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Phytoplankton composition, biodiversity and a pilot survey of toxic cyanoprokaryotes in a large cascading reservoir system (Tietê basin, Brazil)

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Introduction

Eutrophication has been one of the most apparent threats to surface waters throughout the world. It has become an acute problem in many tropical countries. Demographic growth and social problems have created unplanned and huge cities without sanitation facilities and expanding industrial parks with ineffective environmental protection laws which, among many other factors, have contributed to the degradation of almost all available fresh water (ROCHA et al. 1997).

Starting at the upper-middle Tietê river just before its entrance into the Paraná river (SE Brazil), the reservoirs of Barra Bonita, Bariri, Ibitinga, Promissão, Nova Avanhandava, Três Irmãos, Jupiá and Ilha Solteira form a linked cascade of large (>100 km^2 except Bariri) and mostly shallow man-made lakes (8–40 m depth) built from the late 1960s to deal mainly with the rapidly growing energy demand of this region. This region possesses the highest demographic density in the country, and includes, in the State of São Paulo alone, 2,300 industries demanding 113 $m^3 s^{-1}$ of water. This amount is further increased by irrigation to supply an ever-expanding agriculture industry – cattle-raising (AGOSTINHO et al. 1995) – and also to cater for other uses such as flood regulations, navigation, recreation, and water supply.

As a consequence of inadequate soil management, heavy use of agricultural chemicals and elimination of considerable areas of natural vegetation, there has been an increasing degradation of the waters with still unknown consequences on the natural resources. These reservoirs receive considerable loads of nutrients mainly due to discharges of untreated sewage and industrial effluents, amounting to 25 $m^3 s^{-1}$ (TUNDISI et al. 1993). Despite exhibiting secondary thermoclines and potentially being able to stratify during summers depending on their depths, permanent stratification is unlikely to occur. This is mainly due to operational routines (flushing out) associated with wind action, and is facilitated by relative shallowness and a lack of wind barriers along their shores. Most of these reservoirs were characterised as polymictic (TUNDISI 1990).

The aim of this study was to assess phytoplankton biomass and biodiversity changes along the reservoirs. Barra Bonita, the first of this reservoir cascade, is one of the aquatic systems in Brazil which have been thoroughly studied recently. It is characterised as polymictic, and is in an advanced state of eutrophication (OLIVEIRA 1997). Little information was available about the phytoplankton of the other reservoirs. Until the early 1990s, phytoplankton of the Barra Bonita Reservoir had been repeatedly reported as being dominated by cryptophytes, green algae and diatoms (HENRY et al. 1985, OLIVEIRA 1997). More recently, proliferation of cyanobacteria was observed not only in this reservoir but also in some others downstream. For this reason, a preliminary survey of actual and potential cyanotoxicity was also carried out.

Study sites, materials and methods

Vertically integrated (whole water column) samples were taken from between four and eight sampling stations at each reservoir between 2 and 11 February 1998. Dissolved oxygen, conductivity and temperature were measured with a portable profile sensor. Averages of records are used in this paper. Phytoplankton samples were preserved with formaldehyde up to 3–4% end concentration. Samples for chlorophyll *a* and toxin analyses were filtered through GF/F glass fibre and nucleopore (pore size: 0.2 µm) filters and kept frozen until subsequent laboratory analyses. Dense cyanoprokaryote samples were collected by means of a plankton net (10 µm mesh size), deep frozen and subsequently freeze-

dried for toxicity tests. Such freeze-dried material was collected from reservoirs Promissão, Ibitinga, Bariri and Barra Bonita, however, only materials from the latter two reservoirs proved to be sufficient for comparative toxicity tests. Freeze-dried material from the Paranoá Reservoir (Brasília, Federal District, date of collection: 28 January, 1998) was also tested for toxicity using a plant test.

Phytoplankton was counted under an inverted microscope. A minimum of 400 settling units was counted in each sample giving a counting accuracy of ±10%. *Microcystis* spp. were counted in separate sedimentation chambers after 15 s ultrasonication. Diversity was calculated by the Shannon–Weaver function (SHANNON 1948) based on $\log_2$.

Cyanobacterial isolates were established from plankton samples by frequent transfers using combined nitrogen free (-NO_3) liquid (ALLEN 1968) medium and/or media solidified with agar. The isolates were deposited into the cyanobacterial strain collection BGSD of the Botanical Department of the Kossuth University, Debrecen. The isolates were cultivated in Erlenmeyer flasks (100 mL) on orbital shakers or in 5–10 L cultures bubbled with sterile air at 25 °C and 100–150 μE m^{-2} s^{-1} PAR.

For cyanotoxin analysis, lyophilised seston or thawed filters were extracted with 1.5 mL of 75% 50–50 aqueous methanol according to the method described by FASTNER et al. (1998). Dried extracts were stored at –20 °C until HPLC analysis. Prior to HPLC analysis, the extracts were resolved in 50% (v/v) aqueous methanol. The detection of microcystins was performed with a 616 Waters solvent delivery system, a 717 WISP autosampler and a 991 photo diode array detector (Waters, Eschborn, Germany). Extracts were separated on a LiChrospher® 100, ODS, 5 μm, LiChroCART® 250-4 cartridge system (Merck, Darmstadt, Germany) using a gradient of aqueous acetonitrile (with 0.05% trifluoroacetic acid) with a flow of 1 mL min^{-1}. UV-spectra were obtained from 200–300 nm, and microcystins identified by their retention time, characteristic UV-spectra (UV_{max} 240 nm) and coinjection of standard microcystins. Microcystins were quantified at 240 nm using microcystin-LR, -YR and -RR (Calbiochem, Bad Soden, Germany) as external standards.

For toxicity tests primary rat hepatocytes were obtained by perfusion of fresh rat livers to test specifically for hepatotoxicity, and a CHO-K1 cell line was used for assessing general cytotoxicity. Cells were seeded into microtiter well plates and exposed to aqueous extracts of seston for 20 h. Viability was assessed with the MTT test as described by HEINZE (1996) and LC_{50} values were calculated.

Blue–green's *Sinapis* toxicity test (BGST, mustard test) was performed as described by KÓS et al. (1995). Briefly, mustard seeds (*Sinapis alba* L.) were sterilised with 5% H_2O_2 solution and washed several times with sterile distilled water. The sterile seeds were deposited onto the surface of a plant nutrient solution in a cupped test tube solidified with 0.6% agar (Bacto) and supplemented with cyanotoxin-containing samples (e.g. freeze-dried samples and whole cells suspension after freezing at –20 °C). Seeds were grown at least in three parallels and were kept at 25 ± 1 °C in darkness. The mean length of seedlings, hypocotils and roots were measured after 3 days and the IC_{50} (IC, inhibitory concentration) were calculated, respectively. Since the fresh weight of the isolated cyanobacterial strains might be different, the IC_{50}s were calculated on the basis of chlorophyll *a*.

The Thamnotoxkit F™ assay is based on the species *Thamnocephalus platyurus*, a freshwater crustacean that can be stored in cyst form. Before the Thamnotox test was taken, the cysts had been hatched in water under continuous illumination and at 25 °C. Immediately prior to toxicity assays a dilution series was prepared from the freeze-dried samples. Repeated freezing and melting (twice) were used as treatment for the water soluble cyanotoxins. The concentration series used for each sample were 0.1, 0.3, 0.5, 1.0 and 3.0 mg mL^{-1}. Mortality was determined after 24 h of exposure in darkness. Three parallels based on each sample were observed, containing 1 mL of the sample solution with ten larvae in each. The result was counted as negative when mortality was below 10%.

Results

Physical and chemical variables

In February 1998, the waters ranged between slightly acidic to alkaline (pH 6.20–8.46), average conductivity for the Tietê reservoirs was between 105 and 182 μS cm^{-1} but much lower for Jupiá and Ilha Solteira (43–51 μS cm^{-1}) and oxygen levels showed a smooth decrease with depth, although oxygen was still present within the bottom waters in all the reservoirs. Within the upper layers (0–5 m) its values ranged as follows: 4.4–5.0 mg L^{-1} (Barra Bonita), 3.3–7.0 mg L^{-1} (Bariri), 2.6–5.6 mg L^{-1} (Ibitinga), 9.0–9.3 mg L^{-1} (Promissão), 5.9–6.8 mg L^{-1} (Nova Avanhandava), 6.8–6.9 mg L^{-1} (Três Irmãos), 7.6–8.0 mg L^{-1} (Jupiá), and 0.0–7.6 mg L^{-1} (Ilha Solteira). This last reservoir exhibited complete oxygen depletion within the upper layers (0–4 m depth) with water temper-

atures oscillating between 29.8 °C and 32.4 °C throughout the day. On the other hand, the layers in between 4 and 13 m depth showed oxygen levels ranging from 7.6 to 5.5 mg L^{-1} at water temperatures oscillating between 29.1 and 29.7 °C, and even near to the bottom (17 m) traces of oxygen such as 0.30 mg L^{-1} were recorded.

Well-defined stratification patterns were not recorded in any of these reservoirs although the presence of secondary thermoclines was evident. Temperature differences between surface and bottom ranged from 0.5 to 3.4 °C, including even rather shallow waters, since a difference of only 3.4 °C over 17 m was recorded for Ilha Solteira.

In February 1998, the total phosphorus levels within these reservoirs ranged from 22.8 µg L^{-1} (Três Irmãos) to 63.6 µg L^{-1} (Ibitinga). Considering the reservoir cascade, an interesting feature is evident, the higher levels within the upper three reservoirs (48.9–63.6 µg L^{-1}) and lower levels within the final three (22.8–27.9 µg L^{-1}) are probably a consequence of specific watershed differences in morphometric features, retention time and external loads.

The chlorophyll *a* concentrations (not corrected for phaeophytin *a*, Fig. 1b) show a similar pattern, with values ranging from 43 to 55 µg L^{-1} in the upper reservoirs and a considerable decrease in the lower ones (3–7 µg L^{-1}).

Phytoplankton flora

Species numbers (Table 1) obtained during quantitative investigations do not show marked differences (42–66 species per reservoir). During the supplementary qualitative microscopic studies a number of additional species were found in reservoirs Ilha Solteira, Jupiá and Promissão. These additional species belonged almost exclusively to Chlorococcales and Desmidiales. It has to be noted that among the *Microcystis* spp. predominant in the three uppermost reservoirs nine morphologically different forms were found, however, only *M. wesenbergii* was readily identifiable.

Shannon diversity (Table 1) was highest (3.18) in Barra Bonita, the first in the cascade, then dropped to a low level (1.64–1.78) in the three subsequent reservoirs. An increase in compositional diversity can be observed in Nova Avanhandava (2.53). In Três Irmãos, the lowest level (1.09) was found. In adjacent reservoirs, Ilha Solteira and Jupiá, of the Paraná basin, compositional diversity increased (2.53).

A marked change in dominant species can be found along the reservoir cascade (Fig. 1a, Table 1). In the Barra Bonita Reservoir unicellular centric diatoms dominated (38% of biomass) with a considerable *Microcystis* (34%) population and with many subdominants (*Planktothrix* 4%, *Aphanizomenon issatschenkoi* 3%, *Cylindrospermopsis raciborskii* 3%, *Eutetramorus tetrasporus* 3% and *Aulacoseira granulata* 5%). In the adjacent Bariri Reservoir, overwhelming dominance of *Microcystis* spp. (75%) is characteristic with some *Aulacoseira* (4%). In the Ibitinga, *Microcystis* reached the highest dominance (78%) and there was no other species that exceeded the 3% contribution to total biomass. Quite a marked change occurred in the Promissão Reservoir where *Microcystis* spp. (12%) were replaced by *Coelastrum reticulatum* var. *cubanum* (73%) and an unidentified *Staurastrum* sp. (6%) was subdominant. Records of the Nova Avanhandava Reservoir are quite similar (*Microcystis* 9%, *Cylindrospermopsis* 4%, *Coelastrum* 86% and *Staurastrum* sp. 7%). Cyanobacteria became negligible in the Três Irmãos Reservoir where *Coelastrum* reached an overwhelming dominance (86%) with *Eutetramorus policoccus* being subdominant (5%). In the two reservoirs (Jupiá and Ilha Solteira) belonging to the Paraná basin *Coelastrum* also predominated in the phytoplankton (61% and 48%). *Eutetramorus policoccus* were subdominant in both reservoirs (9% and 4%). Contributions of different desmids (*Cosmarium reniforme* in the Jupiá; *Staurastrum chaetoceras* and *S. planktonicum* in the Ilha Solteira) were also significant. Besides, *Peridinium cinctum* (Jupiá, 6%) and *Aulacoseira granulata* (Ilha Solteira, 4%) reached higher amounts.

Microcystin content and toxicity tests

In the four freeze-dried materials from the four upstream reservoirs with a large or relatively large fraction of cyanoprokaryota, microcystin-

Table 1. Morphometric parameters and phytoplankton data from the Tietê and Paraná basin reservoirs (species numbers were obtained from both qualitative and quantitative investigations).

Reservoir	Barra Bonita	Bariri	Ibitinga	Promissão	Nova Avanhandava	Três Irmãos	Jupiá	Ilha Solteira
Area (km^2)	310	63	114	741	210	817	330	1195
Average depth (m)	10.1	8.6	8.6	14.0	13.0	17.2	11.2	17.6
Year of completion	1964	1969	1969	1975	1985	1991	1974	1978
Retention time (days)	37–137	7–24	12–43	124–458	32–119	166–615		
TP µg L^{-1}	51.8	48.9	63.6	27.9	23.1	22.8	24.6	
Species number								
Chroococcales	9	12	11	12	8	6	13	7
Oscillatoriales	8	8	8	8	5	3	7	8
Nostocales	6	4	5	3	3	1	2	2
Tetrasporales	1	3	1	1	2	2	2	1
Phytomonadina				1			1	1
Chlorococcales	21	20	24	32	18	21	45	26
Desmidiales	1	1	1	12	8	5	31	12
Ulothrichales		1		2	1	1	2	2
Chamaesiphonales							1	
Euglenophyta				4		1	1	2
Xanthophyceae		1	1					
Chrysophyceae	1	2	1	2	1	2	2	1
Cryptophyceae	3	2	3	3	2	2	2	2
Pyrrhophyta		1		1	1		2	2
Centrales	6	5	6	7	5	5	7	7
Pennales	1	5	4	7	10	2	11	8
Total species number	57	65	65	95	64	51	129	81
Species number from quantitative studies	53	59	58	48	52	49	66	42
Shannon diversity	3.18	1.88	1.78	1.64	2.53	1.09	2.53	2.53
Chlorophyll *a* (µg L^{-1})	42.57	55.77	54.9	17.11	10.98	6.70	4.52	3.02
Contribution of dominant (>3%) species to total phytoplankton biomass								
Microcystis spp.	34	75	78	12	9			
Planktothrix sp.	4							
Aphanizomenon issatschenkoi	3							
Cylindrospermopsis raciborskii	3				4			
Coelastrum reticulatum var. *cubanum*				73	67	86	61	48
Eutetamorus policoccus						5	9	4
E. tetrasporus	3							
Cosmarium reniforme							4	
Staurastrum chaetoceras								22
S. planktonicum								8
Staurastrum sp.				6	7			
Peridinium cinctum							6	
Aulacoseira granulata	5	4						4
Unicellular centric diatoms	38							

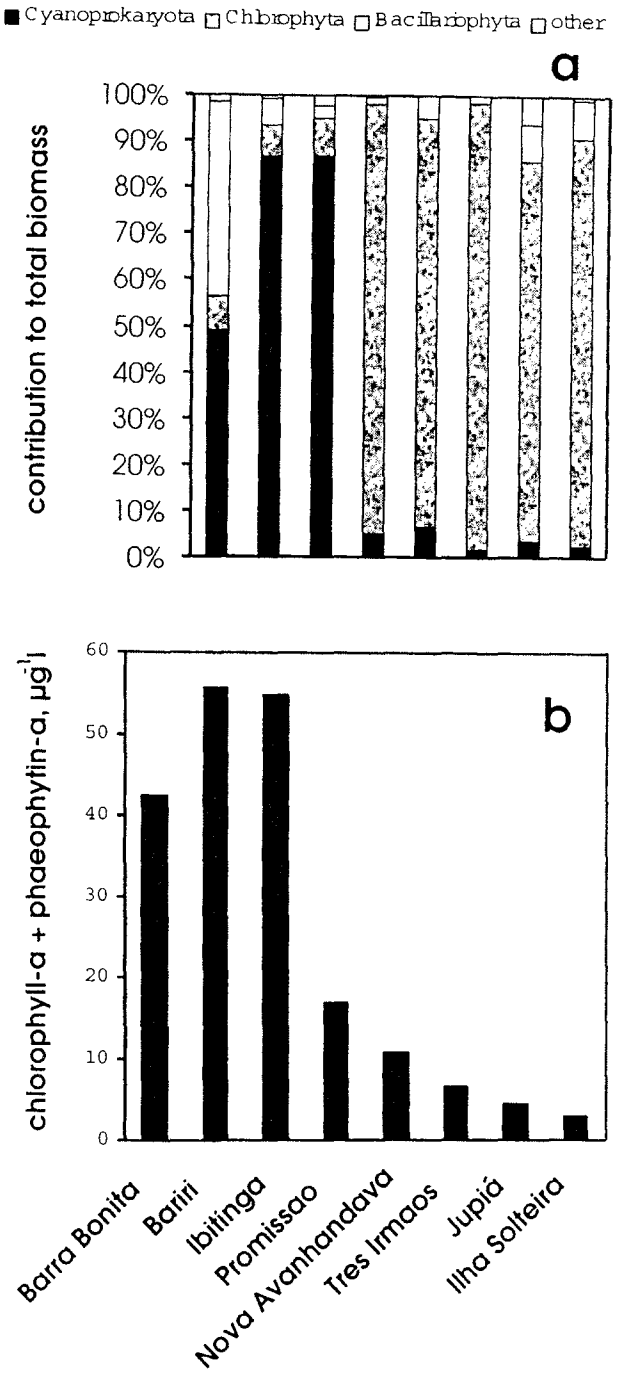

Fig. 1. (a): Percentage distribution of phytoplankton biomass in main algal groups; (b): chlorophyll *a* (not corrected for phaeophytin) content along the reservoir cascade in February 1998.

RR was found in the highest concentration (12.5–18.8 mg g^{-1}), concentrations of microcystin-LR (0.0–0.2 mg g^{-1}) and microcystin-YR (1.6–3.0 mg g^{-1}) were considerably lower according to HPLC analyses (Table 2).

The freeze-dried material had a definite toxic effect for rat hepatocyte-, *Thamnocephalus*- and mustard (BGST)-tests, but no cytotoxicity on the CHO-K1 cell line was registered (Table 2).

For the isolated cyanobacterial strains the IC_{50}s from the BGST-test were calculated on the base of chlorophyll *a* and according to the data all strains had an inhibitory effect (Table 3).

Discussion

Phytoplankton floras of rivers are usually dominated by diatoms and chlorococcalean green algae (REYNOLDS et al. 1994), and South American rivers often support a rich planktonic desmid flora. Cyanobacteria, outside Australia, are not characteristic elements of river phytoplankton. Dominance of different species of the genus *Aulacoseira* has been repeatedly reported from turbid South American rivers (e.g. O'FARRELL et al. 1996 and literature cited within) and even from river impoundments (LUDWIG et al. 1997). Almost none of these characteristics were observed along the cascade of reservoirs in the basin of the Tietê river. The river carries a phytoplankton dominated by unicellular Centrales into the Barra Bonita, the first reservoir in the series. In this reservoir, as experienced in other cases (PRYGIEL & LEITAO 1994), the development of an assemblage dominated by cyanobacteria (in this case *Microcystis* spp.) occurs as a consequence of the combined effect of 1) reduced water flow which results in a retention time of at least 37 days, 2) increased sedimentation of inorganic turbidity, therefore, a better light climate and 3) nutrient-rich headwaters. Proliferation of cyanobacteria becomes even more prominent in the two subsequent reservoirs (Bariri and Ibitinga). A transition occurs in the two middle-stretch reservoirs (Promissão and Nova Avanhandava) where *Microcystis* spp. are outcompeted by the tropical, chlorococcalean green alga, *Coelastrum reticulatum* var. *cubanum*. In the last reservoir (Três Irmãos) belonging exclusively to the Tietê basin, this species reaches an overwhelming dominance. The Ilha Solteira Reservoir, first in the Upper Paraná River, was also dominated by *C. reticulatum* var. *cubanum* with a significant amount of planktonic desmids being co-dominant. These two assemblages are mixed in the

Table 2. Toxin analytical data and toxicity in different tests of freeze-dried materials deriving from different Brazilian reservoirs (n.t.: not tested).

	Barra Bonita	Bariri	Ibitinga	Promissão	Paranoá
Microcystin-LR (mg g^{-1}), HPLC	0.0	0.0	0.2	0.2	n.t.
Microcystin-RR (mg g^{-1}), HPLC	12.5	14.8	18.8	16.0	n.t.
Microcystin-YR (mg g^{-1}), HPLC	2.2	2.7	3.0	1.6	n.t.
IC_{50} (mg mL^{-1}) in BGST test	2.0	2.0	n.t.	n.t.	4.0
LC_{50} (mg mL^{-1}) in rat hepatocyte test	0.05	0.14	0.05	0.19	n.t.
Microcystin-RR (mg g^{-1}),as calculated from rat hepatocyte test	16.00	5.71	16.0	4.21	n.t.
Cytotoxicity in cell-line CHO-K1 in 0–1.67 mg mL^{-1} conc. range	non-toxic	non-toxic	non-toxic	non-toxic	n.t.
LC_{50} (mg mL^{-1}) in *Thamnocephalus* test	0.55	0.40	n.t.	n.t.	n.t.

Table 3. IC_{50} of cyanobacterial strains isolated from Rio Tietê region in BGST tests on the basis of chlorophyll *a* content.

Isolated species	Origin of isolate	Strain N°	IC_{50} (μg mL^{-1})
Microcystis spp.	Ibitinga	BGSD 300	100
Microcystis spp.	Promissão	BGSD 301	40
Microcystis spp.	Nova Avanhandava	BGSD 302	30
Cylindrospermopsis raciborskii	Nova Avanhandava	BGSD 303	15
C. raciborskii	Promissão	BGSD 304	20
Aphanizomenon aphanizomenoides	Bariri	BGSD 305	30
A. issatchenkoii	Bariri	BGSD 306	60

Jupiá Reservoir, which shows the richest flora in the area. Compositional diversities indicate two monospecific equilibrium stages (sensu SOMMER et al. 1993) with minimal diversities: one is dominated by *Microcystis* spp. (Bariri, Ibitinga) the other by *Coelastrum reticulatum* var. *cubanum* (Três Irmãos). Diversities are higher in reservoirs where either different floras are mixing (Barra Bonita, Jupiá) or that are in transitional position (Nova Avanhandava). Available literature (DOS SANTOS & CALIJURI 1997, OLIVEIRA 1997) suggests that phytoplankton assemblages in other seasons and/or in other years might be remarkably different. However, late summer data are of special importance since this period can be qualified as the low-disturbance state of the system with low resources and more opportunity for temporal stratification (MATSUMURA-TUNDISI & TUNDISI 1997).

Comparing TP of different reservoirs to the OECD (1982) scale, the first three (Barra Bonita, Bariri and Ibitinga) can be qualified as eutrophic, the others as mesotrophic. These TP data do not indicate very eutrophic conditions, thus suggesting the occurrence of nutrient losses, probably precipitation to the sediments. This hypothesis was suggested by PEDROSO et al. (1988) who postulate a precipitation of ferric phosphate onto the sediments, facilitated by the high rates of erosion of lateritic soils, estimated as being as high as 20 tons ha^{-1} $year^{-1}$. Supporting this hypothesis, TUNDISI et al. (1993) suggested denitrification processes within the wetlands associated with the reservoirs or in the littoral macrophyte stands as a possible source of nitrogen depletion. Nevertheless, because heterocytic cyanoprokaryota were negligible by amount, N-deficiency was not likely to occur. Furthermore, high levels of phosphate in the sediments (513.6 ppm) were recorded by ESTEVES (1983) in Barra Bonita Reservoir.

However, there was only a three-fold difference between the lowest and highest TP concentrations, while the difference increases to 18-fold in terms of chlorophyll *a*. These data allow us to conclude that in the upper reservoirs

the major part of TP is found either dissolved or bound to algal cells and that in the lower ones a richer and more complex trophic structure is supported by primary producers.

In addition to the general negative impact of eutrophication on water quality and options for water use, the presence of dense cyanobacterial populations implies danger of cyanotoxicity with its consequences for human and animal health if the water is consumed.

In natural samples, different combinations of microcystins can be found. For example, in a *Microcystis* bloom from Homer Lake USA, 19 different microcystins were characterised (NAMIKOSHI et al. 1992, 1995). Microcystin-LR is often mentioned as the most frequently occurring microcystin. It has been reported to be the major toxin in bloom and strain samples from Portugal (VASCONCELOS et al. 1995, 1996), France (VEZIE et al. 1997), Canada (KOTAK et al. 1993) and frequently co-occurring with microcystin-RR and -YR in Japan (WATANABE et al. 1988, 1989). In Hungary the situation is similar to Japan where microcystin-LR are accompanied by microcystin-RR and -YR (TÖRÖKNÉ et al. 2000). However, in an Australian bloom of *Microcystis aeruginosa*, 23 microcystins were quantified by HPLC, none of which were microcystin-LR (JONES et al. 1995), and in Finland, other microcystins dominate over microcystin-LR (SIVONEN & JONES 1999). The Brazilian samples also contained very little or no microcystin-LR. SIVONEN & JONES (1999) assume different microcystin patterns to reflect regional differences in dominance of cyanobacterial species or strains.

The microcystin levels found in the Brazilian samples were remarkably high: a recent literature review reported 7 mg g^{-1} as the highest published concentration (SIVONEN & JONES 1999). The highest recorded total microcystin level in the sample collected from Lake Velencei, Hungary was 2.21 mg g^{-1} (microcystin-LR 0.71 mg g^{-1}, microcystin-YR 1.50 mg g^{-1}; TÖRÖKNÉ et al. 2000). A German survey of 51 samples dominated by *Microcystis* spp. showed maximal concentrations of 2.2 mg g^{-1} and a median value of 0.8 mg g^{-1} (FASTNER et al. 1999). In contrast, the microcystin levels of 12.5–18.8 mg g^{-1} reached in the upstream reservoirs along the Rio Tietê were extremely high. However, these consisted largely of microcystin-LR, which is distinctly less toxic than the other two variants typical for *Microcystis* spp., microcystin-LR and microcystin-YR. This does not only apply for mouse bioassays, but also for isolated primary rat hepatocytes: after 20 h incubation, microcystin-RR shows an LC_{50} of 0.8 µg mL^{-1}, whereas microcystin-LR shows an LC_{50} of 0.05 µg mL^{-1} and microcystin-YR shows an LC_{50} of 0.08 µg mL^{-1} (HEINZE 1996).

All of the isolates of Brazilian origin (Rio Tietê region) were toxic in plant tests. For *Microcystis* and *Cylindrospermopis* strains (BGSD 300–304), IC_{50} data are comparable to those isolated from different waters in Central Europe (KÓS et al. 1995). It is worth noting that the BGSD 303 and 304 *Cylindrospermopsis* strains isolated from reservoirs Nova Avanhandava and Promissão were more toxic than the BGSD 266 isolated from Lake Balaton, Hungary (BORBÉLY et al. 1997). *Aphanizomenon aphanizomenoides* and *A. issatschenkoi* were found to be toxic first time. In rat hepatocyte tests the toxicity of the samples from Ibitinga and Barra Bonita was very high in relation to many samples from Germany (HEINZE et al. 2001), however, this corresponds well to the high content of microcystin-RR. For these two reservoirs, calculation of the microcystin-RR content from the LC_{50} of these samples and the LC_{50} of pure microcystin-RR shows good agreement with the concentrations determined by HPLC (Table 2).

This reservoir cascade plays an important role not only in providing the aforementioned services but the reservoirs also function as effective "storing agents" of considerable loads of nutrients, particularly at the upper-middle Tietê stretch, thus contributing to the better water quality downstream of the cascade, as demonstrated by total phosphorus decrease recorded for Promissão, as compared to the levels recorded for Barra Bonita, Bariri and Ibitinga, upstream. AGOSTINHO et al. (1995) pointed out a reduction of ca. 60% of the total P concentration comparing levels upstream and down-

stream of Itaipu Reservoir, the last reservoir in the Paraná River within Brazilian territory, following this cascade. However, water quality of upstream reservoirs including prevalent blooms of toxic cyanobacteria must not be disregarded.

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