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Water Toxicity Assessment of the Ceará River Estuary (Brazil)

J. NILIN,¹ C. B. DE CASTRO,¹ M. F. PIMENTEL,¹ W. FRANKLIN JÚNIOR,¹
R. F. G. DE MATOS,¹ T. M. C. LOTUFO² & L. V. COSTA-LOTUFO^{1*}

¹Laboratório de Ecotoxicologia Marinha, Instituto de Ciências do Mar (Labomar),
Universidade Federal do Ceará, Fortaleza, Ceará, Brasil

²Laboratório de Ecologia Animal, Instituto de Ciências do Mar (Labomar),
Universidade Federal do Ceará, Fortaleza, Ceará, Brasil

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ABSTRACT

The Ceará River Basin is one of the three major water sources of the Metropolitan Region of Fortaleza, Ceará State, Brazil. As an urban river, it receives domestic and industrial effluents, but the impacts of such discharges were not properly investigated. This research focused on the evaluation of the quality of the Ceará river estuary using the sea urchin *Lytechinus variegatus* embryo-larval toxicity test. Four sampling stations were distributed along the estuary, and the water samples were collected monthly from March 2004 through February 2005. The physical-chemical analyses showed a gradient of salinity and pH from the inner part of the estuary towards the river mouth, while dissolved oxygen (DO) and temperature values were very similar among the stations. Toxicity occurred in three of four surveyed stations, especially at S1 and S2, where the effects were more evident. The higher toxicity at these stations may be related to the proximity to the confluence between Ceará River and Maranguapinho River, which carries effluents from Maracanaú's Industrial District.

Keywords: *Lytechinus variegatus*, toxicity test, Ceará River, water quality.

RESUMO

Avaliação da toxicidade da água do estuário do rio Ceará (Brasil)

A bacia do rio Ceará compreende um dos três principais corpos hídricos da região metropolitana de Fortaleza, Estado do Ceará, Brasil. O rio Ceará e seus afluentes recebem efluentes domésticos e industriais cujos impactos não foram adequadamente investigados. Esta pesquisa enfocou a avaliação da qualidade das águas do estuário do rio Ceará, usando o teste de toxicidade embrio-larval do ouriço-do-mar *Lytechinus variegatus*. Quatro estações de coleta foram estabelecidas ao longo do estuário, e amostras de água foram coletadas mensalmente de março de 2004 até fevereiro de 2005. Na análise físico-química foi evidenciado um gradiente de salinidade e pH da parte interna do estuário em direção à foz, enquanto o oxigênio dissolvido e a temperatura foram similares para todas as estações. A análise das amostras mostrou toxicidade em três das quatro estações examinadas, especialmente em S1 e em S2. A maior toxicidade nestas estações provavelmente esteve relacionada à proximidade da confluência entre o rio Ceará e o rio Maranguapinho, que recebe efluentes do distrito industrial de Maracanaú.

Palavras-chave: *Lytechinus variegatus*, teste de toxicidade, rio Ceará, qualidade das águas.

*Corresponding author: Letícia Veras Costa-Lotufo, e-mail: lvcosta@secrel.com.br.

INTRODUCTION

Estuaries are very important ecosystems, which present high biological productivity and economical value, associated to intensive human activities. Such activities are related to the discharge of contaminants all along the river basin, leading to a reduction in the estuaries environmental quality (Khelebovich *et al.*, 1997).

The Brazilian coast has many examples of pollution and degradation due to the human action (Braga *et al.*, 2000; Abessa *et al.*, 2001; Lacerda & Gonçalves, 2001; Silva *et al.*, 2001; Borrelly *et al.*, 2002; Silva *et al.*, 2002; Kehrig *et al.*, 2003; Aguiar *et al.*, 2004; Carreira *et al.*, 2004; Machado *et al.*, 2004; Marins *et al.*, 2004; Medeiros *et al.*, 2005). Domestic and industrial effluents, treated or not, are directly discharged in many estuarine systems, altering their natural conditions (Borrelly *et al.*, 2002).

The Ceará River Basin (CRB) (Figure 1) is one of the three major water sources of the Metropolitan Region of Fortaleza (MRF), the Ceará State capital, located on the Northeast coast of Brazil. Despite its ecological and economic importance to the MRF, this river has suffered considerable degradation, caused by intensive and continuous industrial and domestic contamination (Escouto, 1996; Juvêncio, 1997; Marques, 2002; Aguiar *et al.*, 2004; Marins *et al.*, 2004; Vaisman *et al.*, 2005). The economy of the State of Ceará is still strongly dependent on the fisheries, and many communities rely on resources from the aquatic environments.

According to Brandão (1998), the CRB fluvial-marine plain was created from sediments deposition, mostly clay and sand, with considerable amounts of organic matter, giving origin to a suitable substrate for the mangrove vegetation development. The marine influence on the estuary reaches up to about 8 km and is also affected by the rainfall regime, which determines the river outflow (Juvêncio, 1997). The rainy season lasts from February to May, while during the rest of the year the rainfall is almost absent. The pluviometric indices also vary from year to year, but they usually range from 1200 to 1400 mm/year (Brandão, 1998).

Maranguapinho River is one of the main tributaries of Ceará River, and discharges in the estuary region, at about 5

km from the river mouth. It brings the contaminants released by the Industrial District of Maracanaú, working as an important secondary source of pollution to the Ceará River. A multitude of industrial plants manufacturing plastics, oils, textiles, chemicals, along with leather tanneries and slaughter-houses discharge their waste waters in the river, containing high concentrations of phenols, sulfite substances, oils, grease and residues containing metals like cadmium, lead, copper, chromium, mercury, zinc, iron, nickel and manganese (SEMACE, 1990). According to Juvêncio (1997), a large number of industries do not treat their effluents, releasing them into the public sewage system or directly into the water bodies.

This study aimed to evaluate the quality of the waters from the Ceará river estuary, using the sea urchin *Lytechinus variegatus* embryo-larval toxicity test. Moreover, toxicity parameters were used to rank sites (make a classification among sites) and to take into account possible intra-annual variations (rainy-dry season sampling campaigns) at each site.

METHODOLOGY

Water samples collection

The samples were collected once a month, from March 2004 through February 2005, in a depth of 0.3 m, during low spring tides, at four stations in the Ceará River estuary (Table 1). The water was collected directly in 100 ml amber glass bottles, which were properly identified and kept in ice until the analysis.

Physical-chemical analyses

The physical-chemical parameters were determined *in situ* at each station. Dissolved oxygen (DO) was measured with a portable oximeter (Quimis, model Q408P, São Paulo, SP, Brazil, precision 0.3 mg L⁻¹); salinity was determined using a hand refractometer (Biobrix, precision 1); and temperature using analogical thermometers. The pH reading was made for each replica with a pH meter (Quimis model Q400A, São Paulo, SP, Brazil, precision 0.01 pH), upon arrival on the lab. Ammonia was determined by the titrimetric method as described in the Standard Methods for Examination of Water and Wastewater, (APHA/ AWWA/ WEF, 1995).

Table 1 – Sampling stations and geographic coordinates.

Station	Locality	Geographic co-ordinates	
		Latitude (S)	Longitude (W)
1	Salina dos Pombos	03°43'150	038°37'342
2	Rio Maranguapinho	03°42'949	038°37'225
3	Croa do Mosquito	03°42'140	038°36'162
4	Espigão da Barra do Ceará	03°41'640	038°35'240

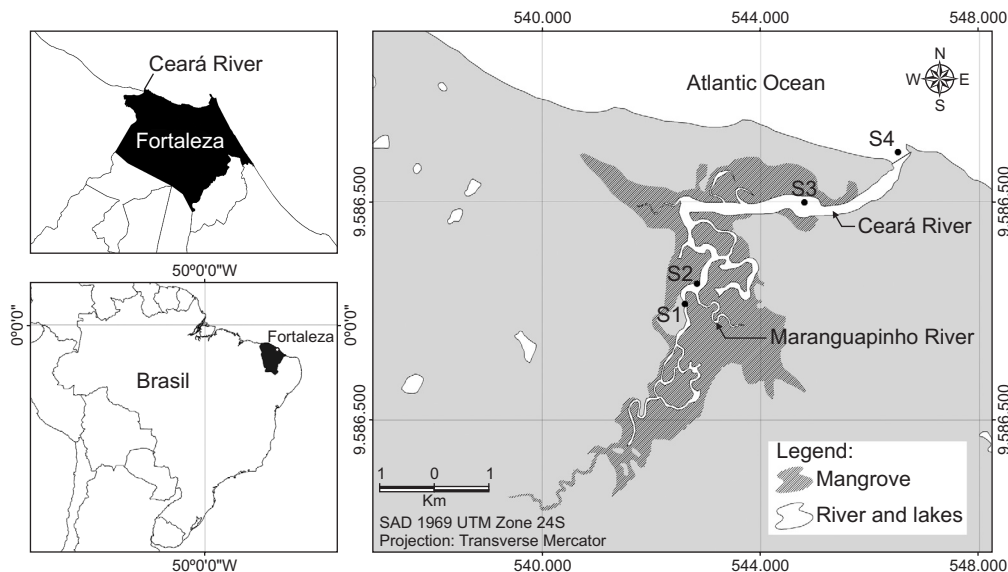


Figure 1 – Map of the study area showing the sampling stations at the Ceará River Estuary.

Bioassay

The toxicity tests were performed monthly (except in October) and, in parallel to each assay, reference substance tests were also used for validation. The toxicity test with *L. variegatus* embryos followed the method described by CETESB (1999) modified to the use of 24 multiwell plates.

The samples were tested in 5 different dilutions: 100, 80, 60, 40, 20%, and 0% with dilution water. The dilution water was collected at Lagoinha beach, Paraipaba, Ceará, where the adult sea-urchins were collected. This water was filtered (0.45 μm membrane) before the experiments. According to USEPA (2002), to attain reliable results, each concentration was tested in 3 replicates. When the sample salinity was lower than 35‰, brine was added for salinity correction. This procedure led to dilutions from 2.8 to 20% for the samples collected at S1 and S2, and from 1.6 to 10% for the samples collected at S3.

Gametes were obtained as described by Costa-Lotufo *et al.* (2002). Fertilization was performed by mixing activated sperms (0.05 mL of sperm in 2.45 mL seawater) to the egg cell suspension under slight shaking for 5 minutes. After this interval, the samples were checked for fertilization under the microscope.

In each cavity containing the sample, 100 μL of seawater containing 500 eggs were added. They were then incubated on a final volume of 2.5 mL per cavity. After 24h a 10 μL aliquot was taken to verify the embryos development. When the control showed 80% of well-developed *Pluteus* larvae, 125 μL of formaldehyde were introduced in each cavity to fix the embryos. Afterwards, for each replicate, 100 embryos

were counted, and the percentage of normal embryos was calculated.

The experiment negative controls were conducted using dilution water or distilled water, which had the salinity adjusted by brine addition. In all assays the controls showed normal plutei values greater than 80% (87.7 ± 0.3 , $n = 61$, C.V. = 7.5%). Physical-chemical analysis of the dilution water demonstrated that DO, pH, salinity, temperature and ammonia were inside the desirable range for use in toxicity testing. Zinc, used as a reference toxicant (positive control), confirmed that, throughout the experiments, the embryo toxicity test showed good repeatability, with a mean EC_{50} of $0.077 \pm 0.009 \text{ mg L}^{-1}$ (C.V. = 32.8%, $n = 8$).

Statistical analysis

Toxicity data were expressed as EC_{50} (as percentage of water sample) and NOEC (Non Observed Effect Concentration) values. EC_{50} values with respective 95% confidence limits were calculated by non-linear regression using GraphPad Prism 3.01 (GraphPad Software, Inc.). The NOEC values were obtained by the comparison of the number of normal pluteus among control and tested concentrations using ANOVA followed by Dunnett test, using a significance level of 5%. Moreover, the data expressed as EC_{50} were also transformed into Toxicity Units ($TU = 1/EC_{50} \times 100$) (USEPA, 2002). EC_{50} values were compared using Student's T test (for two stations, in May, August, November and February) or one-way ANOVA followed by Student Newman Keuls (for three stations, in March, April, June and July), both with a 5% significance level.

RESULTS AND DISCUSSION

The present study intended to measure the toxicity of water samples collected at four stations along the Ceará river estuary using the short-term chronic toxicity test with embryos of *L. variegatus*. As previously mentioned, there are evidences that this estuary is impacted (Escouto, 1996; Juvêncio, 1997; Marques, 2002; Aguiar *et al.*, 2004; Marins *et al.*, 2004; Vaisman *et al.*, 2005), but this is the first water quality assessment based on ecotoxicological analyses.

The results of the physical-chemical analysis are presented in Table 2. Generally, there is a gradient of salinity and pH from the inner part of the estuary towards the river mouth, while dissolved oxygen (DO) and temperature values were very similar among the stations. The lowest DO values were registered in the end of the rainy season for all stations. The temperature on the Ceará River estuary showed little variability, remaining itself around $29 \pm 2^\circ\text{C}$. The pH values were all between the limits established in the CONAMA resolution no. 357 (Brasil, 2005) concerning this type of environment (class I – recommended values from 6.5 to 8.5). The salinity presented the highest variations along the stations, as expected for an estuary, but all tests were run with the salinity previously adjusted. Highest salinities at all stations were observed from November to February, in the end of the dry season, whereas the lowest values were always obtained on April/March. Station 4 (river mouth) presented the smallest range of variation in salinity (from 25 on March to 40 on November).

Toxicity was detected in samples from three of the surveyed stations, especially at S1 and S2, where the effect was more evident (Table 3). Samples collected at S1 showed toxic effects in 8 out of 11 analyzed months. The rainy season samples were more toxic at this station. The station S2 was overall the most impacted, with samples consistently toxic, except in January. When the average toxicity (EC_{50}) between S1 and S2 was compared, the differences were significant only in August. For S3, the toxicity was noticed only in four months (March, April, June and July), but higher EC_{50} values were observed. Finally, the samples collected at S4 had shown no toxicity during this study.

The Toxic Units (TU) for the stations where EC_{50} was calculated ranged from 1.09 to 3.38 (Table 3). According to Environmental Canada (1999), TU values greater than 1 are associated to the presence of toxic compounds in a mixture. Thus, present data indicate that 50% of the analyzed samples probably presented toxic compounds.

According to Carr *et al.* (2006), ammonia is commonly a contributing factor in toxicity tests using sea urchin embryological development endpoint, since this test seemed to be strongly sensitive to unionized ammonia, with a LOEC (lowest observed effect concentration) of $90 \mu\text{g L}^{-1}$ and a NOEC of $30 \mu\text{g L}^{-1}$. In the analyzed samples, the unionized ammonia concentration ranged from 28 to $1,420 \mu\text{g L}^{-1}$, however the highest values were always related to S4 station, which presented

the lowest toxicity values. Thus, the toxicity of the samples seems to be only slightly influenced by this parameter.

The toxicity in S2 should be related to the proximity to the confluence of Ceará and Maranguapinho rivers, which receives effluents of the Industrial District in Maracanaú. Escouto (1996) registered higher concentrations of nitrogenous and phosphate compounds at this confluence during the rainy period. The presence of metals, especially Pb, Cr and Cd, is probably related to industrial discharges, while Ni, Cu and Zn seem to come from a strong contribution of the atmospheric pollution (Juvêncio, 1997). Additionally, the presence of mercury in sediments of the Maranguapinho and Ceará rivers was registered by Marques (2002), Marins *et al.* (2004) and Vaisman *et al.* (2005). Another important aspect is the presence of an operating shipyard in the vicinities of S3, which may have influenced locally the toxicity at this station. Aguiar *et al.* (2004) demonstrated higher concentrations of Zn and Cu in sediments samples collected at this area compared with upstream and downstream stations along Ceará river estuary.

Generally, it was observed a toxicity gradient from S1 and S2 towards the river mouth, which suggests that upstream pollutants have remained on the water column, becoming progressively less concentrated as they approached to the sea. In fact, several factors could influence the fate of contaminants in the environment, including dilution, evaporation or volatilization, adsorption/precipitation, biodegradation, and photo-oxidation (Khlebovich *et al.*, 1997; OGP, 2005). Individually or collectively, these processes tend to reduce the concentrations of compounds in the water column along the estuary. A striking feature of the distribution of most dissolved constituents in an estuary is that they appear to reflect the mixture between freshwater and seawater in a conservative and a non-conservative manner (Muller *et al.*, 1994; Wen *et al.*, 1999; Wang & Liu, 2003). Furthermore, the salinity changes the valency in some metallic ions, causing variation in dissolved ions, sedimentation of basic mass of clayey particles and flocculation of colloids, since it changes the electrical properties of the water (Khlebovich *et al.*, 1997; Hatje *et al.*, 2003). Thus, the salinity gradient is evidently highly responsible for the fate of contaminants in the estuarine ecosystem.

A study at Santos Bay (Braga *et al.*, 2000) demonstrated a clear dilution of the nutrients by the seawater. Nitrite and nitrate, for example, presented values 40 times higher at the head of the estuary than at the river mouth, while for phosphate, the dilution was 24 times. On the basin of Trancão River, near Lisbon, Portugal, it was registered a reduction of the metal concentration towards the mouth due to the flocculation as well as the dilution by mixture with seawater (Araújo *et al.*, 1998). Nevertheless, in tropical estuaries, there is also evidence of compositional variations due to changes in the magnitude of estuarine input and removal processes; and the intensity of these processes is modified by freshwater discharge or, more specifically, the water residence time (Khlebovich *et al.*, 1997).

Table 2 – Physical-chemical parameters of the waters samples from the Ceará River Estuary, between Mar/2004 and Feb/2005.

Month	DO (mg L ⁻¹)				Salinity				Temp. (°C)				pH			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Mar	7.1	8.0	8.2	4.8	2	2	28	25	27	28	28	29	7.39	7.40	7.43	8.13
Apr	4.3	5.2	3.3	2.3	3	2	17	36	28	28	28	28	7.30	7.31	7.56	8.07
May	7.1	4.0	3.0	1.8	2	8	28	38	28	29	28	30	7.39	7.85	7.43	8.61
Jun	4.3	1.9	4.8	6.7	3	3	22	38	28	27	29	28	7.30	7.46	7.56	8.12
Jul	3.8	8.2	10.4	15.0	12	3	22	36	29	27	28	27	7.95	7.42	8.48	8.23
Aug	1.7	5.3	5.9	6.1	9	10	29	33	28	27	27	27	7.47	7.38	7.62	8.18
Sep	8.6	5.2	6.1	6.5	5	24	34	35	28	28	27	28	7.40	7.36	7.66	8.11
Nov	5.5	3.3	13.6	16.7	20	16	35	38	27	29	28	27	7.35	7.44	7.77	8.15
Dec	5.6	3.5	11.7	16.5	30	12	36	40	28	30	27	30	7.45	7.62	8.00	8.27
Jan	6.2	3.5	5.0	6.2	25	35	37	39	28	30	29	30	7.48	7.40	7.77	8.10
Feb	7.9	6.1	6.5	3.5	31	8	23	32	30	29	30	31	7.46	7.48	8.10	8.23

Table 3 – Toxicity of water samples collected between Mar/2004 and Feb/2005 at stations 1-4 on the estuary of Ceará River. The values are expressed as EC₅₀ (%) ± standard error of the mean (S.E.M.) from three replicates; the Toxic Units (TU) obtained by the mean EC₅₀; and NOEC (Non Observed Effect Concentration, expressed as dilution percentage).

Mouth	EC ₅₀ (%) ± S.E.M.				T.U.				NOEC			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Mar	69.4 ± 7.2	77.3 ± 1.8	87.5 ± 7.6	>100	1.44	1.29	1.14		54.2	53.1	75.9	100
Apr	36.9 ± 2.2	31.7 ± 1.8	61.7 ± 1.4 ¹	>100	2.71	3.15	1.62		16	16	54	100
May	71.2 ± 1.7	49.9 ± 7.5	>96.7	>100	1.40	2.00			69.4	33.3	96.7	100
Jun	36.8 ± 0.4	32.6 ± 0.8	80.2 ± 1.9 ¹	>100	2.71	3.06	1.25		n.d.	n.d.	74.7	100
Jul	39.6 ± 0.5	29.6 ± 6.0	82.8 ± 0.8 ¹	>100	2.52	3.38	1.20		17.6	17.6	57.6	100
Aug	72.8 ± 1.3	38.1 ± 1.2 ²	>98.4	>100	1.37	2.62			55.9	18	98.4	100
Sep	>97.2	80.6 ± 1.0	>100	>100		1.24			97.2	58.3	100	100
Nov	91.5 ± 0.9	53.7 ± 7.9	>100	>100	1.09	1.86			76	37	100	100
Dec	>97.2	51.7 ± 4.3	>100	>100		1.93			97.2	36.5	100	100
Jan	>100	>93.8	>100	>100					100	93.8	100	100
Feb	60.5 ± 3.5	53.5 ± 0.4	>100	>100	1.65	1.65			54.3	35.4	100	100

The EC₅₀ were obtained by non-linear regression using the GraphPad Prism. ¹p < 0.05, ANOVA followed by Student Newman-Keuls and ²p > 0.05, *t* of Student comparing data from S1 and S2. The NOEC values were obtained by the comparison of the number of normal puletus among control and tested concentrations using ANOVA followed by Dunnet test, using a significance level of 5%. N.d. = not determined.

Despite the importance of the Ceará river estuary to the Metropolitan region of Fortaleza, studies describing the dynamics of this ecosystem are scanty, but according to Brandão (1998) the rhythm of the deposition on this estuary is determined mainly by tidal influence. This author also observed the entire mixture of the water column all along

the estuary. On the other hand, in the rainy season, there is a considerable increase in the leaching of superficial residues, along with domestic and industrial sewages that are drained to the estuary, carrying contaminants and a great organic load. In fact, the toxicity was more evident in samples collected during the rainy season.

CONCLUSION

This study is the first report on the water quality of Ceará River estuary, based on toxicity tests. The results showed the presence of toxicity on sea-urchin embryos in the internal portion of this estuary, suggesting that the Maracanaú's Industrial District is an important contributor to the pollution of this important ecosystem.

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