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Acute toxicities of four metals on the early life stages of the crab *Chasmagnathus granulata* from Bahía Blanca estuary, Argentina

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Abstract

Acute semistatic toxicity tests were carried out for 96 h with first zoeae and young crabs of *Chasmagnathus granulata*. Survival curves and LC_{50} (lethal concentration 50, the concentration which produces the death of 50% of the exposed population) indices for copper, zinc, cadmium, and lead were determined. Furthermore, mixture toxicity tests (Cd/Cu and Cd/Zn) with first-stage larvae were also carried out. The LC_{50}-96 h values determined in this study were 1093.4 (881–1319) $\mu g\,Pb^{2+}\,L^{-1}$, 219.2 (188.9–248.9) $\mu g\,Cu^{2+}\,L^{-1}$, 172.1 (141.3–203.6) $\mu g\,Zn^{2+}\,L^{-1}$, and 47.8 (37.9–58.0) $\mu g\,Cd^{2+}\,L^{-1}$ for zoeae I and 130.1 (121.7–139.0) $mg\,Cu^{2+}\,L^{-1}$, 51.0 (41.9–61.6) $mg\,Zn^{2+}\,L^{-1}$, and 35.7 (30.1–41.9) $mg\,Cd^{2+}\,L^{-1}$ for young crabs. The LC_{50}-96 h indices for mixture tests with zoeae I were 260.6 (227.3–286.3) $\mu g\,Cd^{2+}/Zn^{2+}\,L^{-1}$ and 41.3 (37.4–60.7) $\mu g\,Cd^{2+}/Cu^{2+}\,L^{-1}$. Cadmium presented the highest acute toxicity for both stages of the life cycle examined. The toxicity of the metals analyzed followed the order cadmium > zinc > copper > lead. First zoeae were more sensitive than young crabs to acute exposure to all metals analyzed. The young crabs were considered potentially dangerous agents of transference to the associated trophic chain because of their relatively elevated resistance and their capacity to bioaccumulate heavy metals in their tissues. Mixed toxicity tests carried out on first-stage larvae showed different kinds of interactions. Cadmium/copper presented an additive interaction trend while the mixture cadmium/zinc showed an antagonistic interaction.

Keywords: Cu; Zn; Cd, and Pb acute toxicities; Larvae and young crabs toxicity bioassays; Mixture toxicity tests

1. Introduction

The main objective of ecotoxicology is the evaluation of risk for an ecosystem exposed to environmental stress, including contamination. Although physical–chemical parameters are essential for risk determination, during the past decade the results of biological response to chemical stress have been used as references to determine the expected biological damage (Axiak, 1991).

Estuaries and coastal zones receive pollutant inputs from both specific and nonspecific sources, especially such ecosystems as seaports, cities, or other industrialized coastal areas that receive chronic inputs of metals. Since many species of crustaceans inhabit estuaries, numerous studies have aimed at examining the bioaccumulation and effects of various toxicants in these animals (Weis et al., 1992; Weis and Weis, 1994).

Acute lethal toxicity bioassays are useful for providing a measurement of the relative toxicity of substances, for assessing the sensitivity of the species' different stages of life to a particular substance, and for determining concentrations of chronic toxicity so as to assess water quality criteria.

Moreover, mixed toxicity bioassays provide information on the global effects of mixtures present in environments and allow evaluation of the magnitude of the effects by determining additive, synergistic, and/or antagonistic responses.

The Bahía Blanca estuary is located in the south-eastern section of Buenos Aires province, Argentina (38°45′–39°40′S and 61°45′–62°30′W). It is a coastal environment with a human population exceeding 350,000 inhabitants, where many ports, industries (oil

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Table 1
Heavy metal values in different compartments within Bahía Blanca estuary ($\mu g\,g^{-1}$, d.w.), (estuarine water, $\mu g\,L^{-1}$), (range in experiments with larvae, $\mu g\,L^{-1}$; with juvenile crabs, $mg\,L^{-1}$)

	Cu	Zn	Cd	Pb	Reference
Sediments	18.1±1.88	60.2±4.86	2.23±0.31	19.82±2.23	Marcovecchio and Ferrer (1999)
Suspended particulate matter	63.12±4.86	163.4±13.4	3.71±0.73	56.12±3.96	Ferrer et al. (2000), Andrade (2001)
Estuarine water	9.63±1.17	12.11±0.73	0.65±0.11	3.01±0.61	Ferrer et al. (2000), Andrade (2001)
Crabs	14.3±2.03	74.12±5.61	5.15±0.77	3.06±0.72	Ferrer et al. (2000), Ferrer (2001)
Range in experiments:					
Larvae	0–1280	0–1600	0–640	0–12800	This study
Juvenile	0–200	0–480	0–640	—	

refineries and terminals, petrochemical industries, and plastic factories), leather plants, textile plants, fish and meat factories, silos, and cereal mills are located. Moreover, fishing boats, oil tankers, and cargo vessels use the area extensively; thus it requires regular dredging.

It constitutes an estuarine environment with a very particular behavior, and it includes a tidal plain with an area close to 1150 km^2; it has a relatively small input of inland water and several marginal areas that function seasonally as hypersaline areas (Freije et al., 1981; Freije and Asteasuain, 1997). The middle-littoral is characterized by beaches with shallow slope and broad surfaces abundantly covered by “espartillar” (the halophytes *Spartina* sp. and *Salicornia* sp.) and crab burrows or “cangrejales” (mainly populations of *Chasmagnathus granulata* crab). The estuary’s main characteristics are the presence of various channels, fine sand, and silt–clay sediments together with a shallow depth. Tidal oscillations of 4 m and predominantly northwesterly winds create strong tidal currents, which facilitate water mixture and lead to a uniform vertical distribution of the main oceanographic parameters (Piccolo and Perillo, 1990; Perillo and Piccolo, 1991).

The *C. granulata* (Brachyura, Grapsidae) crab is widely spread on the Atlantic coast of South America, from Rio de Janeiro (Brazil) to Patagonia (Argentina) (Boschi, 1964). The abundant populations of this species play important roles as links in the trophic web, and every stage of the crab’s life cycle becomes a relevant food component for many fish species.

Previous studies on the presence and distribution of heavy metals in surface sediments, the fine-grained size fraction of sediments ($<63\,\mu m$), the dissolved and suspended particulate matter, and the geochemical partitioning in sediments were carried out in the Bahía Blanca estuary (Andrade et al., 1996; Ferrer et al., 1996; Marcovecchio and Ferrer, 1999). Thus, copper, zinc, cadmium, and lead were selected to develop toxicity bioassays, bearing in mind that they have been recognized as a potential risk for this environment (Table 1) (Ferrer, 2001).

This study included information on the acute toxic effects of Cu, Cd, Zn, and Pb on young crabs and on first-stage larvae in addition to mixed toxicity tests (Cd/Cu and Cd/Zn) on first stage larvae.

2. Materials and methods

Young crabs and ovigerous female specimens of *C. granulata* were collected in the intertidal zone at Puerto Cuatreros, Bahía Blanca estuary, Argentina during the spring and summer of 1999–2000.

Once in the laboratory, ovigerous females of similar maturity and carapace widths were selected. Young crabs in intermolting stage and with complete appendixes were selected.

They were placed in glass containers and acclimatized under environmentally controlled conditions selected for the bioassays that would be carried out later. During the acclimatization period, the specimens were fed twice a week, before the water was changed.

Naturally aged estuarine water was used after being sifted through a 0.45-μm pore filter and activated charcoal to remove dissolved organic matter and trace metals. Distilled water was then added to obtain the water dilution used for both acclimatization and assays at the desired salinity of 35±1 psu and pH 8±0.4. Both a constant temperature (22±1 °C) and controlled photoperiodism (12 L:12 D; fluorescent light) were maintained during the experiments.

The acute semistatic toxicity test was carried out according to the standard methodology for this kind of study such as those of the FAO (Ward and Parrish, 1982; Reish and Oshida, 1987) and the American Public Health Association (APHA, 1992).

A probit analysis was used to estimate the concentration and 95% confidence limits of copper and zinc that kills 50% of the exposed zoeae (LC_{50}) (Finney, 1971).

A comparison was carried out between LC_{50} major and LC_{50} minor ratio. Differences were significant when the corresponding statistics turned were higher than the critical value given by APHA (1992).

The evaluation of responses to mixed toxicity tests were carried out with regard to the toxic unit of each component in the mixture (Kagan, 1985). The additive rate and 95% confidence limits were estimated to determine the additive, synergistic, or antagonistic responses (Marking, 1997).

The solutions were prepared from $Cl_2Cu \cdot 2H_2O$, Cl_2Zn, $Cl_2Cd \cdot 2\frac{1}{2}H_2O$, and $Pb_2(NO_3)_2$, and concentrations were expressed with regard to cation.

2.1. First-stage larvae toxicity bioassay

All females were checked daily to detect hatched eggs or loss of clutches; also, posthatching and dead females were removed. Immediately after hatching, the first zoeae were exposed to Cu, Zn, Cd, Pb, Cd/Cu, and Cd/Zn for 96 h. Freshly hatched larvae (less than 8 h after hatching) were examined under a stereomicroscope to select those with the highest viability. Approximately 400 larvae were removed to carry out the toxicity tests.

Semistatic toxicological bioassays were carried out for 96 h, and all test solutions were replaced every 24 h, enable to calculation of the acute lethal toxicity in different concentrations of heavy metals. A series of six concentrations, 0 (control), 80, 160, 320, 640 and 1280 $\mu g\,Cu^{2+}\,L^{-1}$, six concentrations, 0 (control), 100, 200, 400, 800, and 1600 $\mu g\,Zn^{2+}\,L^{-1}$, seven concentrations, 0 (control), 20, 40, 80, 160, 320, and 640 $\mu g\,Cd^{2+}\,L^{-1}$, and seven concentrations, 0 (control), 400, 800, 1600, 3200, 6400, and 12 800 $\mu g\,Pb^{2+}\,L^{-1}$, were assayed on the basis of previous preliminary assays. Moreover, a series of six concentrations, 0 (control), 31, 62,5, 125, 250, and 500 $\mu g\,Cd^{2+}/Cu^{2+}\,L^{-1}$, and six concentrations, 0 (control), 200, 330, 550, 920, and 1530 $\mu g\,Cd^{2+}/Zn^{2+}\,L^{-1}$, were assayed in a mixed toxicity test based on several preliminary analyses developed to fix the range of exposure concentrations to these metal mixtures.

Three replicates of at least 20 animals were exposed to the above-stated concentrations. The criterion to determine death was the absence of movement once the animals were gently touched with a glass rod. Mortality was recorded every 24 h, a period of time after which dead zoeae were removed.

2.2. Young crabs toxicity bioassay

Once in the laboratory, young crabs were acclimatized for 14 days. The size at the onset of sexual maturity in *C. granulata* to be considered a young crab entails an 18.5-mm carapace width for females and a 12 mm carapace width for males (López Greco et al., 2001).

Semistatic toxicological bioassays were carried out for 96 h, and all test solutions were replaced every 24 h, enable to calculation of the acute lethal toxicity in different concentrations of heavy metals. A series of six concentrations, 0 (control), 81.9, 102.4, 128, 160, and 200 $mg\,Cu^{2+}\,L^{-1}$, six concentrations, 0 (control), 30, 60, 120, 240, and 480 $mg\,Zn^{2+}\,L^{-1}$, and seven concentrations, 0 (control), 20, 40, 80, 160, 320, and 640 $mg\,Cd^{2+}\,L^{-1}$, were assayed on the basis of previous preliminary assays. Lead toxicity tests could not be carried out because this metal precipitates in the corresponding solutions due to the high level required to produce lethal effects on this stage of life of the crab; this problem has not occurred with larvae exposure solutions, where the lower Pb levels which produced lethal effects on crab larvae did not produce any precipitation in the solution.

Three replicates of at least 10 animals were exposed to the above-stated concentrations. The criteria to determine death was the complete laxity of appendixes and the absence of movement once the animals were gently touched with a glass rod. Mortality was recorded every 24 h, a period of time after which dead crabs were removed.

The experimental conditions (temperature, salinity, and pH) of the toxicity test were similar to those found in the environment during the period when zoeae I are abundant (spring and summer). To match the environmental conditions, an average of these parameters was used. This is due to the changes in environmental variable conditioning of metal speciation, affecting their availability and toxicity (Adema et al., 1980; Luoma, 1983; Förstner, 1993; Bourg and Loch, 1995).

3. Results and discussion

Coastal habitats of Bahía Blanca estuary are subjected to various environmental perturbations including water pollution with high concentrations of metals generated from industry and municipal effluent discharges, dredging operations and recovery of the extracted sediments, harbor operations, etc. (Marcovecchio, 2000). The study of the effects of heavy metals on key organisms within this environment seems to be an adequate tool with a sensitive approach to the corresponding pollution risk.

Table 2 summarizes the LC_{50} values for zoeae I obtained for the four metals at different times of exposure. In the control tests, zoeae I mortality after 96 h was 8.1% for lead, 8.8% for copper, 7.5% for zinc, and 8.9% for cadmium. The validity of the tests was possible because the mortality in the control tests was less than 10% in all of the cases.

The LC_{50}-96 h value of cadmium registered for zoeae I of *C. granulata* at 30 psu was larger than that reported earlier in this study, although no significant differences were found (64.95 $\mu g\,L^{-1}$ (32.11–92.29)). Moreover, the LC_{50}-96 h value of copper was significantly less

Table 2
LC_{50} values, 95% confidence limits, and probit line parameters of lead, copper, zinc, and cadmium for first zoeae of the crab *Chasmagnathus granulata*

Metal	Exposure (h)	CL_{50} ($\mu g\,L^{-1}$)	95% confidence limits	Slope	Correlation coefficient
Pb	24	5653.6	(4560–7270)	2.45	0.70
	48	3145.5	(2634–3716)	4.10	0.69
	72	2415.3	(1970–2906)	3.49	0.84
	96	1093.4	(881–1319)	3.44	0.90
Cu	24	666.0	(597.1–742.6)	7.48	0.93
	48	371.3	(325.1–422.8)	5.23	0.61
	72	303.4	(270.0–340.0)	4.37	0.78
	96	219.2	(188.9–248.9)	7.42	0.99
Zn	24	848.6	(699.7–1069.9)	2.56	0.62
	48	671.8	(552.2–827.3)	2.64	0.72
	72	250.4	(208.6–295.5)	3.48	0.92
	96	172.1	(141.3–203.6)	3.64	0.96
Cd	24	287.2	(239.1–355.9)	2.87	0.74
	48	141.6	(122.9–161.8)	5.30	0.78
	72	66.8	(36.1–117.3)	3.41	0.82
	96	47.8	(37.9–58.0)	2.99	0.86

Table 3
LC_{50} values, 95% confidence limits, and probit line parameters of mixture cadmium–copper and cadmium–zinc for first zoeae of the crab *Chasmagnathus granulata*

Metal mixture	Exposure (h)	CL_{50} ($\mu g\,L^{-1}$)	95% confidence limits	Slope	Correlation coefficient
Cd–Zn	24	992.3	(873.5–1138)	5.79	0.68
	48	457.4	(422.7–501.9)	11.25	0.99
	72	284.3	(241.6–305.8)	12.36	0.99
	96	260.6	(227.3–286.3)	8.37	0.99
Cd–Cu	24	253.1	(215.2–298.9)	4.66	0.71
	48	138.1	(118.3–161.3)	5.08	0.73
	72	76.6	(69.5–115.8)	5.08	0.78
	96	41.3	(37.4–60.7)	2.92	0.86

($P<0.05$) than that reported in this study (110.62 $\mu g\,L^{-1}$ (78.13–153.66)) (López Greco et al., 2001).

Likewise, these results showed that cadmium toxicity was the highest (40 times) and that lead toxicity was similar to the values registered for zoeae I of the Southern King Crab *Lithodes santolla*, LC_{50}-96 h 2.07 $mg\,L^{-1}$ and 1.66 $mg\,L^{-1}$ for cadmium and lead, respectively (Amín et al., 1998).

Table 3 summarizes the LC_{50} values for zoeae I obtained from a mixture of metals at different times of exposure. In the control tests, zoeae I mortality after 96 h was 8.0% for cadmium/zinc and 3.6% for cadmium/copper. Once again, the validity of the tests was possible because the mortality in the control was less than 10% in both cases.

Table 4 summarizes the LC_{50} values for young crabs obtained for three metals at different times of exposure. In the control tests, young crabs mortality after 96 h was practically 0% for all metals, which showed the validity of the tests.

The LC_{50}-96 h value for cadmium reported in this study for young crabs is significantly high ($P<0.05$) compared to those stated for both stages of *C. granulata* at 12 psu, for adults (LC_{50}-96 h, 25.33 $mg\,L^{-1}$ (20.40–31.45); Bigi et al., 1996) and for young crabs (LC_{50}-240 h, 24.10 $mg\,L^{-1}$ (14.88–39.34); López Greco et al., 2001).

It is important to emphasize that our experiments have been conducted under lower salinity conditions those in than previous tests, which were mentioned as references. A considerable body of evidence indicates that metal bioavailability is a function of not only the total metal concentrations but also their free ion activity, which is affected by factors such as salinity and complexation capacity (Forbes, 1991; Bjerregaard and Depledge, 1994; Blaudez et al., 2000; Philp, 2001). In this sense, De Wolf et al. (2004) have reported that, in this kind of bioassay, the organism's time to death increases with higher salinity levels or—which is the same—mortality increases with lower salinity levels. The

Table 4
LC_{50} values, 95% confidence limits, and probit line parameters of copper, zinc, and cadmium for young crab *Chasmagnathus granulata*

Metal	Exposure (h)	CL_{50} ($mg\,L^{-1}$)	95% confidence limits	Slope	Correlation coefficient
Cu	24	178.8	(160.0–200.0)	—	—
	48	150.4	(141.4–160.1)	15.45	0.94
	72	138.5	(129.4–148.3)	12.61	0.96
	96	130.1	(121.7–139.0)	13.13	0.99
Zn	24	147.8	(118.7–184.6)	3.71	0.96
	48	101.9	(83.9–123.9)	4.87	0.92
	72	75.0	(60.9–92.1)	4.32	0.88
	96	51.0	(41.9–61.6)	5.29	0.95
Cd	24	283.2	(246.2–325.8)	6.51	0.98
	48	106.8	(89.5–127.4)	3.90	0.74
	72	64.6	(54.2–77.0)	3.94	0.89
	96	35.7	(30.1–41.9)	4.73	0.98

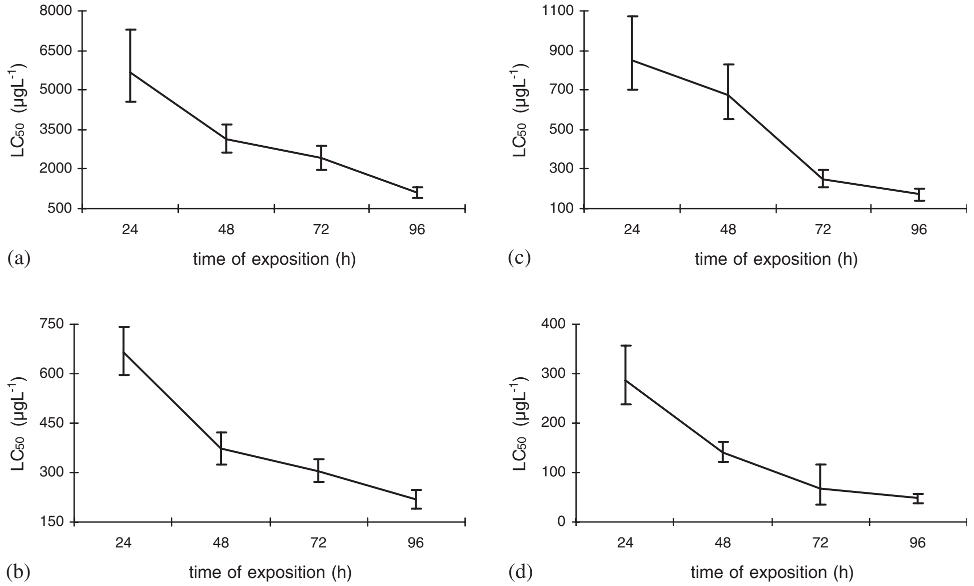

Fig. 1. LC_{50} values of Pb (a), Cu (b), Zn (c) and Cd (d) for the first zoeae of the crab *Chasmagnathus granulata* in relation to exposure time (average ± 95% confidence limits).

"salinity effect" may be explained with regard to the strong regulation it produces on metals uptake and toxicity (Blust et al., 1992; Rainbow et al., 1993; Jackson et al., 2000). Indeed, salinity-increased results are found, for instance, in ionic strength and osmolarity, and in calcium and chloride concentrations (De Wolf et al., 2004). Moreover, since Ca^{2+} and Cd^{2+} may be taken up by the same transport systems (Philp, 1999; Blaudez et al., 2000), the competition for uptake between both ions will depend on salinity (Bjerregaard and Depledge, 1994; Heugens et al., 2001; De Wolf et al., 2004). Finally, considering that the Bahía Blanca estuary environment is often subjected to wide variations in salinity during the summer period (it can reach values between 12.1 and 40.2 psu), it seems that the relationship between metals toxicity and salinity should be carefully considered to better understand the results as recorded.

Copper assays showed the sharpest gradients, a fact that indicates a rapid absorption of this metal and an immediate increase of toxic effect with regard to the rest of the metals.

Cadmium presented the highest acute toxicity for both stages of life cycle considered. Toxicity of the analyzed metals followed the order cadmium > zinc > copper > lead. This trend of toxicity depends on long-life species and life-cycle stages as the conditions of assays. The first-stage larvae of *L. santolla* showed the following toxicity order: copper > lead > cadmium = zinc (Amín, 1995).

The toxicity curves (LC_{50} values in relation to exposure time) presented an evident asymptotic trend in all cases, a fact that corroborates the validity of assays (Figs. 1–3). This validity includes the correct selection of concentrations and trial organisms, the good health of organisms, and sufficient time of

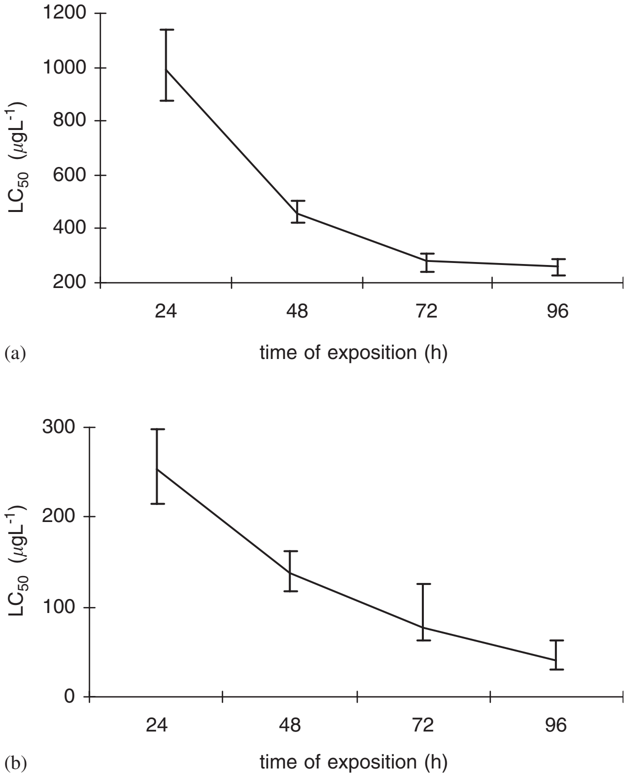

Fig. 2. LC_{50} values of mixture Cd–Zn (a) and Cd–Cu (b) for the first zoeae of the crab *Chasmagnathus granulata* in relation to exposure time (average ± 95% confidence limits).

acclimatization in the laboratory (FAO, Reish and Oshida, 1987).

The comparison of LC_{50} values in relation to the exposure time allowed determines the incipient lethal threshold. The absence of significant differences between LC_{50}-72 h and LC_{50}-96 h indicates the stabilization of mortality toward the end of the bioassay; hence LC_{50}-96 h may be considered the lethal incipient threshold (APHA, 1992). On the contrary, significant differences between the values of LC_{50} at 72 and 96 h make it impossible to determine the lethal incipient threshold of each metal.

The lethal incipient threshold for zoeae I can be determined only for cadmium (47.8 $\mu g\,L^{-1}$), while in the mixed toxicity test 260.6 $\mu g\,L^{-1}$ was determined for cadmium/zinc. In the case of young crabs, the lethal incipient threshold was determined only for copper (130.1 $mg\,L^{-1}$).

Table 5 shows the effects of mixed toxicity tests for zoeae I of *C. granulata*. Mixed toxicity tests with first-stage larvae showed different kinds of interactions; a cadmium/copper mixture presented an additive trend (after 48 h it showed antagonistic interaction) while the mixture cadmium/zinc showed an antagonistic interaction.

Weis et al. (1992) reported the same antagonistic interaction to cadmium and zinc for the crab *Uca pugilator*.

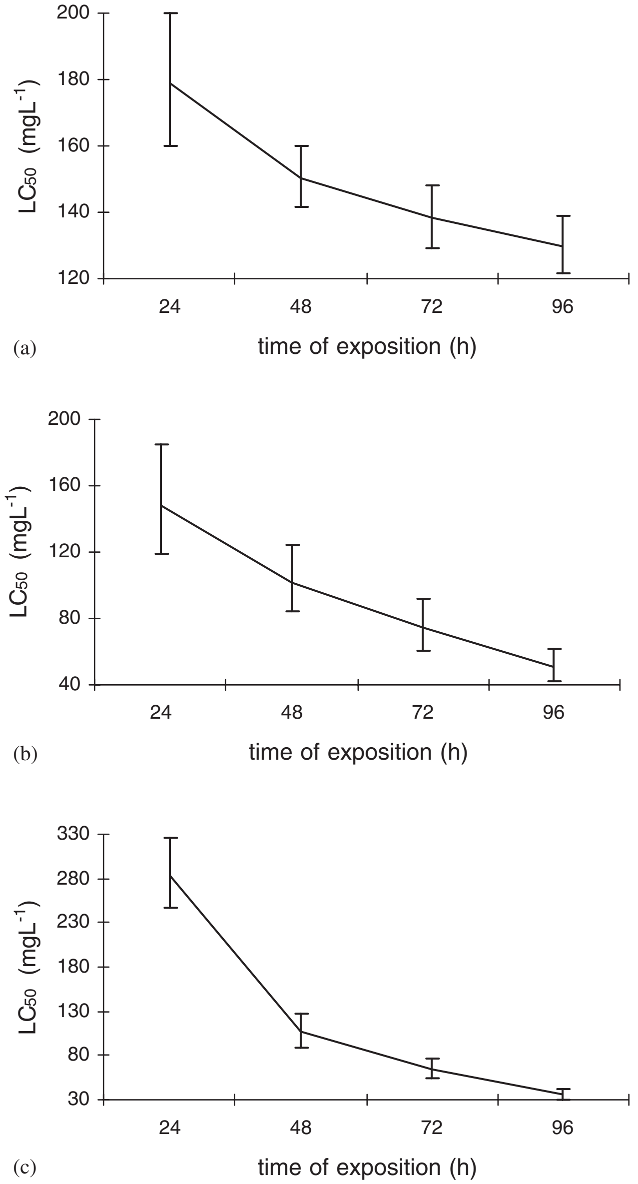

Fig. 3. LC_{50} values of Cu (a), Zn (b) and Cd (c) for young crab *Chasmagnathus granulata* in relation to exposure time (average ± 95% confidence limits).

From an ecotoxicological point of view the understanding of the interaction of two kinds of pollutants under particular environmental conditions is very important, and the fact that pollution in aquatic environments includes the mixture of the substances must be taken into account.

The LC_{50}-96 h value of the cadmium/copper mixture reported in this paper was one order of magnitude lower than that registered for zoeae I of *L. santolla* at 30 psu (452 $\mu g\,L^{-1}$ (290–690): Amín, 1995). Nevertheless, the consequences of the interactions for this mixture were similar to those reported for *L. santolla*, which presented additive and antagonistic responses.

Table 5
Effects of mixture toxicity tests for zoeae I of *Chasmagnathus granulata* (AR, additive rate)

Mixture	Exposure (h)	AR	95% confidence limits of AR	Effects
Cd/Cu	24	−0.26	−0.75; 0.11	Additive
	48	−0.34	−0.80; −1.10E-2	Antagonistic
	72	−0.39	−2.63; 0.25	Additive
	96	−5.27E-2	−0.92; 0.25	Additive
Cd/Zn	24	−3.62	−5.38; −2.27	Antagonistic
	48	−2.91	−3.99; −2.12	Antagonistic
	72	−4.38	−8.94; −1.87	Antagonistic
	96	−5.96	−8.58; −4.03	Antagonistic

Table 6
Comparisons between LC_{50}-96 h values for metals in both *Chasmagnathus granulata* crab life history stages analyzed

LC_{50}-96 h comparisons	Statistical value	95% Critical value	LC_{50}-96 h quotient
Zoeae I			
Zn–Cd	3.60	1.32	3.6*
Cu–Cd	4.58	1.29	4.6*
Pb–Cd	22.8	1.34	22.9*
Cu–Zn	1.27	1.25	1.3*
Pb–Zn	6.35	1.31	6.4*
Pb–Cu	4.98	1.27	5.0*
Young crab			
Zn–Cd	1.42	1.28	1.4*
Cu–Cd	3.64	1.19	3.6*
Cu–Zn	2.55	1.22	2.6*

*Significant differences ($P<0.05$).

Table 7
Comparisons between LC_{50}-96 h values for both *Chasmagnathus granulata* crab life history stages analyzed

Metal	Life history stages	LC_{50}-96 h (mgL^{-1})	95% Critical value	Relative toxicity
Cu	Zoeae I	0.22	1.16	593.5*
	Young crab	130.1		
Zn	Zoeae I	0.17	1.29	296.3*
	Young crab	51.0		
Cd	Zoeae I	0.05	1.48	746.9*
	Young crab	35.7		

*Significant differences ($P<0.05$).

Table 6 presents comparisons between LC_{50}-96 h values for metals in both analyzed life stages of the crab *C. granulata*. The biggest zoeae I acute toxicity relationship (ratio between LC_{50} values at 96 h) was 22.9 times for lead/cadmium ($P<0.05$). Moreover, at the end of exposure (96 h) the rest of the ratios presented were statistically significant ($P<0.05$).

A higher toxicity of cadmium than of copper for zoeae I of *C. granulata* at 30 psu was also observed, although to a lesser degree (1.7 times). These results show that, due to the nature of cadmium, which is not a physiological element, it produces a progressive accumulation in organisms with regard to copper, which can be regulated between a wide range of environmental concentrations (López Greco et al., 2001).

Likewise, young *C. granulata* crabs at 12 psu presented a higher toxicity of cadmium than of copper (10 times higher). As previously stated, cadmium toxicity has a strong dependence on salinity; thus, this increase is significantly associated with low salinity levels (Blaudez et al., 2000; De Wolf et al., 2004.

First zoeae were more sensitive than young crabs to acute exposure to all the metals analyzed (Table 7); presumably this is related to the weight and development differences between these stages. Comparisons between LC_{50}-96 h values for both stages considered in the test showed a difference of three orders of magnitude. The same relative toxicity was found between first zoeae and *C. granulata* young crabs in bioassays at 12 and 30 psu (López Greco et al., 2001).

On the one hand, the *C. granulata* young crabs can be considered good indicators of heavy metal pollution because they are highly resistant, abundant, widely distributed, and easy to sample (Margaleff, 1983). These characteristics make *C. granulata* a good species to evaluate in its natural environment, both and for lethal (distribution, abundance, diversity, recruit) and for sublethal (growth rate, reproductive variables) effects (Wenner, 1988).

On the other hand, young crabs were considered potentially dangerous agents for transference along the associated trophic chain, such as fish and bird species that inhabit the Bahía Blanca estuary, because of their relative elevated resistance and their capacity to bioaccumulate heavy metals in their tissues (Ferrer, 2001).

Finally, the *C. granulata* zoeae I LC_{50}-96 h values determined for Cd, Cu, Zn and Pb were two order of magnitude higher than the corresponding metal concentrations in the Bahía Blanca environment (Table 1;

Andrade, 2001); thus it can be stated that crab larvae were significantly far from being an acute toxicity hazard.

4. Concluding comments

Metals toxicity response of the crab *C. granulata* from Bahía Blanca estuary was studied for the first time. It is a remarkable point, considering that, although several toxicological studies of the mentioned crab species have been previously developed in other South American environments (i.e., Vitale et al., 1999; López Greco et al., 2001; Rodríguez Moreno et al., 2003), these kind of studies have still not been developed in Bahía Blanca estuary, which is a recognized man-impacted system within Argentina's marine coastal system.

Cadmium presented the highest acute toxicity for both considered stages of life cycle. The toxicity of the analyzed metals followed the order: cadmium > zinc > copper > lead.

The lethal incipient threshold for zoeae I can be determined only for cadmium (47.8 $\mu g\,L^{-1}$), while the mixed toxicity test was determined for cadmium/zinc (260.6 $\mu g\,L^{-1}$). In the case of young crabs, the lethal incipient threshold was determined only for copper (130.1 $mg\,L^{-1}$). However, it should be noted that these LC_{50} values were higher than the corresponding metal concentrations measured in the Bahía Blanca environment. Last but not least, the assessment of acute lethal toxicity is the first step to determine the chronic effects on estuarine organisms, which will be evaluated in a future time.

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