

**Environmental cadmium
and lead concentrations
in the Bahía Blanca
Estuary (Argentina).
Potential toxic effects of
Cd and Pb
on crab larvae***

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Abstract

The Bahía Blanca Estuary includes a large tidal plain with an area close to 1150 km². Mud is predominant in its sediments, where a significant population of the crab *Chasmagnathus granulata* lives during the whole year. Moreover, there are important urban and industrial discharges into this environment.

Cd and Pb concentrations were determined in samples of water (for both dissolved and suspended particulate matter) and surface sediments (total and < 63 μm fractions). Organic matter was analysed in the sediments, while temperature, salinity, pH and dissolved oxygen were measured in the estuarine water.

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The metal concentrations determined in this study were: $1.47 \pm 1.08 \mu\text{g Cd g}^{-1}$ and $14.68 \pm 4.31 \mu\text{g Pb g}^{-1}$ in surface sediments; $2.21 \pm 1.33 \mu\text{g Cd g}^{-1}$ and $25.70 \pm 7.09 \mu\text{g Pb g}^{-1}$ in the $< 63 \mu\text{m}$ sediment fraction; $0.18 \pm 0.11 \mu\text{g Cd g}^{-1}$ and below the analytical detection limit of Pb in suspended particulate matter. Furthermore, dissolved Cd and Pb were lower than the norms for marine and estuarine waters ($2 \mu\text{g dm}^{-3}$ for Cd and $5 \mu\text{g dm}^{-3}$ for Pb, E.P.A.).

Simultaneously, the effects of Cd and Pb were studied on recently hatched larvae of *Ch. granulata*, through 96-hour semi-static acute assays. Viability was the criterion assessed in the assays. LC₅₀ (96 h) for Cd was $46.43 \mu\text{g dm}^{-3}$ ($36.92 - 56.34 \mu\text{g dm}^{-3}$), whilst that for Pb was 1.00 mg dm^{-3} ($0.79 - 1.23 \text{ mg dm}^{-3}$), which demonstrates that Cd is more toxic towards larvae. Finally, both LC₅₀ values determined for Cd and Pb were higher than the corresponding metal concentrations measured in the Bahía Blanca environment.

1. Introduction

Estuaries and coastal zones receive inputs of pollutants from point and non-point sources, most of which are eventually diluted at sea or deposited in estuarine sediments. Pollution by heavy metals deserves special attention because of their high toxicity and persistence in the aquatic environment, especially with respect to such ecosystems as seaports or other industrialised coastal areas that receive chronic inputs of these 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). In this sense, the present work constitutes the first integral study of trace metal distributions and their effects in the Bahía Blanca Estuary.

The Bahía Blanca Estuary is located between $38^{\circ}45' - 39^{\circ}40'S$, and $61^{\circ}45' - 62^{\circ}30'W$, on the south-eastern coast of Buenos Aires Province in Argentina. Elongated in shape and 80 km in length, it is characterised by the presence of various channels, fine sand and silt-clay sediments. Tidal ranges of 4 m and prevailing north-westerly winds create strong tidal currents facilitating water mixing, which in turn leads to a uniform vertical distribution of the main oceanographic parameters. At low tide this estuary encompasses a water surface area of 400 km^2 , while at high tide this area rises to nearly 1300 km^2 . Bahía Blanca is an estuarine environment with a very particular behaviour, which includes a relatively small input of inland water, and with a number of marginal areas seasonally functioning as hypersaline ones (Freije *et al.* 1981).

At the northern boundaries of the estuary various ports, cities (with a population of over 300 000 inhabitants) and industries are located, and several streams and other watercourses discharge into the area. Oil refineries and terminals, petrochemical industries, meat factories, leather plants, fish

factories, textile plants, silos and cereal mills discharge their effluents into the streams or directly into the estuary. The area is extensively used by fishing boats, oil tankers and cargo vessels and therefore requires regular dredging.

The crab *Chasmagnathus granulata* (Grapsidae) is distributed widely 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 role as a link in the trophic web, and all the stages of the crab's life cycle become a relevant food component for many fish species.

The present study includes information on the occurrence and distribution of cadmium and lead in surface sediments, the finest grain size fraction of sediments ($< 63 \mu\text{m}$), dissolved and suspended particulate matter, and the acute toxic effects of Cd and Pb on crab larvae.

2. Materials and methods

2.1. Environmental parameters and trace metals

The study area and the location of the sampling station at Puerto Cuatros, Bahía Blanca Estuary, Argentina, are shown in Fig. 1. The samples were taken once a month from a sampling station located in the inner zone of the estuary between May 1997 and June 1998. Temperature, salinity, pH and dissolved oxygen were measured *in situ* with a Horiba U-10 sensor.

Surface water samples (0.5–1 m) were collected with a Van Dorn oceanographic bottle on deck from a position in the bows, which minimised contamination from the ship. The water samples were passed through Millipore HA filters (0.45 μm pore size) for the determination of suspended and dissolved metals. Before use, the filters were soaked overnight in dilute HCl and stored in redistilled water. The filtered samples were acidified using 3 ml of HNO_3 (analytical quality). All fractions were frozen until analysis.

Surface sediments were obtained with a sledge and stored in a freezer at -20°C prior to their analysis in the laboratory. These samples were oven-dried at $45 \pm 5^\circ\text{C}$ to constant weight, subsamples were sifted, and the fraction smaller than 63 μm 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 Koirtyohann & Wen (1973). All extracted water samples were combined to ensure homogeneity and to provide a sufficient volume of matrix for the preparation of blanks and standards. Several

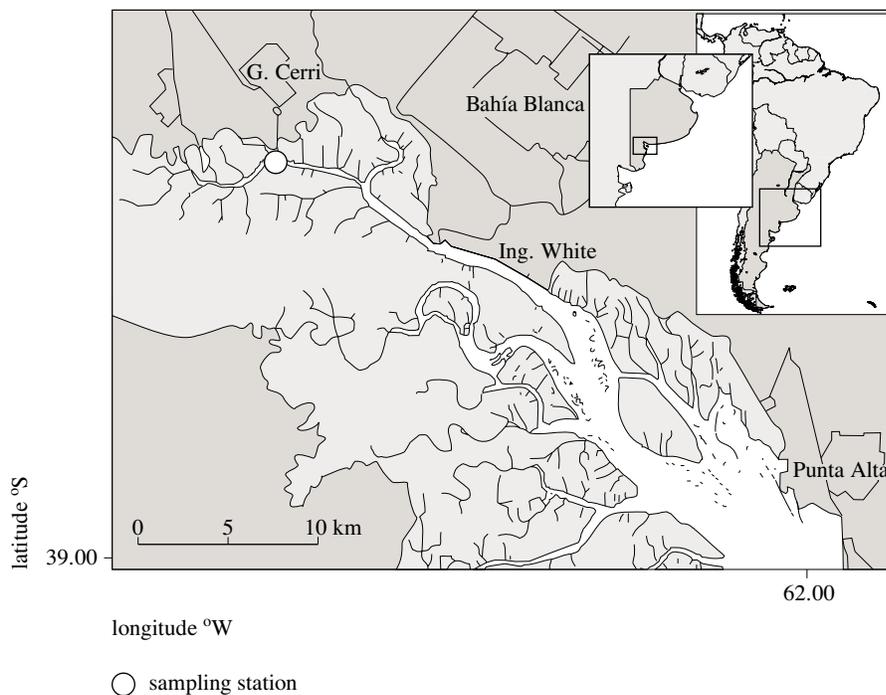


Fig. 1. Location of sampling station in the study area, Bahía Blanca Estuary (Argentina)

aliquots of this matrix were treated along with the samples to obtain analytical blanks. The standards were prepared by adding increasing amounts of Cd and Pb to the matrix.

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

Heavy metal concentrations (Cd and Pb) were determined with a Perkin–Elmer Model 2380 atomic absorption spectrophotometer with an air/acetylene flame. 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).

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

Metal analysed	Estuarine water	Pond sediment
Cd	89.4–94.2%	91.4–99.3%
Pb	92.1–97.5%	94.7–98.8%

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

2.2. Toxicity bioassay

Ovigerous female specimens of *Ch. granulata* were collected in the intertidal zone, Puerto Cuatrerros, Bahía Blanca Estuary, Argentina (Fig. 1) during the spring of 1998. Once in the laboratory, ovigerous females of similar maturity and carapace widths were selected. They were placed in glass containers, and acclimatised under environmentally controlled conditions selected for the bioassays that would be carried out later.

Naturally aged estuarine water was used after being passed through a 0.45 μm pore filter and active carbon to remove dissolved organic matter. It was then added to distilled water to obtain the water dilution used both for acclimatisation and assays, at the desired salinity of 35 ± 1 PSU and pH 8 ± 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 being removed.

Immediately after hatching, the first zoeae were exposed to Cd and Pb 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 & 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 being replaced every 24 h in order to calculate the acute lethal toxicity from different concentrations of Cd ($\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$) and Pb ($\text{Pb}(\text{NO}_3)_2$). A series of seven concentrations 0 (control), 20, 40, 80, 160, 320 and 640 $\mu\text{g Cd}^{2+} \text{ dm}^{-3}$, and eight concentrations 0 (control), 200, 400, 800, 1600, 3200, 6400 and

12 800 $\mu\text{g Pb}^{2+} \text{ dm}^{-3}$, 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 cadmium and lead that kills 50% of the exposed zoeae (Finney 1971).

3. Results and discussion

3.1. Environmental analysis

Temperature, salinity, pH and dissolved oxygen were measured monthly *in situ* at a sampling station in the inner zone of the estuary during one year (May 1997–June 1998). The distributions of these parameters are presented in Fig. 2.

Fluctuating between 4.7 and 23.5°C, the annual cycle of water temperature recorded followed a distinct unimodal pattern, irrespective of the semi-arid conditions.

Salinity also varies seasonally (22.08–41.20 PSU); decreases in salinity are related to precipitation and freshwater run-off.

The annual average temperature and salinity were in agreement with historical values for this area (Freije & Asteasuain 1997).

The pH values (average 7.9) presented a homogeneous pattern during the sampling period.

Dissolved oxygen showed a seasonal distribution pattern, with the maximum in winter owing to low temperatures and the production from the phytoplankton bloom (Gayoso 1998); variations lay between 6.0 and 11.7 mg dm^{-3} .

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

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

The average value of Cd in surface sediments, the fine-grain size fraction ($< 63 \mu\text{m}$) and SPM reported here are slightly greater than those reported as the natural background for similar regions (*i.e.* 0.4 $\mu\text{gCd g}^{-1}$ in the fine fraction of sediments by Palenque & Díaz 1994) and were similar to those reported from industrialised areas (*i.e.* 2.39 $\mu\text{gCd g}^{-1}$ in surface sediments by Ferreira *et al.* 1996).

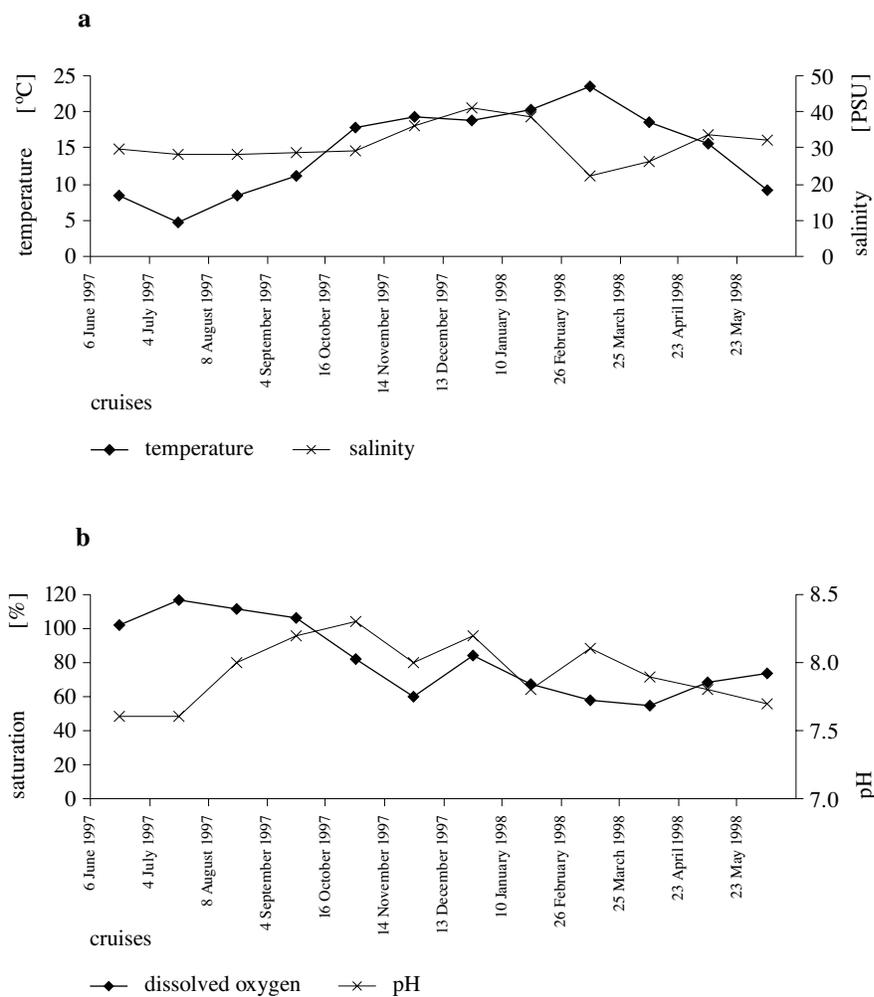


Fig. 2. Distribution of oceanographic parameters: temperature and salinity (a), dissolved oxygen (% saturation) and pH (b)

On the other hand, the average values of Pb in both fractions of sediments and SPM reported in this study are similar to those reported as natural background for similar regions (*i.e.* $25 \mu\text{gPb g}^{-1}$ in surface sediments by Lacerda *et al.* 1999).

In addition, the Cd values were higher than those previously reported by Pucci (1988) for surface sediments from the same area, which varied between 0.57 and $0.64 \mu\text{g g}^{-1}$ dry wt.

Furthermore, dissolved Cd and Pb were lower than those levels accepted for marine and estuarine waters ($2 \mu\text{g dm}^{-3}$ for Cd and $5 \mu\text{g dm}^{-3}$ for Pb, USEPA 1989).

Table 2. Cadmium and lead concentrations (average \pm standard deviation) in samples from the Bahía Blanca Estuary (n = 12)

Sample type	Units	Cd	Pb
sediments			
surface	$\mu\text{g g}^{-1}$ dry wt.	1.47 ± 1.08	14.68 ± 4.31
< 63 μm	$\mu\text{g g}^{-1}$ dry wt.	2.21 ± 1.33	25.70 ± 7.09
water			
dissolved	$\mu\text{g dm}^{-3}$	0.18 ± 0.11	N.D.
SPM	$\mu\text{g g}^{-1}$ dry wt.	1.39 ± 2.35	3.27 ± 0.98

N.D. = non-detectable.

Organic matter contents in surface sediments varied between 3.15 and 6.63 mgC g^{-1} dry wt. Organic matter in estuarine sediments has been widely recognised as a significant trapper of pollutants, trace metals included. Furthermore, there was also a strong, highly significant, linear correlation for the other metals studied – Cu: $r = 0.7599$, $p < 0.01$, $n = 70$; Zn: $r = 0.7529$, $p < 0.01$, $n = 70$; Cr: $r = 0.7278$, $p < 0.01$, $n = 70$ – (Ferrer *et al.* 1999), which indicated the affinity of these elements for the organic fraction of the sediments (Stephenson & Mackie 1988, Coquery & Welbourn 1995).

On the other hand, the determined values of Cd and Pb did not show the above relationship, with correlation coefficients much lower than the ones mentioned. Both metals showed non-significant correlation coefficients at the 99.9% confidence level, with $r = 0.1760$, $n = 70$ for Cd, and $r = 0.3061$, $n = 70$ for Pb. In this way, it was clearly established that the stability constants for Cd or Pb-humic complexes are lower than those for other metals. So both Cd and Pb are less likely to form stable organic complexes, although the extent of Cd or Pb interaction with humic substances also depends on the abundance and composition of competing ligands (Campbell *et al.* 1988). This kind of relationship has occasionally been reported for other estuarine environments (*i.e.* Mantoura *et al.* 1978, Koblitz *et al.* 1997).

The lack of a relationship between OM and the Cd and Pb contents in the sediments indicates an elevated proportion of labile forms of these metals. This could govern their bioavailability in the system.

3.2. Toxicity bioassay

The experimental conditions of the toxicity test were similar to those found in the environment (temperature, salinity and pH). Table 3 summarises the LC_{50} values obtained for both metals at different times of

exposure. In the controls, zoeae I mortality at 96 h was 7% for cadmium, and 8% for lead.

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
Cd [μg dm ⁻³]	24	370.6	(319–434)	4.99	0.60
	48	142.0	(123–162)	5.34	0.80
	72	77.6	(66–89)	5.08	0.78
	96	46.4	(37–56)	2.92	0.86
Pb [mg dm ⁻³]	24	6.02	(4.71–8.15)	2.08	0.63
	48	3.31	(2.81–3.85)	4.99	0.61
	72	2.65	(2.20–3.13)	4.34	0.80
	96	1.00	(0.79–1.23)	2.85	0.85

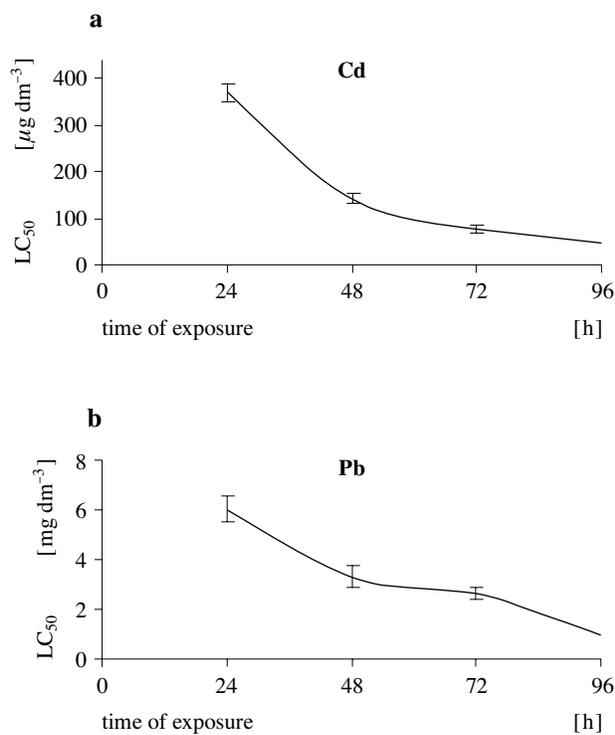


Fig. 3. LC₅₀ values of Cd (a) and Pb (b) in relation to exposure time (average ± standard deviation)

At the end of exposure (96 h) cadmium was found to have been significantly ($p < 0.05$) more toxic (21 times) than lead towards zoeae I.

Comparison of LC₅₀ values in relation to exposure time showed significant differences between 24 and 48 h, and between 72 and 96 h, while no significant differences between 48 and 72 h were observed in the lead assay, even though there was an evident asymptotic trend. In the case of the cadmium assay, the same comparison showed significant differences between 24 and 48 h, 48 and 72 h, 72 and 96 h; here again there was an evident asymptotic trend (Fig. 3).

The 96 h LC₅₀ values for cadmium reported in this study are similar to those reported in *Ch. granulata* zoeae I at 12 PSU ($64.9 \mu\text{g dm}^{-3}$ (32.1–92.3), Rodríguez *et al.* 1998). However, our results showed a higher toxicity for cadmium than those reported for the other crab and were similar to those reported for lead (*i.e.* king crab *Lithodes santolla* zoeae I yielded 96 h – LC₅₀ 2.07 mg dm^{-3} for Cd and 1.66 mg dm^{-3} for Pb, Amín *et al.* 1998).

The LC₅₀ values determined for Cd and Pb were higher than the corresponding metal concentrations measured in the Bahía Blanca environment; thus it can be stated that crab larvae – zoeae I of *Ch. granulata* – were far from being a hazard due to acute toxicity.

4. Concluding comments

The annual average of oceanographic parameters agreed well with historical values for the area, and did not present any evidence of abnormality in their distributions. This could support the notion that no gross change in the chemical species of trace metals occurred during the time of the measurements.

Cadmium concentrations in sediments and SPM are higher than those previously reported from the same environment, and are similar to those reported from other industrialised areas. On the other hand, the average value of Pb in both fractions of sediments and SPM reported in this study are similar to those reported as natural background for similar regions. Analysis of the results appears to suggest an elevated proportion of labile forms of these metals. This presumably could govern their bioavailability within the system.

Dissolved cadmium and lead were lower than those levels assumed for marine and estuarine waters, and this point is important considering that the dissolved fraction is the main input to aquatic ecosystems.

Cadmium displayed a significantly higher acute toxicity than lead (21 times) towards *Ch. granulata* zoeae I at the end of exposure (96 h).

Finally, it should be noted that both LC₅₀ values determined for Cd and Pb were higher than the corresponding metal concentrations measured in

the Bahía Blanca environment. Nevertheless, the assessment of the acute lethal toxicity is the first step to determine the chronic effects on estuarine organisms, which will be evaluated in the future.

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