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RESEARCH ARTICLE

The influence of ph and waterborne metals on egg fertilization of the blue mussel (*Mytilus edulis*), the oyster (*Crassostrea gigas*) and the sea urchin (*Paracentrotus lividus*)

Inmaculada Riba [1] · **Bardukh Gabrielyan** [2] · **Alla Khosrovyan** [1] · **Angel Luque** [3] · **T. Angel Del Valls** [1]

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Abstract This study evaluated the combined effect of pH and metals on the egg fertilization process of two estuarine species, the blue mussel (*Mytilus edulis*), the oyster (*Crassostrea gigas*) and a marine species, the sea urchin (*Paracentrotus lividus*). The success of egg fertilization was examined after exposure of gametes to sediment extracts of various degrees of contamination at pH 6.0, 6.5, 7.0, 7.5 and 8.0. At the pH levels from 6.5 to 8.0, the egg fertilization of the different species demonstrated different sensitivity to metal and/or acidic exposure. In all species, the results revealed that egg fertilization was almost completely inhibited at pH 6.0. The egg fertilization of the blue mussel *M. edulis* was the least sensitive to the exposure while that of the sea urchin *P. lividus* demonstrated a concentration-dependent response to the pH levels from 6.5 to 8.0. The results of this study revealed that acidity increased the concentration of several metal ions (Cr, Ni, Cu, Zn, Cd, and Pb) but reduced its availability to the organisms, probably related to the reactivity of the ions with most non-metals or to the competition among metals and other waterborne constituents.

Keywords Acidification · Sediment elutriate · Metal · Egg fertilization · Mussel · Oyster · Sea urchin

Responsible editor: Thomas Hutchinson

✉ Alla Khosrovyan
alkhosrov@yahoo.com

Inmaculada Riba
inmaculada.riba@uca.es

Bardukh Gabrielyan
gabrielb@sci.am

Angel Luque
angel.luque@ulpgc.es

T. Angel Del Valls
angel.valls@uca.es

[1] UNESCO/UNITWIN WiCop, Department of Physical Chemistry, University of Cadiz, Poligono Río San Pedro, s/n, 11510, Puerto Real, Cádiz, Spain

[2] Scientific Center of Zoology and Hydrobiology, 7 Paruir Sevak, Yerevan 0014, Armenia

[3] Department of Biology, University of Las Palmas de Gran Canaria, Tafira, Las Palmas, Spain

Introduction

Contaminants flowing into marine environments from rivers can be deposited and incorporated into estuarine sediment. In an estuary, tidal activity, wind action or river flow may cause abrupt changes to important physical and chemical parameters of the environment such as salinity, pH, dissolved oxygen, temperature, nutrients, and particulate matter (Herman and Heip 1999; Feely et al, 2010: Chapman et al. 2013; Mukherjee 2014). Salinity and pH influence the partitioning of metals between sediment and water (Riba et al. 2004) and alteration of sorptive characteristics of sediment particles influences metal ion composition in the particulates (Turner and Rawling 2001). Thus, a complex interplay of many factors affect concentrations, chemical reactivity and distribution patterns of trace contaminants bound to aquatic sediments, especially in estuaries.

With limited mobility, benthic species may be one of the most exposed and vulnerable groups in coastal ecosystems. While adult organisms may resist acidification, e.g. by increasing the calcification rate at the expense of muscle growth (Wood et al. 2008), their gametes, embryos and larval stages may have less tolerance and thus population stability may be perturbed during low pH events (Dupont et al. 2010; Halsband

and Kurihara 2013). Several studies showed detrimental effects of sea water acidification on fertilization of marine organisms either related to natural variations of pH level or CO_2-driven ocean acidification (e.g., Wong and Wessel 2005; Kurihara et al. 2007; Moulin et al. 2011; Barros et al. 2013).

In this work, the blue mussel (*Mytilus edulis*), the oyster (*Crassostrea gigas*) and a marine species, the sea urchin (*Paracentrotus lividus*) were selected as model species to study the combined effect of acidification and metal pollution on the success of egg fertilization and to compare sensitivities of egg fertilization to the combined exposure. Egg fertilization was subjected to the acidification range varying from the predicted decrease of ocean pH by 0.3–0.4 units by 2100 (Intergovernmental Panel on Climate Change IPCC 2007) to pH 6.0 which may occur due to a leak of CO_2 gas from subsurface geological storage sites (Huesemann et al., 2002; Barry et al., 2004). The species selected have been widely used in standard toxicity assessment (Bellas et al. 2005; Volpi Ghirardini et al. 2005; Baussant et al. 2011; Moulin et al. 2011; Khosrovyan et al. 2013) and are important model organisms in environmental science (Wei et al. 2015).

Experiment

Sediment sampling

Two different environments were selected for investigating the influence of acidified sediment elutriates on the toxicity of metals: an estuarine area affected by mining activity and a coastal area (Fig. 1).

Huelva estuary (H1, H2) is a heavily industrialized area located at the mouth of two estuaries defined by the rivers, Tinto and Odiel. The area historically receives acidic fluvial discharges with high concentrations of metals (Riba et al. 2002). The Bay of Cadiz (Ca) is relatively clean with metal loads usually below the harmful thresholds for chemical concentrations (Riba et al. 2004; Rodriguez-Romero et al. 2013).

Sediments were collected with a 0.025-m^2 Van Veen grab and transferred to the laboratory in a cooler. Sediments were homogenized with a Teflon spoon until no colour or textural differences could be detected and stored at 4 °C in the dark until elutriate preparation within 2 weeks.

Elutriate

Sediment elutriates were obtained using a modification of the United States Environmental Protection Agency (US EPA) method (US EPA 1998). Sediments were homogenized and mixed with clean seawater in a proportion 1:4 *v*/*v* (sediment/water) for 30 min at approximately 20 °C. The mixture was left to settle overnight, and then the supernatant was siphoned. The sediment elutriates were kept in the dark at 4 °C until they were used in the toxicity tests (within two days). On the test day, elutriates were transferred to test chambers and left to reach the test temperature without additional aeration before the addition of the test organisms.

Elutriates were subjected to different pH levels (6.0, 6.5, 7.0, 7.5, 8.0) and dilution with clean water (50 %) of comparable acidity to analyze the effect of varying metal concentrations on egg fertilization. A detailed description of pH adjustment of elutriates can be found elsewhere (Riba et al. 2004). Briefly, the pH was adjusted by HCl and NaOH solutions (Merck, Darmstadt, Germany) with prior increase of the buffering capacity of seawater by adding 10 mM of $HCO^-{}_3$ (Mount and Mount 1992). Subsamples of elutriates were taken for chemical characterization.

Prior to elutriate preparation and use in the tests, all glassware was thoroughly cleaned with acid (10 % HNO_3) and rinsed with double-deionized (Milli-Q) water.

Bioassays

The molluscs *M. edulis* and *C. gigas* were purchased from an aquaculture facility and held in clean aerated running seawater until use in the tests, within 24 h of acquisition. The method of artificial egg fertilization, described in detail by Bellas et al. (2005), was used with a small modification. Briefly, gametes were obtained from mature molluscs by thermal stimulation (a series of cold/warm cycles differing by around 10 °C) in separate vessels. Prior to use, egg and sperm suspensions were filtered to 75 μm to remove debris.

Adult sea urchins (*P. lividus*) were collected from a clean area in the Bay of Cadiz (Ca) (Casado-Martinez et al. 2007; Khosrovyan et al. 2015) and held in clean aerated running seawater until testing later on the same day. Gametes were obtained from sexually mature females and males by careful extraction by a pipette after dissection of the animals. Prior to use, sperm and eggs were held in clean seawater. Detailed methods used in egg fertilization of sea urchin were described by Khosrovyan et al. (2013).

For all tests, sperm mobility and egg quality were checked under microscope before use. Fertilization was performed by mixing 1 ml of pooled egg suspension with 1 ml of pooled sperm suspension in 20-ml elutriate with adjusted pH. The vials were incubated at 20 °C for oyster and sea urchin gametes and at 18 °C for *M. edulis*, being occasionally and gently stirred to allow fertilization. After incubation, samples were preserved by adding 0.5–1 ml of 40 % formaldehyde. Five replicates per treatment were assayed for the sea urchin tests, and three replicates per treatment were assayed for the mollusc tests. In all tests, a negative control consisted of clean seawater used for preparation of elutriates (salinity 34 ‰, pH 8.0).

The percentage of unfertilized eggs (those not surrounded fully or partially by a fertilization membrane), or failure rate, in 100 eggs was selected as toxicity endpoint.

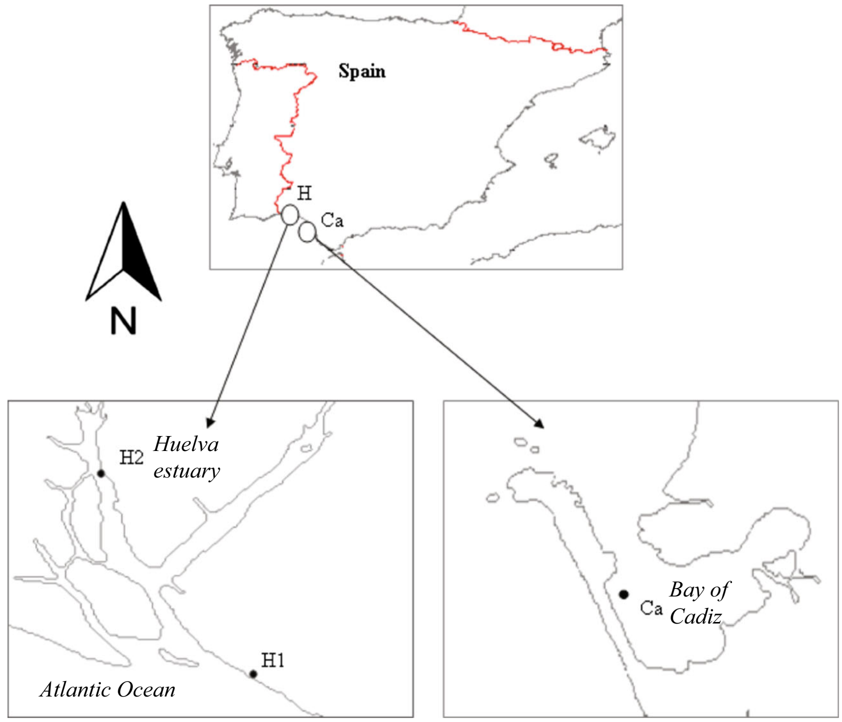

Fig. 1 Map of Iberian Peninsula showing sediment sampling locations in Huelva estuary (*left*) and Bay of Cadiz (*right*). Site H1 of Huelva estuary located near the town of Mazagon and H2 near Muelle Levante

Chemical and statistical analysis

Total dissolved concentrations of select elements (Cr, Ni, Cu, Zn, As, Cd, Pb) in elutriates were measured at each acidity level by the "Servicios Centrales de Ciencia y Tecnología" of University of Cádiz. The accuracy of metal analysis was checked by the SLRS-4 River Water Reference Material for Trace Metals (certified reference material). The analytical detection limits were 0.5 μg l^{-1} for Cu, 0.9 μg l^{-1} for Zn and 0.01 μg l^{-1} for Cd, and the mean recovery was 100 ± 10 %.

The distributions of all toxicity responses were normal and one-way ANOVA was used to identify the significant differences between these responses ($p < 0.05$). The selection of the post hoc test was based on the homogeneity of variance assumption (Tukey test, if homogeneity is assumed and Games-Howell otherwise).

Multivariate, principal component analysis (PCA) on 25 cases (treatments) and 10 variables (toxicity and chemical data) were performed to explore and interpret patterns among toxicity and chemical data and treatments. The method identifies the principal components that group the variables such that the variables are correlated within the component, and the components are not (or weakly) correlated with each other. Correlations of variables within the component are determined by component loadings. Loadings of more than 0.4 (by absolute value) were considered per component. Linear combinations of the value of a variable with its corresponding loading per component (positive scores) were interpreted to determine patterns between toxicity and chemical data and treatments. Statistical procedures were performed by SPSS 16 software.

The linear interpolation method was used to calculate the toxic parameter LpH50, defined as the pH that causes lethal effects in 50 % of the exposed population (Basallote et al. 2014). The method was incorporated into the software programme recommended by US EPA Environmental Research Laboratory.

Results

Chemical analysis

Mean total concentrations of dissolved metals (Cr, Ni, Cu, Pb, Zn) and metalloid As at each pH and control treatment in Ca elutriate are shown in Fig. 2, in H1 elutriate in Fig. 3 and in H2 elutriate in Fig. 4 (mean ± SD). In all elutriates, Cd concentrations were below the detection limit.

The concentrations of some metals (Cu, Pb and Zn) and As in Huelva elutriates (H1, H2) were greater compared to that of Cadiz Bay (Ca). Between Huelva samples, metal levels were, in general, greater in H2.

The metal partitioning behaviour from sediment into elutriate at different acidity levels showed metal and site specificity. Cr and Ni were highly mobilized by a change in the acidity level, compared to the controls, whereas the partitioning behaviour of Pb and As was clearly promoted by higher acidity (with an exception at pH 8.0 in H1). In Ca and H2, concentrations of all chemicals in the pH treatments were higher, compared to the controls (except for Pb and Zn in Ca). However, concentrations of Cu and As remained almost

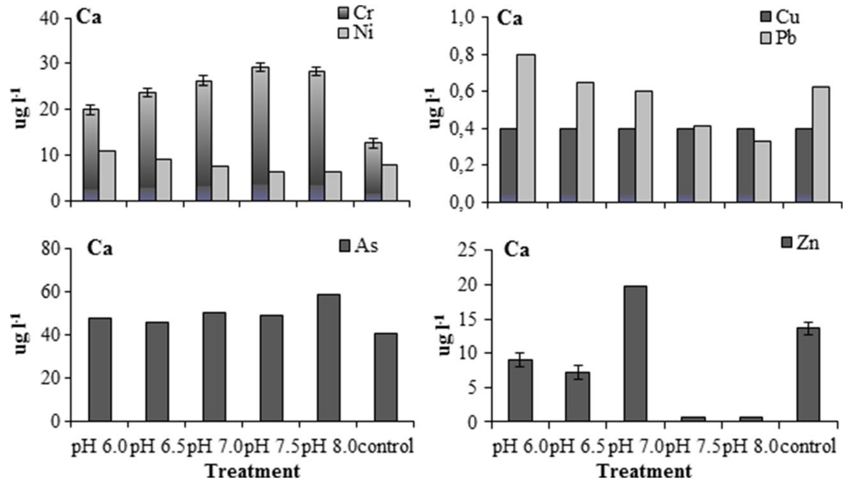

Fig. 2 Total concentrations (mean ± SD) of dissolved metals (Cr, Ni, Cu, Pb, Zn) and metalloid As in the sediment elutriates from the Bay of Cadiz (Ca) per treatment (pH 6.0, 6.5, 7.0, 7.5, control)

unchanged in Ca, and concentrations of Zn, Cu, Pb and As decreased in pH treatments in H1, compared to the control (Figs. 2, 3 and 4).

Toxicology analysis

The results of egg fertilization tests (percentage of unfertilized eggs) by treatment for *M. edulis* are shown in Fig. 5, for *C. gigas* in Fig. 6 and *P. lividus* in Fig. 7. The percentage of unfertilized eggs in *M. edulis* was negligible compared with the other species at all pH levels except for pH 6.0 (Fig. 5). In *C. gigas*, unfertilized eggs were almost equal across treatments in each elutriate. While the number of unfertilized eggs was the lowest in Ca, in more contaminated H2 elutriate, it was lower than in relatively less contaminated H1 (Fig. 6). In *P. lividus*, the fertilization success across elutriates demonstrated a concentration dependence pattern (in 50 %-diluted elutriate fertilization, success was significantly higher than in undiluted ones). The highest failure rates were observed in H1 and the lowest in Ca. The number of unfertilized eggs was higher at relatively stronger acidity (pH 6.5) and was the lowest at pH 7.5 in all elutriates (Fig. 7).

In all species, the fertilization failure at the pH 6.0 was significantly higher ($p < 0.05$) than that at the other treatments. At pH levels (6.5–8.0), the failure in more contaminated H1 and H2 elutriates was greater compared to that in less contaminated Ca, although not always significantly. For all species, fertilization failure in the controls was lower than in any pH treatment.

LpH50, which estimates the pH level that may be able to cause lethal effects in 50 % of the population in low contaminated Ca elutriate, showed pH 7.64 for *C. gigas*, pH 6.41 for *P. lividus* and pH 6.26 for *M. edulis* (Figs. 5, 6 and 7).

Egg fertilization failure in *C. gigas* did not show a concentration-dependent response. In 50 %-diluted H1 elutriate, the failure was significantly lower only at pH 6.5 and 8.0, compared to undiluted H1. At pH 7.0, the failure increased and at pH 7.5, it decreased, although not significantly. In 50 %-diluted H2 elutriate, the failure at pH 7.5, 8.0 and control significantly increased

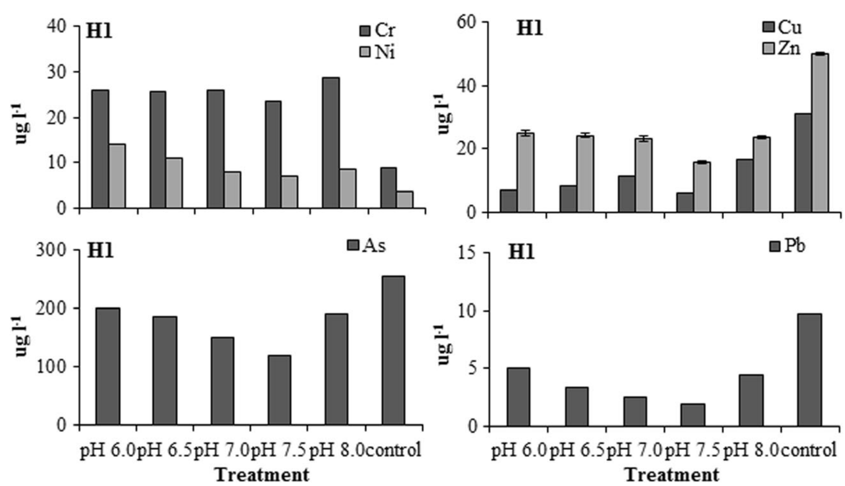

Fig. 3 Total concentrations (mean ± SD) of dissolved metals (Cr, Ni, Cu, Pb, Zn) and metalloid As in sediment elutriates from the Huelva estuary (H1) per treatment (pH 6.0, 6.5, 7.0, 7.5, control)

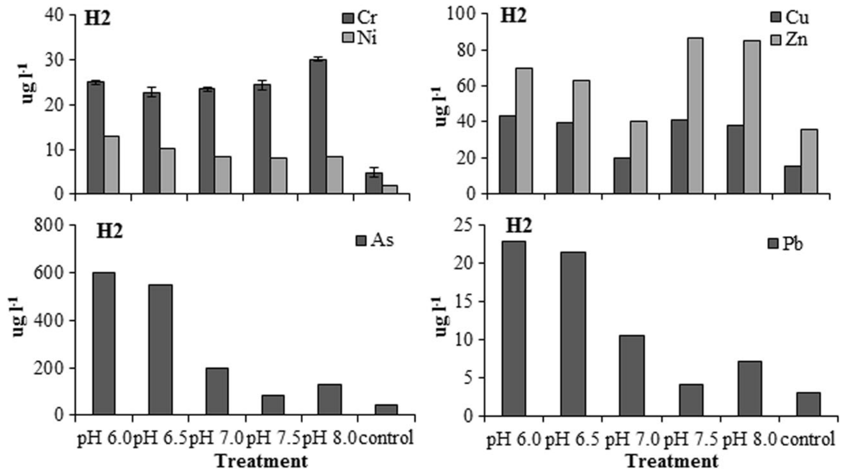

Fig. 4 Total concentrations (mean ± SD) of dissolved metals (Cr, Ni, Cu, Pb, Zn) and metalloid As in sediment elutriates from the Huelva estuary (H2) per treatment (pH 6.0, 6.5, 7.0, 7.5, control)

compared to undiluted ones ($p < 0.05$). They decreased at pH 6.5 although not significantly and remained almost unchanged at pH 7.0 (Fig. 6).

In 50 %-diluted H1 and H2 elutriates, the egg fertilization failure of *P. lividus* was significantly lower ($p < 0.05$)

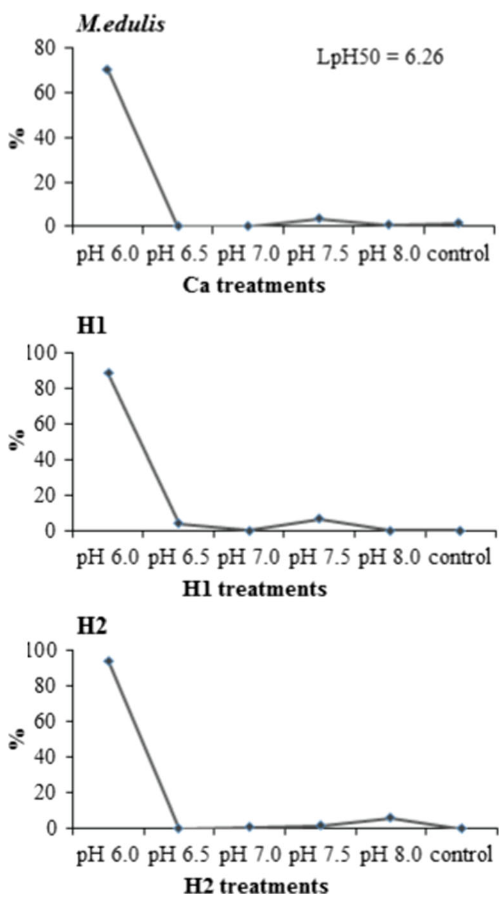

Fig. 5 The percentage of unfertilized eggs in blue mussel (*Mytilus edulis*) exposed to sediment elutriates from the Bay of Cadiz (Ca) and Huelva (H1, H2) per treatment (pH 6.5, 7.0, 7.5, 8.0 and control). LpH50: pH level that may cause fertilization failure in 50 % of population

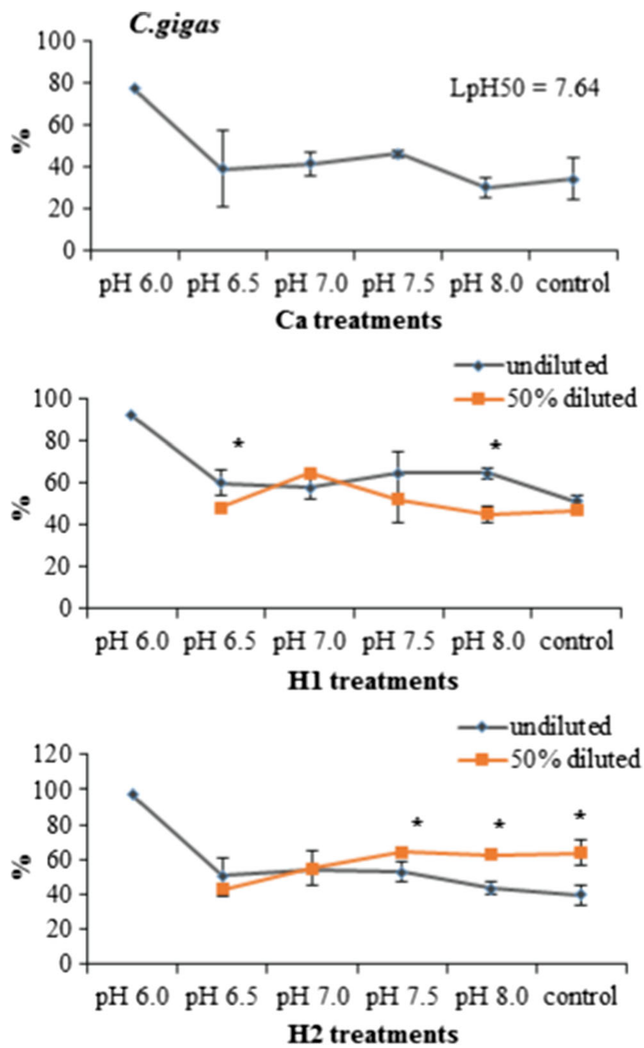

Fig. 6 The percentage of unfertilized eggs in oyster (*Crassostrea gigas*) (mean ± SD) exposed to sediment elutriates from the Bay of Cadiz (Ca) and Huelva (H1, H2) per treatment (pH 6.5, 7.0, 7.5, 8.0 and control). LpH50: pH level that may cause fertilization failure in 50 % of population. *Significant differences in responses between diluted and 50 %-diluted elutriates ($p < 0.05$)

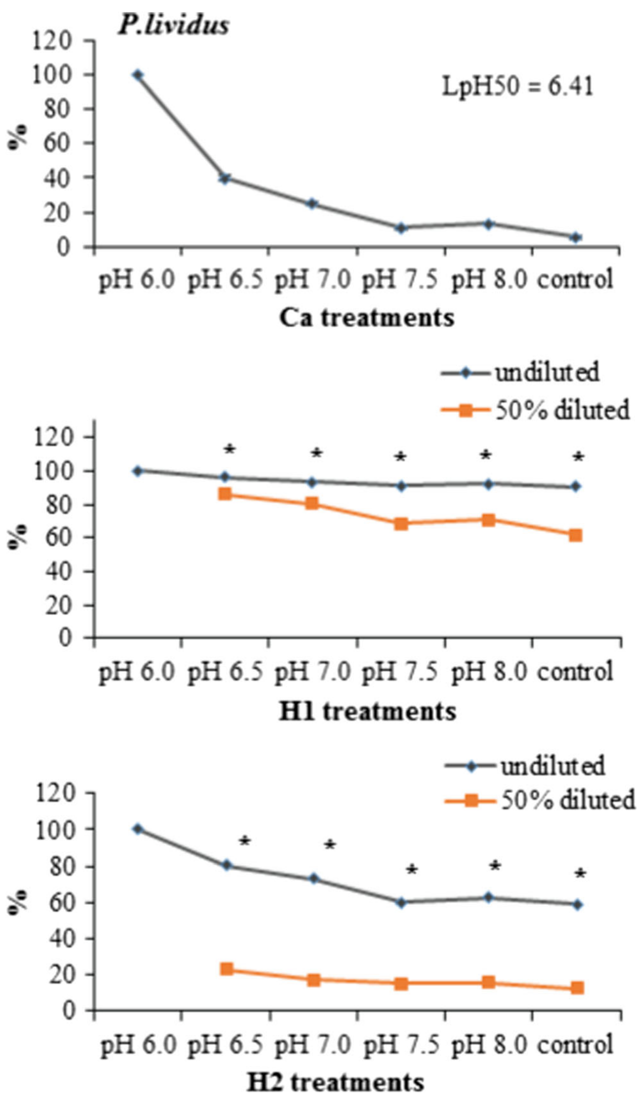

Fig. 7 The percentage of unfertilized eggs in sea urchin (*Paracentrotus lividus*) exposed to sediment elutriates from the Bay of Cadiz (Ca) and Huelva (H1, H2) per treatment (pH 6.5, 7.0, 7.5, 8.0 and control). LpH50: pH level that may cause fertilization failure in 50 % of population. *Significant differences in responses between diluted and 50 %-diluted elutriates ($p < 0.05$)

compared to undiluted ones, confirming concentration-dependent response of the egg fertilization in this species (Fig. 7).

Linking chemical and toxicological data

The results of PCA indicated three principal components (explaining totally 73 % of variance); however, none of them linked toxicity responses with the metal load in elutriates. The plot of variable loadings and scores in the coordinates of the first and second principal components is shown in Fig. 8. According to the plot, toxicants containing As, Zn, Cu and Pb may be associated with H2 elutriates at pH ≥6.5, whereas Ca and H1 elutriates could be related with Ni and Cr loads.

Discussion

The analysis of toxicology responses obtained in this study has been done in relation with the water quality criteria for aquatic life defined by US EPA (2012). In all elutriates (Ca, H1 and H2), at least one metal load exceeded the chronic effect level, sometimes even in the control solution. In both Huelva elutriates, Cu and As levels were far above acute effect levels (4.8 and 69 μg l^{-1}, respectively) and remained high even after 50 % dilution. In H2, chronic effect concentrations of Zn (81 μg l^{-1}) was exceeded at pH 7.5 and 8.0 and Pb (8.1 μg l^{-1}) at pH ≤7.0. Concentration of Pb exceeded chronic effect level also in the H1 control. In Ca, metal concentrations were far below chronic effect levels, except for Ni (at pH 6.0 and 6.5) and metalloid As (all pH levels). In fact, Ni chronic levels were exceeded at pH 6.5 and 6.0 in all elutriates.

Despite the difference in the contaminant load, all elutriates at pH 6.0 were extremely toxic for the egg fertilization of all species resulting in high or complete inhibition of fertilization (Figs. 5, 6 and 7). A detailed analysis of the egg fertilization failures showed species-specific sensitivity. *P. lividus* eggs showed a concentration-dependent fertilization failure across elutriates (from low contaminated Ca to highly contaminated H2) and pH levels (from pH 6.5, where As and Ni concentration were high, to the control, where only As chronic threshold had been exceeded). A concentration-dependent response of the egg fertilization was also demonstrated by a significant decrease in the fertilization failure after 50 % dilution of H1 and H2 elutriates (Fig. 7), although Cu and As loads were still far above acute effect levels. Pagano et al. (1982a) showed that sperm exposure to As(III) or As(V) can cause mitotic abnormalities in cleaving *P. lividus* embryos. Furthermore, LpH50 6.41, calculated for Ca elutriate, also demonstrated that if the contaminant impact is not severe, acidity as low as pH 6.4 may impede *P. lividus* egg fertilization process (Fig. 7). Thus, it can be assumed that the toxicity of elutriates to the egg fertilization in *P. lividus* had been stipulated by dissolved metal load and not the pH levels (6.5, 7.0 and 7.5) tested in the work. Boudouresque and Verlaque (2013) also reported that low pH had no effect on egg fertilization success and larval development of *P. lividus* and that adult species have low sensitivity to many environmental factors including ocean acidification.

In general, fertilized eggs of *P. lividus* are known to activate protective mechanisms that prevent egg death, e.g. generation of hydrogen peroxide (Wong and Wessel 2005). Geraci et al. (2004) reported that heat shock proteins, cellular protection mechanisms (Riabowol et al. 1988), were synthesized in blastula embryos when Ni or Pb was administered at the beginning of egg fertilization, while in the control embryos, these proteins were absent. Pagano et al. (1982b) reported that sperm pretreated with a low level of Cd^{2+} resulted in an increase in the fertilization rate; although at high cadmium load,

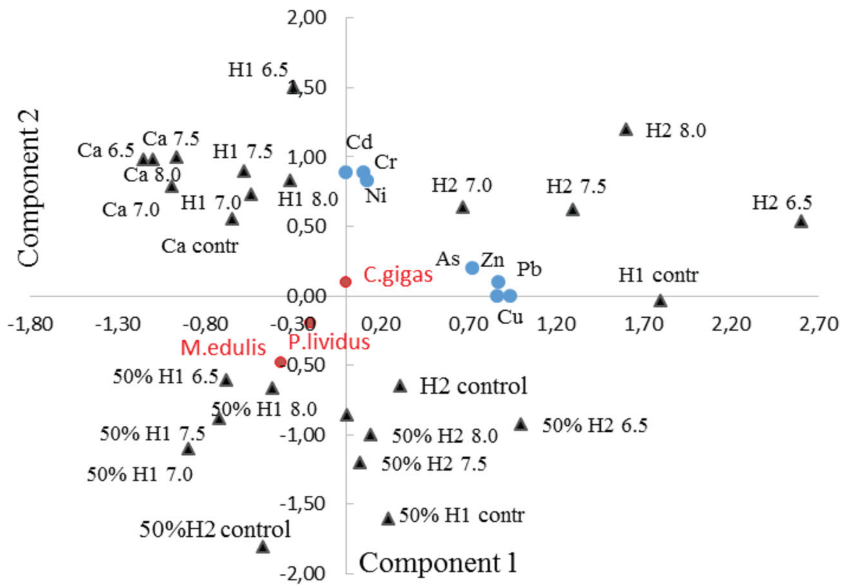

Fig. 8 Loadings and scores of the toxicity responses of *C. gigas*, *M. edulis* and *P. lividus* (egg fertilization failure) and metal concentrations in different treatments in the coordinate system of the two main principal components

a depression of fertilizing capacity occurred. The egg fertilization in *P. lividus* was also shown to be less sensitive to a contaminant mix compared to embryos (Khosrovyan et al. 2013; Wong and Wessel 2005). Nevertheless, at elevated metal levels, despite protective measures and insensitivity to environmental changes, damage to fertilized eggs may occur.

The fertilization failure in *C. gigas* was also lower in less contaminated Ca, compared to H1 and H2. However, in all three elutriates, a pH dependence of the fertilization failure was more obvious than a dependence on metal concentrations, in contrast to *P. lividus*. The LpH50 value for the Ca elutriate was 7.64, also demonstrating that acidity may play a significant role in egg fertilization failure. Indeed, Barros et al. (2013) has also shown that in *C. gigas*, a reduction of pH from 8.0 to 7.76 yielded 80 % of fertilized eggs while reduction to pH 7.37 yielded only 41 % of fertilized eggs after 4 h of exposure. Kurihara et al. (2007) reported shell mineralization failure after 2 days of exposure of fertilized eggs of *C. gigas* to CO_2-acidified water at pH 7.4. However, in a multiple-stressor environment (e.g. contaminants and acidity), acidity effect may be variable. In this study, 50 % dilution of H1 elutriate at pH 6.5 and 8.0 resulted in a significantly lower fertilization failure compared to undiluted elutriate. However, in 50 %-diluted H2 elutriate, the failure significantly increased at pH 7.5 and 8.0 and in the control (Fig. 6). Such an increase may point to the complexity of metal-pH interaction in natural environment. Acidity is an adverse factor affecting organisms in a variety of ways, e.g. influencing internal ion balance in organs of aquatic animals (Paquin et al. 2002). However, a combined effect of metal and acidity may result in a non-linear metal and proton uptake behaviour. For example, the competition exist between metal and hydrogen ions (H+) for binding sites in water and on biological surfaces (Santore et al. 2002). Whilst free metal ion is considered as the most bioavailable species (Bervoets and Blust 2000), presence of inorganic or organic ligands in solution may alter exchange or adsorption success of metal cations. Paquin et al. (2002) noted a reduction of free metal ion activity at the presence of ligands in water (e.g. hardness cations, dissolved organic carbon, calcium). The reduction of the activity could be related to the formation of complexes, which affect the mobility of metals and hence their bioavailability and toxicity (US EPA 1992). These complexes may have different electrical charges or be neutral altering their adsorption efficiency to the biological binding sites of which many are pH dependent. At more acidic pH, the number of negative sites for cation adsorption decreases (US EPA 1992); however, the amount of hydrogen ions increases. Hence, cationic metals (e.g. Pb, Ni, Zn, Cu) compete with H+ for the same uptake sites at the cell surface (Bervoets and Blust 2000). Furthermore, metal ions compete with each other as many essential and toxic metals share common uptake routes (Komjarova and Blust 2009). At low concentrations, adsorbed metal cannot be removed by competing cations; however, at higher concentration, when adsorption sites with strong binding capacity are saturated, exchange of metal ions at weak binding sites is enhanced affecting metal mobility (US EPA 1992).

As the pH increases, decreased competitive activities result in the increased metal toxicity. However, at higher pH values, further formation of complexes may occur (such as zinc hydroxide complexes at pH 8.0–8.5) which reduce metal ion activity and thereby binding to the biotic ligand (Santore et al. 2002). Perhaps, the competition between metal and hydrogen ions for biological binding sites in *C. gigas* can explain lower fertilization failure at higher acidity (pH 6.5), in contrast to higher or similar failure at lower acidity (pH 7.0 and above) in diluted H1 and H2 elutriates (Fig. 6). Significantly higher fertilization failure in diluted H2, compared to undiluted elutriate, may be explained by effective binding of metals to biological sites in *C. gigas* in a less (metal) competitive environment (Fig. 6). Enhanced complexation processes between metals and ligands present in undiluted H2 at pH 7.5 and 8.0 could be attributed to lower fertilization failure rate in *C. gigas*, compared to higher rate at more acidic conditions (Fig. 6). In less contaminated undiluted H1, the complexation processes did not seem to be as effective as in H2 to reduce the bioavailability of metals to both *C. gigas* and *P. lividus* (Figs. 6 and 7).

Hence, competition among different dissolved species controls the bioavailability of metals and in less loaded environments, favorable conditions might be created for the uptake of an individual metal. Perhaps of equal importance are the physiological pH levels in different organs of an organism (Amiard et al. 2007) and the reactivity of a metal (Paquin et al. 2002). The predominant form of Cu in bulk water is its divalent form (Cu2+); however, in order to be transported across biological membranes, it is likely to be reduced to Cu+ (Paquin et al. 2002). Thus, bioavailability and toxicity of metals in acidic environments cannot be simply related to total metal concentrations or to the acidity level but to metal reactivity and physiological pH level of a particular organ. Perhaps, this can explain the lack of correlations between egg fertilization responses and total dissolved metal concentrations indicated by PCA (Fig. 8).

The egg fertilization in *M. edulis* demonstrated vulnerability to pH 6.0 only. At higher pH levels (6.5 and above), neither metal nor acidity effects could be concluded. The percentage of unfertilized eggs was remarkably lower (with the highest failure of 6 % at H1 pH 7.5), compared to that of the other two species (Fig. 5). The calculated LpH50 6.26 also assumes that toxicity to the eggs or sperm could be caused by a relatively stronger acidity (equal or below pH 6.26) compared to the other two species. The blue mussel (*M. edulis*) is known to bioaccumulate Cd (Amachree et al. 2013) and Cu even at lower environmental concentrations (<9 $\mu g\ l^{-1}$) and regulate bioaccumulation of Zn (Allison et al. 1998; Grout and Levings 2001) through storage and elimination, in contrast to *C. gigas* in which this ability is limited (Amiard et al. 2007). Such accumulating ability could also be relevant to other metals. Although these bivalves have a variety of uptake routes (e.g. ingestion of particles, compartmentalization through shell and gills) (Turner et al. 2009), the bioaccumulation and effective removal capability increases their tolerance to many chemicals and factors in the environment.

Previous studies showed elevated concentration of many metals in the tissues of *C. gigas* compared to *M. edulis* which were stipulated by metabolic differences in these species (Thomson 1985; Amiard et al. 2007). Remarkably, in the present study, fertilization failure in the blue mussel was also lower compared to the oyster, possibly suggesting that in the zygotes of blue mussel, special metal detoxification mechanisms could be present.

The analysis of egg fertilization responses across pH levels did not demonstrate a clear vulnerability to acidity, and this raises concerns about the suitability of these species in the acidification studies. Ecological risk for acidity-sensitive biota may be underestimated if these species are to be used as a model.

Concluding remarks

In this study, the egg fertilization responses of different species to the combined effect of acidity and metals (including metalloid) and metal fractionation behaviour from sediment to the overlying water were compared. Egg fertilization of the bivalves *M. edulis* and *C. gigas* and sea urchin *P. lividus* cannot take place at pH 6.0 in a metal-contaminated environment. The egg fertilization failure in *P. lividus* in a metal-contaminated acidified environment increases in the concentration-dependent manner. The egg fertilization in *M. edulis* is not sensitive to an acidity-metal impact in the pH range 6.5–8.0. In *C. gigas*, a decreased metal load in acidified medium led to a higher egg fertilization failure indicating a complex interaction between acidity and metal.

While metals in sediments were mobilized in the acidified environment, a complex interplay of competing agents (metal-metal, metal-pH, metal-other constituents) for available binding sites affected metal toxicity. Besides, blue mussels and sea urchins demonstrated low vulnerability to acidity. Thus, care should be applied when selecting species and methods for risk assessment in acidified environments.

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