

Analysis of data on cyanobacteria and cyanotoxins in public supply reservoirs (São Paulo, Brazil)

by

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Abstract

The incidence of cyanobacterial blooms in reservoirs intended for human supply represents a serious health risk due to the ability of these organisms to produce cyanotoxins. The study aimed to collect and summarize information related to variables associated with eutrophication of water sources of the Sorocaba River basin (São Paulo, Brazil). The objective was to assess the occurrence of cyanotoxins in treated water supplied to approximately 800,000 people. The study analyzed data from different abstraction, treatment, and distribution stations, emphasizing the supply from the Itupararanga and Ipanema dams, as well as the Sorocaba River. Data were obtained from the Drinking Water Quality Surveillance Information System (SISAGUA), the Autonomous Water and Sewage Service (SAAE), and the Environmental Company of the State of São Paulo (CETESB). A total of 4719 data points for cyanobacteria, chlorophyll-*a*, and cyanotoxins were analyzed between 2014 and 2021. The results indicate a deterioration in the trophic state of the Sorocaba River and the Itupararanga Dam, revealing a correlation between cyanobacteria and saxitoxins, with a predominance of the genus *Raphidiopsis*. A microcystin concentration of 0.40 µg l⁻¹ was recorded at the Itupararanga Dam in treated water.

Key words: eutrophication, cyanobacteria, cyanotoxins, reservoirs, public health

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1. Introduction

Cyanobacteria can colonize most aquatic ecosystems on our planet. Over time, they have developed adaptations, including tolerance to the incidence of ultraviolet rays and the formation of specialized structures, including i.a. aerotopes, heterocysts, akinetes (Molica, Azevedo 2009). The process of eutrophication has favored the dominance of cyanobacteria in the phytoplankton community (Schopf 1995; Carneiro et al. 2005; Molica, Azevedo 2009).

Cyanobacterial blooms have brought serious problems to reservoirs and water bodies used to supply drinking water to the public, due to the alteration of water quality, not only through the production of cyanotoxins, but also through the formation of active secondary compounds by certain genera of cyanobacteria, which cause a change in the taste and odor of drinking water. The cyanotoxins produced can be classified as neurotoxins, hepatotoxins, or dermatotoxins (WHO 2003; Carvalho et al. 2013; Sonobe et al. 2019; Chorus, Welker 2021).

Several studies have reported poisonings and deaths of humans and animals due to cyanotoxins. In his article "Poisonous Australian Lake", published in the journal *Nature*, George Francis (1878) was the first to report the death of animals after consuming water from a source with proliferating cyanobacteria in South Australia (Huisman et al. 2018). Harada et al. (1996) investigated the levels of microcystins in drinking water from the Haimen reservoir in China between 1992 and 1994, and observed a higher incidence of hepatocellular carcinoma among individuals consuming water from the reservoir compared to those using well water.

The first confirmed case of death from cyanotoxins occurred in 1996 in Caruaru, Pernambuco, Brazil, involving hemodialysis patients who developed liver lesions after intravenous exposure to cyanotoxins. Of the total 124 patients affected, 51 did not survive (Jochimsen et al. 1998). Following this incident, Brazil has distinguished itself in implementing regulations to control cyanobacteria and cyanotoxins in drinking water (Bittencourt-Oliveira et al. 2014; Dos Santos Machado et al. 2022).

In addition to affecting the use of water resources, the proliferation of cyanobacteria also impairs compliance with the regulations established by the Ministry of Health, which defines guidelines and responsibilities related to the control and supervision of the quality of water intended for human consumption (Brazil 2021).

Therefore, given the importance of continuous

research to understand the effects of cyanobacteria and artificial enrichment on water bodies, this study collected and summarized data on parameters associated with eutrophication in the Sorocaba River basin (São Paulo, Brazil). The density of cyanobacteria and concentrations of cyanotoxins and chlorophyll-*a* were also assessed.

2. Materials and methods

2.1. Study area

The Sorocaba River basin (BHS), located in the central-southeastern part of the State of São Paulo (Brazil), comprises the lower, middle, and upper Sorocaba sub-basins (Fig. 1). The BHS is located in Water Resources Management Unit 10 (UGRHI 10), which includes the Sorocaba River basin and the middle Tietê River basin, with a drainage area of 11,829 km². This region is characterized by the presence of large rivers, such as the Sorocaba River, which crosses 28 municipalities in the basin, and the Tietê River, which runs through almost the entire State of São Paulo (FABH-SMT 2023).

The Sorocaba River, about 230 km long, is the largest left-bank tributary of the Tietê River, originating from the Sorocamirim and Sorocabuçu streams and receiving water from tributaries such as the Sarapuí, Tatuí, Pirajibu and Ipanema rivers (Smith 2003). The Itupararanga Dam, located upstream of the Sorocaba River, is its main reservoir, with a length of 40 km and a total estimated capacity of 355,000,000 m³ of water (Leite et al. 2018). According to Dos Santos Machado et al. (2022), 63% of the reservoir's water is used to supply about 800,000 people.

This study analyzed SISAGUA data from the Itupararanga and Ipanema dams, as well as the Sorocaba River, along with information from six CETESB monitoring points (Fig. 1). Four of these points are located along the Sorocaba River, two in the middle Sorocaba sub-basin (SORO 02100 and SORO 02200), and two in the lower Sorocaba sub-basin (SORO 02900 and SORO 02500). At the Itupararanga Dam, two points (SOIT 02900 and SOIT 02100), located in the upper Sorocaba sub-basin, were evaluated. There are no CETESB monitoring points for the Ipanema Dam, therefore only data from SISAGUA were included in the analyses.

2.2. Data collection

Data were acquired through the federal government's open data portal, known as dados.

of cyanobacteria reaches 20,000 cells ml⁻¹, weekly analyses of cyanotoxins in raw water are required. Cyanotoxin analyses at the WWTP outlet must be conducted with the same frequency if intake point analyses show that permitted values are exceeded. Chlorophyll-*a* monitoring must be conducted monthly, with adjustments based on cyanobacteria density or cyanotoxin analysis in raw water.

2.3. Data analysis

First, an exploratory analysis was conducted, generating a graph of the percentage of cyanobacteria in samples collected from the studied reservoirs from January 2014 to December 2021 (Fig. 2). The results were categorized into three ranges: results ≤ 10,000 cells ml⁻¹; 10,000 < results ≤ 20,000 cells ml⁻¹; results ≥ 20,000 cells ml⁻¹, following the guidelines of Brazilian regulations, CONAMA Resolution No. 357 of 2005, and Ordinance No. 888 of 2021 (Brazil 2005; Brazil 2021).

Using SISAGUA data, annual average cyanobacteria densities and chlorophyll-*a* concentrations were calculated for each water source, accounting for different data collection periods. To better understand the results for the Sorocaba River and the Ituparanga Dam, annual averages of the Trophic State Index (TSI) from the CETESB Reports (2021) were consulted, along with annual averages of total phosphorus and chlorophyll-*a* concentrations from six CETESB monitoring points in the study region. The TSI was determined using the formula from the modified Carlson model and presented using appropriate colors, as proposed by Lamparelli (2004).

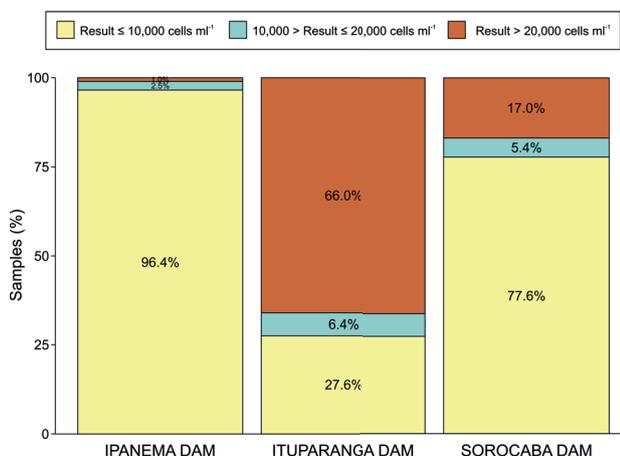


Figure 2

Percentage of cyanobacterial density by ranges of values at the Ituparanga Dam, the Ipanema Dam, and Sorocaba River from January 2014 to December 2021.

To assess the diversity of cyanobacteria in the water sources, 1440 SISAGUA records were analyzed. After excluding zero-density cases, 1100 data were selected to determine the frequency of genera at each site. SISAGUA compiled a list of 16 relevant genera for rivers and water sources, including *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*, *Geitlerinema*, *Jaaginema*, *Lyngbya*, *Microcystis*, *Planktothrix*, *Planktolyngbya*, *Pseudanabaena*, *Radiocystis*, *Raphidiopsis*, *Sphaerospermopsis*, *Synechococcus*, *Synechocystis* and *Tychonema*, categorizing as “Other genera” those not listed or unidentified.

To verify whether the concentrations of cyanotoxins were within the Maximum Permitted Values (MPVs) set by Annex 10 of Ordinance No. 888/2021 (Brazil 2021), the concentrations of cyanotoxins [saxitoxins, microcystins, cylindrospermopsins, and anatoxin-a(S)] were analyzed by collection date, both at the collection points and in the treated water from each source. A total of 1765 data were analyzed for the intake point, 241 of which corresponded to anatoxin-a(S), 387 to cylindrospermopsins, 491 to saxitoxins, and 646 to microcystins. A total of 1230 data were analyzed for the treated water, of which 150 related to anatoxin-a(S), 259 to cylindrospermopsins, 359 to saxitoxins, and 462 to microcystins.

Ninety data sets were collected for Principal Component Analysis (PCA), following Kaiser’s rule (1958), to assess the relationships between the studied variables. The selection involved identifying, for each water source, the collection dates that included the largest number of relevant parameters for the analysis. The parameters retained were cyanobacteria, chlorophyll-*a*, saxitoxins, and microcystins. Within this set, four observations are from the Ituparanga Dam, 61 from the Sorocaba River, and 25 from the Ipanema Dam. R software version 3.4.0 (R Development Core Team 2023) and PAST version 4.03 (Hammer et al. 2001) were used for statistical analyses and graphing.

3. Results

3.1. Cyanobacterial density and trophic state of water sources

The highest densities of cyanobacteria were recorded at the Ituparanga Dam and in the Sorocaba River, with results above 20,000 cells ml⁻¹, representing 66% and 17% of their data, respectively, while the Ipanema Dam recorded only 1% in this range (Fig. 2).

For the Ituparanga Dam for 2014, there was only one record of cyanobacteria density in SISAGUA from

January, and there were no records for 2015. From 2016 to 2021, results were documented monthly, except for March and June 2017. Annual average cyanobacterial densities in 2014, 2016, and 2017 were below 50,000 cells ml⁻¹. From 2018, there was an increase, reaching 76,085 cells ml⁻¹ (± 17,416). In 2019, the average was 72,636 cells ml⁻¹ (± 33,310). In 2020, it increased to 121,448 cells ml⁻¹ (± 48,229), and in 2021 it reached 123,794 cells ml⁻¹ (± 27,857). The monthly maximum average was approximately 189,435 cells ml⁻¹ recorded in November 2017 (Table 1). The occurrence of cyanobacteria was observed throughout the year.

The TSI used to assess the trophic state of the dam's water classified the SOIT 02900 point as mesotrophic from 2014 to 2021, with a maximum phosphorus concentration of 0.02 mg l⁻¹ and a maximum chlorophyll-*a* concentration of 18.0 µg l⁻¹. On the other hand, the SOIT 02100 point showed eutrophic conditions in 2015 and 2021, with phosphorus concentrations reaching 0.04 mg l⁻¹ and chlorophyll-*a* concentrations up to 36.0 µg l⁻¹ (Fig. 3) (Table 2).

In the Sorocaba River in 2014, cyanobacteria densities were recorded only in May and July. In 2015, there were no records for January, March, April, and July, while in 2016, the months without records were February, June, July, August, and September. In the following years, analyses were recorded monthly. Annual average densities of cyanobacteria remained below 10,000 cells ml⁻¹ from 2014 to 2018. However, the annual average increased to 11,393 cells ml⁻¹ (± 10,633) in 2019, to 50,690 cells ml⁻¹ (± 58,730) in 2020, and reached 104,501 cells ml⁻¹ (± 156,496) in 2021, with a maximum monthly average of 565,018 cells ml⁻¹ in January 2021 (Table 1).

Changes in TSI have occurred over the years at the Sorocaba River monitoring points, located in the lower Sorocaba sub-basin. Point SORO 02900 shifted in classification from oligotrophic state in 2014 to mesotrophic by 2020, and reached eutrophic state in 2021, recording an annual average of 0.35 mg l⁻¹ phosphorus and 31.0 µg l⁻¹ chlorophyll-*a*. Point SORO 02500 showed improvement between 2016 and 2017,

Table 1

Statistical summary of SISAGUA data on annual cyanobacterial density (cell ml⁻¹) in the Itupararanga and Ipanema dams and the Sorocaba River from 2014 to 2021.

years	Itupararanga Dam					Ipanema Dam					Sorocaba River				
	N.	Min.	Max.	Mean	SD	N.	Min.	Max.	Mean	SD	N.	Min.	Max.	Mean	SD
2014	1	35,180	35,180	35,180	0	2	1	5449	2725	3852	2	1	3616	1809	2556
2015	-	-	-	-	-	8	1	6389	813	2253	8	1	3444	445	1212
2016	12	1950	54,445	22,427	17,583	8	0	1	1	1	7	0	66	12	24
2017	10	0	189,435	39,662	58,239	12	0	5588	1045	1629	12	0	7566	1382	2108
2018	12	49,695	104,576	76,085	17,416	12	1	2415	379	710	12	0	3838	687	1334
2019	12	31,712	147,685	72,636	33,310	12	0	39,823	4426	11,223	12	0	33,812	11,393	10,633
2020	12	64,012	188,709	121,448	48,229	12	1	68,201	6248	19,522	12	7	157,186	50,690	58,730
2021	12	93,856	185,263	123,794	27,857	12	0	6750	1023	1980	12	0	565,018	104,501	156,496

"-" – analysis not performed; SD – Standard Deviation; Min – minimum density; Max – maximum density; N – number of registered months

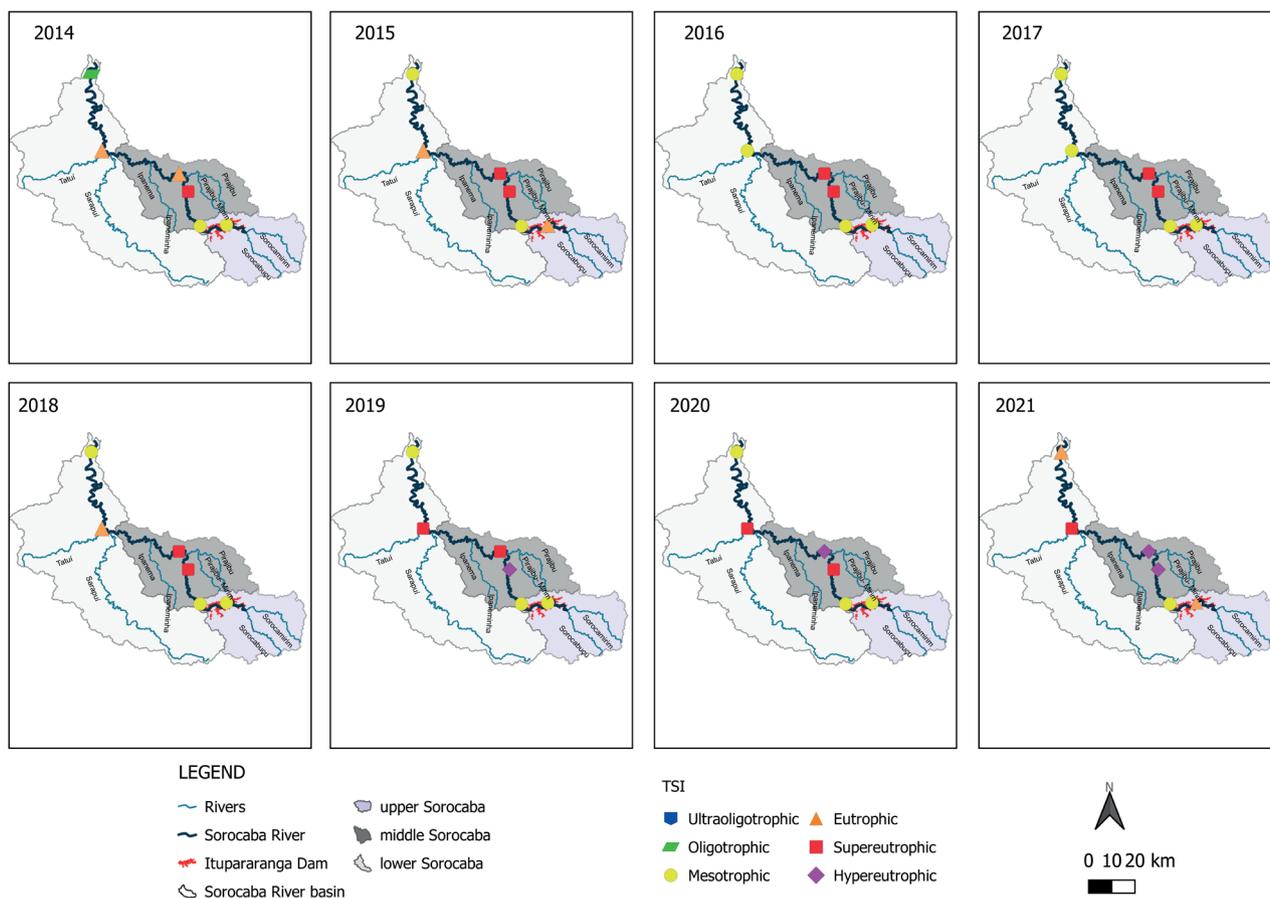
Table 2

Annual averages for total phosphorus (PT) and chlorophyll-*a* (Chl-*a*) at six CETESB monitoring points from 2014 to 2021.

years	SORO 02100		SORO 02200		SORO 02500		SORO 02900		SOIT 02100		SOIT 02900	
	PT mg l ⁻¹	Chl- <i>a</i> µg l ⁻¹	PT mg l ⁻¹	Chl- <i>a</i> µg l ⁻¹	PT mg l ⁻¹	Chl- <i>a</i> µg l ⁻¹	PT mg l ⁻¹	Chl- <i>a</i> µg l ⁻¹	PT mg l ⁻¹	Chl- <i>a</i> µg l ⁻¹	PT mg l ⁻¹	Chl- <i>a</i> µg l ⁻¹
2014	0.20	12.0	0.37	-	0.19	3.8	0.82	0.9	0.03	24.0	0.02	12.0
2015	0.18	11.0	0.35	8.2	0.20	3.7	0.23	0.8	0.04	36.0	0.02	13.0
2016	0.19	9.1	0.25	5.9	0.18	1.5	0.20	0.6	0.02	14.0	0.02	7.4
2017	0.13	13.0	0.22	8.8	0.19	2.5	0.17	0.7	0.03	20.0	0.01	12.0
2018	0.13	13.0	0.22	5.9	0.19	6.5	0.17	3.3	0.03	28.0	0.01	13.0
2019	0.23	15.0	0.41	9.7	0.24	8.7	0.24	1.0	0.02	20.0	0.02	12.0
2020	0.22	13.1	0.53	10.5	0.40	9.3	0.28	2.3	-	-	0.02	14.8
2021	0.39	16.0	0.63	17.0	0.32	13.0	0.35	31.0	0.03	34.0	0.02	18.0

"-" indicates analysis was not performed (adapted from CETESB 2021)



**Figure 3**

Map of the annual TSI for CETESB monitoring points on the Sorocaba River and the Itupararanga Dam from 2014 to 2021.

changing from eutrophic to mesotrophic. However, its trophic state has deteriorated since 2018, reaching a supereutrophic state in 2019–2021, with a maximum annual average of 0.40 mg l^{-1} phosphorus and $13.0 \text{ } \mu\text{l}^{-1}$ chlorophyll-*a* (Fig. 3; Table 2).

The monitoring points in the middle Sorocaba sub-basin (SORO 02200 and SORO 02100) have alternated between supereutrophic and hypereutrophic status since 2019. In 2021, both points were classified as hypereutrophic, with point SORO 02200 recording a maximum annual average of $0.63 \text{ } \mu\text{l}^{-1}$ phosphorus and $17.0 \text{ } \mu\text{l}^{-1}$ chlorophyll-*a*, while point SORO 02100 showed $0.39 \text{ } \mu\text{l}^{-1}$ phosphorus and $16.0 \text{ } \mu\text{l}^{-1}$ chlorophyll-*a* (Fig. 3; Table 2).

The density of cyanobacteria at the Ipanema Dam in 2014 was recorded in SISAGUA in May and July. In 2015, no analysis was recorded in January, March, April, and July, and in 2016 in June, July, August, and September. Monthly measurements were conducted from 2017 to 2021. The annual average cyanobacterial

density at the Ipanema Dam remained below $10,000 \text{ cells ml}^{-1}$. In November 2019 and December 2020, however, the monthly maximum averages reached $39,823 \text{ cells ml}^{-1}$ and $68,201 \text{ cells ml}^{-1}$, respectively (Table 1).

3.2. Chlorophyll-*a*

A total of 283 chlorophyll-*a* samples from SISAGUA were analyzed between 2016 and 2021. Of these, 198 were from the Sorocaba River, 62 from the Ipanema Dam, and 23 from the Itupararanga Dam (Table 3). In 2016, only one chlorophyll-*a* analysis from the Sorocaba River was recorded in November. Between 2017 and 2021 no analyses were conducted at the Itupararanga Dam in January, February, August, and December. During this period, analyses were conducted monthly in both the Sorocaba River and the Ipanema Dam, with multiple analyses conducted on some collection dates.

Table 3

Statistical summary of SISAGUA data on chlorophyll-*a* concentrations in the Itupararanga and Ipanema dams and the Sorocaba River for 2016–2021.

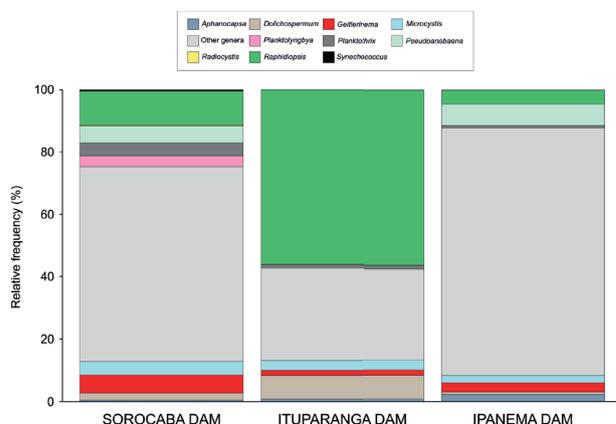
Source of water	N	Mean ($\mu\text{g l}^{-1}$)	SD
Ipanema Dam	62	2.2	0.6
Itupararanga Dam	23	6.8	2.3
Sorocaba River	198	3.3	0.4

N – number of samples; SD – Standard Deviation

The highest average concentration of chlorophyll-*a* was determined at the Itupararanga Dam, reaching $6.80 \mu\text{g l}^{-1}$ (± 2.30). The average concentrations in the Sorocaba River and the Ipanema Dam were $3.3 \mu\text{g l}^{-1}$ (± 0.40) and $2.20 \mu\text{g l}^{-1}$ (± 0.60), respectively.

3.3. Descriptive analysis of cyanobacterial composition

Among the identified cyanobacterial genera, *Raphidiopsis* dominated (Fig. 4). The most frequent genera in the Itupararanga Dam included *Raphidiopsis* (56%), *Dolichospermum* (7%) and *Microcystis* (3%). Seven genera were identified in the Ipanema Dam, with the highest percentage recorded for “Other genera” (80%) and for *Pseudanabaena* (7%). The highest diversity of genera was determined in the Sorocaba River – 10 genera, with *Raphidiopsis* (11%), *Geitlerinema* (6%), and *Pseudanabaena* (5%) being the most abundant.

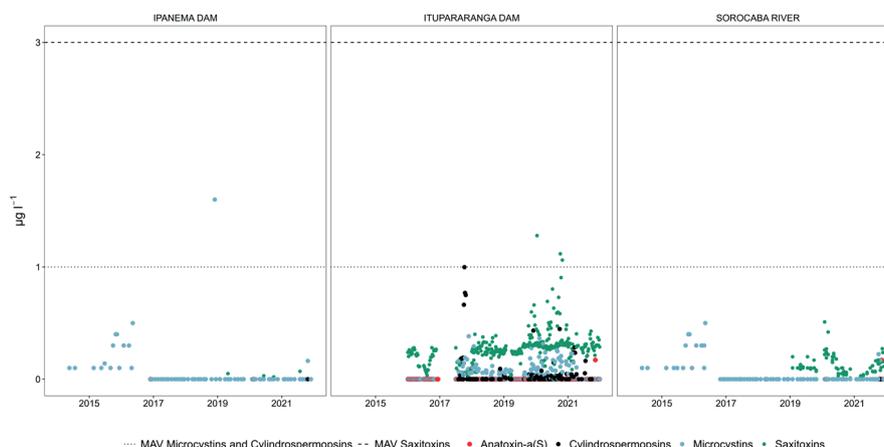
**Figure 4**

Relative frequency of cyanobacterial genera identified from January 2014 to December 2021 at the Itupararanga Dam, the Ipanema Dam, and in the Sorocaba River.

3.4. Cyanotoxins

At the Itupararanga Dam intake point, microcystin concentrations were below the MPV of $1.00 \mu\text{g l}^{-1}$ (Brazil 2021), with an increase in saxitoxins in 2020 and 2021, reaching $1.30 \mu\text{g l}^{-1}$. At the end of 2017, there was a peak in cylindrospermopsins reaching $1.00 \mu\text{g l}^{-1}$, and in 2021 the concentration of anatoxin-a(S) was $0.17 \mu\text{g l}^{-1}$ (Fig. 5).

At the Ipanema Dam intake point, there was an increase in the concentrations of microcystins at the end of 2015 and the beginning of 2016, reaching $0.50 \mu\text{g l}^{-1}$. At the end of 2018, these concentrations

**Figure 5**

Results of cyanotoxin analyses at the point of water intake at the Itupararanga Dam, the Ipanema Dam, and in the Sorocaba River, from January 2014 to December 2021, with the limits of saxitoxins, microcystins, and cylindrospermopsins established by Ordinance No. 888 of 4 May 2021.



reached $1.60 \mu\text{g l}^{-1}$. The maximum concentration of saxitoxins was $0.07 \mu\text{g l}^{-1}$, with no detection of cylindrospermopsins and anatoxin-a(S) (Fig. 5).

At the Sorocaba River intake point, there were an increase in saxitoxins concentrations in 2019, 2020 and 2021, with the highest value of $0.51 \mu\text{g l}^{-1}$ recorded at the beginning of 2020. The concentrations of microcystins ranged between $0.10 \mu\text{g l}^{-1}$ and $0.50 \mu\text{g l}^{-1}$, showing an increase at the end of 2015 and the beginning of 2016. Anatoxin-a (S) reached a value of approximately $0.17 \mu\text{g l}^{-1}$ (Fig. 5).

After water treatment at the Itupararanga Dam, the maximum concentrations recorded were about $0.30 \mu\text{g l}^{-1}$ for saxitoxins and $0.15 \mu\text{g l}^{-1}$ for cylindrospermopsins, with the concentrations of microcystins exceeding $0.40 \mu\text{g l}^{-1}$ in 2020. The highest values at the Ipanema Dam were $0.05 \mu\text{g l}^{-1}$ for saxitoxins and $0.11 \mu\text{g l}^{-1}$ for microcystins. In the Sorocaba River, the maximum concentrations after treatment were $0.20 \mu\text{g l}^{-1}$ for saxitoxins and $0.16 \mu\text{g l}^{-1}$ for microcystins. No concentrations of anatoxin-a(S) were detected in the treated water from any of the water sources (Fig. 6).

3.5. Principal Component Analysis (PCA)

PCA explained 70.02% of the data variation, with 44.95% associated with the first axis and 25.07% with the second. The variables 'saxitoxins', 'chlorophyll-*a*' and 'cyanobacteria' showed a more significant correlation with each other. The Sorocaba River and the Itupararanga Dam showed a stronger association with the first axis, indicating a greater correlation between cyanobacteria and saxitoxins. The Ipanema Dam occupied an intermediate position in relation to axes 1 and 2, suggesting a weaker correlation with the variables (Fig. 7).

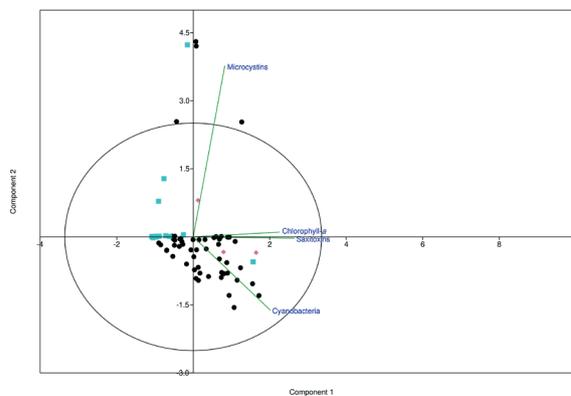


Figure 7

PCA biplot of correlations between the variables determined as cyanobacteria, chlorophyll-*a*, saxitoxins, and microcystins for the Itupararanga Dam, the Ipanema Dam, and Sorocaba River (Black circle: Sorocaba River; Green square: Ipanema Dam; Pink diamond: Itupararanga Dam).

4. Discussion

The study revealed a deterioration in the trophic status of the Sorocaba River basin (BHS), especially in the middle Sorocaba sub-basin. As of 2019, the SORO 02100 and SORO 02200 monitoring points began to oscillate between supereutrophic and hypereutrophic (CETESB 2021). This increase in eutrophication was attributed to excess phosphorus detected at both the monitoring points of the Sorocaba River and at the Itupararanga Dam, where annual averages exceeded the limits established by CONAMA Resolution No. 357/2005, i.e. 0.10 mg l^{-1} for Class I waters in lotic

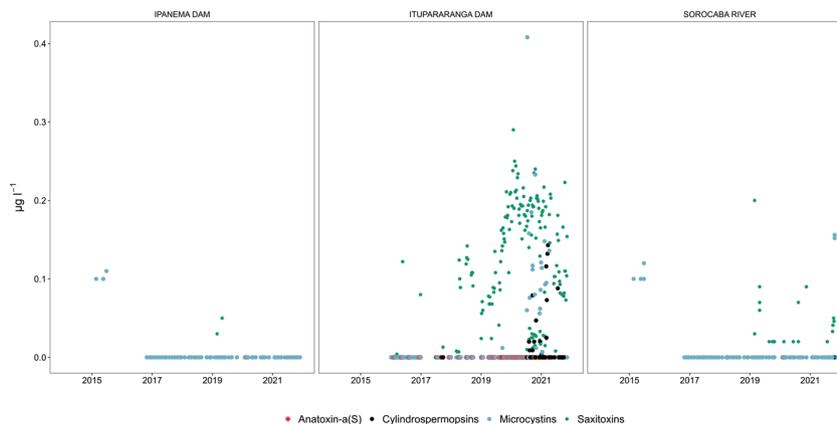


Figure 6

Results of cyanotoxin analyses of treated water from the Itupararanga Dam, the Ipanema Dam, and the Sorocaba River, from January 2014 to December 2021.

environments and 0.02 mg l^{-1} in lentic environments (Brazil 2005). Previous studies have also confirmed the relationship between phosphorus levels and eutrophication (Kitsiou, Karydis 2011; Trindade, Mendonça 2014; Abreu, Cunha 2016).

In addition, the Water Quality Report of the Interior of the State of São Paulo (CETESB 2021) indicated that UGRHI 10 recorded annual rainfall volumes below historical averages in 2014, 2018, 2020, and 2021. The report also highlighted that in 2021, approximately 80% of the phytoplankton community in the Itupararanga Dam consisted of cyanobacteria. Smith (2003) and Casali (2014) relate the degradation of water quality in the Itupararanga reservoir to improper use of the reservoir. This includes the production of electricity for the Votorantim Metals industry, water supply for the towns of the upper and middle Sorocaba sub-basins, recreational activities, and pollution caused by waste from residential condominiums. Importantly, a significant portion of BHS consists of rural areas and small communities devoid of sewage treatment systems. According to IAS (2023), in the municipality of Ibiúna (SP), which covers most of the territory of the Itupararanga Dam, about 65% of the population lives in rural areas, while 35% reside in urban zones. Alarmingly, approximately 81% of urban residents are not connected to wastewater treatment services, leading to the direct discharge of this waste into the environment. These factors resulted in the increased density of cyanobacteria observed in 2020 and 2021, both in the Sorocaba River and in the Itupararanga Dam. There is evidence that cyanobacterial blooms in river waters are associated with prolonged winter periods (dry seasons), during which the lack of rainfall and low water runoff create favorable conditions for the growth of these microorganisms, especially when combined with an adequate balance of nutrients and elevated temperatures (Jardim et al. 2014; Nichetti et al. 2022). This observation was supported by a study conducted by Hur et al. (2013), which identified a negative correlation between the abundance of *Aphanizomenon* and rainfall levels, highlighting the role of phosphorus in changes in cyanobacterial communities, which may have positive or negative correlations in response to this factor.

Among the genera potentially producing cyanotoxins, the following were mainly observed: *Raphidiopsis* – a producer of cylindrospermopsins and saxitoxins (Paerl, Otten 2013; Chorus, Welker 2021; Šuikaitė et al. 2023); *Dolichospermum* – a producer of cylindrospermopsins, saxitoxins, anatoxin-a(s), and microcystins (Li et al. 2016; Carvalho et al. 2013); and *Microcystis* – a producer of microcystins

(Sant'Anna et al. 2008). Other studies have also reported the abundance of the genera *Raphidiopsis* and *Dolichospermum* in Brazilian reservoirs (Moschini-Carlos et al. 2009; Sant'Anna et al. 2011; Rodrigues et al. 2019; Dos Santos Machado et al. 2022). According to Bittencourt-Oliveira and Molica (2003), Brazilian strains of *Raphidiopsis*, isolated in different regions of the country, were identified as producers of saxitoxins. In this study, 56% of the genus *Raphidiopsis* was identified in the Itupararanga Dam. Dos Santos Machado et al. (2022) report that the prevalence of the Cyanophyceae class in this reservoir is mainly attributed to the abundance of the species called "*Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno". The authors found a significant correlation between *R. raciborskii* biomass and saxitoxins content; however, the linear effect was classified as moderate ($R^2 = 0.21$; $p < 0.05$). In the present study, we also found a correlation between cyanobacterial density and saxitoxin concentrations in the Itupararanga Dam and the Sorocaba River. According to Šuikaitė et al. (2023), *R. raciborskii* shows a clear preference for environments enriched with high concentrations of phosphorus. However, the authors also point to its ability to thrive in oligotrophic lakes, where nutrients are scarce. The study conducted by Xiao et al. (2020) furthers this discussion, highlighting that different strains within the same population of *R. raciborskii* adopt different strategies to deal with phosphorus storage, showing adaptations to the availability of this nutrient in aquatic environments. Additional research, such as that by Chislock et al. (2014) and Burford et al. (2016), offers complementary insight into the biochemical and physiological mechanisms employed by the species to optimize the utilization of phosphorus when it is scarce. This remarkable adaptability poses a major challenge to effective control of species proliferation in the reservoirs studied, especially when considering strategies based solely on reducing phosphorus concentrations.

The levels of saxitoxins, microcystins, and cylindrospermopsins at the Sorocaba River intake point were in accordance with the standards established by both Brazilian legislation and WHO (2020a, b). The concentrations of saxitoxins were below $3.0 \text{ } \mu\text{g l}^{-1}$, while the concentrations of microcystins remained within the $1.00 \text{ } \mu\text{g l}^{-1}$ limit. It should be noted that Ordinance No. 888 of 4 May 2021 recommends a value of $1.00 \text{ } \mu\text{g l}^{-1}$ for cylindrospermopsins, while WHO (2020a) stipulates a lower limit of $0.70 \text{ } \mu\text{g l}^{-1}$. However, the Itupararanga Dam intake point exceeded the value of $0.70 \text{ } \mu\text{g l}^{-1}$ established by WHO for cylindrospermopsins in October 2017. In



addition, the Ipanema Dam exceeded the limit of $1.00 \mu\text{g l}^{-1}$ for microcystins in November 2018, failing to meet both national and international criteria for these cyanotoxins (Brazil 2021; Chorus, Welker 2021). According to the study by Li et al. (2011), it is estimated that a child consumes on average about 1.20 l of water daily. According to WHO (1999) guidelines, the recommended daily intake of microcystins for children weighing 10 kg is $0.40 \mu\text{g}$. WHO (2020b) establishes different reference values for this toxin in drinking water intended for adults: $1.00 \mu\text{g l}^{-1}$ for long-term continuous use, $12.00 \mu\text{g l}^{-1}$ for short-term use, and $24.00 \mu\text{g l}^{-1}$ in water used for recreational purposes (Chorus, Welker 2021). After water treatment, the analyzed water sources met the standards established for adults, maintaining microcystin concentrations below $1.00 \mu\text{g l}^{-1}$. However, in July 2020, the Itupararanga Dam recorded a concentration of $0.40 \mu\text{g l}^{-1}$, approaching the WHO (2020b) recommended maximum limit for children.

A study conducted by Mrdjen et al. (2022) examined the effect of chronic low-dose microcystin-LR (MC-LR) exposure through drinking water on the development of liver tumors. Zeng et al. (2021) investigated the potential impact of MC-LR on cognitive function and memory capacity in children. The study aimed to explore whether serum levels of MC-LR were associated with cognitive performance. The results indicated a positive association between serum MC-LR concentrations, particularly in the range between 0.80 and $0.95 \mu\text{g l}^{-1}$, and higher cognitive and neurobehavioral performance. Several studies have reported bioaccumulation of these toxins at different trophic levels of the food chain, including fish, shellfish, and plants (Zhang et al. 2009; Schmidt et al. 2014; Arman, Clarke 2021). In this context, the ingestion of water with concentrations of microcystins higher than $0.40 \mu\text{g l}^{-1}$, especially when combined with contaminated food, can exacerbate the adverse effects of this toxin on the body, resulting in long-term negative health effects.

Based on the study by Humpage and Falconer (2003), WHO (2020a) sets interim limits of $0.70 \mu\text{g l}^{-1}$ for the presence of cylindrospermopsins in drinking water for long-term use, $3.00 \mu\text{g l}^{-1}$ in drinking water for short-term use, and $6.00 \mu\text{g l}^{-1}$ in water intended for recreational purposes (Chorus, Welker 2021). The results of this study indicated that the concentrations of cylindrospermopsins in the treated water were below $0.20 \mu\text{g l}^{-1}$.

Chorus and Welker (2021) mention that due to the lack of available data, it was not possible to determine the level of adverse effects observed for anatoxin-a(S), resulting in the absence of a reference

value for tolerable daily intake. The authors reported that New Zealand adopted a provisional limit of $1.00 \mu\text{g l}^{-1}$ as the maximum acceptable value for this toxin in drinking water. Data from this study indicated that all concentrations of this cyanotoxin were zero after water treatment.

The study by Farrer et al. (2015) addressed a reference value of $0.20 \mu\text{g l}^{-1}$ for saxitoxins in drinking water in the state of Ohio, in the Midwestern USA, to prevent chronic effects in the population. O'Neill et al. (2017) analyzed the effects of saxitoxins on neurite outgrowth in neuronal cell models. They observed that even at low doses, specifically at a concentration of $0.25 \mu\text{g l}^{-1}$, saxitoxins caused a significant reduction in the percentage of axons. Other studies also indicate that prolonged, low-dose exposure to saxitoxins can have adverse effects on neurite outgrowth (Testai et al. 2016; Metcalf et al. 2021; Sun et al. 2021). The concentrations of saxitoxins in approximately 11% of the treated water samples from the Itupararanga Dam exceeded this limit, raising concerns about the possible ineffectiveness of conventional treatment in removing these toxins and the potential long-term effects on these concentrations. A similar situation was recorded in the Sorocaba River in February 2019.

According to SAA reports (SISAGUA 2023), water treatment stages for water collected from the studied sources include pre-oxidation, coagulation, flocculation, sedimentation, slow filtration, disinfection, and fluoridation. Some water treatment plants perform flotation without filtration. Karner et al. (2001) suggest that between 50% and 95% of the toxic compounds produced by cyanobacteria accumulate intracellularly and can be easily removed during conventional treatment, provided the integrity of the cell wall is maintained. Chorus and Bartram (1999) note that conventional water treatment techniques fail to remove dissolved cyanotoxins due to the inefficiency of coagulants in destabilizing and precipitating these compounds. This compromises the efficient removal of cyanotoxins in subsequent stages of the purification process. Chorus and Welker (2021) highlight the high chemical stability of microcystins, which remain toxic even after prolonged boiling and can last for years in dry storage at room temperature, resisting chemical hydrolysis and oxidation. Moreover, the authors observe that saxitoxins tend to degrade more rapidly in alkaline environments and under the influence of factors such as microbial activity and increased temperature. Consequently, the effectiveness of conventional methods for eliminating cyanotoxins is questionable and calls for other techniques, such as activated carbon column filtration, advanced oxidative processes, photocatalysis, ozonation, and

new nanofiltration technologies (Leal, Soares 2004; Albuquerque et al. 2020).

5. Conclusions

Analysis of the data revealed a progressive deterioration of the trophic status of the BHS rivers and reservoirs during the study period. Between 2019 and 2021, there was a considerable increase in the average annual density of cyanobacteria, in both the Sorocaba River and the Itupararanga Dam, due to high concentrations of phosphorus in the water sources. These conditions resulted in increased concentrations of cyanotoxins such as saxitoxins and microcystins. The dominant genera identified as producing these toxins were *Raphidiopsis* and *Dolichospermum*.

In this regard, the high percentage of samples collected from the two water sources that contained cyanotoxins even after treatment was of concern. Water from both the Itupararanga Dam and the Sorocaba River contained concentrations of saxitoxins above the limits set by the state of Ohio (0.20 µg l⁻¹) to address chronic effects of contaminated water. Moreover, concentrations of microcystins above 0.40 µg l⁻¹, i.e. close to the maximum limit recommended by WHO (2020b) for a 10 kg child, were recorded at the Itupararanga Dam. This could pose a future risk to the most vulnerable population, potentially causing long-term hepatotoxic effects. Therefore, it is necessary to improve the techniques used in the treatment of water for public supply and to intensify inspections of the service providers responsible for these processes.

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