

Environmental factors associated with toxic cyanobacterial blooms across 20 drinking water reservoirs in a semi-arid region of Brazil

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ABSTRACT

Cyanobacteria are known to produce a wide variety of bioactive, toxic secondary metabolites generally described as hepatotoxins, neurotoxins, cytotoxins, or dermatotoxins. In Brazil, the regular monitoring of cyanobacterial toxins has intensified after the death of 65 patients in a hemodialysis clinic in Caruaru in the state of Pernambuco due to microcystin exposure. The primary objective of this study was to use multivariate statistics that incorporated environmental parameters (both biotic and abiotic) to forecast blooms of cyanobacteria and their toxic secondary metabolites in 20 drinking water reservoirs managed by the Water Treatment Company of Ceará (CAGECE) in the semi-arid region of Ceará, Brazil. Across four years (January 2013 to January 2017), 114 different phytoplankton taxa were identified, including 24 cyanobacterial taxa. In general, Ceará reservoirs were dominated by cyanobacteria due to eutrophication but also because of the dry and warm climate found throughout the region. Interestingly, specific cyanobacterial taxa were influenced by different biotic and abiotic factors. For example, nitrogen-to-phosphorus (N:P) and evaporation were positively related to saxitoxin-producing taxa, especially *Raphidiopsis raciborskii*, while temperature, electrical conductivity, total phosphorus, and transparency (measured as Secchi depth) were positively associated with microcystin-producing taxa, such as *Microcystis aeruginosa*. Climate forecasts predict higher evaporation and temperatures in the semi-arid Ceará region, which will likely magnify droughts and water scarcity as well as promote toxic cyanobacterial blooms in reservoirs in the future. Therefore, understanding the factors associated with algal blooms dominated by specific taxa is paramount for water resource management.

1. Introduction

Cyanobacteria are gram-negative, prokaryotic organisms widely distributed in aquatic and terrestrial environments from the poles to the equator, often times dominating other planktonic and benthic species in aquatic systems (Sompong et al., 2005; Kleinteich et al., 2012; Paerl and Otten, 2013). Cyanobacteria are known to produce a wide variety of bioactive secondary metabolites (Sivonen and Jones, 1999; Smith et al., 2008) that are not essential for cyanobacterial growth or metabolism (Vanek et al., 1981), but are capable of causing unpleasant tastes and odors and acute and/or chronic damage and mortality to animal health (Van Apeldoorn et al., 2007; Stewart and Falconer, 2008). Previous studies have reported more than 40 species of cyanobacteria known to produce toxins (Stewart and Falconer, 2008; Gkelis and Zaoutos, 2014). Moreover, fifty-one cyanobacterial species have been

reported to produce taste and odor compounds, such as 2-methylisoborneol (MIB) and geosmin, that can negatively affect the palatability of drinking water and farmed fish (Izaguirre and Taylor, 1995; Zimba and Grimm, 2003; Suurnäkki et al., 2015). Additionally, some cyanobacteria have the ability to produce the neurotoxic compound, beta-methylamino-L-alanine (BMAA) (Cox et al., 2009; Downing et al., 2011).

Natural and anthropogenic nutrient pollution, in concert with increasing global temperatures, are known to promote toxic cyanobacterial blooms (Paerl and Paul, 2012). Several studies have shown that temperatures around 20 °C cause stable or reduced growth rates for many freshwater eukaryotic phytoplankton taxa, including diatoms, chlorophytes, cryptophytes, and dinoflagellates, while growth rates of cyanobacteria tend to increase, thus providing cyanobacteria with a competitive advantage (Paerl and Huisman 2008; Paerl and Huisman,

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2009; O'Neil et al., 2012; Huisman et al., 2018). Elevated temperatures also affect the vertical gradient of water density causing longer and more stable stratification periods (Wetzel, 2001). Reduced mixing can be advantageous for some cyanobacteria that have gas pseudovacuoles allowing for flotation to access available sunlight (Huisman et al., 2006). In addition, some cyanobacteria produce photoprotective accessory pigments (e.g., carotenoids) and other compounds that absorb UV radiation that enable prolonged survival during longer periods of exposure to high intensities of sunlight (Paul, 2008; Carreto and Carignan, 2011). Due to their adaptations to high temperatures, longer stratification periods, and increased exposure to UV light, cyanobacteria often have a competitive advantage over other phytoplankton components during adverse climate conditions (Carey et al., 2011).

According to the Intergovernmental Panel on Climate Change (IPCC, 2007), temperatures may increase from 4 to 6° C in the tropics, making climate change a constant concern for equatorial communities (Corlett, 2012; Trewin et al., 2014; Mowe et al., 2015). In Ceará state (Northeastern Brazil), the average temperature has already increased by an average of at least 1° C since the 1960's (INMET, 2016), increasing concerns about drinking water safety and risks to the water industry.

In Brazil, monitoring the frequency and concentration of cyanobacterial toxins has intensified after the death of 65 patients in a hemodialysis clinic in Caruaru in the state of Pernambuco due to microcystin poisoning (Carmichael et al., 2001). Consequently, the monitoring of drinking water sources for cyanobacteria and their toxins has been made mandatory in Brazil since 1996. In Ceará state, CAGECE (2010) reports that neurotoxic, cyanobacterial blooms and their toxins have been increasing in frequency in reservoirs that supply the most populated cities in the state. Concomitantly, agriculture, untreated sewage, and intensive fish farming within the reservoirs continue to compromise water quality in Ceará (COGERH, 2008).

With the potential to increase cyanobacterial blooms and toxin frequency and intensity, there is a pressing need to monitor drinking water reservoirs in poorly studied, semi-arid regions. Identifying toxic cyanobacteria in environmental samples can be a labor-intensive method that requires proper training (Kim et al., 2006). Therefore, multivariate statistical analyses, which do not require restrictive assumptions (Basille et al., 2013), can be used to explore data without defined hypotheses (Everitt and Hothorn, 2011; Yang et al., 2012). In addition, these methods are widely used in aquatic systems and ecological modeling where large, complex datasets of biotic and abiotic data are common (Bengraïne and Marhaba, 2003; Yang et al., 2016). With these factors in mind, the main objectives of this project were to study the distribution, frequency, and dominance of cyanobacterial taxa found across 20 drinking water reservoirs in a semi-arid region of Brazil and use statistical modeling to elucidate the environmental parameters that are associated with cyanobacterial blooms and their toxins.

2. Material and methods

2.1. Study site

The Ceará state is located in northeastern Brazil between latitudes S2° 30' 00" and S8° 52' 00" and longitudes W37° 14' 00" and W41° 30' 00" (Fig. 1). The study site belongs to the semi-arid region of Brazil characterized by a low, irregular annual rainfall extending from early January to May (IPECE, 2017; Neto et al., 2014). Besides low precipitation, the Ceará region has several conditions that are ideal for cyanobacterial growth, such as high solar radiation (5 kWh m⁻² day⁻¹) for approximately 8 h day⁻¹, surface water accumulation in reservoirs with a prolonged retention time (> 12 months), a high average annual temperature (≈32° C), and intensive anthropogenic activity leading to the eutrophication of its reservoirs (Barros et al., 2017; COGERH, 2017; FUNCEME 2017). Data included in this study were from 20 drinking water reservoirs located in nine hydrographic basins and managed by CAGECE (Appendix 1).

2.2. Sampling and analytical methods

Duplicate samples were collected monthly or weekly (Table 1) from the water treatment plant intake at a depth of 30 cm from the surface of 20 reservoirs managed by the CAGECE from January 2013 to January 2017 for a total of 2,489 samples. According to Brazilian law PCR n° 5/2017, when cyanobacterial densities exceeds 20,000 cells mL⁻¹, analysis of cyanobacterial toxins in the raw and treated water are required (Brasil, 2017). Therefore, additional samples were collected for sites that had high cyanobacterial densities. On multiple occasions, water levels were too low to collect samples via boat and resulted in fewer samples at some reservoirs (Table 1). Two types of samples were collected: one fixed with Lugol's solution (0.3 to 0.5% for oligotrophic environments and 0.5 to 1.0% for eutrophic environments) and another *in vivo* sample. Both sample types were stored in a chilled container until processed in the laboratory.

Cell counts were performed using an inverted microscope (Zeiss Axio A1) with a Sedgewick-Rafter chamber by CAGECE. On average, fields were counted at multiple magnifications (200x & 400x depending on the size of the phytoplankton taxon) until at least 100 individuals of the most frequent species or 400 individuals were counted in random fields (APHA, 2012). Large colonies/filaments (≥ 4 cells) were observed for each sample to insure sufficient coverage of ambient phytoplankton diversity (Jeong et al., 2018; Yoo et al., 2018). Cyanobacteria were classified into four orders: Chroococcales, with the presence of unicellular or colonial stems; Oscillatoriales - homozygous filamentous stems (without heterocytes and akinetes); Nostocales - filamentous stems, heterocytes (with heterocytes and/or akinetes), without branching or with false ramifications; Stigonematales - Heterocytes filamentous stems (rare akinetes), with true ramifications. All classifications followed existing keys for cyanobacteria (Komárek and Anagnostidis, 1989, 1998, 2005; Anagnostidis and Komárek, 1990). Cell densities were converted to algal biovolume by multiplying cell densities by the average taxa-specific cell biovolume (DELWP, 2018).

Cyanobacterial toxin analyses were performed using enzyme-linked immunosorbent assays (ELISA; Abraxis, LLC, Warminster, Pennsylvania). Aliquots were frozen and thawed three times and then filtered. Quality control included positive and negative controls, laboratory replicates, and cyanotoxin-spiked laboratory samples (cylindrospermopsin at 1.0 µg L⁻¹, microcystin-LR at 1.0 µg L⁻¹ and saxitoxins at 0.20 µg L⁻¹, according to Loftin et al., 2016). All samples were analyzed according to the manufacturer directions: microcystin (Abraxis LLC, 2007), cylindrospermopsin (Abraxis LLC, 2009a), and saxitoxin (Abraxis LLC, 2009b). The minimum reporting level (MRL) for the assays were 0.10, 0.05, and 0.02 µg L⁻¹ for microcystin-LR equivalents, cylindrospermopsin, and saxitoxin, respectively.

Nutrients concentrations were analyzed following the persulfate method for simultaneous determination of total nitrogen and total phosphorus (APHA, 2012), as well as chlorophyll analysis following standard methods (APHA, 2012). Total nitrogen (TN) and phosphorus (TP) were measured as these values include both dissolved and particulate organic and inorganic forms that are stable, representative measures of eutrophication (Ostrofsky, 2012). Moreover, Downing et al. (2001) and Ghaffar et al. (2016) concluded that TN and TP are better predictors of cyanobacterial abundance when compared to other nutrient derivatives.

Meteorological data, including temperature, precipitation, evaporation, and irradiation, were collected at weather stations monitored by the Foundation of Meteorology and Water Resources of Ceará (FUNCEME) and National Institute of Meteorology of Brazil (INMET). All the parameters studied and the methods used to collect these data are available in Table 2.

2.3. Statistical analyses

Analysis of variance (ANOVA) followed by Tukey's multiple

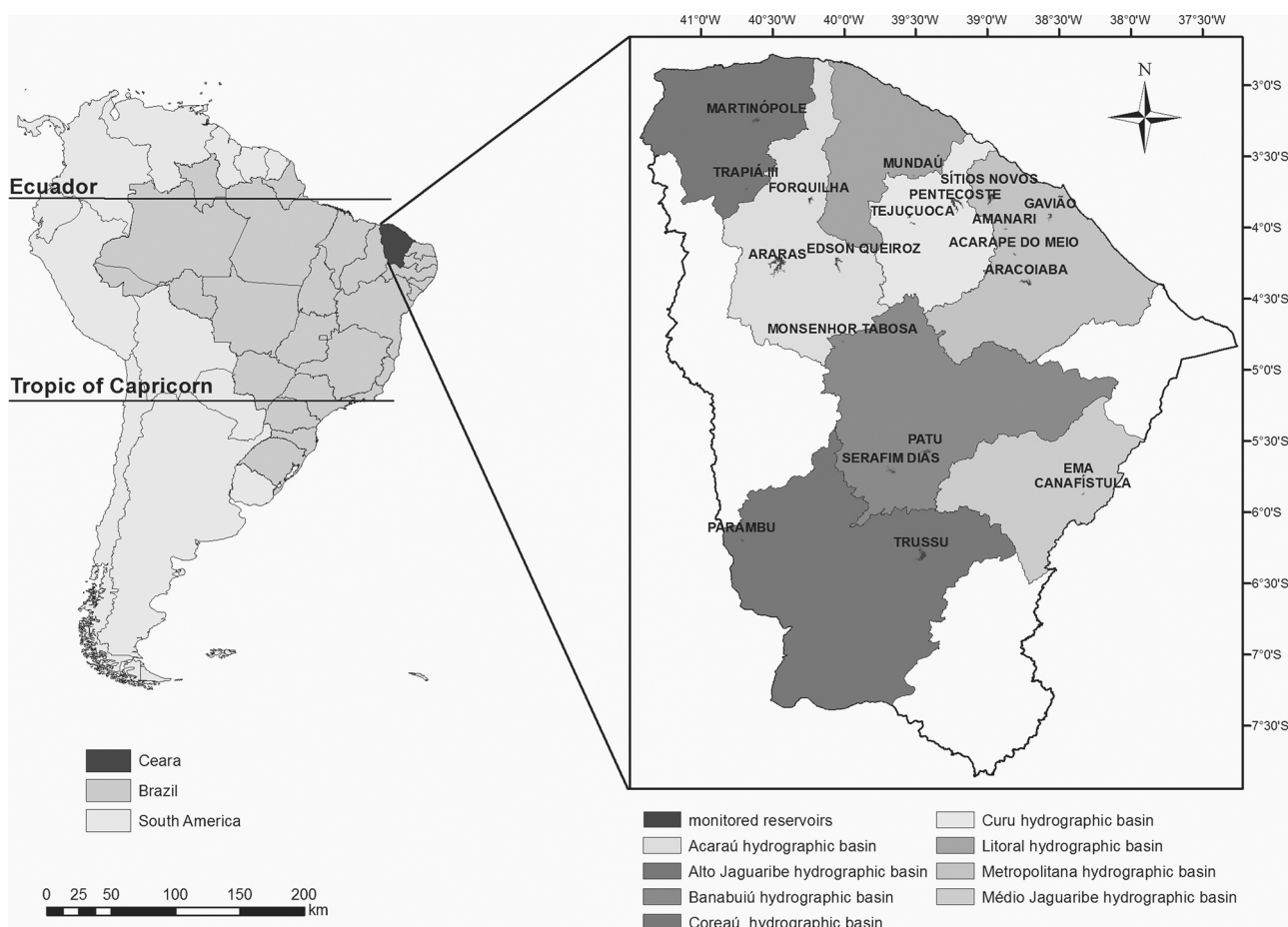


Fig. 1. Map of Ceará state, Brazil, showing the geographical location of the 20 studied reservoirs.

Table 1

Number of samples collected in each drinking water reservoir across 2013-2017. Reservoirs are denoted by abbreviations. Reservoir names are available in Appendix 1.

Year	AM	AMR	AR	BO	CN	AT	EQ	E	FQ	G	MRT	MT	MU	PT	PS	PM	PU	SD	SN	T
2013	46	40	37	43	6	1	47	13	0	46	37	12	18	39	46	17	37	39	45	41
2014	30	26	31	28	28	28	28	18	0	42	23	23	27	27	26	27	6	24	29	23
2015	46	20	45	17	44	47	44	39	25	49	45	42	44	40	43	36	24	39	46	45
2016	29	40	51	19	21	44	34	18	32	52	32	31	30	20	30	27	10	22	22	26
2017	0	0	0	0	0	0	4	0	4	5	2	0	0	0	0	0	0	0	0	0
Total samples	151	126	164	107	99	120	157	88	61	194	139	108	119	126	145	107	77	124	142	135

Table 2

Parameters measured during each sampling event.

Parameter	Analysis method
Phytoplankton	APHA, 2012, section 10200
Microcystin (MC)	ELISA Abraxis, LLC, 2007
Saxitoxin (STX)	ELISA Abraxis, LLC, 2009b
Cylindrospermopsis (CYN)	ELISA Abraxis, LLC, 2009a
Total phosphorus (TP)	APHA, 2012, section 4500-P J
Total nitrogen (TN)	APHA, 2012, section 4500-P J
Chlorophyll (Chl)	APHA, 2012, section 10200 H
Transparency	Secchi Disc
Electrical conductivity (CE)	Probe YSI-EXO-1
Temperature	Maximum and minimum thermometers
Irradiation	Piche evaporimeter
Evaporation	Pyrography
Precipitation	Pluviometer

comparison tests were used to compare reservoir mean water quality parameters. Canonical correspondence analysis (CCA), a non-linear multivariate statistical technique, was used to determine the relationships between environmental parameters (abiotic and biotic) and cyanobacteria. All parameters were natural log transformed ($\ln(x + 1)$) to normalize the data prior to analyses. Statistical analyses were conducted using R v3.4.1, and CCA graphics were created using the *vegan* R package. For the CCA graphic, the dataset was composed of the top 10 dominant cyanobacterial taxa (listed in Appendix 1), including *Aphanizomenon* sp. (APHAZ), *Aphanocapsa* sp. (APHAC), *Chroococcales* (CHR), *Planktothrix agardhii* (PA), *Microcystis aeruginosa* (MA), *Geitlerinema* sp. (GEI), *Pseudanabaena* (PSEU), *Raphidiopsis raciborskii* (RR) (previously called *Cylindrospermopsis raciborskii*, Aguilera et al., 2018), *Merismopedia* sp. (MER), and *Planktolyngbya* (PLANK), and 12 environmental and water quality variables, including microcystin concentration (MC), saxitoxin concentration (STX), cylindrospermopsis concentration (CYN), precipitation (rain), temperature (temp),

Table 3

List of cyanobacterial taxa identified in the 20 studied reservoirs. Reservoirs are denoted by abbreviations. Reservoir names are available in Appendix 1.

Cyanobacterial taxa	AM	AMR	AR	BO	CN	AT	EQ	E	FQ	G	MRT	MT	MU	PT	PS	PM	PU	SD	SN	T
<i>Anabaenopsis</i> sp.		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	
<i>Anathece</i> sp.		X								X	X			X						X
<i>Aphanizomenon</i> sp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Aphanocapsa</i> sp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Artrospira</i> sp.		X		X	X			X						X				X		
Chroococcales	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Coelomorion</i> sp.	X		X			X	X			X		X	X	X	X	X	X		X	X
<i>Cuspidothrix</i> sp.		X	X					X		X		X		X	X					X
<i>Raphidiopsis raciborskii</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Dolichospermum</i> sp.		X	X	X	X	X	X	X		X		X		X	X	X				X
<i>Eucapsis</i> sp.						X	X			X					X					X
<i>Geitlerinema</i> sp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Merismopedia</i> sp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Microcystis aeruginosa</i>			X	X	X	X	X	X					X							X
Phormidiaceae	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Phormidium</i> sp.										X							X	X		
<i>Planktolyngbya</i> sp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Planktothricoides</i> sp.	X	X			X			X							X		X	X	X	
<i>Planktothrix agardhii</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Planktothrix isothrix</i>										X										
<i>Pseudanabaena</i> sp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Romeria</i> sp.	X	X	X	X	X	X	X		X	X				X	X	X	X	X	X	
<i>Shaerocavum</i> sp.	X	X	X				X	X						X		X	X			
<i>Synechocystis</i> sp.	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

evaporation (evap), irradiation (sunlight), chlorophyll (Chl), total phosphorus (TP), total nitrogen (TN), transparency (measured as Secchi depth; Secchi) and electrical conductivity (CE). The significance of the CCA was tested using the Monte Carlo test with 999 unrestricted permutations (5% of significance level) using *faraway* and *ade4* packages.

3. Results

During the four-year study period, 114 phytoplankton taxa were identified. Despite the large phytoplankton biodiversity observed across the 20 reservoirs, non-cyanobacterial phytoplankton taxa accounted for less than 1% of the total phytoplankton biovolume. Twenty-four cyanobacterial taxa were observed during this study including representatives from the Chroococcales order (CHR) and the Phormidiaceae family (PHOR) as well as *Aphanizomenon* sp. (APHAZ), *Aphanocapsa* sp. (APHAC), *Geitlerinema* sp. (GEI), *Merismopedia* sp. (MER), *Planktolyngbya* sp. (PLANK), *Pseudanabaena* sp. (PSEU), *Synechocystis* sp. (SYNE), *Raphidiopsis raciborskii* (RR), and *Planktothrix agardhii* (PA) (Table 3). Despite a wide variety of cyanobacteria being present, the filamentous species, *Raphidiopsis raciborskii* (RR), dominated 8 of the 20 reservoirs (AM, BO, CN, EQ, FQ, MT, PU and T; Tukey's $P < 0.05$) and accounted for approximately 52 to 88% of the total cyanobacterial biovolume (Fig. 2B). These 8 reservoirs were also characterized with elevated saxitoxin concentrations ranging from 0.49 to $3.51 \mu\text{g L}^{-1}$ (Fig. 3B). The colony-forming order, Chroococcales, dominated three reservoirs (MRT, PM, and PT; Tukey's $P < 0.05$), and accounted for approximately 52 to 95% of total cyanobacterial biovolume (Fig. 2B). *Aphanocapsa* sp. was dominant in a single reservoir, AT (Tukey's $P < 0.05$). Elevated microcystin was associated with Chroococcales dominance (Fig. 4) and ranged from 0.22 to $2.33 \mu\text{g L}^{-1}$ (Fig. 3A). Chroococcales and *Raphidiopsis raciborskii* were not statistically different from each other in four reservoirs (AMR, AR, MRT and SN reservoirs; Tukey's $P \geq 0.09$).

Detectable microcystins ($> 0.15 \mu\text{g L}^{-1}$) were found in 13 reservoirs (Fig. 3A). Seven reservoirs presented microcystin concentrations higher than $1 \mu\text{g L}^{-1}$, which is the maximum concentration allowed by the Brazilian potability ordinance PCR n° 5/2017 (Brasil 2017). Saxitoxin concentration was greater than $0.11 \mu\text{g L}^{-1}$ in 9 reservoirs (Fig. 3B). The highest average saxitoxin concentration ($3 \mu\text{g L}^{-1}$, PCR n° 5/2017) was measured in reservoir BO.

Raphidiopsis raciborskii represented approximately 80% of the cyanobacteria in that reservoir (Fig. 2B). Lastly cylindrospermopsin was detected ($> 0.05 \mu\text{g L}^{-1}$) in only five reservoirs (Fig. 3C), with concentrations above the recommended threshold ($> 1 \mu\text{g L}^{-1}$) in most of the reservoirs but was concentrated at three sites (AR, E and PT; Fig. 3C). Interestingly, multiple cyanotoxins were recorded in all of the study reservoirs during the four year study period, although MC and STX were more commonly detected than CYN (Fig. 3; Graham et al., 2010).

Canonical correspondence analysis (CCA) highlighted the association of specific environmental factors (black arrows) on cyanobacterial taxa composition (grey letters) at the study reservoirs. Canonical axes 1 and 2 explained 38.31% and 24.39% of the dataset variance (accumulated constrained eigenvalues), respectively (Fig. 4). There was a significant correlation between cyanobacterial taxa and environmental parameters ($F = 2.2656$; $p = 0.002$). Monte Carlo test has shown that STX, CYN, Chl, and Secchi were highly meaningful (Table 4).

Microcystin (MC) was directly correlated with electrical conductivity (CE), temperature (Temp), total phosphorus (TP) and weakly associated with transparency (Secchi). There was also a strong inverse relationship between microcystin and N:P ratio (NP) and evaporation (Evap), which in turn were strongly associated with saxitoxin (STX). This may be an indication that the relationship of these two measurements are intrinsically linked to the production of saxitoxin. Besides that, saxitoxin (STX) was associated with rainfall (rain), and chlorophyll concentration. Microcystin and cylindrospermopsin were associated with the same environmental variables (Fig. 4).

To further explore cyanobacterial taxa and toxin relationships, CCA showed that *Raphidiopsis raciborskii* (RR) was positively associated with saxitoxin. For example, in reservoir BO the average biovolume of RR ($> 10 \text{mm}^3 \text{L}^{-1}$) and saxitoxin concentration ($3.51 \mu\text{g L}^{-1}$) were high (Fig. 3B). *Pseudanabaena* sp. (PSEU) and *Planktolyngbya* sp. (PLANK) were positively associated with saxitoxin as well, but there is no report in the literature of saxitoxin production by either of these taxa (Appendix 2). Several taxa, including *Aphanizomenon* sp., Chroococcales order (CHR), *Geitlerinema* sp. (GEI), and *Microcystis aeruginosa* (MA), were associated with CYN and MC (Fig. 4). Among the environmental parameters tested, *Geitlerinema* sp. was associated with electrical conductivity and total phosphorus.

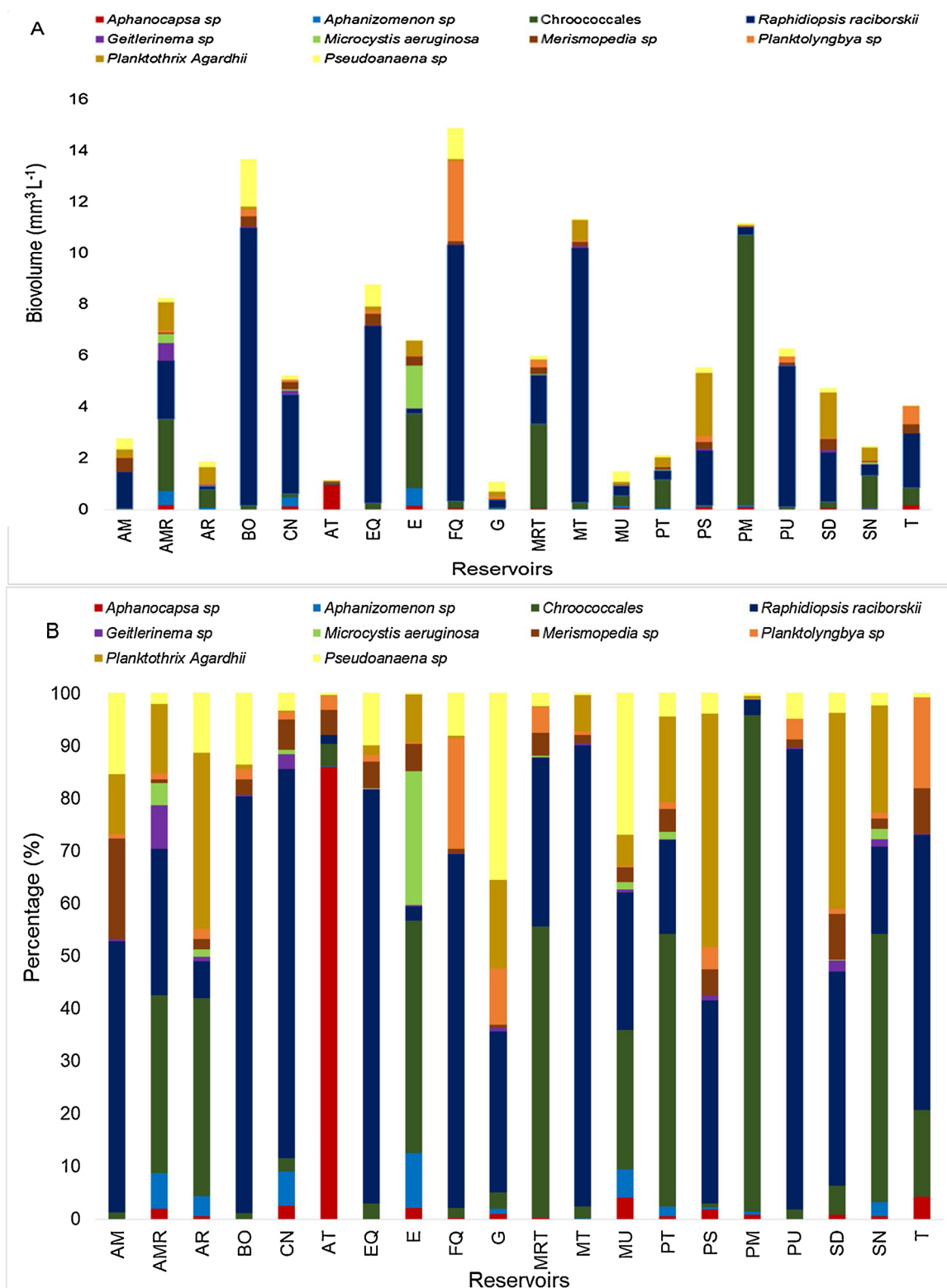


Fig. 2. (A) Absolute ($\text{mm}^3 \text{L}^{-1}$) and (B) relative (%) biovolume for the main cyanobacterial taxa observed across 20 Brazilian reservoirs.

4. Discussion

Data from 20 reservoirs used for drinking water production showed that non-cyanobacterial phytoplankton accounted for less than 1% of the total biovolume. Furthermore, *Raphidiopsis raciborskii* dominated 8 reservoirs, accounting for up to 88% of the total cyanobacterial biovolume in those reservoirs. Lins et al. (2016) also found that in the

semi-arid region of Brazil, more than 90% of the total phytoplankton community was composed of cyanobacteria. For example, *Pseudanaena limnetica*, *Raphidiopsis raciborskii*, *Aphanocapsa incerta*, *Microcystis aeruginosa*, and *Planktothrix agardhii* coexisted and alternated dominance. Costa et al. (2006), who studied cyanobacteria in another Brazilian semi-arid region, found that *R. raciborskii*, *Microcystis* sp., and *Aphanizomenon* sp. were persistent and accounting for 90 to 100% of

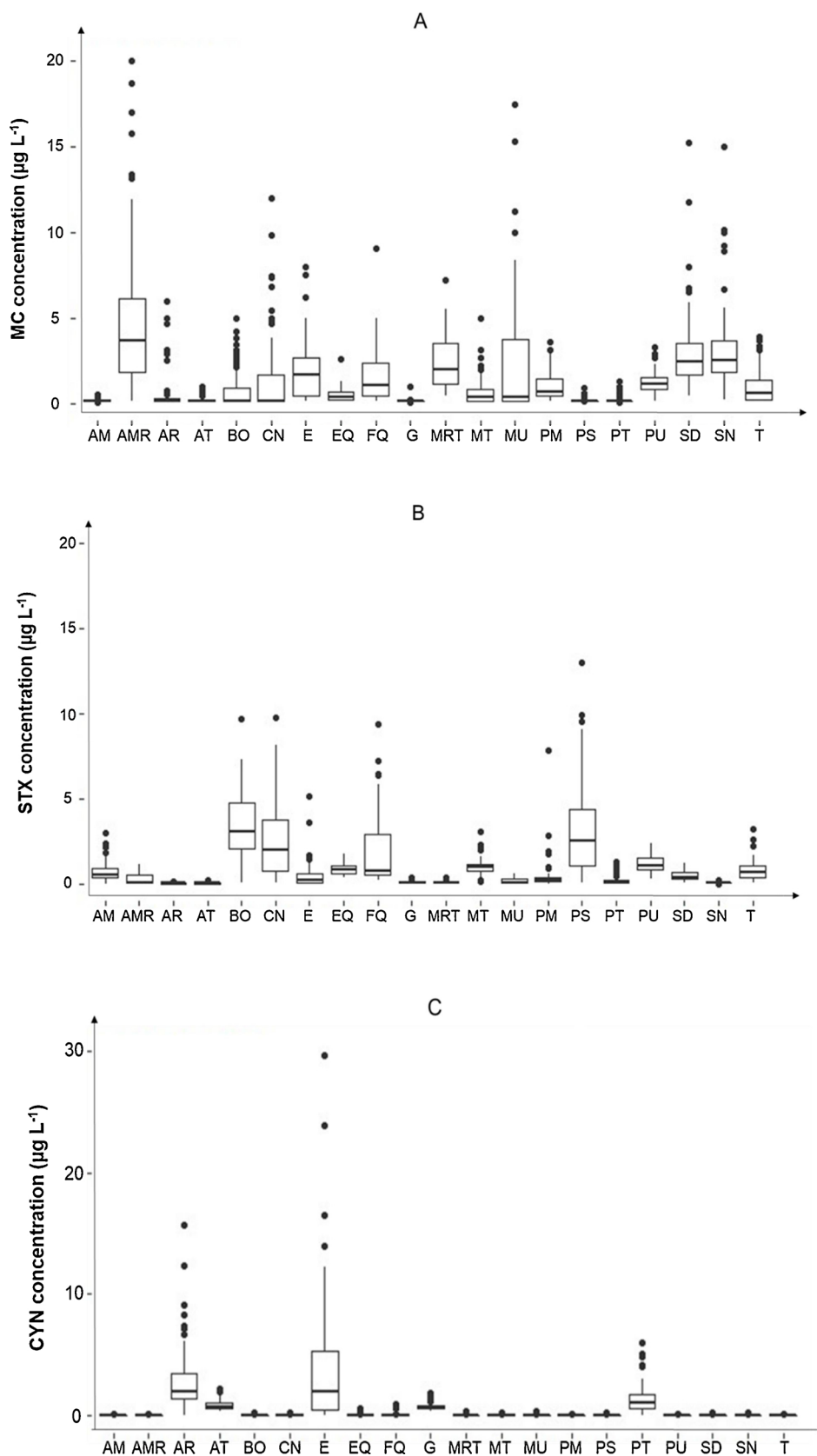


Fig. 3. Boxplots of concentrations of (A) microcystin (MC; detection limit method = $0.15 \mu\text{g L}^{-1}$), (B) saxitoxin (STX; detection limit method = $0.11 \mu\text{g L}^{-1}$), and (C) cylindrospermopsin (CYN; detection limit method = $0.05 \mu\text{g.L}^{-1}$).

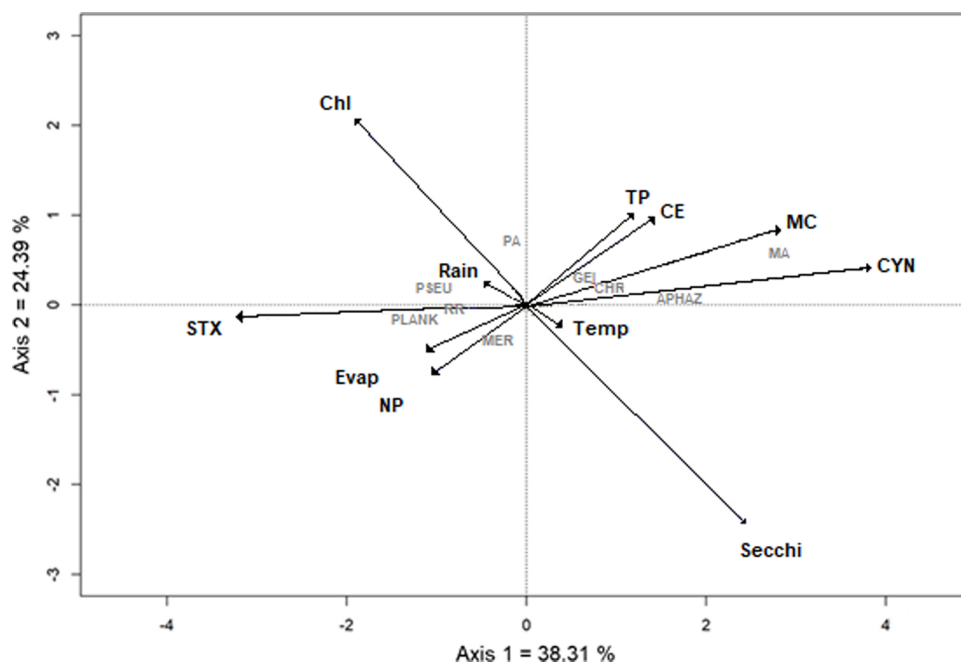


Fig. 4. Canonical correspondence analysis (CCA) ordination diagram of the cyanobacterial community (APHAZ = *Aphanizomenon* sp.; APHAC = *Aphanocapsa* sp.; CHR = Chroococcales; RR = *Raphidiopsis raciborskii*; GEI = *Geitlerinema* sp.; MER = *Merismopedia* sp.; PA = *Planktothrix Agardhii*; PSEU = *Pseudanabaena* sp., PLAN = *Planktolyngbya* sp.) in relation to studied environmental parameters.

Table 4
Monte Carlo test results after 999 permutations.

	CCA1	CCA2	r ²	p-value	VIF*
Temp	0.78374	-0.62109	0.0069	0.905	2.052
Evap	-0.87647	-0.48146	0.0467	0.535	9.219
Rain	-0.80444	0.59403	0.0089	0.886	7.006
MC	0.94550	0.32562	0.2462	0.065	4.041
STX	-0.99999	0.00461	0.2996	0.039	4.559
CYN	0.99665	0.08182	0.4218	0.035	2.194
TP	0.64651	0.76290	0.0779	0.407	16.083
NP	-0.71895	-0.69506	0.0567	0.474	9.258
Chl	-0.58394	0.81180	0.3221	0.029	13.008
CE	0.74549	0.66652	0.0939	0.337	4.016
Secchi	0.61292	-0.79014	0.4788	0.006	7.405

* VIF – Variance inflation factors.

the total phytoplankton cell counts; similar trends were found in this study (Figs. 2 and 3).

Several environmental factors have been shown to promote cyanobacteria in semi-arid regions, including high temperature, long photoperiods, alkaline pH, shallower water, and nutrient pollution (Yang et al., 2012; Lins et al., 2016; Yang et al., 2016; Moura et al., 2017). Due to its equatorial climate, the Ceará state in Brazil offers optimal conditions for the growth of cyanobacteria, such as high light incidence (~12 h light day⁻¹ throughout the year), a restricted rainy season, and surface waters confined in dams with long retention times (Lins et al., 2016; Moura et al., 2017). In addition, most of the Ceará reservoirs are shallow (average depth 10 m), which further enhances the effects of elevated ambient temperatures (COGERH, 2008).

Microcystin (MC), saxitoxin (STX), and cylindrospermopsin were found in a measurable concentration in 13, 9 and 5 reservoirs, respectively. Although CCA showed that *Raphidiopsis raciborskii* (RR), *Pseudanabaena* sp., and *Planktolyngbya* sp. were positively correlated to saxitoxin, it is believed that RR is responsible for its production since there are no instances in the literature of saxitoxin production by either of these last two taxa (Appendix 2). As expected, *Microcystis aeruginosa* (MA) was correlated to microcystin production. On the other hand, there was a strong inverse relationship between microcystin and N:P ratio (NP) and a direct correlation between saxitoxin (STX) and N:P ratio (NP).

It is well-known that excess nutrients stimulate phytoplankton

growth, especially cyanobacteria. Phosphorus and nitrogen are considered the main nutrients that limit primary productivity in aquatic environments (Downing et al., 2001; Dolman et al., 2012) although some species of cyanobacteria are capable of fixing atmospheric nitrogen (Dolman et al., 2012; Monchamp et al., 2014; Woodland and Cook, 2014; Vico et al., 2016). Nitrogen can be present in several forms and influence the success of many STX-producers considering that several are nitrogen-fixers. For example, Barros et al. (2015) cultivated the diazotroph, *Cylindrospermopsis raciborskii* (now called *Raphidiopsis raciborskii*), with reduced nitrogen which negatively affected its growth rate because of the relatively high energetic demands placed on nitrogen fixation. In that study, the culture reached stationary phase slower and with a lower cell density compared to phosphorus limited treatment. Moreover, Saker and Neilan (2001) evaluated intraspecific variation in growth of seven strains of *C. raciborskii* from Australia exposed to different sources of nitrogen and found that ammonia amendments supported faster growth than nitrogen limited environments that required energetically costly nitrogen fixation. Moreover, Briand et al. (2004) found that different nitrogen sources can cause changes at the molecular level in cyanobacteria, including gene expression and toxin production in *C. raciborskii* (also see Stucken et al., 2014).

These results show that N:P ratio strongly influenced saxitoxin producers, mainly by *Raphidiopsis raciborskii*. Smith (1983) compiled data from 17 lakes worldwide and found that cyanobacteria were dominant in lakes with low N:P ratios. Their results conclude that lakes having an epilimnetic N:P ratio higher than 29 by weight will exhibit a low proportion of cyanobacteria. Nevertheless, Chislock et al. (2014) also experimentally showed that *R. raciborskii* can dominate under very high or very low N:P conditions. Miotto (2016) verified in Brazil that two *R. raciborskii* strains exhibited varied growth under three different N:P ratios (4.5, 10, and 40) but that the highest growth rates was observed at an N:P of 4.5. Bouvy et al. (1999, 2000) observed the dominance of *R. raciborskii* in reservoirs located in the semi-arid regions of northeastern Brazil and concluded that its dominance was related to high temperatures and pH values and low N:P in addition to the absence of efficient consumers.

Canonical correspondence analysis (CCA) reinforced the importance of evaporation to STX-producers and showed that *Raphidiopsis raciborskii* was the main taxon associated with STX. Carneiro et al. (2013)

demonstrated that *R. raciborskii* was successful because of multiple traits, including its ability to fix nitrogen, high affinity for ammonia and phosphorus, and ability to regulate its buoyancy and form akinetes. According to these authors, the focal semi-arid reservoirs showed that *R. raciborskii* dominated the phytoplankton communities. Brentano et al. (2016) found that in a subtropical lagoon in Brazil, dissolved nitrogen lead to elevated STX concentrations. Lastly, Vico et al. (2016) contended that *C. raciborskii* could alternatively use atmospheric nitrogen (N₂) thus allowing it to develop in low dissolved nitrogen concentration systems. On the other hand, when nitrate is high, *C. raciborskii* produces less heterocysts, suggesting that it prefers to use dissolved nitrogen forms, when available (Vico et al., 2016).

Phosphorus has been reported as an important limiting nutrient for phytoplankton growth (O'Neil et al., 2012; Chislock et al., 2014; Jacoby et al., 2015). Yet, Dolman et al. (2012) found a saturation function of cyanobacterial biovolume across increasing concentrations of TP indicating that other factors limit phytoplankton growth when phosphorus concentration is high. In this research, there was a strong positive relationship between total phosphorus and two cyanobacterial toxins (MC and CYN; Fig. 4).

According to Komárek and Anagnostidis (1998) and Sant'anna et al. (2004), the order Chroococcales is comprised of all unicellular or colonial cyanobacteria, which do not form true filaments with direct physiological interference between cells. *Microcystis aeruginosa*, a member of the order Chroococcales, is one of the dominant bloom-forming cyanobacterial species in freshwater environments. In addition to producing microcystin, *M. aeruginosa* is capable of producing taste and odor compounds, including geosmin and 2-methylisoborneol (Wang et al., 2016). Bartoli et al. (2014) found seven microcystin variants (MC-RR, MC-LR, MC-YR, MC-LF, MC-LW, dm-MC-RR, and dm-MC-LR) in reservoirs from São Paulo, Brazil. Gkelis and Zaoutsos (2014) showed that the highest concentrations of MC were produced by *Microcystis viridis*, *Microcystis novacekii* and *Microcystis aeruginosa* compared to *Microcystis wesenbergii*, which is considered a non-toxic species. These studies show that most of the morphological types of *Microcystis* include toxic and non-toxic strains and that higher MC concentrations were found when *Microcystis aeruginosa* was the dominant species. *Microcystis aeruginosa* showed positive relationships with CYN; however, this species is not reported as a cylindrospermopsin-producer but is well known as a producer of MC (Bartoli et al., 2014; Gkelis and Zaoutsos, 2014).

MC was positively correlated with temperature (Fig. 4). Cyanobacterial maximum growth rate varies has been shown to vary within and across species (Wilson et al., 2006). For example, the maximum growth rate for *Aphanizomenon flos-aquae* and *Planktothrix agardhii* is ~20 °C, while *Microcystis aeruginosa* prefers ~28 °C and some species of the genus, *Synechococcus*, can thrive in temperatures above 30 °C (Reynolds, 1989, 2006). According to Carey et al. (2011), the acceleration rate, commonly measured as Q₁₀ (change in growth between 10 °C temperature shifts; usually 10–20 °C) was 2.6 for *Synechococcus* sp. but 3.7x higher for *Microcystis aeruginosa* (Q₁₀ = 9.6). This value was the highest recorded among the cyanobacteria and eukaryotic phytoplankton; therefore, *Microcystis aeruginosa* species should have a physiological advantage when the water temperature is > 20 °C. Moreover, Merel et al. (2013) confirmed that cyanobacterial blooms are primarily regulated by three environmental factors; temperature, light exposure, and nutrients. In this study, duration of light exposure was more important for growth than light quality (Fig. 4).

Gkelis and Zaoutsos (2014) identified MC- and STX-producing taxa in freshwater waterbodies of Greece, but did not identify CYN-producing taxa. They also found that strains of *Cylindrospermopsis raciborskii* (now called *Raphidiopsis raciborskii*) in Greece carry the genes for STX and CYN production but were only reported to produce STX. Several studies have shown that strains of *C. raciborskii* found in temperate lakes are unable to produce cylindrospermopsin (Fastner et al., 2003; Yilmaz et al., 2008). While other studies have shown that several

Aphanizomenon species can produce CYN (Banker et al., 1997; Wörmer et al., 2008; Preußel et al., 2009; Brient et al., 2009; Cires et al., 2011). In this study, there was no relationship between RR and CYN; however, there was a positive correlation between APHAZ and CYN (Fig. 4), which support findings in these past studies (Appendix 2).

As expected, chlorophyll and Secchi depth were inversely correlated in this study (Fig. 4). Secchi depth is a direct measure of waterbody transparency that may be influenced by organic (phytoplankton) and inorganic (suspended sediment) components (Gillion and Bortleson, 1983). Saxitoxin-producing cyanobacteria were most associated with chlorophyll. Since 2012, according to the Foundation of Meteorological Studies of Ceará (FUNCEME), Ceará state has experienced below average precipitation that has influenced phytoplankton dynamics, including more frequent and intense cyanobacterial blooms. The correlation between rainfall and saxitoxin corroborates with Barros et al. (2017) results, who noticed that precipitation below the historical mean favors the proliferation of cyanobacteria in reservoirs in the same region. Lastly, Reichwaldt and Ghadouani (2012) investigated the effect of rain patterns on toxic cyanobacterial blooms and determined that increased nutrient concentration associated with high evaporation (and concomitant less waterbody volume) and prolonged stratification processes favor the proliferation of toxic cyanobacteria.

5. Conclusions

Reservoirs in Ceará state used for drinking water were dominated by cyanobacteria due to a variety of factors, including nutrient pollution and a semi-arid climate. The study period included a cycle of prolonged drought, high evaporation, and elevated temperature associated with water scarcity; factors that favor toxic cyanobacterial blooms in waterbodies around the world, including Ceará reservoirs. Throughout the region, there was a predominance of colonial and filamentous cyanobacterial taxa, including representatives belonging to the order, Chroococcales, and the species, *Raphidiopsis raciborskii*.

Canonical correspondence analysis (CCA) showed to be a valuable tool for identifying environmental factors associated with cyanobacteria and their associated toxins. Using CCA, it was suggested that N:P was associated with the development of STX-producers, especially *R. raciborskii*, while phosphorus was strongly related to MC and CYN producers. Water transparency was negatively correlated with STX-producing cyanobacteria, including *R. raciborskii*. In this study, *R. raciborskii* dominated in eight reservoirs where STX and Chl were high. Transparency, electrical conductivity, and elevated temperature favored the proliferation of toxigenic species, *Aphanizomenon* sp. and *Microcystis* sp., and the order Chroococcales that were strongly correlated with CYN and MC. Since *Aphanizomenon* is not known to produce microcystin, *Microcystis* sp., is likely responsible for its presence while *Aphanizomenon*, known to produce CYN, it is likely to be responsible for CYN in the study systems.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.hal.2019.05.006>.

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