

Population dynamics of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju, a Cyanobacteria toxic species, in water supply reservoirs in São Paulo, Brazil

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ABSTRACT - (Population dynamics of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju, a Cyanobacteria toxic species, in water supply reservoirs in São Paulo, Brazil). The Guarapiranga and Billings reservoirs are main sources of public water supply to millions of people in the city of São Paulo, Brazil. They have been under intense anthropic action as a result of domestic, industrial, farm and livestock waste being dumped in the reservoirs. Cyanobacteria develop very well in such an environment, producing blooms that are most often toxic. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju is a toxic species which is rapidly spreading all over the world and is abundant in the studied reservoirs. The goal of the study was to follow the year-round variation of the *C. raciborskii* population density and to correlate it with selected environmental factors. Samples were collected monthly on the surface of the water column and studied under a binocular optic microscope, whereas quantitative studies were carried out under an inverted microscope, according to the Utermöhl method. Among the phytoplankton community, organisms of the Cyanobacteria Class were represented by the greatest population density (cells mL⁻¹). *Cylindrospermopsis raciborskii* was one of the abundant species in the Billings reservoir, both in the dry and rainy season. The principal environmental factors that influenced *C. raciborskii* population dynamics were water temperature, high pH values and low euphotic zone values.

Key words: bloom, *Cylindrospermopsis raciborskii*, eutrophic reservoirs, phytoplankton

RESUMO - (Dinâmica populacional de *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju, uma espécie tóxica de Cyanobacteria em reservatórios de abastecimento, SP, Brasil). Os Reservatórios Guarapiranga e Billings integram uma das principais fontes de abastecimento público da cidade de São Paulo, suprindo água para milhões de pessoas. Esses reservatórios estão sofrendo intensa ação antrópica devido a despejos domésticos, industriais e agropecuários. Em tais ambientes, Cyanobacteria desenvolvem-se intensamente, formam florações que, na maioria das vezes são tóxicas. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju é uma espécie tóxica e em expansão em todo o planeta, sendo uma das espécies abundantes nos reservatórios estudados. O objetivo do trabalho foi acompanhar a variação da densidade da população de *C. raciborskii* ao longo do ano, relacionando com os fatores ambientais estudados. As coletas mensais, realizadas na superfície da coluna d'água, foram analisadas ao microscópio óptico binocular e a análise quantitativa foi feita ao microscópio invertido, conforme método de Utermöhl. A maior densidade (cel mL⁻¹) da comunidade fitoplanctônica observada, foi representada por organismos da classe Cyanobacteria, em ambos os reservatórios. *Cylindrospermopsis raciborskii* foi uma das espécies abundantes no Reservatório Billings, nas estações seca e chuvosa. Os principais fatores ambientais que influenciaram na dinâmica de *C. raciborskii* foram: temperatura da água e pH elevados, assim como baixos valores da zona eufótica.

Palavras-chave: *Cylindrospermopsis raciborskii*, fitoplâncton, florações, reservatórios eutróficos

Introduction

The Guarapiranga and Billings reservoirs are components of one of the main sources of public water supply in the metropolitan area of the city of São Paulo, providing water to millions of people and hundreds of industrial plants. Those reservoirs, like

all reservoirs located near large urban centers, suffer intense anthropic action due mostly to the discharge of household and industrial waste water and fertilizer, and to deforestation of surrounding areas.

According to Zagatto *et al.* (1997), high nutrient concentration, mostly phosphates and nitrogen compounds, added to light intensity, water temperature

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(between 15 and 30 °C) and pH values between 6 and 9, together favor the multiplication of phytoplankton species, leading to blooming.

Cyanobacteria blooms have several environmental, social and economic consequences on continental waters around the globe and their ability to produce toxins also causes important losses to the public health sector. The competitive success of Cyanobacteria is based on their adaptive strategies, both physiologic and ecologic, which enable them to remain in the euphotic zone and to regulate their position within the water column in order to take greater advantage of light and nutrients (Klemer 1991).

Cylindrospermopsis raciborskii (Woloszynska) Seenayya & Subba Raju, in particular, has been widely studied (Isvánovics *et al.* 2000, Padisák 1997, Padisák *et al.* 2003, Sant'Anna *et al.* 2006) because it produces blooms that interfere with water utilization and contains hepatotoxins (cyclic guanidinic alkaloid) and neurotoxins (carbamate-type alkaloids). The cylindrospermopsin-type hepatotoxin inhibits protein synthesis, thus damaging the structure and leading to necrosis of the liver, as well as of the kidneys, heart, lungs and gastric mucosa. The saxitoxin-type neurotoxin (known as Paralytic Shellfish Poison or PSP in short) blocks sodium channels in nerve cells, leading to paralysis, hypotension and respiratory failure (Kuiper-Goodman *et al.* 1999, Sant'Anna *et al.* 2006).

Padisák (1997) analyzed data about the occurrence, ecology, migratory patterns and distribution of *Cylindrospermopsis raciborskii* on four continents, and observed its dominance in different environments, resulting from its many abilities to compete and to resist herbivore organisms. According to Padisák (1997), the ecologic success of *C. raciborskii* is attributed to different factors such as floatability, tolerance to low light levels and high capacity to assimilate ammonium and phosphate. The occurrence and migratory patterns of *C. raciborskii* suggest that its wide distribution through tropical, subtropical and temperate zones is due to its great ability to disperse through water bodies and through birds and to its resistant akinetes. In view of the above and of the environmental and public health problems *C. raciborskii* has been causing, Komárek & Komárková (2003) emphasize the importance of carrying out detailed studies of the autoecology of this species.

Several studies show *Cylindrospermopsis raciborskii* is widely distributed in Brazil but studies of its population dynamics are lacking. Among them

it can be found the researches of Branco & Senna (1991, 1994), which studied taxonomic aspects of *Cylindrospermopsis raciborskii* in Paranoá Lake (center-west region of Brazil), Bouvy *et al.* (1999), which registered the dynamics of this species in an eutrophic reservoir in Pernambuco (northeast of Brazil), Komárková *et al.* (1999) which studied its morphology in a south Brazil coastal lagoon, and finally, Tucci & Sant'Anna (2003) which studied *C. raciborskii* weekly variation in an eutrophic reservoir in São Paulo.

Regarding the reservoirs analyzed in the course of this study there are even fewer studies on *C. raciborskii* dynamics and the only existing work is that of Souza *et al.* (1998). In that study the phytoplankton of a branch of the Billings reservoir was analyzed and the authors observed that *C. raciborskii* was the dominant species throughout the period studied.

Thus, in view of the problems caused by this species and the importance of the reservoirs as sources of water provision to millions of people, the goal of this study was to analyze the population dynamics of *C. raciborskii* during a full seasonal cycle in two tropical water supply reservoirs, and to determine its relationship to the environmental factors that influenced the development of the organism.

Material and methods

The Guarapiranga and Billings reservoirs are part of the High Tietê basin and are located in the São Paulo, SP metropolitan area, in Brazil (table 1).

Samples of material were collected on the surface of the water, every month, from February 2002 to January 2003. A specific sampling point was selected for each reservoir, at the location where water is captured for supplying to the consumer public. The following measurements were made: water temperature, transparency (Secchi Disk), euphotic zone (Cole 1994), turbidity (Turbiditymeter), dissolved oxygen (Oxymeter OXI-197 TWT), conductivity (Conductivitymeter) and pH. The collected material was analyzed as follows: ammonium - NBR 10560, ABNT (1988); nitrate - 4500NO₃⁻, APHA (1998); nitrite - 4500NO₂⁻, APHA (1998); total phosphorus 4500P, APHA (1998) and chlorophyll *a* (CETESB 1990).

Samples used for qualitative analysis of the phytoplankton were collected by means of a plankton net with a 20 µm mesh size, and fixed in 4% formaldehyde. Analysis of the material was made

Table 1. General characteristics of the Guarapiranga and Billings Reservoirs (municipality of São Paulo).

	Guarapiranga	Billings
Coordinates	23°43'S and 46°32'W	23°47'S and 46°40'W
Area	33 Km ²	120 Km ²
Maximum profundity	13 m	18 m
Water retention time	185 days	720 days

under a binocular microscope whose optical train was fitted with a light chamber, a measuring eyepiece and an epifluorescence device. The following classification systems were used: Komárek & Anagnostidis (1989, 1999, 2005) for Cyanobacteria, Van den Hoek *et al.* (1995) for Chlorophyceae and Bourrelly (1985) for the remaining classes.

Samples gathered for quantitative analysis of the phytoplankton were collected by means of a Van Dorn bottle and preserved in a 1% acetic lugol solution. Cell counts were established according to the method described by Utermöhl (1958), using an inverted Carl Zeiss microscope, under 400 × magnification. Sedimentation time of the samples was three hours for each centimeter of height of the chamber, according to the criterion proposed by Lund *et al.* (1958). The sedimentation chamber used was a 2 mL one. Counting was carried out by means of horizontal and vertical transects and the minimum number of counted fields per sedimentation chamber followed the stabilization curve of the number of species, which was obtained based on new species added to each counted field.

The dominant species were considered with superior densities to 50% of the total density of the

sample. The abundant species were those with superior densities to the medium density of each sample (Lobo & Leighton 1986).

Statistical analysis of results was made by multivariate analysis of data. Principal Components Analysis (PCA) was used in order to determine the variability of environmental data, in relation to the months studied (McCune & Mefford 1997).

Results

Abiotic variables - Data on physical and chemical variables are shown in figure 1 and 2 for the Guarapiranga and Billings reservoirs, respectively. They are transparency, euphotic zone, rainfall, water temperature, dissolved oxygen, pH, conductivity, turbidity, ammonium, nitrates, nitrites and total phosphorus.

The Principal Components Analysis (PCA), applied to both reservoirs studied herein, showed the two aquatic environments as two different units, grouping 68% of the total variability in the two first axes (it represents table 2, figure 3). Axis 1 summarized the variability for both systems, separating the reservoirs,

Table 2. Pearson and Kendall correlation between environmental variables on the two first axes of the PCA ordination, as observed in the two reservoirs, Guarapiranga and Billings, during the period covered by the study ($n = 24$).

Environmental variables	Abbreviations	Principal Components	
		Axis 1	Axis 2
Water temperature	WT	-0.152	-0.958
Euphotic zone	Euphz	0.894	-0.133
Turbidity	Turb	-0.804	0.139
Conductivity	Cond	-0.882	0.235
pH	pH	-0.868	-0.164
Dissolved oxygen	DO	-0.688	0.025
Ammonium	NH ₄ ⁺	0.431	0.345
Nitrate	NO ₃ ⁻	0.767	0.132
Total phosphorus	TP	-0.590	-0.053
Chlorophyll <i>a</i>	Chlo <i>a</i>	-0.839	0.099

according to the environmental variables, as well as in gradient seasonal tracking the axis 2: on the positive side of Axis 1 (53% of explained variance) the sampling units of June, July, August and October were ordered, associated to the high values of nitrite and euphotic zone, in the Guarapiranga reservoir. On the negative side of Axis 1 the sampling units of February, June, August, October, November, December and

January, during which the highest of conductivity, turbidity, dissolved oxygen, total phosphorus and pH values, in the Billings reservoir. On the negative side of Axis 2 (15% of explained variance) the sampling units of March, April and January associated to the high values of temperature of the water, in both reservoirs.

Phytoplankton community - Quantitative

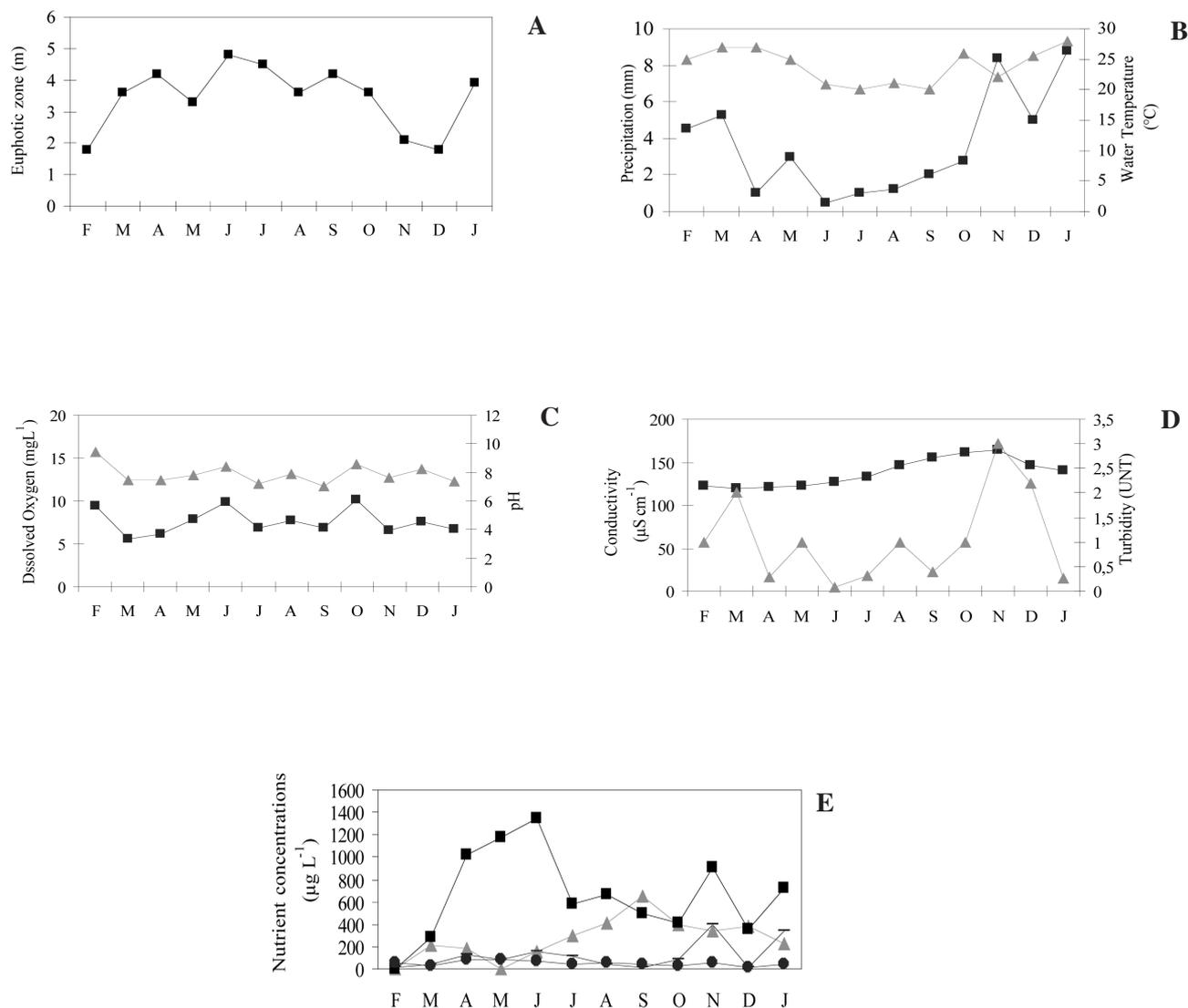


Figure 1. Monthly variation of physical and chemical parameters of Guarapiranga reservoir: euphotic zone (m, -■-); precipitation (mm, -■-) and water temperature (°C, -▲-); dissolved oxygen (mg L⁻¹, -■-) and pH (-▲-); conductivity (µS cm⁻¹, -■-) and turbidity (UNT, -▲-) in A, B, C and D; nutrient concentrations (-▲- ammonium, -■- nitrate, -■- nitrite, -●- phosphorus total) in E. Months are represented from February (F) through December (D) 2002 and January (J) 2003.

analyses of samples from the Guarapiranga reservoir yielded 178 taxa distributed among nine classes. In the Billings reservoir 142 taxa were identified, distributed among eight classes. Chlorophyceae was the class qualitatively best represented in both Reservoirs, with 88 taxa (49%) in the Guarapiranga Reservoir and with 61 taxa (42%) in the Billings Reservoir.

Cyanobacteria was the second best represented class in each of the Reservoirs, with 34 taxa (20%) in the

Guarapiranga and 35 taxa (26%) in the Billings reservoir. Bacillariophyceae was represented by 10% of the identified taxa in each of the reservoirs. Xanthophyceae was present only in the Guarapiranga Reservoir.

From the quantitative standpoint, Cyanobacteria is the class presenting the greatest annual density in cells mL^{-1} in both reservoirs. Figure 4 show the percentage of phytoplankton classes in relationship to chlorophyll *a* in the studied reservoirs.

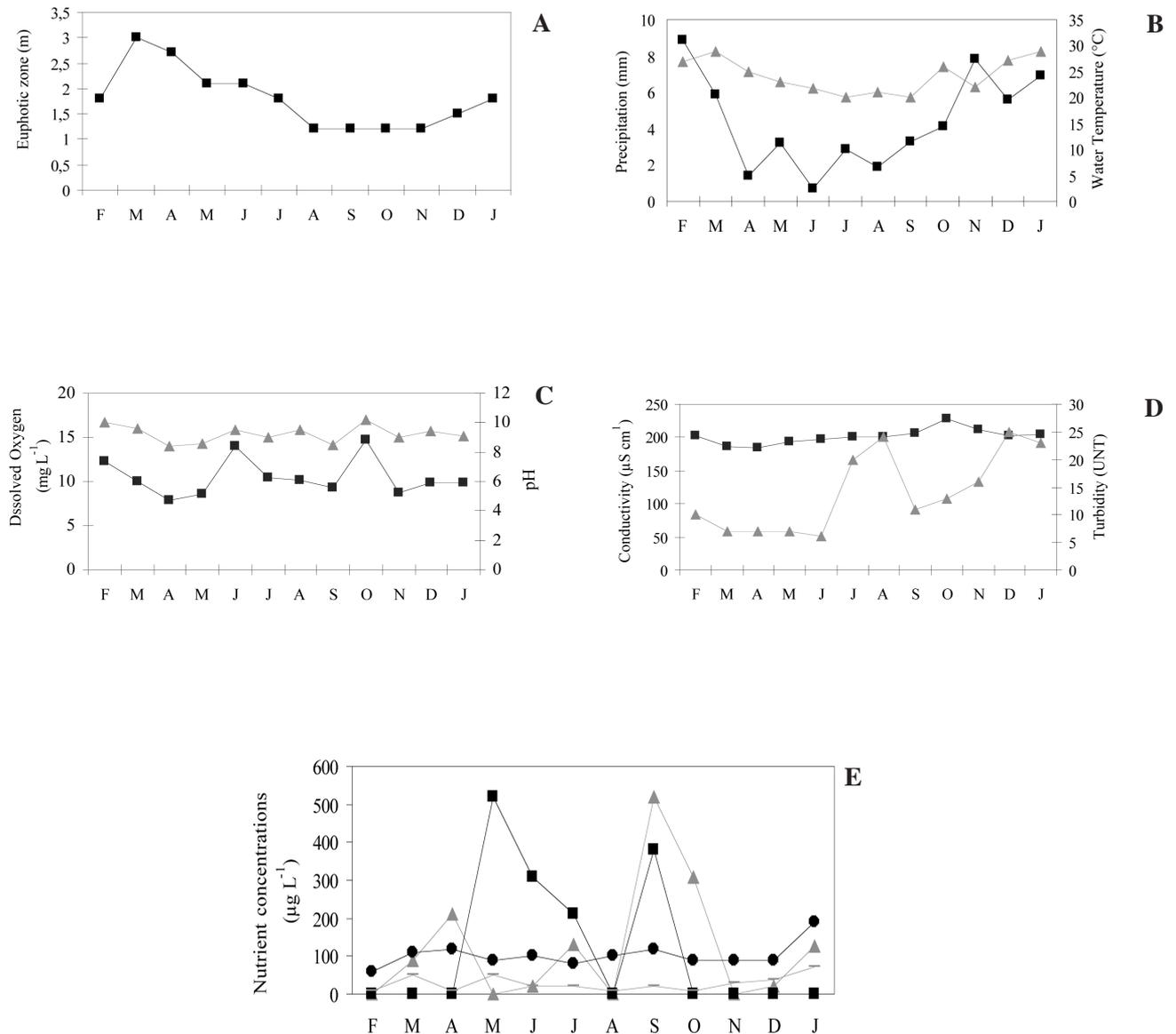


Figure 2. Monthly variation of physical and chemical parameters of Billings reservoir: euphotic zone (m; -■-) and precipitation (mm; -■-) and water temperature (°C, -▲-) in A, B, C and D; dissolved oxygen (mg L^{-1} , -■-) and pH (-▲-) in A, B, C and D; conductivity ($\mu\text{S cm}^{-1}$, -■-) and turbidity (UNT, -▲-) in A, B, C and D; nutrient concentrations (-▲- ammonium, -■- nitrate, -■- nitrite, -●- phosphorus total) in E. Months are represented from February (F) through December (D) 2002 and January (J) 2003.

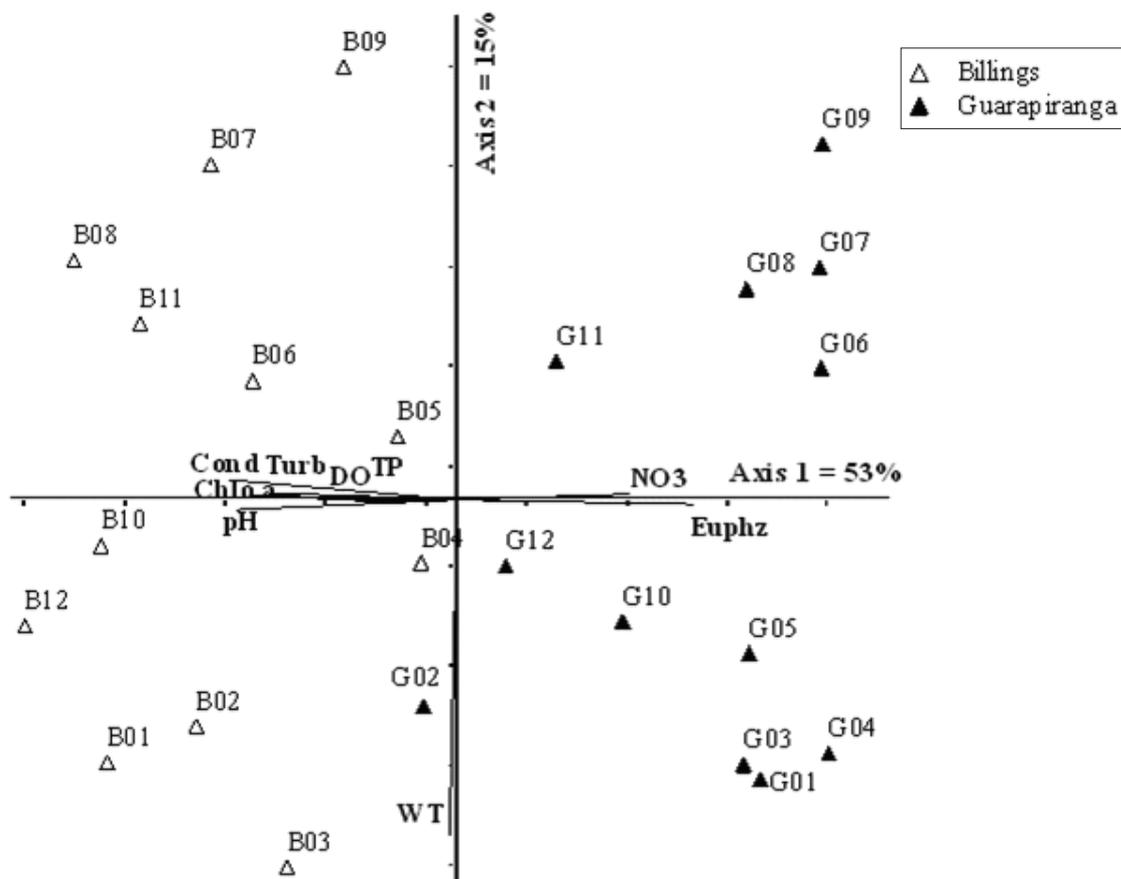


Figure 3. Biplot ordination resulting from the PCA applied to the sampling units with the physical, chemical and biological variables, in the Billings and Guarapiranga reservoirs. Months are represented from B02=February, B03=March, B04=April, B05=May, B06=June, B07=July, B08=August, B09=September, B10=October, B11=November, B12=December of 2002 and B01=January of 2003; G02=February, G03=March, G04=April, G05=May, G06=June, G07=July, G08=August, G09=September, G10=October, G11=November, G12=December of 2002 and G01=January of 2003. Abbreviations for environmental variables are presented in table 2.

Cylindrospermopsis raciborskii (Woloszynska) Seenayya & Subba Raju (figure 5) is characterized by single, straight or curved trichomas, cells with aerotopes, terminal heterocytes and cylindrical, subterminal akinetes.

When comparing the density of *C. raciborskii* (figure 6) with densities of other Cyanobacteria in the Guarapiranga reservoir, *C. raciborskii* occurred in February, May, November, December 2002 and January 2003 and was one of the abundant species in February, reaching 20% of the total density of all Cyanobacteria. In the Billings reservoir it occurred during the entire period of the study and was abundant in the dry (May, June and August 2002) and rainy (November and December 2002, and January 2003) seasons (figure 6). The figure 6 show a comparison of *C. raciborskii* density with that of other Cyanobacteria

species and of chlorophyll *a*, for both reservoirs. In figure 7, the annual densities of phytoplankton classes are compared to the density of *C. raciborskii*, the latter being larger than the density of all other phytoplankton classes.

Discussion

The composition of the phytoplankton community in the two reservoirs, Guarapiranga and Billings, revealed that the Chlorophyceae class, mostly represented by the Chlorococcales order, was the one with the largest number of taxa. These results seem to apply to other eutrophized reservoirs in Brazilian tropical regions and are consistent with those from several other authors: Beyruth (1996), Bouvy *et al.* (1999), Matsuzaki (2004), Sant'Anna

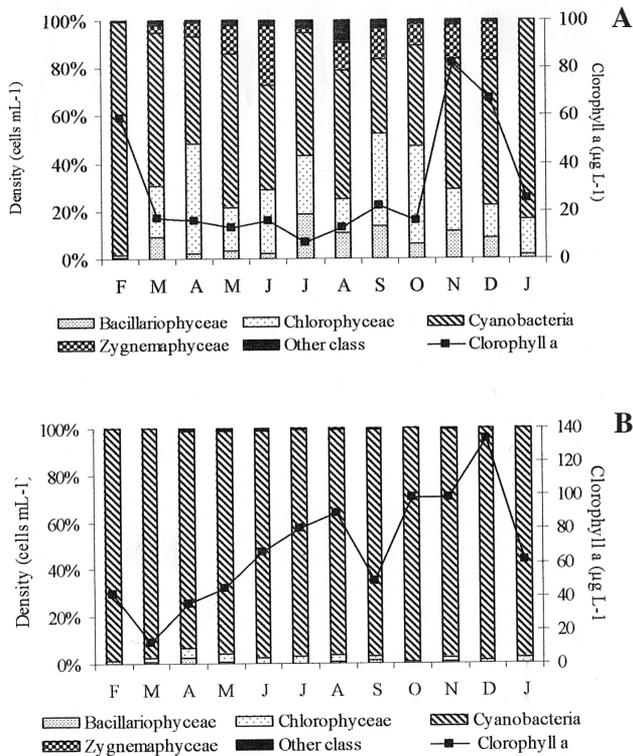


Figure 4. Contribution of phytoplankton classes to total density and chlorophyll *a* in the Guarapiranga (A) and Billings (B) reservoirs. Months are represented from February (F) through December (D) 2002 and January (J) 2003.

et al. (1989, 1997), Silva (1999) and Tucci & Sant'Anna (2003).

The Principal Component Analysis (PCA) showed that the physical and chemical variables that interfered in the temporal reservoirs dynamics were: water temperature, euphotic zone, turbidity, conductivity, pH and dissolved oxygen. Moreover, high nitrate and total phosphorus were available, allowing ideal conditions to phytoplankton blooms on both reservoirs. The PCA has also confirmed the difference between the reservoirs, showed by their separation through axis 1, also by a seasonal gradient through axis 2.

Moreover, the observed high availability of total phosphorus, ammonium, nitrate and nitrite, help create ideal conditions for the formation of blooms by Cyanobacteria. Similar results were obtained by Huszar *et al.* (2000) in eight tropical lakes situated in different tropical regions of Brazil.

The results of the present study confirm the high population density of the phytoplankton community in the Guarapiranga reservoir, the same observation

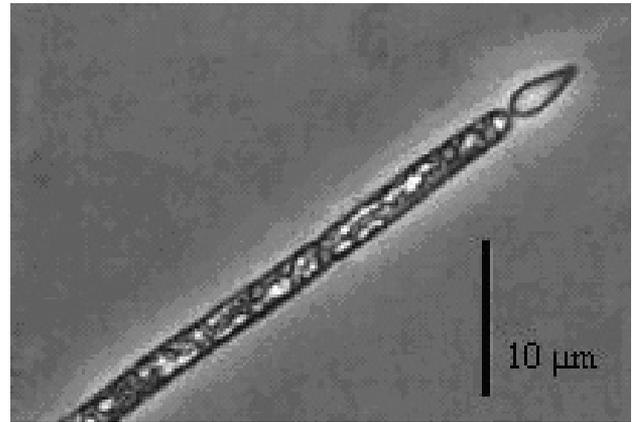


Figure 5. Photomicrograph of *Cylindrospermopsis raciborskii* with terminal heterocyst.

having been made by Beyruth *et al.* (1997), who attributed the problem to the eutrophication of the water body. In our study, values upwards of 10^6 cells mL⁻¹ were detected, mainly for Cyanobacteria. Important results obtained in the present study show that, starting in 2002, the density of *Cylindrospermopsis raciborskii* (Wolosz.) Seen. & Subba Raju, was high. This species had not been found in this reservoir before 2000 (C. L. Sant'Anna, unpublished data).

The present research also shows an increase in the number of taxa of the Cyanobacteria class in relation to the number found by Beyruth (1996), who identified 14 taxa of that class. This present study shows that the number of taxa have risen to 34 and including the presence of *Cylindrospermopsis raciborskii*. This fact is probably due to urban human settlements in the reservoir area, a phenomenon that has been growing in the last years and leads to eutrophication (Beyruth *et al.* 1997). Urbanization, as well as other human activities, when carried out improperly or in the absence of adequate planning, cause sometimes irreversible damage to the capacity of water sources (Capobianco & Whately 2002). Moreover, due to water shortages affecting Guarapiranga reservoir, it was necessary to build a canal linking the Taquacetuba branch of the Billings reservoir to the Guarapiranga reservoir, in order to solve the issue of water deficit in the latter. Pumping operations in the canal started in August 2000 (Beyruth 2000, Capobianco & Whately 2002, Tundisi 2003).

This study shows that subsequently to the pumping of the water, several species of Cyanobacteria were found in Guarapiranga Reservoir, including *Cylindrospermopsis raciborskii*, which had not been reported in that Reservoir.

In the Billings Reservoir, Cyanobacteria were dominant and abundant throughout the period of study. This fact is related to the physiologic and ecologic strategies employed by Cyanobacteria and that offer great competitive advantage where the environment is highly charged with phosphorus (Padisák 1997). Chorus & Bartram (1999) cited the different strategies allowing for the survival of those organisms, such as nutrient availability that leads to their rapid development, the presence of a mucilaginous sheath and of aerotopes for better floatability within the water column, thus facilitating light exposure, temperatures above 25 °C and low light tolerance in order to prevent the growth of other species.

The Billings reservoir has been considered eutrophic and hipereutrophic for decades, with frequent Cyanobacteria bloom formation (Branco 1986). Souza *et al.* (1998) studied one of the branches of the Billings Reservoir called Rio Pequeno, and observed the dominance of *Cylindrospermopsis raciborskii*. Lagos *et al.* (1999) cultivated *C. raciborskii* from water samples of the Taquacetuba branch of Billings Reservoir and showed its ability to produce saxitoxin. *Cylindrospermopsis raciborskii* is considered a species whose distribution is expanding in different regions of the world (Padisák 1997). Apparently, the release of toxins by *C. raciborskii* and by other Cyanobacteria species has an allelopathic effect in the water, thus inhibiting the growth of other species of the phytoplankton community and allowing for the rapid development of *C. raciborskii* and for its impact on the utilization of the water supply reservoirs (Yunes *et al.* 2003).

There seems to be no nitrogen fixation by *Cylindrospermopsis raciborskii* in either of the studied reservoirs since in the Guarapiranga reservoir no heterocyte (the cells type where nitrogen fixation occurs) was found on the analyzed trichomas. In the Billings reservoir only 1.4% of trichomas showed heterocytes. The fact that only a few trichomas presented heterocytes may be explained by the high concentrations of nitrogen observed in the studied environment (figures 1E, 2E) which makes this nutrient a non-limiting factor, in this case, to the development of *C. raciborskii*.

It was observed that, in the Guarapiranga reservoir, the environmental factors that most interfered with the dynamics of *Cylindrospermopsis raciborskii* were high values for water temperature (between 20 and 28 °C), rainfall (between 0.5 and 9 mm) and pH (between 7 and 10), as well as low values for euphotic zone

(between 1.8 and 4.8 m), those conditions coinciding with the periods of greatest density of *C. raciborskii* (figure 6).

High values for water temperature, pH and conductivity, as well as low numbers for euphotic zone were also found in the Billings reservoir (figure 2). An important factor to be considered is the water retention time (table 1, which is much higher for the Billings Reservoir and which certainly favored dominance by Cyanobacteria, including *Cylindrospermopsis raciborskii*, during the analyzed period. A comparison of the data here reported with those reported by Bouvy *et al.* (1999), Branco & Senna (1994), Huszar *et al.* (2000), Komárková *et al.* (1999), Souza *et al.* (1998) and by Tucci & Sant'Anna (2003) shows there is the similarity among data from several different regions in Brazil.

Due to eutrophic conditions of Paranoá Lake, in Brasília, in the center-west region of Brazil, *C. raciborskii* was responsible for bloom formation in that lake (Branco & Senna 1994). Bouvy *et al.* (1999) analyzed the species making up the phytoplankton, as well as their seasonal succession, of an eutrophized

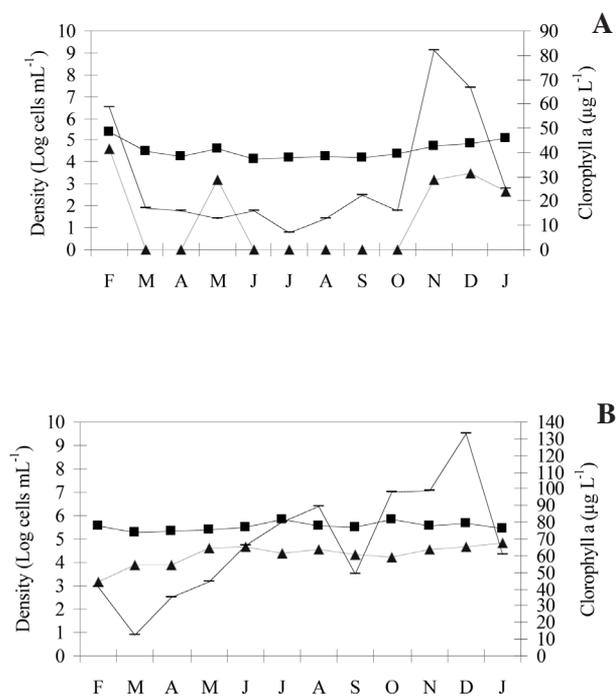


Figure 6. Density of *Cylindrospermopsis raciborskii* (-▲-), other Cyanobacteria (-■-) and chlorophyll *a* (-●-) in the Guarapiranga (A) and Billings (B) reservoirs. Months are represented from February (F) through December (D) 2002 and January (J) 2003.

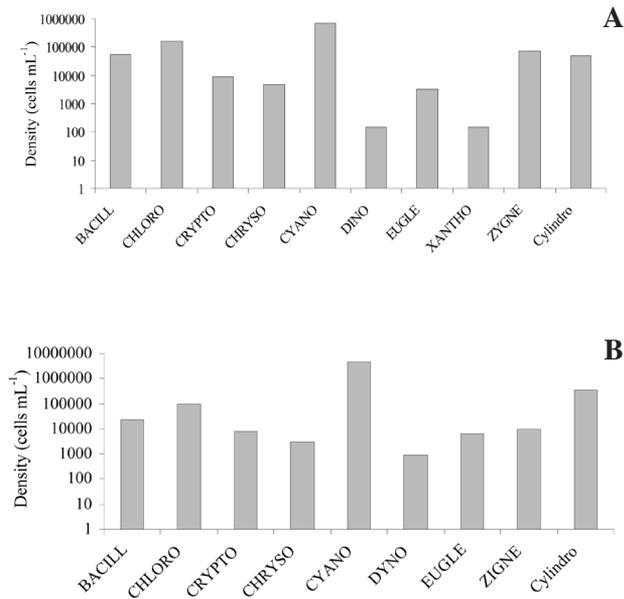


Figure 7. Annual density of the different phytoplankton classes and of *Cylindrospermopsis raciborskii* in the Guarapiranga (A) and Billings (B) reservoirs.

reservoir in Pernambuco, in the northeast region of Brazil. The authors observed that neurotoxic *C. raciborskii* was the dominant species throughout the period of the study. Komárková *et al.* (1999) observed, while studying a coastal lagoon in the south of Brazil, that *C. raciborskii* may present great morphologic variety (straight, curved or spiral shapes) and that the species was the dominant one during periods of high temperature, low nitrogen concentration and high phosphorus concentration. Tucci & Sant'Anna (2003) studied the weekly variation of *C. raciborskii* in an eutrophic reservoir in São Paulo, in Brazil, and correlated the high density of that organism to high temperature and pH, high concentration of chlorophyll *a*, low water transparency, low concentrations of free carbonic gas and ammonium.

The present study, results shows that the ideal conditions for the development of *Cylindrospermopsis raciborskii* were water temperature above 20 °C, low levels of transparency (between 0.4 and 1.0 m) and euphotic zone (between 1.2 and 4.5 m), pH values around 8 and high concentrations of available ammonium ions (700 µg L⁻¹) and nitrate (1,400 µg L⁻¹). Besides the above ideal conditions, the transfer of water from the Billings reservoir to the Guarapiranga reservoir certainly favored the appearance of *C.*

raciborskii in the Guarapiranga reservoir, since there are reports about the occurrence of the species in the Billings reservoir dating back the 1990s (Carvalho *et al.* 1997). In Guarapiranga reservoir, the previous studies, do not mention the occurrence of *C. raciborskii* (Matsuzaki 2007).

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