Cyanobacteria Occurrence in Photosynthetic Stabilization Ponds

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Abstract

Photoautotrophic organisms, particularly cyanobacteria, have great ecological importance due to their photosynthetic capacity, and biosynthetic versatility in diverse and extreme environments. However, photosynthetic ponds, they may be serious and dangerous producers of potentially toxic toxins. Their release and bloom in treated effluent receiving bodies are a major concern because of the negative consequences on aquatic biota and the risks to public health. The aim of this study is to analyze the occurrence, composition, density and spatio-temporal distribution of cyanobacteria in sewage treatment plants by photosynthetic ponds in ten cities located in the central region of the São Paulo State, Brazil. The results recorded high densities of Microcystis sp. with a maximum average of 9.4x105 cells per millilitre (cells/mL); Synechococcus sp., with an average of 7.8x105; Synechocystis aquatilis with 7.2x105; Merismopedia tenuissima with 4.8x105; and Phormidium sp. with 1.9x105. Among these species found, the highest occurrence was M. tenuissima. The high densities show that these ponds are an aquatic environment conducive to the development of cyanobacteria and, potentially, an important source of cyanotoxin production. Therefore, studies and monitoring of the effects on the receiving water bodies are recommended by determining their cyanobacteria densities and investigating the possible presence of cyanotoxins.

Keywords: Cyanobacteria; sewage treatment plants; photosynthetic stabilization ponds.

1. Introduction

Planktonic cyanobacterial flora communities are distributed in a range of ecologically diverse habitats of estuarine and marine water, soil, as well as extreme habitats such as hot springs and Antarctic and Arctic environments (Sompong et al., 2005; Taton et al., 2006).
They are primarily distributed in freshwater aquatic ecosystems in almost all tropical, subtropical, temperate, polar and sub-polar latitudes around the world (Kosten et al., 2012; Vincent & Quesada, 2012;

Paerl & Otten, 2013).

Some genera of cyanobacteria, such as Dolichospermum and Aphanizomenon have a cosmopolitan distribution (Sheath et al., 1996; Sheath Muller, 1997; Tang et al., 1997); others are apparently more restricted to cold waters in temperate regions, such as most Oscillatoria sp., or to tropical and subtropical waters, such as Cylindrospermopsis and Spirulina (Padisak, 1997; Whitton & Potts, 2000; Karadžić et al., 2013).

Cyanobacteria are collectively referred to as those that constitute an old group of photosynthetic, highly adaptable and abundant prokaryotic phytoplankton organisms, which are recognized as important causes of environmental issues, having serious implications for human health and water-related economic activities (Azevedo & Vasconcelos., 2006; Tsukamto & Takahashi, 2007; Santos & Bracarense, 2008). In surface fresh waters, their abundance, biomass, and species composition are widely known for varying greatly in time and space (Knoppers et al., 1984; Graham et al., 2006; Prentice, 2008; Sarika et al., 2010).

Excessive proliferation of cyanobacteria in the form of intense blooms is generally correlated with various combined environmental factors, such as nutrient availability, especially nitrogen and phosphorus compounds (Chorus & Bartram, 1999; Whitton & Potts, 2000), a wide range of seasonal variations of light intensity, temperature, hydrographic and hydrological conditions (Sangita Ganesh et al., 2014; Lorena et al., 2015). Many of these factors are the result of anthropogenic activities, which are much more important than the natural causes of cyanobacteria proliferation.

It is known that the increased accumulation or availability of phosphatic and nitrogenous compounds in temperate, mainly tropical continental environments, provide an increase in the primary productivity (Smith, 1983) and play a key role in regulating the composition of the cyanobacteria community (Andersson et al., 2015). Consequently, the environmental factors may benefit species selection through competitive mechanisms (Calijuri et al., 2006).

The main concern with the occurrence of high densities of cyanobacteria in water bodies, especially in water sources, is the fact that some of these organisms are known to produce and release more than one type of cyanotoxin into the waters. Moreover, there may be several strains producing toxins within the same species (Furey et al., 2005; Soltero-Santos et al., 2005). The Brazilian legislation on surface water quality, CONAMA Resolution No. 357/2005, establishes that the limit for the density of cyanobacteria in Classes 1, 2 and 3 water bodies is 20,000 cells/mL, 50,000 cells/mL and 100,000 cells/mL, respectively (Brasil, 2005). Class 1 and 2 water bodies are not very polluted and are generally used as springs.

In Brazil, facultative photosynthetic stabilization ponds have been widely used to treat sanitary and industrial sewage due to the fact that they are simple to construct, operate and maintain. They are also inexpensive and efficient in removing pollutants. However, these ponds, that have high nutrient contents, constitute a habitat which favours conditions for the intense development of phytoplankton, mostly cyanobacteria, having high concentrations of bacteria, algae and cyanobacteria in the final effluent, which interact mutually (Pearson, 1987; de Oliveira, 1990; Abdel-Raouf et al., 2012).

The high densities of cyanobacteria, although contributing significantly to the aquatic primary production and to the process of removing atmospheric CO2 and its conversion into organic matter and O2, playing a relevant role in the plankton. Together with eukaryotic microalgae, are an inconvenience insofar as they may cause problems concerning toxicity, colour, smell, taste and altered appearance in the supply water, as well as operational problems in the water treatment plants, leading to limitations for drinking water treatment or similar issues (Smith et al., 2008; Rosales et al., 2008, Abdel-Raouf et al., 2012).

2. Methods

2.1 Study Subject

The object of study consisted of ten sanitary sewage treatment plants by facultative photosynthetic stabilization ponds (LF1 to LF10), from cities in the central region of the São Paulo State, southeast Brazil near the city of São Carlos. The criteria for choosing the cities/ ponds were the proximity to São Carlos and easy access to the sampling points. A sampling point was established for each pond at the outlet of its effluent and the ponds were georeferenced.

Table 1 lists the ten cities and their respective ponds, the location of the sampling points considering the geographic coordinates and the respective receiving water bodies of treated sewage and their Class.

Table 1. Sampling point coordinates and receiving water bodies.

City	Pond	Location	Receiving water body
Analândia	LF1	S 22°08’36,9” and W 47°39’81,5”	Corumbataí River, Class 2
Brotas	LF2	S 22°17’28” and W 48°08’72”	Jacaré Pepira River, Class 3
Itirapina	LF3	S 22°24’59,4” and W 47°50’27,5”	Água Branca Brook, Class 2
Charqueada	LF4	S 22°35’24,5” and W 47°42’20,3”	Corumbataí River, Class 2
Ipeúna	LF5	S 22°26’52,6” and W 47°42’46,8”	Lavadeiras Brook, Class 2
Corumbataí	LF6	S 22°14’06” and W 47°36’95,2”	Corumbataí River, Class 2
Guariba	LF7	S 27°21’04,2” and W 48°09’4,85”,	Guariba Brook, Class 4
Santa Lúcia	LF8	S 22°40’02,4” and W 48°05’98,5’	Ponte Alta Brook, Class 2
Santa Eudóxia	LF9	S 21°46’60,2” and W 47°47’17”	Quilombo River, Class 2
Ibaté	LF10	S 21°56’70,7” and W 48°01’72,3”	São José das Correntes Brook, Class 2

Figure 1 shows the location of the ten ponds studied and their respective cities in the State of São Paulo and in Brazil.

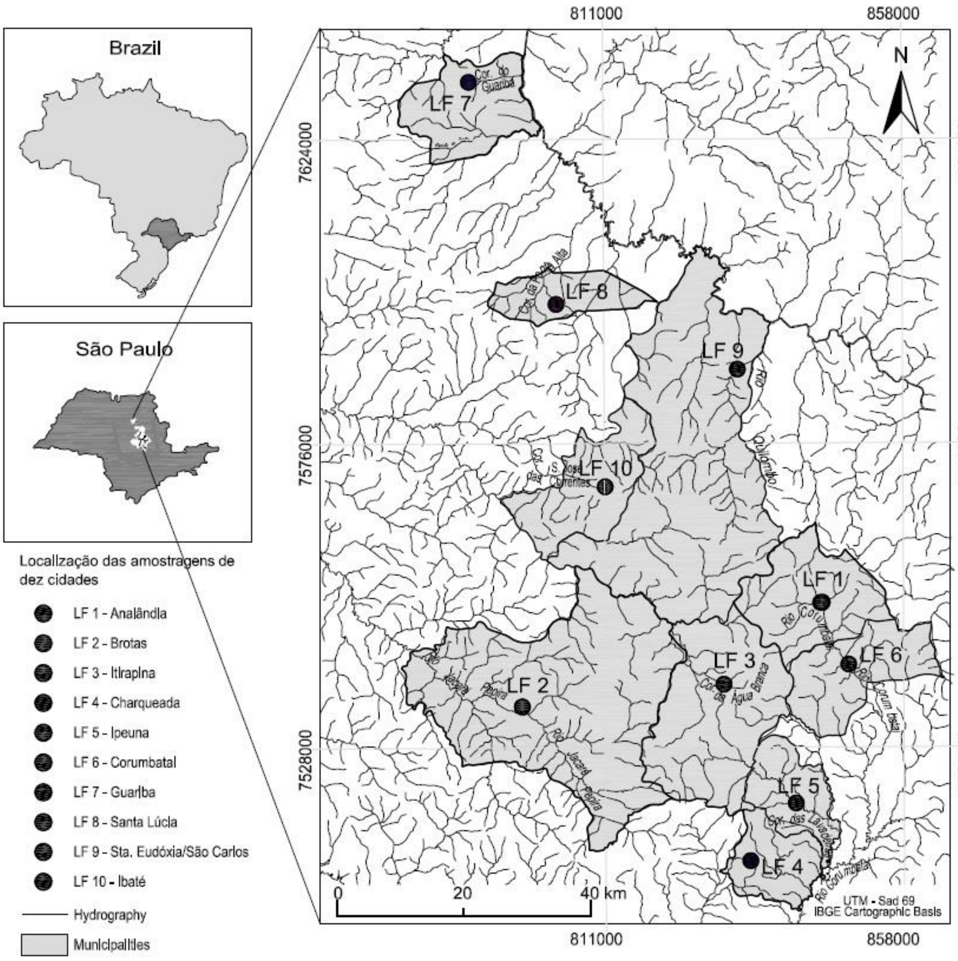

Figure 1. Location of the ponds studied and their respective cities.

2.2 Field Sampling Procedures

From May, 2012 to April, 2013 monthly samples were taken to identify and characterize the distribution of cyanobacteria qualitatively and quantitatively. For the qualitative analysis, the samples were collected and filtered through a 20 μm nylon conical plankton net, dragged horizontally at each collection site on the subsurface (approximately 0.5 m deep).

For the quantitative analyses, the samples were collected using a bucket (capacity 5 litres) and a cup (capacity 1 litre) made of stainless steel - AISI316. Immediately after the samples were taken, the seston was packed in 250 ml polyethylene plastic bottles containing 4% formaldehyde solution to fix the samples and preserve them, which were stored in polystyrene boxes with crushed ice and transported to the Sanitation Laboratory of the Civil Engineering Department at the Federal University of São Carlos in order to be analysed.

2.3 Identification and Quantitative Evaluation of Cyanobacteria

For the traditional classical taxonomic identification of the network samples, even at the species level, they were analyzed using a common optical binocular microscope with a magnification of 400 to 1000x, coupled to a clear and ocular camera. The identification was primarily based on the following identification keys adopted by Desikachary (1959); Anagnostidis & Komárek (1988); Komárek & Anagnostidis (1989); Komárek & Anagnostidis (1999); Komárek & Anagnostidis (2000); Komárek & Anagnostidis (2005); Komárek & Cronberg (2001); and Sant'Anna & Azevedo (2000).

The Utermöhl method was used to estimate the density of the cyanobacteria by numerical counting. The technique using transparent cylindrical acrylic sedimentation chambers of different known volumes was adopted, as described by Utermöhl (Utermöhl, 1958), using a Coleman binocular inverted microscope N/B100, with an ocular micrometer scale coupled to a microscope with a magnification of 400 to 1000x (Anderson & Thröndsen, 2003). The results were recorded in cells/mL.

2.4 Statistical Data Analysis

Due to the possibility of extreme results and the occurrence of a non-normal distribution, the data were statistically analysed using the nonparametric Kruskal-Wallis test (Viali, 2008). In the present study, the significance level of 5% was set for all tests in order to record the similarity or difference between the groups of samples.

3. Results and Discussion

Cyanobacteria were identified in all the stabilization ponds and their cell concentrations were determined for each sample in their spatio-temporal distribution during the twelve-month study period.

Observations concerning the periodicity of cyanobacteria occurrence show that conditions of low annual temperature variations, characteristic of tropical regions and non-thermally stratified waters are optimal conditions for the occurrence and development of the main cyanobacteria genera. (Calijuri et al., 2006; Reynolds, 2006). This was the case of the temperatures observed in the ten ponds, which were generally within the range of 25° to 30° C. However, more detailed studies are needed to verify some temporary stratification.

The genera of cyanobacteria found in the present study differ little from those recorded by Aquino (2010) and Aquino (2011) in photosynthetic stabilization ponds in the Ceará State as well as those observed by Konig et al. (1999) in the Paraíba State, Brazil.

Six species of cyanobacteria were common in the ten studied ponds: M. tenuissima, Aphanocapsa sp, Lyngbya sp, Pseudanabaena sp, Microcystis sp e Spirulina sp. On average, M. tenuissima appeared more, suggesting that this species best adapts to the hypereutrophic environment of the ponds. Lyngbya sp was the second most abundant species. The genus Merismopedia, to which belongs the species M. tenuissima, adapts very well to different environments/habitats (Brettum, 1989, Blomqvist, 2001, Tian et al., 2012). The genera Microcystis and Planktothrix are considered to be toxin producers by Sivonen & Jones (1999). Furtado et al. (2009) report the coexistence and even the alternation between Microcystis sp. and Merismopedia sp. with Planktothrix sp. and Cylindrospermopsis sp. in sewage treatment ponds.

The densities of the cyanobacteria found in the present study are presented in Table 2.

Table 2. Densities of the cyanobacteria recorded in the facultative ponds studied (cells/mL).

Month	LF1	LF2	LF3	LF4	LF5	LF6	LF7	LF8	LF9	LF10
05/2012	121,139	17,289	3,956	31,875	62,334	3,093	*	949,119	361,300	12,048
06/2012	98,462	10,510	14,510	4,704	77,823	52,925	0	437,034	138,310	75,730
07/2012	442,218	7,745	6,704	8,468	*	12,898	230,322	27,613	451,962	112,867
08/2012	16,038	9,409	0	9,408	130,547	32,930	2,352	11,516	137,605	34,107
09/2012	174	56,667	27,639	36,851	48,220	53,631	2,303	2,205	87,522	29,403
10/2012	28,227	43,516	1,176	11,761	197,586	58,217	106	4,410	109,378	3,528
11/2012	13,819	31,093	4,234	19,942	801,518	18,999	5,881	1,764	48,367	865
12/2012	3,458	28,227	33,208	19,994	186,794	114,082	4,704	17,642	19,759	14,113
01/2013	2,487	152,012	248,747	34,107	*	137,605	10,364	576,293	100,190	20,582
02/2013	2,211	41,458	11,761	52,925	301,084	255,804	37,312	717,426	91,207	100,408
03/2013	6,910	6,910	148,823	108,960	223,234	154,138	113,067	963,030	10,364	134,206
04/2013	6,495	351,068	152,012	107,099	150,542	77,623	171,013	884,433	981,168	18,524
Average	61,803	62,992	54,398	37,175	217,968	80,995	52,493	382,707	211,428	47,365

* No sampling.

As can be seen in the previous table, the densities or concentrations and distribution of cyanobacteria were very varied in the ponds and over time, the following ponds stood out: Ipeúna - LF5, Santa Lúcia - LF 8 and Santa Eudóxia - LF 9 for their high values with averages above 200,000 cells/mL and maximum values greater than 800,000 cells/mL (see Table 2).

High densities can be produced by a number of combined biotic and abiotic factors resulting from eutrophication and other processes: low turbulence, low water transparency, low or high pH values, high water temperatures, thermal stratification, high light incidence and availability of nutrients, although many of their species are able to fix atmospheric nitrogen and transform it into assimilable forms (ammonia and nitrate), and are also able to store phosphorus in the form of polyphosphates (Reynolds, 1984; Reynolds, 1987; Reynolds, 1998; Crayton & Sommerfield, 1979; Sant'Anna et al., 2008). Chorus & Bartram (1999) state that as the decomposition of the sewage occurs throughout the stabilization ponds, the number of species in their effluents released into the receiving bodies of water generally decrease.

According to (Harsha & Malammanavar, 2004), the high turbidity and high contents of dissolved solids favour the growth of cyanophytes. The presence of the high density of cyanophytes indicates high pollutant load and a nutrient rich condition (Muhammad et al., 2005; Tas & Gonulol, 2007). According to Chorus & Bartram (1999), flowering densities above 10000 cells/mL of cyanobacteria can be considered.

Figures 2 to 4 show the seasonal distribution of densities in the effluents of the richest cyanobacterial ponds: Ipeúna - LF5, Santa Lúcia - LF8 and Santa Eudoxia - LF9, for the twelve months of sampling. In the Ipeúna pond, there was no sampling from July, 2012 to January, 2013.

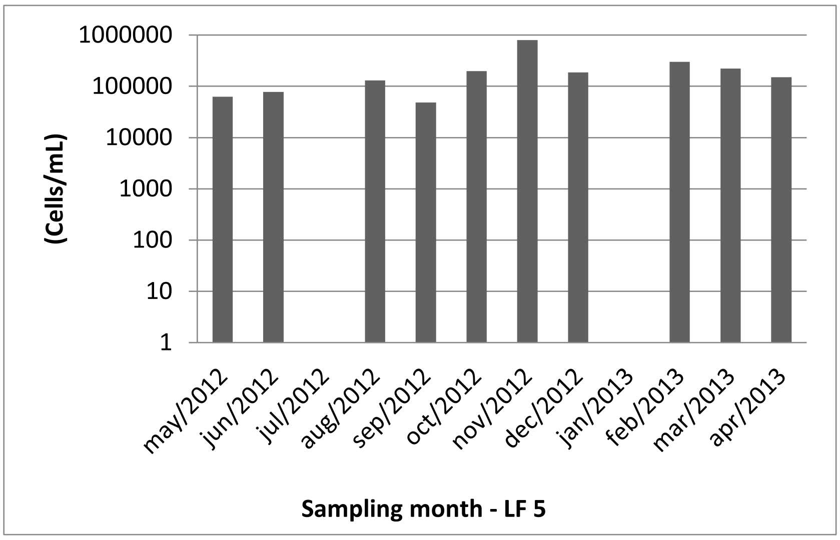

Figure 2 - Densities of cyanobacteria in the effluent of the Ipeúna pond.

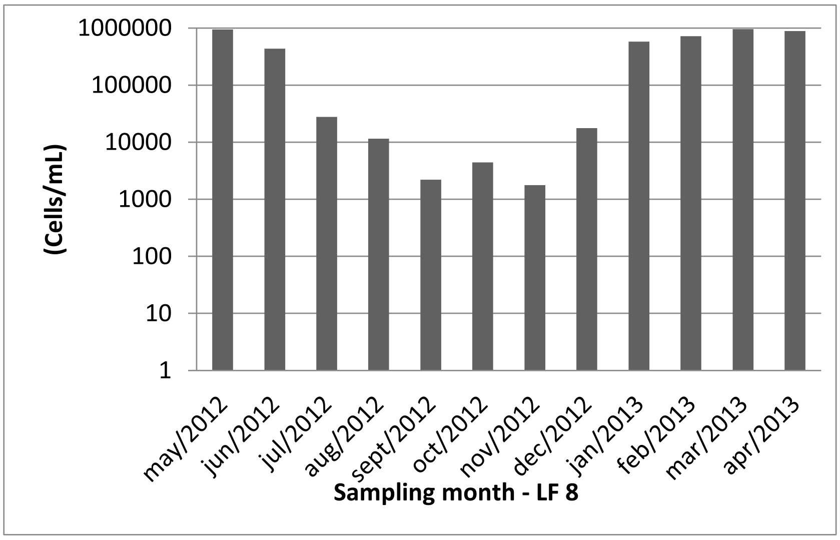

Figure 3 - Densities of cyanobacteria in the effluent of the Santa Lúcia pond.

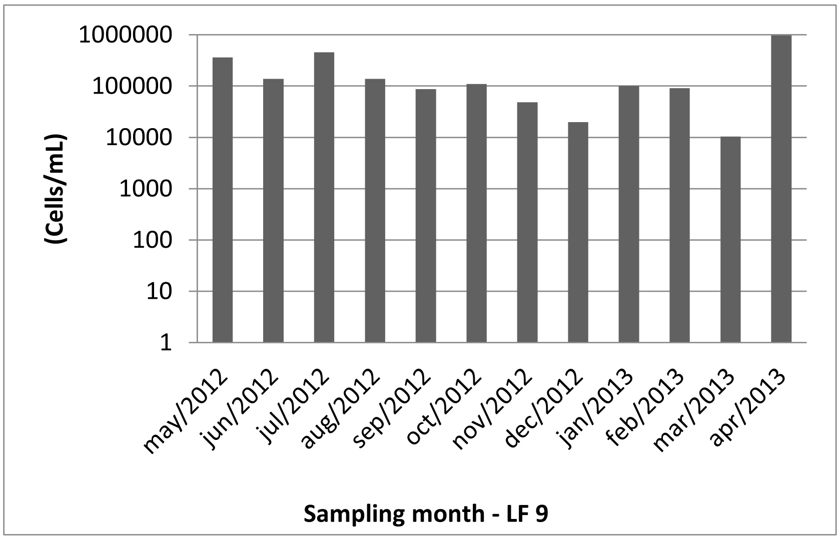

Figure 4 - Densities of cyanobacteria in the effluent of the Santa Eudóxia pond.

High densities of more than 20,000 cells/mL in the treated effluents deserve attention and systematic and detailed research. Depending on the flow rate and the dilution and environmental conditions (potential for flowering) of the receiving water bodies, they can result in values above the legal limits set forth in the CONAMA Resolution 357/2005, whose lowest density is 20,000 cells/mL for water bodies belonging to Class 1, with low pollution (Brasil, 2005).
Seasonal variations of cyanobacteria concentrations with maximum values in the sunny months were observed in the studies carried out by Oswald (1988) and Zulkifli (1992). It can be observed that there is currently a large proliferation of cyanobacterial blooms occurring in freshwater ecosystems at all latitudes leading to growing concerns for scientists and water resource managers (Wilhelm et al., 2011). Damas (1964) believes that in the intertropical zone, plankton can appear in any season of the year. According to Paerl & Otten (2013), to explain the true causes of fluctuations in algae and mixofícea populations, appropriate and case-specific studies would be required.

5. Conclusions

The results show the occurrence of various species of cyanobacteria at high concentrations, above 20,000 cell/mL, indicating the risk of contamination of the receiving water bodies beyond the limits of the Brazilian legal standards of water quality for these organisms.
In general, cyanobacteria dominated the growth of the flora in the ten ponds studied, emphasising more the ponds in the cities of Ipeúna, Santa Lucia, and Santa Eudóxia. The three main genera found were Merismopedia, Pseudanabaena and Limnothrix, and the presence of Merismopedia is considered common in photosynthetic ponds around the world.
Among other registered genera, it is worth mentioning Planktothrix and Microcystis as they are related to the production of potentially toxic cyanotoxins, showing evidence of effluents at a greater risk to the receiving bodies and public health. However, in the present study no significant or dangerous concentrations of cyanotoxins were detected.
Thus, detailed studies involving not only the stabilization ponds and their operational conditions, but also their receiving bodies and continuous and systematic monitoring are of the utmost importance concerning the presence of cyanobacteria and, if necessary, cyanotoxins.

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