

Copper and zinc concentrations in Bahía Blanca Estuary (Argentina), and their acute lethal effects on larvae of the crab *Chasmagnathus granulata*

Laura D. Ferrera^a, J. Santiago Andrade^a, Elena T. Contardia^a, Raúl O. Asteasuain^a and Jorge E. Marcovecchio^{a,b}*

^aLaboratorio de Química Marina, Instituto Argentino de Oceanografía (IADO). C.C. 804, 8000 Bahía Blanca, Argentina

^bFacultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMDP). Dean Funes 3350, 3° piso, 7600 Mar del Plata, Argentina

ABSTRACT

Bahía Blanca is an estuarine environment with a very particular behavior, which includes a large tidal plain with an area close to 1150 km², a relatively small input of inland water, and with several marginal areas that seasonally function as hypersaline. Mud is predominant in the sediments, where a significant population of the crab *Chasmagnathus granulata* lives during the whole year. There are several cities along the estuary as well as important harbors and a large industrial nucleus which discharge their effluents within this environment.

Cu and Zn concentrations were determined in samples of water (for both dissolved and suspended particulate matter) and surface sediments (total and <63 μ m fractions). Organic matter was analyzed in the sediments, while temperature, salinity, pH and dissolved oxygen were measured in the estuarine water.

The metal concentrations determined in this study were: $16.01 \pm 1.85 \mu\text{g Cu g}^{-1}$ and $52.96 \pm 5.67 \mu\text{g Zn g}^{-1}$ in surface sediments; $111.05 \pm 42.78 \mu\text{g Cu g}^{-1}$ and $105.66 \pm 19.16 \mu\text{g Zn g}^{-1}$ in the <63 μg surface sediment fractions. While the concentrations in suspended particulate matter were $35.61 \pm 9.77 \mu\text{g Cu g}^{-1}$ and $205.03 \pm 70.68 \mu\text{g Zn g}^{-1}$, and $4.65 \pm 2.27 \mu\text{g Cu L}^{-1}$ and $15.82 \pm 6.14 \mu\text{g Zn L}^{-1}$ in dissolved fraction. Furthermore, dissolved Cu was similar than the quality criteria concentration, while Zn was lower than the norms for marine and estuarine waters ($4.8 \mu\text{g L}^{-1}$ for Cu and $90.0 \mu\text{g L}^{-1}$ for Zn, USEPA, 1999).

Simultaneously, the effects of Cu and Zn were studied on recently hatched larvae of *C. granulata*, through 96 hours semi-static acute assays. Viability was the applied criterion within the assays. LC₅₀-96h for Cu was $219.2 \mu\text{g L}^{-1}$ ($188.9\text{--}248.9 \mu\text{g L}^{-1}$), whilst that for Zn was $172.1 \mu\text{g L}^{-1}$ ($141.3\text{--}203.6 \mu\text{g L}^{-1}$), which demonstrates that Zn is more toxic towards larvae. Finally, both LC₅₀-96h values determined for Cu and Zn were higher than the corresponding metal concentrations measured in the Bahía Blanca environment.

Keywords: estuarine copper and zinc, toxicity test, estuarine crab larvae

INTRODUCTION

The study of occurrence, fate and distribution of trace metals in estuarine environments has deserved to receive

major attention, mainly because of their persistent toxic effects, as well as their ability to be accumulated within compartments of these environments (Salomons, 1995; 1996). Thus, knowledge of both the physical and physico-chemical processes controlling the behavior of heavy metals is a basic requirement for the understanding of their potential toxicity in the environment.

Estuaries and coastal zones receive inputs of pollutants from both point and non-point sources, especially con-

*To whom correspondence should be addressed. E-mail: jorgemar@criba.edu.ar

cerning with such ecosystems as seaports, cities or other industrialized coastal areas which receive chronic inputs of these metals. Since many crustacean species inhabit estuaries, numerous studies have aimed at examining the bioaccumulation and effects of various toxicants in these animals (*i.e.*, Weis *et al.*, 1992). In this sense, the present work constitutes the first integral study of copper and zinc distributions and their effects in the Bahía Blanca Estuary.

The Bahía Blanca Estuary is located in the south-eastern of Buenos Aires province, Argentine (38°45'–39°40' S and 61°45'–62°30' W). It is an estuarine environment having unusual aspects, which include a large tidal plain with an area close to 1150 km², a relatively small input of inland water, and with several marginal areas that seasonally function as hypersaline ones (Freije *et al.*, 1981). The middle-littoral is characterized by beaches with scarce slope and broad surface and abundantly covered by halophyte vegetation or “espartillar” (*Spartina* sp. and *Salicornia* sp.) and crab caves or “cangrejales”, basically from populations of the crab *Chasmagnathus granulata*.

The estuary is also characterized by the presence of various channels, fine sand and silt-clay sediments and low depth. Tidal oscillations of 4 m and predominant northwesterly winds create strong tidal currents, which facilitate water mixture, leading to a uniform vertical distribution of the main oceanographic parameters (Piccolo and Perillo, 1990; Perillo and Piccolo, 1991).

At the northern boundaries of estuary various ports, towns (with a population exceeding 350,000 inhabitants) and industries are located and several streams discharge within the area. Oil refineries and terminals, petrochemical industries, meat factories, leather plants, fish factories, textile plants, wool washing plants, silos and cereal mills discharge their effluents into the estuary with or without treatment. Moreover, this area is extensively used by fishing boats, oil tankers and cargo vessel and therefore requires regular dredging.

The crab *Chasmagnathus granulata* (Grapsidae) is widely distributed on the Atlantic coast of South America, from Rio de Janeiro (Brazil) to Patagonia (Argentina) (Boschi, 1966). The abundant populations of this species play an important linking role within the corresponding trophic web, and all stages of the crab's life cycle become a relevant food component for many fish and shellfish species.

The present study includes information on the distribution of oceanographic parameters and the occurrence and distribution of copper and zinc in surface sediments, the finest grain size fraction of sediments (<63 µm), dissolved and suspended particulate matter, as much as the acute toxic effects of Cu and Zn on crab larvae.

MATERIALS AND METHODS

Environmental parameters, organic matter and trace metals

Both, the study area as well as the location of the sampling station at Puerto Cuatros, Bahía Blanca Estuary (Argentina) are shown in Figure 1. Samples were taken monthly at a sampling station located at the inner zone of the estuary between May 1999 and September 2000. Temperature, salinity, pH and dissolved oxygen were *in situ* measured using an Horiba U-10 multisensor device.

Surface water samples (0.5–1 m depth) were collected using a Van Dorn oceanographic bottle on deck, from a position in the bow which minimized contamination from the ship. Obtained water samples were filtered through Millipore HA filters (0.45 µm mesh) for the determination of suspended and dissolved metals. Before use, filters were soaked overnight in dilute HCl and stored in redistilled water. The filtered samples were acidified using 3 ml of HNO₃ (analytical quality) in order to be stored. All fractions were frozen (at –20°C) until corresponding analysis.

Surface sediments were obtained with a sledge and stored in a freezer (at –20°C) up to their analysis in the laboratory. These samples were oven-dried (at 45 ± 5°C) up to constant weight; subsamples were carefully sifted, and the < 63 µm fraction was removed.

The dissolved metals were simultaneously extracted from filtered water samples by chelation with ammonium pyrrolidine-dithiocarbamate (APDC) followed by methyl isobutyl ketone (MIBK) extraction according to the method described by Koirtzmann and Wen (1973). All extracted water samples were combined to ensure homogeneity as well as to provide an adequate volume of matrix for the preparation of blanks and standards. Several aliquots of this matrix were treated along with the samples to obtain analytical blanks. The standards were prepared by adding increasing amounts of Cu and Zn to the matrix.

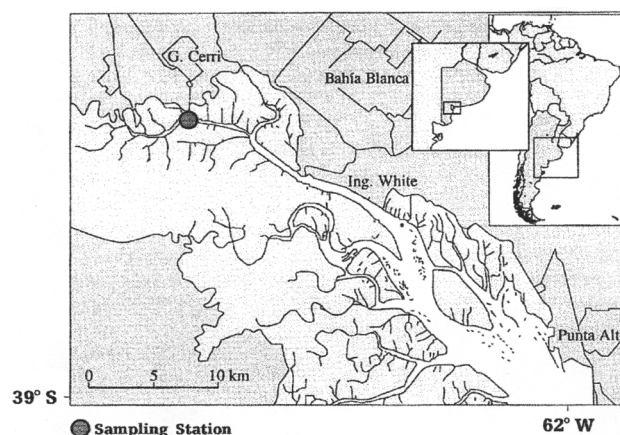


Figure 1 Location of sampling station in the study area, Bahía Blanca Estuary (Argentina).

Sediment samples, sediment finest grain size fraction and suspended particulate matter (SPM) were fully digested in a concentrated acids mixture, according to the method described by Dalziel and Baker (1983) and modified by Marcovecchio *et al.* (1988). Subsamples of 500 ± 50 mg were removed, and mineralized with a 1:3 perchloric-nitric acid mixture in a thermostatic bath (at $90 \pm 10^\circ\text{C}$) up to minimum volume (< 1 ml). Solutions were made up to 10 ml with 0.7% nitric acid, and corresponding absorbances of each metal were measured by atomic absorption spectrophotometry.

Concentrations Cu and Zn were determined with a Perkin-Elmer AA-2380 atomic absorption spectrophotometer with an air/acetylene flame and deuterium background correction (D₂BGC). Analytical grade reagents were used to make up the relevant blanks and calibration curves, and the analytical quality (AQ) was tested against reference materials (Table 1) provided by The National Institute for Environmental Studies (NIES), from Tsukuba (Japan).

Organic matter contents within sediments were determined following the method modified by Strickland and Parsons (1968). This involves the wet oxidation of carbon by acidic dichromate, and adaptation to spectrophotometry. A Beckman DU2, UV-visible spectrophotometer at wavelength 440 nm was used to determine the organic matter concentrations.

Toxicity bioassay

Ovigerous female specimens of *Chasmagnathus granulata* were collected in the intertidal zone at Puerto Cuatros, Bahía Blanca Estuary, Argentina (Figure 1) during the spring of 1999. Once in the laboratory, ovigerous females of similar maturity and carapace widths were selected. They were placed in glass containers, and acclimatized under environmentally controlled conditions selected for the bioassays which would be carried out later.

Naturally aged estuarine water was used after being passed through a $0.45 \mu\text{m}$ mesh filter as well as active carbon in order 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 $\text{pH } 8 \pm 0.4$. A constant temperature ($22 \pm 1^\circ\text{C}$) and controlled photoperiod (12 L:12 D, fluorescent light) were maintained during the experiments.

All females were checked daily in order to detect hatched eggs or loss of clutches; post-hatching and dead

females were removed. Immediately after hatching, the first zoeae were exposed to Cu and Zn for 96 h, and the acute semi-static toxicity test was carried out according to the standard methodology for this kind of studies – the FAO (Reish and Oshida, 1987) and the American Public Health Association (APHA, 1992).

Recently hatched larvae were examined under a stereomicroscope in order to select larvae with the highest viability. Approximately four hundred larvae were removed to carry out the toxicity tests. Semi-static toxicological bioassays were carried out for 96 h; all test solutions have been replaced every 24 h, in order to calculate the acute lethal toxicity from different concentrations of Cu ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) and Zn (ZnCl_2). A series of six concentrations 0 (control), 80, 160, 320, 640 and $1280 \mu\text{g Cu}^{2+} \text{L}^{-1}$, and six concentrations 0 (control), 100, 200, 400, 800 and $1600 \mu\text{g Zn}^{2+} \text{L}^{-1}$, were assayed on the basis of previous preliminary assays. Three replicates of at least twenty animals were exposed to the above concentrations. The criterion for death was the absence of movement after the animals had been gently touched with a glass rod. Mortality was recorded every 24 h, after which dead zoeae were removed.

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).

RESULTS AND DISCUSSION

Environmental parameters

Temperature, salinity, dissolved oxygen and pH, were measured monthly *in situ* at a sampling station in the inner zone of the estuary during one and a half-year (May 1999–September 2000). Corresponding distribution of these parameters are presented in Figure 2.

The recorded annual cycle of water temperature has fluctuated between 4.9 and 22.9°C , followed a distinct unimodal pattern, irrespective of the semi-arid conditions. Salinity has also seasonally varied, with a range of 14.50 – 40.34 psu, and its recorded decreases were related to precipitation and freshwater run-off.

The annual average temperature and salinity were in agreement with historical values previously reported for this area (Piccolo and Perillo, 1990; Freije and Asteasuain, 1997).

Dissolved oxygen showed a seasonal distribution pattern, with the maximum in winter owing to low temperatures and production from the phytoplankton bloom (Gayoso, 1998), and variations laying between 5.4 and 11.6 mg L^{-1} . These results allow to support that this environment does not show oxygen deficiency, because values of “severe hypoxia” ($< 4 \text{ mg L}^{-1}$) or below “minimum acceptable level” ($< 2 \text{ mg L}^{-1}$) for coastal and estuarine waters (Windsor, 1985; Rabalais *et al.*, 1991) have never been recorded.

Table 1 Percentages of recovery in the analysis of reference materials to assess analytical quality (ranges)

Metal analyzed	Estuarine water	Pond sediment
Cu	91.1–95.3%	95.5–99.4%
Zn	92.9–96.5%	97.4–101.5%

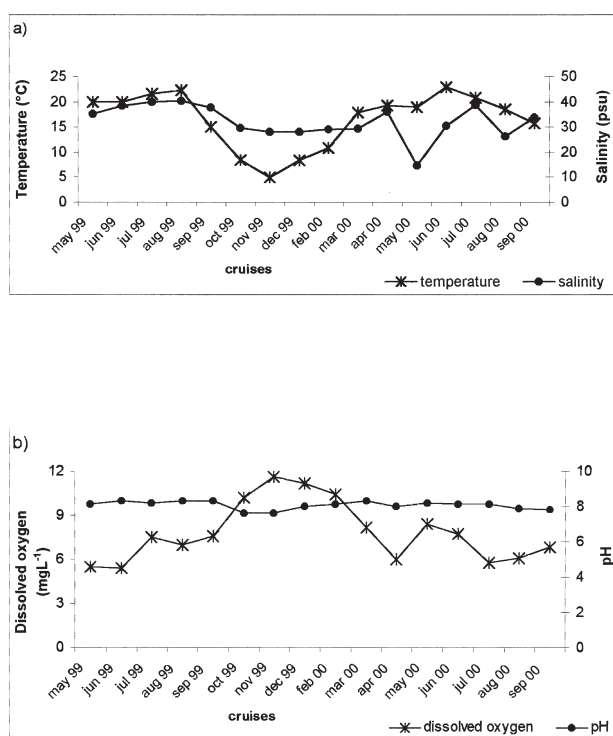


Figure 2 Distribution of oceanographic parameters: temperature and salinity (a), dissolved oxygen and pH (b).

On the other hand, the pH values (average 8.06 ± 0.23) presented a homogeneous pattern during the sampling period. It is important emphasize that a narrow range of pH variation, like as the recorded in this study, implicate that metals desorption and solubilization processes from particles do not take place (Bourg and Loch, 1995).

The annual averages of these parameters were in agreement with historical values for this area, and their distributions did not show any signs of abnormality.

Trace metals and organic matter

Table 2 presents the average concentrations of copper and zinc in surface sediments (both, total and fine-grain size fraction) and estuarine water (dissolved and particulate) of the inner zone of the Bahía Blanca Estuary.

Generally the concentrations of Cu and Zn in surface sediments were lower than those measured for different coastal areas, which were previously characterized as strongly contaminated by significant point sources of these two metals (*i.e.*, Dassenakis *et al.*, 1996; Lacerda *et al.*, 1998; Hayes *et al.*, 1998). In addition, these Cu and Zn values were similar than those previously reported by Marcovecchio *et al.* (1986) and Pucci (1988) for surface sediments from the same area.

Metal contents in the fine fraction ($< 63 \mu\text{m}$) of sediments were highest than total sediment fraction (at least in one order of magnitude). This was fully in

Table 2 Copper and zinc concentrations (average \pm standard deviation) in samples from the Bahía Blanca Estuary ($n = 16$).

Sample type	Units	Cu	Zn
<i>Sediments</i>			
Total fraction	$\mu\text{g g}^{-1}$ dry wt	16.01 ± 1.85	52.96 ± 5.67
$< 63 \mu\text{m}$ fraction	$\mu\text{g g}^{-1}$ dry wt	111.05 ± 42.78	105.66 ± 19.16
<i>Water</i>			
Dissolved	$\mu\text{g L}^{-1}$	4.65 ± 2.27	15.82 ± 6.14
SPM	$\mu\text{g g}^{-1}$ dry wt	35.61 ± 9.77	205.03 ± 70.68

agreement with previous studies, on account of the high content of metals in the finest fraction of sediments, which are due to biggest surface area and more places of union (Förstner and Wittmann, 1983; Salomons and Förstner, 1984). On the other hand, the average value of Cu and Zn in the fine-grain size fraction ($< 63 \mu\text{m}$) and SPM as reported here were slightly greater than those reported as the natural background for other coastal and estuarine regions, while were similar to those reported from industrialized areas (González, 1991; González and Ramírez, 1995).

Furthermore, dissolved Cu was similar than the quality criteria concentration, while Zn was lower than the norms for marine and estuarine waters ($4.8 \mu\text{g L}^{-1}$ for Cu and $90.0 \mu\text{g L}^{-1}$ for Zn, USEPA, 1999). This point is very important in the case of copper, where the concentration of dissolved fraction (the best bioavailable fraction) is near the limit of acceptable concentration for marine and estuarine waters.

Organic matter contents in surface sediments varied between 3.15 and 5.77 mg C g^{-1} , dry wt. Organic matter in estuarine sediments has been widely recognized as a significant trapper of pollutants, including trace metals (Coquery and Welbourn, 1995). Furthermore, the determined values of Cu and Zn showed a strong, highly significant correlation with the OM concentrations, which indicated the affinity of these elements for the organic fraction of the sediments: Cu $r = 0.7598$, $P < 0.01$, and Zn $r = 0.7529$, $P < 0.01$. These results were in agreement with previous studies, on account of these metals form strong complexes with humic substances in natural waters (Mantoura *et al.*, 1978; Nriagu, 1979).

The same relationship was found for chromium (Cr: $r = 0.7278$, $P < 0.01$; Ferrer *et al.*, 1999), while Cd and Pb showed non-significant correlation at the 99% confidence level, so both metals are less likely to form stable organic complexes (the stability constants for Cd or Pb – humic complexes are lower than those for other metals) (Koblitz *et al.*, 1997; Ferrer *et al.*, 2000).

The relationship between OM and Cu and Zn contents in the sediments indicates a smallest proportion of labile forms of these metals (Campbell *et al.*, 1988). This could participate in govern of their bioavailability within the system.

Toxicity bioassay

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 was abundant (spring and summer). In order to be a good fit of environmental conditions, an average of these parameters was used. This is on account of changes in environmental variable conditioning metal speciation, affecting their availability and toxicity (Adema *et al.*, 1980; Luoma, 1983; Förstner, 1993; Bourg & Loch, 1995).

Table 3 summarizes the LC₅₀ values as obtained for both metals at different times of exposure. In the controls, zoeae I mortality at 96 h was 8.8 % for copper, and 7.5 % for zinc.

Copper assay showed most emphasized slopes, which has indicated a rapid absorption of this metal and an immediate turn up of toxic effect respect to zinc. Although, at the end of exposure (96 h) zinc was found to have been significantly ($P < 0.05$) more toxic (1.3 times) than copper towards zoeae I. It is notable to point out that the same toxic trend (zinc > copper, 2.6 times) was registered for young crabs of *Chasmagnathus granulata* (Ferrer, 2001). Unlike this, Amín (1995) has established for zoeae I of king crab *Lithodes santolla*, at the end of exposure (96 h) that copper was found to be more toxic than zinc.

Comparisons of LC₅₀ values in relation to exposure time have shown significant differences between 24 and 48 h, 48 and 72 h and between 72 and 96 h in copper assay, even though there was an evident asymptotic trend. In the case of the zinc assay, the same comparison has shown no significant differences between 24 and 48 h, while significant differences between 48 and 72 h, 72 and 96 h have been observed, again with an evident asymptotic trend (Figure 3). Significant differences between the values of LC₅₀ at 72 and 96 h have made impossible to determine the lethal incipient threshold for these metals.

The LC₅₀-96 h value for copper reported in this study is significantly higher ($P < 0.05$) than those previously reported for *Chasmagnathus granulata* zoeae I at 30 psu (110.62 $\mu\text{g L}^{-1}$; range: 78.13–153.66 $\mu\text{g L}^{-1}$) (López Greco *et al.*, 2001).

The LC₅₀-96 h values determined for Cu and Zn were higher than the corresponding metal concentrations as determined within the Bahía Blanca environment; thus, it can be stated that larvae/zoeae I of the crab *Chasmagnathus granulata* were far to be under hazard due to Cu or Zn acute toxicity.

CONCLUSIONS

The annual average of oceanographic parameters has well agreed with historical values for the area, and has not presented any evidence of abnormality in its distribution. This could support the concept that no gross changes in the chemical species of trace metals have occurred during the time of the measurements.

Copper and zinc concentrations in surface sediments were similar than those previously reported from the same environment (twenty years ago), and were similar to those reported as natural background for similar regions. On the other hand, the average of copper and zinc in both fine fraction of sediments (< 63 μ) and SPM reported in this study, were similar than those reported from other industrialized areas. Analysis of the relationship between OM and Cu or Zn has appeared to suggest a smallest proportion of labile forms of these metals. This presumably could govern their bioavailability within the system.

Dissolved Cu was similar than the quality criteria concentration, and this was important considering that the dissolved fraction is the main input to aquatic ecosystems, while Zn was lower than the norms for marine and estuarine waters.

Zinc displayed a significantly higher acute toxicity than Copper (1.3 times) towards *Chasmagnathus granulata* zoeae I at the end of exposure (96 h).

Finally, it should be noted that both LC₅₀ values determined for Cu and Zn were higher than the corresponding metal concentrations measured in the Bahía Blanca environment. Nevertheless, the assessment of acute lethal toxicity is the first step to determine the chronic effects on estuarine organisms, which will be carefully evaluated in the future.

Table 3 LC₅₀ values, 95% confidence limits and probit line parameters for the first zoeae of the crab *Chasmagnathus granulata*.

Metal	Exposure (h)	LC ₅₀	95% confidence limits	Slope	Correlation coefficient
Cu ($\mu\text{g L}^{-1}$)	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 ($\mu\text{g L}^{-1}$)	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

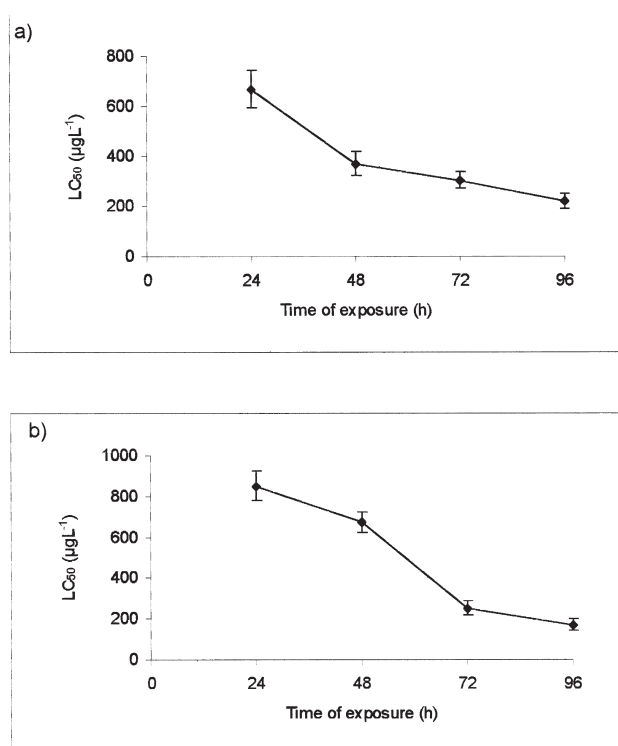


Figure 3 LC₅₀ values of Cu (a) and Zn (b) in relation to exposure time (average \pm 95% confidence limits).

ACKNOWLEDGEMENTS

This work was partially funded by CONICET (Consejo Nacional Investigaciones Científicas y Técnicas) and ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica), both from Argentina, through corresponding grants.

REFERENCES

- Adema, D., Hanstveit, A., Hooftman, R. & Vink, G. 1980. Determination of toxicity. In: *Degradability, Ecotoxicity and Bioaccumulation. The determination of the possible effects of chemicals and wastes on the aquatic environment*, Chap 5. Government Publishing Office, The Netherlands.
- Amín, O. 1995. Toxicidad para invertebrados marinos de algunos metales pesados detectados en la zona costera próxima a Ushuaia, Tierra del Fuego. Doctoral Thesis, Universidad de Buenos Aires, Argentina, 144 pp.
- APHA 1992. *Standard methods for the examination of water and wastewater*. 18th Ed, American Public Health Assoc., Am. Water Works Assoc. And Water Poll. Contr. Fed. (APHA, AWWA, WCPF), Washington DC, 1200 pp.
- Boschi, E. 1966. Preliminary note on the geographic distribution of the decapod crustaceans of the marine waters of Argentina (South West Atlantic Ocean). *Proc. Symp. Crustacea*, **1**: 449–456.
- Bourg, A. and Loch, J. 1995. Mobilization of heavy metals as affected by pH and redox conditions. In: *Biogeochemistry of Pollutants in soils and sediments*. Salomons W. and Stigliani, W. (eds.), Springer, pp. 87–102.
- Campbell, P., Lewis, A., Chapman, P., Crowder, A., Fletcher, W., Imber, B., Luoma, S., Stokes, P. & Winfrey, M. 1988. *Biologically available metals in sediments*. NRCC No. 27694, Ottawa, 126 pp.
- Coquery, M. and Welbourn, P. 1995. The relationship between metal concentration and organic matter in sediments and metal concentration in the aquatic macrophyte *Eriocaulon septangulare*. *Water Res.*, **29**(9): 2094–2102.
- Dalziel, J. and Baker, C. 1983. Métodos analíticos para medir la presencia de metales mediante espectrofotometría de absorción atómica. *FAO Doc. Tecn. Pesca*, **212**: 15–22.
- Dassenakis, M., Kloukiniotou, A. and Pavlidou, S. 1996. The influence of long existing pollution on trace metal levels in a small tidal mediterranean bay. *Mar. Pollut. Bull.*, **32**(3): 275–282.
- Ferrer, L. 2001. Estudio de diversos metales pesados en sedimentos del estuario de Bahía Blanca y sus efectos tóxicos sobre el cangrejo *Chasmagnathus granulata*. Doctoral Thesis, Universidad Nacional del Sur, Argentina, 212 pp.
- Ferrer, L., R. Asteasuain and Marcovecchio, J. 1999. Heavy metals distribution in estuarine sediments: a possible trapper role of organic matter. *4th Open Sci. Meeting – Land Ocean Interact. Coast. Zone (LOICZ-IGBP)*, LOICZ, Argentine, 113 pp.
- Ferrer, L., Contardi, E., Andrade, S., Asteasuain, R., Pucci, A. and Marcovecchio, J. 2000. Environmental cadmium and lead concentrations in the Bahía Blanca estuary (Argentina). Potential effects of Cd and Pb on crab larvae. *Oceanologia*, **42**(4): 493–504.
- Finney, D. 1971. Probit analysis: a statistical treatment of the sigmoid response curve. 3rd Ed., Cambridge Univ. Press., London, 702 pp.
- Förstner, U. 1993. Metal speciation – an overview. *Intern. J. Environ. Analyt. Chem.*, **51**: 5–27.
- Förstner, U. and Wittmann, G. (eds.) 1983. *Metal pollution in the aquatic environment*. Springer-Verlag, Heidelberg, 486 pp.
- Freije, R., Asteasuain, R., Schmidt, A. and Zavatti, J. 1981. Relación de la temperatura del agua con las condiciones hidrometeorológicas en la porción interna del estuario de Bahía Blanca. *Instituto Argentino Oceanografía, Contribución Científica 57*, 20 pp.
- Freije, R. and Asteasuain, R. 1997. La clorofila a en la zona interna del estuario de Bahía Blanca y su relación con la salinidad y temperatura del agua entre 1975–1997. *X Coloquio Argentino Oceanografía*, International Association Physical Science Ocean (IAPSO), Argentina, 46 pp.
- Gayoso, A. 1998. Long-term phytoplankton studies in the Bahía Blanca estuary, Argentina. *ICES J. Mar. Sci.*, **55**: 655–660.

- González, H. 1991. Heavy metal surveys in sediments of five Cuban Bays. *Biogeochemistry*, **14**: 113–128.
- González, H. and Ramírez, M. 1995. The effect of nickel mining and metallurgical activities on the distribution of heavy metals in Levisa Bay, Cuba. *J. Geochem. Explor.*, **52**: 183–192.
- Hayes, W., Anderson, I., Gaffoor, M. and Hurtado, J. 1998. Trace metals in oysters and sediments of Botany Bay, Sydney. *Sci. Total Environment*, **212**(1): 39–47.
- Koblitz J., Andreato, J., Tinoco, C., De Mello, V., Mayer, C. and David, S. 1997. Análise dos metais pesados, granulometria e matéria orgânica dos sedimentos recentes da lagoa Rodrigo de Freitas, Rio de Janeiro. *Proc. VII Congresso Latinoamericano sobre Ciencias do Mar (COLACMAR)*, Chap. 2, Martins Paiva A. & R. Weber (Eds.), Brazil, 50–52.
- Koirtiyohann, S. and Wen, J. 1973. *Critical study of the APDC-MIBK extraction system for atomic absorption*. *Analyt. Chem.*, **45**(12): 1986–1989.
- Lacerda, D. de, Huertas, R., Moresco, H., Carrasco, G., Viana, F., Lucas, R. and Pessi, M. 1998. Trace metal concentrations and geochemical partitioning in Arroyo Carrasco, Montevideo, Uruguay. *Geochem. Brasiliensis*, **12**: 63–74.
- López Greco, L., Sánchez, M., Nicoloso, G., Medesani, D. and Rodríguez, E. 2001. Toxicity of cadmium and copper on larval and juvenile stages of the estuarine crab *Chasmagnathus granulata* (Brachyura, Grapsidae). *Arch. Environmental Contamin. Toxicol.*, **41**: 333–338.
- Luoma, S. 1983. Bioavailability of trace metals to aquatic organisms – A review. *Sci. Total Environ.*, **28**: 1–22.
- Mantoura, R., Dickson, A. and Riley, J. 1978. The complexation of metals with humic materials in natural waters. *Estuar., Coast. Mar. Sci.*, **6**: 387–408.
- Marcovecchio J., Lara, R. and Gómez, E. 1986. Total mercury in marine sediments near a sewage outfall. Relation with organic matter. *Environ. Technol. Lett.*, **7**: 501–507.
- Marcovecchio, J., Moreno, V. and Perez, A. 1988. Determination of heavy metal concentrations in biota of Bahía Blanca, Argentina. *Sci. Total Environ.*, **75**: 181–190.
- Nriagu, J. 1979. Copper in the environment. *Part 1: Ecological cycling*. Wiley, New York.
- Perillo, G. and Piccolo, M. 1991. Tidal response in the Bahía Blanca estuary, Argentina. *J. Coast. Res.*, **7**(2): 437–449.
- Piccolo, M. and Perillo, G. 1990. Physical characteristics of the Bahía Blanca estuary (Argentina). *Estuar., Coast. Shelf Sci.*, **31**: 303–317.
- Pucci, A. 1988. Heavy metals in water and sediments of the Blanca Bay, Argentina. *Metals in coastal of Latin America*. pp. 9–15. Seeliger U., D. Lacerda de & Patchineelam (eds.), Springer-Verlag, Berlín.
- Rabalais, N., Turner, R., Wiseman, W. & Boesch, D. 1991. A brief summary of hypoxia on the northern Gulf of Mexico continental shelf: 1985–1988. *Geol. Soc. Special Pub. n° 58*. Geol. Society. London, 470 pp.
- Reish, D. & Oshida, P. 1987. *Manual of methods in aquatic environment research. Part 10: Short-term static bioassays*. *FAO Fish. Tech. Paper*, **247**: 62 pp.
- Salomons, W. 1995. Long-term strategies for handling contaminated sites and large-scale areas. In: *Biogeochemistry of pollutants in soils and sediments*. Salomons W. and Stigliani, W. (eds), Springer, 1–30.
- Salomons, W. 1996. Some remarks on processes affecting metal concentrations in estuaries. In: *Pollution Processes in Coastal Environments*. Marcovecchio, J. (ed.), UNMdP, Ch. I, 107–111.
- Salomons W. and Förstner, U. (eds.) 1984. *Metals in the Hydrocycle*. Springer-Verlag, Heidelberg, 346 pp.
- Strickland J. & Parsons, T. 1968. *A practical handbook of seawater analysis*. Fish. Research Board Canada, Canada, 432 pp.
- U.S.EPA, United States Environmental Protection Agency 1999. *National Recommended Water Quality Criteria – Correction*. Office of Water, EPA 822-Z-99-001, 25 pp.
- Weis, J., Cristini, A. & Rao, K. 1992. Effects of pollutants on molting and regeneration in Crustacea. *Am. Zool.*, **32**: 495–500.
- Windsor J. (1985). *Nationwide review of oxygen depletion and eutrophication in estuarine and coastal waters*. NOAA/OAD. Rockville, Maryland, 177 pp.

