ORIGINAL ARTICLE

Spatio-temporal variation of cyanobacteria and cyanotoxins in public supply reservoirs of the semi-arid region of Brazil

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ABSTRACT

Cyanobacteria harmful algal blooms (CyanoHABs) have become increasingly frequent and intense in public supply reservoirs as a result of eutrophication and global climate change. The semi-arid region of Brazil has a well documented history of CyanoHABs but the underlying factors that control the excessive proliferation of these organisms and the production of their bioactive secondary metabolites are not comprehensively understood. This study aimed to identify the environmental factors that explain the spatial and temporal variations in the abundance of cyanobacteria and the concentration of cyanotoxins (microcystins, saxitoxins, and cylindrospermopsin) in semi-arid reservoirs. The following hypotheses were tested: i) the largest biovolumes of potential toxin producing cyanobacteria occur when cyanotoxin concentrations are highest; and ii) the environmental factors that explain variations in biovolume of cyanobacteria also explain changes in cyanotoxins concentrations. Samples were taken from four reservoirs located in the Northeast region of Brazil, over a three-month period (October 2016 and February and June 2017). Of the 24 species of cyanobacteria identified, 13 were potentially toxin-producing. Physicochemical variables such as water volume of the reservoir, water transparency, soluble reactive phosphorus, and total phosphorus explained the abundance of cyanobacteria and the levels of cyanotoxins. These results corroborate the hypothesis that similar physicochemical conditions influence the abundance and diversity of cyanobacteria and cyanotoxins. Cyanobacterial blooms composed of more than one potential toxin producing species were observed in the studied reservoirs, where potential microcystin-producing species were the most common. Microcystins and saxitoxins were detected in all the reservoirs studied, while cylindrospermopsin and the cyanobacterium *Cylindrospermopsis raciborskii* were simultaneously recorded in only one reservoir (Camalaú Reservoir). Cylindrospermopsin was only detected in a reservoir for the first time in the State of Paraíba. Canonical redundancy analysis showed that the cyanotoxins were related to potential toxin producing species. These results corroborate the proposed hypothesis that there is a correlation between cyanotoxins and the biomass of potential producers. Also, there were situations where cyanotoxins were detected without the presence of potential producers. These results demonstrate the need for reassessment of potential toxin producing species of cyanobacteria in semi-arid reservoirs. This may lead to the identification and characterization of novel producers of these bioactive secondary metabolites.

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Key words: Eutrophication; cylindrospermopsins; microcystins; saxitoxins; tropical reservoirs.

Edited by: Andrea Lami, *CNR-IRSA Verbania, Italy.*

Contributions: JSS, MAC, JELB, study concept; JSS, RDSS, DAO, CFM, VVB, data curation; RDSS, DAO, CFM, VVB, formal analysis; RDSS, CFM, VVB, methodology; JSS, MAC, JELB, resources and supervision; RDSS, original manuscript draft; RDSS, JSS, MAC, JELB, manuscript review and editing. All authors read and approved the final version to be published.

Conflict of interests: the authors declare no potential conflict of interests

Received: 11 February 2019.
Accepted: 16 September 2019.

Licensee PAGEPress, Italy
J. Limnol., 2020; 79(1): 13-29
DOI: 10.4081/jlimnol.2019.1893

INTRODUCTION

In reservoirs used for public water supply, cyanobacteria may be a serious public health threat (Te and Gin, 2011). This is because they can synthesize and release bioactive secondary metabolites (a.k.a. cyanotoxins) that are hepatotoxic, neurotoxic, cytotoxic, and dermatotoxic (Funari and Testai, 2008; Sierosławska *et al*., 2010; Buratti *et al*., 2017).

Several studies have discussed the factors that cause the development of cyanobacterial blooms. Among these factors, high concentrations of nutrients such as nitrogen and phosphorus (Dolman *et al*., 2012; Figueredo *et al*., 2016; Chia *et al*., 2017), high light intensity (Kaebernick and Neilan, 2001; Bittencourt-Oliveira *et al*., 2010), high temperatures (Paerl and Otten, 2013; Chia *et al*., 2017), low water transparency, and high pH values (Gao *et al*., 2012) stand out. Furthermore, turbulence and stratification (Fortin *et al*., 2010) and biotic interactions such as competition and herbivory (Wang X. *et al*., 2010) influence successional processes of cyanobacteria in aquatic ecosystems (Chia *et al*., 2018).

Increasing global temperatures and light intensities associated with changing global climatic conditions have been shown to support the excessive proliferation of several noxious bloom forming cyanobacteria, leading to their persistence and wide geographic distribution (Paerl *et al*., 2011; O'Neil *et al*., 2012; Paerl and Paul, 2012; Mantzouki *et al*., 2018). In particular, cyanobacteria appear to be well adapted to take advantage of these changing climatic conditions (Paerl and Huisman 2009).

According to the report of the Intergovernmental Panel on Climate Change (IPCC, 2013), the semi-arid regions will be most affected by the negative effects of climate change. In this region, the dynamics of aquatic ecosystems are strongly affected by the frequent periods of drought, high temperatures, high evaporation rates, and water residence time (Barbosa *et al*., 2012). Together, these factors cause a reduction in water volume, which leads to higher nutrient concentrations. Climate changes are predicted to increase the frequency and intensity of droughts, which will lead to reductions in water availability in semi-arid regions (Ragab and Prudhomme, 2002; Krol and Bronstert, 2007; Huang *et al*., 2016). Semi-arid reservoirs appear to be model examples of possible near future effects of climate change on aquatic ecosystems worldwide (Mowe *et al*., 2015).

Cyanobacterial blooms persist throughout the year in most semi-arid reservoirs and, unfortunately, several of these blooms comprise cyanobacteria species that produce cyanotoxins such as microcystins, cylindrospermopsin, saxitoxins and anatoxins (Bittencourt-Oliveira *et al*., 2014; Lorenzi *et al*., 2018; Moura *et al*., 2018).

The ecological roles of cyanotoxins are not comprehensively understood (Carmichael, 1992; Downing *et al*., 2015; Omidi *et al*., 2018), but experimental evidence suggest they lead to the dominance of cyanobacteria and influence successional processes in aquatic ecosystems (Ger *et al*., 2014; Chia *et al*., 2018). They act as a defense mechanism against herbivory (Freitas *et al*., 2014) and as allelochemicals by altering the growth of macrophytes and phytoplankton species during competition for nutrients and light (Rojo *et al*., 2013; Mohamed, 2017).

Studies have shown that there is a direct relationship between cyanobacteria biomass and cyanotoxin production (Pawlik-Skoworoska and Toporowska, 2016), but cyanotoxins do not always increase with increasing abundance of cyanobacteria (Piccin-Santos and Bittencourt-Oliveira, 2012; Walls *et al*., 2018). This means that the production of these compounds may have no relation to the ability of cyanobacteria to dominate water bodies.

Most studies on cyanobacteria and cyanotoxins of semi-arid reservoirs have rarely considered how they are influenced by spatial and temporal variations in physicochemical conditions. For example, Lorenzi *et al*., (2018) showed seasonal and annual changes in cyanotoxins in public supply reservoirs in the semi-arid region of Brazil, but as with the case with other studies, the relationship between physicochemical conditions and biological parameters (cyanobacteria and cyanotoxins) was not described in quantitative terms. Therefore, the objective of the present study was to identify the environmental factors that explain the spatial and temporal variation in cyanobacteria abundance and the concentration of the cyanotoxins; microcystin, saxitoxin and cylindrospermopsin in semi-arid reservoirs. The following hypotheses were tested: i) the largest biovolumes of potential toxin producing cyanobacteria occur when cyanotoxin concentrations are highest; and ii) the environmental factors that explain variations in biovolume of cyanobacteria also explain changes in cyanotoxins concentrations. The results of the present study contribute to the understanding of key factors that control the excessive proliferation of bloom-forming cyanobacteria and their toxins, and will enable the establishment of preventive measures for CyanoHABs in semi-arid public supply reservoirs.

METHODS

Study area

The study areas were the Epitácio Pessoa (commonly called the Boqueirão Reservoir) (7º28’9’’S; 36º8’2’’W), Camalaú (7º53’10’’S; 36º49’25’’W), Poções (7º53 ‘45’’S, 37º0’50’’W) and Mucutú (7º07’7,8’’S; 36º39’2,3’’W) reservoirs. They are located in the Paraíba River Basin, Paraíba State, Brazil (Fig. 1). The reservoirs provide potable water to 29 cities in Paraiba State and are used for animal watering, recreation, and irrigation.

The region has a tropical climate (BSh) and an annual rainfall of *ca.* 400 mm in the driest areas (Alvares *et al*., 2013). The highest precipitation is recorded between February and May (Araújo *et al*., 2009), but a prolongated drought occurred between 2014 and 2017, when rainfall was below the historical average of the region (Walter *et al*., 2018; Jovem-Azevêdo *et al*., 2019). This prolonged drought resulted in a drastic reduction in the water volume of the reservoirs; consequently, water supply shortages (Martins *et al*., 2015; Walter *et al*., 2018).

From April 2017, the reservoirs Boqueirão, Camalaú and Poções received water from the São Francisco River. The Brazilian government project named the “São Francisco River Integration Project”, fed more than 700 kilometers of concrete channels in two major axes (north and east) with water from São Francisco River passing through Pernambuco, Paraíba, Ceará and Rio Grande do Norte states.

Sampling and data analysis

Samplings were conducted in October 2016 and February and June of 2017 (*i.e.* the dry season of the region), at three locations in the reservoirs, between the dam and the point of entry of the Paraíba River, namely: site 1, point of entry of the Paraíba River to the reservoirs; site 2, region between the dam and the point of entry of the Paraíba River; and site 3, located on the dam (Fig. 1). Samples (32 L) were collected under the water surface with the aid of a bucket for nutrients, chlorophyll-a, cyanobacteria, and cyanotoxins analyses.

Climatic variables, abiotic variables, and chlorophyll-a

Data on water volume of the reservoirs and average precipitation during the sampling dates were obtained from the Agência Executiva de Gestão das Águas (AESA; Paraíba State).

Water temperature (ºC), pH, and turbidity (NTU) were measured in situ with the HORIBA© U-50 multi-parameter probe. Water transparency was determined using the Secchi disk.

Samples for nutrient analysis and chlorophyll-a were stored in plastic bottles and transported on ice to the laboratory. The concentration of ammonia (NH_4-N), nitrate (NH_3-N), nitrite (NH_2-N), soluble reactive phosphorus (SRP), and total phosphorus (TP) were measured according to the techniques described in APHA (2012). Dissolved inorganic nitrogen (DIN) content was determined by summing the concentrations of NH_4-N, NH_3-N and NH_2-N. Chlorophyll-a was extracted with 96% ethanol per the method of Jespersen and Christoffersen (1987) and measured spectrophotometrically (Lorenzen, 1967).

Total phosphorus and chlorophyll-a data were used to calculate the Trophic State Index (TSI) as described by Carlson (1977) and modified for tropical environments by Toledo Jr. *et al*., (1983). The trophic classification followed the criterion: oligotrophic TSI <44, mesotrophic $44 < \text{TSI} > 54$ and eutrophic TSI > 54.

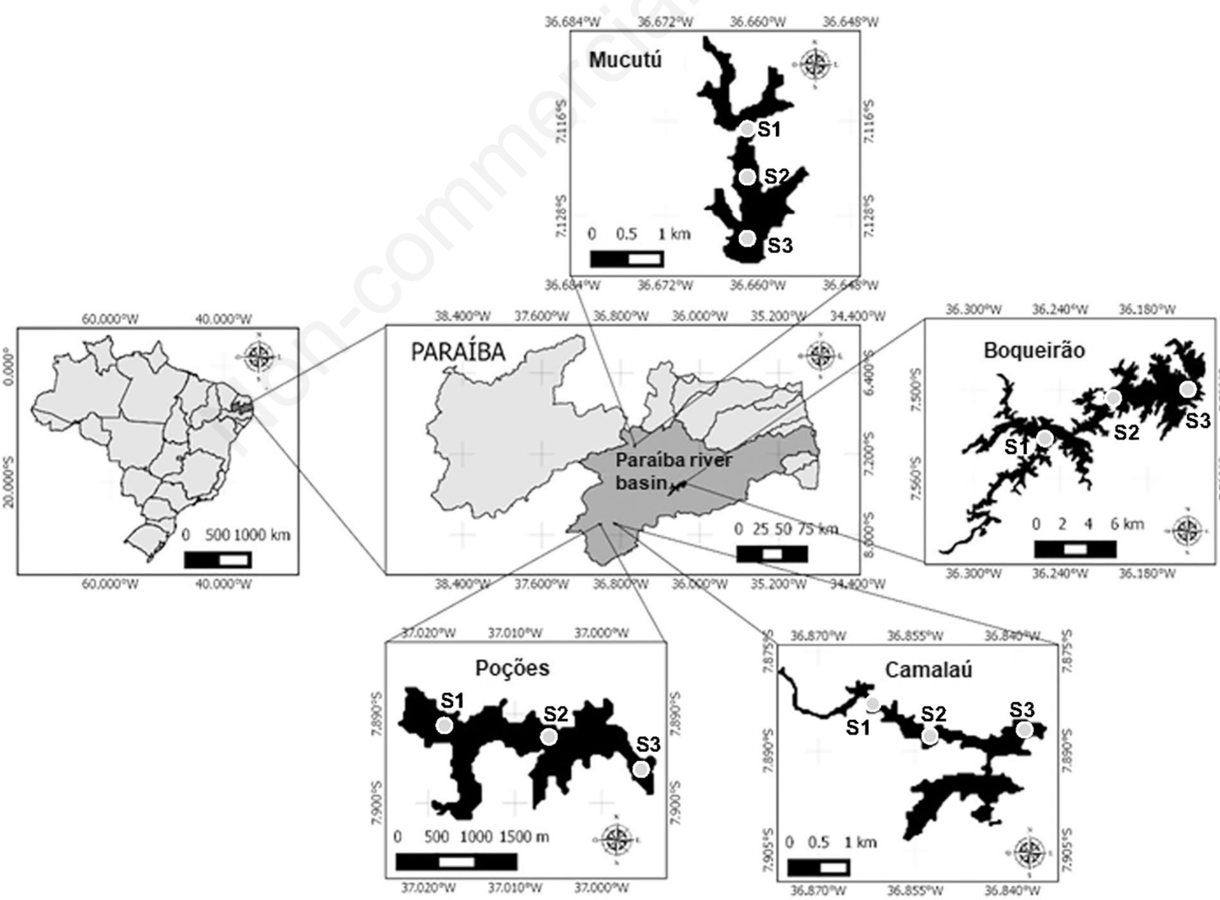

Fig. 1. Location of Boqueirão, Camalaú, Mucutú and Poções reservoirs in the State of Paraíba, Brazil. ●, Sampling sites; S1, sampling site 1; S2, sampling site 2; S3, sampling site 3.

Cyanobacteria biovolume

Samples for analysis of cyanobacteria were collected directly from the water, stored in amber flasks and fixed with 1% Lugol. Semipermanent slides of cyanobacterial samples were prepared, viewed under a Zeiss Axioskop 40 optical microscope (Carl Zeiss, Jena, Germany), and the taxa identified using identification keys provided by Komárek and Anagnostidis (1989, 1999, 2005), Komárek *et al*. (2002), and Komárek (2013). Quantitative analysis of cyanobacteria was performed using an inverted microscope (Zeiss Axiovert 40°C) at 400X magnification (Uthermöhl, 1958). At least 400 individuals of the most abundant species or 100 fields per sample were enumerated to minimize count errors. Density (individuals mL^{-1}) was obtained using the formula described by Ross (1979) and the biovolume ($mm^3 L^{-1}$) was estimated by multiplying the density of the species by the average volume of the cells (~20 individuals). The volume of the cell was calculated from geometric models close to the shape of the species - spheres, cylinders, cones, parallelepipeds, pyramids, ellipsoids and others - as described by Hillebrand *et al*. (1999).

Cyanotoxins

For the analysis of cyanotoxin concentrations, thirty liters of the water from the reservoirs (per sampling station) were filtered and concentrated through a plankton net (20 µm mesh size), stored in 250 mL bottles, and transported on ice to the laboratory in a styrofoam box.

The total concentrations (µg L^{-1}) of microcystin, saxitoxin and cylindrospermopsin were determined by the Enzyme-Linked Immuno Sorbent Assay (ELISA) method using kits an Abraxis plate (Warminster, Pa) specific to each toxin, per manufacturer's instruction. To extract the toxin from the cells, three freeze/thaw cycles were carried out on the samples at 40 ºC. The absorbance of the color reaction of the ELISA runs was measured with an ASYS A-5301 microplate reader (ASYS Hitech GmbH, Eugendorf, Austria).

Statistical analysis

In order to test for significant differences in the physical and chemical variables, cyanobacteria biovolumes, and cyanotoxin concentrations between the months (October, February, and June) and sampling sites, a Linear Mixed Effects Model was used. This was followed by the Holm-Sidak post hoc test to separate means that were significantly different. The normality and homoscedasticity of the data were evaluated *via* the Kolmogorov-Smirnov and Mauchly's Sphericity tests, respectively. Where Sphericity was violated, the Greenhouse-Geisser and Huynh-Feldt corrections were employed.

The Pearson correlation test (r) was applied to verify the correlation between the analyzed cyanotoxins and potential cyanotoxin-producing species. The potential species that produce microcystin, saxitoxin, and cylindrospermopsin were determined according to Bernard *et al*. (2016).

Redundancy analysis (RDA) was performed to identify the relationship between physical and chemical variables and cyanobacteria and cyanotoxins. The criterion for selection of the RDA was based on the length of the axes of the Detrended Correspondence Analysis (DCA), which ratifies the performance of this analysis when the length of axis 1 is lower than that of axis 3 (Ter Braak and Prentice, 1988). To test if the RDA model was significant, a factorial ANOVA was run.

Two RDAs were performed, one containing cyanobacteria as the dependent data and the other cyanotoxins. The matrix with the abiotic variables was standardized using Standard Deviation. To identify the abiotic variables that made independent and significant contributions to the variation of cyanobacteria and cyanotoxins, selection of variables was performed stepwise using permutation tests, and then selection by variance inflation factor (VIF) from the inclusion of variables with VIF <20. In the cyanobacteria data matrix, only species with biovolumes equal to or greater than 1% of the total phytoplankton biovolume were included, and the data log transformed (log [x + 1]).

Correlation and Redundancy analyses were performed at a significance level of 5% using the R software for Windows. Linear Mixed Effects Model was performed using IBM SPSS version 24 for MacOS.

RESULTS

Abiotic variables and trophic state index

During the study period, it rained only in June 2017 at Boqueirão, Camalaú and Poções reservoirs. Maximum rainfall (39.10 mm) was recorded in Poções Reservoir (Fig. 2). The volume of water in these reservoirs was *ca.* 6% in October 2016, followed by an increase in June 2017 to the highest value of 14.90% in Poções Reservoir (Fig. 2 A,B,D). In the Mutucú Reservoir, rainfall occurred from February 2017 (17.4 mm), but its water volume continued to decline in the following months, reaching 0.65% in June 2017 (Fig. 2C).

The TSI varied between 55 and 73 in all the reservoirs, which led to their classification as eutrophic (Fig. 2). Changes in physical and chemical parameters were significantly different ($P<0.05$) between the sampled months, while those observed between the sampling sites were not (Tab. 1). In the Boqueirão Reservoir, water temperature not significantly ($P=0.354$) higher in

February 2017, while water transparency (P=0.01) and pH (P=0.002) were lowest in June 2017. Similarly, the highest turbidity (p>0.05), nitrate (p=0.039), orthophosphate (P>0.05), and total phosphorus (P>0.05) levels were recorded in June 2017 (Tab. 1). In June 2017, the Camalaú Reservoir had the lowest water transparency (P=0.001) and pH (P<0.001), while nitrate (P=0.014) and total phosphorus (P=0.043) concentrations were highest in the same month (Tab. 1).

Physicochemical conditions such as water temperature (P=0.022), turbidity (P<0.001), and total phosphorus (P=0.002) were highest in Poções reservoir in February 2017, while water transparency was lowest (p=0.004) (Tab. 1). In addition, in June 2017, the lowest pH was recorded (P<0.001) in Poções reservoir. In Mucutú Reservoir, in June 2017, the highest turbidity (P=0.011), ammonia (P<0.001), nitrite (P>0.05), DIN (P<0.001), and total phosphorus (P<0.016) levels were found (Tab. 1). The highest water temperature was recorded in Februrary 2017 (P=0.03) in Mucutú reservoir.

Cyanobacteria

Twenty-four species of cyanobacteria were identified in the reservoirs studied, out of which 10 are potential toxin producers. Specifically, seven of the species were potential producers of microcystins, four of saxitoxins, and two of cylinderspermopsin (Tab. 2).

The total biovolume of cyanobacteria ranged from 0.03 to 16.57 x10^2 mm^3 L^{-1} in Boqueirão reservoir, 0.01 to 23.77 x10^2 mm^3 L^{-1} in Camalaú reservoir, 0.69 to 40.15 x10^2 mm^3 L^{-1} in Mucutú reservoirs and 0.02 to 310.76 x10^2 mm^3 L^{-1}in Poções reservoir (Tab. 3). A significant increase in total cyanobacterial biomass was recorded in February 2017 in Camalaú (p=0.003) and Mucutú (P=0.000) reservoirs. Cyanobacteria biomass data revealed that *Coelomorum tropicale* had the highest biomass contribution in Poções Reservoir, and *Dolichospermum solitarium* and *Cylindrospermopsis raciborskii* in Camalaú Reservoir (Fig. 3).

The RDA showed that the influence of abiotic

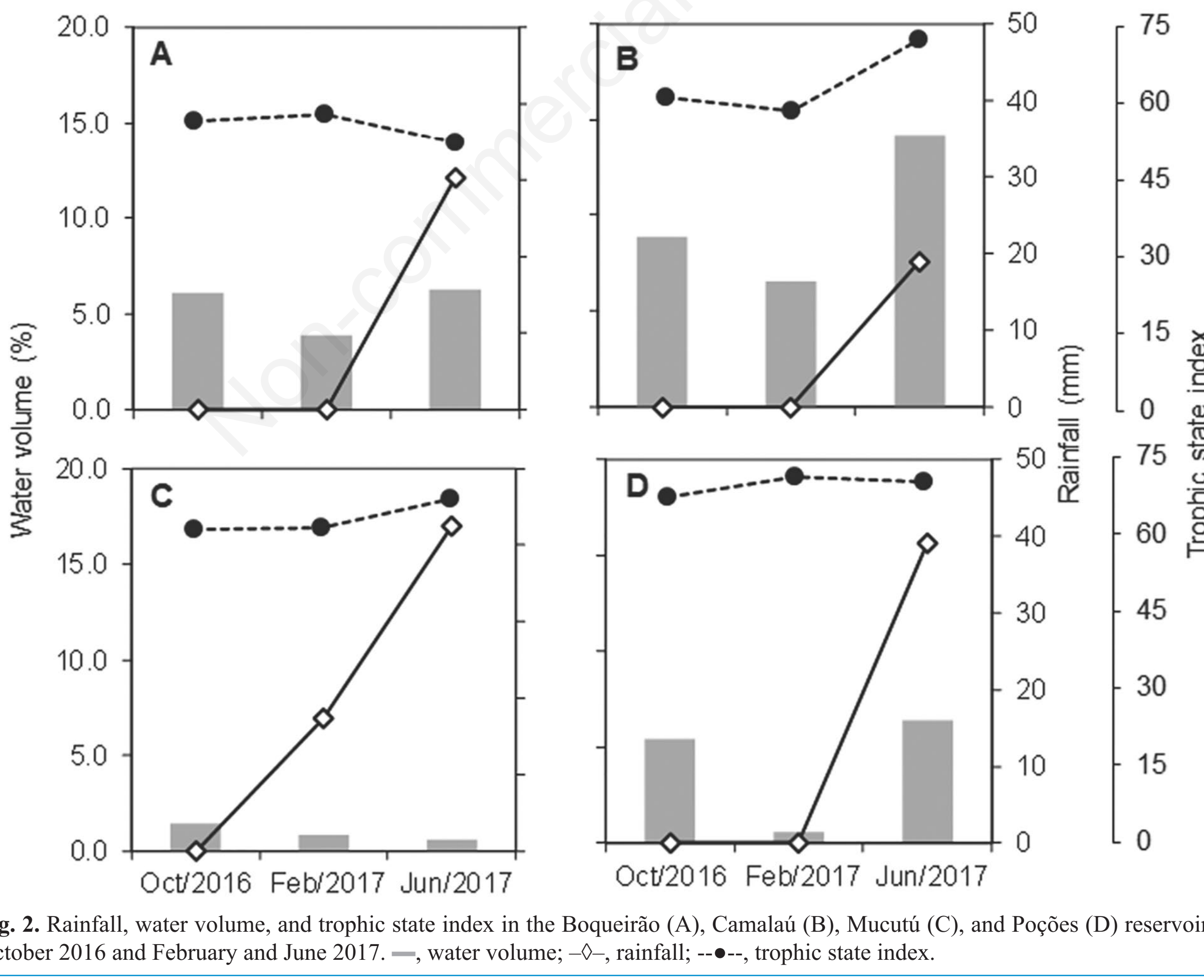

Fig. 2. Rainfall, water volume, and trophic state index in the Boqueirão (A), Camalaú (B), Mucutú (C), and Poções (D) reservoirs in October 2016 and February and June 2017. ▬, water volume; –◊–, rainfall; --●--, trophic state index.

Tab. 1. Physical and chemical characteristics of Boqueirão, Camalaú, Poções, and Mucutú reservoirs.

Reservoirs/variables	Temp (C°)	Secchi (cm)	Turb (UNT)	pH	N-NH_4 (µg L^{-1})	N-NH_2 (µg L^{-1})	N-NH_3 (µg L^{-1})	DIN (µg L^{-1})	SPR (µg L^{-1})	TP (µg L^{-1})
Boqueirão reservoir										
October/2016										
S1	24.57	1.39	18.50	8.91	248.00	5.60	96.34	349.94	33.00	160.33
S2	24.92	1.50	16.80	8.54	12.69	0.00	25.43	38.12	43.00	120.00
S3	24.57	1.08	7.65	8.64	198.2	2.24	24.22	224.66	13.00	53.66
February/2017										
S1	25.95	1.40	2.36	8.47	23.97	0.00	33.91	57.88	3.00	43.66
S2	26.57	1.19	36.10	8.52	13.39	15.67	8.48	37.54	28.00	33.66
S3	26.23	1.78	18.50	8.49	6.34	7.84	14.53	28.71	28.00	60.33
June/2017										
S1	24.71	0.40	194.00	7.45	47.24	25.75	193.79	266.78	83.00	300.33
S2	24.80	0.45	9.50	7.56	43.71	24.63	218.01	286.35	93.00	340.33
S3	25.14	1.10	228.00	7.91	40.19	4.48	69.04	113.71	3.00	80.33
Camalaú reservoir										
October/2016										
S1	24.81	1.46	19.00	9.20	64.87	97.39	190.16	352.42	153.00	273.66
S2	24.52	-	34.80	8.80	11.98	12.24	24.22	48.44	23.00	304.00
S3	23.86	-	29.70	8.69	14.80	0.00	53.29	68.09	18.00	67.00
February/2017										
S1	26.2	0.65	136.00	8.97	9.87	4.48	13.22	27.57	38.00	57.00
S2	25.53	0.78	116.00	8.92	2.82	14.55	10.9	28.27	28.00	57.00
S3	25.13	0.81	37.40	8.55	7.75	5.60	6.05	19.4	28.00	60.33
June/2017										
S1	22.79	0.34	178.00	7.64	47.94	42.54	171.99	262.47	73.00	633.66
S2	24.65	0.34	90.00	7.42	25.38	3.36	164.72	193.46	143.00	370.33
S3	25.09	0.34	79.80	7.61	46.53	0.00	171.99	218.52	43.00	253.66
Mucutú reservoir										
October/2016										
S1	23.6	0.24	29.10	8.26	110.70	5.60	60.65	176.95	28.00	180.33
S2	19.7	0.63	6.30	6.70	14.78	4.48	24.89	44.15	38.00	98.00
S3	25.07	0.50	4.40	8.36	16.21	4.48	29.07	49.76	63.00	173.66
February/2017										
S1	25.3	0.27	136.00	7.99	703.21	0.00	10.9	714.11	3.00	157.00
S2	25.51	0.31	56.00	7.61	626.15	3.36	8.48	637.99	18.00	133.66
S3	25.71	0.31	62.80	7.73	731.21	0.00	4.84	736.05	58.00	167.00
June/2017										
S1	23.16	0.23	231.00	7.41	1107.05	23.51	31.49	1162.05	193.00	423.65
S2	23.61	0.23	188.00	7.67	1170.51	23.51	41.18	1235.20	183.00	377.00
S3	23.85	0.23	192.00	7.57	906.97	24.63	42.39	973.99	188.00	397.00
Poções reservoir										
October/2016										
S1	25.97	0.47	48.90	7.60	456.00	0.00	20.59	476.59	5.00	318.00
S2	24.8	0.39	54.80	8.39	24.67	8.96	47.24	80.87	38.20	207.00
S3	25.66	0.41	50.50	8.28	50.60	14.10	80.60	145.30	158.00	638.78
February/2017										
S1	29.65	0.10	325.00	8.46	14.10	11.19	16.96	42.25	913.00	883.66
S2	29.75	0.16	394.00	8.70	62.06	320.15	218.01	600.22	633.00	1070.33
S3	27.12	0.16	297.00	8.71	239.74	226.12	186.52	652.38	638.00	870.33
June/2017										
S1	25.51	0.41	12.50	7.43	98.71	53.73	147.76	262.47	133.00	377.00
S2	25.14	0.41	190.00	7.35	45.83	10.07	125.96	193.46	103.00	243.66
S3	24.37	0.41	151.00	7.23	86.02	8.96	140.50	181.86	153.00	443.66

Temp, water temperature; Secchi, water transparency; Turb, turbidity; NH4-N, ammonia; NH2-N, nitrite; NH3-N, nitrate; DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus; TP, total phosphorus; S1, sampling site 1; S2, sampling site 2; S3, sampling site 3.

variables on cyanobacteria species was significant ($F_{Boqueirão}$=2.76, P=0.003; $F_{Camalaú}$= 2.47, P=0.003; $F_{Mucutú}$= 8.48, P=0.005; $F_{Poções}$= 3.46; P=0.007). In Boqueirão Reservoir, the RDA explainability was 59.78%. Water transparency had the most significant positive contribution to axis 1, where it grouped *D. solitarium*, *Eucapis densa*, *Plankthotrix isothrix*, *Pseudanabaena catenata*, *P. galeata*, *Spirulina subsalsa,* and *Synechocystis aquatilis* in October 2016 and February 2017 (Fig. 4A).

In the Camalaú Reservoir, the proportion explained by the RDA was 43.45%, where water volume had a positive relationship with axis 1 and was grouped with *Aphanocaspa annulata*, *Chroococcus dispersus*, *E. densa,* and *Merismopedia minima* in June 2017. On the other hand, water transparency had a negative association with axis 1 and was grouped with *Aphanocaspa delicatissima*, *Chroococcus minutus,* and *Dolichospermim circinale* in October 2016 (Fig. 4B). The second axis of the RDA for Camalaú reservoir grouped *Cuspidothrix tropicalis*, *Cylindrospermopsis raciborskii*, *Plankthotrix agardhii*, *P. galeata,* and *P. isothrix* in February 2017.

The explanability of the RDA for Mucutú Reservoir was 54.79%, where SRP had a positive relationship with *Geitlerinema amphibium* in June 2017 (Fig. 4C). In the Poções Reservoir, the proportion explained by the RDA was 47.74%, and TP was the explanatory variable having a positive contribution to axis 1 and a negative relationship with *A. annulata*, *Aphanocapsa incerta,* and *C. dispersus* in June 2017 (Fig. 4D).

Tab. 2. Species of cyanobacteria identified in the studied reservoirs.

Species	Reservoirs Boqueirão	Camalaú	Mucutú	Poções	Potencial cyanotoxins producers
Chroococcales					
Chroococcus dispersus	x	x		x	-
Chroococcus limneticus	x				-
Chroococcus minutus		x			-
Microcystis aeruginosa		x			Mic
Oscillatoriales					
Geitlerinema amphibium			x		Sax
Planktothrix agardhii	x	x	x		Mic
Planktothrix isotrix	x	x	x		Mic
Spirulina subsalsa	x				-
Nostocales					
Aphanizomenon gracile	x				Cyl/Sax
Cylindrospermopsis raciborskii		x			Cyl/Sax
Cuspidothrix tropicalis		x	x		-
Dolichospermum solitarium	x	x	x		-
D. circinale		x			Mic/Sax
Synechococcales					
Aphanocapsa annulata	x	x		x	-
Aphanocapsa delicatissima		x			-
Aphanocapsa incerta	x	x		x	-
Coelomoron tropicale		x		x	-
Eucapis densa	x	x	x	x	-
Limnococcus limneticus	x			x	-
Merismopedia glauca	x				-
Merismopedia tenuissima				x	Mic
Pseudanabaena catenata	x		x		Mic
Pseudanabaena galeata	x	x	x		Mic
Synechocystis aquatilis	x	x			-
Species richness	15	16	8	7	

Mic, microcystins; Sax, saxitoxins; Cyl, cylindrospermopsin; -, there is no record of toxicity.

Cyanotoxins

Microcystin was detected throughout the investigation in Boqueirão, Camalaú, Mucutú and Poções reservoirs, with the exception of the samples obtained in February 2017 from Camalaú Reservoir (Fig. 5). Saxitoxin was also present in all the reservoirs, but less frequently than microcystin, while cylindrospermopsin was only recorded in Camalaú Reservoir in February 2017. The maximum concentrations recorded were 2.28, 1.98 and 2.89 μg L^{-1} for total microcystins, total saxitoxins and total cylindrospermopsins, respectively.

The PCA for potential toxic cyanobacteria and cyanotoxins of Boqueirão Reservoir indicated that microcystin was positively related to *P. galeata*, *P. agardhii* and *P. isothrix*, while saxitoxin was positively associated with *Synechocystis aquatilis* and *Aphanizomenon gracile*. In the Camalaú Reservoir, microcystin and cylindrospermopsin had a positive relationship with *P. galeata*, *P. agardhii*, *P. isothrix* and *C. raciborskii*, while saxitoxin was positively correlated with *D. circinale*, *Dolichospermum solitarium* and *Microcystis aeruginosa*. In the Mucutú Reservoir, microcystin had a positive relationship with *P. galeata*, *P. agardhii*, and *P. isothrix,* while saxitoxin was related to *G. amphibium*. In Poções Reservoir, the only potential toxin producing species identified was *Merismopedia tenuissima*, which also had a positive relationship with saxitoxin (Fig. 6).

The RDA for the influence of the abiotic variables on the cyanotoxins was significant ($F_{Boqueirão}$= 8.53, P=0.045; $F_{Camalaú}$= 18.15, P=0.004; $F_{Mucutú}$= 5.17, P=0.040; $F_{Poções}$= 5.65, P=0.043). Transparency, TP, SRP and water volume significantly influenced the cyanotoxin concentrations of Boqueirão, Camalaú, Mucutú and Poções, respectively (Fig. 7). However, no clear pattern was found in the distribution of cyanotoxins, since they remained close to the origin of the ordination.

DISCUSSION

In this study, the cyanotoxins, microcystin and saxitoxin were detected in all the investigated reservoirs, while cylinderspermopsin was limited to a single reservoir (Camalaú Reservoir). This is the first record of cylindrospermopsin in a reservoir in the State of Paraíba, Northeast of Brazil. To date, most cylindrospermopsin detections have been in the State of Pernambuco in the northeast of Brazil (Bittencourt-Oliveira *et al*., 2011; Lorenzi *et al*., 2018).

Several of the identified potential toxin producing species had a linear relationship with the presence of specific cyanotoxins and these results corroborate with those of other studies (Costa *et al*., 2006; Yang *et al*., 2006; Naselli□Flores *et al*., 2007; Sinang *et al*., 2015; Lorenzi *et al*., 2015; Lorenzi *et al*., 2018). In contrast to known hypotheses, and field and experimental evidence (Dolman *et al*., 2012; Monchamp *et al*., 2014), saxitoxin was detected in the absence of known potential producers of this bioactive secondary metabolite in Poções Reservoir, which probably indicates the presence of yet to be identified producers of this toxin. These results suggest that using only the identification of potential toxin-producing species as an indicator of the presence of cyanotoxins in monitoring programs for water quality assessment can be problematic and inadequate.

Another possible explanation for the detection of saxitoxins in the absence of potential producers may be that sampling was performed after the breakdown of

Tab. 3. Biovolume of cyanobacteria (x10^2 mm^3 L^{-1}) in Boqueirão, Camalaú, Mucutú, and Poções reservoirs, in October 2016, and February and June 2017.

Months and Sites	Reservoirs			
	Boqueirão	Camalaú	Mucutú	Poções
October/2016				
S1	0.14	1.14	40.15	0.02
S2	9.14	1.33	5.02	0.21
S3	16.57	0.68	1.47	1.79
February/2017				
S1	5.46	18.48	0.69	310.76
S2	1.41	23.77	1.12	127.70
S3	12.49	14.44	6.63	39.03
June/2017				
S1	0.03	0.01	3.27	0.07
S2	2.68	0.14	2.32	0.23
S3	9.50	0.01	9.80	0.07
Total biovolume	6.38±5.88	6.66±9.48	7.83±12.48	53.32±105.37

S1, sampling site 1; S2, sampling site 2; S3, sampling site 3.

blooms of known producers. This is because these compounds tend to remain chemically stable for weeks in the environment at higher concentrations after cell lysis, making it impossible for the detection of potential toxin producing species (Jones and Orr, 1994; Lahti, 1997; Chorus and Bartram, 1999; Funari and Testai, 2008).

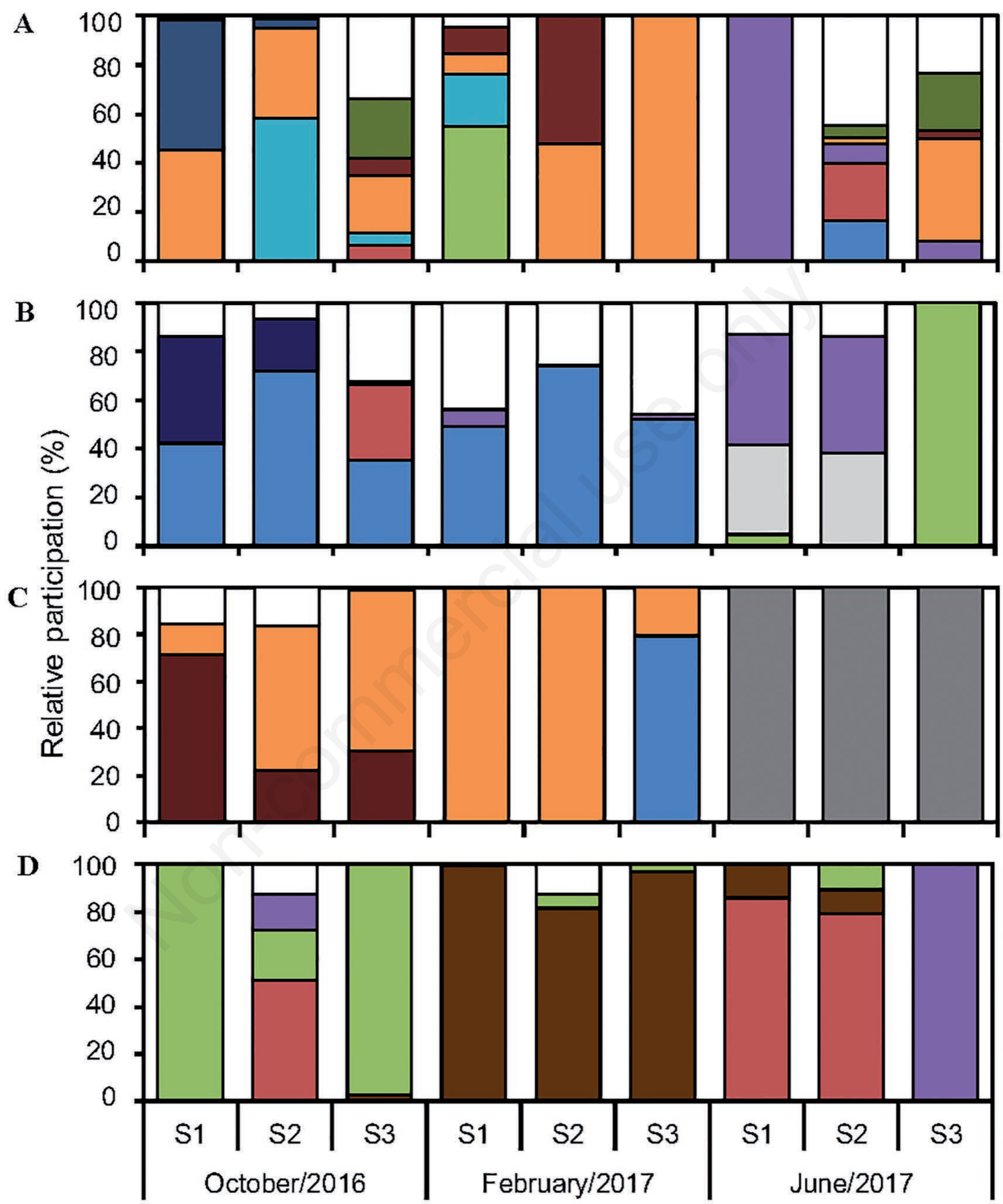

Fig. 3. Relative abundance of Cyanobacteria in the Boqueirão (A), Camalaú (B), Mucutú (C) and Poções (D) reservoirs in October 2016 and February and June 2017. Species with biovolume equal to or less than 1% of the total biovolume of Cyanobacteria were classified in the category "Other Cyanobacteria". ■, *Aphanocapsa annulate*; ■, *A. incerta*; ■, *Chroococcus dispersus*; (■, *Coelomoron tropicale*; ■, *Dolichospermum circinale*; ■, *D. solitatium*; ■, *Eucapsis densa*; ■, *Merismopedia minima*; ■, *Microcystis aeruginosa*; ■, *Planktothrix isothrix*; ■, *P. agardhii*; ■, *Pseudanabaena catenata*; □, Other Cyanobacteria. "Other Cyanobacteria" in the Boqueirão Reservoir were *Chroococcus dispersus*, *Eucapis densa,* and *Spirulina subsalsa*; in the Camalaú Reservoir were *C. dispersus*, *C. minutus*, *Coelomoron tropicalis* and *E. densa*; in the Mucutú Reservoir was *E. densa*; and in the Poções Reservoir were *C. tropicale*, *C. disperses,* and *E. densa*. S1, sampling site 1; S2, sampling site 2; S3, sampling site 3.

Potential microcystin-producing species had the highest relative abundance in the studied reservoirs, which correlated with the frequent detection of the cyanotoxin. This result is consistent with the assertion that this is the most common and frequently detected cyanotoxin in lakes and reservoirs (Mowe *et al*., 2015; Paerl and Otten, 2016). However, as reported by Merel *et al*., (2013), it can not be ruled out that the high frequency of microcystin detection in water bodies published is because they are much more studied and monitored than other cyanotoxins. Therefore, it is important to monitor other cyanotoxins, in addition to microcystins, to comprehensively determine the risk of cyanobacterial metabolites in aquatic ecosystems.

Microcystin production has been frequently associated with the presence of *Microcystis* spp., particularly *Microcystis aeruginosa*, a highly toxic cyanobacterium that commonly forms blooms in lakes and reservoirs throughout the world (Gkelis and Zautsos, 2014; Mowe *et al*., 2015; Hayes and Vanni, 2018). However,

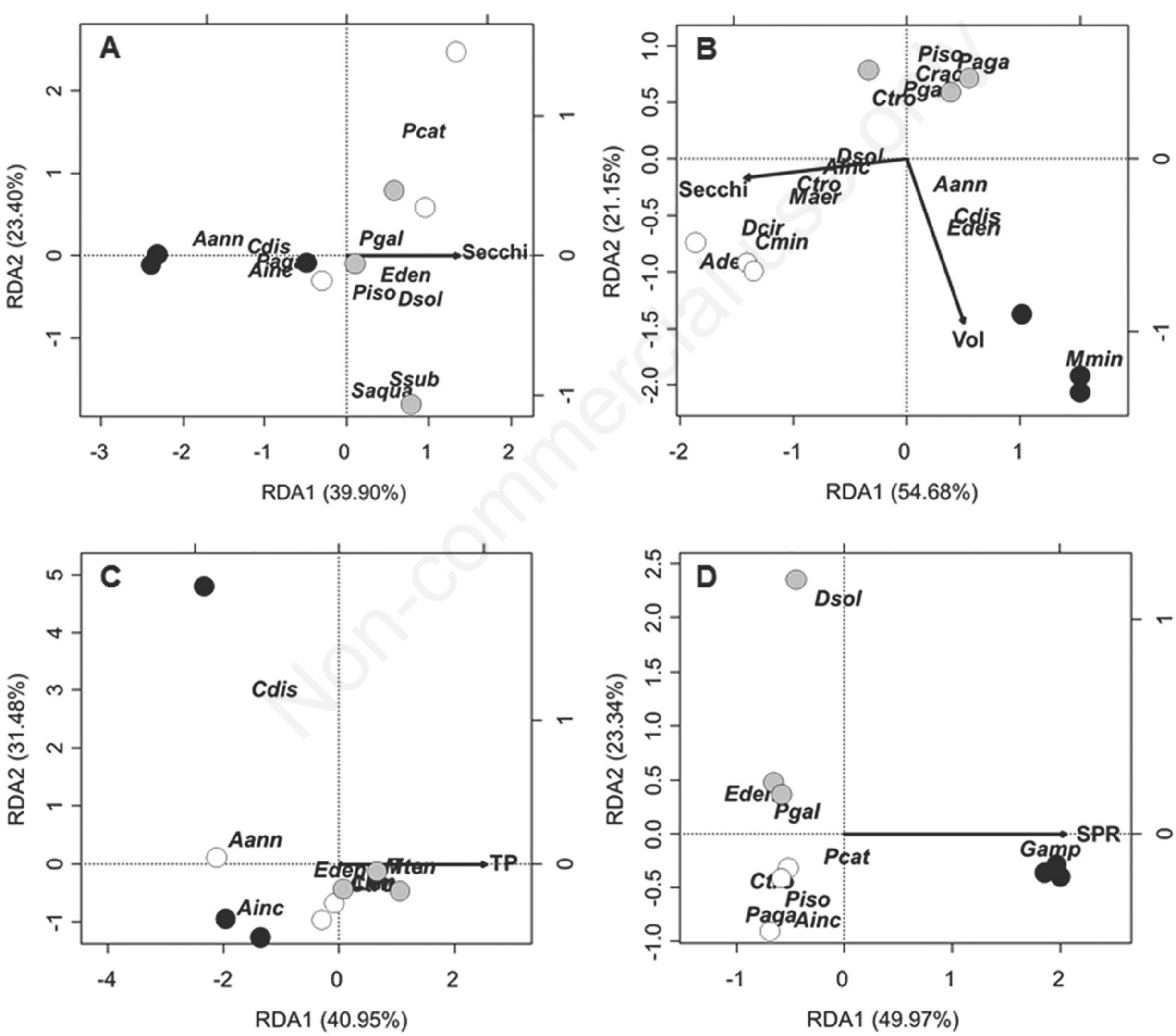

Fig. 4. RDA biplot showing the relationship between abiotic variables and Cyanobacteria in Boqueirão (A), Camalaú (B), Mucutú (C) and Poções (D) reservoirs. ○, October 2016; ●, February 2017; ●, June 2017; Aann, *Aphanocaspa annulata*; Adel, *Aphanocaspa delicatissima*; Ainc, *Aphanocaspa incerta*; Cdis, *Chroococcus dispersus*; Crac, *Cylindrospermopsis raciborskii*; Ctro, *Cuspidothrix tropicalis*; Dcir, *Dolichospermum circinale*; Dsol, *Dolichospermum solitarium*; Eden, *Eucapis densa*; Gamp, *Geitlerinema amphibium*; Llim, *Limnococcus limneticus*; Maer, *Microcystis aeruginosa*; Mmin, *Merismopedia minima*; Mten, *Merismopedia tenuissima*; Paga, *Plankthotrix agardhii*; Pcat, *Pseudonabaena catenata*; Pgal, *Pseudanabaena galeata*; Piso, *Plankthotrix isothrix*; Saqua, *Synechocystis aquatilis*; Ssub, *Spirulina subsalsa*; Secchi, water transparency; Vol, water volume; SRP, soluble reactive phosphorus; TP, total phosphorus.

microcystins are produced by other cyanobacterial species of genera such as *Oscillatoria*, *Aphanizomenon*, *Merismopedia*, *Nostoc*, *Anabaena*, *Phormidium*, *Planktothrix,* and *Anabaenopsis* (Bernard *et al*., 2016).

In this study, a linear correlation between microcystin and potential producing species such as *P. agardhii*, *P. isothrix,* and *P. galeata* was found in Boqueirão, Camalaú and Mucutú reservoirs. *Microcystis aeruginosa* was identified in the Camalaú Reservoir, but had a negative relationship with microcystin content. *Planktothrix* species are known to form intense blooms in semi-arid reservoirs, and the detection of microcystin has been mainly associated with the presence of *P. agardhii* (Bittencourt-Oliveira *et al*., 2014; Fonseca *et al*., 2015; Lorenzi *et al*., 2018).

The positive relationship between *C. raciborskii* and microcystin in the Camalaú reservoir was unexpected, since to date there is no record of strains of this species producing this cyanotoxin. Recently, Panou *et al*. (2018) detected fragments of the microcystin synthetase gene in *C. raciborskii* strains isolated from Greece, suggesting a need for further studies on the metabolic diversity of this cyanobacterium.A survey of the tropical zone of America showed that about 47% of cyanoHABS (cyanobacterial harmful algal blooms) are formed by members of the genus *Cylindrospermopsis*, especially *C. raciborskii* (Mowe *et al*., 2015). Phytogeographic studies have demonstrated the tropical origin of this cyanobacterium and emphasized its expansion in the last decades to temperate regions, as an invasive species that form dense blooms (Antunes *et al*., 2015).

Cylindrospermopsin is a key secondary metabolite produced by *C. raciborskii* (Ohtani *et al*., 1992), which explains the significant positive relationship between them in the Camalaú reservoir. This cyanotoxin is produced by other filamentous cyanobacteria such as *Anabaena bergii*, *Aphanizomenon ovalisporum*, *A. flos-aquae*, *Oscillatoria* sp. PCC6506, *Sphaerospermopsis aphanizomenoides,* and *Umezakia natans* (Armah *et al*., 2013; Rzymski and Poniedziałek, 2014). The low frequency of cylindrospermopsin detection in the reservoirs we investigated agrees with the findings of studies on reservoirs in the semi-arid region of the Northeast of Brazil (Costa *et al*., 2006; Bittencourt-Oliveira *et al*., 2014).

In the present study, saxitoxin was the second most frequent cyanotoxin and the second cyanotoxin with the highest number of potential producers. The presence of the toxin was significantly correlated with the filamentous cyanobacterium *G. amphibium* in Mucutú Reservoir. Saxitoxins are the most potent natural toxins known with a lethal dose 50 (LD_{50}) (*i.e.* the dose that causes the death of 50% exposed individuals) of 10 µg Kg^{-1}. There are 57 identified analogues of saxitoxins (Wiese *et al*., 2010)

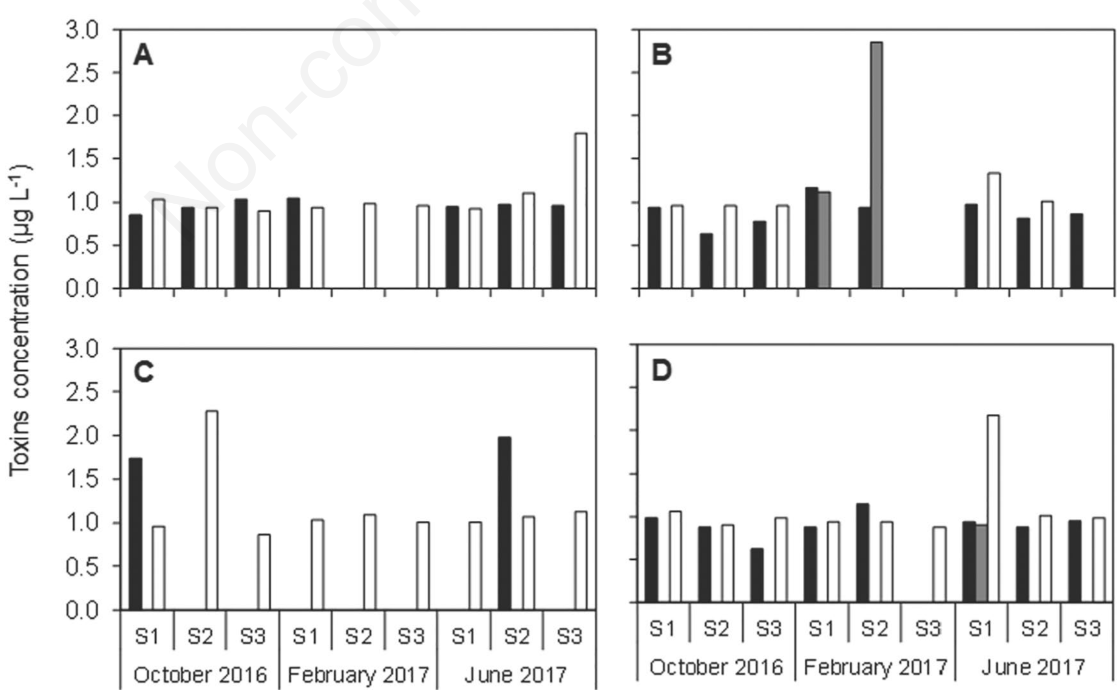

Fig. 5. Cyanotoxin concentrations in Boqueirão (A), Camalaú (B), Mucutú (C) and Poções (D) reservoirs in October 2016 and February and June 2017. □, Microcystin; ■, Saxitoxin; ■, Cylindrospermopsin. S1, sampling site 1; S2, sampling site 2; S3, sampling site 3.

produced by freshwater cyanobacteria (*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Planktothrix*, *Raphidiopsis,* and *Scytonema*) and marine dinoflagellates (Hackett *et al*., 2012; Boopathi and Ki, 2014). Although it does not form blooms, the saxitoxins producer *Geitlerinema* is one of the most commonly occurring genera in semi-arid reservoirs (Aragão-Tavares *et al*., 2013). In Ingazeira, *Geitlerinema amphibium* was found in 100% of the samples obtained from a monthly survey conducted between 2012 and 2013 in Ipojuca and Pedra reservoirs, State of Pernambuco-Brasil (Aragão-Tavares *et al*., 2017).

The abiotic variables that explained the variations in the biomass of cyanobacteria and the concentration of cyanotoxins in our study were nutrients (total and dissolved forms of phosphorus), the water volume of the reservoirs, and the availability of light in the water column (transparency and turbidity). These results corroborate the

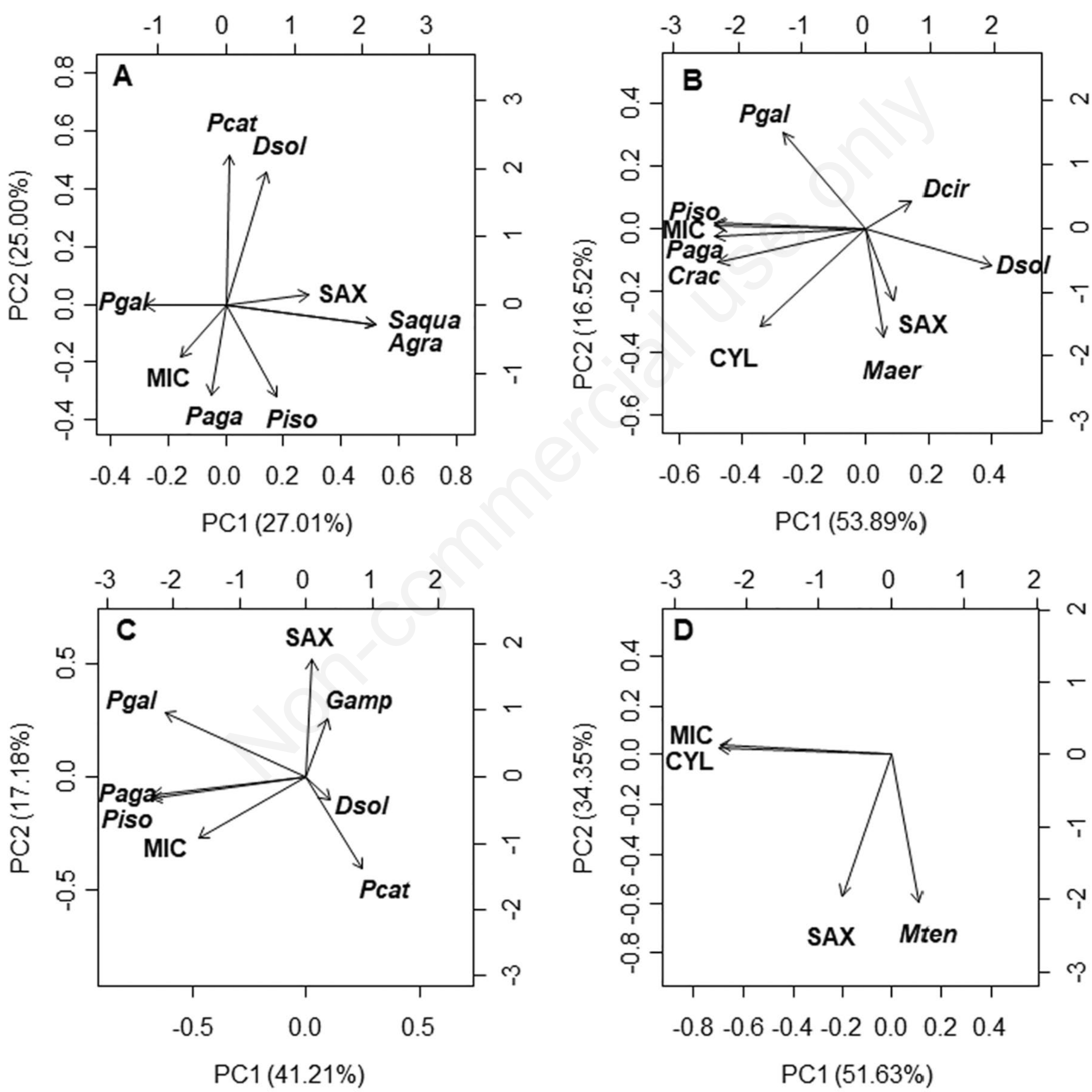

Fig. 6. PCA biplot with the Cyanobacteria species and cyanotoxins of the Boqueirão (A), Camalaú (B), Mucutú (C) and Poções (D) reservoirs. Species with biovolume equal to or less than 1% of the total biovolume of cyanobacteria were not included in the analysis. Aann, *Aphanocaspa annulata*; Ainc, *Aphanocaspa incerta*; Cdis, *Chroococcus dispersus*; Dcir, *Dolichospermum circinale*; Dsol, *Dolichospermum solitarium*; Eden, *Eucapis densa*; Gamp, *Geitlerinema amphibium*; Maer, *Microcystis aeruginosa*; Mmin, *Merismopedia minima*; Paga, *Plankthotrix agardhii*; Piso, *Plankthotrix isothrix*; Pcat, *Pseudonabaena catenata*; Pgal, *Pseudanabaena galeata*; MIC, total microcystins; SAX, total saxitoxins; CYL, total cylindrospermopsins.

hypothesis that similar physicochemical conditions influence the abundance and diversity of cyanobacteria and cyanotoxins. These results are in agreement with those observed in lakes that have a strong relationship between cyanotoxins and cyanobacteria biovolume/biomass (Graham *et al*., 2004; Wang Q. *et al*., 2010).

In this study, the variables described as predictors of cyanobacteria and cyanotoxins have been implicated in facilitating the formation of cyanoHABs in semi-arid reservoirs in Brazil (Dantas *et al*., 2011; Rocha *et al*., 2018). Nutrients are important triggers for the development of cyanobacterial blooms (Merel *et al*., 2013), since excessive nutrient loads cause alterations in the trophic state and, consequently, increase in algal biomass (Cao *et al*., 2016). Phosphorus is a major limiting nutrient of cyanobacteria, particularly the dissolved form (Kotak *et al*., 2000; Brasil *et al*., 2016; Descy *et al*., 2016) that is directly assimilated by primary producers. This explains the significant positive correlation found

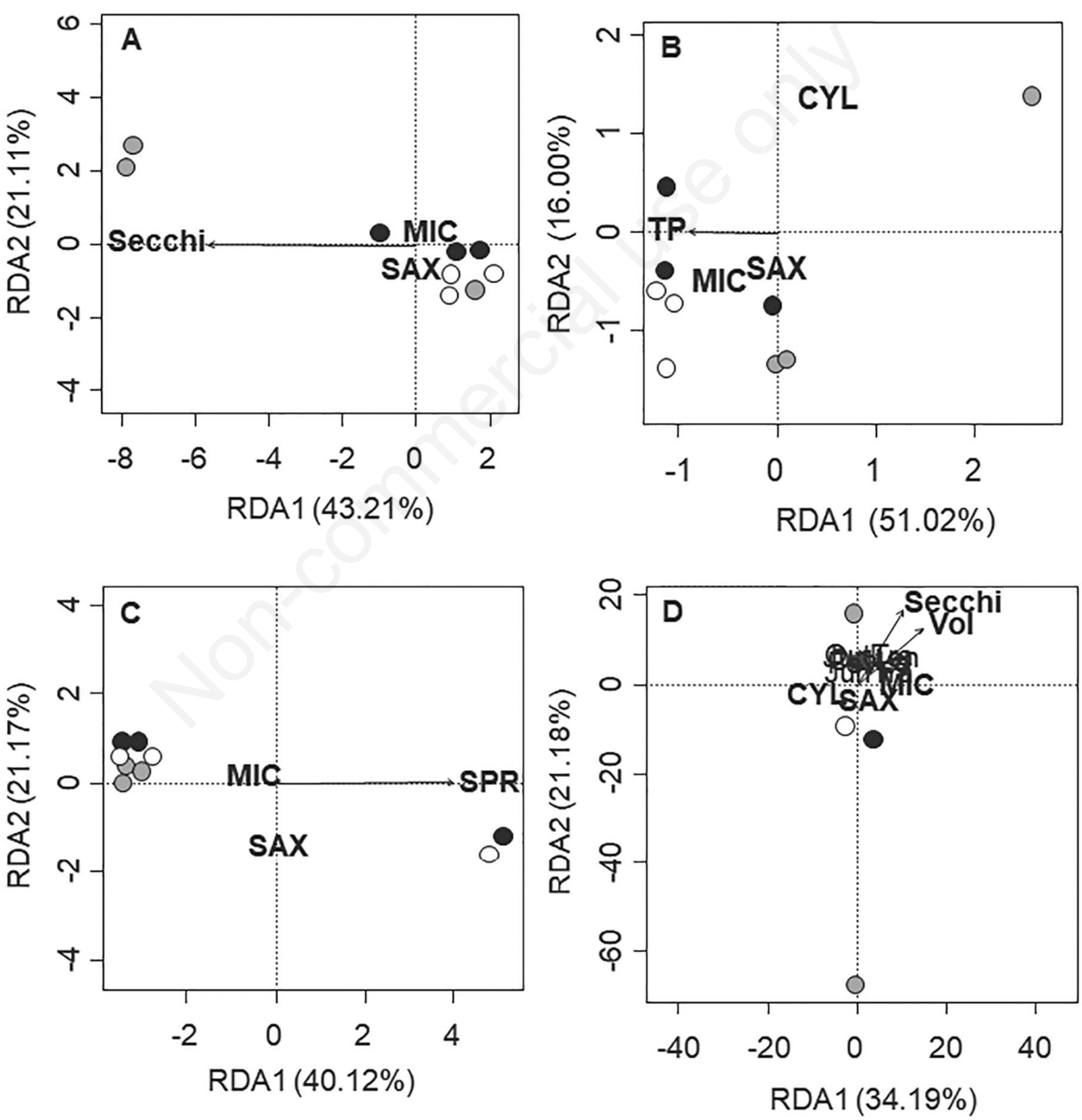

Fig. 7. RDA biplot with abiotic variables and cyanotoxins of the Boqueirão (A), Camalaú (B), Mucutú (C) and Poções (D) reservoirs. ○, October 2016; ●, February 2017; ●, June 2017; Mic, total microcystins; Sax, total saxitoxins; Cyl, total cylinderspermopsins; Secchi, water transparency; Vol, water volume; SRP, soluble reactive phosphorus; TP, total phosphorus.

between this nutrient and several potential toxin producing species of cyanobacteria (*G. amphibium* and *M. tenuissima*) in our study.

In addition to nutrients, studies have also highlighted the importance of temperature as a trigger for increased cyanobacterial abundance and cyanotoxin concentration (Rigosi *et al*., 2015; Cunha *et al*., 2018). In our study, the effect of temperature on cyanobacterial biomass and cyanotoxins content was not significant, and this is due to the fact that the investigated reservoirs have high temperatures (>25°C) throughout the year, a situation that is characteristic of semi-arid aquatic ecosystems. This condition favors the development of cyanobacteria and formation of perennial blooms (Bouvy *et al*., 1999; Moura *et al*., 2011; Moura *et al*., 2018; Portella *et al*., 2018).

The association between cyanobacteria and light availability (water transparency and turbidity) is common in eutrophic reservoirs with high suspended organic matter in the form of cyanobacteria cells (Graham *et al*., 2004; Dalu and Wasserman, 2018). Thus, cyanobacterial blooms cause an increase in the concentration of suspended particles in the water column and, consequently, reduction of transparency, increase in turbidity, and reduction in the quality and quantity of light available for competiting species. While the reduction in light availability is detrimental to the growth of most phytoplankton species, bloom-forming cyanobacteria have ecophysiological strategies that allow them to remain suspended in the region of the water column with the most photon irradiation using gas vesicles to increase their buoyancy, and are well adapted to changing light conditions (Reynolds *et al*., 1987).

The influence of the water volume on the cyanobacteria diversity and abundance observed in our study is in agreement with findings from other semi-arid reservoirs. The reduction of water volume intensifies the formation of cyanoHABs (Brasil *et al*., 2016; Rocha Junior *et al*., 2018), but the effects on cyanotoxins are still poorly understood. Thus, further investigations are needed on the effects of water volume on cyanotoxins production, as semi-arid reservoirs in Brazil are often exposed to prolonged droughts (Rocha Junior *et al*., 2018), which may increase the risks of human exposure to these bioactive metabolites.

CONCLUSIONS

There was a significant correlation between cyanotoxins and the biomass of potential producing species, but there were situations where cyanotoxins were detected without the presence of potential producers. These results demonstrate the need for reassessment of potential toxin producing species of cyanobacteria in semi-arid reservoirs. This may lead to the identification and characterization of novel producers of these bioactive secondary metabolites.

Also, total and dissolved phosphorus, the water volume of the reservoirs, and availability of light (water transparency and turbidity) in the water column significantly influence the community structure and dynamics of cyanobacteria and their toxins in the investigated aquatic ecosystems.

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