

***Icyano*: a cyanobacterial bloom vulnerability index for drinking water treatment plants**

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ABSTRACT

Managing freshwater systems has become a challenge for global water utilities given that cyanobacterial blooms have been increasing in frequency and intensity. Consequently, a water quality index that uses conventional measurements to assess toxic cyanobacterial hazards and guide the selection of proper treatment technologies could benefit water resource managers about water quality parameters routinely analyzed in line with environmental changes. An index model, called *Icyano*, showed that chlorophyll-*a*, cyanobacterial concentration, and total nitrogen were most important for the index. All reservoirs classified as good by *Icyano* used direct filtration water treatment technology. Many of the medium *Icyano*-classified reservoirs used a pre-treatment unit followed by a direct filtration unit. Two reservoirs that were classified as bad or very bad have been utilizing pre-treatment + direct filtration or a complete cycle technology, respectively. As the *Icyano* index increases, water treatment plants should switch from direct filtration to using a pre-treatment to improve finished water quality. Findings from this project suggest that the direct filtration technology initially used in water treatment plants is not capable of meeting the current water quality guidelines in reservoirs that contain adverse water quality conditions, mostly related to an increase in toxic cyanobacterial blooms. As such, based on our findings, we recommend prioritizing financial resources towards pre-treatment technology or changes to more advanced technologies when *Icyano* index values increase.

Key words | harmful algae, water quality, water quality tool, water treatment plants, water treatment technologies

HIGHLIGHTS

- A new index for assessing cyanobacterial hazards (*Icyano*) in drinking water reservoirs was developed.
- Chlorophyll, total nitrogen, and cyanobacterial concentration were the three most important parameters to the *Icyano* index.
- A high N:P may best explain the dominance of cyanobacteria in the reservoirs.
- As *Icyano* increases, water treatment plants should switch to more complex treatments.

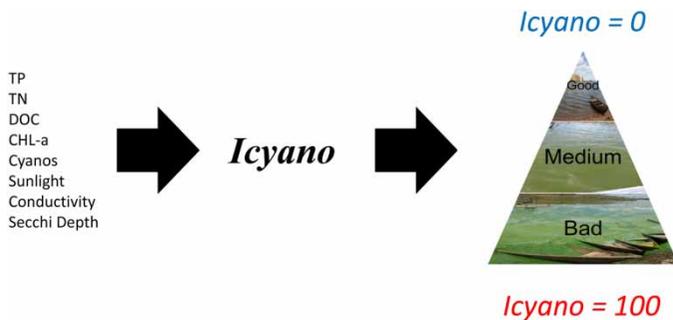
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GRAPHICAL ABSTRACT



INTRODUCTION

Anthropogenic eutrophication and changing climate, such as increasing drought or flooding, have increased toxic cyanobacterial bloom (cyanoHABs) frequency and intensity worldwide (Paerl & Paul 2012; Bakker & Hilt 2016; Zhao *et al.* 2019). The increase in cyanoHAB events has greatly affected surface drinking water supplies in many parts of the world, including northeastern Brazil (CAGECE 2019), and led to difficulties in managing water resources. Certain species of cyanobacteria may produce a wide variety of toxic secondary metabolites (e.g. microcystins, saxitoxins, nodularins, cylindrospermopsins) (Carmichael 1994; Mohamed *et al.* 2006; Van Apeldoorn *et al.* 2007), which are linked to a variety of deleterious effects on the liver, kidney, heart, gonads, and nervous system of both fish and mammals (Zhao *et al.* 2019). Furthermore, cyanobacteria may produce taste and odor compounds, such as 2-methylisoborneol (MIB) and geosmin, which are non-toxic but make the water unpalatable to consumers and give the perception of overall poor water quality (Parinet *et al.* 2010; Pestana *et al.* 2014; Edwards *et al.* 2017).

According to the United Nations (2011), drylands (otherwise known as arid and semi-arid regions) occupy about 41.3% of the world's land surface and are populated by approximately two billion people. Moreover, the majority of people who lack access to clean drinking water reside in semi-arid regions from sub-Saharan Africa, Asia, and Latin America (Walter *et al.* 2018; WHO 2019). The semi-arid region classification is mainly based on rainfall irregularity, in which annual rainfall ranges from 300 to 500 mm. Ceará State, in northeastern Brazil, is

characterized by an annual rainfall average below 600 mm (FUNCEME 2017), the majority of which is distributed between February and May. However, precipitation is often irregular, leading to prolonged droughts and frequently resulting in the drying-out of waterbodies (Walter *et al.* 2018). Additionally, this region has a precipitation/evapotranspiration ratio between 0.2 and 0.5 (FUNCEME 2017), and high temperatures (23–27 °C) and ambient sunlight (2,800 hr·year⁻¹). Due to its equatorial climate, harmful algal blooms are more likely to occur in freshwater reservoirs from semi-arid regions in Brazil. CyanoHABs are favored by surface water accumulation in shallow reservoirs with high hydraulic retention time, high average temperature, high solar radiation, and overall intensive anthropogenic activities that lead to elevated nutrient inputs. Furthermore, prolonged thermal stratification conditions that coincide with a rainy season that promotes excessive enrichment of nutrients have also been reported as a main cause of CyanoHABs in northeastern Brazil (Dantas *et al.* 2011; Barros *et al.* 2019; Zhang *et al.* 2020). There is a serious threat to potable drinking water supplies in semi-arid regions, like Ceará State, that exhibit elevated temperatures, high sunlight, and persistent drought conditions due to a changing climate (Marengo 2009) that are linked to current and forecasted cyanobacterial bloom events (Brasil *et al.* 2016; Moura *et al.* 2017).

Reports of cyanobacterial intoxication (Falconer 1993; Carmichael *et al.* 2001; Backer *et al.* 2010) have led to several policy decisions focused on protecting freshwater systems worldwide, especially those related to drinking water

supplies (Leigh *et al.* 2010). However, toxic cyanobacteria in water are the result of associations among anthropogenic nutrient pollution and other factors, such as geographical features and disturbances stemming from climate change (Barros *et al.* 2019; Fadel *et al.* 2019; Mukundan *et al.* 2020). Assessing water quality parameters individually can be a complicated practice involving numerous chemical, physical, and biological measurements that are often interacting and/or unexplored (UNEP 2007; Bharti & Katyaj 2011; Srebotnjak *et al.* 2011; Srivastava & Kumar 2013; Garcia *et al.* 2018), especially considering the relatively ease to access large environmental datasets. As an alternative, water quality indices that utilize an algorithm to synthesize different types of data into a single number are becoming more common and useful for classifying water quality trends over time and in different geographic areas (Poonam *et al.* 2013).

Early water quality indices, such as that proposed by Horton (1965), have been refined to improve their accuracy and modified for targeted locations (Ewaid & Abed 2017), especially in areas that are subject to deterioration due to natural or anthropogenic impacts (Poonam *et al.* 2013). In general, these approaches consist of summarizing a set of interconnected parameters by a single numerical value situated on a fixed scale that allows users to quickly evaluate and rank water quality for efficient management decisions. Due to its reductionist character, in which several quality items are converted into a score or single evaluation, indices are controversial, since they can may overlook important conditions that occur in a waterbody (Nasiri *et al.* 2007). Despite its intrinsic limitations, the synthesizing capacity provided by an index, is of great importance to the public and managers who need simple tools for making important management decisions and to prioritize financial resources (Srebotnjak *et al.* 2011; Srivastava & Kumar 2013; Garcia *et al.* 2018). However, limited published studies on indices that evaluate water quality regarding the potential hazards of cyanobacteria and their metabolites exist. This knowledge gap makes decision-making in the water quality industry more challenging, especially when it comes to selecting the proper technology for water treatment.

Conventional water treatment, composed of chemical coagulation/flocculation, sedimentation, and granular media filtration processes, is the most used technology for

municipal water treatment. However, some developing countries, including Brazil, regularly utilize direct and double filtration due to its low cost of construction and operation. Direct filtration is composed of chemical coagulation followed by a single pass through a granular media filter while in double filtration water goes through two granular media filters after chemical coagulation. Direct filtration has been demonstrated to be able to meet drinking water guidelines for source waters with low cyanobacterial concentrations, low turbidity, and apparent color. However, when water quality decreases, one should consider using double filtration or, in the worst-case scenario, conventional treatment. The challenge for water companies is to identify what technology to adopt given their current water quality parameters while considering water quality guidelines and economic aspects. Over the last 40 years in semi-arid region of Brazil, current water treatment plant (WTP) technologies have become outdated, and it has often been necessary to add a pre-treatment step to manage the increase in cyanobacteria and their associated toxins (CAGECE 2019; COGERH 2019). Institutional managers must rely on technical and synthesized data to support the allocation of funds and resources regarding the status and management of water quality (Srebotnjak *et al.* 2011). Therefore, the goals of this work were to develop a new water quality index, called *Icyano*, for assessing cyanobacterial and their associated toxins in artificial reservoirs used for drinking water production as well as to identify which water treatment plant processes are most vulnerable to cyanobacterial hazards to inform water treatment companies where to prioritize resources.

MATERIALS AND METHODS

Study region

This study used data collected from 2013 to 2017 in 20 drinking water reservoirs within the Ceará State, northeastern Brazil (2° 30' 00" - 8° 52' 00" South and 37° 14' 00" - 41° 30' 00" West; Figure 1 and Table 1). The climate of this area is considered tropical hot and semi-arid (type 'BSh', Köppen climate classification; NETO *et al.* 2014; IPECE 2017). The water reservoirs studied in the Ceará State are mainly used

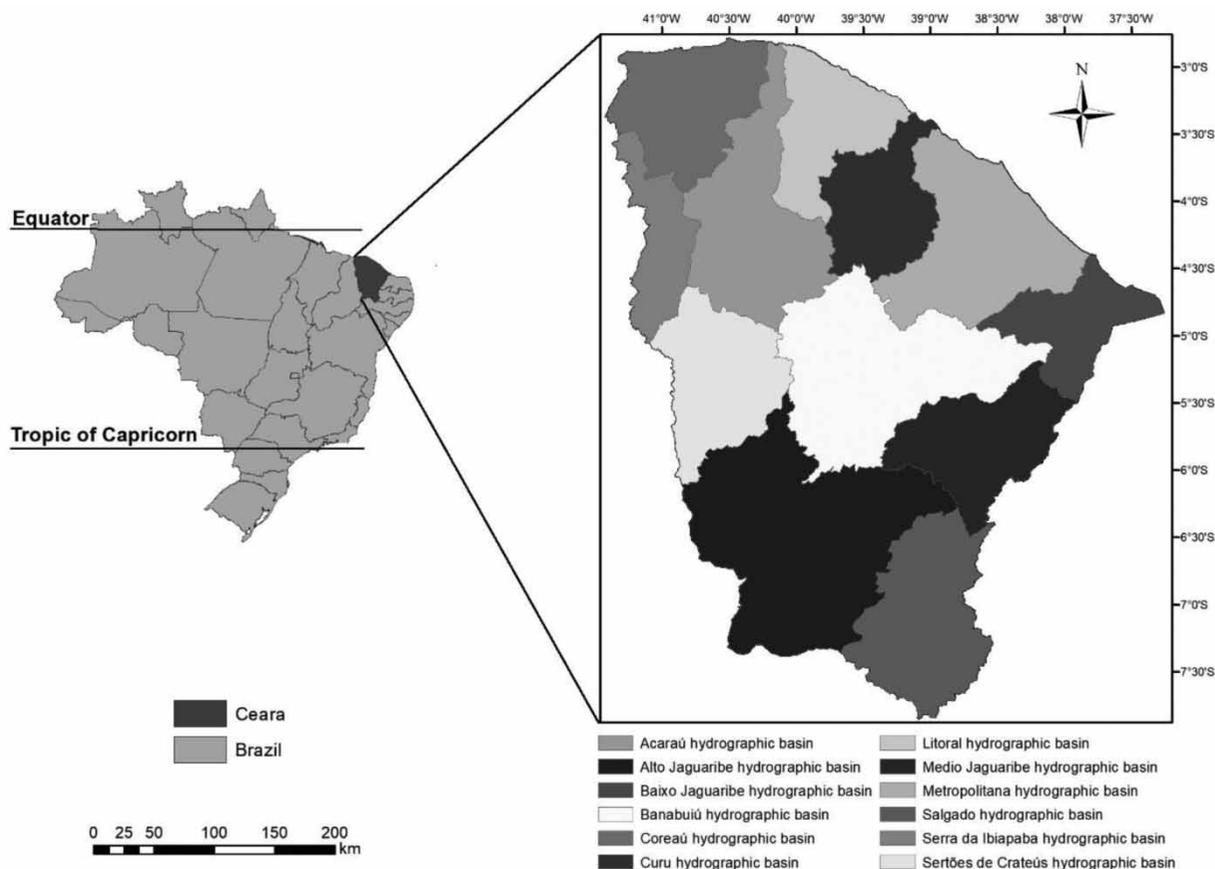


Figure 1 | Map of Ceará State, northeastern Brazil.

for human and animal consumption, but also fish farming, agriculture, industrial demands, and flow regulation (COGERH 2019).

Field sampling and analytical methods

From 2013 to 2017, samples were collected in duplicate (monthly or weekly) at water intakes 30 cm below the surface at water treatment plants (WTP) used by the Water and Wastewater Company of Ceará (CAGECE). Sampling frequency was based on the prevalence of cyanobacteria, as Brazilian legislation calls for standard monthly sampling increased to weekly sampling when cyanobacterial concentrations in reservoirs exceed 20,000 cells mL⁻¹. Although many water quality parameters have been used in the water industry, temperature, evaporation, sunlight, electrical conductivity, chlorophyll, total nitrogen, total phosphorus, Secchi depth, and concentration of cyanobacteria were

adopted for the proposed model since they have been commonly monitored by the local water companies and are readily available. As a criterion for sample selection in this model, we included samples that contained concentrations of microcystin and saxitoxin >1 µg L⁻¹, which resulted in 2,489 samples used in the model (total samples analyzed 24,169).

For phytoplankton analyses, samples were preserved with 1% Lugol's solution, while biological material used *in vivo* was kept refrigerated to decrease organism metabolism and oxygen consumption in the absence of light. Phytoplankton cell counts were carried out with a Sedgewick-Rafter counting chamber. On average, fields were counted at multiple magnifications (200x and 400x, depending on the size of the phytoplankton taxon) until at least 100 individuals of the most frequent species or 400 individuals in total were counted (Lawton 1999; APHA 2012; Barros et al. 2019).

Table 1 | Characteristics of the studied reservoirs including trophic classification according to COGERH (2019)

Code	Reservoir	Trophic classification ^a	Capacity (m ³)	Hydraulic basin (ha)
AM	Acarape do Meio	Mesotrophic	31,500,000	220
AMR	Amanari	Eutrophic	11,010,000	271
AR	Aracoiaba	Eutrophic	170,700,000	1,506
AT	Trussu	Eutrophic	301,000,000	5,509
BO	Boqueirão	Hypereutrophic	28,110,000	512
CN	Canafístula	Eutrophic	13,110,000	315
E	Ema	Hypereutrophic	10,390,000	284
EQ	Edson Queiroz	Hypereutrophic	254,000,000	2,660
FQ	Forquilha	Hypereutrophic	50,132,000	923
G	Gavião	Eutrophic	32,900,000	618
MRT	Martinopole	Hypereutrophic	23,200,000	647
MT	Monsenhor Tabosa	Hypereutrophic	12,100,000	185
MU	Mundaú	Eutrophic	21,300,000	123
PM	Pereira de Miranda	Hypereutrophic	395,638,000	5,700
PS	Paulo Sarasate	Hypereutrophic	891,000,000	9,600
PT	Patú	Hypereutrophic	71,829,000	856
PU	Puiu (Parambú)	Eutrophic	8,530,000	159
SD	Serafim Dias	Hypereutrophic	43,000,000	688
SN	Sítios Novos	Hypereutrophic	126,000,000	2,010
T	Trapia III	Eutrophic	5,510,000	130

^aTrophic classification based on Carlson (1977) adapted for tropical reservoirs by Toledo *et al.* (1983).

Although testing for cylindrospermopsin is only recommended by Brazilian guidelines, the lakes monitored were also tested for this toxin and no significant concentrations were found, as reported by Barros *et al.* (2019). Therefore, cylindrospermopsin was not considered a factor of concern in our region. Two cyanobacterial toxins, microcystin and saxitoxin, were measured using enzyme-linked immunosorbent assays (ELISA) according to the manufacturer directions (Abraxis LLC 2007; Abraxis LLC 2009). These ELISA kits recognize saxitoxin or a specific antibody moiety (-ADDA) found in many microcystin variants. The minimum reporting level (MRL) for the assays were 0.02 and 0.15 µg L⁻¹ for saxitoxin and microcystin-LR equivalents, respectively (Abraxis, LLC, Warminster, Pennsylvania). Nutrient concentrations were analyzed following the persulfate method for the simultaneous determination of total nitrogen and total phosphorus (APHA 2012). 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).

Modeling the cyanobacterial index (*Icyano*)

The cyanobacterial index, *Icyano*, was calculated for each reservoir using a three-pronged approach. First, Pearson's correlation coefficients were used as an estimate of the strength and direction of the relationship between each environmental parameter. Secondly, the Kaiser-Meyer-Olkin (KMO) adequacy test was performed to check whether the correlation structure of the dataset was suitable for factorial analysis (third and final approach) and index development (i.e. KMO >0.6; Hair *et al.* 1987; Parinet *et al.* 2010; Te & Gin 2011). The KMO value for this study was 0.62, so a factorial analysis was used to identify important latent variables (i.e. common factors loads).

A factorial analysis is a widely used technique for the construction of indexes (Meireles *et al.* 2010). This multivariate statistical technique aims to summarize and reduce data dimensions while analyzing the relationships between variables and defining a set of common latent variables. Through this technique, it is also possible to calculate the weight represented by each variable for the index composition (W_i) (Hair *et al.* 1998; Johnson & Wichern 2007). The cyanobacterial index (*Icyano*) was calculated according to the following equation:

$$Icyano = \sum_{i=1}^n Q_i W_i$$

where, *Icyano* is a dimensionless parameter ranging from 0 to 100; Q_i represents a standardized variable (0–100), it is the quality of the i_{th} parameter and a function of its concentration or measurement; W_i is the normalized weight of the i_{th} parameter and a function of its importance in explaining the global variability in water quality. W_i is described according to the following equation:

$$W_i = \frac{\sum_{j=1}^k F_j A_{ij}}{\sum_{j=1}^k \sum_{i=1}^n F_j A_{ij}}$$

where, F_j is component 1 eigenvalue; A_{ij} is the explanatory power of parameter i by factor j ; i is the number of parameters selected by the model, ranging from 1 to n ; j is the number of factors selected in the model, varying from 1 to K .

Statistical tests and modeling were performed using the software *R* with the *corrplot* and *psych* packages. An analysis of variance (ANOVA) followed by a Tukey test were also used to further test relationships observed with the *Icyano* index. Based on the calculated *Icyano* index score, each reservoir was then classified into one of four water quality classification categories according to Shweta *et al.* (2013) and Ewaid & Abed (2017). The classification categories were *Icyano* range from 0 to 25: Good (G); 26–50: Medium (M); 51–75: Bad (B); 76–100: Very Bad (VB). Each reservoir *Icyano* index was then compared with the technology used at the WTP adjacent to that reservoir.

RESULTS

Environmental variables

Relatively little variability (i.e. $\mu > \sigma$) was observed in the climatological variables across the 20 study reservoirs (Appendix A in the supplementary material provides the descriptive statistics of the analyzed parameters). Such findings are not surprising considering the inland area of northeastern Brazil consistently exhibits high temperatures and low thermal amplitudes (INMET 2016; FUNCEME 2017; IPECE 2017). In contrast, water quality varied widely across the 20 reservoirs, including in cyanobacterial diversity (Figure 2). Additionally, two analyzed cyanobacterial toxins, microcystins (MC) and saxitoxins (STX), differed greatly across the reservoirs (Figures 3 and 4). Despite 13 reservoirs having detectable microcystins $>0.15 \mu\text{g L}^{-1}$, seven (AMR, E, FQ, MRT, PU, SD and SN reservoirs) had MC concentrations $>1 \mu\text{g L}^{-1}$ ($p > 0.05$; Figure 3(a)), a limit that exceeds World Health Organization guidelines for microcystin-LR (WHO 2017). The colony-forming order Chroococcales dominated in the MRT reservoir (Turkey's $P < 0.05$; Figure 2), while FQ and PU reservoirs contained high abundances of *Raphidiopsis raciborskii*. Interestingly, this cyanobacteria is not known to produce microcystins. Furthermore, these two reservoirs also contained detectable concentrations of saxitoxin. There seems to have been a competition or succession in the cyanobacterial community, and it may have influenced toxin production favoring microcystins. Although nine reservoirs had detectable saxitoxins greater than $0.11 \mu\text{g L}^{-1}$ (AM, BO, CN, EQ, FQ, MT, PS, PU and T; $p > 0.05$; Figure 3(b)), only one reservoir (BO) surpassed WHO guidelines for this toxin ($>3 \mu\text{g L}^{-1}$; WHO, 2019; $P > 0.05$; Figure 3(b)). *R. raciborskii* represented approximately 80% of cyanobacteria in that reservoir and was the dominant species in seven additional reservoirs (AM, CN, EQ, FQ, MT, PU and T; Turkey's $P < 0.05$). *Planktothrix agardhii* and *R. raciborskii* dominated in the PS reservoir; this time, saxitoxin production was clearly prevalent (Figure 3(b)).

As expected, chlorophyll concentration showed a moderate inverse correlation with Secchi depth ($r = -0.574$; $p = 0.008$) and was positively correlated with cyanobacterial

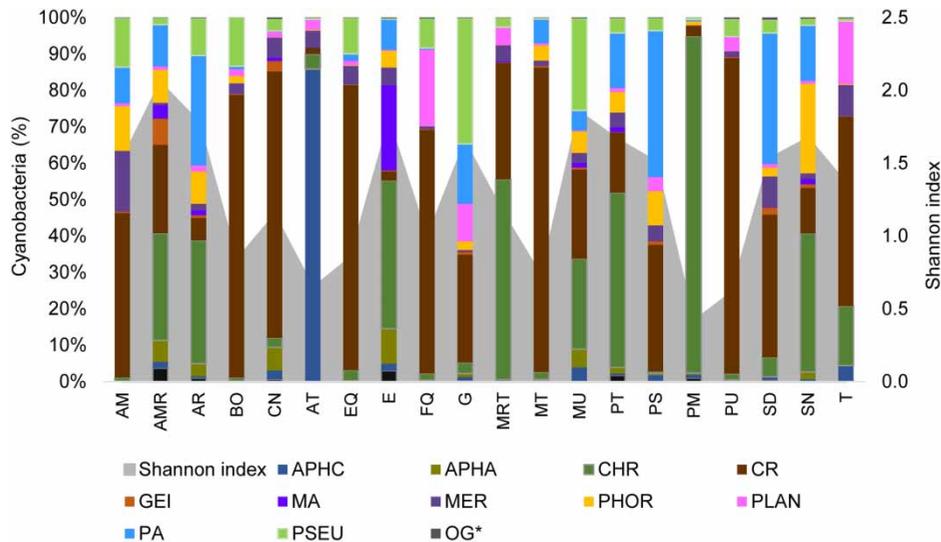


Figure 2 | Relative abundances of 12 phytoplankton taxa or groups (APHA = *Aphanizomenon* sp.; APHC = *Aphanocapsa* sp.; CHR = Chroococcales; CR = *Cylindrospermopsis raciborskii*; GEI = *Geitlerinema* sp.; MA = *Microcystis aeruginosa*; MER = *Merismopedia* sp.; PA = *Planktothrix agardhii*; PHOR = Phormidiaceae family; PSEU = *Pseudanabaena* sp.; PLAN = *Planktolynghya* sp.; OG* = other phytoplankton groups), and Shannon index values observed in 20 Ceará reservoirs.

concentration ($r = 0.588$; $p = 0.006$). A positive correlation was observed between total nitrogen (TN) and chlorophyll-*a* (Chl) ($r = 0.811$; $p = 0.000014$) as well as cyanobacterial density (Cyano) ($r = 0.549$; $p = 0.012$), but was inversely correlated with Secchi depth ($r = -0.586$; $p = 0.006$). Lastly, a strong positive correlation was observed between temperature (temp) and evaporation (Evap) ($r = 0.753$; $p = 0.00013$). The complete Pearson correlation matrix is provided in Table 2. A Bartlett's test ($p = 2.2 \times 10^{-16}$) suggested that variances differed among the reservoirs studied (see factorial results in Table 3).

Icyano classifications

Reservoirs AM, AT, G, and MU were classified as good (G), while FQ and MT were classified as very bad (VB) by *Icyano* (Table 4). The calculated *Icyano* index generally followed the trophic classification assigned to the reservoir with a few exceptions; three reservoirs given a eutrophic classification were deemed 'good' by *Icyano*. On the other hand, water treatment technology robustness (direct filtration < double filtration < conventional treatment) did not follow the worsening of water quality, apparently because the company responsible for the treatment does not have a proper procedure to guide the selection of the necessary treatment technology during the WTP design and construction phases.

This means that although some facilities were capable of using higher-quality treatment methods, they chose not to due to a lack of awareness of apparent water quality. For example, as *Icyano* increases (i.e. water quality deteriorates), the treatment technology involved did not change from a simple treatment technology (direct filtration) to a more robust one (conventional treatment) to meet drinking water guidelines (Table 4), as would be expected.

Although the reservoirs classified as good were dominated by cyanobacteria, reservoirs AM, G, and MU had higher cyanobacterial diversities (Figure 2). *Aphanocapsa* sp. represented more than 85% of the cyanobacterial composition of reservoir AT (Figure 2). Reservoirs classified as bad (BO, MRT, PS) or very bad (FQ and MT) showed low cyanobacterial biodiversity (Figure 2). *Raphidiopsis raciborskii* was the predominant species in FQ and MT reservoirs ($P < 0.05$) composing 67 and 84% of the phytoplankton cell counts, respectively. FQ and MT reservoirs also presented high MC concentrations, exceeding the Brazilian guideline ($1 \mu\text{g L}^{-1}$). BO and PS reservoirs exceeded the concentrations determined for STX ($3 \mu\text{g L}^{-1}$). Most reservoirs were classified as M (medium), from which only the reservoirs PT, EQ, and AR were within the guideline tolerance for both MC and STX (Figure 3). Among MC detected reservoirs, reservoir E and MRT have been using direct filtration, and AMR and SN reservoirs have already changed the

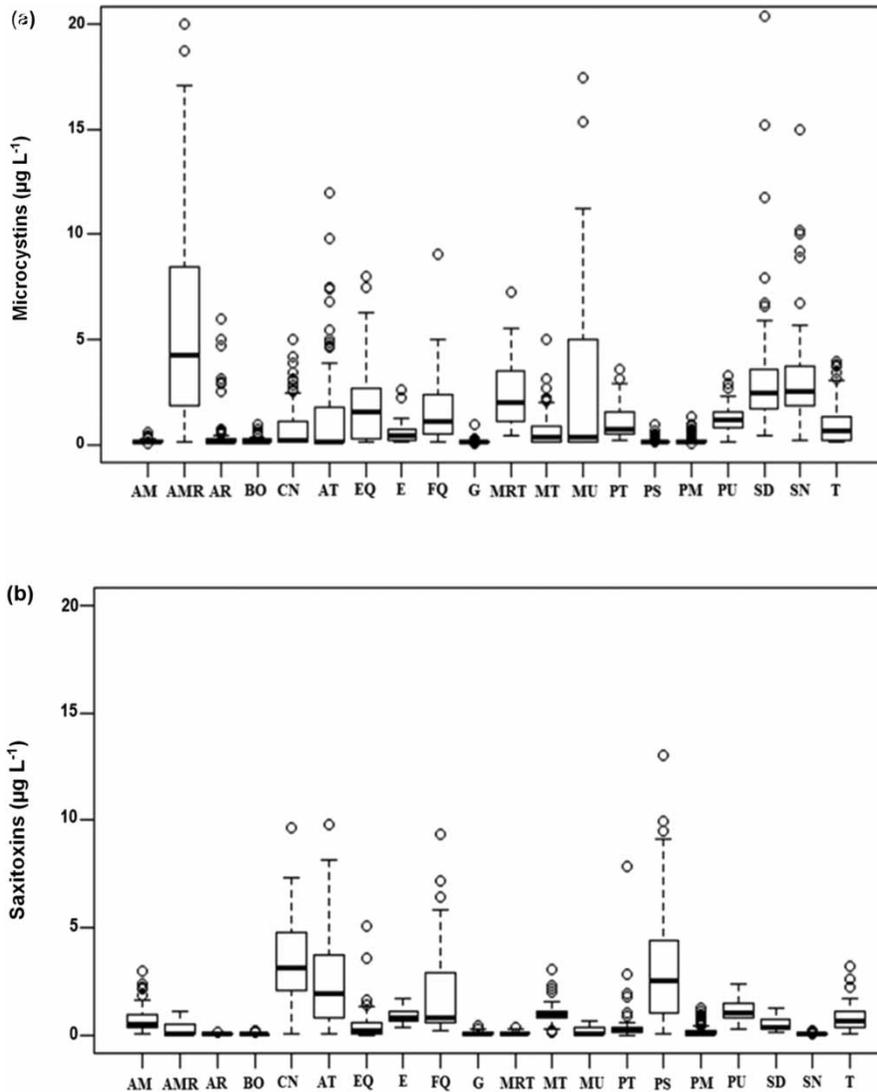


Figure 3 | Boxplots (median; 25 and 75% quartiles) of ELISA cyanotoxins concentration analyzed in the 20 reservoirs studied. (a) Microcystin concentration (detection limit method = $0.15 \mu\text{g L}^{-1}$). (b) Saxitoxin concentration (detection limit method = $0.11 \mu\text{g L}^{-1}$).

technology initially designed (direct filtration) for double filtration. The PU, FQ and SD reservoirs, being newer WTPs, have already been designed with greater robustness with more treatment steps (conventional treatment).

In this study, we found that the best model to predict dissolved microcystin concentration incorporated chlorophyll-*a*, TP, TN, DOC, and the interaction between temperature and chlorophyll-*a* among others. These findings reinforce the model developed by Walls *et al.* (2018) on overall water quality; however, we were able to increase the power of our index with the inclusion of variables, like

evaporation, sunlight, conductivity, Secchi depth, and cyanobacterial concentration. Additionally, we were able to rank the strength of the relationships between the observed variables from strongest to weakest using Pearson's correlation coefficients: TN & Chl ($r=0.811$), Temp & Evap ($r=0.753$), Chl & Cyano ($r=0.588$), TN & Secchi ($r=-0.586$), Chl & Secchi ($r=-0.574$), TP & CE ($r=-0.526$), and TP & Sunlight ($r=-0.526$) (Table 2). Furthermore, it should be noted that total nitrogen, chlorophyll-*a*, and cyanobacterial concentration accounted for 60% of the model composition.

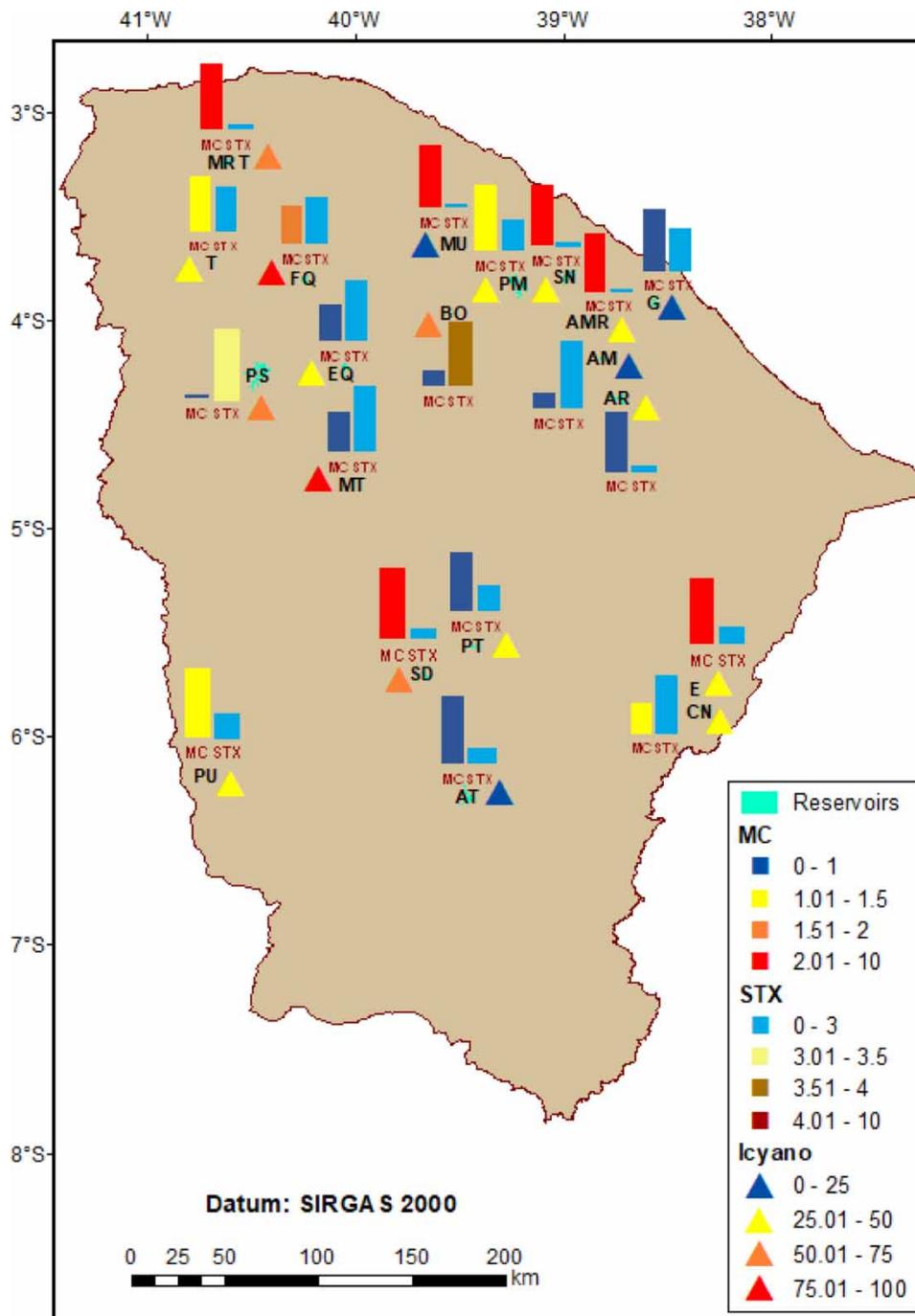


Figure 4 | Location of each reservoir studied, classification of the mean concentration of microcystin (MC), saxitoxin (STX), and *Icyano* classification. *Results of cyanotoxins are provided in $\mu\text{g L}^{-1}$.

DISCUSSION

According to Huang *et al.* (2016), global warming trends are particularly intensified in semi-arid regions, with droughts

becoming longer and more severe with higher evaporation and lower precipitation. The most noticeable consequences of high temperatures and the other climatic forecasts in reservoirs, like those studied in the Ceará region, is the

Table 2 | Pearson correlation matrix for the variables studied for *Icyano*

	Temp	Evap	Sunlight	TN	TP	Chl	CE	Sec	Cyano
Temp	1								
Evap	0.753	1							
Sunlight	0.158 ^a	0.362 ^a	1						
TN	0.169 ^a	0.369 ^a	-0.115 ^a	1					
TP	-0.317 ^a	-0.158 ^a	-0.526	0.359 ^a	1				
Chl	0.248 ^a	0.426 ^a	-0.113 ^a	0.811	0.428	1			
CE	-0.109 ^a	0.251 ^a	0.036 ^a	0.444	0.526	0.391 ^a	1		
Sec	-0.313 ^a	-0.220 ^a	0.299 ^a	-0.586	-0.399 ^a	-0.574	-0.299 ^a	1	
Cyano	0.456 ^a	0.334 ^a	0.063 ^a	0.549	-0.163 ^a	0.588	-0.022 ^a	-0.394 ^a	1

Temp = temperature; Evap = evaporation; Sunlight = irradiation; TN = total nitrogen; TP = total phosphorus; Chl = chlorophyll; CE = electrical conductivity; Sec = Secchi depth, and Cyano = Cyanobacterial concentration. Units for each measurement are provided in Table 3.

^aVariables are independent ($\alpha > 0.05$).

Table 3 | Matrix for factorial loads estimating commonality and the weight of each variable (W_i)

Variables	Factor 1	Factor 2	Factor 3	W_i
Temp (°C)	0.289	0.755	-0.106	0.073
Evap (mm)	0.243	0.936	0.243	0.061
Sunlight (h)	-0.128	0.488	-0.263	0.032
TN (mg L ⁻¹)	0.759	^a	0.392	0.191
TP (mg L ⁻¹)	0.166	-0.418	0.793	0.042
Chl (mg L ⁻¹)	0.800	0.133	0.439	0.202
CE (µS cm ⁻¹)	0.155	^a	0.655	0.039
Sec (m)	-0.577	^a	-0.341	0.145
Cyano (cell mL ⁻¹)	0.851	0.209	-0.277	0.214
Loadings for each factor	2.483	1.934	1.738	
Variance (%)	0.276	0.215	0.193	
Accumulated variance (%)	0.276	0.491	0.684	

Temp = temperature; Evap = evaporation; Sunlight = irradiation; TN = total nitrogen; TP = total phosphorus; Chl = chlorophyll; CE = electrical conductivity; Sec = Secchi depth and Cyano = Cyanobacterial concentration.

^aInsignificant values.

significant decrease in water availability and quality. Studies conducted by The Inter-American Institute for Cooperation on Agriculture in 2002 affirmed that evaporation in semi-arid environments may reach 1,000 mm yr⁻¹ in coastal regions and more than 3,000 mm yr⁻¹ in inland areas. Bakker & Hilt (2016) also concluded that decreases in reservoir levels during the summer often lead to longer retention times as well as an increase in the accumulation of nutrients that may promote cyanobacterial blooms. Brasil *et al.* (2016)

affirmed that this phenomenon may increase salinity (measured in this study as electrical conductivity). Low conductivity favors the presence of diazotrophic cyanobacteria, while high salinity typically favors the growth of taxa that do not produce heterocysts (Srivastava *et al.* 2009). All of which point to the importance and necessity for an index tool that can easily and accurately inform resource managers how to best alter their treatment methods to keep the distributed water at the highest quality possible.

The high, positive correlation between chlorophyll-*a* and total nitrogen is not surprising and could be because cyanobacteria have a higher nitrogen requirement when compared to eukaryotic algae (Allen 1984). According to Dolman *et al.* (2012), nitrogen may have a stronger influence than phosphorus on eutrophication and concomitant shifts in phytoplankton community composition. According to these authors, the correlation between total cyanobacterial biovolume and TP became less significant at high TP concentrations but continued to increase linearly across a broader range of TN. Moreover, Lewis & Wurtsbaugh (2008) argued that nitrogen fixation is not always sufficient to overcome nitrogen limitation due to limitations in light or other microelements. Previous research also suggests a saturation between phosphorus concentration and cyanobacterial biovolume (Dolman *et al.* 2012) and biomass (Watson *et al.* 1997). All of which reinforce why total nitrogen and chlorophyll-*a* comprise such a large part of the model.

Generally, TN:TP ratios were proportional to cyanobacterial concentrations. Not only were cyanobacteria heavily

Table 4 | Type of treatment used in the WTP of each reservoir; calculated value of the cyanobacterial index (*Icyano*) and classification of each reservoir

Reservoir	Current water treatment technology	Trophic classification	<i>Icyano</i> index	<i>Icyano</i> classification	Action to be taken
AT	Direct filtration	Eutrophic	6.36	G	KT
G	Direct filtration	Eutrophic	22.13	G	KT
MU	Direct filtration	Eutrophic	23.39	G	KT
AM	Direct filtration	Mesotrophic	24.02	G	KT
AR	Direct filtration	Eutrophic	28.14	M	CT
PT	Direct filtration	Hypereutrophic	32.63	M	CT
AMR	Double filtration ^a	Eutrophic	36.55	M	KT
PU	Conventional	Eutrophic	38.22	M	KT
SN	Double filtration ^a	Hypereutrophic	38.42	M	KT
CN	Direct filtration	Eutrophic	40.08	M	CT
EQ	Double filtration ^a	Hypereutrophic	44.19	M	KT
E	Direct filtration	Hypereutrophic	44.29	M	CT
PM	Direct filtration	Hypereutrophic	45.94	M	CT
SD	Conventional	Hypereutrophic	48.32	M	KT
T	Double filtration ^a	Eutrophic	48.72	M	KT
BO	Direct filtration	Hypereutrophic	50.47	B	CT
MRT	Direct filtration	Hypereutrophic	52.65	B	CT
PS	Double filtration ^a	Hypereutrophic	52.89	B	CT
FQ	Conventional	Hypereutrophic	77.59	VB	KT
MT	Direct filtration	Hypereutrophic	77.64	VB	CT

^aAdapted for double filtration, but initially designed for direct filtration (G = Good, M = Medium, B = Bad, and VB = Very Bad). KT = Keep WTP Technology; CT = Change WTP Technology.

abundant, but they were incredibly dominant in our study sites (Figure 2). This observation contradicts the findings of Smith (1983) who compiled data from 17 lakes worldwide and concluded that cyanobacteria tended to be rare when TN:TP exceeded 29. Furthermore, a similar study conducted by Havens *et al.* (2003) examined a 28-year long dataset from Lake Okeechobee, FL, USA. They found that as TN:TP decreased through the decades, cyanobacterial dominance increased. However, a recent study published by Li *et al.* (2018) examined a Swedish drinking water lake and found cyanobacteria dominated the phytoplankton community despite inflow TN:TP ratios being measured at upwards of 40. The findings of Li *et al.* (2018) could explain the patterns found in the reservoirs in this study. Slow-growing cyanobacteria are typically less palatable to grazers than eukaryotic green algae and this coupled with a constant source of high concentrations of N and P (among other nutrients) could encourage cyanobacterial dominance and the negative effects that are associated with them. Despite

the importance of the TN:TP ratio, it was not added into the model because it exhibited high multicollinearity with TN and TP. In other words, there is very high intercorrelations or inter-associations among these independent variables (TP, TN, and TN:TP), which generates a disturbance within the model that can cause statistical inferences that make the model less reliable.

When warnings related to high cyanobacterial biomass and consequently potentially high cyanotoxin concentrations in the water supply are signaled, additional measures are required to ensure water quality and protect consumers (He *et al.* 2016). Additionally, when these warnings become increasingly frequent, water treatment technologies should be evaluated. With that being said, as this study was being carried out no such evaluation criteria existed. Therefore, many of the 20 WTPs associated with the studied reservoirs in this project used a less robust water treatment technology (direct filtration), and when their water quality was evaluated with the *Icyano*

index, scores closely represented the trophic state of the reservoir. This means that most of the WTPs in this study were likely undertreating their water when samples were collected. The proposed index could be an effective tool not only for selecting water treatment plant technology but also for the management of the multiple uses of water resources in the semi-arid regions. Although this index was developed and used data from semi-arid regions, harmful algal blooms are a global issue and the parameters that govern harmful blooms in semi-arid regions likely do so similarly around the world and may be used in other regions with success.

CONCLUSIONS

The parameters that best explained the proposed *Icyano* index were temperature, evaporation, sunlight, electrical conductivity, chlorophyll, TN, TP, Secchi depth, and concentration of cyanobacteria. However, chlorophyll-*a*, total nitrogen, and cyanobacterial concentration accounted for ~60% of the model composition, suggesting that water resource managers with limited infrastructure can still monitor their water quality effectively.

Across the study reservoirs, there was a low diversity of phytoplankton and an absolute dominance of cyanobacteria (greater than 90% of phytoplankton composition) highlighted by *R. raciborskii*. Apparently, competition between cyanobacteria species may be associated with the production of cyanotoxins, MC being the most common cyanotoxins found in studied reservoirs.

In this study, the direct filtration technology utilized in many WTPs was incapable of providing drinking water that met Brazilian potability and WHO standards due to the presence of cyanobacteria. WTPs initially designed to operate only with direct filtration now use pre-treatment technology (double filtration) or a conventional treatment to address this deficiency after being evaluated with the *Icyano* index. Moreover, *Icyano* has shown promising results in helping to identify technological and infrastructural improvements in WTPs associated with cyanobacterial blooms in raw water. Additionally, the use of *Icyano* proved to be effective in assisting water resource management in the semi-arid region of Brazil and may have some application

in other semi-arid regions of the world and, quite possibly, in other regions as well.

ACKNOWLEDGEMENTS

We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FUNCAP (Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico) for providing the funding that allowed the development of this research. This study was supported by the Alabama Agricultural Experiment Station and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture. We also thank CAGECE (Companhia de Água e Esgoto do Ceara) and COGERH (Companhia de Gestão dos Recursos Hídricos) workers and managers for the use of their facilities. Lastly, we would like to thank three anonymous reviewers for constructive feedback that improved the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

- Abraxis, L. L. C. 2007 *Microcystins-ADDA ELISA (Microtiter Plate)*, Product No. 520011. Available from: <http://www.abraxiskits.com/wp-content/uploads/2014/08/Microcystin-PL-ADDA-Users-Guide-ETV-R082714.pdf/> (accessed May 2019).
- Abraxis, L. L. C. 2009 *Saxitoxin (PSP) ELISA (Microtiter Plate)* Product No. 52255B. Available from: <http://www.abraxiskits.com/wpcontent/uploads/2014/04/STXplateinsertR042414.pdf/> (accessed May 2019).
- Allen, M. M. 1984 *Cyanobacterial cell inclusions*. *Annual Review in Microbiology* **38**, 1–25.
- APHA 2012 *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
- Backer, L., McNeel, S. W., Barber, T., Kirkpatrick, B., Williams, C., Irvin, M., Zhou, Y., Johnson, T. B., Nierenberg, K., Aubel, M., LePrell, R., Chapman, A., Foss, A., Corum, S., Hill, V. R., Kieszak, S. M. & Cheng, Y. S. 2010 *Recreational exposure to*

- microcystins during algal blooms in two California lakes. *Toxicon* **55** (5), 909–921.
- Bakker, E. & Hilt, S. 2016 Impact of water-level fluctuations on cyanobacteria blooms: options for management. *Aquatic Ecology* **50**, 485–498.
- Barros, M. U. G., Wilson, A. E., Leitão, J. I. R., Pereira, S. P., Buley, R., Fernandez-Figueroa, E. G. & Capelo-Neto, J. 2019 Environmental factors associated with toxic cyanobacterial blooms across 20 drinking water reservoirs in a semi-arid region of Brazil. *Harmful Algae* **86**, 128–137.
- Bharti, N. & Katyal, D. 2011 Water quality indices used for surface water vulnerability assessment. *International Journal of Environmental Sciences* **2** (1), 154–173.
- Brasil, J., Attayde, J. L., Vasconcelos, F. R., Dantas, D. D. F. & Huszar, V. L. M. 2016 Drought-induced water-level reduction favors cyanobacteria blooms in tropical shallow lakes. *Hydrobiologia* **770**, 145–164.
- CAGECE, Companhia de Água e Esgoto do Ceará 2019 (*Water and Wastewater Company of Ceará*). Phytoplankton monitoring of human supply reservoirs of the state of Ceará. Available from: <https://www.cagece.com.br/> (accessed 2019).
- Carlson, R. E. 1977 A trophic state index for lakes. *Limnology and Oceanography* **22** (2), 361–369.
- Carmichael, W. W. 1994 The toxins of cyanobacteria. *Scientific American* **270**, 78–86.
- Carmichael, W. W., Azevedo, S. M. F. O. O., An, J. S., Molica, R. J. R., Jochimsen, E. M., Lau, S., Rinehart, K. L., Shaw, G. R. & Eaglesham, G. K. 2001 Human fatalities from cyanobacteria, chemical and biological evidence for cyanotoxins. *Environmental Health Perspectives* **109**, 663–668.
- COGERH, Companhia de Gestão de Recursos Hídricos do Ceará 2019 (*Water Resources Management Company of Ceará*). Water quality monitoring network. Available from: <http://www.hidro.ce.gov.br/> (accessed 2019).
- Dantas, E. W., Moura, A. N. & Bittencourt-Oliveira, M. C. 2011 Cyanobacterial blooms in stratified and destratified eutrophic reservoirs in semi-arid region of Brazil. *Anais da Academia Brasileira de Ciências* **83** (4), 1327–1338.
- Dolman, A. M., Rucker, J., Pick, F. R., Fester, J., Rohrlack, T., Mischke, U. & Wiedner, C. 2012 Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. *PLoS ONE* **7** (6), e. 38757.
- Edwards, C., Mckenzie, C., Pestana, C. J., Yates, K. & Lawton, L. A. 2017 Rapid analysis of geosmin and 2-methylisoborneol from aqueous samples using solid-phase extraction and GC-MS. In: *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis* (J. Meriluoto, L. Spoof & G. A. Codd, eds). John Wiley & Sons, Ltd, Chichester, UK, pp. 475–480.
- Ewaid, S. H. & Abed, S. A. 2017 Water quality index for Al-Gharraf River, Southern Iraq. *Egyptian Journal of Aquatic Research* **43**, 117–122.
- Fadel, A., Sharaf, N., Siblini, M., Slim, K. & Kobaiissi, A. 2019 A simple modelling approach to simulate the effect of different climate scenarios on toxic cyanobacterial bloom in a eutrophic reservoir. *Ecology & Hydrobiology* **19**, 359–369.
- Falconer, I. 1993 *Algal Toxins in Seafood and Drinking Water*. Academic Press, New York, NY, p. 224.
- FUNCEME 2017 *Fundação Cearense de Meteorologia e Recursos Hídricos. (Ceará Foundation of Meteorology and Water Resources of Ceará)*. Available from: <http://www.funceme.br/> (accessed November 2017).
- Garcia, C. A. B., Silva, I. S., Mendonça, M. C. S. & Garcia, H. L. 2018 *Evaluation of Water Quality Indices: Use, Evolution and Future Perspectives, Advances in Environmental Monitoring and Assessment*.
- Hair, J. F., Anderson, R. E. & Tatham, R. L. 1987 *Multivariate Data Analysis with Readings*, 2nd edn. Macmillan Publishing Company, New York, NY.
- Hair, J. F., Black, W. C., Babin, B. J. & Anderson, R. E. 1998 *Multivariate Data Analysis*, 7th edn. Pearson, London, UK.
- Havens, K. E., James, R. T., East, T. L. & Smith, V. H. 2003 N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environmental Pollution* **122** (3), 379–390.
- He, X., Liu, Y., Conklin, A., Westrick, J., Weavers, L. K., Dionysiou, D. D., Lenhart, J. J., Mouser, P. J., Szlag, D. & Walker, H. W. 2016 Toxic cyanobacteria and drinking water: impacts, detection, and treatment. *Harmful Algae* **54**, 174–193.
- Horton, R. K. 1965 An index number system for rating water quality. *Journal of the Water Pollution Control Federation* **37**, 300–306.
- Huang, J., Ji, M., Xie, Y., Wang, S., He, Y. & Ran, J. 2016 Global semi-arid climate change over the last 60 years. *Climate Dynamics* **46**, 1131–1150.
- Instituto Interamericano de Cooperação para Agricultura 2018 (*Inter-American Institute for Cooperation on Agriculture*). Available from: <http://www.iica.org.br/> (accessed April 2018).
- INMET 2016 *Banco de dados meteorológicos da ensino e pesquisa. (Meteorological Database of Teaching and Research)*. Available from: <http://www.inmet.gov.br/> (accessed December 2016).
- IPECE 2017 *Instituto de Pesquisa e Estratégia Econômica do Estado do Ceará. (Institute of Research and Economic Strategy of the State of Ceará)*. Available from: <http://www.ipece.ce.gov.br/> (accessed February 2017).
- Johnson, R. A. & Wichern, D. W. 2007 *Applied Multivariate Statistical Analysis*, 6th edn. Pearson, Upper Saddle River, NJ, USA.
- Lawton, L. 1999 Determination of cyanobacteria in the laboratory. In: *Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management* (I. Chorus & J. Bartram, eds). E&FN Spon, London, UK, p. 416.
- Leigh, C., Burford, M. A., Roberts, D. T. & Udy, J. W. 2010 Predicting the vulnerability of reservoirs to poor water quality and cyanobacterial blooms. *Water Research* **44**, 4487–4496.
- Lewis, W. M. & Wurtsbaugh, W. A. 2008 Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *International Review of Hydrobiology* **93**, 446–465.
- Li, J., Hansson, L. A. & Persson, K. 2018 Nutrient control to prevent the occurrence of cyanobacterial blooms in a

- eutrophic lake in Southern Sweden, used for drinking water supply. *Water* **10** (919), 1–11.
- Marengo, J. A. 2009 Vulnerabilidade, Impactos e adaptação as mudanças de clima no semiárido do Brasil, In *Parecerias Estratégicas/Centro de Gestão de Estudos Estratégicos-Ministerio da Ciencia e Tecnologia*, 1 (1), pp. 149–176.
- Meireles, A. C. M., Andrade, E. M., Chaves, L. C. G., Frischkorn, H. & Crisostomo, L. A. 2010 A new proposal for the classification of irrigation water. *Revista Ciência Agrônômica* **41** (3), 349–357.
- Mohamed, Z. A., El-Sharouny, H. M. & Ali, W. S. M. 2006 Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals, Egypt. *Toxicon* **47**, 584–590.
- Moura, A. N., Aragao-Tavares, N. & Amorim, C. A. 2017 Cyanobacterial blooms in freshwater bodies from a semi-arid region, Northeast Brazil: a review. *Journal of Limnology* **77** (2), 179–188.
- Mukundan, R., Hoang, L., Gelda, R. K., Yeo, M.-H. & Owens, E. M. 2020 Climate change impact on nutrient loading in a water supply watershed. *Journal of Hydrology* **586**, 124868.
- Nasiri, F., Massood, I., Huang, G. & Fuller, N. 2007 Water quality index: a fuzzy river-pollution decision support expert system. *Journal of Water Resources Planning and Management* **133** (2), 95–105.
- Neto, J. R. A., Andrade, E. M., Meireles, A. C. M., Guerreiro, M. J. S. & Palácio, H. A. Q. 2014 Salinity index approach of the surface water from reservoirs in Ceará, Brazil. *Revista Agroambiente* **8**, 184–193.
- Paerl, H. W. & Paul, V. J. 2012 Climate change: links to the global expansion of harmful cyanobacteria. *Water Research* **46** (5), 1349–1363.
- Parinet, J., Rodriguez, M. J. & Serodes, J. 2010 Influence of water quality on the presence of off-flavor compounds (geosmin and 2-methylisoborneol). *Water Research* **44**, 5847–5856.
- Pestana, C. J., Robertson, P. K. J., Edwards, C., Wilhelm, W., McKenzie, C. & Lawton, L. A. 2014 A continuous flow packed bed photocatalytic reactor for the destruction of 2-methylisoborneol and geosmin utilizing pelletized TiO₂. *Chemical Engineering Journal* **235**, 293–298.
- Poonam, T., Tanushree, B. & Sukalyan, C. 2013 Water quality indices – important tools for water quality assessment: a review. *International Journal of Advances in Chemistry* **1** (1), 15–28.
- Shweta, T., Bhavtosh, S., Prashant, S. & Rajendra, D. 2013 Water quality assessment in terms of water quality index. *American Journal of Water Resources* **1**, 34–38.
- Smith, V. H. 1983 Low nitrogen to phosphorus ratios favors dominance by blue-green algae in lakes: phytoplankton. *Science* **221**, 669–671.
- Srebotnjak, T., Carr, G., Sherbinin, A. & Rickwood, C. 2011 A global water quality index and hot-deck imputation of missing data. *Ecological Indicators* **17**, 108–119.
- Srivastava, G. & Kumar, P. 2013 Water quality index with missing parameters. *International Journal of Research in Engineering and Technology* **2** (4), 609–614.
- Srivastava, A. K., Bhargava, P., Kumar, A., Rai, L. C. & Neilan, B. A. 2009 Molecular characterization and the effect of salinity on cyanobacterial diversity in the rice fields of Eastern Uttar Pradesh, India. *Saline Systems* **5**, 4.
- Te, S. H. & Gin, K. Y. 2011 The dynamics of cyanobacteria and microcystin production in a tropical reservoir of Singapore. *Harmful Algae* **10**, 319–329.
- Toledo, A. P. J., Talarico, M., Chinez, S. J. & Agudo, E. G. 1983 A aplicação de modelos simplificados para a avaliação do processo da eutrofização em lagos e reservatórios tropicais (The application of simplified models for the evaluation of the eutrophication process in tropical lakes and reservoirs). In: *Congresso Brasileiro de Engenharia Sanitária e Ambiental*, 12., Balneário Camboriú, Santa Catarina. ABES – Associação Brasileira de Engenharia Sanitária e Ambiental, pp. 1–34.
- UNEP GEMS/Water Programme 2007 *Global Drinking Water Quality Index Development and Sensitivity Analysis. Report*. UNEP GEMS/Water Programme, Burlington, Ontario.
- United Nations 2011 *Resolution Adopted by General Assembly on 20 December 2010*. United Nations, New York, NY.
- Van Apeldoorn, M. E., Egmond, H. P., Speijers, G. J. A. & Bakker, G. J. I. 2007 Toxins of cyanobacteria. *Molecular Nutrition Food Research* **51**, 57–60.
- Walls, J. T., Wyatt, K. H., Doll, J. C., Rubenstein, E. M. & Rober, A. R. 2018 Hot and toxic: temperature regulates microcystin release from cyanobacteria. *Science of the Total Environment* **610–611**, 786–795.
- Walter, J. M., Lopes, F. A. C., Lopes-Ferreira, M., Vidal, L. M., Leomil, L., Melo, F., Azevedo, G. S., Oliveira, R. M. S., Medeiros, A. J., Melo, A. S. O., Rezende, C. E., Tanuri, A. & Thompson, F. L. 2018 Occurrence of harmful cyanobacteria in drinking water from a severely drought-impacted semi-arid region. *Frontiers in Microbiology* **9**, 176.
- Watson, S. B., McCauley, E. & Downing, J. A. 1997 Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. *Limnology and Oceanography* **42**, 487–495.
- WHO, World Health Organization 2017 *Guidelines for Drinking-Water Quality*, 4th edn. Incorporating the first Addendum. p. 178. World Health Organization, Geneva, Switzerland.
- WHO, World Health Organization 2019 *Guidelines for Drinking-Water Quality and Guidelines for Safe Recreational Water Environments, Version for Public Review*, 15, November 2019. World Health Organization, Geneva, Switzerland.
- Zhang, H., Yan, M., Huang, T., Huang, X., Yang, S., Li, N. & Wang, N. 2020 Water-lifting aerator reduces algal growth in stratified drinking water reservoir: novel insights into algal metabolic profiling and engineering applications. *Environmental Pollution* **266** (1), 115384.
- Zhao, C. S., Shao, N. F., Yang, S. T., Ren, H., Ge, Y. R., Feng, P., Dong, B. E. & Zhao, Y. 2019 Predicting cyanobacteria bloom occurrence in lakes and reservoirs before blooms occur. *Science of The Total Environment* **670**, 837–848.