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Cyanobacterial occurrence and detection of microcystin by planar chromatography in surface water of Billings and Guarapiranga Reservoirs, SP, Brazil

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ABSTRACT – (Cyanobacterial occurrence and detection of microcystins by planar chromatography in surface water of Billings and Guarapiranga Reservoirs, SP, Brazil). Billings and Guarapiranga Reservoirs were deeply affected by environmental disturbances, which more evident consequence are the cyanobacterial blooms. Microcystins are the most common cyanotoxin in freshwaters and more than 70 types are known. Different methods for microcystins analysis in water can be used, among which ELISA and HPLC are the most frequently employed. However, less sophisticated and more economic methods can also be used. This is the case of planar chromatography (thin-layer chromatography) method previously used in cyanotoxins purification but gradually replaced by others. Posterior optimization of the microcystin chromatography conditions and because of its simplicity, rapidity, efficiency and low cost, this method is again considered an option for the analysis of microcystins and nodularins. Considering the importance of Billings and Guarapiranga Reservoirs for drinking water supplies and the few scientific data about cyanobacteria and cyanotoxins in these water bodies, the aims of this work are to analyze the biodiversity of cyanobacteria in the Billings and Guarapiranga Reservoirs and the detection of dissolved microcystins in the water. It was possible to identify 17 species of cyanobacteria, 9 of them being potentially toxic. In Billings Reservoir *Microcystis aeruginosa* (Kützing) Kützing and *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju are the most common species, while in Guarapiranga Reservoir only *M. aeruginosa* was considered as a common species. Microcystins were detected in all Billings Reservoir samples and in only one sample from Guarapiranga Reservoir.

Key words - cyanobacteria, drinking water, microcystins, planar chromatography, reservoirs

RESUMO – (Ocorrência de cianobactérias e detecção por cromatografia planar de microcistinas dissolvidas nas águas superficiais das represas Billings e de Guarapiranga, SP, Brasil). As represas Billings e Guarapiranga foram profundamente afetadas por fatores ambientais cuja conseqüência mais evidente são as florações de cianobactérias. As microcistinas formam a classe de cianotoxinas mais freqüente em água doce e são comumente analisadas por ELISA ou CLAE. No entanto, processos menos sofisticados e mais econômicos também podem ser usados. Este é o caso da cromatografia planar (cromatografia em camada delgada), método anteriormente usado em trabalhos de purificação de cianotoxinas, mas que foi gradualmente substituído por outros. Assim, considerando a importância das represas Billings e Guarapiranga para o abastecimento público e a carência de informações científicas sobre as cianobactérias e as cianotoxinas que nelas ocorrem, nossos objetivos foram o estudo da composição desses organismos e a detecção de microcistina dissolvida na água. Foi possível identificar 19 espécies de cianobactérias, das quais 9 são consideradas potencialmente tóxicas. *Microcystis aeruginosa* (Kützing) Kützing e *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju são as espécies mais comuns no Reservatório Billings e, na Guarapiranga, apenas *M. aeruginosa* foi considerada como espécie de ocorrência comum. Microcistina-LR foi detectada em todas as amostras da Represa Billings e em somente uma amostra da Represa Guarapiranga.

Palavras-chave - água de abastecimento, cianobactérias, cromatografia planar, microcistinas, reservatórios

Introduction

The twentieth century was marked by heavy damages caused to the environment and water eutrophication is one of the worst consequences. The eutrophication process is more intense in water bodies near great urban centers as the Metropolitan Region of São Paulo City where over 15 million people live and hundreds of industries are located (Cetesb 1998).

For many decades the water bodies in this region have been intensely impacted by domestic and industrial waste input, deforestation, soil erosion and irregular land use in the surrounding areas (Sendacz & Kubo 1999). Billings and Guarapiranga Reservoirs, that make part of this complex system, were also deeply affected by environmental impacts.

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Few scientific articles on phytoplankton in Billings and Guarapiranga Reservoirs describe the transformations occurred in these water bodies, including the development of cyanobacterial blooms.

In the beginning of the eighties, Xavier (1981) studied the phytoplanktonic community in Billings Reservoir and did not report the presence of cyanobacterial blooms. At the end of the nineties, Souza *et al.* (1998) analyzed the water quality of Billings for one year, and observed that *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya & Subba Raju (Cyanobacteria) was the dominant species. Carvalho (2003) studied the phytoplanktonic community in Billings and its use as a tool for monitoring water quality and found out that cyanobacteria were the dominant group throughout the studied period.

Concerning Guarapiranga Reservoir, Zagatto *et al.* (1996) evaluated water quality based on ecological and toxicological aspects and detected toxic blooms of cyanobacteria which poisonous effects were confirmed by mice testing. Beyruth (1996) studied the Guarapiranga phytoplanktonic community and also detected blooms of cyanobacteria. The direct correlation between these blooms and the nutrient input was demonstrated.

Sant'Anna & Azevedo (2000) studied the potentially toxic Brazilian cyanobacteria from different reservoirs, including Billings and Guarapiranga. The authors showed that *Microcystis*, *Anabaena, Aphanizomenon*, *Cylindrospermopsis*, *Raphidiopsis* and *Planktothrix* are the most widely distributed genera in Brazilian reservoirs. The blooms of these genera are toxic in more than 65% of occurrences (Costa & Azevedo 1994).

Regarding cyanotoxins, microcystin is the most common toxin in freshwaters and around 70 different types are known (Spoof *et al.* 2003). It is possible to use several methods for the microcystin analysis but ELISA and HPLC are the most frequently used. However, less sophisticated and less expensive methods can also be employed as discussed in the present paper. This is the case of planar chromatography, or the thin-layer chromatography, a method previously used in hepatotoxins purification (Poon *et al.* 1987, Harada *et al.* 1888a, b) but gradually replaced by others. Based on its simplicity, rapidity, efficiency and low cost, the thin-layer chromatographyc method has been improved (Ojanperä *et al.* 1995, Pelander *et al.* 1996, 1997, 1998, 2000) and it is considered a good procedure to detect microcystins and nodularins. The use of Amberlite XAD-2 for clean-up and solid-phase extraction during the microcystin purification process is also described (Pietrzy 1989, Lingeman & Tjandem 1990).

Clean-up and solid-phase extraction (SPE) are common steps in the cyanobacterial and water sample preparation and are usually performed with the hydrophobic chemically modified silica C_{18}. In this process, the analytes are adsorbed onto the hydrophobic material by means of van der Waals interactions and to some extent by hydrophobic bonding or dipole-dipole interactions (Lingeman & Tjaden 1990). The styrene divinylbenzene copolymer XAD-2 is an hydrophobic sorbent too, that is applied for clean-up and concentration of protein-rich matrix (Lingeman & Tjaden 1990). The suitability of the use of XAD-2 in qualitative analyses of microcystin-LR was evaluated.

The aims of this work are to analyze the biodiversity of cyanobacteria in the Billings and Guarapiranga Reservoirs and to detect the dissolved microcystin in the water.

Material and methods

Eighteen 20-liter samples (9 from each reservoir) were collected monthly from the surface of Billings and Guarapiranga Reservoirs, since December/2000 till August/2001. Table 1 presents the main characteristics of these reservoirs.

Biological studies – From each sample, 100 mL of water was preserved in formaldehyde for taxonomic studies under light microscope; other similar aliquot was kept alive for isolating and culture of cyanobacteria strains; the remaining sample was used for the microcystin analysis.

The culture conditions were: BG-11 and/or AMS-1 media, temperature 22 °C ± 1 °C, irradiances 15-20 µmol photons m^{-2} s^{-1} and 10-14 hours light-dark cycle (Azevedo & Sant'Anna 2003). All culture strains studied, including the strain SPC 686 used for the microcystin purification, belong to the "Instituto de Botânica" Algae Culture Collection (SPC) - SMA.

Table 1. Main characteristics of Billings and Guarapiranga Reservoirs, according to Beyruth (2000) and Carvalho (2003).

	Reservoir	
	Billings	Guarapiranga
Location	23°47' S and 46°40' W	23°43'S and 46°32'W
Mean depth (m)	10	5.7
Area (km^2)	127	33.981
Transparency (m)	1.20	2.00
Retention time (days)	538	185
Conductivity (µS cm^{-1})	178.70	168.70
Chlorophyll a (µg L^{-1})	41.20	28.60

The classification system of Anagnostidis & Komárek (1988) and Komárek & Anagnostidis (1989, 1999) was adopted for cyanobacteria identification.

Chemical essays – Study on material from culture and on water samples using Amberlite XAD-2 (Supelco) for microcystins solid-phase extraction.

Culture – About 780 mg of lyophilized cells of the *Microcystis panniformis* SPC 686 strain were extracted (3x) with MeOH/H_2O (75:25 v/v) by exposure to ultrasound (4 x 30 sec; 50 W) and centrifugation at 1045 x *g* for 60 min. (Ramanan *et al.* 2000). The supernatants were combined and evaporated under reduced pressure to remove the organic solvent; the aqueous residue was lyophilized (dried weight = 0.175 *g*), dissolved again in deionized water and applied to an Amberlite XAD-2 column (Supelco, 10 × 2.5 cm). The eluting solvents were H_2O (20 mL), MeOH/ H_2O (20:80, v/v) (20 mL) and MeOH (30 mL) (Pietrzyk 1989, Lingeman & Tjandem 1990). These three eluates were taken to dryness (96.0, 9.0, and 51.4 mg, respectively) and suspended again in 1 mL of 0.9% (w/v) NaCl solution. A small aliquot of each one was applied to silica gel thin-layer chromatography plates that were developed with the solvent systems: $CH_3CO_2C_2H_5$/$(CH_3)_2CHOH$/H_2O (9:6:5, v/v/v, upper layer) and $CHCl_3$/MeOH/H_2O (65:35:10, v/v/v, lower layer) and were used in all chromatographic runs performed. The visualization of microcystin spots of the samples and of the purified microcistin was done with short wavelength UV light, with iodine vapors and by derivatization with TMB (3, 3, 5, 5-tetramethylbenzidin) (Harada *et al*. 1988b, Pelander *et al*. 2000). The toxicity testing was carried out with an i.p. injection into Swiss-Webster male mice (20-25 g body weight). Two animals were used for the first eluate, one for the second and two for the third. The time of death was recorded and the remaining mice, including the control animal, were observed for 24 hours.

Water sample – each water sample (10 L) from Billings and Guarapiranga reservoirs was filtered through a glass-fiber filter (GF/C Whatman) and applied directly to an Amberlite XAD-2 (Supelco) vacuum column (10 × 2.5 cm) before elution decreased, thereby providing an indication of maximum sample load; the loaded column was washed with water (30 mL) followed by H_2O/MeOH (80:20, v/v) (30 mL) and subsequently by methanol 100% (30 mL). The column was regenerated (Pietrzyk 1989, Lingeman & Tjandem 1990) and the procedure was repeated for the whole sample. The methanolic eluates were combined, evaporated under reduced pressure and then the residues were reconstituted with methanol (0.5 mL) and applied to silica gel plates (20 × 20 cm, 0.25 mm, Kieselgel 60GF_{254}, E. Merck) that were developed with the same solvent systems as described by Harada *et al.* (1988b). The microcystin spots of the samples and purified microcystin were visualized with short-wavelength UV, iodine vapor and by derivatization with TMB (3, 3, 5, 5-tetramethylbenzidin) (Harada *et al*. 1988b, Pelander *et al*. 2000).

Analysis of microcystins in water samples from the reservoirs using silica gel planar chromatography – For evaluating toxin concentration, ten-liter water samples were filtered through a glass-fiber filter GF/C Whatman and applied directly to an Amberlite XAD-2 vacuum column (10 × 2.5 cm) and eluted as previously described for water samples. The combined eluate in 100% methanol was evaporated under reduced pressure and then the residue was suspended again in 0.5 mL of methanol. This suspension was subjected to a silica gel column (E. Merck, Kieselgel 60, 230-400 mesh, 10 × 2.5 cm) preconditioned with MeOH-100%. After washing with 20 mL of MeOH 100%, microcystins were eluted with 25 mL of the solvent system H_2O/TFA/MeOH-(10: 0.1:89.9, v/v/v) (Tsuji *et al.* 1994, Harada 1996). This eluate containing microcystins was evaporated to dryness.

Planar Chromatography – The dried microcystin fraction was dissolved in a small volume of methanol and applied to silica gel plates (E.Merck, Kieselgel 60GF_{254}, 20 × 20 cm, 0.25 mm) according to Harada *et al.* (1988b). The positions of the microcystin spots in the plates were evaluated (Pelander *et al*. 2000). The microcystin-LR was isolated from a strain of *Microcystis panniformis* SPC 686 ("Coleção de Culturas de Algas e Cianobactérias do Instituto de Botânica"), purified and recognized by its mass spectrum (figure 1). ESI-MS analyses were carried out on a Q-TOF Micro (Micromass Ltd, Manchester, UK) mass spectrometer fitted with an electrospray ion source, operating in positive ion mode. The capillary voltage was 3-3.5 kV and sample cone voltages were 30-40 V.

Hepatotoxic water bloom in Guarapiranga reservoir – Cyanobacterial cells were obtained from a surface bloom collected in August 2001. The toxin extracted from freeze-dried cells in 0.9% (w/v) with NaCl solution (25.5 mg cells mL^{-1}) by repeated bath ultrasonication (4 x 30 sec, 50W). The extract was centrifuged (1045 x *g* 60 min) and analyzed in silica gel thin-layer chromatography, as previously described. The obtained supernatant was i.p. injected in seven Swiss-Webster male mice weighing 20 to 25 *g*. The doses ranged from 869.05 to 346.47 mg dry weight cyanobacteria kg^{-1} b.w. and the signs of poisoning and survival times were recorded. Postmortem necropsies were carried out.

Derivatization reaction with TMB – The developed plate was exposed to chlorine gas for 20 minutes in a glass tank. The chlorine gas was generated by mixing equal volumes of 10% HCl and 5% KMn O_4. After exposure, the plate was sprayed with TMB (3, 3, 5, 5-tetramethylbenzidin) solution that was prepared by dissolving 8 mg of TMB in 1.5 glacial CH_3COOH and 25 mL of H_2O and 50 mg of KI were added (Pelander *et al.* 2000). All chemicals were analytical-reagent grade.

Results and Discussion

Cyanobacterial biodiversity – The results showed that cyanobacterial biodiversity as well as the number of potentially toxic species are greater in Billings than in

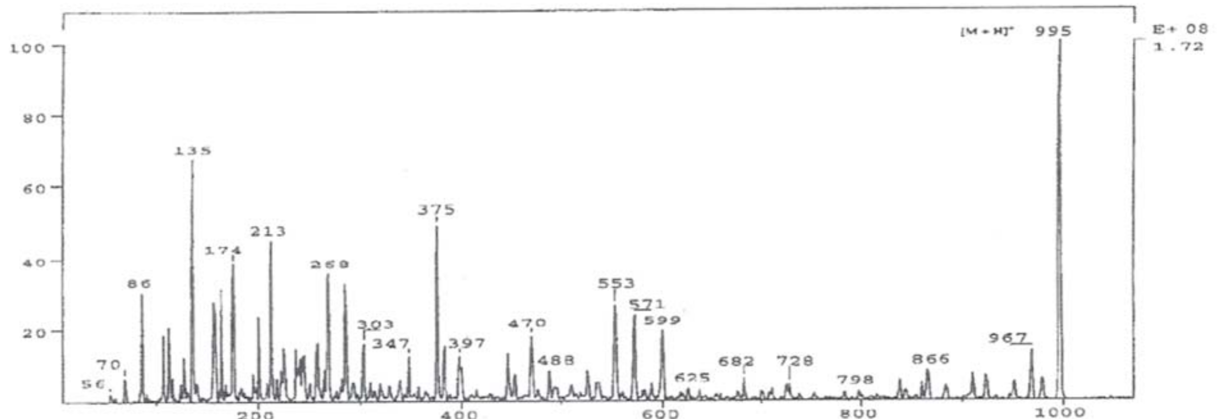

Figure 1. ESI-Q-TOF/MS spectrum of microcystin-LR. Molecular ion $[M+H]^+$ and characteristic fragmentation pattern can be observed.

Guarapiranga Reservoir (table 2). A first consequence is the strong probability to detect cyanotoxins in Billings water considering that 67% of the species collected in this reservoir are potentially toxic. In relation to the number of cyanobacteria species, in the Guarapiranga reservoir this possibility decreases to 50%. Furthermore, the cyanobacterial abundance is always high in Billings water and cyanobacterial blooms were always observed. The microcystin analyses confirm these results: presence of dissolved microcystin in Billings Reservoir during the whole studied period (table 3). On the other hand, in Guarapiranga reservoir the presence of dissolved microcystin was detected only once, in the sample containing *Microcystis* in 01/August/2001 (table 4).

Blooms of *Microcystis* species were found in

Table 2. Cyanobacteria species found in samples of Billings and Guarapiranga Reservoirs. * Potentially toxic species, according to Chorus & Bartram (1999).

Species	Billings	Guarapiranga
CHROOCOCCALES		
Aphanocapsa delicatissima West & G. S. West	+	+
Chroococcus sp.	+	+
*Microcystis aeruginosa** (Kützing) Kützing	+	+
*M. botrys** Teiling	+	
*M. novacekii** (Komárek) Compère	+	
*M. panniformis** Komárek	+	+
*M. protocystis** Crow	+	
*M. wesenbergii** (Komárek) Komárek	+	
OSCILLATORIALES		
Geitlerinema unigranulatum Komárek & M. T. P. Azevedo	+	+
Limnothrix planctonica (Woloszynska) Meffert	+	
Phormidium sp.		+
*Planktothrix agardhii** (Gomont) Anagnostidis & Komárek	+	+
Pseudanabaena galeata Böcher	+	
P. mucicola (Naumann & Huber-Pestalozzi) Schwabe	+	+
Romeria gracilis (Koczwara) Koczwara ex Geitler	+	
NOSTOCALES		
*Anabaena planctonica** Brunnthaler		+
Cylindrospermopsis raciborskii (Woloszynska) Seenayya & Subba Raju	+	+
Total	15	10

Table 3. Potentially toxic cyanobacteria isolated from Billings Reservoir and microcystins presence. *Most common species.

Date	Species	Presence of microcystins
20/Dec./2000	*Cylindrospermopsis raciborskii*, *Microcystis* sp., *Planktothrix agardhii*	+
17/Jan./2001	*Cylindrospermopsis raciborskii**, *Microcystis aeruginosa**, *Planktothrix agardhii*	+
14/Feb./2001	*Cylindrospermopsis raciborskii**, *Microcystis* sp.	+
10/Apr./2001	*Cylindrospermopsis raciborskii**, *Microcystis panniformis*	+
25/Apr./2001	*Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*	+
23/May/2001	*Cylindrospermopsis raciborskii**, *Microcystis aeruginosa*	+
06/Jun./2001	*Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *M. panniformis*	+
04/Jul./2001	*Microcystis aeruginosa**, *M. panniformis*, *M. protocystis*	+
01/Aug./2001	*Cylindrospermopsis raciborskii*, *Microcystis aeruginosa**, *M. panniformis**, *M. protocystis*, *M. novacekii*	+

Table 4. Potentially toxic cyanobacteria isolated from Guarapiranga Reservoir and microcystins presence. *Most common species.

Date	Species	Presence of microcystins
20/Dec./2000	*Cylindrospermopsis raciborskii*, *Microcystis* sp., *Planktothrix agardhii*	-
17/Jan./2001	*Anabaena planctonica*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *Planktothrix agardhii*	-
14/Feb./2001	*Cylindrospermopsis raciborskii*, *Phormidium* sp.	-
10/Apr./2001	*Phormidium* sp.	-
25/Apr./2001	-	-
23/May/2001	*Planktothrix agardhii*	-
06/Jun./2001	*Cylindrospermopsis raciborskii*, *Microcystis* sp.	-
04/Jul./2001	*Microcystis panniformis*	-
01/Aug./2001	*Anabaena planctonica*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa**, *M. panniformis*, *M. protocystis*, *Planktothrix agardhii*	+

Billings Reservoir throughout the studied period, as also previously reported by Carvalho (2003). Different results were obtained for Guarapiranga Reservoir where *Microcystis* species did not form blooms. The only exception was the *M. aeruginosa* bloom formed in August 2001, probably favored by the low level of water during the dry season and high nutrients concentration (Cetesb 2003).

Table 5 shows the *Microcystis* species associated with oscillatorian cyanobacteria found in the samples with positive results for microcystins, mainly in Billings Reservoir. It is worth mentioning that, except for *Planktothrix agardhii*, it is still unknown whether the other Oscillatoriales identified are able to produce toxins.

Based on its biodiversity, it is possible that the development of cyanobacteria is much more favored by physical and chemical conditions in Billings than those in Guarapiranga. Our results show clear differences between both reservoirs concerning the biodiversity of cyanobacterial species. However, it is difficult to predict how long this condition will remain because since 2000 the reservoirs have been connected by a channel that carries water from Billings to Guarapiranga (Cetesb 2003). Certainly this fact will change the species composition and density of the phytoplanktonic community of Guarapiranga Reservoir.

Chemical essays – Solid-phase extraction of microcystins from the culture and the water samples using Amberlite XAD-2.

To date, the clean-up and concentration of samples were mostly performed in octadecylsilanized silica (C_{18}) (Harada 1996, Meriluoto 1997) but the potential of stationary phases other than C_{18} has been evaluated for

Table 5. Cyanobacteria species found in samples with positive results for microcystins. *Microcystins producers species, according to Chorus & Bartram (1999),**Species kept in culture.

Species	Reservoirs	
	Billings	Guarapiranga
CHROOCOCCALES		
Aphanocapsa delicatissima	+	
Microcystis aeruginosa*	+	+
M. botrys*	+	
**M. novacekii*	+	
M. panniformis*	+	+
M. protocystis*	+	+
**M. wesenbergii*	+	
OSCILLATORIALES		
Geitlerinema unigranulatum	+	+
Limnothrix planctonica	+	+
Planktothrix agardhii*	+	+
Pseudanabaena galeata	+	
P. mucicola	+	+
Romeria gracile	+	

these procedures. Therefore, some phases like Oasis[â] HLB (Waters) and immunoaffinity columns were compared to C_{18} cartridges, with better performances than the latter (Rapala *et al.* 2002, Aranda-Rodriguez *et al.* 2003).

In the current work, the sorbent XAD-2 (Pietrzyk 1989), a neutral hydrophobic polymer, was tried as stationary phase for microcystins concentration. After experiments, the concentrating conditions were established for culture and water samples and a solvent elution scheme similar to that preconized by Pietrzyk (1989) was adopted. The solvent series are: deionized water; MeOH/ H_2O (20:80, v/v) and MeOH 100%.

Toxicity testing with fractions from culture of *M. panniformis* SPC 686: mice tested with aqueous and methanolic-aqueous (20:80, v/v) eluates from the Amberlite XAD-2 column did not show any sign of poisoning but methanolic (100%) fraction caused typical signs of microcystin poisoning (weakness, paralysis, piloerection, diarrhea, pallor of extremities and heavy breathing) and death after an average time of 1hour and 3 minutes.

Planar chromatography with material from culture and water sample: microcystins were detected in the methanolic eluates obtained from the XAD-2 columns, for culture and water sample, using silica gel thin-layer chromatography, under the conditions previously related.

These results appear to offer high potential for concentrating water samples when the purpose is to detect microcystin-LR.

The identity of the toxin from *M. panniformis* SPC 686 (microcystin-LR) is supported by the molecular ion at m/z 995.6 and by the characteristic series of ions present in the spectrum (Diehnelt *et al.* 2005).

Analysis of microcystins from reservoir water samples by planar chromatography (silica gel thin-layer chromatography) – Environmental samples contain a multitude of interfering contaminants. The clean-up process with octadecilsilanized silica is not always sufficient to eliminate the impurities and avoid background interferences in chromatograms (Harada 1996, Pelander *et al.* 2000). So an additional clean-up process is required and the treatment with silica gel is very effective for this purpose (Harada 1996).

Thin-layer chromatographic methods have been widely employed for the analysis of microcystins and for the isolation and purification of authentic microcystin standards. It is a rapid, simple and inexpensive procedure that effectively enables the detection of these toxins (Poon *et al.* 1987, Harada *et al.* 1988b, Meriluoto 1997, Pelander *et al.* 1998, 2000). The detection limit for the purified microcystins is 1 ng. For microcystins in water or culture samples it was found to be 1 mg L^{-1}; this higher limit is due to interference from other substances, in natural samples (Harada *et al.* 1988b, Meriluoto 1997, Pelander *et al.* 1998, 2000).

Figures 2-3 and table 5 show the results obtained from qualitative planar chromatographic analysis of water samples from Guarapiranga and Billings Reservoirs. Microcystins were detected in all Billings Reservoir samples and in only one sample from Guarapiranga Reservoir.

Hepatotoxic water bloom occurred in August 2001, in Guarapiranga Reservoir – All animals injected with the toxic extract presented symptoms of poisoning similar to those described for microcystins and died in a time ranging from 50 minutes to two hours and five minutes. Upon autopsy, swollen and blood-engorged livers were observed. These results confirm the toxicity of this bloom.

This study showed a constant incidence of microcystins in Billings Reservoir and a single occurrence (August 2001) in Guarapiranga Reservoir (table 2) and these results are coincident with the biological studies, indicating the toxicity of *Microcystis* species in both reservoirs. It was also possible to identify 17 species of cyanobacteria, 9 of them considered potentially toxic. *Microcystis aeruginosa* and

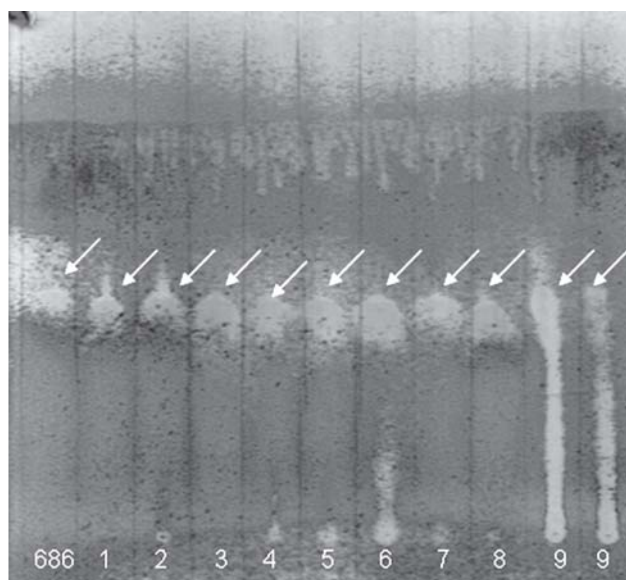

Figure 2. Chromatogram (planar chromatography) of 686 (purified microcystin-LR) and of Billings samples; 1 (20/Dec./2000), 2 (17/Jan./2001), 3 (14/Feb./01), 4 (10/Apr./2001), 5 (25/Apr./2001), 6 (23/May/2001), 7 (06/Jun./2001), 8 (04/Jul./2001) and 9 (01/Aug./2001). Mobile phase: $CHCl_3$/MeOH/H_2O (65:35:10, v/v/v, lower layer). Detection: TMB (3, 3, 5, 5-tetramethylbenzidin). Arrows indicate the microcystin-LR spots.

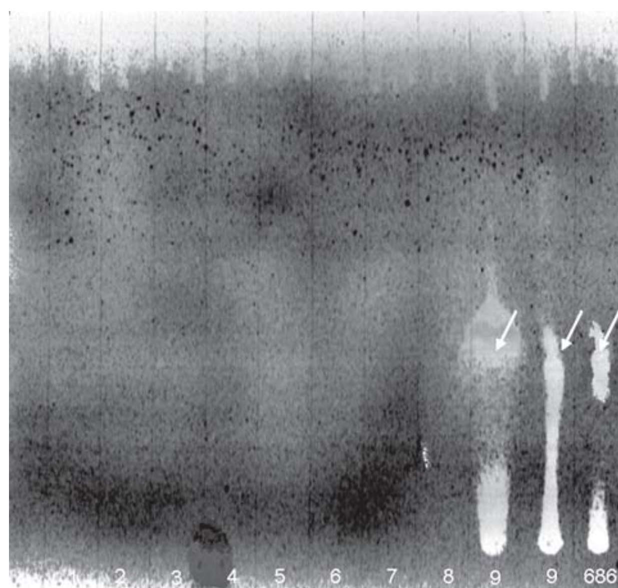

Figure 3. Chromatogram (planar chromatography) of Guarapiranga samples; 1 (20/Dec./00), 2 (17/Jan./01), 3 (14/Feb./01), 4 (10/Apr./01), 5 (25/Apr./01), 6 (23/May/01), 7 (06/Jun./01), 8 (04/Jul./01) and 9 (01/Aug./01) and of 686 (purified microcystin-LR). Mobile phase: $CHCl_3$/MeOH/H_2O (65:35:10, v/v/v, lower layer). Detection: TMB (3, 3, 5, 5-tetramethylbenzidin). Arrows indicate the microcystin-LR spots.

Cylindrospermopsis raciborskii were the most common species in Billings Reservoir, while Reservoir only *M. aeruginosa* was considered a common species in Guarapiranga.

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