for the assays. During this period, clams were fed on a mixture of microalgae (*Tetraselmis chuii*, *Isocrhysis galbana*, and *Chaetoceros gracilis*).

Different values of salinity (10, 20, and 35) and pH (6.5, 7.5, and 8.5) were spiked in overlying water before the exposure of the organisms to the sediment samples. The salinity of this clean overlying water, used in the toxicity assays and in the controls, was adjusted by diluting natural seawater (S = 36) with distilled water (Milli-Q, Millipore Iberia, Madrid, Spain). The overlying seawater used in the pH-dependent toxicity assays was set up to permit a pH control by using the buffer capacity of the carbonate system in oceanic waters as reported by Mount and Mount [19]. Briefly, we manipulated the carbon dioxide concentration in the atmosphere over the assay solutions, as well as added approximately 10 mM of HCO_3^- to increase the buffer capacity of seawater. The pH was adjusted with HCl and NaOH (Suprapur, Merck, Darmstadt, Germany). Once the various pH and salinity values selected for each assay treatment had been separately fixed, this seawater was used during the acclimation period (15 d) of the organisms before performing the assays. During this period, the animals were maintained in tanks of approximately 20 L capacity and fed on a mixture of microalgae as described above, and the water was continuously aerated and replaced (80%, v/v) every 3 d with fresh seawater of salinity and pH values adjusted to those required. The pH, salinity, temperature (20°C), and the concentration of dissolved oxygen (>5 mg/L, 60% saturation) were measured and controlled every day.

After the acclimation period, two different bioassays were performed in duplicate by using different values of pH and salinity. Both assays were conducted in whole sediment (2 L per assay) by using a water to sediment ratio of 1:4 (v/v), at constant temperature (20°C), as reported by DelValls et al. [20] with 15-L vessels. Briefly, 40 organisms previously acclimated to each particular set of salinity and pH values were added to each vessel and exposed for 10 d. Two different endpoints were selected to assess the toxic effects. The sublethal endpoint was the percentage of clams buried during the first 48 h of exposure (median effective reburial time [ET50]), and the lethal endpoint was the percentage of mortality (median lethal concentration [LC50]) at the end of the experiment (after 10 d). Clean sediment (as used for the diluted toxic mud assays) was used as negative control, and the highest percentage of toxic mud dilution was used as positive control of toxicity for the clam mortality bioassay. Water replacement (80%) was performed on day 5 of the experiment, except for those assays where mortality was measured, in which it was performed on the same day as the mortality detection [20].

Chemical analysis

Sediment. For sediment grain size, an aliquot of wet sediment was analyzed with a Fristch (model Analysette 22) laser particle sizer, by following the method reported by DelValls et al. [21]. The remaining sediment was dried at 60°C before chemical analysis. Dried sediments were gently homogenized. For trace metal analysis, the sediments were digested as described by Loring and Rantala [22]. Zinc and Cu concentrations in the extracts were determined with a Perkin-Elmer 2100 flame atomic absorption spectrophotometer (Norwalk, CT, USA). The other trace metals were measured by graphite furnace atomic absorption spectrophotometry (4100 ZL, Perkin-Elmer). Results are expressed as mg/kg dry sediment. The analytical procedures were checked with reference material (Marine Sediment References Material for Trace Metals-1, National Research Council, Certified Reference Material, 277 BCR) and present agreement to more than 90% with the certified values.

Water analysis. The pH (seawater scale) was measured with a potentiometric analyzer (670, Metrohm, Herisau, Switzerland) with a glass combination electrode (ref. 6.0210.100, Metrohm). Salinity and oxygen concentration were measured as reported by Gómez-Parra and Forja [23,24]. The concentration of metals (Zn, Cd, Pb, and Cu) in the water samples was determined using differential pulse anodic stripping voltametry. Measurements were taken with static drop mercury electrode, with a Metrohm 693 processor, as reported by Ponce et al. [25]. The analytical procedures were checked with reference material (CASS-3) and reference solutions of the corresponding metals (Titrisol, Merck). The detection limit was 1 μg/L with an accuracy of >90%. This procedure presents agreement to more than 90% with the certified values.

Table 1. Summary of the concentrations of four heavy metals (mg/kg dry wt) measured in sediments used in the toxicity assays, for the control (C), the toxic mud dilutions (0.3% and 7.9%, respectively), and from the environmental stations (Spain), the Huelva estuary (H) and Guadalquivir River estuary (GR), determined at various different values of pH and salinity (S)

Treatments	[Cd]	[Pb]	[Cu]	[Zn]	[Cd]	[Pb]	[Cu]	[Zn]	[Cd]	[Pb]	[Cu]	[Zn]
	pH = 8.5				pH = 7.5				pH = 6.5			
C	0.68	20.97	18.24	69.03	1.01	19.26	22.21	95.18	1.02	24.72	21.90	68.83
0.3%	0.36	41.17	29.94	112.3	0.37	38.59	25.95	88.95	0.46	41.79	21.43	107.1
7.9%	2.95	404.1	94.87	562.2	3.23	412.4	113.9	596.9	4.02	436.7	162.2	849.4
H	19.48	2,959	2,905	2,946	21.44	2,986	2,910	3,675	20.74	2,798	2,963	3,457
GR	0.79	90.42	85.66	253.8	0.59	65.99	54.99	252.8	0.48	67.36	48.11	160.4
	S = 35				S = 20				S = 10			
C	0.42	20.59	13.25	49.57	0.43	22.38	13.17	46.08	0.58	19.60	15.26	50.37
0.3%	0.25	22.35	24.66	86.31	0.43	28.95	22.35	80.36	0.43	35.39	24.0	89.98
7.9%	2.58	499.4	148.8	925.9	3.67	614.3	142.4	1,086	3.77	630.0	147.4	978.1
H	20.64	3,385	2,916	3,217	23.59	2,747	3,150	3,956	27.55	3,061	3,098	3,936
GR	0.74	48.62	57.95	225.3	0.65	42.31	52.89	221.4	0.93	46.34	50.55	223.7

Data calculation and statistical analysis

The partitioning coefficient (K_d) of four heavy metals (Cd, Pb, Cu, and Zn), defined as the ratio of the heavy metal concentration in the sediment (μg/kg dry wt) to that dissolved in the overlying water (μg/L), was used to establish the mobility of heavy metals from the sediment to the water and to indicate their potential bioavailability versus the salinity and pH values. The units of K_d are L/kg, although for convenience it is assumed that a volume of 1 L of seawater is equivalent to a mass of 1 kg of sediment, rendering K_d dimensionless. The hypothesis is that low K_d values are related to those contaminants that may feasibly transfer from the sediment to the water, and thus have a potentially higher bioavailability and toxicity than those with high K_d values.

The sublethal endpoint ET50 selected in the toxicity assays was the time required for the reburial of 50% of the population. The ET50 was calculated by linear regression of log toxicant time on declining probit values, by using a probit modified from the classic methodology following the method reported by DelValls et al. [26].

The lethal endpoint (LC50) was determined by using the percentage of mortality after 10 d of exposure and was associated with the dilution of toxic mud. Three different dilutions of toxic mud with control sediment were used (0.3%, 7.9%, and 10.8%) to define the concentration (percentage of toxic mud) that provokes mortality of 50% of the exposed population. This was derived by linear regression of log toxicant dilution of toxic mud on declining probit values.

The resulting parameters (ET50 and LC50) from the duplicated assays and the controls were compared by using analysis of variance and Scheffe's F tests to identify significant differences in sensitivity between media ($p < 0.05$).

Adequate quality assurance and quality control measures were followed in all aspects of the study, from field sampling through to laboratory and data entry as described by Chapman [27] and the American Society for Testing and Materials [28,29].

RESULTS AND DISCUSSION

Chemistry

A summary of the concentrations of selected heavy metals analyzed in sediments is given in Table 1. Sediment samples had relatively similar texture, and were dominated by the clay fraction with a dark color and with the percentage of fine grains (<1 μm) ranging between 75 and 88%.

pH dependence. The partitioning coefficient for Cd, Pb, Cu, and Zn in the negative control, in two mud dilutions (0.3% and 7.9%), and in the Huelva estuary and the Guadalquivir River estuary stations against the pH values used in the assays are shown in Figure 2. The acidification of the samples tends to release those metals less strongly associated with sediments to the overlying water, and increases their potential bioavailability. All the K_d values are higher than 1, so the concentrations of heavy metal in sediments are higher than in the overlying water. The highest values of K_d for all the metals are those calculated for the environmental stations and the controls, with those from the Huelva estuary being the highest (Cd, 2,192; Pb, 1,670; Cu, 6,452; and Zn, 4,785). The partitioning coefficients calculated for the dilution of toxic mud analyzed are the lowest at the dilution of 7.9% (Cd, 16; Pb, 1,180; Cu, 49; and Zn, 39). This demonstrates that heavy metals bound to the toxic mud are more easily mobilized from sediments to water than those from the environmental samples and are potentially more bioavailable and toxic. Low K_d values were calculated for Cd, Zn, and Cu in toxic mud. Although the K_d values calculated for Pb in toxic mud are lower than in sediment from environmental stations, they are higher than those obtained for the other three metals.

In general, the mobility of all the metals from the sediment to the water increases when the pH value decreases, and their concentrations in water at pH 6.5 are higher than at pH 7.5 and 8.5. These metals could be more bioavailable at lower pH than at higher pH values, especially for Cd, Zn, and Cu in the toxic mud.

Salinity dependence. The partitioning coefficients for Cd, Pb, Cu, and Zn in the negative control, in two mud dilutions (0.37percnt; and 7.9%), and in the sediments from the Huelva estuary and the Guadalquivir River estuary against the salinity values used in the assays are shown in Figure 3. The values of K_d obtained for all the metals are higher in environmental stations than in toxic mud dilutions, with sediment from the Huelva estuary (Cd, 1,856; Pb, 266,919; Cu, 50,316; and Zn, 8,924) at low salinity being the highest, and the mud dilution of 7.9% at high salinity being the lowest for Cd (5) and the mud dilution of 0.3% at low salinity being the lowest for the other three metals (Pb, 2,761; Cu, 117; and Zn, 62). Again,

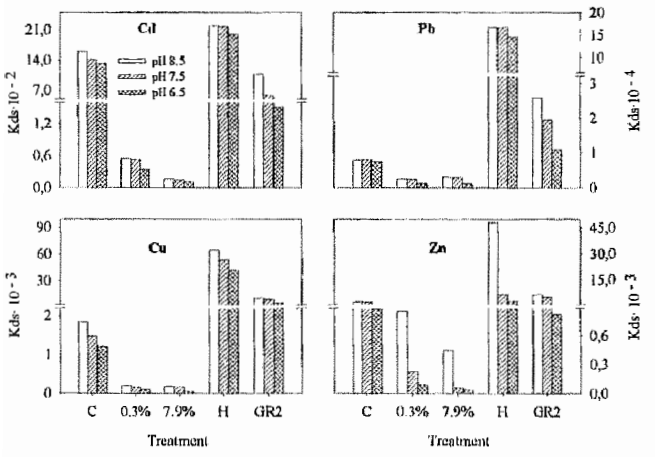

Fig. 2. The partitioning coefficient (K_d) values (L/kg) of Cd, Pb, Cu, and Zn for the three pH values tested in the toxicity assays (8.5, 7.5, and 6.5) in the control sediment (C), the dilutions of toxic mud (0.3% and 7.9%), and sediment from the environmental stations (Spain), the Huelva estuary (H) and the Guadalquivir River estuary (GR).

all the K_ds are higher than 1, so the concentrations in sediment are higher than in water.

The effect of the salinity values on the K_d behavior for the metals studied is variable and depends on the particular metal. The mobility of heavy metals from the sediment to the water when the salinity varies will depend on the relative importance of the two counteracting groups of processes [30–32], with one being desorption due to increased complexation with seawater anions (Cl^- and SO_4^{2-}) or increasing competition for particle sorption sites with seawater cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}), and the other group being the processes of coagulation, precipitation, and flocculation. In this sense, K_ds for metals may decrease, increase, or be constant depending on the nature of the sediments and the estuarine conditions. The values of K_d for Cd increase in all assays when salinity decreases, indicating that this metal tends to be more efficiently trapped by low-salinity sediments and it presents a higher concentration in high-salinity water compared with low salinity. The trends of K_d values for Pb, Cu, and Zn are similar to those of Cd in control sediments and in sediments from the Huelva estuary, but are different in the dilutions of toxic mud and in the sediments from the Guadalquivir River estuary, where K_ds decrease when salinity decreases.

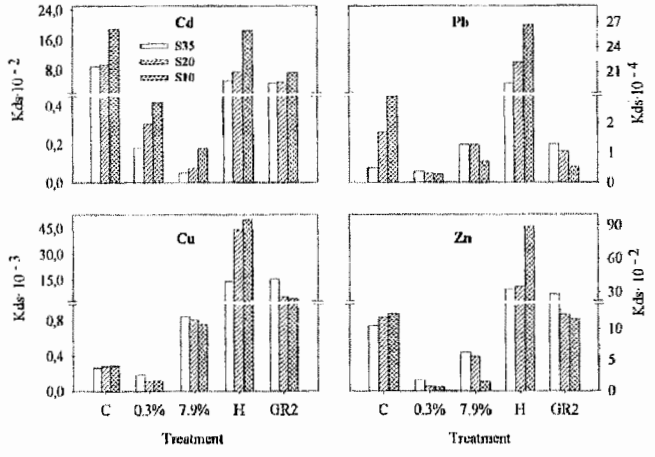

Fig. 3. The partitioning coefficient (K_d) values (L/kg) of Cd, Pb, Cu, and Zn for the three salinity values tested in the toxicity assays (30, 20, and 10) in the control sediment (C), the dilutions of toxic mud (0.3% and 7.9%), and sediment from the environmental stations (Spain), the Huelva estuary (H) and the Guadalquivir River estuary (GR).

Table 2. Clam mortality results (%) measured in the control (C), the toxic mud dilutions (0.3%, 7.9%, and 10.8%) and the environmental stations (Spain), the Huelva estuary (H) and Guadalquivir River estuary (GR), measured in each pH and salinity toxicity assay and in each duplicate (suffix A or B). The values of the calculated median lethal concentration (LC50; % of toxic mud that provokes mortality of 50% of clams in dry wt) also is shown for each pH and salinity toxicity assay

	pH			Salinity		
Treatments	8.5	7.5	6.5	35	20	10
C A	0	2.5	2.2	0	0	7.5
C B	2.5	0	2.5	0	2.5	2.5
0.3% A	15	25	45	15	15	35
0.3% B	10	15	55	15	5	25
7.9% A	45	50	65	35	55	97.5
7.9% B	40	45	75	30	45	82.5
10.8% A	80	90	100	87.5	100	100
10.8% B	100	100	100	92.5	100	100
H A	5	5	5	0	0	10
H B	0	5	5	0	5	10
GR A	0	5	5	0	0	5
GR B	0	0	0	0	0	0
LC50 A	4.09	2.10	0.50	4.33	2.21	0.49
LC50 B	3.43	2.66	0.22	4.31	3.76	0.94

In general, low values of K_d are calculated for heavy metal concentrations measured at the low salinity (10) and low pH (6.5) values tested in the bioassay, except for Cd in the salinity bioassay. These metals may be more bioavailable at lower rather than at higher values of both salinity and pH, and thus may be more toxic to the exposed organisms.

Toxicity

Clam reburial. The sediment toxicity results for each pH and salinity test are summarized in Figures 4 and 5, showing the clam reburial during the first 48 h of exposure. Figure 6 shows the summarized results of the calculated ET50, and identifies significant differences between stations, dilutions, and controls for each salinity and pH value used in the toxicity assays. For all the salinity and pH values, the ET50 values were highest for controls compared with the rest of treatments. No trends are measured for ET50 as a function of pH. However, for the salinity toxicity assays, smooth increases of ET50 values are observed as the salinity decreases. The lowest ET50 values were associated with the toxic mud dilution of 7.9%, in which only 30% of the population was buried after 48 h; this is significantly different from the rest of the treatments assayed, at all the pH and salinity values. Intermediate values of ET50 were measured in sediments from the Huelva estuary; this is significantly different from the control for all the treatments except for salinity of 10 ($p < 0.05$). The highest ET50 values were measured in the Guadalquivir River estuary and the 0.3% dilution; these responses are not significantly different from the control ($p < 0.05$).

No or few differences in the toxicity ranking of treatments are identified as the pH decreases (Fig. 6). This indicates that pH values do not affect the rate of burial of the organisms exposed under the conditions tested. In the salinity assays, the rankings at salinity values 35 and 20 are similar and show few differences (similar to those detected in the pH assays, and related to Guadalquivir River estuary and the 0.3% dilution) in the ranking of nontoxic treatments. However, at a salinity

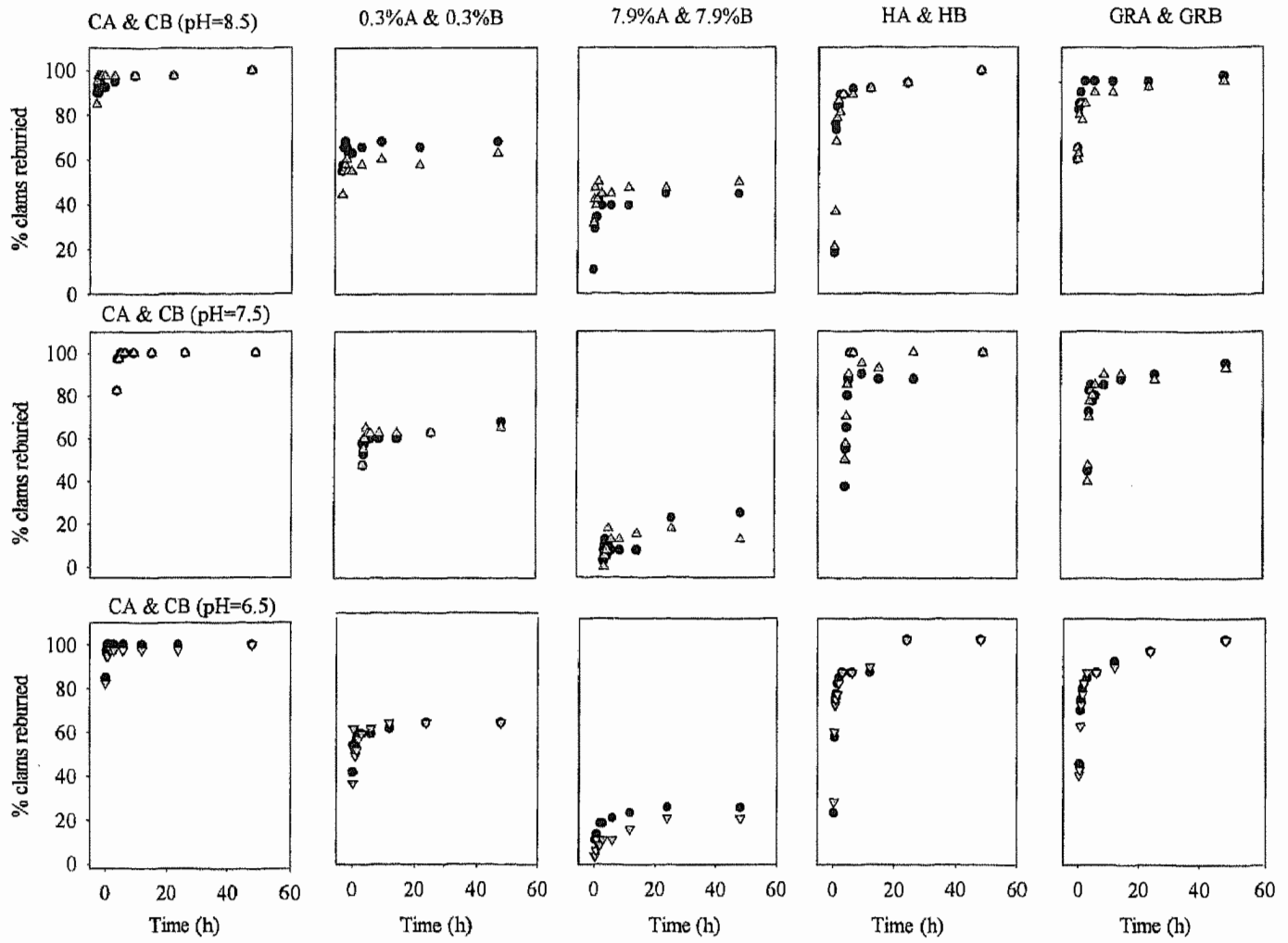

Fig. 4. Clam reburial results of the toxicity assays for the control sediment (C), the dilutions of toxic mud (0.3% and 7.9%), and sediment from the environmental stations (Spain), the Huelva estuary (H) and the Guadalquivir River estuary (GR) at different values of pH (8.5, 7.5, and 6.5). The percentage of clams buried is represented for each replicate versus the time of exposure (up to 48 h). Triangles and full circles show different percentages of burial for each replicate assay performed (A and B).

of 10, an increase of toxicity was measured (lower values of ET50 than at the higher salinity values) in the treatments with toxic mud dilutions (0.3% and 7.9%).

Clam mortality. The percentage of clam mortality for each duplicated assay (A and B) in every treatment is shown in Table 2. In general, for each particular treatment (environmental station or toxic mud dilution), the mortality increases as both the pH and salinity values decrease. The highest mortality was measured in the positive control at a toxic mud dilution of 10.8%, ranging from 90% at a pH of 8.5 and salinity of 35 to 100% for the lowest values of pH (6.5) and salinity (10). The mortality of the clams increases when the toxic mud dilution increases. In sediment from the environmental stations, no significant differences were found in the toxic responses compared with those measured in the control ($p < 0.05$), so no lethal toxicity was measured at these stations. Nevertheless, the percentages of mortality at low salinity and pH were higher than at higher pH and salinity values.

As a means of quantifying the effect of the salinity and pH on the mortality of clams, a concentration of the contaminated mud sufficiently lethal to provoke the death of 50% of the population of clams in 10 d (LC50) was devised. These calculated LC50 concentrations (in %) are shown in Table 2. The lowest LC50 value was associated with a pH value of 6.5 (0.29%) and the highest LC50 was associated with a salinity value of 35 (2.24%). At the lowest values of both pH (6.5) and salinity (10), lower concentrations of toxic mud were required to produce mortality of 50% of clams (Table 2). The LC50 values were significantly different at the lowest pH and salinity values with respect to the rest of the treatments ($p < 0.05$).

During the monitoring of the impact of the Aznalcóllar mining spill in the Guadalquivir River estuary, the heavy metals Zn and Cd were identified as those of most concern in sediments of the estuary [16,33–35]. To assess the implications of the differences in lethal toxicity at low pH and salinity values, we have derived sediment quality values for two metals (Zn and Cd) in the toxic mud. From the LC50 values obtained (Table 2) and by using the concentration of Zn and Cd (Cd, 45.7 mg/kg dry wt and Zn, 21,618 mg/kg dry wt) in the toxic mud (100%) previously reported by Riba et al. [18], we can derive sediment quality values for these two metals at different values of pH and salinity; these are defined as the concentrations of Zn and Cd associated with the mortality of 50% of the clams after 10 d of exposure. These results are shown in Table 3, where it can be observed that lower concentrations of Zn and Cd are required to produce the measured toxicity

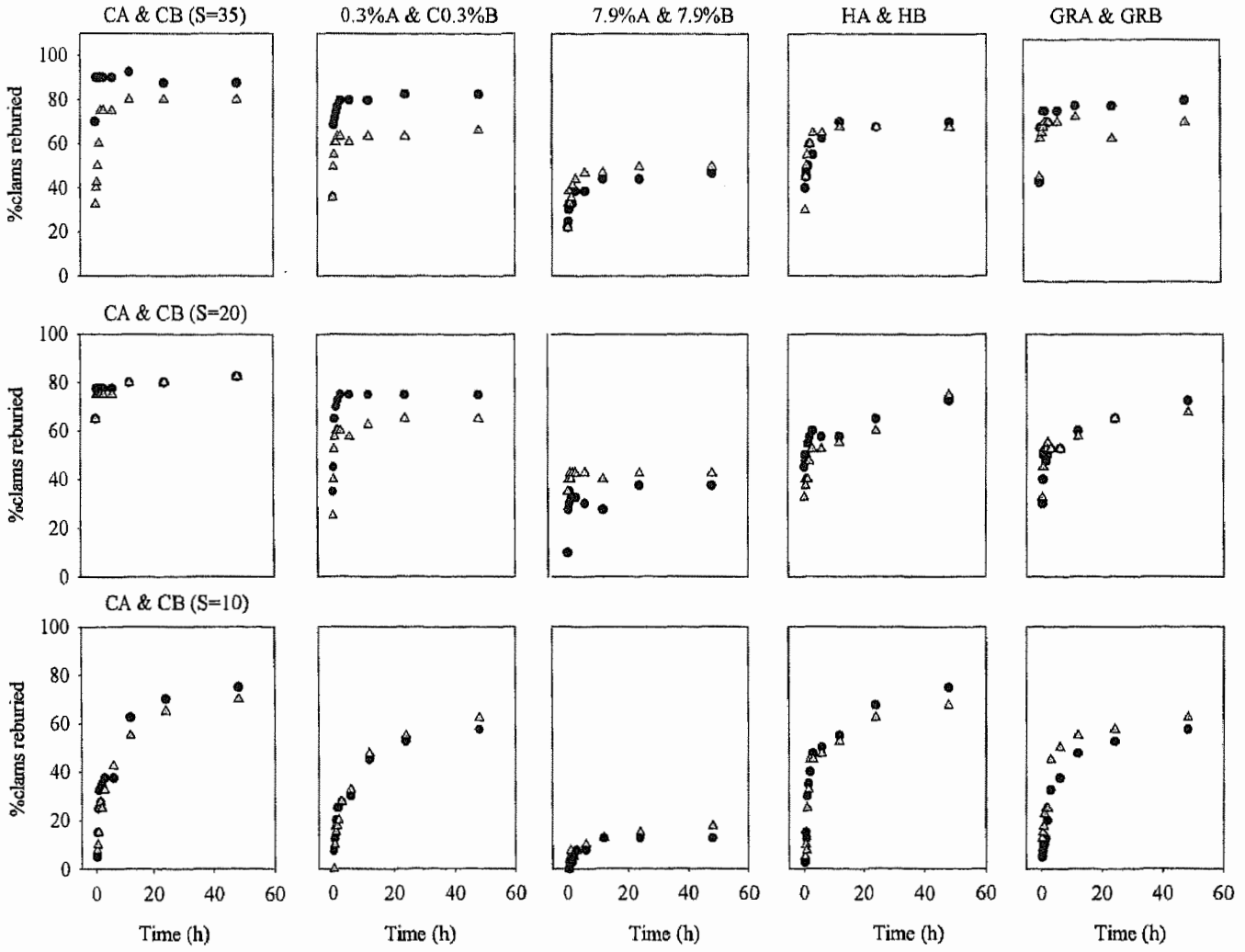

Fig. 5. Clam reburial results of the toxicity assays for the control sediment (C), the dilutions of toxic mud (0.3% and 7.9%), and sediment from the environmental stations (Spain), the Huelva estuary (H) and the Guadalquivir river estuary (GR) at different values of salinity (35, 20, and 10). The percentage of clams buried is represented for each replicate versus the time of exposure (up to 48 h). Triangles and full circles show different percentages of burial for each replicate assay performed (A and B).

in clams at low pH and salinity, compared with those at high pH and salinity values. On this basis, the toxic mud from the Aznalcóllar spill can be considered more toxic at low pH and salinity values. It is important to note that the sediment quality values calculated here are not intended to be more widely applied, not even in this estuary; they are presented merely to show the deficiency of previous models in which these values were derived without taking into account the effects of pH and salinity. Based on the results obtained in this study, the recommendation is made that the influence of the salinity and pH values should be taken into account when deriving sediment quality values for estuarine environments. These values can increase the toxic effects for identical concentrations of contaminants in sediments located in areas with pH and salinity values equal or lower than 6.5 and 20, respectively.

Salinity variations previously have been reported to change the route of exposure of the heavy metals bound to sediments. Because of this, the partitioning of heavy metals to particles favors their uptake by sediment feeders, whereas desorption to the overlying water favors their uptake via dermal exchange surfaces such as gills and their uptake by water (including interstitial water) feeders. Clams are filter-deposit feeders and the food should be filtered through the overlying water. This could explain the differences in the toxic responses between a behavioral endpoint (ET50) and a lethal endpoint (LC50) measured in the bioassays performed. These differences may be related to the heavy metal concentration in sediments and their mobility to the overlying water if changes in pH and salinity are occurring. The ET50 was toxic ($p < 0.05$) for those stations with high concentrations of heavy metals in sediments: The Huelva estuary, and for the 7.9% dilution of toxic mud. Nevertheless, LC50 was only toxic ($p < 0.05$) in the stronger dilutions of toxic mud (≥7.9%) and was not toxic ($p < 0.05$) in sediments from the Huelva estuary, which have the highest concentration of heavy metals (Table 1). Furthermore, the mobility of metals from the 7.9% dilution of toxic mud was higher than that from sediments from Huelva estuary (Figs. 2 and 3). This suggests that the LC50 is related to those heavy metals more easily mobilized from sediments to water and that are bioavailable through the overlying water. Sediments with K_ds lower than the others (toxic mud dilution, for all the metals) have provoked mortality, even with heavy metal concentrations in sediment lower than in sediments from the Huelva estuary that had the highest concentration of metals and in which no significant mortality was measured ($p < 0.05$).

Table 3. Sediment quality guideline values for Zn and Cd (dry wt) obtained from the median lethal concentration of toxic mud (LC50) and derived for each value of pH and salinity used in the sediment toxicity tests and for each duplicate (A and B)

	Cd	Zn
pH		
8.5 A	1.87	884.1
8.5 B	1.57	741.5
7.5 A	0.96	454
7.5 B	1.22	575.0
6.5 A	0.23	108.0
6.5 B	0.10	47.6
S		
35 A	1.98	936.1
35 B	1.97	931.7
20 A	1.01	477.8
20 B	1.72	812.8
10 A	0.22	105.9
10 B	0.43	203.2

CONCLUSION

Chapman and Wang [2] reported that, to their knowledge, no studies directly assessing the effects of adjusting interstitial salinities on bioavailability and toxicity have been published. Similarly, few studies have been published of attempts to replicate in the laboratory the interstitial and overlying water exposures found in situ. These authors recommended conducting studies to determine the effect of changing interstitial salinities on contaminant bioavailability and toxicity to develop realistic toxicity testing procedures for estuaries that experience large-scale seasonal salinity changes. The study described here presents the results of an evaluation of the influence of selected pH and salinity values typically measured in most estuaries (6.5–8.5 and 10–35, respectively) on the bioavailability and toxicity of heavy metals bound to sediments. The assays described here represent an approach based on the determination of the heavy metal bioavailability, measured as the mobility from sediments to overlying water, and on the assessment of the heavy metal toxicity using a true estuarine species (*R. philippinarum*) [14,15]. Two different endpoints (sublethal [ET50] and lethal [LC50]) were determined in different kind of sediments, environmental estuarine samples, and controlled dilutions of a previously tested toxic mud. The main conclusions obtained in this study of the role of pH and salinity in estuarine environments on bioavailability and toxicity of heavy metals are summarized as follows.

The mobility from sediment to water of the four heavy metals studied increases when the pH decreases in all treatments. The effect of the salinity varies from metal to metal depending on the relative importance of two counteracting processes, desorption from sediments to water or coagulation, flocculation, and precipitation. Sediments collected in an area affected by chronic heavy metal contamination (the Huelva estuary) tend to be more efficient in trapping Zn, Cu, and Pb at low salinity values. However, sediments affected by the acute contamination event (the Guadalquivir River estuary) associated with the Aznalcóllar mining spill, and the dilution of toxic mud from the accidental spill, show a different pattern of higher concentrations of these metals in water when the salinity values decrease. The heavy metal Cd shows a similar mobility in sediments from both stations, becoming more mobile as the salinity increases.

The observation was made that the toxicity of heavy metals

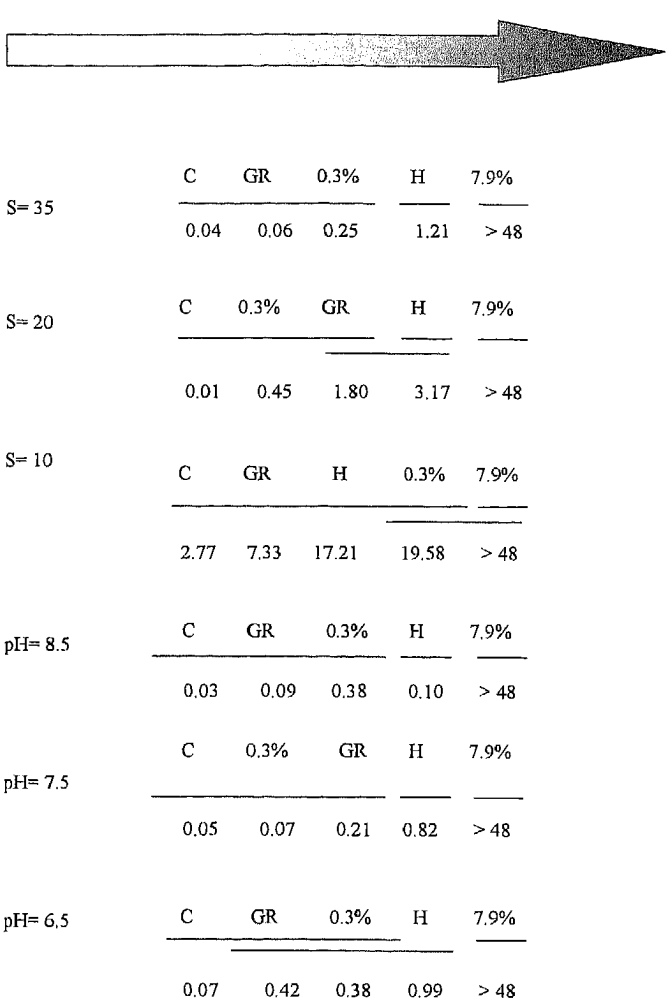

Fig. 6. Summary of the behavioral results (average effective burial time) obtained for the control sediment (C), the dilutions of toxic mud (0.3% and 7.9%), and sediment from the environmental stations (Spain), the Huelva estuary (H) and the Guadalquivir River estuary (GR) at the different salinity and pH values. Treatments not underlined by the same line are significantly different at $p < 0.01$ (Scheffe's F tests).

from the sediments studied is affected by the pH and salinity values associated with lethal effects (LC50), whereas these variables do not affect the results of the toxicity when using the behavioral endpoint (ET50). Low values of pH and salinity show mortality associated with heavy metals in the dilution of toxic mud (7.9% or higher) that was the only treatment that was significantly toxic. The clam mortality is related to the K_d values of the metals rather than to the concentration of heavy metal in the sediments. The behavioral toxic endpoint (ET50) is more related to the concentration of heavy metal in sediments detecting sublethal toxicity in one station without lethal mortality (Huelva estuary). These differences in the toxic responses can be explained by the route used by this organism in ingesting food and heavy metals from the overlying water.

The heavy metals from the mining spill studied are more bioavailable at low pH and salinity values, and this could explain previous results that identified toxicity in low hydrodynamic areas of the estuary with pH and salinity values similar to those tested in this study. Furthermore, this may explain why the concentrations of heavy metals in the dilutions of

toxic mud are associated with lethal toxicity despite being much lower than those monitored in the Huelva estuary. This gives an interesting indication of the fundamental difference between the long-term effects of continuous heavy metal discharge over centuries and the effect of an isolated, albeit very large, single discharge.

The assay results reported in this paper are an attempt to approach the determination of the effects of changing salinity and pH values on the toxicity effects of heavy metals bound to sediments. Further studies on the relationship of heavy metal speciation and their toxic effects under estuarine conditions should be performed in the near future by comparing smaller differences in pH and salinity values. Furthermore, studies that combine the effects of the influence of both variables are recommended. From the results obtained, no influences of pH and salinity separately were measured at values of pH of 7.5 and salinity of 20 or higher. Although the experimental design described is merely a new approach to true environmental conditions, it may be considered a further step in conducting estuarine studies that rigorously mimic conditions typical of estuaries.

Acknowledgement—Thanks to John Roitz, Peter Chapman, Almut Gerhardt, and three anonymous reviewers for critically reading an earlier version of the manuscript. We thank Rosa Vázquez and the staff of the aquaculture facility at the Faculty of Marine and Environmental Sciences, University of Cádiz. This research was supported by grant REN2002-01699 from the Spanish National Plan for research, innovation, and development (Ministerio de Ciencia y Tecnología).

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