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Phytoplanktonic Structure and Chemistry of the Water in the Monjolinho Reservoir (SP, Brazil) During a Cyanobacterial Bloom Episode

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ABSTRACT

The composition of the phytoplankton and parameters of the water quality were studied in a tropical reservoir during cyanobacterial bloom episode. Samples were collected at two sites during October 2004. The phytoplankton community consisted of 69 taxa, distributed into 9 classes. Most taxa belonged to Cyanobacteria (17), Chlorophyceae (21), Bacillariophyceae (8), Conjugatophyceae (7), Dinophyceae (1), Chrysophyceae (4), Euglenophyceae (9), Cryptophyceae (1) and Xanthophyceae (1). *Anabaena circinalis* Rabenhorst *ex* Bornet et Flahault e *Anabaena spiroides* Klebahn were the dominant species. Statistical analyses revealed significant spatial variation ($p < 0.05$) between sampling points only for N compounds and chlorophyll *a*. Most limnological variables analyzed showed no significant temporal variation ($p > 0.05$). Cyanobacteria bloom was concomitant with the following environmental conditions, warm water temperatures (19 to 22 ºC), periods of water column stability and high concentrations of N (724.9 $\mu g.L^{-1}$) and P (90.9 $\mu g.L^{-1}$) in the reservoir and NH_4 as being the predominant nitrogen compounds. It is speculated that those conditions could play a role in the dominance of cyanobacteria.

Keywords: Anabaena circinalis, Anabaena spiroides, cyanotoxins, phytoplankton community, *toxic bloom*.

RESUMO

Estrutura Fitoplanctônica e Química da Água na Represa do Monjolinho (SP) Durante uma Floração de Cianobactérias

A composição do fitoplâncton e parâmetros da qualidade da água foram estudados em um reservatório tropical durante uma floração de cianobactérias. As amostras foram coletadas em dois pontos do reservatório durante o mês de outubro de 2004. A comunidade fitoplanctônica consistiu de 69 taxa, distribuídos em 9 classes. A maioria dos taxa pertenceu à Classe Cyanobacteria (17) e Chlorophyceae (21), seguidas por Bacillariophyceae (8), Conjugatophyceae (7), Dinophyceae (1), Chrysophyceae (4), Euglenophyceae (9), Cryptophyceae (1) e Xanthophyceae (1). *Anabaena circinalis* Rabenhorst *ex* Bornet et Flahault e *Anabaena spiroides* Klebahn foram as espécies dominantes no reservatório. As análises estatísticas indicaram variação espacial significante ($p < 0,05$) entre os pontos de amostragem apenas para os compostos nitrogenados e clorofila-*a*. A maioria das variáveis limnológicas analisadas não indicou qualquer variação temporal ($p > 0,05$). A floração de cianobatérias foi concomitante com as seguintes condições ambientais: valores relativamente elevados na temperatura da água (19 a 22 °C), períodos de estabilidade na coluna d'água e altas concentrações de N (724,9 $\mu g.L^{-1}$) e P (90,9 $\mu g.L^{-1}$) na represa e NH_4 como a forma predominante entre os compostos nitrogenados. Especula-se que estas condições poderiam favorecer a dominância das cianobactérias.

Palavras-chave: Anabaena circinalis, Anabaena spiroides, cianotoxinas, comunidade fitoplanctônica, floração tóxica.

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INTRODUCTION

The growth of cyanobacterial populations, especially those attaining a high biomass, has been increasingly reported around the world. There is general concern about public health and the environment given the likely effects of global warming, especially regarding unpredictable responses of those species that produce toxins (Aboal; Puig 2005) considering that both moderate and high temperatures tend to favour the occurrence of blooms. Cyanobacteria often dominate phytoplankton communities under certain conditions such as: nutrient-enriched waters and warm temperatures (ranging from 15 to 30 °C). Additionally water column stability, mild or absent winds, low turbulence are other favourable environmental conditions to cyanobacterial blooming. Morphometry of aquatic systems, low grazing rates by large zooplankton have been considered advantageous conditions for the development of those kinds of bloom as well (Mur *et al*., 1999; Zurawell *et at*. 2005).

Blooms of various species of cyanobacteria occur frequently as a consequence of eutrophication of aquatic environments. Cyanobacterial blooms have adverse effects on aesthetics and recreation. They are also linked with a number of other water-related problems including bad-smelling, non-potability, and fish kills due to oxygen depletion and ammonia release as the cyanobacteria decomposition proceeds (Chorus *et al*., 2000; Smith *et al.,* 2008). Moreover, seasonal growth of cyanobacteria also interferes with drinking water treatment processes (Alam *et al*. 2001; Watson *et al.* 2007).

Freshwater blooms of cyanobacteria commonly produce toxins which can be classified in five functional groups: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (lipopolysaccharides) (Wiegand; Pflugmacher, 2005). Among the hepatotoxins, the microcystins are the most frequently detected. The chemical structures and mode of action of these cyanotoxins, mainly microcystins, nodularin and anatoxins have been extensively described in the literature (Zurawell *et al*., 2005; Wiegand; Pflugmacher 2005). Similarly, several observations of the effects of these toxins to various animals (including humans) have also been broadly reported (Zurawell *et al*. 2005; Wiegand; Pflugmacher, 2005; Ibelings; Chorus, 2007).

Cyanobacterial blooms are a common occurrence in Brazilian reservoirs since most of them are characterized as eutrophic or hypereutrophic (Calijuri *et al*., 2002; Dellamano-Oliveira *et al*., 2008; Tundisi *et al*., 2008). Despite the frequency of episodes of cyanobacterial blooms in Brazil, there is few data or information about the structure of the phytoplankton, zooplankton communities and toxicological features during the occurrence of toxic blooms (Sotero-Santos *et al*., 2006). Additionally, the means by which environmental factors influence cyanotoxin production are weakly understood. Though nutrients have long been assumed as a contributing factor to both cyanobacterial abundance and toxin production, surprisingly few information has been published on how, for example, alterations in phosphorus and nitrogen may change microcystin production (Kotak *et al*., 2000).

The present study described some aspects of the structure of the phytoplankton community and chemistry of water in Monjolinho Reservoir (21° 59' 8.4'' S and 47° 52' 45'' W) during a brief episode of *Anabaena* bloom. Toxin analyzes and toxicity to aquatic organisms (cladocerans) and to mammals (mice) were also investigated and confirmed. Details about the toxicological features of bloom were described previously (Sotero-Santos *et al*., 2008). The Monjolinho Reservoir is considered a eutrophic system which receives continuous nutrient inputs directly through a poultry industry and indirectly from agricultural fields. This reservoir was studied previously as reported by Hino *et al.* (1984), Nogueira and Tundisi (1994) and Seleghim and Godinho (2004). The present study reports the first occurrence of cyanobacteria bloom in this productive system.

MATERIALS AND METHODS

Study site description

The site of this study is a shallow, artificial reservoir located in the São Carlos city, São Paulo State, Brazil. It was formed by the damming of the Monjolinho River. The main morphometric and hydrological characteristics of Monjolinho Reservoir are: area = 47.157 m^2, volume = 73.251 m^3, max depth = 3 m, mean depth = 1.54 m, max length = 510 m, max width = 150 m and water retention time varying between about 2 (rainy season) and 23 days (dry season) (Nogueira; Tundisi, 1994).

Properties of reservoir water and phytoplankton sampling

Sampling was carried out three times per week during October 2004, at two sites in the reservoir: S-1 (near inflow of river) and S-2 (near the dam) from 10:00 – 12:00 hours in the morning. To evaluate the water quality, samples for laboratory analysis were collected at the surface of the water. Simultaneously, *in situ* measurements of temperature, pH, conductivity and dissolved oxygen (DO) were performed in the water column, using a Horiba U-10 multi-probe (Horiba, Co., Japan). Water transparency was estimated from Secchi Disk readings.

Analyses of nitrate (NO_3), nitrite (NO_2), ammonium (NH_4) concentrations, total dissolved phosphate (TDP), organic (TOP) and inorganic phosphate (TIP), and total organic nitrogen (TN) concentrations were performed in the laboratory following the procedures described in Koroleff (1976), Golterman *et al*. (1978) and Mackereth *et al*. (1978), while total organic phosphorus (TP), was determined as recommended by APHA (1995). Chlorophyll *a* and silicon as soluble reactive silicon (SRSi) were determined as by Nush (1980) and Golterman *et al.* (1978), respectively.

Qualitative analysis of phytoplankton was performed on samples collected with conical plankton net of 20 µm *mesh* size and samples were preserved in 4% formaldehyde solution. Taxonomic identification was performed down to the lowest possible level, mostly to species, using an Axiovert

Zeiss microscope with 2560 maximum magnification and consulting specialized literature. The classification system used for diatoms (Bacillariophyceae) was that of Round *et al.* (1990); for Cyanophyceae, Anagnostidis & Komárek (1988, 1989) and Komárek & Anagnostidis (1999); and for other groups, Hoek *et al.* (1995).

For quantitative analysis, samples were collected in Van Dorn bottles at the surface level (± 50 cm depth, considering that the water column was not thermally stratified) and fixed in Lugol's preservative. Populations were counted under an inverted microscope (Zeiss) at a magnification of 400 X, using Utermöhl's method (Utermöhl, 1958). Settled sample volumes varied from 2 to 10 mL, depending on organism density. Sedimentation time was at least 3 hours (Wetzel; Likens, 1991). The individuals (cells, colonies, cenobia and filaments) were counted in random fields and densities were calculated as recommended by APHA (1995). Relative abundance was estimated in accordance with criteria proposed by Lobo and Leighton (1986) and community richness was evaluated as the total number of species found during the whole study. The specific diversity calculation was based on the Shannon and Wiener index (Shannon; Weaver, 1963). The equitability or uniformity (J) was evaluated by the method described by Pielou (1975). In order to identify significant temporal and spatial differences between the limnological variables (abiotic and biotic) at the sampling stations (S-1 and S-2) the Tukey parametric test was employed when the distribution was normal (ANOVA one-way) or the Kruskall-Wallis non-parametric, if not (with significant level, $\alpha = 0.05$).

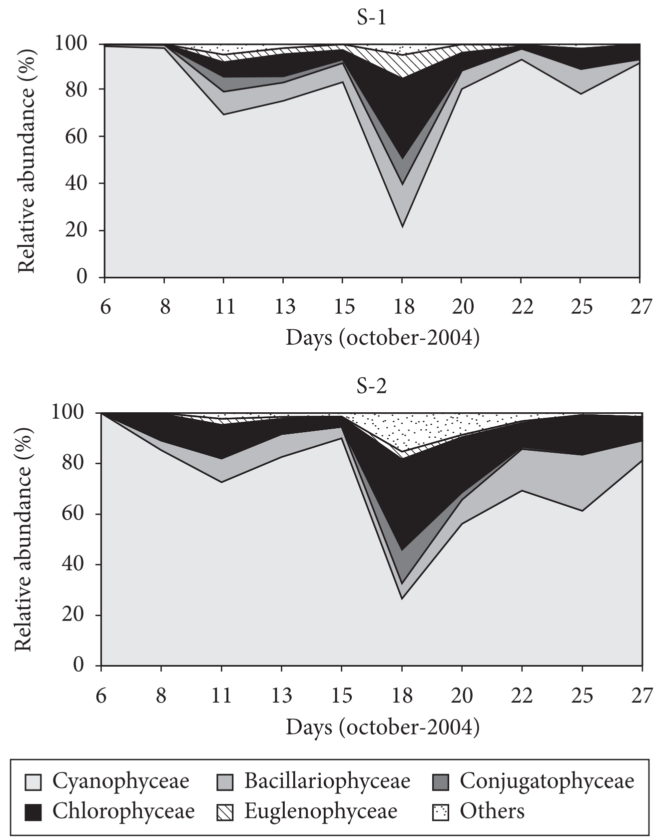

Figure 1 - Relative abundance of phytoplankton at site 1 (near the river inflow) and site 2 (near the dam) of Monjolinho reservoir, expressed as percentages.

RESULTS AND DISCUSSION

In the current study, changes in the color of the reservoir water were first noticed in the beginning of October, 2004 and an inspection of phytoplankton net-samples revealed the presence of a cyanobacterial bloom that was dominated by *Anabaena circinalis* Rabenhorst *ex* Bornet et Flahault and *Anabaena spiroides* Klebahn. Neither phytoplankton nor physical and chemical variables had regularly been monitored in the previous months. For this reason the interpretation of reservoir conditions was limited to the period of establishment, full development and decay of the *Anabaena* bloom, a period extending from October 6th to November 10th, 2004.

The phytoplankton community consisted of 69 taxa distributed in 9 classes. Most taxa belonged to the following taxonomic classes: Cyanobacteria (24.6%), Chlorophyceae (30.4%), Euglenophyceae (13.0%), Bacillariophyceae (11.6%) and Conjugatophyceae (10.1%). Other groups poorly represented were: Chrysophyceae (8.8%), Dinophyceae (1.4%), Cryptophyceae (1.4%) and Xanthophyceae (1.4%) (Figure 1). The mean value of the total phytoplankton density varied between 3.4×10^5 ind.mL^{-1} (site 1) and 1.3×10^6 ind.mL^{-1} (site 2). *Anabaena circinalis* and *A. spiroides* Klebahn were dominant (in the majority of samples) in the phytoplankton community during October 2004 (Figure 2). Phytoplankton succession in this reservoir was first described by Nogueira and Tundisi (1996) and Seleghim and Godinho (2004) which reported the dominance of diatoms (*Aulacoseira granulata* and *A. italica*) during the dry season, replaced by chrysophyceans (mainly *Dinobryon* sp and *Synura elegans*) in October-November and a later replacement by chlorophyceans after the heavy rainfalls in December and January months.

Figure 3 shows that during the period of this study the climate in the region was characterized by moderate precipitation (total precipitation = 128 mm and mean = 4.2 mm), low mean wind velocities (mean value = 3.1 m.s^{-1}) and warm temperatures (17.5 - 22.0 ºC). Precipitation ranged from 0 to 14 mm per day and maximum wind velocity was 6.8 m.s^{-1}. Typical conditions of a tropical spring (October) in the Southeast of Brazil include a transition period between the dry and wet periods with low turbulence in the water and relatively higher residence time when compared to winter (highest wind velocities) and summer conditions (higher temperatures and intense rainfalls). In concordance with our observations, Tundisi *et al.* (2008) reported that the hydrology of the basin, retention time of the reservoir and cold fronts have had an impact in the vertical and horizontal structure of the Barra Bonita Reservoir which promoting rapid changes in the planktonic community and in the succession of species. In that reservoir, blooms of *Microcystis* sp. have been recurrent during periods of stability.

Cyanobacteria were the best represented group in terms of relative abundance in the majority of the samples collected in both sampling sites. On the other hand, the occurrence of the first rainfalls in October induced changes in the abundance

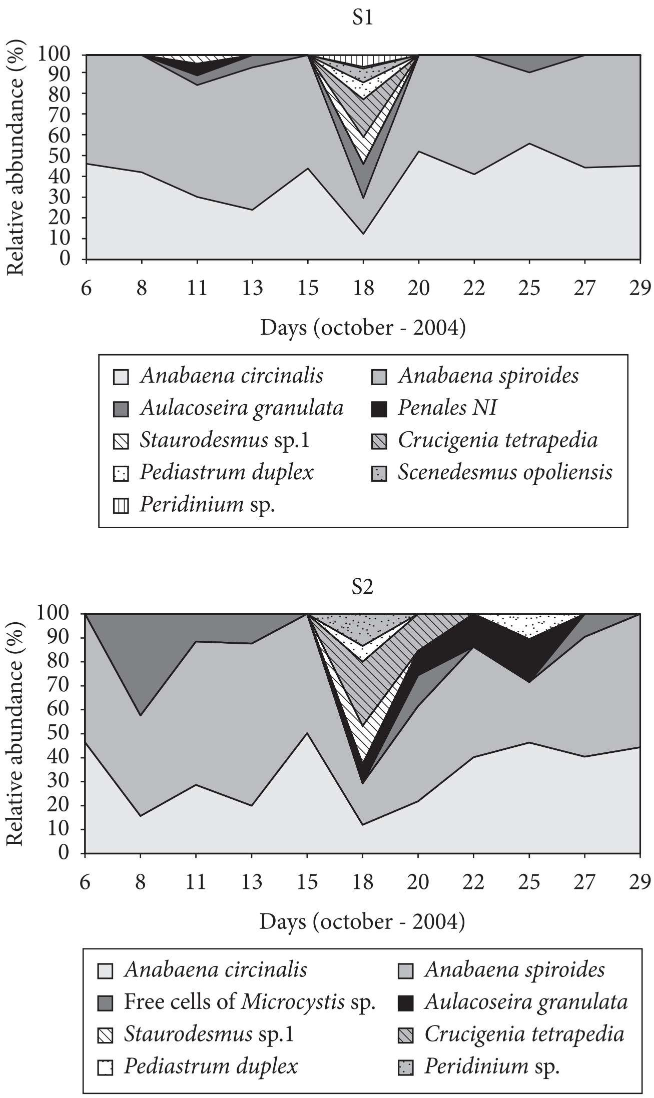

Figure 2 - Relative abundance (%) of the dominant species in the phytoplankton community in Monjolinho Reservoir (sites 1 and 2) during October 2004.

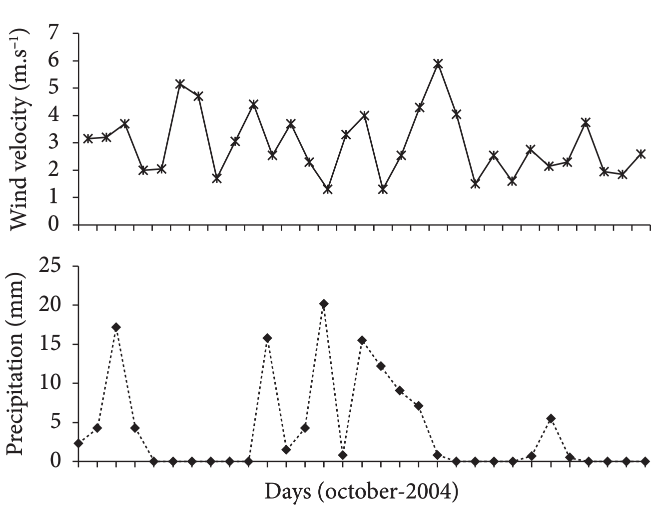

Figure 3 - Wind velocities (m.s^{-1}) and precipitation (mm) registered in a Monjolinho Reservoir.

Cyanobacteria (Figure 1-3). However, the dominant total algal densities decreased markedly after consecutive days of rainfall, particularly in the period between 11th and 18th of October (Figure 3). The number of species (richness) and the diversity indices are presented in Figure 4. The highest richness and diversity occurred at site 1.

Data about quality of water show that temperature of the water in the reservoir varied from 19 to 22 ºC, slowly rising over the spring. Warm water temperatures (about 20 ºC) are typical for the beginning of the rainy season in this tropical area and are considered favourable for the growth of phytoplankton. Water pH varied widely, from slightly acid to basic (5.86 to 9.45). Similarly, dissolved oxygen concentrations varied from 4.89 to 14.43 mg.L^{-1} (data not presented). High concentrations of DO are also common during the occurrence of bloom due to intense photosynthetic activity. Both pH and DO values were expected because these parameters are related to photosynthetic activities of the cyanobacterial bloom and other green-algae as well (Sotero-Santos *et al*., 2008). Cyanobacteria and phytoplankton blooms are likely to increase the pH when they take up CO_2.

Table 1 displays the mean values and standard deviations for nutrients (N and P compounds), chlorophyll *a* and SRSi concentrations in the surface water of Monjolinho Reservoir, from the two sampling sites (S-1 and S-2). Measurements of these variables were performed only at the surface level since the water column was homogeneous during most of the study period as evidenced by the temperature profile (Figure 5) and the reservoir is shallow (max depth = 3 m). Seleghim and Godinho (2004) have stated that there is no persistent thermal or density stratification in a shallow reservoir, such as Monjolinho.

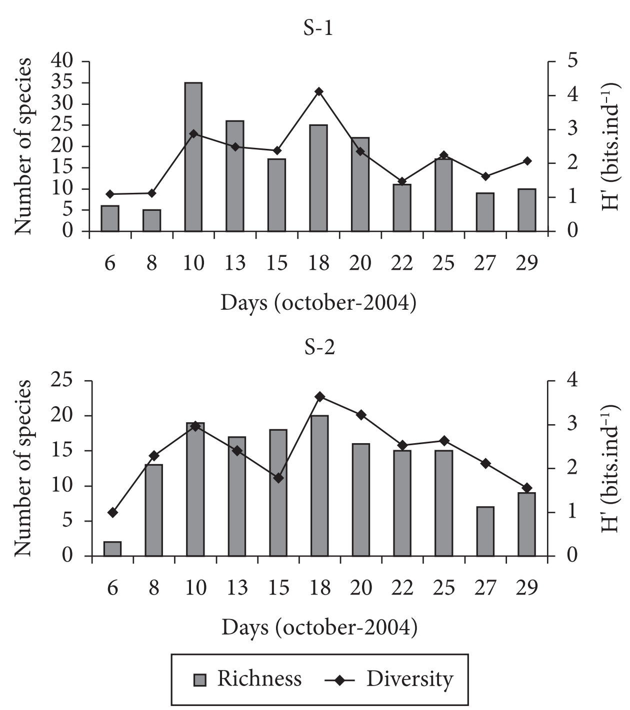

Figure 4 - Richness and diversity (bits.organisms^{-1}) variation in the phytoplankton community in Monjolinho Reservoir (site 1 and 2) during October 2004.

High nutrient concentrations were observed in the reservoir. Elevated nitrogen and phosphorus loadings revealed its intense trophic state, which is comparable to other eutrophic reservoirs such as Barra Bonita (Tundisi *et al*., 2008). In Monjolinho reservoir, nutrient inputs have been attributed to punctual (organic-industrial effluents) and non-punctual (agricultural runoff) sources. Additionally, untreated domestic sewage was being accidentally discharged into the reservoir due the rupture of sewage pipe and obviously that fact also contributed to increase the nutrient levels in the reservoir in October 2004. Concentration of nutrients available for algal or cyanobacterial growth exhibited a temporal and significant variation and it showed difference among the sampling sites.

Table 1 - Mean values and standard deviations (SD) for nutrients (N and P compounds, in µg.L^{-1}), chlorophyll *a* (µg.L^{-1}) and soluble reactive silica (mg.L^{-1}) concentrations in the surface water of Monjolinho Reservoir during October 2004, in the sites S-1 and S-2. (NO_2: nitrite; NO_3: nitrate; NH_4: ammonium; TN: total nitrogen; TDP: total dissolved phosphate; TIP: total inorganic phosphate; OP: total organic phosphate; TP: total phosphorus; SRSi: soluble reactive silica; TN/TP: total nitrogen and total phosphorus ratio and Chl *a*: chlorophyll *a*).

Variables	Sites	Mean and SD
NO_2^-	S1	15.4 ± 6.6
	S2	8.2 ± 2.6
NO_3	S1	49.9 ± 19.3
	S2	28.9 ± 12.5
NH_4	S1	128.7 ± 93.3
	S2	39.2 ± 25.6
TN	S1	724.9 ± 158.0
	S2	881.5 ± 289.1
TDP	S1	31.3 ± 12.0
	S2	27.5 ± 6.7
TIP	S1	21.0 ± 12.0
	S2	13.5 ± 4.5
TOP	S1	10.3 ± 9.4
	S2	14.0 ± 2.9
TP	S1	90.9 ± 25.8
	S2	104.1 ± 27.3
SRSi	S1	6.1 ± 1.1
	S2	6.5 ± 0.7
TN/TP	S1	8.2 ± 1.7
	S2	8.4 ± 1.5
Chl *a*	S1	39.1 ± 38.1
	S2	91.7 ± 54.9

Ammonium and nitrate mean concentrations in the upper part of the reservoir, at site S-1 (128.7 ± 93.3 and 49.9 ± 19.3 µg.L^{-1}, respectively) were much higher than the values recorded in the lower part of the reservoir near the dam, at site S-2 (39.2 ± 25.6 and 28.9 ± 12.5 µg L^{-1}, respectively). The nitrogen analyses indicated that ammonium was the predominant nitrogen form in the Monjolinho reservoir in both sites. It is speculated that both *Anabaena* species could take advantage over other phytoplanktonic species through their ability to uptake ammonium. Cyanobacteria have been considered able to use nitrate, nitrite and ammonium as inorganic source of nitrogen by the so as N2 from N-fixing Cyanobacteria. However, when ammonium is available, it seems that these organisms do not assimilate others inorganic source of nitrogen (Ochoa-de-Alda *et al.,* 1996). According to Von Rückert and Giani (2004) Cyanobacteria may compete with eukaryotes for nitrate and they also have better ability to the rapid use of ammonium when nitrate is no longer available in the environment, thus allowing them to suddenly increase their population and develop into a bloom.

Variations in phosphate data were not as clear as observed for nitrogen compounds. Inorganic phosphate mean concentration at site S-1 (21.0 ± 12.0 µg.L^{-1}) was higher than that observed at site S-2 (13.5 ± 4.5 µg.L^{-1}), but that tendency was not verified for organic phosphate where both sites exhibited concentrations relatively similar (10.3 ± 9.4 µg.L^{-1} at site S-1 and 14.0 ± 2.9 µg.L^{-1} at site S-2). On the other hand the total nitrogen (724.9 ± 158.0 µg.L^{-1} at site S-1 and 881.5 ± 289.1 µg.L^{-1} at site S-2) and total phosphorus (90.9 ± 25.8 µg.L^{-1} at site S-1 and 104.1 ± 27.3 µg.L^{-1} at site S-2), which reflect both, nutrient available in the water plus the amount incorporated in biomass were only slightly lower in the upper part than near the dam.

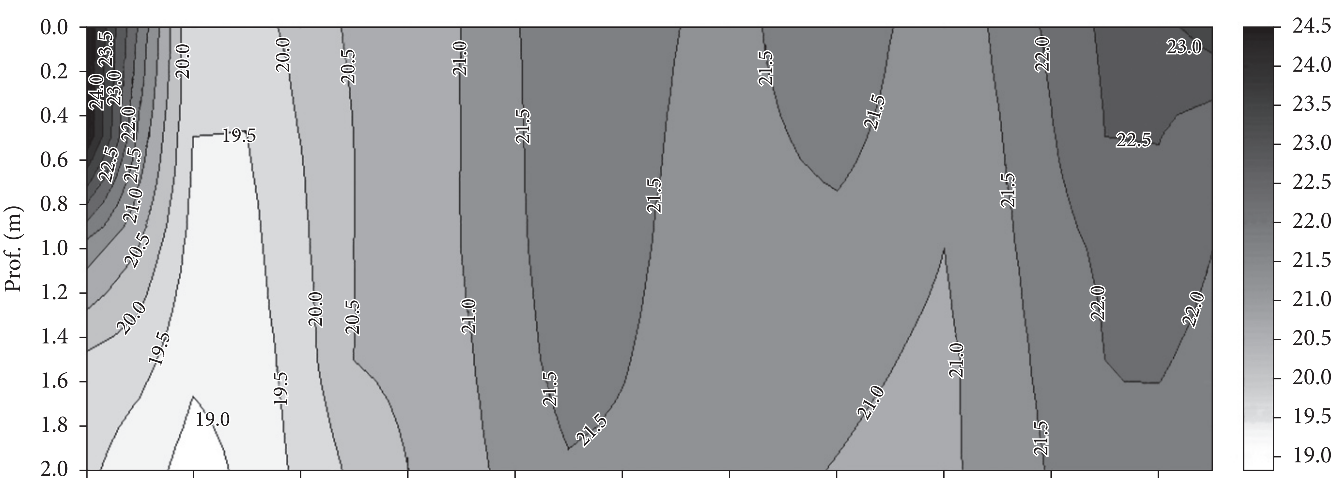

Figure 5 - Isopleths of temperature (°C) registered in a Monjolinho Reservoir during October 2004 in the S-2 station.

Total nitrogen/total phosphorus ratio greatly changed along the bloom occurrence (Table 1). The ratios were low at the beginning of the bloom, decreasing further after the occurrence of rainfalls triggering a change in the cyanobacteria dominance and returned to the initial levels at the end of the bloom (Figure 6). Phosphorus concentrations were particularly high at the time of the bloom establishment with N:P ratios around 8.0 (Table 1). Blooms of cyanobacteria are usually associated with stable conditions and high nutrient concentrations (Giani *et al.,* 2005). Nitrogen-fixing species such as those of *Anabaena* genus have been found to be especially associated with high phosphorus availability (Smith, 1983; Downing *et al.,* 2001). Low N:P ratio has been considered as a stimulatory factor to cyanobacterial blooms among other conditions such as water column stability and presence of gas vacuoles in the majority of cyanobacteria cells (Mur *et al*., 1999). According to Reynolds (1999), the crucial factor for cyanobacterial dominance, comes from its ability to remain in suspension by regulating buoyancy and responding rapidly to favourable nutritional conditions.

Chlorophyll mean concentrations at site S-1 (39.1 ± 38.1 μg.L^{-1}) were much lower than at site S-2. (91.7 ± 54.9 μg.L^{-1}). That fact was expected because cyanobacterial scum tend to be concentrated near to the shores and around the dam due water flux and wind action. Consequently, chlorophyll-*a* concentrations at S-2 could be overestimated when compared with to other parts of the reservoir. Statistical analyses revealed significant

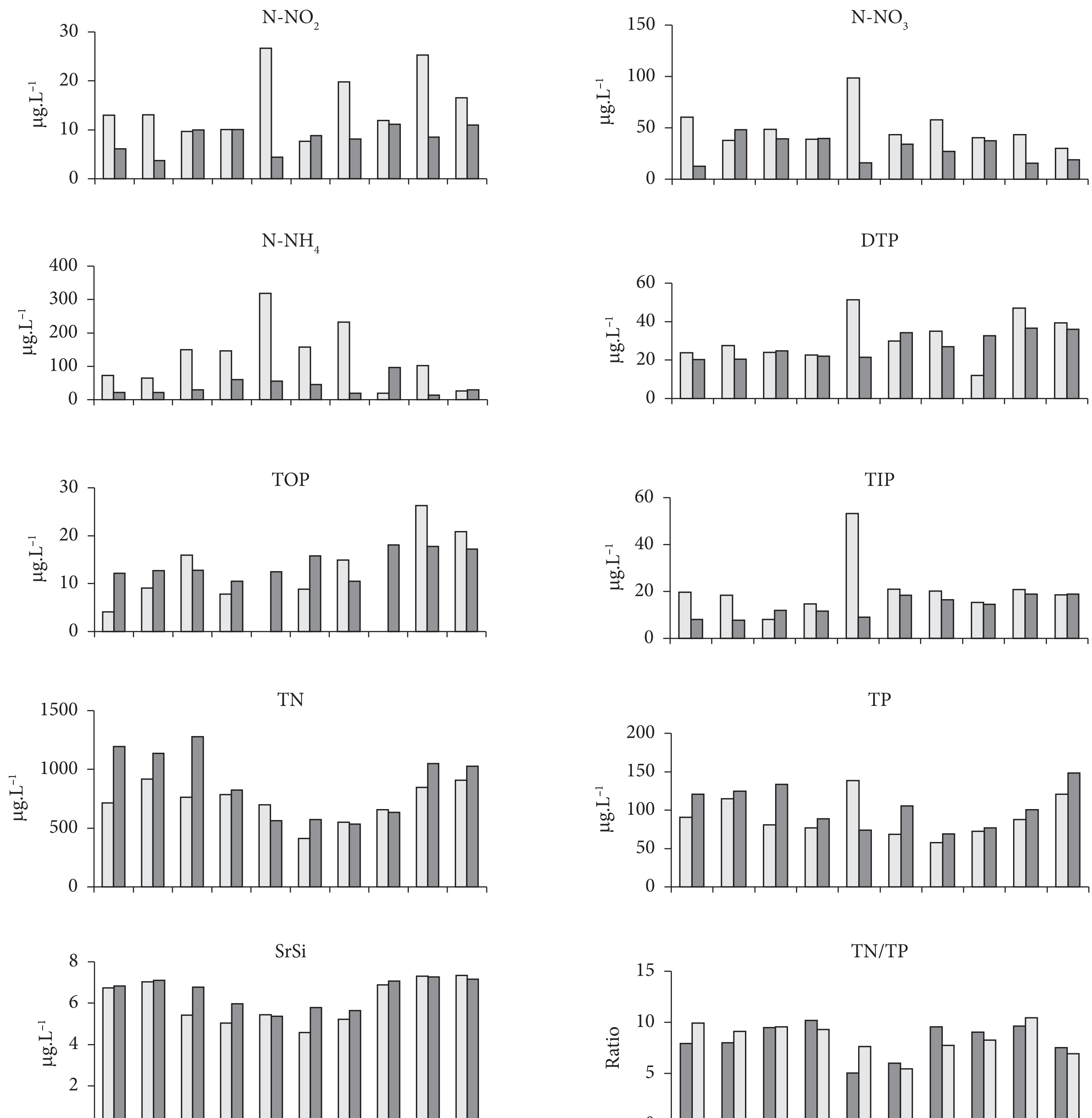

Figure 6 - Concentrations of nutrients (μg.L^{-1}) and SRSi (mg.L^{-1}) in the surface water of Monjolinho Reservoir during October 2004, in stations S-1 and S-2 (S-1= bar black, near the river inflow and S-2= bar gray, near the dam).

spatial variation ($p < 0.05$) between sampling points only for nitrogen compounds and chlorophyll *a*. Most limnological variables analyzed showed no significant temporal variation ($p > 0.05$), except for temperature, pH, DO and SRSi. Water transparency varied between 20 and 50 cm (data not presented) but no correlation was found between Secchi readings and chlorophyll *a* concentrations.

Climate events and physical and chemical conditions driven by them appear as forcing functions that favor the bloom. Historically, the dominant climate conditions in Monjolinho Reservoir during August and September months has been characterized by the highest wind velocities in the year and rising temperatures from September onwards. A wind force of 6 m/s has been suggested as sufficient to promote turbulence in the whole water column, or at least in a great volume of the reservoir with important mixing consequences on the vertical distribution of dissolved oxygen, turbidity, and water temperature (Tundisi *et al.*, 2002). In the present study, climate events were somewhat different in 2004 (see Figure 3). It is speculated that a combination of moderate winds and increasing temperatures in early October allowed thermal stratification development in the Monjolinho Reservoir, possibly favoring the growth of *Anabaena*. Moreover, the Monjolinho Reservoir has been considered plenty of nutrients round the year (Bianchini; Antônio, 2000), as also observed for the majority of the shallow eutrophic and tropical systems. Our results suggested that nutrient loading, warm water temperature and water column stability were relevant environmental factors that influenced cyanobacterial dominance in the Monjolinho Reservoir. *Anabaena* species are also favored in low mixing depth ratios ($Zm < 1$).

Anabaena species are S-strategists (Reynolds, 1997) being stimulated to grow under high light intensities and thermal stability. A delay of the rainfalls with prolonged reservoir stability coupled with nutrient availability and increasing temperature seem to be the combination of weather events and/or physical and chemical factors that triggered the unusual growth of *Anabaena* populations in Monjolinho Reservoir. The arrival of rainfalls by middle October induced temperature drop, nutrient dilution and wash-out and broke up the thermal stratification. Although *Anabaena* spp. was still dominant, other groups of algae such as chlorophyceans and diatoms increased their abundance. On the other hand, the *Anabaena* bloom was quickly re-established as soon as rainfall stopped and stable conditions returned in late October.

Regarding the toxins analyses, neurotoxins (saxitoxin and gonyautoxin) were not detected in cyanobacterial samples collected during October 2004. On the other hand, microcystin was found in both water (ranging from 28 - 45 $\mu g.L^{-1}$) and natural cyanobacterial samples (138 to 445 $\mu g.g^{-1}$ dry weight freeze-dried cyanobacteria). *Anabaena* crude extracts prepared from the cyanobacterial material collected in the reservoir caused acute toxicity to both cladocerans and mice (Sotero-Santos *et al.*, 2008), indicating that toxins released are potentially capable of killing aquatic invertebrates, fish and eventually other animals such as capybaras, herons and other birds which could drink water from reservoir. Currently the Monjolinho Reservoir has as function to improve or beautify the landscape of the UFSCar campus and it has been used as water supply only for wildlife consequently the water quality should be environmentally monitored and preserved. Detecting the occurrence of a toxic bloom in an aquatic ecosystem is only a first step in researching this phenomenon. Additional efforts must be made to develop environmentally correct strategies for eutrophication control in reservoirs such as Monjolinho.

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