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Cylindrospermopsis raciborskii (Wolosz.) Seenaya and Subba Raju (Cyanophyceae) Dominance and a Contribution to the Knowledge of Rio Pequeno Arm, Billings Reservoir, Brazil

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ABSTRACT: From November 1992 to October 1993, Billings Reservoir in São Paulo State was analyzed to identify its environmental quality. Several physical, chemical, biological, and microbiological parameters were evaluated, phytoplankton being among them. Phytoplankton samples were taken monthly at eight sites, including one located on the Rio Pequeno arm. From the samples taken at Rio Pequeno, a total of 69 taxa were recorded. Cylindrospermopsis raciborskii (Wolosz.) Seenaya and Subba Raju was the dominant species from November 1992 to May 1993. Then Scenedesmus quadricauda (Turp.) Bréb. and S. opoliensis P. Richt (Chlorophyceae) became dominant. Another Cyanophyceae that was frequent but less expressive in terms of density and biomass was Oscillatoria sp. Physical and chemical water parameters, including pH, dissolved oxygen, transparency (Secchi disk), conductivity, water and air temperatures, depth, total organic nitrogen, nitrate, total phosphorus, aluminum, and iron also were analyzed. Water temperature, pH, dissolved oxygen, and depth were positively correlated with C. raciborskii density, while aluminum was negatively correlated. S. quadricauda and S. opoliensis densities were inversely correlated to the same factors. There was no indication of correlation between Oscillatoria sp. density and the abiotic factors analyzed. C. raciborskii seems to be favored by physical and chemical characteristics of Rio Pequeno, such as high pH and high levels of iron, and also by biological characteristics such as the capability to migrate on the water column and to fix atmospheric nitrogen. The species toxicity is another possible explanation for the dominance of C. raciborskii, through the growth suppression of other phytoplanktonic groups and protection against grazing by zooplankton. The toxicity of these algal blooms could be responsible for fish kills which occurred at the same place in 1990. C. raciborskii dominance at Rio Pequeno during most of the year advises against the use of this water for domestic supply, due to its potential toxicity, which represents risks to human health, requiring appropriate treatment. © *1998 by John Wiley & Sons, Inc.* Environ Toxicol Water Qual **13:** 73-81, 1998

Keywords: phytoplankton; *Cylindrospermopsis raciborskii*; water quality; toxicity; reservoir

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INTRODUCTION

The Billings Reservoir, situated at 23°42′ and 23°45′S, 46°27′ and 46°42′W (Fig. 1) was constructed in the 1920s near the city of São Paulo, Brazil, and has a maximum area of 127 km^2 (Rocha, 1984). With a drainage area of 560 km^2 and a volume of 1.2×10^9 m^3, the reservoir receives contributions from the Grande, Pequeno, Bororé, Taquacetuba, and other rivers. The air temperature in this region is higher between December and March, with the highest average of 21 and 22°C. Temperatures decline from April on, and the lowest average of 14 and 15°C is between June and July. January is the rainiest month, with the highest rainfall average recorded of 250 mm, and July is the driest one, with an average of 30–45 mm precipitation (DAEE, 1972). Thermal stratification of the water column occurs between the end of spring and the middle of autumn (CETESB, 1979), and affects other factors such as the phytoplankton concentration and the oxygen transportation to deep layers.

The reservoir has been used for recreation, fishing, water supply for the neighborhood, and the generation of energy to the industrial complex of Cubatão. To maintain the water level, the reservoir has received pumped water from the Pinheiros river, which is highly polluted due to industrial effluents and domestic sewage inputs from the metropolitan area (CETESB, 1991). Hence, to recover the reservoir from problems due to eutrophication, fish mortalities, and unpleasant odors, the São Paulo State government deterred the pumping from Pinheiros River. Project "SOS-Billings" was developed from November 1992 to October 1993 for the purpose of characterizing its water, sediment quality, and biotic communities, as well as its ecological recovery after the pumping stopped. As a part of this project, the phytoplankton community was analyzed and special attention was given in Rio Pequeno because of a government proposal to isolate this arm for utilization in public water supply.

The Rio Pequeno arm shows different characteristics from the main water body, since it is located far

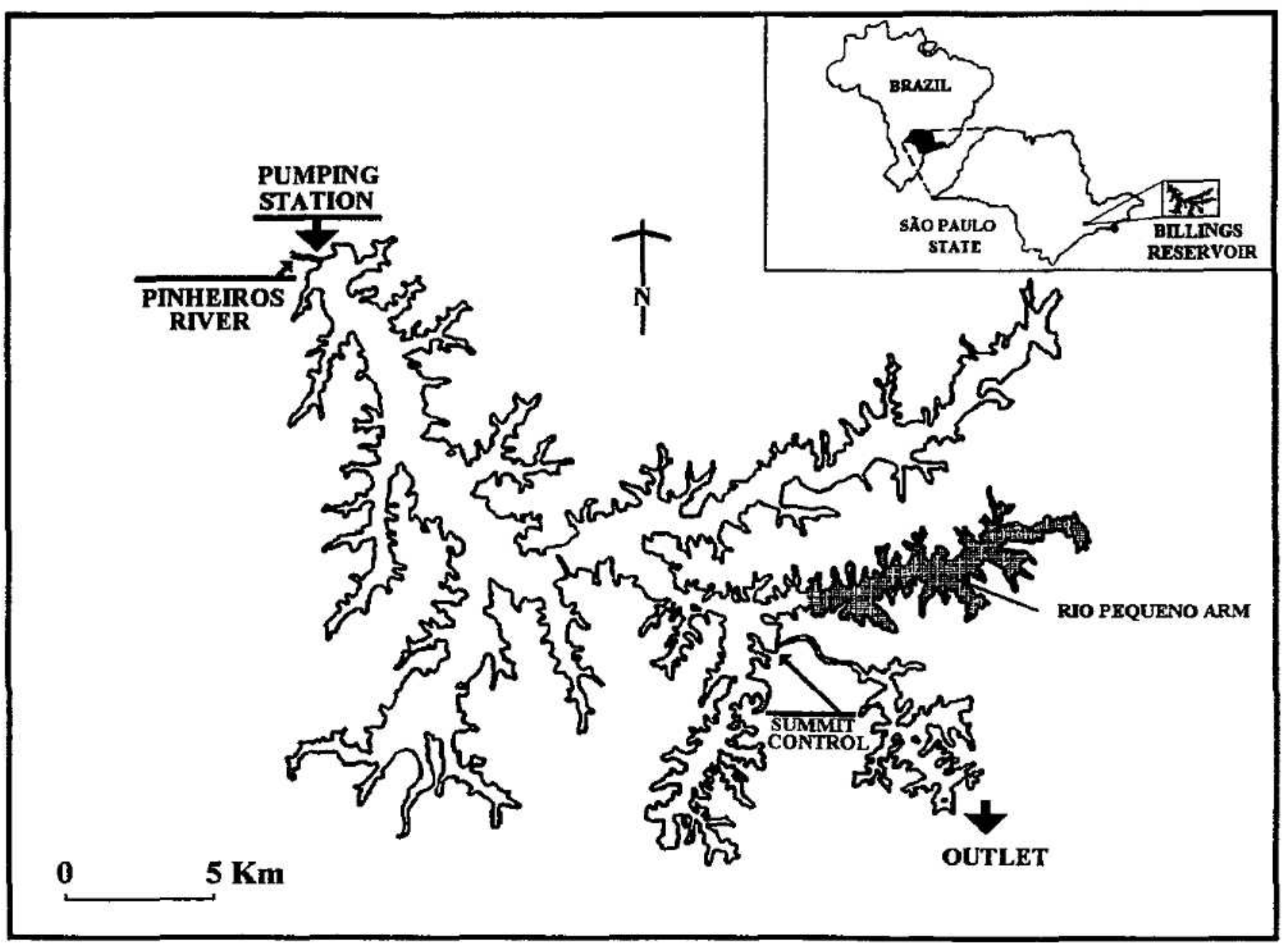

Fig. 1. Map showing the Billings Reservoir and location of the Rio Pequeno arm.

(approximately 21 km) from organic matter input at Pedreira's pumping station (Agudo et al., 1975). Differences in physical and chemical characteristics of Rio Pequeno water and other sampling stations include nutrient levels, conductivity, pH, turbidity, and iron and aluminum concentrations. The phytoplankton was dominated by a Cyanophyceae species, *Cylindrospermopsis raciborskii*, over a period of 7 months. The present study aims to relate physical and chemical characteristics of Rio Pequeno water to the phytoplankton community and *Cylidrospermopsis raciborskii* dominance.

MATERIALS AND METHODS

Phytoplankton samples were taken monthly. The water samples were collected from the surface in 135 mL glass flasks and preserved in formaldehyde at a 2% final concentration. At the laboratory, following sedimentation for a minimum of 24 h in Utermöhl's 2 mL settling chambers, the phytoplanktonic organisms were identified, counted, and measured using a Zeiss phase contrast inverted microscope (magnification 400x), and an eyepiece (Whipple) grid to estimate biomass, in agreement with Utermöhl (1958; modified by CETESB, 1978).

Phytoplankton identifications were made according to Bourrely (1968, 1970, 1972), Komarek and Horeka (1979), Komarek and Fott (1983), Philipose (1967), and Prescott (1962). *Cylindrospermopsis raciborskii* identification was confirmed by Dr. Célia Sant'Anna, from the Institute of Botany of São Paulo, Brazil.

At the site, pH, transparency (Secchi disk), depth, and water and air temperatures were measured. Water samples were collected concomitantly and analyzed for dissolved oxygen, conductivity, total phosphorus, nitrate, total organic nitrogen, iron, and aluminum, according to APHA (1989).

To measure the degree of association between the physical and chemical water parameters (water and air temperatures, transparency, pH, dissolved oxygen, conductivity, total phosphorus, nitrate, total organic nitrogen, iron, aluminum, and depth and the biotic variables (*C. raciborskii*, *Scenedesmus quadricauda*, *S. opoliensis*, and *Oscillatoria* sp. densities), Spearman correlation coefficients were applied. Due to the number of observations performed in the study and because the usual product-moment correlation coefficient is very sensitive to extreme points (Siegel, 1956; Hotelling and Pabst, 1936; Conover, 1980; Lehmann, 1975), a nonparametric analysis was used.

Due to high values of aluminum found from April 1993 on and possible interference of this metal in other physical and chemical water parameters, its correlation with these factors was also calculated.

RESULTS

A total of 69 taxa of phytoplanktonic organisms was recorded at the Rio Pequeno arm from November 1992 to October 1993 (Table I). Cyanophyceae was the dominant group and was responsible for more than 50% of the organisms' total density in 58.3% of the samples (Fig. 2).

Cylindrospermopsis raciborskii was the predominant phytoplanktonic organism, from November 1992 to May 1993, when it was replaced by *Scenedesmus quadricauda* and *S. opoliensis*. *C. raciborskii* reached a maximum density of 21,182 organisms per milliliter in March 1993, with a biomass of 27,809.5 ASU (area standard unit). The other Cyanophyceae recorded was *Oscillatoria* sp., which was constant, but less important in terms of density and biomass. Chlorophyceae showed low density values until June, when *Scenedesmus quadricauda* began to dominate. Bacillariophyceae (diatoms), dinoflagellates, other flagellates, and Xantophyceae were recorded in lower levels.

Physical and chemical data for Rio Pequeno during the study period are summarized in Table II. High pH levels were recorded, with a maximum of 9.4 in March 1993. Total phosphorus levels ranging from 0.030 mg/L in March 1993 and 0.120 mg/L in June 1993 were found. Minimum water temperature was 16°C in July/August and maximum was 25°C in January. Dissolved oxygen values between 7.0 (December 1992) and 9.8 mg/L (September 1993) were found.

Iron was another relevant factor, with values between 0.23 mg/L in January 1993 and 0.98 mg/L in June 1993, exceeding the limits for aquatic life preservation (0.3 mg/L, according to Brazilian regulation; CONAMA, 1986) and higher than the values found in other points of the Billings Reservoir. A range of < 0.20 mg/L in November 1992 to 1.16 mg/L in September 1993 (CONAMA limit is 0.1 mg/L) was recorded for aluminum.

Water temperature, pH, dissolved oxygen, aluminum, and depth were the variables that presented stronger correlation coefficient to biotic variables except for *Oscillatoria* sp., whose density did not show significant correlation to the analyzed abiotic variables. The results described above, with correlation coefficient values and associated p values, are summarized in Table III. Aluminum showed strong correlation with pH ($r = -0.71$; $p = 0.0190$), total phosphorus ($r = 0.62$; $p = 0.0389$), and depth ($r = -0.77$; $p = 0.0108$).

DISCUSSION AND CONCLUSIONS

The dominance of lakes by cyanobacterial species is characteristic of a water body that has nonlimiting

TABLE 1. Phytoplankton taxa present in the Rio Pequeno arm from November 1992 to October 1993

Chlorophyceae	Cyanophyceae
Ankistrodesmus fusiformes	*Anabaena* sp.
Ankistrodesmus sp.	*Aphanocapsa* sp.
Chlorella sp.	*Chroococcus* sp.
Chlorococcum sp.	*Cylindrospermopsis raciborskii*
Closteriopsis sp.	*Merismopedia tenuissima*
Coelastrum microporum	*Merismopedia* sp.
Coelastrum reticulatum	*Microcystis* sp.
Coelastrum sp.	*Oscillatoria* sp.
Coenochloris sp.	*Oscillatoria quadripunctulata*
Cosmarium sp.	*Synechococcus* sp.
Crucigenia tetrapedia	
Dictyosphaerium pulchelum	Bacillaryophyceae
Dictyosphaerium sp.	*Achnantes* sp.
Diplochloris sp.	*Aulacoseira granulata*
Euastrum sp.	Nonidentified Centrales
Golenkinia paucispina	*Cyclotella meneghiniana*
Golenkinia radiata	*Melosira* sp.
Golenkinia sp.	*Navicula* sp.
Micractinium pusillum	*Nitzschia* sp.
Micractinium sp.	Nonidentified Pennales
Monoraphidium contortum	*Synedra* sp.
Monoraphidium minutum	
Monoraphidium sp.	Dinoflagellates
Mougeotia sp.	*Gymnodinium* sp.
Oocystis sp.	
Pediastrum duplex	Flagellates
Pediastrum tetras	Nonidentified form
Scenedesmus acuminatus	*Chlamydomonas* sp.
Scenedesmus bicaudatus	*Cryptomonas* sp.
Scenedesmus opoliensis	*Trachelomonas bacillifera*
Scenedesmus quadricauda	*Trachelomonas* sp.
Scenedesmus spinosus	*Trachelomonas volvocina*
Staurastrum gracile	Nonidentified Volvocalles
Staurastrum sp.	
Staurastrum volans	Xanthophyceae
Tetraedron gracile	*Goniochloris mutica*
Tetraedron regulare	*Tetraplekton* sp.
Tetraedron sp.	
Treubaria sp.	
Westella sp.	

nutrient concentrations, high pH levels, regular mixing of the water column, and a shallow euphotic zone (Harding, 1992). Since 1962, *C. raciborskii* dominance has been related to environments that, besides high pH and high organic matter, have low redox potential, i.e., alkaline and reducing waters (Singh, 1962). Rio Pequeno showed the smallest values of conductivity and hardness in the Billings Reservoir, in addition to the highest pH values (Vargas Boldrini et al., 1995).

The high pH registered in places where high densities of the species occurred could be related to the increase of *C. raciborskii* metabolism (Branco, 1991), that accentuates the water bicarbonates absorption and diminishes the CO_2 during the rainy season. This could explain the low levels of hardness recorded at Rio Pequeno.

Pinto-Coelho and Giani (1985), Branco (1991), and Tóth and Padisák (1986) observed that *C. raciborskii* dominance is also related to high temperatures (summer) and rainy seasons, but low winds. These observations confirm the results obtained in this study, since *C. raciborskii* density showed significant correla-

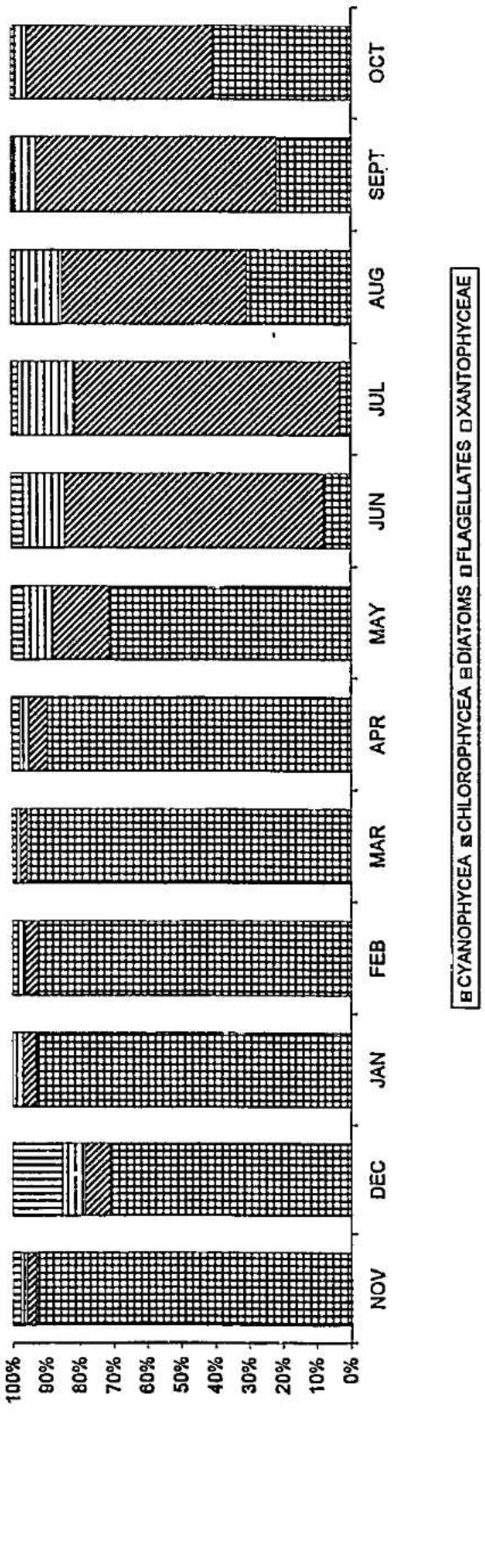

Fig. 2. Phytoplanktonic groups present in the Rio Pequeno arm from November 1992 to October 1993.

tion to water temperature and the species disappeared in autumn, during a dry period.

The Billings Reservoir's depth decreased because the water input from the Pinheiros River stopped at the same time that turbining to Cubatão industries (output) continued. The water level depletion was accentuated by the low rainfall registered during this period. Since *C. raciborskii* dominance occurred in periods of higher depth, it was not possible to determine the causes of changes in phytoplankton composition that begin in June 1993, when the species disappeared; this could be due to the physical and chemical changes resulting from the shallow depth as well as to natural conditions of the algae biological cycle.

Another factor that could have contributed to *C. raciborskii* dominance—still related to the water quality of Rio Pequeno—is its high iron levels. Blue-green algae have a particularly high demand for iron, due to nitrogen fixation. These algae are known to excrete iron-selective chelators which suppress the growth of other competing algae species (Murphy et al., 1976). Iron stimulates enzymes (nitrate and nitrite reductase and glutamine synthetase) involved in nitrogen metabolism in blue-green algae, but inhibits their activity in green algae. It was observed also that iron is important in the early stages of heterocysts' formation by blue-green algae (Storch and Dunham, 1986). On the other hand, the strong positive correlation found between *Scenedesmus quadricauda*, *S. opoliensis* densities, and the aluminum levels that, on the contrary, was negative for *C. raciborskii*, indicates that this metal concentration increase from April 1993 was of fundamental importance to the changes of Rio Pequeno phytoplankton community composition. This is also evidenced by a strong correlation among aluminum, total phosphorus, pH, and depth.

Aluminum solubility is a function of pH and concentrations of complexing ligands (Hongve, 1993). At Rio Pequeno, aluminum seemed to be resuspended and resolubilized when the water level was low. Since the reservoir was very shallow, resuspension of particles and erosion of bottom sediment could take place because of wind influence.

The genera *Scenedesmus* is considered resistant to cadmium and copper concentrations (Mouchet, 1986). The results from this study indicated that *S. quadricauda* and *S. opoliensis* are also resistant to high aluminum concentrations. *Scenedesmus* dominance is associated with extremely high mean values of total phosphorus, orthophosphates, and Kjeldahl nitrogen and alkalinity lakes (Nicholls et al., 1992). However, nutrients concentration of Rio Pequeno water did not show significant correlation with the biotic variables analyzed, and they are not the probable cause of the changes observed on phytoplanktonic composition.

TABLE 2. Physical and chemical water parameters for the Rio Pequeno arm from November 1992 to October 1993

Parameters	Month and Year											
	Nov. 92	Dec. 92	Jan. 93	Feb. 93	Mar. 93	Apr. 93	May 93	Jun. 93	Jul. 93	Aug. 93	Sept. 93	Oct. 93
Air temperature (°C)	17	19	26	25.5	24	23	20.5	18.5	17	13	20	27
Water temperature (°C)	21	22	25	24.5	26	23	21	18.5	16	16	17.5	24
Transparency (m)	0.40	0.80	0.40	0.40	0.50	0.40	0.35	0.40	0.40	0.80	0.50	0.60
pH	9.0	6.7	9.0	9.0	9.4	7.8	8.6	7.2	6.7	7.5	5.4	6.7
Dissolved oxygen (mg/L)	7.8	7.0	8.0	7.6	8.7	7.8	9.4	9.0	8.8	8.8	9.8	8.3
Conductivity (μs/cm)	109	76	73	49	71	57	35	46	49	55	74	64
Total phosphorus (mg/L)	0.040	0.040	0.045	0.045	0.030	0.060	0.050	0.120	0.050	0.045	0.080	0.035
Nitrate (mg/L)	0.02	0.04	< 0.02	0.04	< 0.02	< 0.02	0.14	0.05	< 0.02	< 0.02	0.30	0.44
Total organic nitrogen (mg/L)	2.0	0.6	0.8	0.8	0.7	0.8	1.7	a	a	a	1.2	0.8
Iron (mg/L)	0.34	1	0.23	0.54	0.43	0.88	0.91	0.98	0.65	0.89	1.21	2.81
Aluminum (mg/L)	< 0.20	< 0.20	0.25	0.24	< 0.20	0.57	0.34	0.55	0.92	0.47	1.16	0.59
Depth (m)	9.0	7.0	6.0	5.0	5.0	4.5	5.0	2.0	3.0	2.0	3.0	3.5

[a]Analytical problems.

TABLE 3. Spearman correlation coefficients and associated *p* values for the major phytoplanktonic species and abiotic variables for the Rio Pequeno arm, November 1992 to October 1993

Abiotic Variables	Biotic Variables (Density)			
	C. raciborskii	*S. quadricauda*	*S. opoliensis*	*Oscillatoria* sp.
Air temperature	0.30 $p = 0.317$	−0.23 $p = 0.440$	−0.24 $p = 0.417$	0.41 $p = 0.169$
Water temperature	0.65 $p = 0.030$	−0.66 $p = 0.027$	−0.68 $p = 0.023$	0.30 $p = 0.321$
Depth	0.61 $p = 0.042$	−0.80 $p = 0.008$	−0.76 $p = 0.011$	0.04 $p = 0.874$
pH	0.77 $p = 0.011$	−0.59 $p = 0.052$	−0.67 $p = 0.027$	0.27 $p = 0.376$
Dissolved oxygen	−0.50 $p = 0.095$	0.74 $p = 0.014$	0.67 $p = 0.026$	0.003 $p = 0.990$
Conductivity	0.27 $p = 0.371$	−0.46 $p = 0.125$	−0.42 $p = 0.161$	0.20 $p = 0.496$
Transparency	−0.34 $p = 0.247$	0.01 $p = 0.950$	−0.01 $p = 0.970$	−0.12 $p = 0.690$
Total phosphorus	−0.26 $p = 0.387$	0.49 $p = 0.101$	0.52 $p = 0.088$	0.12 $p = 0.681$
Nitrate	−0.35 $p = 0.240$	0.38 $p = 0.202$	0.47 $p = 0.114$	0.04 $p = 0.896$
Total organic nitrogen	−0.19 $p = 0.595$	0.47 $p = 0.176$	0.63 $p = 0.075$	0.17 $p = 0.612$
Iron	−0.53 $p = 0.079$	0.47 $p = 0.118$	0.52 $p = 0.082$	−0.07 $p = 0.825$
Aluminum	−0.67 $p = 0.025$	0.85 $p = 0.005$	0.86 $p = 0.004$	0.08 $p = 0.778$

C. raciborskii dominance seemed to be favored by the natural conditions of Rio Pequeno before water level depletion, such as iron availability and high pH as well as by its capability to migrate on the water column and to fix nitrogen from the atmosphere. In a steady water column, green algae and diatoms tend to sink, leaving the photic zone and being unable to return. Blue-green algae, however, are able to leave the photic zone when gas vacuoles collapse and return when new vacuoles are formed. They are, therefore, adapted to utilization of environments with a strong vertical separation between optimum depths for light and nutrients (Ganf and Oliver, 1982). The occurrence of the species blooms in earlier years on Rio Pequeno supports the idea that this occurrence is not eventual. Xavier (1981) also registered the species occurrence, although not in high densities.

Another reason proposed for the dominance of cyanobacteria in eutrophic waters is the growth suppression of other phytoplanktonic groups by cyanobacterial excretion products and possible protection against grazing by zooplankton due to cyanobacterial toxins (Codd et al., 1985).

Two cases of fish kills occurred in March 1990 at Rio Pequeno and can be related to the high density of *C. raciborskii* observed at that time. The toxin of this algae (cylindrospermopsin) was described as an unusual alkaloid that is hepatotoxic and has symptoms indistinguishable from those originally described for the cyanobacterial extract (Ohtani et al., 1992).

It appears that environmental factors such as nutrients, light, and temperature not only influence bloom production, but they also enhance the toxicity of some noxious cyanobacteria (Runnegar et al. apud Bourke and Hawes, 1983). In the same way, metals concentration and pH can affect toxin production (Lukac and Aegerter, 1993).

The occurrence of toxic blooms or dense growths of cyanobacteria has been related to fish and other animals mortalities and human illness (Bourke and Hawes, 1983; Carmichael, 1992; Codd et al., 1985). Although the toxicity of a number of cyanobacteria is well documented, there are no reports of human intoxication by *C. raciborskii* except for a domestic water supply reservoir in Australia, where a bloom was cited as the cause of hepatoenterities in 148 people (Hawkins et al., 1985). In São Paulo state, high toxicity was demonstrated in mice bioassays that were carried out by intraperitoneal injections of an algae cell suspension (Zagatto et al., 1995). Hepatotoxins from blue–green algae are increasingly recognized as a potential hazard in drinking water supplies. The clinical consequences of ingestion include acute or chronic liver injury, with the possibility of enhanced susceptibility to and growth of liver tumors (Falconer et al., 1994).

The evidence points to *C. raciborskii* toxicity as the cause of those cases of fish kills which occurred in 1990 at Rio Pequeno. The highest densities of the species in 1993 occurred in March/April, the same period of former fish kills. Other factors associated with algal blooms, like dissolved oxygen depletion, elevated pH levels, and ammonia toxicity following declines of blooms, do not seem to be responsible. Although high values of density were registered for a long period during the SOS Billings project, fish mortalities were not registered. This sporadic toxicity could be explained by the fact that the same species may produce both toxic and nontoxic varieties. Moreover, the same strain can produce toxins and molecules that are similar, but show an opposite stereochemical configuration and are therefore harmless (Harada et al., 1994).

The variability between toxicity of blooms and the simultaneous occurrence of different toxic strains makes the identification of these strains and any prediction about when and where toxicity will occur very difficult. This hazard assessment is important with regard to popular recreational areas or potential domestic supply reservoirs such as Billings. Undetected toxicity may threaten the health of people and domestic animals. Furthermore, cyanobacterial blooms in drinking waters sources are harmful not only because of their potential toxicity, but also because of their capability to produce unpleasant tastes and odors (Palmer, 1980; Persson, 1982), which increases the costs of water treatment.

Whereas the Rio Pequeno arm has characteristics that seem to favor *Cylindrospermopsis raciborskii* and other Cyanophyceae dominance, any project with the purpose of its water utilization for public supply should be carefully studied, taking into consideration the potential hazards and/or high cost of treatment required.

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