Cadmium concentrations in the Bahía Blanca Estuary (Argentina). Potential effects of dissolved cadmium on the diatom *Thalassiosira curviseriata*[*]

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Abstract

Concentrations of dissolved and particulate cadmium were measured in the water of the Bahía Blanca Estuary, one of the most highly industrialised regions in Argentina. Simultaneously, Cd contents were also determined in samples of phytoplankton. Thus, the estuary was characterised from the viewpoint of Cd occurrence. Additionally, a microscale environment was selected within the estuary in order to carry out an ecotoxicological study. Thus, environmental parameters (*i.e.* temperature, salinity, nutrients, chlorophyll *a*) were measured weekly at Puerto Cuatreros (in the inner estuary) during winter, when the phytoplankton bloom occurs; during the other seasons sampling was done twice a week. In addition, cells of the diatom *Thalassiosira curviseriata* – the dominant species

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in the phytoplankton bloom – were isolated and cultivated in the laboratory. The monoclonal cultures obtained were exposed to increasing concentrations of Cd under controlled environmental conditions and the growth rate inhibition was measured. As a preliminary conclusion it was found that Cd levels in the Bahía Blanca Estuary are significantly lower than the EC50 value for *T. curviseriata*.

1. Introduction

The behaviour of trace metals in aquatic systems is highly complex due to the large number of possible interactions with ill-defined dissolved and particulate components and non-equilibrium conditions (Salomons & Förstner 1984).

Cadmium is toxic, even at very low concentrations and has hazardous effects on humans (Clarkson 1986). Several of the many inorganic cadmium compounds, *e.g.* chloride and sulphate, are quite soluble in water. This metal is easily complexed with some organic compounds, *e.g.* thiocarbamates, and this property is the basis of several analytical methods. There are some synthetic organometallic compounds, but these have not been found in the general environment since they are rapidly decomposed (Förstner & Wittman 1983).

Temperate bays and estuaries are characterised by great variability. Phytoplankton populations are highly dynamic and recurrent seasonal blooms usually occur (Smayda 1983, Sournia 1987).

Nearly 80 km long, with depths ranging from 15 to 20 m, the Bahía Blanca Estuary is situated on the Atlantic coast at 38°45′S, 62°22′W (south-eastern Buenos Aires Province, Argentina) and is a well mixed body of water. Small streams enter it throughout its length, discharging a total volume of nearly 5 m^3 s^{-1}. At low tide this estuary covers an area of 400 km^2, but at high tide the total area rises to nearly 1300 km^2. This area receives a large quantity of raw sewage from the nearly 300 000 inhabitants of Bahía Blanca city, not to mention untreated industrial effluent and run-off water from almost 4200 km^2 of cultivated land.

Diatoms dominate the phytoplankton, with a major bloom during winter and early spring (Gayoso 1989, 1998); *Thalassiosira* species are its most important components. The succession pattern of the *Thalassiosira* species since 1980 has been described by Gayoso (1983); *Thalassiosira curviseriata* is the dominant phytoplankton species in this environment (Gayoso 1989, 1998, Popovich & Gayoso, 1999).

The levels and distribution of cadmium in the seawater of the Bahía Blanca Estuary were studied in order to obtain reference values for the future detection of changes caused by contamination. An increase in the contamination of these waters is to be expected because water exchange in the upper part of the estuary is very prolonged; the study area is thus a potential depositional area for contaminants, including cadmium (Pucci 1988).

An algal toxicity test was conducted to evaluate the toxic effects of increasing cadmium concentrations on the growth of *T. curviseriata.*

2. Materials and methods

2.1. Sampling and analysis

The study area and the location of sampling stations are shown in Fig. 1. Samples of seawater from six sampling stations along the Bahía Blanca Estuary were collected each month so that the spatial distribution of dissolved and suspended fractions of cadmium in the surface water could be examined.

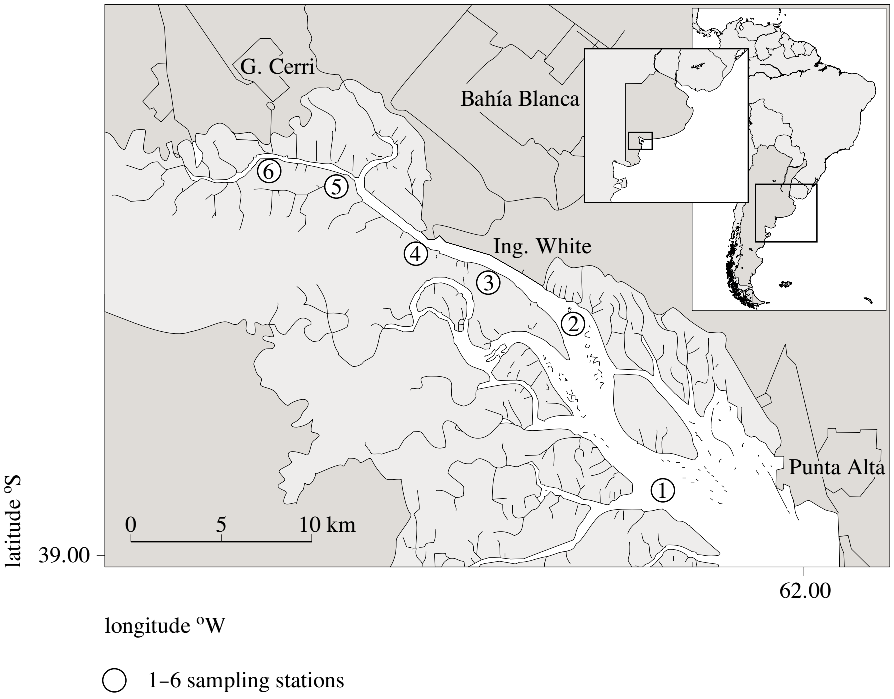

Fig. 1. Location of sampling stations (1–6) in the study area

The inner zone of the estuary has an average depth of 7 m and tidal currents and winds dominate the circulation. The turbidity is high, and the salinity is subject to fluctuations dependent on tide, rainfall, evaporation and runoff. In order to understand the main hydrographic characteristics of this inner area, a monitoring programme was established which included weekly sampling of estuarine water from 1997 to 1999, at high tide, at station 6. During the cruises, corresponding values of temperature, salinity, pH, turbidity and dissolved oxygen were measured *in situ*. Further samples were collected for the determination of concentrations of cadmium and inorganic micronutrients (NO_2^-, NO_3^-, PO_4^{3-}, SiO_2^{2-}), the quantification of suspended particulate matter (SPM) and the concentration of chlorophyll *a* in SPM.

2.2. Analytical methodology

Nutrients

Immediately on return to the laboratory, water samples were passed through a GF/C Whatman filter. They were subsequently stored in the dark at 4°C to minimise deterioration. Nutrient concentrations were determined with a four-channel Technicon® Auto Analyzer TMII, and the methods described by Treguer & Le Corre (1975), Grasshoff *et al.* (1983), Eberlein & Kattner (1987), and Technicon® (1973) were followed to quantify nitrate, nitrite, phosphate and silicate respectively.

Chlorophyll *a*

Water samples were kept cool and in the dark, and were filtered as soon as possible after collection. Chlorophyll *a* was extracted from the filters with 90% acetone, and the concentration of chlorophyll *a* in the extract was measured in a Beckman DU–2 spectrophotometer. The amount of chlorophyll *a* was calculated from the spectra, after Lorenzen (1967) formula.

Cadmium

Seawater samples were collected with a Van Dorn oceanographic sampling bottle and were passed through Millipore HA filters (0.45 μm pore size). These filtered seawater samples were acidified with 2 cm^3 of concentrated HNO_3 (analytical quality) per 1 dm^3 of sample and stored at 4°C prior to the determination of dissolved cadmium. The filters with samples of suspended particulate matter were kept in a freezer at −20°C until the determination of particulate cadmium. Before use, the filters were soaked overnight in dilute HCl, then dried at 75°C to constant weight.

Net samples were collected using a 40 μm mesh phytoplankton net and were passed through Millipore HA filters (0.45 μm pore size). The filters were kept in a freezer at −20°C until the determination of cadmium concentration.

The dissolved cadmium was simultaneously extracted from the filtered water by chelation with ammonium pyrrolidine-dithiocarbamate (APDC) followed by methyl iso-butyl ketone (MIBK) extraction according to the method described by Koirtyohann & Wen (1973). All extracted seawater samples were combined to ensure homogeneity and to provide a sufficient volume of matrix for the preparation of blanks and standards. Several aliquots of the matrix were treated along with the samples to obtain analytical blanks. The standards were prepared by adding increasing amounts of cadmium to the matrix.

The suspended particulate matter and the filters with phytoplankton were digested in a 3:1 mixture of concentrated nitric and perchloric acids, according to the method of Dalziel & Baker (1983), modified by Marcovecchio *et al.* (1988). A Perkin-Elmer Model 2380 atomic absorption spectrophotometer with an air/acetylene flame was used to determine cadmium concentrations. The relevant blanks and calibration curves were made up with analytical grade reagents. Reference materials provided by the National Institute for Environmental Studies (NIES) from Tsukuba (Japan) were used to check the efficiency and accuracy of the methods. The results given in Table 1 show that the recovery efficiency was 91.8% for dissolved cadmium and 95.3% for cadmium in the suspended sediments.

Table 1. Mean ± 1 standard deviation of cadmium concentrations in certified reference materials: estuarine water and pond sediment (R. M. No. 2, NIES)

Sample	Dissolved Cd [μg dm^{-3}]	Cd in suspended sediments [μg g^{-1} dry wt.]
measured	0.79 ± 0.044	1.19 ± 0.14
certified	0.86 ± 0.078	1.25 ± 0.11

Toxicity test

A clonal culture of *T. curviseriata* was obtained from the inner zone of the Bahía Blanca Estuary, during the phytoplankton bloom (winter–early spring).

The culture was kept in f/2 medium (Guillard 1975) using the requisite sterile technique. The media utilised in the experiments were prepared from natural aged seawater taken from the Bahía Blanca Estuary. The water (with a cadmium concentration below the detection limits of the method) was first passed through 0.45 μm Millipore HA filters and then through a column of active charcoal to remove dissolved organic matter.

An algal toxicity test of 96 h exposure was conducted. The stock culture was maintained in f/2 medium under controlled conditions (as in the natural environment) in a culture chamber at 12 ± 1°C and pH 8.1 ± 0.1 in 500 cm^3 borosilicate bottles containing 100 cm^3 of 30 PSU seawater medium. Cells were grown under cool-white fluorescent lighting (OSRAM L 18W/20) at an intensity of 55 ± 5 μmol photons m^{-2} s^{-1} on a 12:12 L/D cycle. The inoculum was prepared from the stock culture in exponential growth and the initial cell density was 6×10^3 cells cm^{-3} in treated and control flasks. The test solutions consisted of seawater medium with the addition of different concentrations of cadmium (0.033, 0.1, 0.3, 0.9, 2.7, 8.1 and 24.3 mg Cd dm^{-3}). Control and treated cultures were grown under the same conditions of temperature and photoperiod as the stock culture and were done in triplicate.

The cells were counted daily using a Sedgwick-Rafter chamber and the growth rate estimated.

A one-way statistical analysis of variance ($p < 0.01$) in conjunction with Dunnett's test was performed to assess the observed differences between control and cultures exposed to different concentrations of cadmium. The EPA Program Version 1.5 with the Spearman-Karber method was employed to calculate the EC50 value at 96 h and 95% confidence intervals. Dunnett's test (archsen transformation) was used to calculate the NOEC (no observed effect concentration), defined as the highest concentration of a toxicant in which the values of the parameters in question are not statistically significantly different from the control, and the LOEC (low observed effect concentration), defined as the lowest concentration of a toxicant in which the values for the relevant parameters are statistically significantly different from the control. CV (chronic value), defined as the square root of the product of NOEC and LOEC, was calculated according to U. S. Environmental Protection Agency (USEPA 1989) and American Public Health Association (APHA 1992) guidelines.

3. Results and discussion

Oceanographic parameters

Temperature, salinity, pH, turbidity and dissolved oxygen were measured *in situ* twice weekly at station 6 (internal) for three years (1997, 1998 and 1999). The distributions of these parameters are presented in Fig. 2.

The annual averages of temperature and salinity are in agreement with the historical values for this area (Freije & Asteasuain 1997). The annual

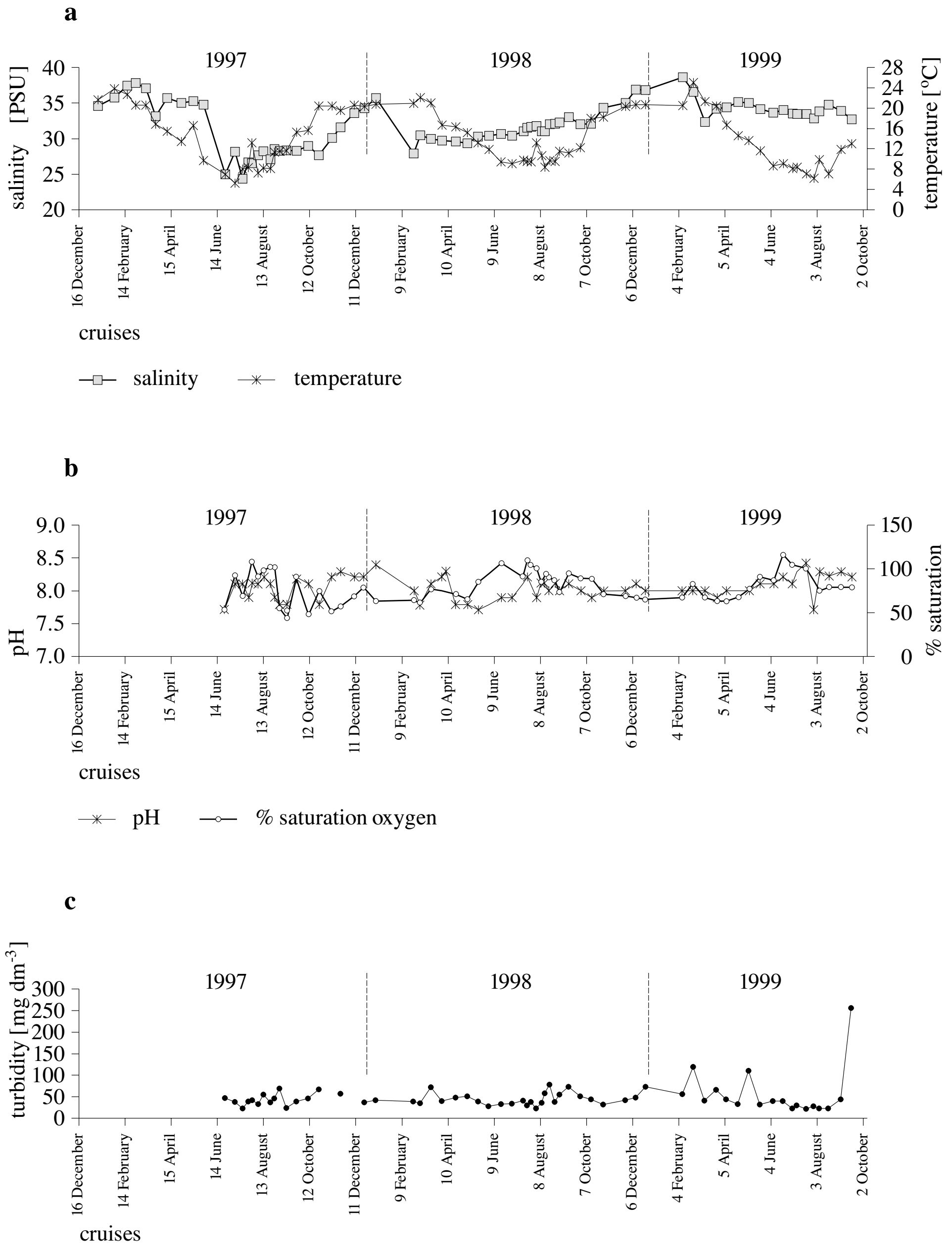

Fig. 2. Distribution of oceanographic parameters: salinity and temperature (a), pH and dissolved oxygen (b), turbidity (c)

cycle of the water temperature recorded in the inner zone of the estuary follows a distinct unimodal pattern, corresponding to semi-arid conditions, with oscillations between 5.6 and 24.5°C.

Salinity oscillations also occur seasonally; decreases in salinity are generally related to precipitation and freshwater run-off, and range between 20 and 38 PSU.

The pH values presented a homogeneous pattern during the sampling period, with an average of 8.1.

Dissolved oxygen shows a seasonal distribution pattern, with a maximum in winter due to low temperatures (which facilitates solubility of the gas) and a greater production due to the phytoplanktonic bloom, with variations between 4.5 and 11.5 mg dm^{-3}.

The turbidity pattern is undefined; it is influenced by wind intensity, tidal currents and the turbulence of the water column, and values have ranged between 20 and 75 mg dm^{-3}.

Nutrients

Analyses of samples collected from the inner zone of the Bahía Blanca Estuary indicated at least three types of nutrient behaviour: biological removal, biological regeneration and interactions caused by inorganic or abiotic processes. Nutrient distributions (nitrates, nitrites, phosphates and silicates) were highly variable in time (Fig. 3).

During the study period, nutrient concentrations were cyclic: nutrient maxima were recorded at the end of each summer (March), when chlorophyll *a* values were low. Nitrates rose to 27.41, 17.76 and 25.64 μmol N dm^{-3}; nitrites rose to 8.89, 5.11 and 9.45 μmol N dm^{-3}; phosphates rose to 3.5, 2.43 and 3.27 μmol P dm^{-3} and silicates also reached high values – 130, 115 and 131 μmol Si dm^{-3} – in 1997, 1998 and 1999 respectively. The lowest concentrations of all the nutrients, were recorded in 1998.

On the other hand, the results obtained during each winter season (July–August) pointed to nutrient removal from the estuary. They showed a sharp decrease in nitrates, nitrites and phosphates that was synchronous with the bloom. There were no differences in minimum nutrient values during the years in question: nitrate – 0.24 μmol N dm^{-3}, nitrite – 0.10 μmol N dm^{-3} and phosphate – 0.4 μmol P dm^{-3}.

Silicate concentrations dropped to 34.18, 66.39 and 58.12 μmol Si dm^{-3} in 1997, 1998 and 1999 respectively. This decrease in concentrations was due to consumption by diatoms – the principal component group of the bloom (Gayoso & Popovich 1992, Popovich & Gayoso 1999).

The data obtained during each spring (October–November) indicated a slow increase in nutrients by regeneration. This may be explained by zooplankton excretion from copepods and other zooplankton groups whose growth rate rises in spring (Hoffmeyer 1994).

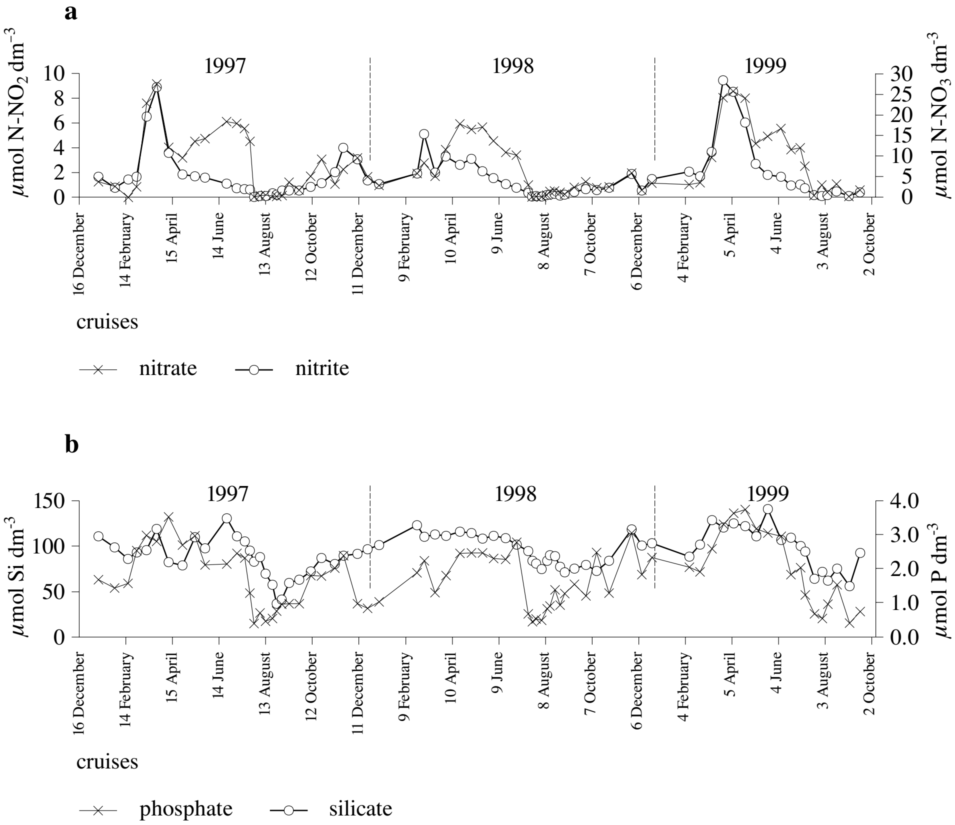

Fig. 3. Distribution of nutrients: nitrate and nitrite (a), phosphate and silicate (b)

Chlorophyll *a*

Fig. 4 illustrates the changes in chlorophyll *a*. Maximum values were recorded in each winter season (up to 39.64, 32.37 and 21.09 mg chl *a* m^{-3}

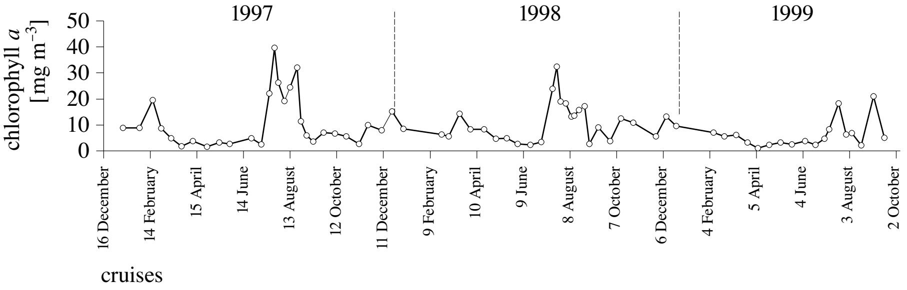

Fig. 4. Distribution of chlorophyll *a*

in 1997, 1998 and 1999 respectively), followed by an appreciable reduction during spring (2–10 mg chl *a* m^{-3}). Data analysis of chlorophyll *a* in the water showed that the highest phytoplankton densities occur in the Bahía Blanca Estuary in winter, when the phytoplankton bloom occurs (Gayoso 1981, 1983, 1988, Gayoso & Popovich 1992, Popovich & Gayoso 1999).

The distributions of both chlorophyll *a* and dissolved nutrients are further indications that a major bloom occurs in winter.

Cadmium

The results of dissolved and particulate cadmium measured along the main channel of the Bahía Blanca Estuary are shown in Fig. 5.

Dissolved cadmium values varied from undetectable levels (< 0.05 μg dm^{-3}) to 1 μg dm^{-3}, but the highest values were obtained during March–May 1998, reaching a maximum value of about 5 μg dm^{-3} at sampling station 4 (Fig. 5a). These concentrations were similar to those reported by Villa & Pucci (1987) in the same area.

Particulate Cd concentrations (Fig. 5b) ranged from undetectable levels (< 0.2 μg g^{-1}) to 10 μg g^{-1} dry wt. As in the case of dissolved Cd, the highest value (40 μg g^{-1}) was obtained at station 4 in May–June 1998.

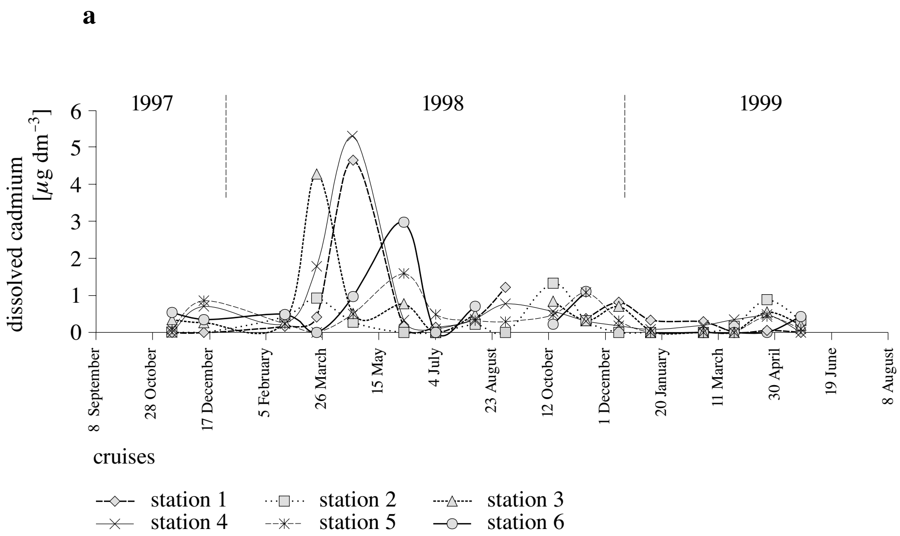

Fig. 5. Distribution of cadmium concentrations in the main channel: dissolved cadmium (a), particulate cadmium (b)

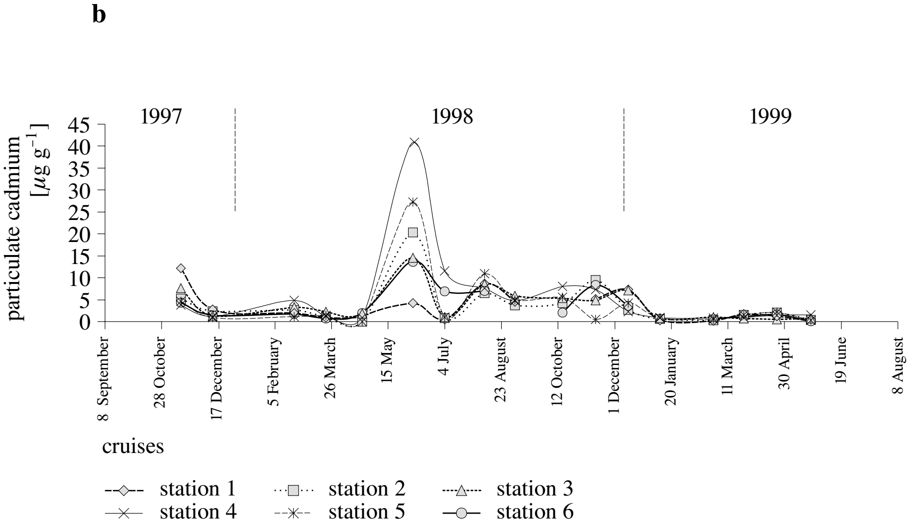

Fig. 5. (continued)

The cadmium distribution in water samples from the Bahía Blanca Estuary seems to be influenced by local effluents and the water circulation pattern of this area, and show little evidence of any abnormality in the distribution of this trace metal.

Cadmium was measured in dissolved, particulate and phytoplankton net sample fractions biweekly at station 6 (the inner zone of the Bahía Blanca Estuary) during three years (1997, 1998 and 1999).

There was no clearly-defined distribution pattern of the dissolved cadmium concentrations. Nevertheless, these are significant data, considering the lability of this dissolved element within the estuary. Dissolved cadmium values varied from below the detection limit ($< 0.05\ \mu\text{g dm}^{-3}$) to 0.4 $\mu\text{g dm}^{-3}$, while particulate Cd ranged from below the detection limit ($< 0.2\ \mu\text{g g}^{-1}$) to 8 $\mu\text{g g}^{-1}$ dry wt. Cadmium in the phytoplankton net samples varied from below the detection limit to 3 $\mu\text{g g}^{-1}$ dry wt. (Fig. 6).

None of these concentrations displayed a definite temporal distribution pattern. Cadmium levels in the phytoplankton net samples were of the magnitude as those detected in suspended particulate matter.

The plot of dissolved cadmium concentrations versus salinity is shown in Fig. 7. The cadmium-salinity correlation was worse ($r = 0.211$; $p > 0.05$; $n = 90$), indicating non-conservative behaviour. This may be due to the influence of coastal biogeochemical processes on cadmium distribution. The behaviour of dissolved cadmium observed during this study agree well with previous findings by Kremling & Hydes (1988) and Apte & Day (1998).

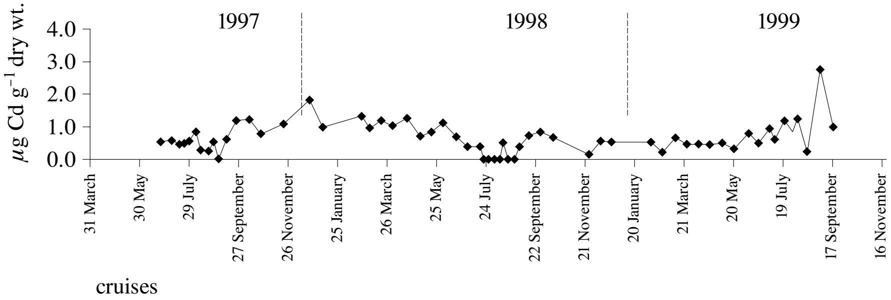

Fig. 6. Distribution of cadmium concentrations in samples of phytoplankton net at station 6

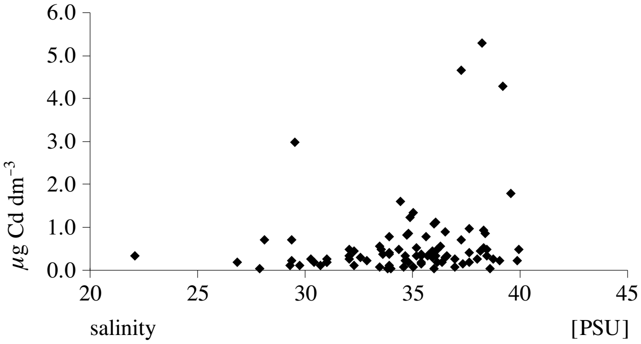

Fig. 7. Plot of concentration of dissolved cadmium versus salinity

Toxicity test

The experimental conditions of the toxicity test were similar to those found in the environment (temperature, salinity and pH). The growth rate inhibition of *T. curviseriata* was measured (Fig. 8). There were clear differences between the control curve and most of the curves obtained from the experiments with added cadmium. No significant inhibition was found between the control and cultures exposed to 0.033, 0.1, 0.3, 0.9 and 2.7 mg Cd dm^{-3} for 48 h, neither was there any significant growth inhibition with respect to the culture exposed to 0.033 mg Cd dm^{-3} throughout the test. However, a significant decrease in growth as compared to the control was recorded in the cultures exposed to concentrations higher than 0.1 mg Cd dm^{-3} for 96 h. At this time, there were significant differences in the growth of the exposed cultures except among those exposed to 0.3 and 0.9 mg Cd dm^{-3}.

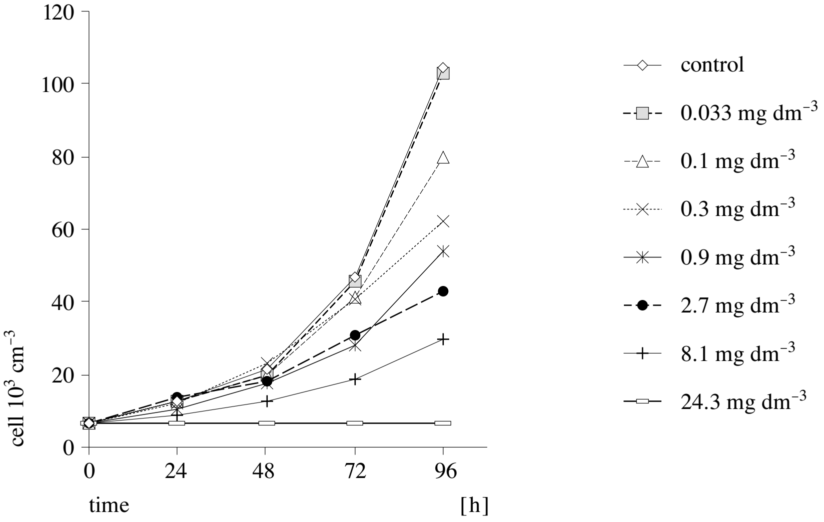

Fig. 8. Growth rate inhibition of *Thalassiosira curviseriata* exposed to cadmium

The 96-h toxicity endpoints of cadmium – EC_{50}, LOEC, NOEC, VC – with respect to *T. curviseriata* under our experimental conditions were obtained. The 96-h EC_{50} was 3.47 mg Cd dm^{-3} and the 95% confidence interval was 2.83–4.27 mg Cd dm^{-3}. NOEC was 0.033 mg Cd dm^{-3}, and LOEC was 0.1 mg Cd dm^{-3}. The CV (chronic value) of cadmium for *T. curviseriata* was 0.057 mg Cd dm^{-3}; this value is more useful for assessing strict regulatons of permissible limits than the interval defined by LOEC–NOEC values.

In view of the fact that the chronic value (57 μg Cd dm^{-3}) was significantly ($p < 0.01$) higher than the environmental concentrations determined (maximum dissolved cadmium: 5 μg Cd dm^{-3}), *T. curviseriata* appeared to be far from a potential risk.

4. Concluding comments

A marked phytoplankton bloom occurs in the inner zone of the Bahía Blanca Estuary during the winter, when chlorophyll *a* levels reach a maximum. On the other hand, the results also indicated nutrient removal from the estuary. They showed a sharp decrease in nitrate and phosphate that was synchronous with the bloom. The spring data indicated a slow increase in nutrients by regeneration.

The most important feature of the bloom is the predominance of the genus *Thalassiosira*, the most abundant species being *T. curviseriata* (up to 40%).

As a preliminary conclusion it can be stated that Cd levels in the Bahía Blanca Estuary are significantly lower than the endpoints calculated for *T. curviseriata* from this environment.

Taking into account the CV and the environmental concentrations of cadmium, *T. curviseriata* seems to be a long way from being a potential hazard.

Nevertheless, other processes should be taken into account in order to evaluate the environmental risk of this trace metal (*i.e.* interaction with other elements, speciation changes, *etc.*), and toxicity tests on different organisms in the trophic chain should be carried out in future.

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