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Toxic blooms of cyanobacteria in the Patos Lagoon Estuary, southern Brazil

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Abstract

The Patos Lagoon is the largest lagoonal system in South America. Its waters are formed by a huge drainage basin (201,600 km^2) situated in the most industrialized areas of the Southern state of Rio Grande do Sul. On its margins more than 3 million inhabitants live in several cities and towns. The lagoon waters are used for leisure, drinking, industry, fisheries, agriculture and navigation. A monitoring and sampling program was developed from February 1994 to January 1996 with emphasis on the estuarine area, aiming to evaluate the occurrence of algal blooms. In the last 15 years, several cyanobacterial (blue-green algal) blooms of the *Microcystis aeruginosa* have been registered in the lagoon estuary. High *M. aeruginosa* biomass (50 to 9,000 μg chl *a* l^{-1}) was observed in the whole region in late summer and autumn 1994, and early summer 1995. The LD50 of toxic bloom samples tested in mice varied from 22 to 250 mg dry weight kg body weight^{-1} while levels of toxicity (LC50) in the brine shrimp varied from 0.47 to 2.44 mg ml^{-1}. Toxicity varied in different blooms, in the distances along the scum and with time, within the same bloom. The hepatotoxin microcystin-LR was identified in almost all samples.

1. Introduction

Blooms of toxic cyanobacteria (blue-green algae), particularly of *Microcystis aeruginosa*, have been registered worldwide and have often been associated with the deaths of domestic and wild animals, birds and fish (Carmichael, 1986; Codd & Beattie, 1991). Several blooms of *M. aeruginosa* have been observed irregularly during the last 15 years throughout the Patos Lagoon (Odebrecht et al., 1987; Torgan, 1989; Yunes et al., 1992). However, no animal deaths are known to have been registered.

The Patos Lagoon (30°20′ S to 32°10′ S) is the second largest inland waterbody in Brazil and the largest lagoonal complex in South America. The Lagoon is approximately 200 miles long and between 2 and 40 miles wide. Along its margins there are more than three million inhabitants living in several cities, towns, fishing villages and summer resorts (Figure 1). To the North, a huge drainage basin of approximately 200,000 km^2 formed by several rivers, provides 75 to 80 percent of the freshwater to the Patos Lagoon and estuary. Lagoon waters are also used for domestic water supply, fisheries, leisure, navigation, agriculture and receive domestic and industrial sewage.

M. aeruginosa blooms in the Patos Lagoon appear to be associated with pH higher or equal to 8.0, water temperatures higher than 20 °C, freshwater and a balance of nutrients with a N/P ratio close to 13:1. This paper reports on the toxicity of the Patos Lagoon bloom material in mouse and brine-shrimp (*Artemia salina*) bioassays and identification of toxins.

2. Materials and methods

The sampling area and collection sites are shown in Figure 1. Samples were collected during 24 cruises in the southern part of Patos Lagoon, at two fixed stations in the navigation channel (10 m deep – Farolete and Barra), at two fixed-station in shallow areas (1 m deep – Marambaia and Torotama), at one fixed station

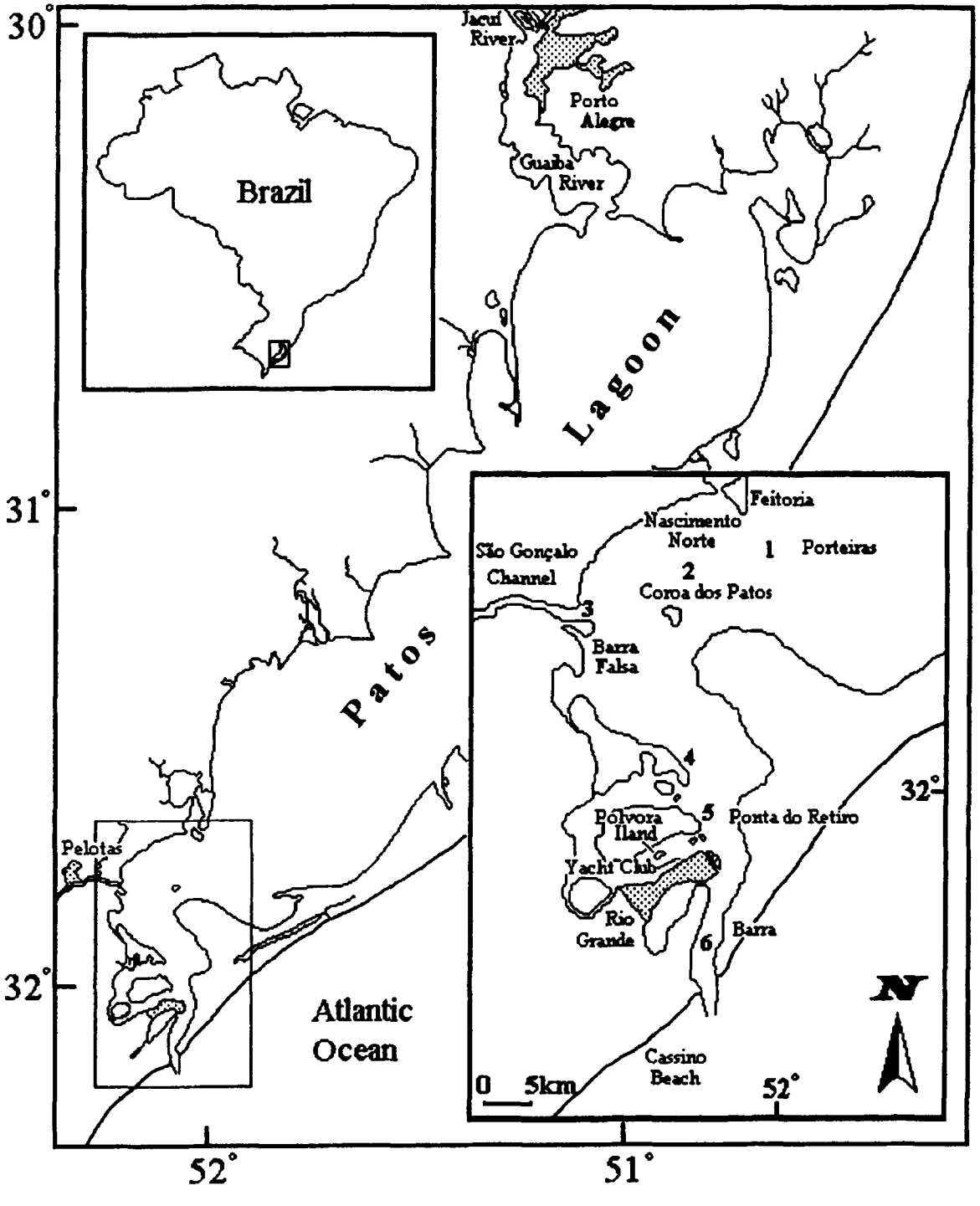

Figure 1. Map of the Patos Lagoon showing sampling stations in the estuarine area (1. Feitoria; 2. Farolete; 3. São Gonçalo; 4. Torotama; 5. Marambaia; 6. Barra) where samples of *Microcystis* bloom and scum were collected during the monthly cruises or during particular blooms.

in the São Gonçalo Channel (6 m deep), at one fixed station at the Feitoria Channel and also from independent stations. Cruises were conducted over one or two days. Independent stations associated with blooms within the estuary were sampled at Pólvora Island, the Rio Grande Yacht Club, Porteiras, Coroa dos Patos, Barra Falsa, Ponta do Retiro and Nascimento Norte (see Figure 1). Samples were collected and returned to the laboratory for immediate examination. Surface water samples were collected using 1.5-l van Dorn bottles and placed in clean 1 l plastic bottles. Samples were preserved in Lugol's solution (100 g KI + 50 g I_2 in 100 ml H_2O) for further analysis of cyanobacterial bloom composition. Scum samples were kept in plastic bags and bloom samples were concentrated by the use of a phytoplankton net (140 μm mesh), brought to the laboratory and frozen at $-20\,^{\circ}C$. Both samples were freeze-dried in the laboratory using a Micromodulyo lyophilizer (Edwards, Crawley, England). The freeze-dried material was resuspended in saline solution (0.9% w/v NaCl) and cells were disrupted by a sequence of at least three freeze-thaw cycles and immersion into a 50 kHz sonic bath (Bandelin, Germany) for 15 min at low temperatures. Intraperitoneal mouse bioassays were performed using samples prepared as above and diluted with saline solution in the range of 15 to 500 mg d.w. kg^{-1} mouse. For each dose, five male Swiss albino mice were injected intraperitoneally with not more than 0.01 ml g b.w.$^{-1}$. For brine-shrimp (*Artemia salina*) bioassays, suspensions containing a mean of 14.5 (sd 3.5) larvae in 200 μl of media were used. A hydrophillic extract of freeze-dried material was obtained as described above, and prepared in the *A. salina* growth medium (Harwig & Scott, 1971). Extracts were centrifuged at 10,000 g in an Eppendorf bench centrifuge model 5415 (Eppendorf, Germany) and the supernatant used for the test. The highest toxicant concentration applied was 45g l^{-1} (for the Barra Falsa extract), 49 g l^{-1} (for the Cassino Beach extract), 5 g l^{-1} (for *M. aeruginosa* PCC7813 extract), 5 g l^{-1} (for the Barra extract), 5 g l^{-1} (for the Marambaia extract) and 90 g l^{-1} (for *M. elabens* M-177, Institute of Applied Microbiology, University of Tokyo), a non hepatotoxic strain; further details are described by Campbell et al. (1994)). The 96-well plates used were covered to reduce evaporation, and results noted after 18 h incubation at 25 °C under cool fluorescent lights (61μE $m^{-2}s^{-1}$). All treatments were carried out in triplicate. The LC50 mortality values were calculated by use of Spearman-Karber software (Hamilton et al., 1997). Further scum samples were lyophilised, methanol-extracted and analyzed for microcystins by high-performance liquid chromatography with photodiode array detection (HPLC-DAD) (Lawton et al., 1994). The HPLC analysis was carried out using Waters instrumentation (Chromatography Division / Millipore Corp.) consisting of a 600E solvent delivery system, a 717 WISP auto sampler with detection using a 991 photodiode array (PDA) detector, 200–300 nm with 3 nm resolution. A Waters μBondapak C_{18}, 3.9 × 300 mm (Millipore Corp.) column was used for all analytical work and run at 40 °C. The eluents used were milliQ water plus 0.05% (v/v) trifluoroacetic acid (TFA) and acetonitrile plus 0.05% (v/v) TFA. Separation was achieved using a linear gradient (water: acetonitrile) at 70% water and 30% acetonitrile, and changing gradually to 100% acetonitrile over a 40 min period. Cyanobacterial cells were extracted in methanol, centrifuged, and 100 μl loaded in the WISP, and 25 μl samples injected onto the column. Microcystin-LR was detected at 238 nm using a deuterium lamp. Samples for chlorophyll *a* (chl *a*) were filtered through 4.7 cm Whatman GF/C filters on board and the filter discs kept refrigerated at +5 °C in dark bottles for further extraction in absolute methanol and analysis. Chlorophyll a values were obtained using the coefficient of Mackinney (1941). Photosynthetic rates of the September, 1994 (Yacht Club and Pólvora Island samples) were determined by the changes in the concentration of O_2 measured with a Pt-Ag O_2 electrode (Rank Brothers, Cambridge, England).

3. Results

Three distinct blooms of *M. aeruginosa* occurred during the twenty-four month monitoring program from February, 1994 to January, 1996 in the Patos Lagoon estuary and caused a clear effect on total chl *a* levels in the whole estuary (Figure 2).

Blooms of *M. aeruginosa* occurred from March to May 1994 with levels of chlorophyll *a* in excess of 9,000 μg l^{-1} with a lower, but still distinct bloom from December to March, 1995. During the May 1994 cruise, a high concentration of cells was observed along the navigation channel between the city of Rio Grande and the Feitoria Channel, a distance of about 45 miles. Scum biomass in the channel reached 5,940 μg chl *a* l^{-1} and cells were also found, concentrated by the wind, at nearby margins, and deposited after some days on the sandy beaches of the South Atlantic Coast.

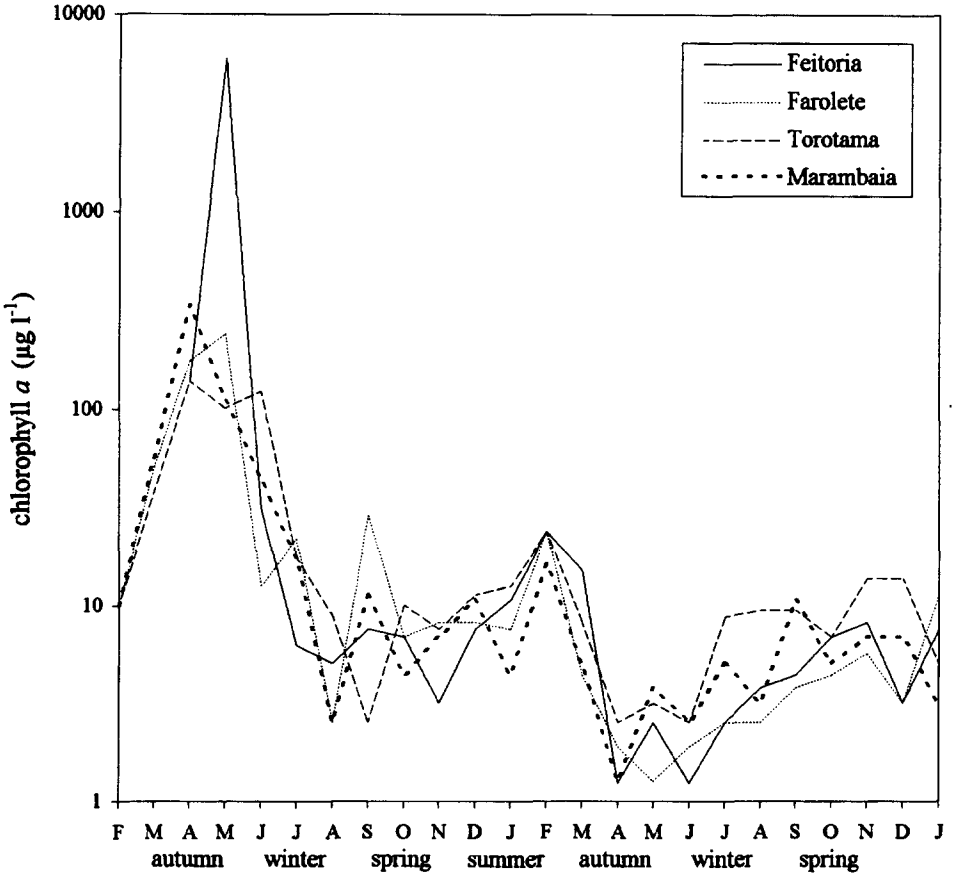

Figure 2. Variation of chlorophyll *a* in 4 sampling stations within the Patos Lagoon estuary during 24 months. Chlorophyll *a* data are expressed on a logarithmic scale.

During the winter months from July to September of 1994, decreased chl *a* levels were observed, however strong Northeast winds in late September provoked resuspension of deposited *M. aeruginosa*. This caused an increase above the normal (10 $\mu g\ l^{-1}$) of the winter chl *a* levels observed at the local Yacht Club and Pólvora Island margins. These concentrated masses were made up of viable cells, as O_2 was liberated under illumination at rates of 8.71 nmoles O_2 chl a^{-1} min^{-1}.

A smaller intensity bloom of *M. aeruginosa* from December 1994 to March 1995 also raised chl *a* levels above the normal winter values. During winter 1995, from April to September, chl *a* levels were much lower than levels from the previous winter as no *M. aeruginosa* blooms occurred in the summer and chl *a* decreased to levels below 10 μg chl $a\ l^{-1}$ (Figure 2).

All cyanobacterial scum samples (and the dried material from the March Feitoria bloom) were toxic by mouse bioassay showing signs of hepatotoxicity typical of cyanobacterial microcystins, with deaths occurring 45 to 60 min after administration, the LD50 ranging from 22 to 250 mg kg b wt^{-1} (Table 1). The toxicity of the scum (May 1994 sample) according to mouse bioassay, showed some variation between Rio Grande and the Feitoria Channel within the same bloom, however the highest toxicity was detected during the *M. aeruginosa* bloom at Barra on 20th December 1994. No apparent correlation was found between the intensity of each blooming event (as determined by chl *a*) and toxicity.

The Barra Falsa *M. aeruginosa* scum, and samples subsequently collected from the Cassino Beach on the South Atlantic coast were also found to be toxic by brine shrimp mortality assay (Table 2). Toxicity was also present in samples of *M. aeruginosa* blooms collected at Marambaia margins and at surface waters of Barra on 20th December 1994. Crude extracts of a microcystin-containing laboratory culture, *M. aeruginosa* PCC 7813 were used for comparison. Microcystin

Table 1. Toxicity of several *M. aeruginosa* bloom samples, collected at different sites along the Patos Lagoon estuary to mouse and analysis for microcystins by high-performance liquid chromatography with diode array detection.

Site/Sample	chl *a* (μ l^{-1})	24h-LD50[a]	Microcystin concentration[b]	Sampling date
Feitoria margin	674	32	n.t.	03/06/94
Porteiras	292	250	n.d.	05/24/94
Coroa dos Patos	241	207	n.d.	05/24/94
Near Barra Falsa	101	59	0.437	05/24/94
Ponta do Retiro	5,195	250	0.270	05/24/94
Barra	41	22	1.121	12/20/94
Marambaia	16	24	0.858	12/20/94
Farolete	15	n.t.	0.330	12/20/94
Nascimento	4,787	n.t.	0.276	01/10/95

[a] All samples were hepatotoxic by mouse bioassay: LD50s were calculated after three tests in which dilution range was shortened to a minimum and expressed as mg dry wt per kg body wt mouse intraperitoneal bioassay.
[b] μg microcystin per mg dry wt.
n.t.: not tested.
n.d.: not detected.

Table 2. Toxicity of Patos Lagoon *M. aeruginosa* bloom samples and laboratory cultures of *M. aeruginosa* to the brine shrimp (*A. salina*) and analysis for microcystin by high-performance liquid chromatography

Site/Sample	18h-LC50[a]	Microcystin concentration[b]	Sampling date
Near Barra Falsa	2.44 (±0.83)	0.437	05/24/94
Cassino Beach	0.47 (±0.27)	0.281	05/24/94
Barra	0.70 (±0.11)	1.121	12/20/94
Marambaia	0.48 (±0.10)	0.858	12/20/94
M. aeruginosa PCC 7813	0.12 (±0.02)	3.780	–
M. elabens M177[c]	>40.00	<0.01	–
Microcystin-LR	0.0028 (±0.0003)	1,000	–

[a] mg dry wt per ml. Data in parenthesis are the max. and min. within the cofindence limit of 95 percent.
[b] High performance iquid chromatography with diode array detection (HPLC-DAD) μg microcystin per mg dry wt.
[c] From Campbell et al. (1994).

toxins were analyzed by HPLC-DAD using the purified hepatotoxin microcystin-LR as a standard in all cases. By contrast, extracts of *M. elabens* M177, which do not contain detectable microcystins as analyzed by HPLC-DAD, were non-toxic in the brine shrimp bioassay at high dose levels (Table 2).

4. Discussion

Blooms of *M. aeruginosa* increased the phytoplankton biomass of the Patos Lagoon estuary. The most intense blooming event happened from March to May 1994, with the highest cyanobacterial concentration observed during the May 1994 cruise. At this event, levels of chl a observed in excess of 9,000 μg chl *a* l^{-1} were comparable to levels found in a hypertrophic water reservoir in South Africa (Robarts & Zohary, 1992). At the Patos lagoon estuary, high concentration of cells seem to be correlated with high pH and temperature, calm weather and freshwater conditions (Yunes et al., 1994).

As listed in Table 1, all of the *M. aeruginosa* scum and bloom samples collected in 1994 and 1995 were toxic by bioassay with the poisoning in mice being characteristic of microcystin hepatotoxins. The most toxic sample tested (Barra) was collected from one of the lowest concentrations of *M. aeruginosa* cells

per litre. In fact, high chl *a* scum samples (e.g. Ponta do Retiro and Porteiras) have shown lower toxicity. It is, therefore, difficult to correlate the intensity of the blooming event and toxicity of the material with the few samples collected in the Patos lagoon estuary. However, laboratory studies have indicated that toxicity of cells varies with age of culture and medium pH (Van der Westhuizen & Eloff, 1983), with different strains (Lawton et al., 1990) and at different temperature and illumination conditions (Van der Westhuizen et al., 1986; Utkilen & Gjolme, 1992). In the present case, several factors could be acting simultaneously as samples were collected at different parts of the scum and at different seasons when waters are subjected to different environmental conditions as pH, soluble N/P ratios and salinity (Yunes et al., 1994).

Toxicity was also confirmed by the brine shrimp bioassay with the Barra Falsa and Cassino beach material and chromatographic detection of microcystins with these samples. The most toxic sample to mice (sample from Barra) was also toxic to *A. salina*, although it was not the most toxic to the brine shrimp. In general, scum samples collected at the Patos Lagoon were from 3 to 20 times less toxic than the strain *M. aeruginosa* PCC7813 grown under laboratory conditions (see Table 2). The Patos Lagoon scum material was also much less toxic to the brine-shrimp assay than the eluted microcystin-containing extracts of a *Microcystis* bloom from Rutland Water, Leicestershire, England and contained much less toxin (see Campbell et al., 1994). This finding nevertheless, does not minimize the toxicity and risks of contamination to which the whole estuarine environment is subjected at every *M. aeruginosa* bloom in the Patos Lagoon.

The locations from which the toxic samples were collected included intensively managed shrimp fisheries and recreational beaches, both inside the Lagoon and outside on the nearby Atlantic coast, with heavy recreational and amenity usage. Although no animal deaths or problems on human populations were registered (due possibly to a lack of a specific monitoring program), several problems have been registered worldwide (Skulberg et al., 1984, 1993; Elder et al., 1993) and a diverse list of diseases related to freshwater cyanobacterial toxins has been recently recognised (Carmichael & Falconer, 1993; Bell & Codd, 1994).

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