UNIVERSIDADE FEDERAL DO CEARÁ

CENTRO DE TECNOLOGIA

DEPARTAMENTO DE ENGENHARIA HIDRÁULICA E AMBIENTAL

PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA CIVIL

MÁRIO UBIRAJARA GONÇALVES BARROS

CYANOBACTERIA IN A CLIMATE CHANGE SCENARIO: A NEW APPROACH

FORTALEZA

2018

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Tese apresentada ao Programa de Pós-graduação em Engenharia Civil, da Universidade Federal do Ceará, como requisito à obtenção do título de doutor em Engenharia Civil. Área de Concentração: Saneamento Ambiental.

Orientador: Prof. Dr. José Capelo Neto.

FORTALEZA
2018

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca Universitária
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

B279c Barros, Mário Ubirajara Gonçalves Barros.
Cyanobacteria in a Climate Change Scenario - a New Approach / Mário Ubirajara Gonçalves Barros Barros. – 2018.
120 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Centro de Tecnologia, Programa de Pós-Graduação em Engenharia Civil: Saneamento Ambiental, Fortaleza, 2018.
Orientação: Prof. Dr. José Capelo Neto .

1. Cianobactérias. 2. Nutrientes. 3. Eutrofização. 4. Mudanças climáticas. 5. Semi árido. I. Título.
CDD 628

MÁRIO UBIRAJARA GONÇALVES BARROS

CYANOBACTERIA IN A CLIMATE CHANGE SCENARIO: A NEW APPROACH

Thesis presented to the Graduate Program in Civil Engineering at Federal University of Ceará, as a requirement to obtain a degree of Doctor in Civil Engineering. Concentration Area: Environmental Sanitation.

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To my parents, I am very grateful for all your love and encouragement.

ACKNOWLEGMENTS

Firstly, I thank God for the gift of life, for all the courage, light, determination and strength that guided me, and made it possible to struggle all stages of this research with health and responsibility.

I thank my family, especially my parents José Mário Barros and Tânia Maria Rodrigues Gonçalves, my sister Ana Carolina Gonçalves Barros and my wife Paloma Ribeiro da Cruz, for their teachings, values, incentives and feelings transmitted. For patience and guidance in times of trouble. You will always be part of every my victories.

To Professor José Capelo Neto, for his guidance in carrying out this research, as well as for its readiness that were fundamental for this research. Thank you for believe in my potential and for all the opportunities in those 7 years at Selaqua. Thank you so much.

To Water and Sewage Company of Ceará (CAGECE), for making me available its facilities to carry out this research, mainly, all that are part of the hydrobiological laboratory.

To my SELAQUA's friends: Hélisia Pessoa, Maria Aparecida, Ana Carolina, Diana Moura and Allan Clemente. In particular, Dr. Ismael Kesley, MSc. Samylla Oliveira and Dr. Carlos Pestana for their intellectual contributions that I have used in execution of this work. All of you contributed to the accomplishment of this work, thank you very much.

I would like to thank the Auburn University, especially to the group of reaserches coordinated by Dr. Alan E. Wilson, for accepting me in his laboratory and for enabling me to live one of the most fantastic experiences in my academic and personal life. In specially, I would like to thank everyone who is from Wilson Lab, my friends Alan Wilson, Edna Fernadez-Figueroa, Riley Buley, Zhen Yang, Bettina We, Leeann Johnston and all the undergraduate students who contributed their knowledge to develop my research in Auburn, and everyone in The Auburn University School of Fisheries, Aquaculture and Aquatic Sciences, thank you very much.

To Dr. João Igor R. Leitão and Dr. Fernando José da Silva, for his patience and readiness in the guidelines on multivariate statistics and on the *"R"* program.

To friend Thaís Benevides Aranha, for all support in the geoprocessing area, which were fundamental for understanding the extent of the problem.

To all the colleagues with whom I shared subjects, for the union and struggle to reach our objectives during this phase.

To all the professors from the Department of Hydraulic and Environmental Engineering, especially those with whom I had the opportunity to share knowledge and who contributed their experiences to my intellectual and professional development.

To Professor Wladimir Ronald Lobo Farias (*In memmoriam*), who was fundamental in the beginning of my academic life believing in my potential, thank you.

To all friends from The Water Resources Management Company (COGERH), who contributed their knowledge to complete this reasearch.

To Fundação Cearense de Apoio ao Desenvolvimento Cientifico e Tecnológico (FUNCAP) for giving me a scholarship, fundamental so that it could devote to the construction of this work.

To the Brazilian government, represented by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for giving me financial resources to develop my research in the United States.

To Hugo Leonardo de Brito Buarque, Alan E. Wilson, José Ethan de Lucena Barboza and Silvano Porto Pereira for accepting my invitation and contributing their knowledge to the improvement of my research.

Finally, to all those who, directly or indirectly, contributed to the accomplishment of this research.

“The mind that opens up to a new idea never returns to its original size”.

Albert Einstein.

RESUMO

As cianobactérias produzem uma ampla variedade de metabólitos secundários tóxicos e compostos bioativos, conhecidos como cianotoxinas. No Brasil, o monitoramento da frequência e concentração de cianotoxinas foi intensificado após a morte de 65 pacientes em uma clínica de hemodiálise em Caruaru, no estado de Pernambuco, devido à exposição por microcistinas. O objetivo principal deste estudo foi através do uso de ferramentas de estatística multivariada, realizar estudos que incorporem parâmetros ambientais (bióticos e abióticos) para prever florescimentos de cianobactérias e seus metabólitos secundários tóxicos em 20 reservatórios de água potável utilizados pela Companhia de Água e Esgoto do Ceará (CAGECE) no região semiárida, Ceará, Brasil; como também desenvolver um índice de avaliação de cianobactérias (*Icyano*), levando em consideração, os principais parâmetros monitorados para o controle e gerenciamento de cianobactérias em reservatórios de abastecimento humano. Em quatro anos (janeiro de 2013 a janeiro de 2017), 114 diferentes fitoplanctonos foram identificados, incluindo 24 espécies de cianobactérias. Em geral, os reservatórios do Ceará foram dominados por cianobactérias devido, principalmente, à eutrofização; mas também devido ao clima seco e quente encontrado em toda a região. Curiosamente, as concentrações de cianobactérias específicas foram influenciadas por diferentes fatores bióticos e abióticos. Por exemplo, o nitrogênio total foi relacionado a taxa de produção de saxitoxina, especialmente, a espécie *Cylindrospermopsis raciborskii*, enquanto a temperatura, irradiação e transparência (medida em profundidade de Secchi) foram associadas a taxa de produção de microcistinas, como a espécie *Microcystis aeruginosa*. A abundância de *Planktothrix agardhii* foi relacionada à concentração total de fósforo e chuvas. Considerando que as previsões climáticas prevêem maior evaporação e temperaturas na região semi-árida do Ceará e que tais mudanças provavelmente aumentarão as secas e a escassez de água, bem como promoverão a proliferação de cianobactérias tóxicas em reservatórios no futuro, entender os fatores associados à proliferação de cianobactérias é primordial para a gestão de recursos hídricos. No modelo de índice proposto, temperatura, evaporação, irradiação, condutividade elétrica e fósforo mostram Wi (peso de cada parâmetro) = 0,073, 0,061, 0,032, 0,039 e 0,042, respectivamente. Clorofila (Wi = 0,202), nitrogênio total (Wi = 0,191) e concentração de cianobactérias (W = 0,214) foram os três parâmetros mais importantes para a composição do índice, representando aproximadamente 60% da composição do peso do indice (Wi). Todos os reservatórios classificados com *Icyano* bom (Good) usam a tecnologia de tratamento de água de filtração direta, enquanto, muitos dos

reservatórios com médio *Icyano* (AMR, EQ, PM, PU, SD, SN e T) usam uma unidade de pré-tratamento seguida por uma unidade de filtração direta. Além disso, o reservatório PS (Bad) também tem duas unidades de tratamento (pré-tratamento + filtragem direta), e o reservatório FQ (Very bad) apresentou a tecnologia de ciclo completo. Pode-se observar que à medida que o *Icyano* aumenta, as estações de tratamento de água mudam de sua conformação original (filtragem direta) para um tratamento mais robusto agregando um pré-tratamento para fornecer água bruta à qualidade exigida pela atual legislação brasileira. Os parâmetros que mais contribuíram para a explicação do modelo proposto foram temperatura, evaporação, luz solar, condutividade elétrica, clorofila, nitrogênio total, fósforo total, profundidade de Secchi e concentração de cianobactérias. Uma alta razão de N: P foi observada e pode explicar melhor a dominância de cianobactérias nos reservatórios estudados.Diante disso, concluimos que foi possível montar um índice que relaciona a qualidade da água ao risco de cianobactérias e comparar esse índice com a tecnologia de tratamento de água, auxiliando gestores de recursos hídricos na priorização dos investimentos em estações de tratamento de água.

Palavras-chave: Cianobactérias, nutrientes, Eutrofização.

ABSTRACT

Cyanobacteria are known to produce a wide variety of bioactive, toxic secondary metabolites generally described as hepatotoxins, neurotoxins, cytotoxins, or dermatoxins. In Brazil, the monitoring of the frequency and concentration of cyanotoxins has intensified after the death of 65 patients in a hemodialysis clinic in Caruaru in the state of Pernambuco due to microcystin exposure. The primary objective of this study was to use multivariate statistics that incorporated environmental parameters (both biotic and abiotic) to forecast blooms of cyanobacteria and their toxic secondary metabolites in 20 drinking water reservoirs managed by the Water Treatment Company of Ceará (CAGECE) in the semi-arid region of Ceará, Brazil, as also develop an index for the study of cyanobacteria (*Icyano*) taking into account the main monitored parameters for the control of cyanobacteria in order to provide summary information. Across four years (January 2013 to January 2017), 114 different phytoplankton taxa were identified, including 24 cyanobacterial species. In general, Ceará reservoirs were dominated by cyanobacteria due to eutrophication but also because of the dry and warm climate found throughout the region. Interestingly, specific cyanobacterial taxa were influenced by different biotic and abiotic factors. For example, total nitrogen was related to saxitoxin-producing taxa, especially *Cylindrospermopsis raciborskii*, while temperature, irradiation and transparency (measured as Secchi depth) were associated with microcystin-producing taxa, such as *Microcystis aeruginosa*. *Planktothrix agardhii* abundance was related to total phosphorus concentration and rainfall. Considering that climate forecasts predict higher evaporation and temperatures in the semi-arid Ceará region and that such changes will likely magnify droughts and water scarcity as well as promote toxic cyanobacterial blooms in reservoirs in the future, understanding the factors associated with algal blooms dominated by specific taxa is paramount for water resource management. In the proposed index model, temperature, evaporation, sunlight, electrical conductivity, and phosphorus show Wi (weight of each parameter) = 0.073, 0.061, 0.032, 0.039 and 0.042, respectively. Chlorophyll (Wi = 0.202), total nitrogen (Wi = 0.191), and cyanobacteria concentration (W = 0.214) were the three most important parameters to the index composition, representing approximately 60% of the Wi composition. All reservoirs classified as good *Icyano* used direct filtration water treatment technology, while many of the medium *Icyano* classified reservoirs (AMR, EQ, PM, PU, SD, SN and T) used a pretreatment unit followed by a direct filtration unit. Additionally, PS (Bad) reservoir also has been two treatment units (pretreatment + filtration direct), and the reservoir FQ (Very bad) presented the complete cycle technology. It can be seen that as *Icyano*

increases, water treatment plants switch from their original conformation (direct filtration) to using a pre-treatment to provide raw water to the quality required by current Brazilian legislation. The parameters that most contributed to the explanation of the proposed model were temperature, evaporation, sunlight, electrical conductivity, chlorophyll, total nitrogen, total phosphorus, Secchi depth, and concentration of cyanobacteria. A high ratio of N:P was observed and may best explain the dominance of cyanobacteria in the studied reservoirs, however, it cannot be considered as the only factor favoring the dominance of cyanobacteria. It was possible to set up an index that relates water quality to the risk of cyanobacteria, and comparing the index with the water treatment technology and auxiliary in the prioritization of the investments in water treatment plants.

Keywords: Blue-green algae, nutrients, eutrophication.

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LIST OF ABREVIATIONS AND ACRONYMS

ATX(S)	Anatoxin-a(S)
ATX	Anatoxin
APHAC/APHC	*Aphanocapsa sp*
APHA Z/APHA	*Aphanizomenon sp*
BMAA	*β-N-metilamino-L-alanina*
DELWP	Department of Environmental. Land, Water and Planning
DIC	Dissolved Inorganic Carbon
CCA	Canonical correspondence analysis
CE	Electrical conductivity
CHL	Chlorophyll
CHR	Chroococcales
CR	*Cylindrospermopsis raciborskii*
CYN	Cylindrospermopsins
CAGECE	Companhia de Água e Esgoto do Ceará
COGERH	Companhia de Gestão de Recursos Hídricos
DL_{50}	Lethal dose for 50% of individuals
DIC	Dissolved Inorganic Carbon
ELISA	*Enzyme-linked immunosorbent assay*
Evap	Evaporation
FUNCEME	Fundação Cearense de Meteorologia e Recurso Hídricos
GC-MS	Gas chromatography-mass spectrometry

GEI	*Geitlerinema sp*
HPLC-PDA	High Performance Liquid Chromatography with photodiode array detection
INMET	Instituto Nacional de Meteorologia
IPCC	Intergovernmental Panel on Climate Change
IPECE	Instituto de Pesquisa do Ceará
LC-MS	Liquid Chromatography-mass spectrometry
MA	*Microcytis aeroginosa*
MALDI-TOF	Matrix-assisted laser desorption/ionization
MER	*Merismopedia sp*
MCs	Microcistins
MS	Ministério da Saúde
N_2	Atmospheric Nitrogen
NSTX	Neosaxitoxin
OG	Other plankton groups
Temp	Temperature
TN	Total nitrogen
TP	Total phosphorus
p/ETP	Precipitation/evapotranspiration potential ratio
PA	*Planktothrix Agardhii*
PHOR	Phormidiaceae family
PLANK/PLAN	*Planktolyngbya sp*
PSEU	*Pseudoanabaena sp*

PSP	*Paralict Shellfish Poising*
Rain	Rainfall
ROM	*Romeria sp*
Secchi	Secchi disc
STXs/SX	Saxitoxins
Sunlight	Irradiation
SYNE	*Synechocystis sp*
UFC	Universidade Federal do Ceará
WTP	Water Treatment Plant

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1 INTRODUCTION

Cyanobacteria are the oldest known oxygen-producing photosynthetic prokaryotic organisms with fossil remains dating to 3.5 billion years; they are only diazotrophic photoautotrophic organisms, in other words, these organisms are capable of using atmospheric nitrogen (N_2) for growth (Fogg, 1969; Gallon, 1992, Rzymski *et al*., 2014). Cyanobacteria have wide distribution; they are found in aquatic and terrestrial environments, including extreme environments, ranging from the polar to the tropical regions of the northern and southern hemispheres, where are able to dominate other planktonic and benthic species in primary production (Sompong *et al*., 2005; Kleinteich *et al.*, 2012).

In water bodies characterized by high nutrients concentrations, due to a limited water exchange, that is, an environment in which the water presents a high retention time, associated to high temperatures and thermal stability, cyanobacteria may develop high biomasses, giving rise to bloom formations on the surface, euphotic zone, even in deeper layers of metalimnion (Paerl and Paul 2012). The dominance of some species in a phytoplankton community depends on a complexity of physical, chemical and biological factors. Cyanobacteria are commonly found in many eutrophic reservoirs (Figure 1) and bloom of these microorganisms is responsible for the deterioration in aquatic environment. Identification of factors that promote accelerated growth of these species is a crucial issue for the effective management of reservoirs (COGERH, 2008).

The main concern that motivates the study and knowledge of cyanobacteria is fact of these organisms produce a wide variety of secondary metabolites and bioactive compounds, some of which exhibit toxic properties and are referred to as cyanotoxins, which vary greatly in their chemical structures, and can be divided into three classes of chemical structures: (a) cyclic peptides (e.g., nodularinas and microcystins); (b) alkaloid (e.g., cylinderspermopsin, anatoxin-a, homoanatoxin, anatoxin-a (s) and saxitoxins); and (c) lipopolysaccharides (Van Apeldoorn *et al*., 2007). In addition to variations in chemical structure, Mohamed *et al*. (2006) reported that cyanobacteria can be divided hepatotoxins (including microcystins and nodularins), cytotoxins (cylindrospermopsin), neurotoxins (alkaloids: anatoxin-a, anatoxin-a(s) and paralytic shellfish poisoning), dermatoxins (lyngbyatoxins and aplysiatoxins), irritant toxins (endotoxins) and protease inhibitors. Furthermore, it has been found that cyanobacteria produce a compound known as *β-N-methylamino-L-alanine* (BMAA), which was initially described as being possibly produced by 95% of all cyanobacteria genera that may be

associated with several neurodegenerative diseases such as Amyotrophic Lateral Sclerosis, Parkinson's Dementia Complex or Alzheimer's Disease. (Murch *et al*., 2004). The social and economic impacts of cyanobacteria include negative effects mainly on leisure, due to the closure of affected areas, fisheries reduction and a costs increase related to water treatment, using more advanced treatment techniques that usually require more financial resources. (Hoagland *et al*., 2002; Paerl, 2008; He *et al*., 2016).

Figure 1 - (a) *Planktothrix Agardhii* bloom in Guaíba Lake (Rio Grande do Sul - Brazil) e (B) *Microcytis aeroginosa* e *Dolichospermum circinales* blooms in Bolivar reservoir (South Australia).

Source: FURG (2017) and PESTANA (2017).

According to Paerl and Huisman (2008) in addition to the problem of nutrient enrichment, climate change, in particular, global warming, favors Toxic cyanobacteria blooms (*CyanoHABs*), with higher frequencies and concentrations. For instance, data collected in the FUNCEME database (Ceará Foundation of Meteorology and Water Resources) affirms that in the last 60 years, temperature average in Ceará state has been increasing by around 0.7 ^{o}C, which coincides at increase in numbers of toxic cyanobacteria blooms (INMET, 2016). According to The Intergovernmental Panel on Climate Change IPCC (2007), average global temperatures may increase 1.5 to 5 ^{o}C, in the present century. Adiditionally, in the tropics, the temperature may also increase 4 to 6 ^{o}C, which makes it a constant concern for communities located close to Equator (Corlett, 2012; Trewin, 2014). Mitrovic *et al*. (2011) reported that there is a positive correlation between temperature and cyanobacterial concentration, which may explain the invasion of some species from tropical to subtropical regions. For example, *Cylindrospermopsis raciborskii* has expanded from the tropics to mid-latitude regions of

Europe and North and South America. (Padisak, 1997). Thus, study of these organisms is fundamental for making decision, in several fields, mostly in water treatment companies. This knowledge is of considerable importance, guiding environmental and water resource management plans as well as new projects for drinking water supply systems.

In Ceará, CAGECE (2010) reports that there is an increase in cyanobacteria bloom occurrence of neurotoxins-producing species in the reservoirs that supply the main cities of the state and, according to COGERH (2008), agriculture, sewage and intensive fish farming are compromising the quality of the reservoirs of Ceará. The intensification of eutrophication process on surface water sources in Ceará state has been intensified and confirmed since 2000, when the Water Resources Management Company of Ceará (COGERH) began a more detailed monitoring of the main reservoirs it manages. According Ferreira (2008), due to the climate feature, and the proximity of the equator, Ceará presents a set of optimal conditions for the development of phytoplankton, such as high luminous incidence, with approximately 12 hours of sun light per day, throughout the year, besides that, high temperatures, restricted rainy season; and surface waters confined by dams.

Despite the great importance of cyanobacteria to water management utilities in Ceará, a comprehensive study has not yet been carried out in Ceará on what cyanotoxin-producing species are present and in which reservoirs these species are normaly found. These parameters are extremely important for the understanding of the cyanobacteria blooms and their possible effects on the public supply.

This thesis is divided in four chapters; chapter 1 will include a literature review, which will describe general characteristics of the cyanobacteria as well as the cyanotoxins produced by them, with focus in MC, STX and CYN cyanotoxins. Chapter 2 will present aspects that contemplate climate change and their relationship with increasing occurrence and cyanobacteria predominance. Chapter 3 will explore the evaluation of environmental factors influencing occurrence and distribution of cyanobacteria and their toxins in Ceara reservoirs. Chapter 4 will propose Index classification of drinking water reservoirs for identifying vulnerability to *cyanoHABs*.

2 CYANOBACTERIA: GENERAL CHARACTERISTICS, CYANOTOXINS AND DETECTION METHODS USED FOR CYANOTOXIN QUATIFICATION

2.1 Cyanobacteria

Cyanobacteria are photosynthetic microorganisms adapted to a wide variety of environments such as oceans, freshwater reservoirs, rocks, and soils (Seckbach, 2007). Cyanobacteria are believed to have been the first photosynthetic organisms on Earth and have contributed to oxygen generation in the atmosphere more than 3 billion years ago (Rasmussen *et al*., 2008). Cyanobacteria are prokaryotic organisms, but have historically been grouped as eukaryotic in the algae class and are sometimes referred to as blue green algae, Myxophyceae, Cyanophyceae, or Cyanophyta (Carmichael, 2008).

According to Catherine *et al*. (2013), cyanobacteria are common in aquatic systems, where they grow in the water column (planktonic), aggregates on the water surface (metaphytic), linked to other algae, cyanobacteria or macrophytes (epiphytes), or linked to the substrate (benthic). Planktonic species are classified into four different orders: Chroococcales, Oscillatoriales, Nostocales and Stigonematales (Sant'anna and Azevedo, 2006). Cyanobacteria blooms alter ecosystem functions, reduce water transparency, oxygen concentration, and planktonic biodiversity, and, in addition, they generate toxic compounds, and taste and odor problems in water supply, fish and shellfish (Huisman *et al*., 2006; Ibelings and Mur, 1992). The impacts of toxic cyanobacteria blooms to supply water reservoirs depend on many factors, such as the extent and nature of bloom, specific toxins and climatic conditions (He *et al*., 2016).

According to Stewart and Falconer (2008), there are more than 40 species representing 20 genera of three orders of cyanobacteria known to produce toxins including cyclic peptides and alkaloids. For instance, Sivonen and Jones (1999) said that there are approximately 50 out of 2000 species of cyanobacteria known to produce toxic molecules. According to Van Apeldoorn *et al*. (2007), out of about 150 genera of cyanobacteria, 40 are known to produce toxic compounds that may influence terrestrial organisms and water quality.

In Brazil, monitoring of the frequency and concentration of cyanotoxins has been intensified after the incident of fatal intoxication caused by microcystins in at least 65 patients of a hemodialysis clinic in Caruaru, Pernambuco state, Brazil (Carmichael *et al*., 2001), consequently, the monitoring of cyanobacteria and cyanotoxins in waters used for human

supply has been mandatory in Brazil since ordinance 518/2004 (Health department of Brazil), although the previous ordinance (1469/2000) already provided for a maximum tolerance of 1 $\mu g.L^{-1}$ for the presence of microcystins.

The ordinance to drinking water from Brazilian Health department affirms that to minimize the risks of contamination in water for human consumption with cyanotoxins, cyanobacteria monitoring must be carried out at WTP intake point, in order to identify different genus present in raw water, considering, for changing the monitoring frequency, the result of the last sampling. When the cell density of cyanobacteria exceeds 20,000 cells.mL^{-1}, cyanotoxin analysis must be performed weekly. Treated water microcystin and saxitoxins concentrations shall not exceed the maximum values laid down in the standard, 1 $\mu g.L^{-1}$ for microcistins, and 3 $\mu g.L^{-1}$ for saxitoxins. In addition, in the case of a potentially cylindrospermopsin-producing genus are detected in monitoring, it is recommended to analyze these cyanotoxins, observing the maximum acceptable value of 1.0 $\mu g.L^{-1}$. In case the monitoring detects the presence of cyanobacterial potentially anatoxin-a (s)-producing cyanobacterial genera, it is recommended to analyze the presence of this cyanotoxin.

Studies investigating benthic are few compared to planktonic cyanobacteria studies, and consequently information and guidance on the management of toxic benthic cyanobacteria are still considered incomplete. Benthic cyanobacteria, associated with a substrate used as support for their development, may form blooms ranging from a few millimeters to thick layers of a few centimeters (Catherine *et al*., 2013).

Benthic cyanobacteria identified as dominant are generally from Oscillatoriales order, with *Oscillatoria* and *Phormidium* genera often more abundant (Mez *et al*., 1997; Guggen *et al*., 2005; Heath *et al*., 2011). According to Catherine *et al*. (2013), other genera such as *Lyngbya sp*, *Leptolyngbya sp*, *Microcoleus sp, Tychonema sp, Schizothrix sp* are also commonly observed. The genus *Planktothrix sp*. identified as planktonic may present benthic habits and produce microcystin LR (Wood *et al*., 2010).Chroococcales, belonging to *Aphanothece* and *Synechococcus* genus were also found in benthic biofilms in freshwater (Dasey *et al*., 2005; Krienitz *et al*., 2003; Mohamed, 2008). According to Dasey *et al*. (2005) the aerotopes absence leads *Aphanothece sp* to form a sticky carpet associated with the substrate, some benthic filamentous cyanobacteria belonging to Nostocales group were also identified, including *Anabaena* and *Scytonema sp* (Mohamed *et al*., 2006; Smith *et al*., 2011).

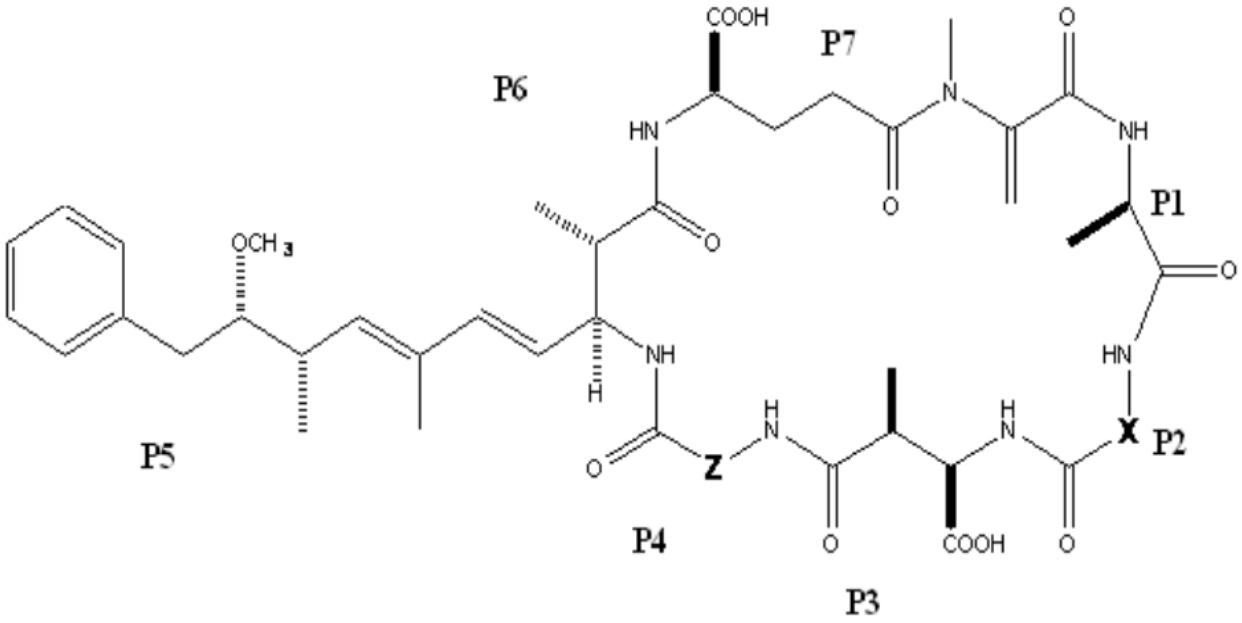
2.2 Cyanotoxins

Many cyanobacterial genera are known to produce a wide variety of bioactive compounds, described as toxic secondary metabolites (Sivonen and Jones, 1999). These toxic secundary metabolites are known as cyanotoxins. They are not essential for cyanobacterial growth or metabolism but are capable of causing acute and / or chronic damage to animal health and in some cases even mortality. Hepatic and neurotoxins are the toxins most frequently produced by cyanobacteria. Hepatotoxins include microcystins and nodularinas, and the three most common types of neurotoxins are anatoxin-a, anatoxin-a (S) and saxitoxins. (Sivonen and Jones, 1999; Klisch and Hader, 2008, Sivonen and Borner, 2008). In addition, Cox *et al*. (2003 and 2005) described the presence of the neurotoxic compound *BMAA* (*Beta-Methylamino-L-alanine*), which in mammals affects the synapse nerve causing diseases such as Amyotrophic Lateral Sclerosis (ALS) of Parkinson's disease. This compound may be produced by 95% of all cyanobacteria genera, thus increasing the potential for human exposure (Cox *et al*., 2003; Murch *et al*., 2004; Cox, 2009).

2.2.1 Microcystins (MCs)

Microcystins (MCs) are toxic secondary metabolites produced by cyanobacteria, classified as cyclic heptapeptides. Microcystins are composed for a short peptides of 7 amino acids (Figure 2) the radicals represent P1: D-alanine; P2: Variable amino acid; P3: D-aspartic acid, P4: Variable amino acid; P5: Adda; P6: Glutamic acid; P7: Methyldehydroalanine. (Pestana, 2012). The amino acid ADDA is often associated with the toxicity of the molecule due to the diene conjugate present (Dawson, 1998; He *et al*., 2016). The presence of MCs in drinking water sources in some water bodies located in southeastern China was associated with an increased risk of hepatocellular carcinoma in the population supplied (WHO, 2010).

Figure 2 - Chemical structure of microcystins with amino acid positions and variables (X and Z).



Source: Author (2018).

Table 1 - 17 analogs amino acids, variable amino acids occupy the X and Z positions, and their respective toxicity in mice.

Microcystin Analogs	***Variable amino acids* (X, Z)**	**Toxicity (LD_{50}) ($\mu g\ kg^{-1}$)**
Microcistina-LA	Leucina, Alanina	50
Microcistina-LAba	Leucina, Acido L-aminobutírico	-
Microcistina-AR	Alanina, Arginina	250
Microcistina-RA	Arginina, Alanina	-
Microcistina-YA	Tyrosina, Alanina	60-70
Microcistina-LF	Leucina, Phenilalanina	-
Microcistina-LR	Leucina, Arginina	50
Microcistina-LY	Leucina, Tirosina	90
Microcistina-HilR	L-homoisoleucina, Arginina	100
Microcistina-FR	Fenilalanina, Arginina	250
Microcistina-M(O)R	Metionina S-oxide, Arginina	700-800
Microcistina-HtyR	L-homotirosina, Arginina	-
Microcistina-YM(O)	Tirosina, metionina S-oxide	56-110
Microcistina-RR	Arginina, Arginina	500-800
Microcistina-YR	Tirosina, Arginina	150-200
Microcistina-(H4)YR	1,2,3,4-tetrahidrotirosina, Arginina	-
Microcistina-WR	Triptofano, Arginina	150-200

Source: Adapted by Pestana (2012).

Microcystins are stable, non-volatile, water-soluble compounds, produced at levels up to 1% of biomass and more linked to the oxidative stress of cyanobacteria (Pimentel and Giani, 2014). Some studies (Sivonen and Jones, 1999; Naselli-Flores *et al*., 2007) affirm that MCs concentrations in lakes and rivers can range from values less than 1 $\mu g.L^{-1}$ to values above 30,000 $\mu g.L^{-1}$, this wide variation can be justified by the large biomass differences of cyanobacteria in the water bodies in different trophic states. On the other hand, the authors affirmed that a significant portion of this variability can be attributed to fraction of toxic and non-toxic genotypes within cyanobacteria species and, to a lesser degree, to physiological variations, both characteristics lead to different cellular quotas of toxins such as toxin production per cell or biomass unit.

According to Salmaso *et al*. (2014), different blooms caused by the same species of cyanobacteria can present different concentrations of toxins, and consequently, different cellular quotas. The authors also claimed that, due to species genotype changes in natural environments, the variability of the MC cell rate exceeds the variability of species grown in laboratories two to three times. Dissolved or extracellular MCs concentrations may be increased into the environmental during blooms decline when cell lysis is triggered. MCs are chemically stable in water bodies and can persist for long periods, ranging from one to six months (Jones, Falconer and Wilkins, 1995). MCs play important physiological roles for cyanobacteria, including a defense mechanism against zooplankton herbivory (Jang *et al*., 2003) and potential intracellular functions such as iron chelation (Fe^{2+}) (Humble *et al*., 1994).

The MCs toxin synthesis is a complex process that may being influenced by environmental conditions, and by the genetic properties of each cyanobacteria strain (Kaebernick *et al*., 2000). The most significant environmental factor for microcystins production is the combination of nitrogen, phosphorus and carbon (N:P:C), which directly influences the rapid increase in MC concentrations per protein cell when exceeding the Redfield rate (Downing *et al*, 2005). According to Watanabe and Oishi (1985), there is a positive correlation between the toxin content produced and the specific growth rate as a function of the light intensity for species of *Microcystis aeruginosa*, thus, this factor may regulate the transcription synthesis of the genes of MCs.

There is still no consensus on the number of variants of MCs. Hotto, Satchwell and Bayer (2007) state that there are more than 80 variants. According to Dai *et al*. (2008), MCs present more than 70 different variants. Recent research developed by Niedermeyer (2015) affirms that this group of toxins can present more than 100 variants. What seems a

consensus is that each variant has different levels of toxicity; in mice bioassays, their lethal dose to 50% of individuals (LD_{50}) may range from 50 to 1200 $\mu g.kg^{-1}$, depending on the cyanobacteria species and the developmental conditions in which the producing cells were exposed (Hotto, Satchwell and Bayer, 2007). The LD_{50} for MC-LR-producing species was reported to be around 50 $\mu g.kg^{-1}$, while for MC-RR producing species this value can reach 600 $\mu g.kg^{-1}$ (Krishnamurthy *et al*., 1986; Watanabe *et al*., 1989). The World Health Organization recommends a safety limit of 1 $\mu g.L^{-1}$ of MC-LR in drinking water (WHO, 2010) and some countries such as Brazil, Australia and New Zealand already adopt these guidelines in their legislation (Burch, 2007).

The production of MC has been related to about 40 genera of benthic and planktonic cyanobacteria, distributed in several climatic areas of the planet. Examples of such genera are *Anabaenopsis sp*, *Aphanocapsa sp*, *Cylindrospermopsis sp*, *Gloeotrichia sp*, *Hapalosiphon sp*, *Nostoc sp*, *Plectonema sp*, *Phormidium sp*, *Pseudoanabaena sp*, *Rivularia sp*, *Synechococcus sp*, *Tolypothrix sp*, and *Woronochinia sp* (Paerl and Otten, 2013; Catherine *et al.*, 2013). According to Dai *et al*. (2008), MCs are continuously produced by some species of freshwater cyanobacteria belonging to the genus *Microcystis*, *Anabaena*, *Pseudanabaena*, *Planktothrix*, *Nostoc* and *Anabaenopsis*. Mez *et al*. (1997) identified the production of MCs by benthic cyanobacteria from an oligotrophic alpine lake and showed that the production of hepatotoxins is not restricted to planktonic species nor to environmental conditions considered typical for the occurrence of cyanobacteria blooms (Table 2).

Table 2 - Main genera and species producing MCs.

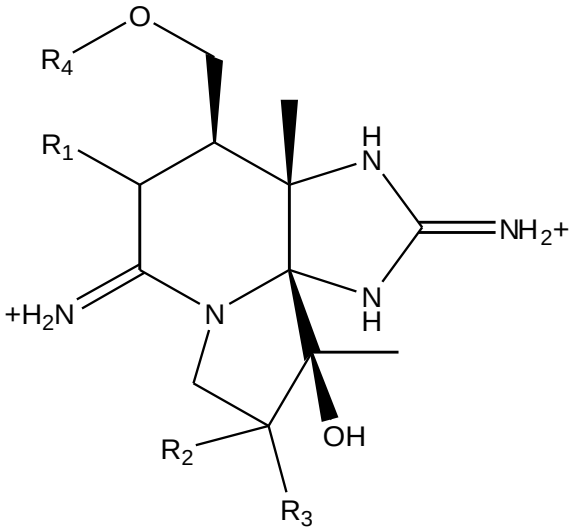
Toxics species	Life Style	MC produced
Anabaena subcylindrica	B*	MC-YR ; MC-LR
Anabaena subcylindrica	B	MC-YR ; MC-LR
Anabaena variables	B	MC-YR ; MC-LR
Anabaena variables	B	MC-YR ; MC-LR
Nostoc spongiaeforme	B	MC-YR ; MC-LR
Nostoc spongiaeforme	B	MC-YR ; MC-LR
Plectonema boryanum	B	MC-YR ; MC-LR
Plectonema boryanum	B	MC-YR ; MC-LR
Phormidium corium	B	MC-YR ; MC-LR
Phormidium corium	B	MC-YR ; MC-LR
Geitlerinema amphibium	P	MC-LR
Synechocystis aquatilis	P	MC-LR
Planktothrix agardhii	P	MC-LR
Microcystis aeruginosa	P	MC-LR
Microcystis aeruginosa	P	MC-LR, MC-RR, MC-LR, MC-YR, MC-LF, MC-LW, dm-MC-RR e dm-MC-LR
Microcystis aeruginosa	P	MC-LR
Microcytis ssp	P	MC-LR; MC-FR; H_4YR; WR
Microcystis aeruginosa	P	MC-LR; MC-FR; H4YR; YR; HtyR
Microcytis ssp	P	MC-LR; MC-RR
Planktothrix agardhii	P	MC-LR; MC-RR

Source: Author (2017). *(P) Planktonic e (B) Benthic

2.2.2 Saxitoxins (STXs)

The saxitoxins (STXs), also known as *Paralitic Shellfish Poison*, belong to an alkaloid group composed of 27 variants (Humpage, 2008), these toxins can be found in marine and freshwater environments. (O'neill *et al*., 2016). PSP analogs all share a 3, 4, 6 - trialkyltetrahydropurine molecule (Figure 3) with two guanidinium ($NH^{+}{}^{2}$) groups (Schantz *et al*., 1975). Variations in the side chains give the analogues varying levels of toxicity being grouped depending on their variations in the side chains. In Table 3, variations of the side chains to the tetrahydropurine for STX and its analogues, as well as the lethal dose in units of mice (20 g) in 15 minutes (units of mice / μmol) are observed.

Figure 3 - General chemical structure of saxitoxins



Source: Author (2018).

STX and neoSTX are not sulphated; Gonyautoxins (GTXs) are monosulfated and disulfated C-toxins. STX are polar and stable in solution (Schantz *et al.*, 1975), while C-toxins and GTXs can degrade to produce other toxic analogues (Fanger *et al.*, 1995). Thus, while the concentration of individual analogues may change, the toxin group may persist in the water for long periods, there being a potential for periods of prolonged exposure. (O'neill *et al.*, 2016).

Table 3 - Types of STXs produced according to the radical position (R1 to R4) and bioassay toxicity.

Radicals		R1	R2	R3	R4	DL50 (μg.Kg^{-1})
Toxins	STX	H	H	H	$OCONH_2$	1656–2483
	neoSTX	OH	H	H	$OCONH_2$	1038–2300
Gonyuatoxins	GTX1	OH	H	OSO3-	$OCONH_2$	1638–2468
	GTX2	H	H	OSO3-	$OCONH_2$	793–1028
	GTX3	H	OSO3-	H	$OCONH_2$	1463–2234
	GTX4	OH	OSO3-	H	$OCONH_2$	1803
	GTX5	H	H	H	$OCONHSO_3^-$	160
	GTX6	OH	H	H	$OCONHSO_3^-$	Not done
C-toxins	C1	H	H	OSO_3^-	$OCONHSO_3^-$	15–17
	C2	H	OSO_3^-	H	$OCONHSO_3^-$	237–329
	C3	OH	H	OSO_3^-	$OCONHSO_3^-$	33
	C4	OH	OSO_3^-	H	$OCONHSO_3^-$	143
Decarbamoylates	dcSTX	H	H	H	H	955–1274
	dcneoSTX	OH	H	H	H	Not done
	dcGTX1	OH	H	OSO_3^-	H	Not done
	dcGTX2	H	H	OSO_3^-	H	1617
	dcGTX3	H	OSO_3^-	H	H	1872
	dcGTX4	OH	OSO_3^-	H	H	Not done

Source: Adapted from Pestana (2012).

These cyanotoxins block the sodium channels (Na^+) in neural cells, preventing nerve transmission and altered homeostasis. Due to the damage caused to membrane permeability, it causes suffocation and death due to progressive respiratory muscle paralysis. Saxitoxins also block calcium (Ca^{2+}) and potassium (K^+) channels in cardiac cells, causing fatal cardiac arrhythmias (Campbell and Hille 1976).

The saxitoxins are produced by several genera of freshwater cyanobacteria, including *Aphanizomenon sp, Anabaena sp, Lyngbya sp* and *Cylindrospermopsis sp* (Lopez *et al*., 2008). According to the most recent literature, STX production is restricted to filamentous species of cyanobacteria belonging to the order Nostocales: *Dolichospermum circinales, Anabaena circinalis, Aphanizomenon gracile, Cuspidothrix issatschenkoi, Cylindrospermopsis raciborskii, Raphidiopsis brookii, Scytonema sp*. and Oscillatoriales *Lyngbya wollei, Geitlerinema sp., Phormidium uncinatum* (Pearson *et al.*, 2010; Borges *et al*., 2015).

The presence of STX has been reported in many countries (Kaas and Henriksen, 2000; Pereira *et al*., 2000; Clemente *et al*., 2010; Ballot *et al*, 2010; Carloto *et al*., 2015).

Wiedner *et al*. (2008) identified strains of the species *Aphanizomenon gracile* producing PSP toxin in two lakes in northeastern Germany, being the first evidence of the STX production by this species in German water bodies. In Table 4, it is possible to show the amplitude of occurrence of these cyanobacteria in the world, as well as the producing and analogous species produced.

Table 4 - Main species STXs-producing.

Country	Toxics species	Life style	PST produced	Reference
Australia	*Dolichospermum sp*	P*	C1, C2, GTX2, GTX3 e STX	Humpage *et al*., (1994)
Brazil	*Cylindrospermopsis raciborskii*	P	neoSTX e STX	Lagos *et al*., (1999)
USA	*Aphanizomenon flos-aquae*	P	neoSTX e STX	Mahmood; Carmichael (1986)
USA	*Lyngbya wollei*	P	dc-GTX2, dc-GTX3, dcSTX e seis análagos indefinidos	Carmichael *et al*. (1997)
Portugal	*Aphanizomenon flos-aquae*	P	STX, neoSTX, GTX5, dcSTX, GTX1,GTX3,GTX4, GTX5 e GTX	Pereira *et al*. (2000) Ferreira *et al*. (2001)
Portugal	*Aphanizomenon gracile*	P	neoSTX e STX	Pereira *et al*. (2004)
Germany	Aphanizomenon gracile	P	GTX5, STX, dcSTX e neoSTX	Ballot *et al*. (2010)
Italy	Planktothrix sp.	P	STX	Pomati *et al*. (2000)
China	Aphanizomenon flos-aquae	P	STX, neoSTX, GTX5, dcSTX e dcGTX3	Liu *et al*. (2006a) Liu *et al*. (2006b)
França	*Aphanizomenon gracile*	P	STX	Ledreux *et al*., 2009
Greece	*Aphanizomenon flos aquae*	P	STX	Gkelis; Zaoutsos (2013)
Greece	*Cylindrospermpsis raciborskii*	P	STX	Gkelis; Zaoutsos (2013)
Brazil	*Dolichospermum spiroides*	P	STX , NEO-STX	Molica *et al*. (2005)

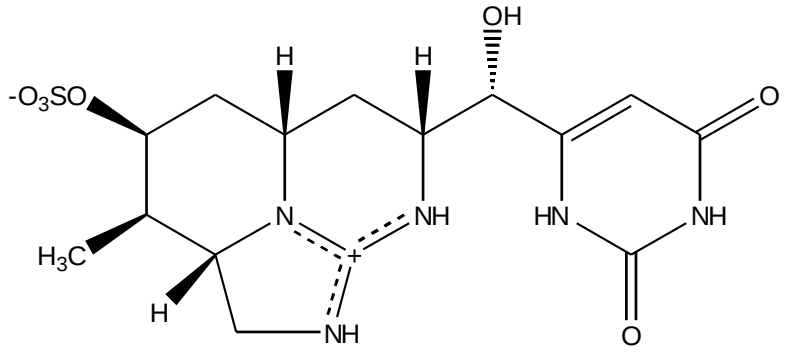
Sources: Author (2017). *(P) Planktonic e (B) Benthic

The toxicity of variants of this group of alkaloids, as well as MC, varies greatly, with saxitoxin being the most potent. According to Chorus (2001), the recommendation for the maximum concentration for saxitoxins is 3 $\mu g.L^{-1}$ for drinking water, a value also used as maximum value by Brazilian potability ordinance. Clinical signs of human intoxication include dizziness, numbness of the mouth and extremities, muscle weakness, nausea, vomiting, thirst, and tachycardia. Symptoms may begin 5 minutes after ingestion and death within 2 to 12 hours. In cases of non-lethal dose intoxication, the symptoms usually disappear from 1 to 6 days (Carmichael, 1994).

2.2.3 Cylindrospermopsin (CYN)

Cylindrospermopsin (CYN) is a cytotoxic alkaloid that inhibits protein and glutathione synthesis and targets the liver primarily (Figure 4). Other organs such as the kidneys, lungs, spleen, adrenal glands, intestinal tract, immune system and heart may also be affected, leading to death with an LD_{50} of 2.1 $\mu g.kg^{-1}$ (Ohtani *et al*., 1992; Runnegar et al., 2002).

Figure 4 - General chemical structure of Cylindrospermopsin (CYN).



Source: Author (2017).

CYN is an important compound for the management of harmful cyanobacteria blooms because, in addition to their high toxicity, there is evidence that they cause chronic effects even when the individual is exposed to concentrations below the toxicity threshold. Initial studies indicate that this toxin inhibits protein synthesis in humans, causes DNA chain breakdown, hepatotoxicity, and changes in cholesterol levels and red blood cell phospholipids (Froscio *et al*., 2003). In the short term, CYN can cause inflammation in the liver, causing hemorrhage. (Wiener *et al*., 2004). Exposure to CYNs can also lead to pneumonia, dermatitis when the subject is exposed for long periods, and is carcinogenic when given continuously at low doses (Runnegar *et al*., 2002, Froscio *et al*., 2003, Pearson *et al*., 2016).

Evidence for the presence of CYN in Western Europe is recent. The first reports date from the beginning of the last decade (Fastner *et al*., 2003; Manti *et al*., 2005), although there have been reports of the presence of these toxins in Australia for several decades (Byth, 1980). The species *Cylindrospermopsis raciborskii* is a cyanobacteria most closely related to the production of CYN, and it was from this species that CYN was initially isolated and

identified (Pearson *et al*., 2016). In previous studies, it was thought that this CYN-producing species originated in tropical environments, such as Australia (Padisák, 1997; Komárek and Komárková, 2003; Schaw *et al*., 1999) or Brazil (Bouvy *et al*., 2000) but now it can be found in many subtropical and temperate lakes in Europe and North America (Hamilton *et al*, 2005; Chapman and Schelske, 1997).

According to Seifert *et al*. (2007), *Lyngbya Wollei* species produces a potent cylindrospermopsin and a deoxy-cylindrospermopsin analogue (deoxy-CYN). The highest concentrations in environmental samples were 20 and 550 $\mu g.g^{-1}$ dry weight for CYN and deoxy-CYN, respectively. According to Wimmer *et al*. (2014), two new CYN analogs produced by the species *Cylindrospermopsis raciborskii* have been discovered, these analogues being described as 7-deoxy-cylindrospermopsin and desulfo-7-deoxy-12-desulfo-acetylcylindrospermopsin, thus totaling, up to now, five CYN analogs.

Other species have also been identified as producers of CYN (Table 5) worldwide, such as *Aphanizomenon flos-aquae* in Germany (Preussel *et al*., 2006), *Aphanizomenon ovalisporum* (Banker *et al*., 1997; Schaw *et al*., 1999; Wormer *et al*., 2008, Yilmaz et al., 2008), *Anabaena bergii* (Schembri *et al*., 2001) and *Lyngbia wollei* in Australia (Seifert *et al*., 2007), *Anabaena lapponica*, Finland (Spoof *et al*. 2006), *Raphidiopsis curvata* in China (Li *et al*., 2001, Sinha, 2015), *Umezakia Natans* in Japan (Harada *et al*., 1994), *Cylindrospermopsis raciborskii* (Li *et al*., 2001; Jiang *et al*., 2014; Sinha et al., 2014; Sinha, 2015).

Table 5 - Main CYN-producing species.

Country	Toxics species	Life style	PST produced	Reference
Portugal	*Aphanizomenon*	P(1)	CYN	Moreira *et al*. (2017)
Israel	*Aphanizomenon ovalisporum*	P	CYN	Guzmán-*Guillén et al*., (2014)
Germany	*Aphanizomenon flos-aquae*	P	CYN	Pruessel *et al*., (2006)
France	*Aphanizomenon flos-aquae*	P	CYN	Brient *et al*., 2008
France	*Aphanizomenon flos-aquae*	P	CYN	Brient *et al*., 2008
France	*Anabaena planctonica/ Aphanizomenon flos-aquae*	P	CYN	Brient *et al*., 2008
France	*Anabaena planctonica*	P	CYN	Brient *et al*., 2008
Germany	*Aphanizomenon flos-aquae*	P	CYN	PruesseL *et al*., 2006
Israel	*Aphanizomenon ovalisporum*	P	CYN	Banker *et al*., 1997
Spain	*Aphanizomenon ovalisporum*	P	CYN	Wormer *et al*., 2008
Australia	*Anabaena bergii*	P	CYN	Schembri *et al*., 2001
Australia	*Cylindrospermpsis raciborskii*	P	CYN	Schembri *et al*., 2001
Japan	*Umezakia natans*	P	CYN	Harada *et al*., 1994
Tailand	*Cylindrospermpsis raciborskii*	P	CYN/ deoxy-CYN	Li *et al*., 2001
Finland	*Anabaena lapponica*	P	CYN	Spoof *et al*., 2006
Australia	*Aphanizomenon ovalisporum*	P	CYN	Schaw *et al*.,1999
USA	*Aphanizomenon ovalisporum*	P	CYN	Yilmaz *et al*., 2008
Australia	*Cylindrospermopsis raciborskii*	P	CYN	Sinha *et al*., 2014
Australia	*Lyngbya wollei*	B	CYN/ deoxy-CYN	Seifert *et al*., 2007

Source: Author (2017). *(1) Planktonics e (2) Benthic

In Germany, CYN is found as frequently as microcystins, with maximum concentrations of 12 $\mu g.L^{-1}$ (Fastner *et al*., 2007). According to McGregor and Fabbro (2000), of the 47 water bodies studied in Australia, 14 presented a mean concentration of 3.4 $\mu g.L^{-1}$ of CYN. Concentrations above 90 $\mu g.L^{-1}$ of CYN were detected in treated water in Florida (Falconer and Humpage, 2006).

The presence of CYN is a particular concern in water treatment plants, since they are primarily in their extracellular form, in contrast to microcystins that remain stored in the cells and can be removed during water treatment (Svrcek and Smith, 2004). Studies by Schaw *et al*. (1999) and Fastner *et al*. (2007) demonstrated the predominance of extracellular CYN

fraction, according to them, about 80% of the total CYN concentration was dissolved when the species *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, and *Aphanizomenon sp*. were present. Considering that CYNs are abundant in the extracellular fraction and that they can remain stable in the water for weeks, the presence of these organisms in waters used for the public supply is a source of concern for the water treatment companies (Wormer *et al*., 2008).

2.3 Detection methods

There is a wide range of cyanotoxin analysis methods, such techniques used in detection and quantification, are divided into physical, biological and physical chemical techniques. However, according to the type of expected results, the samples often require specific preparations prior to analysis. (Merel *et al*., 2013).

Cyanotoxins can be detected and quantified by biological methods such as live bioassays, immunological, or biochemical assays. The method of the bioassay in mice is the most known method used in living organisms. This method was the first developed for the cyanotoxins detection in water and, it consists ot the injection in peritoneal area, in at least 3 mice, and posteriorly necropsy after 24 hours. (Merel *et al*., 2013)

The methodology described by Falconer *et al*. (1993) presents that observation of different symptoms reveals the presence of hepatoxins or neurotoxins. Although it is a very usual method and observation of the effects of cyanotoxians on the living organisms and of affected organs regarding the volume and characteristics of the cells, it is not possible to identify the type of the toxin, not the cyanotoxin species (Merel *et al*., 2013). In addition, due to ethical issues and the development of faster, more sensitive and specific methods for the measurement of cyanotoxins the use of bioassay in mice is limited mainly to toxicological research.

Cyanotoxins can also be detected by recognition and binding to specific antibodies, this method is based on the recognition of peptides by monoclonal antibodies. According to Merel *et al*. (2013), various Enzyme-Linked Immunosorbent Assay (ELISA) kits are commercially available for MCs detection in water. According to Msagati *et al*. (2006), although this method may detect small concentrations of microscitins; ELISA-based detection methods also have some limitations. For example, different MC variants cannot be distinguished and the results must be expressed as MC-LR / L equivalents.

Another limitation of ELISA is cross-reactivity (even limited) with other compounds, which may lead to overestimation of toxin concentration (Chu *et al*., 1989). These authors perceived cross-reactance of MCs variants and found that there were high good cross-reactivities for MC-RR and poor MC-LA. Depending to the antibody and on procedure employed, these methods are extremely sensitive and can reach a detection limit as low as 4 ng. L^{-1} (Lindner *et al*., 2004). Therefore, the ELISA assay is successfully employed for the detection of MCs, and specific antibodies were also designed to apply this method for the detection of CYNs and STXs (Bláhová *et al*., 2009; Campbell *et al*., 2009).

Bláhavá *et al*. (2009) studied water bodies in Czech Republic, and they verified that the presence of CYN determined by ELISA in all samples, and these samples were confirmed by Liquid chromatography-mass spectrometry (LC-MS) method. In this study, there was qualitative agreement between ELISA and LC-MS, and the results were significantly correlated. For these authors ELISA was confirmed as a good detection method for CYN.

Mass spectrometry (MS) has become increasingly common in recent decades because of its high sensitivity compared to other methods and its availability for liquid chromatography (LC-MS) and gas chromatography (GC-MS). In addition, MS detects compounds based on their mass and charge, which limits the potential for interference and improves selectivity. (Merel *et al*., 2013). Futhermore, the development of mass spectrometry also increases specificity through further discrimination of compounds with mass and charge through their specific fragmentation pattern when colliding with the inert gas molecules.

The CYN analysis can be performed by HPLC-MS / MS, being the linearity range of that assay 1-600 $\mu g.L^{-1}$ (Eaglesham *et al.,* 1999). Another approach is the High Performance Liquid Chromatography with photodiode array detection (HPLC-PDA) method, although it is not ideal for systems with complex matrices such as environmental blooms or detection in animal tissues (Welker *et al*., 2002). Several researchers have stated that the insertion of a cleaning step prior to analytical detection has significantly improved the toxin's resolution (Carmichael *et al*., 2001; Norris *et al*., 2001). An ELISA kit for CYN usually has a sensitivity that allows determination in a range of environmental samples such as water, blood or fish tissue samples. Recently, the detection of CYN biosynthesis genes (*cyr* or *aoa*) has been used to infer the potential toxicity of environmental samples; however, the use of these molecular methods has been mostly limited for research. (Mihali *et al*., 2008; Rasmussen *et al*., 2008; Baron-Sola *et al*., 2012).

GC-MS methods were developed and proved successful in the analysis of some cyanotoxins such as MCs, but very complex procedures are required for the preparation of the sample. In fact, GC-MS methods available for MC analysis require even sample oxidation, post-treatment to remove remaining reagents and derivatization. (Merel *et al*., 2013) Consequently, cyanotoxins are mainly detected by LC-MS or LC-MS /MS, following simultaneous detection of a larger quantity of toxins with a sample preparation procedure (Kaushik and Balasubramanian, 2013).

Cyanotoxins can also be detected by MS without preliminary separation chromatography, for example, MALDI-TOF (Matrix-assisted laser desorption/ ionization) instruments can be used to perform toxin analysis in a very small sample volume, such as cell colonies (Kaushik and Balasubramanian, 2013). Molecules included in the solid and dry samples are ionized by a laser beam and identified through the high mass resolution provided by the TOF instrument. However, TOF mass spectrometers generally tend to be less sensitive than other mass spectrometers of the same generation (Merel *et al*., 2013).

3 CLIMATE CHANGE AND ITS RELATION WITH THE PREDOMINANCE OF CYANOBACTERIA.

3.1 Cyanobacteria in a climate change scenario

Climate change contributes to cyanobacteria blooms for the fact to create ideal conditions for cyanobacteria increasing, futhermore, several studies have reported that the enrichment of water bodies with nutrients (mainly nitrogen and phosphorus) together in addition the increase in temperature, carbon dioxide, and changes in hydrological patterns strongly influence frequency, intensity, and duration of cyanobacterial blooms creating a perfect conditions for cyanobacteria growth and blooms (Peeters *et al*., 2007; Jöhnk *et al*., 2008; Paerl and Huisman, 2008, 2009; Wagner and Adrian, 2009; O'neil *et al*., 2012; Paerl and Paul, 2012; Mowe *et al*., 2015, Bennett, 2017). This evidence raises concerns in areas such as drinking water supply, irrigation, fishing, aquaculture, and aquatic recreational activities, according National Oceanic and Atmospheric Administration (NOAA, 2018) *Harmful Algal Blooms* (HABs) cause about US$ 82 million in economic losses to the seafood, restaurant and tourism fields each year. According Dodds *et al* (2009) *CyanoHABs* result in losses of reacreational, drinking and agricultural water resources that are worth higher than US$ 2 billion annually.

Climate change may affect precipitation patterns, especially intensity and duration of droughts and floods around the globe (O'Neil *et a*l 2012). Dry summers are apparently increasing in intensity and duration, which may promote the predominance of cyanobacteria in continental aquatic environments (IPCC, 2007; IPCC 2012). Besides that, precipitations with higher intensities can lead the transport of nutrients to water bodies with elements derived from erosion, or simply, by the surface runoff and discharge of groundwater (Sherman *et al*., 1998; Mitrovic *et al*., 2003). Changes in precipitation patterns can also directly influence water quality by altering the hydrology of inland waters, leading to different dilution rates and changes in physical and chemical conditions and water quality of water bodies (Codd, 1999; IPCC, 2007).

According to The Intergovernmental Panel on Climate Change (2007), the burning of fossil fuels and the consequent increase in the concentration of atmospheric carbon dioxide has caused an increase of approximately 1 ° C in the earth's surface in the 20th century, with a more pronounced increase in the last 40 years. In addition, predictions of temperature

increase for the current century are 1.5 to 5 ° C (Houghton *et al*., 2001; IPCC, 2007). Besides that, Trenberth *et al*. (2007) have researched that in the last century the average global air temperature at the surface increased by 0.75 ± 0.18 ° C. This tendency continues more pronounced in regions of high latitudes than low latitudes. Other authors suggest more alarming values for the tropical regions, with temperature increase from 4 to 6 º C (Corlett, 2012; Trewin, 2014). According IPCC (2014) tropical areas are the most susceptible to global warning, futhermore, for instance Sakai *et al*., (2006) say that in this scenario, tropical semi-arid regions will be affected, because it is increasing in frequency and intesity of droughts.

3.1.1 Cyanobacteria and temperature increasing

Increasing in temperature may promote cyanobacterial proliferation because these microorganisms are prokaryotes, and their growth rates are optimized at high temperature (Paerl and Paul, 2012). Temperature which cyanobacteria present a maximum growth rate depends on the species of cyanobacteria, and can vary from 20º C for the species such as *Aphanizomenon flos-aquae* and *Planktothrix agardhii*, 28º C for *Microcystis aeruginosa*, and higher than 30º C for species of the genus *Synechococcus sp*. (Reynolds, 1989, 2006). According to Carey *et al*. (2011) the acceleration rate, commonly measured as Q_{10} (acceleration along a step of 10 ° C, usually 10 to 20 ° C) was 2.6 for *Synechococcus sp*, whereas for the *Microcystis aeruginosa* species it was 9.6. This value was the highest recorded among the cyanobacteria and eukaryotic phytoplankton; therefore, *Microcystis aeruginosa* species has a physiological advantage when the water temperature is above 20 ºC.

Paerl and Huisman (2008) stated that cyanobacteria exhibit optimal growth rates at relatively high temperatures, usually above 25 ºC. When temperatures are high, cyanobacteria compete more efficiently against eukaryotic microalgae. Several researches suggest that temperatures around 20 ºC modify the growth rates of freshwater eukaryotic phytoplankton (including diatoms, chlorophytes, cryptophytes and dinoflagellates), and generally tend to stabilize or decrease, while growth rates of cyanobacteria tend to increase, providing a competitive advantage (O'neil *et al*., 2012, Canale and Vogel, 1974, Peperzak, 2003, Paerl and Huisman, 2009).

Rising temperatures can not only promote the physiological benefits of cyanobacteria development, but also promote changes in the physical characteristics of aquatic environments so that they can be favorable to cyanobacteria. For example, higher

temperatures may decrease the viscosity of surface water and increase nutrients diffusion to the surface of the cell. (Vogel, 1996; Peperzak, 2003; O'neil *et al*., 2012).Therefore, once again, cyanobacteria will be favored, whereas many of these organisms can regulate their buoyancy in the water column to compensate for the greater tendency of sedimentation. A decrease in viscosity will preferably promote the sinking of most non-mobile phytoplankton, or with weak buoyancy regulation mechanisms, giving an additional advantage to cyanobacteria (Wagner and Adrian, 2009; Paerl and Huisman, 2009).

O'Neil *et al*. (2012) reported that another change resulting from the increase in temperature, which may favor cyanobacteria, is the increase in the frequency, intensity, and duration of thermal lake stratifications. This process reduces nutrients availability in the superficial layers, but increases in the deeper layers, favoring cyanobacteria that can regulate their buoyancy to obtain the nutrients of deeper water or in diazotrophic environments. For these reasons, it is estimated that cyanobacteria blooms may affect increasing numbers, duration and intensity as global temperatures increase. The intense light absorption by dense surface blooms of cyanobacteria can increase the water temperature locally (Kahru *et al*., 1993; Ibelings *et al*., 2003), thus creating a positive feedback that perpetuates the dominance of *CyanoHABs* (Hense, 2007).

In laboratory studies with cyanobacteria producing hepatotoxic metabolites, Gehringer and Wannicke (2014) demonstrated that the temperature increase altered microcystins (MC) production, differently in different species of cyanobacteria. The MC-LR concentrations of *Anabaena sp* strains presented high results at approximately 25ºC, decreasing when the temperature was higher than 30ºC and below 20ºC. Rapalla *et al*. (1997) found that MC-RR concentrations increased steadily between temperatures of 12.5° to 30° C, with concentrations stabilization of this variant from that temperature. Mowe *et al*., (2015) examined the effects of four different temperatures (27, 30, 33 and 36 ºC) on growth rate and microscystin production of five isolated tropical strains: two strains of *Microscystis ichthyoblabe*, one strain of *Microscystis viridis*, one strain of *Microscystis flos-aquae*, and one strain of *Microscystis aeruginosa*. These authors concluded that there were correlations between temperature and growth rate for microscistin production by cell (cellular Quota). The researchers found that the increase in temperature did not significantly affect growth for most of the *Microcystis* strains at higher temperatures. When the temperature was higher than 36º C, all five *Microcytis* strains had lower cell quota. The results show that the increase in the

average water temperature, resulting from the climatic changes in general, may influence the toxin by cells of the *Microcystis* species.

The studies proposed by Briand *et al*., (2004) and Chonudomkul *et al*., (2004), affirm that *Cylindrospermopsis raciborskii* species showed blooms at temperatures ranging from 20 to 35 ° C, this wide temperature range allowed this cyanobacteria to invade temperate zones (Hong *et al*., 2006). In temperate regions, *C. raciborskii* species is found during the summer, when temperatures are around 26ºC (Hamilton *et al*., 2005). Some studies have shown that this species exhibits permanent blooms in tropical lakes throughout the year, while in subtropical lakes they are restricted to the summer (Bouvy *et al*., 2000, Burford and O'donohue 2006; Barros *et al*., 2015, Carlotto *et al*., 2015). The authors hypothesized that either this species is adapting to lower temperatures, or the temperature in those places is increasing, leading to better development at low latitudes.

The studies developed by Padisák and Reynolds (1998) showed that *C. raciborskii* species occurred in the summer of 1982, 1992 and 1994 in Lake Balaton, Hungary, when temperature average increased by approximately 2º C. Other authors such as Hong *et al*. (2006) observed *C. raciborskii* blooms on Lake Mona in Michigan (USA) during August and September, according to these authors, the surface and the bottom temperature of the lake showed increase compared with the historical averages. The studies cited suggest evidence that blooms is a result of climate change, especially when the temperature of the bottom reaches higher temperatures, facilitating the acinetos germination (Sinha *et al*., 2012). Additionaly, at water with higher temperatures, heterotrophic bacterial activity is significantly increased by decreasing the oxygen concentration in the lower extracts of the water column and in the sediment (Turner et al., 1987). Under these conditions the nutrients immobilized in the sediments, mainly phosphorus, are solubilized, feeding cyanobacteria toxics blooms (Paerl 1988).

In adittion, under adverse solar radiation conditions on water surface, cyanobacteria present photoprotective accessory pigments and compounds that absorb UV radiation, guaranteeing survival for long periods of exposure to high irradiance (Castenholz and Garcia-pichel, 2000; Paul, 2008; Carreto and Carignan, 2011). Carotenoids may, for example help protect the surface cyanobacteria blooms from UV rays, also cyanobacteria have been shown to adapt to UV-B radiation for hours or even days after exposure (Paerl *et al*., 1983).

3.1.2 Cyanobacteria and salinity patterns

According to Moisander *et al*. (2002), increasing frequency and severity of droughts has caused salinity increase in aquatic environments such as lakes, reservoirs and estuarine areas around the world. The same authors state that climate change can also affect salinity in estuaries and freshwater systems connected to estuaries. Due to the increase in sea level, an increase in the duration and frequency of droughts, in some regions, thus causing the dominance of *CyanoHABs* due to increase concentration of salts in the water bodies affected.

Increased salinity may also indirectly affect water bodies by increasing stratification in freshwater lakes, due to changes in vertical density, which will benefit cyanobacteria blooms, because genera including *Anabaena sp*, *Microcystis sp* and *Nodularia sp* may regulate their buoyancy in more saline water conditions (Moisander *et al*., 2002). Although many phytoplankton eukaryotic species present tolerance to salted environments, salinity can affect composition of the community as well as distribution of the toxin concentration. According to Ross et al. (2006), intermittent fluctuations in salinity between 15 and 20 $g.L^{-1}$ can still allow survival of *Microcystis* populations, but cause saline stress, leading to the risk of cell disruption and excretion of microcystin. *Anabaena sp* and *aphanizominoides sp* can withstand salt concentrations of up to 15 $g.L^{-1}$, while *Anabaenopsis sp* and *Nodularia spumigena* can tolerate salinities between 0 and 20 $g.L^{-1}$ (Tönk *et al*., 2007). The high tolerance of the cyanobacteria to saline environments is evidenced by the increase in the blooms occurrence in brackish waters (Paerl and Paul, 2012).

3.1.3 Cyanobacteria and CO_2 patterns

According to the IPCC (2007, 2012) one of the main causes of global warming is the increase in the concentration of atmospheric carbon dioxide (CO_2), which is generated by combustion of fossil fuels and biomass burning. Fossil fuels Combustion during the last two centuries has significantly increased the concentration of CO_2 in the atmosphere with projections of increase for the next decades (IPCC, 2007). Cao and Caldeira (2008) affirm that because of these changes, the aquatic chemistry environments are and will continue to be influenced by the increase in the concentration of this compound, either by changes in pH, or decline in carbonate ion concentration.

In waters with a high concentration of nutrients the phytoplankton community, in general, presents high photosynthetic activity and consequently, demand high amounts of CO_2. On the other hand, predominate carbonate (CO_3^{3-}) and bicarbonate (HCO_3^-) forms of carbon. Under these conditions, less than 1% of dissolved inorganic carbon (DIC) is composed of CO_2, making it a limiting factor for phytoplankton growth, except for cyanobacteria that have capacity to regulate their buoyancy and to capture atmospheric CO_2 (Paerl and Ustach, 1982). Low concentrations of DIC and low luminosity are limiting conditions for eukaryotic phytoplankton, ensuring conditions for the dominance of cyanobacteria. The pH of the water bodies is linked to the concentration of Dissolved Inorganic Carbon (DIC), such as CO_2; carbonic acid (H_2CO_3); bicarbonates (HCO_3^-); or carbonates (CO_3^{3-}); many lakes are supersaturated with CO_2, due mainly to the decomposition of organic matter and respiration products (Cole *et al*., 1994). The pH and inorganic carbon concentration in lakes can vary widely, on a daily, episodic, or seasonal scale with variations of up to 2 pH units and 60 μmol.DIC L^{-1}. (Maberly, 1996).

The reduction of the concentration of DIC associated with the algal blooms in eutrophic lakes may cause a reduction of phytoplankton due to the limitation of the carbon concentration. On this, it is hypothesized that cyanobacteria can have an advantage over others phytoplankton, in a sense that it can capture of carbon dioxide (CO_2), which causes the microorganisms to capture CO_2 and diffuse rapidly into the water column, when carbon concentration is a limiting factor for the growth (Paerl and Huisman, 2009).

Some studies such as those proposed by Shapiro and Wright (1990) and Czerny *et al*. (2009) suggest that cyanobacteria decrease cell growth rate in response to low pH conditions. While, studies proposed by Fu *et al*., 2007, 2008; Hutchins *et al*., 2007; Levitan *et al*., 2007; Riebesell *et al*., 2007; Kranz *et al*., 2009 demonstrate that the other cyanobacteria increase the rate of cell division and fixation of carbon, or both mechanisms, when the concentration of CO_2 increases.

In summary, cyanobacteria present a series of ecophysiological adaptations, specific for each genus, that give these organisms competitive advantages over other photosynthetic organisms 1) ability to grow in warmer temperatures, 2) buoyancy due to the production of gas vesicles 3) high affinity and ability to store phosphorus, 4) nitrogen fixers, 5) acinetos production and, finally, the ability to capture light at various wavelengths and intensity (Carey *et al*., 2011).

4. ENVIRONMENTAL FACTORS ASSOCIATED WITH TOXIC CYANOBACTERIAL BLOOMS ACROSS 20 DRINKING WATER RESERVOIRS IN A SEMI-ARID REGION OF BRAZIL

4.1 Introduction

Cyanobacteria are gram-negative, prokaryotic organisms widely distributed in aquatic and terrestrial environments from the poles to the equator, often times dominating other planktonic and benthic species in aquatic systems (Sompong *et al*. 2005, Kleinteich *et al*. 2012, Paerl and Otten 2013). Cyanobacteria are known to produce a wide variety of bioactive secondary metabolites (Sivonen and Jones 1999; Smith *et al*. 2008) that are not essential for cyanobacterial growth or metabolism (Vining 1992), but are capable of causing unpleasant tastes and odors and acute and/or chronic damage and mortality to animal health (Van Apeldoorn *et al*. 2007; Stewart and Falconer 2008, Smith *et al*. 2008). Previous studies have reported more than 40 species of cyanobacteria representing 20 genera in three orders known to produce toxins, including cyclic peptides and alkaloids (Stewart and Falconer 2008; Van Apeldoorn *et al*. 2007; Westrick *et al*. 2010; Glekis and Zaoutsos 2013). Moreover, fifty-one cyanobacterial species have been reported to produce taste and odor compounds, such as 2-methylisoborneol (MIB) and geosmin (Smith *et al*. 2008), that can negatively affect the palatability of drinking water and farmed fish (Izaguirre and Taylor 1995; Zimba and Grimm 2003; Suurnäki *et al*. 2014).

Most cyanotoxins can be divided into hepatotoxins (e.g., microcystin and nodularin), cytotoxins (e.g., cylindrospermopsin), neurotoxins (i.e., anatoxin-*a*, anatoxin-*a(s)* and saxitoxin), dermatoxins (e.g., lyngbyatoxins and aplysiatoxin), and protease inhibitors (Sivonen and Jones, 1999; Klisch and Hader, 2008; Sivonen and Borner, 2008; Smith *et al*. 2008). In addition, cyanobacteria have the ability to produce the neurotoxic compound, *beta-methylamino-L-alanine* (BMAA) (Cox *et al*. 2003, 2005, 2009), which may be produced by most cyanobacterial genera (Downing *et al*. 2011).

In Brazil, monitoring the frequency and concentration of cyanobacterial toxins has intensified after the death of 65 patients in a hemodialysis clinic in Caruaru in the state of Pernambuco due to microcystin poisoning (Carmichael *et al*. 2001). Consequently, the monitoring of drinking water sources for cyanobacteria and their toxins has been made mandatory in Brazil since 1996. In Ceará state, CAGECE (2010) reports that neurotoxic

cyanobacterial blooms have been increasing in frequency in reservoirs that supply the main cities. Concomitantly, agriculture, untreated sewage, and intensive fish farming within the reservoirs continue to compromise water quality in Ceará (COGERH, 2008).

Natural and anthropogenic nutrient pollution in concert with increasing global temperatures are known to promote toxic cyanobacterial blooms (Paerl and Paul, 2012). For example, several studies have shown that temperatures around 20°C cause stable or reduced growth rates for many freshwater eukaryotic phytoplankton taxa, including diatoms, chlorophytes, cryptophytes, and dinoflagellates, while growth rates of cyanobacteria tend to increase at and above 20°C, providing cyanobacteria with a competitive advantage (Dokulil and Teubner 2000; Paerl and Huisman 2008; Paerl and Huisman 2009, O'Neil *et al.* 2012; Huisman *et al*. 2018).

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

According to the Intergovernmental Panel on Climate Change IPCC (2007), average global temperatures may increase in the present century from 1.5 to 5 ºC. In the tropics, temperatures may increase from 4 to 6 ºC, making climate change a constant concern for equatorial communities (Corlett 2012; Trewin 2014; Mowe *et al*. 2015). For example, in Ceará during the last 55 years, the average temperature has increased by an average of at least 1 °C since the 1960's (INMET 2016), similar to the observed temperature shifts recorded by IPCC (IPCC 2007). Such an increase in temperature may explain the invasion of cyanobacterial species from tropical to subtropical regions (Mitrovic *et al*. 2011). For example, *Cylindrospermopsis raciborskii* is thought to have expanded from the tropics to mid-latitude regions of Europe, North America, and South America (Chapman and Schelske 1995; Briand *et al*. 2004). Thus, the study of these organisms is fundamental for water resource managers around the world, and especially those managing drinking water sources (Padisak 1997).

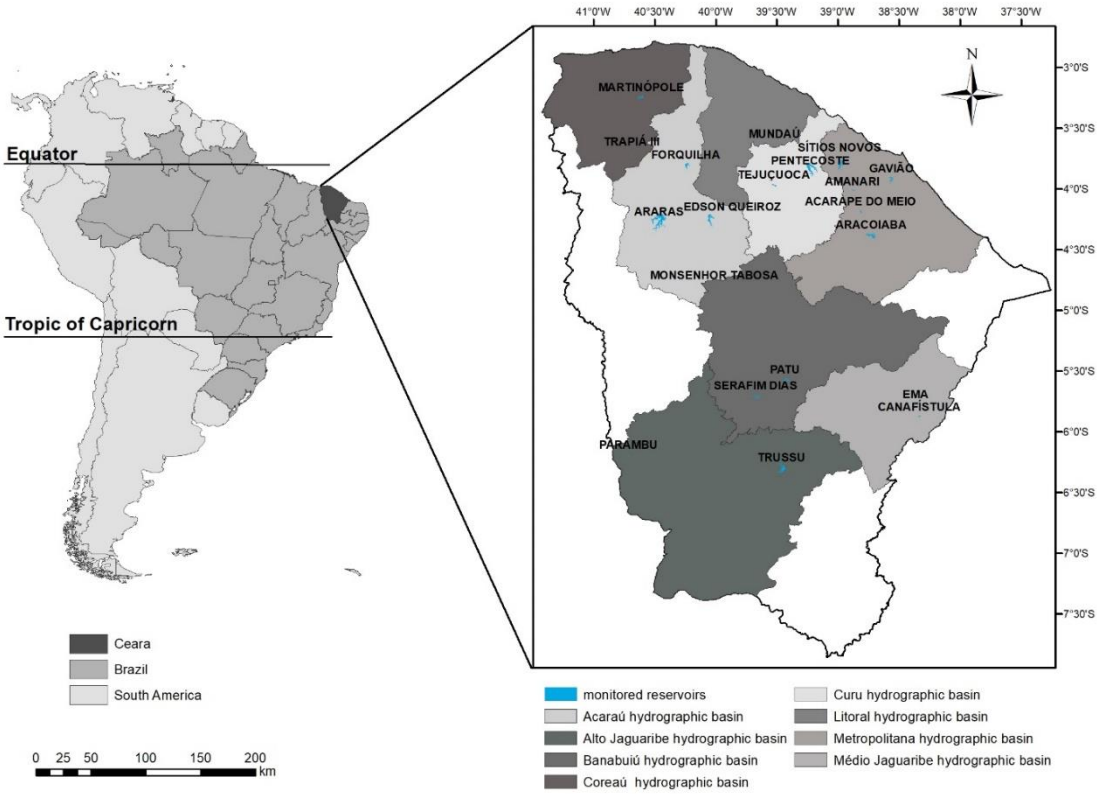
With the increase in water temperature by climate change, which has the potential to increase toxin-producing cyanobacterial blooms, there is a pressing need to monitor drinking water reservoirs in poorly studied, semi-arid regions. However, methods to identify toxigenic cyanobacteria in environmental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

4.2 Material and methods

4.2.1 Study site

The Ceará state is located in northeastern Brazil between latitudes S2 º 30’ 00” and S8º 52’ 00” and longitudes W37 º 14’ 00” and W41º 30’ 00” (Figure 5). The study site belongs to the semi-arid region of Brazil characterized by a low, irregular annual rainfall extending from early January to May (IPECE, 2017; Neto *et al*., 2014). Besides low precipitation, the Ceará region has several conditions that are ideal for cyanobacterial growth, such as a high solar radiation (18 Mj m^{-2} day^{-1}) during approximately 8 hours day^{-1}, surface water accumulation in reservoirs with high retention times, high average annual temperature, and intensive anthropogenic activity leading to the eutrophication of its reservoirs (Barros et al. 2017; COGERH 2017; FUNCEME 2017). Data included in this study were from 20 drinking water reservoirs located in nine hydrographic basins and managed by CAGECE (Table 6).

Figure 5 - Map of Ceará state, Brazil, showing the geographical location of the 20 studied reservoirs.



Source: Author (2017)

Table 6 - General characteristics of the studied reservoirs including trophic classification according to COGERH (2017). N/A = not available.

Code	**Reservoir**	**Trophic Classification**	***Capacity*** (m^3)	***Hydraulic basin (ha)***	***Water Retention time (days)***
AM	Acarape do meio	Mesotrophic	31,500,000	220	326
AMR	Amanari	Eutrophic	11,010,000	271	417
AR	Aracoiaba	Eutrophic	170,700,000	1506	775
BO	Boqueirão	Hypertrophic	28,110,000	512	702
CN	Canafístula	Eutrophic	13,110,000	315	N/A
AT	Trussu	Eutrophic	301,000,000	5509	N/A
EQ	Edson Queiroz	Hypertrophic	254,000,000	2660	N/A
E	Ema	Hypertrophic	10,390,000	284	N/A
FQ	Forquilha	Hypertrophic	50,132,000	923	N/A
G	Gavião	Eutrophic	32,900,000	618	22
MRT	Martinopole	Hypertrophic	23,200,000	647	N/A
MT	Monsenhor Tabosa	Hypertrophic	12,100,000	185	1360
MU	Mundaú	Eutrophic	21,300,000	123	N/A
PT	Patú	Hypertrophic	71,829,000	856	668
PS	Paulo Sarasate	Hypertrophic	891,000,000	9600	1013
PM	Pereira de Miranda	Hypertrophic	395,638,000	5700	792
PU	Puiu (Parambú)	Eutrophic	8,530,000	159	N/A
SD	Serafim Dias	Hypertrophic	43,000,000	688	513
SN	Sítios Novos	Hypertrophic	126,000,000	2010	685
T	Trapia III	Eutrophic	5,510,000	130.2	N/A

4.2.2 Sampling and analytical methods

Samples were collected regularly (weekly or monthly) at each water treatment plant intake from the 20 reservoirs at a depth of 50 cm from the surface by CAGECE from January 2013 to January 2017. According to Brazilian law PCR nº 5/2017, whenever cyanobacterial density exceeds 20,000 cells mL^{-1}, analysis of cyanobacterial toxins in the raw and finished water are required (Brasil 2017). Two types of samples were collected: one fixed with Lugol's solution (0.3 to 0.5% for oligotrophic environments and 0.5 to 1.0% for eutrophic environments) and another sample *in vivo*. Both sample types were stored in a cold container until processed in the laboratory.

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

Enzyme-Linked Immunosorbent Assays (ELISA; Abraxis) were used to quantify three cyanobacterial toxins, including microcystin (MC), saxitoxin (STX), and cylindrospermopsin (CYN). Whole water samples were frozen and thawed three times, filtered, and subsequently analyzed. Nutrient analyses for total phosphorus and total nitrogen as well as chlorophyll analysis followed standard methods (APHA 2005). Meteorological data, including temperature, precipitation, evaporation, and irradiation, were collected at weather stations monitored by Foundation of Meteorology and Water Resources of Ceará (FUNCEME).

4.2.3 Statistical analyses

Analysis of variance (ANOVA) followed by Tukey's multiple comparison tests were used to compare reservoir mean water quality parameters. C*anonical correspondence analysis* (CCA), which is a non-linear multivariate statistical technique, was used to determine the relationships between environmental parameters (abiotic and biotic) and cyanobacteria. All parameters were natural log transformed (log(x +1)) to normalize the data prior to analyses. Statistical analyses were conducted using R v3.4.1, and CCA graphics was created using the vegan R package. In the CCA graphic, the dataset was composed of 10 dominant cyanobacterial taxa (listed in Table 7), including *Aphanizomenon sp.* (APHAZ), *Aphanocapsa sp.* (APHAC), Chroococcales (CHR), *Planktothrix agardhii* (PA), *Microcystis aeruginosa* (MA), *Geitlerinema sp.* (GEI), *Pseudanabaena* (PSEU), *Cylindrospermopsis raciborskii* (CR), *Merismopedia sp.* (MER), *and Planktolyngbya* (PLANK*)*, and 12 environmental and water quality variables, including microcystin concentration (MC), saxitoxin concentration (STX), cylindrospermopsin concentration (CYN), precipitation (rain), temperature (temp), evaporation (evap), irradiation (sunlight), chlorophyll (chl), total phosphorus (TP), total nitrogen (TN), transparency (measured as Secchi depth; Secchi) and electrical conductivity (CE).

4.3 Results

During the four-year study period, 114 phytoplankton taxa were identified. However, despite the large algal biodiversity observed across the 20 reservoirs, non-cyanobacterial phytoplankton taxa accounted for less than 1% of the total phytoplankton biovolume. Twenty-four cyanobacterial taxa representing 1 order, 1 family, 19 genera and 3 species were observed during this study including representatives from the Chroococcales order (CHR) and the Phormidiaceae family (PHOR) as well as *Aphanizomenon sp.* (APHAZ), *Aphanocapsa sp.* (APHAC), *Geitlerinema sp.* (GEI), *Merismopedia sp.* (MER), *Planktolyngbya sp.* (PLANK), *Pseudanabaena sp.* (PSEU), *Synechocystis sp.* (SYNE), *Cylindrospermopsis raciborskii* (CR), and *Planktothrix agardhii* (PA) (Table 6). Despite a wide variety of cyanobacteria being present, filamentous species, such as *Cylindrospermopsis raciborskii* (CR), dominated 7 of the 20 reservoirs (BO, CN, EQ, MT, PS, PU and SD; Tukey's $P < 0.05$) and accounted for approximately 52 to 88% of the total cyanobacterial biovolume (Figure 6B). These 7 reservoirs were also characterized with elevated saxitoxin concentrations ranging from 0.49 to 3.51 µg L^{-1} (Figure 6B).

The colony-forming order Chroococcales dominated 3 reservoirs (PT, PS and PM; Tukey's $P < 0.05$), and accounted for approximately 52 to 95 % of total cyanobacterial biovolume (Figure 6B). *Aphanocapsa sp.* was dominant in a single reservoir, AT (Tukey's $P < 0.05$). Elevated microcystin was associated with Chroococcales dominance and ranged from 0.22 to 2.33 µg L^{-1} (Figure 7A). Chroococcales and *Cylindrospermopsis raciborskii* were statistically different at four reservoirs (AR, MRT and SN reservoirs; Tukey's $P = 0.107$; 0.09; 0.6789 and 0.945; respectively)

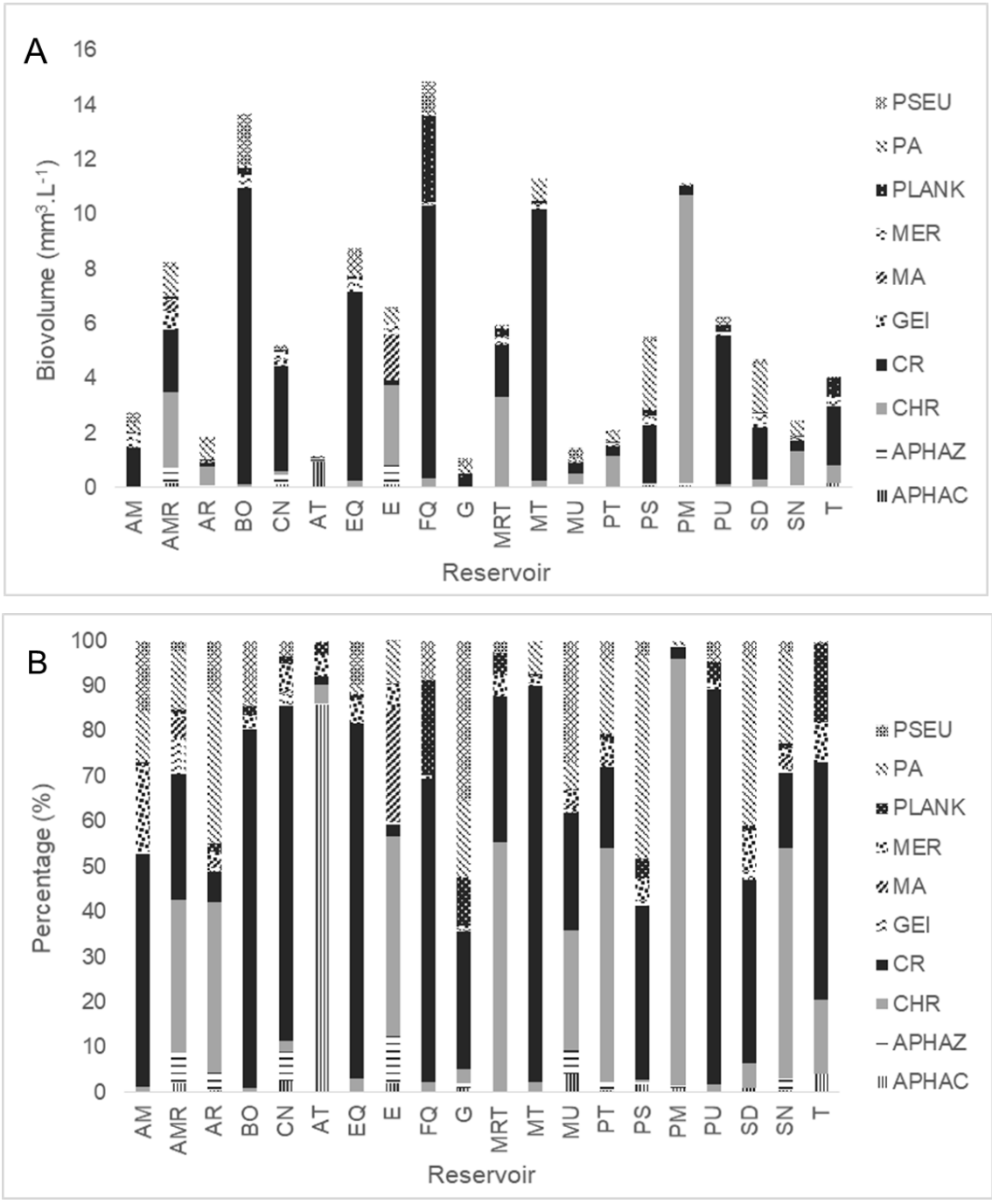
Detectable microcystins (>0.15 µg L^{-1}) were detected in 13 reservoirs (Figure 7A). Seven reservoirs had microcystin concentrations higher than 1 µg L^{-1}, which is the maximum concentration allowed by the Brazilian potability ordinance PCR nº 5/2017 (Brasil 2017). Saxitoxin was measured (>0.11 µg L^{-1}) in 9 reservoirs (Figure 7B). The highest average saxitoxin concentration (3 µg L^{-1}, PCR nº 5/2017) was measured in reservoir BO. *C. raciborskii* represented approximately 80% of the cyanobacteria in that reservoir (Figure 6B). Lastly cylindrospermopsin was detected (>0.05 µg L^{-1}) in only five reservoirs (Fig. 3C), with concentrations higher than the recommended threshold (> 1 µg

L^{-1}) in most of the reservoirs but was concentrated at three sites (AR, E and PT; Figure 7C). Interestingly, multiple cyanotoxins were recorded in all of the study reservoirs during the four year study period, although MC and STX were more commonly detected than CYN (Figure 7; Graham et al. 2010).

Table 7 - List of cyanobacteria taxa identified in 20 studied reservoirs.

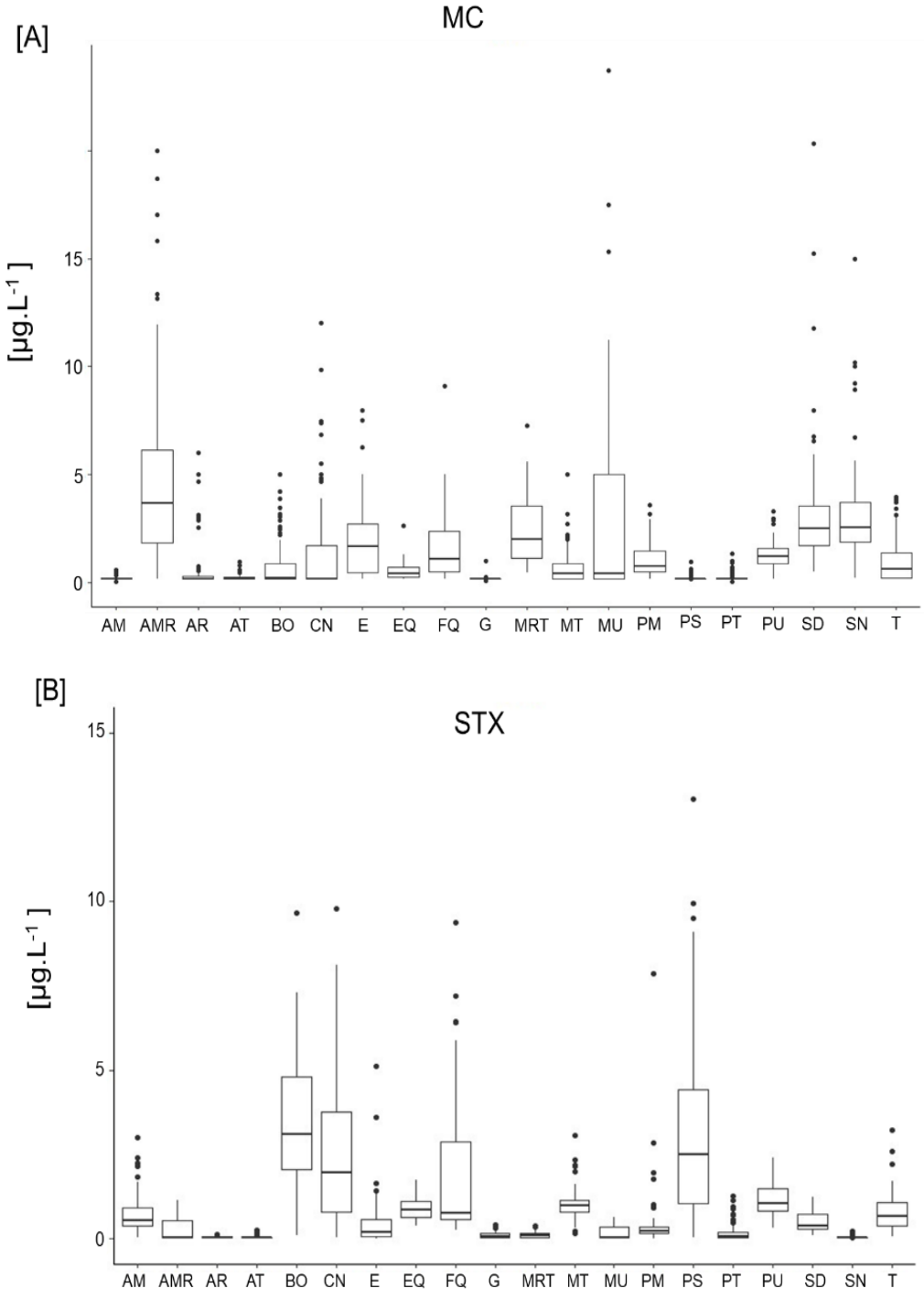
Cyanobacteria Taxa	AM	AMR	AR	BO	CN	AT	EQ	E	FQ	G	MRT	MT	MU	PT	PS	PM	PU	SD	SN	T
Anabaenopsis sp		∗2		∗1	∗1			∗2	∗1			∗1			∗1	∗1				
Anathece sp		∗1									∗1									∗1
Aphanizomenon sp		∗4	∗2		∗3	∗1		∗4		∗1		∗2	∗3	∗2	∗2	∗2			∗2	
Aphanocapsa sp		∗3	∗2		∗3	∗5	∗1	∗2		∗1	∗2	∗1	∗2	∗1	*3	∗3	∗1	∗2	∗2	∗3
Artrospira sp		∗2			∗1			∗3										∗2		
Chroococcales	∗2	∗5	∗4	∗3	∗2	∗2	∗3	∗5	∗3	∗2	∗5	∗3	∗4	∗5		∗6	∗3	∗3	∗5	∗4
Coelomoron sp					∗1							∗1		∗1			∗1			
Cuspidothrix sp		∗1	∗1					∗1				∗1		∗1						
C. raciborskii	∗5	∗5	∗2	∗6	∗6		∗6	∗3	∗6	∗3	∗5	∗6		∗4	∗5	∗3	∗6	∗5	∗4	∗5
Dolichospermum sp		∗2			∗1			∗1		∗1		∗1				∗1			∗1	
Eucapsis sp																				
Geitlerinema sp	∗2	∗4	∗2	∗2	∗3		∗2	∗2	∗2	∗1	∗2	∗2	∗2	∗1	∗2	∗2	∗2	*3	∗2	∗1
Merismopedia sp	∗4	∗2	∗2	∗4	∗3	∗2	∗4	∗3	∗3	∗1	∗3	∗2	∗2	∗3	∗3	∗1	∗3	∗4	∗2	∗3
Microcystis aeruginosa		∗3	∗2		∗2		∗1	∗5		∗1	∗2		∗2	∗2		∗1	∗1	∗1	∗2	
Phormidiaceae	∗4	∗5	∗3	∗3	∗1	∗1	∗2	∗3	∗1	∗2	∗1	∗4	∗3	∗3	∗4	∗3	∗1	∗3	∗5	∗1
Phormidium sp																				
Planktolyngbya sp	∗2	∗3	∗2	∗3	∗3	∗2	∗3	∗2	∗5	∗3	∗3	∗3	∗1	∗2	∗3	∗2	∗3		∗2	∗4
Planktothricoides sp		∗3																		
Planktothrix agardhii	∗3	∗5	∗4	∗2	∗2	∗1	∗3	∗4	∗2	∗3	∗1	∗5	∗3	∗3	∗3	∗2	∗1	∗5	∗4	
Planktothrix isothrix																				
Pseudoanabaena sp	∗4	∗3		∗5	∗3		∗5	∗2	*5	∗4	∗3	∗2	∗4	∗2	∗3	∗2	∗3	∗3	∗2	∗4
Romeria sp																		∗1	∗1	
Shaerocavum sp		∗2	∗2				∗1	∗1						∗2		∗3	∗2			
Synechocystis sp				∗1	∗2		∗1	∗1	∗1		∗2		∗1	∗1	∗1			∗1	∗1	∗1

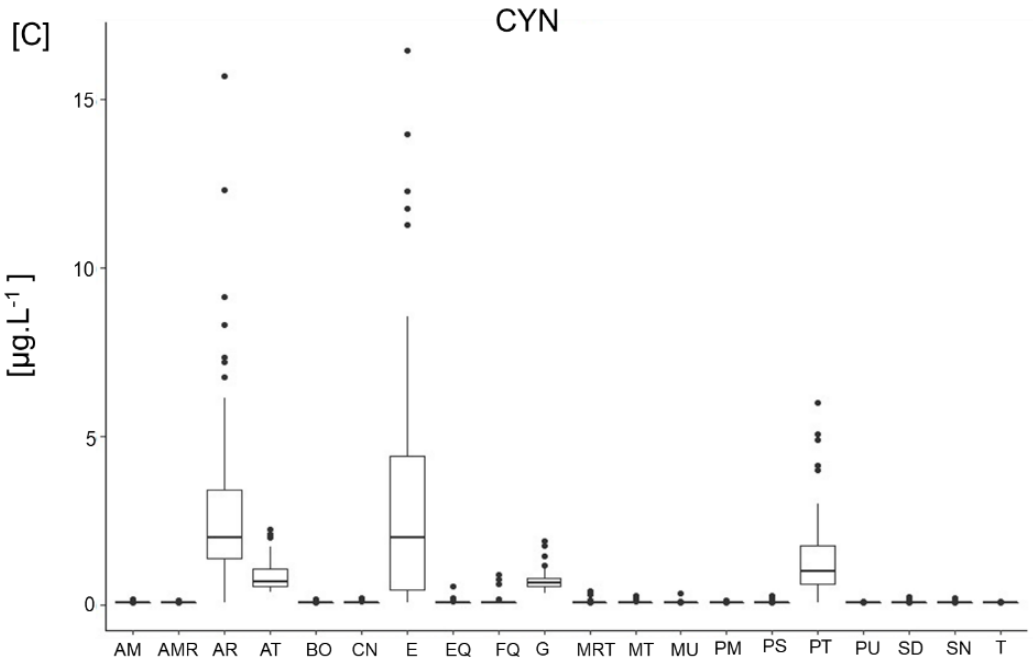
Figure 6 - (A) Absolute (mm3 L-1) and (B) relative (%) algal biovolume for the main cyanobacterial taxa across 20 Brazilian reservoirs.



Source: Author (2018).

Figure 7 - Boxplots of cyanobacterial toxin concentrations for (A) microcystin (MC; detection limit method = 0.15 µg L^{-1}), (B) saxitoxin (STX; detection limit method = 0.11 µg L^{-1}), and (C) cylindrospermopsin (CYN; detection limit method = 0.05 µg.L^{-1}).





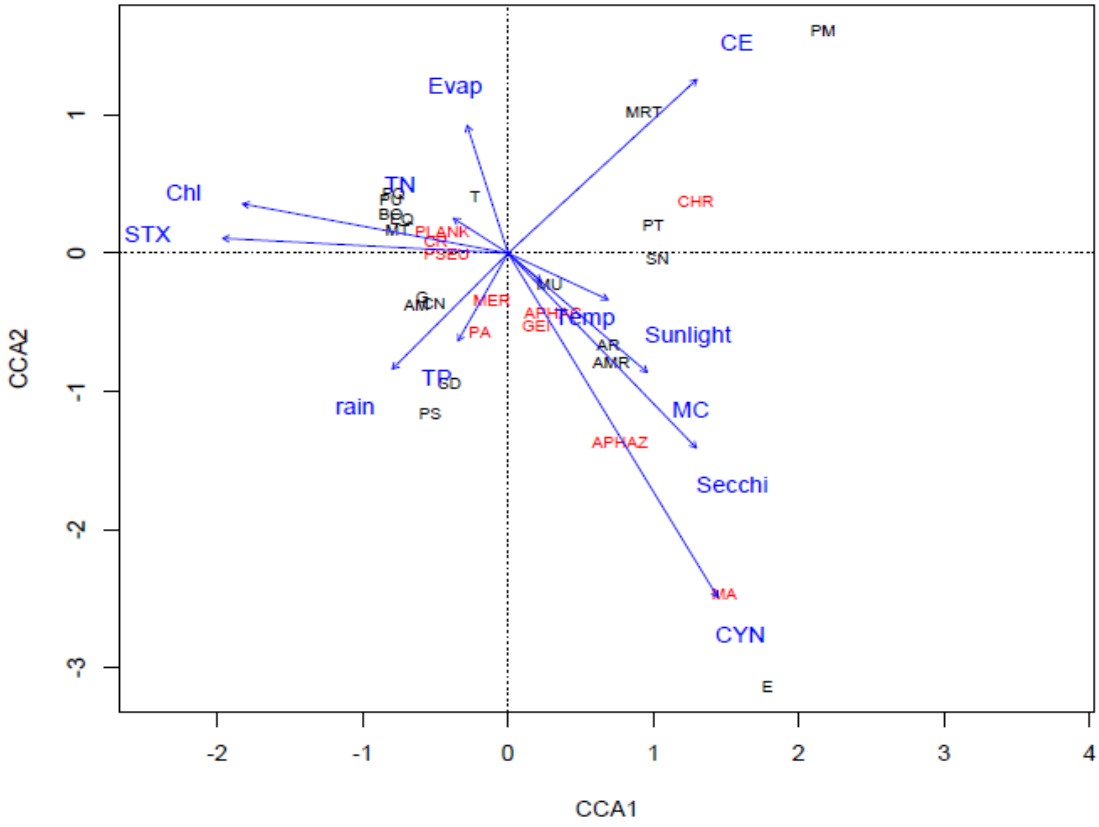
Source: Author (2018).

C*anonical correspondence analysis* (CCA) highlighted the influence of specific environmental factors (blue arrows) on cyanobacterial taxa composition (red letters) at the study reservoirs (black letters). Canonical axes 1 and 2 explained 52.97% and 20.49% of the dataset variance, respectively (Figure 8). Microcystin (MC) was directly correlated with temperature (temp), irradiation (sunlight), and transparency (Secchi) and weakly correlated with total phosphorus (TP). There was also a strong inverse relationship between microcystin and total nitrogen (TN) indicating that this important resource may be intrinsically linked to the production of microcystin. Saxitoxin (STX) was correlated with total nitrogen and chlorophyll concentration (chl) and weakly correlated with evaporation (evap), rainfall (rain) and total phosphorus (TP). Cylindrospermopsin (CYN) was directly correlated with transparency. Microcystin and cylindrospermopsin may have similar environmental triggers considering that both secondary metabolites were impacted by similar environmental variables (Figure 8).

To further explore cyanobacterial taxa and toxin relationships, CCA showed that *Cylindrospermopsis raciborskii* (CR) was positively correlation with saxitoxin. For example, in reservoir BO the average biovolume of CR (>10 mm^3 L^{-1}) and saxitoxin (3.51 µg L^{-1}) were high (Figure 7B). *Pseudanabaena sp.* (PSEU) and *Planktolyngbya sp.* (PLANK) were positively correlated with saxitoxin as well, but there is no report in the literature of saxitoxin production by either of these taxa. Several taxa, including

Aphanizomenon sp., *Aphanocapsa sp*., *Geitlerinema sp*., and *Microcystis aeruginosa* (MA), were correlated with CYN and MC (Figure 8). Among the environmental parameters tested, *Planktothrix agardhii* (PA) and *Merismopedia sp*. (MER), were positively associated with rainfall and total phosphorus, as observed at PS, SD, AM, CN and G reservoirs.

Figure 8 - Canonical correspondence analysis (CCA) ordination diagram of the cyanobacterial community (APHAZ = *Aphanizomenon sp.*; APHAC = *Aphanocapsa sp.*; CHR = Chroococcales; CR = *Cylindrospermopsis raciborskii*, GEI = *Geitlerinema sp.*; MER = *Merismopedia sp.*; PA = *Planktothrix Agardhii*; PSEU = *Pseudanabaena sp.*, PLAN = *Planktolyngbya sp.*) in relation to studied environmental parameters.



Source: Author (2018).

4.4 Discussion

Lins *et al*. (2016) found that in the semi-arid region of Brazil, more than 90% of the total phytoplankton community was composed of cyanobacteria. For example, *Pseudanabaena limnetica*, *Cylindrospermopsis raciborskii*, *Aphanocapsa incerta*, *Microcystis aeruginosa* and *Planktothrix agardhii* coexisted and alternated dominance.

Costa *et al*., (2004) studied cyanobacteria in another Brazilian semi-arid region and found that *C. raciborskii, Microcystis sp.,* and *Aphanizomenon sp.* were persistent throughout the study and accounting for 90 to 100% of the total phytoplankton cell counts; similar trends were found in this study (Figures 6 and 7). Many cyanobacterial species have been reported to adapt to environmental change through phenotypic plasticity and ecological tolerance (Moura *et al.* 2018)

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

It is well known that excess nutrients stimulate phytoplankton growth, especially cyanobacteria. Phosphorus and nitrogen are considered the main nutrients that limit primary productivity in aquatic environments (Downing *et al*., 2001; Dolman *et al*., 2012) although some species of cyanobacteria are capable of fixing atmospheric nitrogen (Paerl *et al*., 2011; Carey *et al*., 2011; Paerl and Paul, 2012; Boopathi and KI, 2014). In this study, the presence of saxitoxin showed a positive correlation with total nitrogen (TN) (Figure 8). Nitrogen can be present in several forms and influence the success of STX-producers in several ways. For example, Barros *et al*. (2015) cultivated the diazotroph, *Cylindrospermopsis raciborskii*, with reduced nitrogen, which negatively affected its growth rate. The culture reached the stationary phase slower and with lower cell number when compared to the experiment with phosphorus limitation. Moreover, Saker and Neilan (2001) evaluated intraspecific variation in growth of seven species of *C. raciborskii* from Australia exposed to different sources of nitrogen and found that ammonia amendments supported caused faster growth than nitrogen limited environments that required energetically costly nitrogen fixation. Briand *et al*. (2004) found that different nitrogen sources can cause changes at the molecular level in cyanobacteria, including gene expression and toxin production in *C. raciborsii* (also see Stucken *et al*. 2014).

C*anonical correspondence analysis* (CCA) reinforced the importance of nitrogen to STX-producers and showed that *Cylindrospermopsis raciborskii* was the main taxon associated with STX. Carneiro *et al*. (2013) demonstrated that *C. raciborskii* was successful because of multiple traits, including its ability to fix nitrogen, high affinity for ammonia and phosphorus, and ability to regulate its bouyancy and form akinetes. According to these authors, the focal semi-arid reservoirs showed that *C. raciborskii* dominated the phytoplankton communities. Brentano *et al*. (2016) found that in a subtropical lagoon in Brazil, dissolved nitrogen lead to elevated STX concentrations. Lastly, Vico *et al*. (2016) contended that *C. raciborskii* could alternatively use atmospheric nitrogen (N_2) thus allowing it to develop in low dissolved nitrogen concentration systems. On the other hand, when nitrate is high, *C. raciborskii* produces less heterocysts, suggesting that it prefers to use dissolved nitrogen forms, when available.

Phosphorus has been reported as an important limiting nutrient for phytoplankton growth (O'Neil *et al*. 2012, Chislock *et al*. 2014, Jacoby *et al*. 2015). However, Dolman *et al*. (2012) found a saturation function of cyanobacterial biovolume across increasing concentrations of TP indicating that other factors limit phytoplankton growth when phosphorus concentration is high. In this research, there was a weak positive relationship between total phosphorus and all three cyanobacterial toxins (MC, STX and CYN; Figure 8). However, climate change scenario forecast longer and more intense precipitation events, which could cause higher run-off from terrestrial systems that could enhance the release of phosphorus from sediment to waterbodies (Andersson *et al*. 2015. Such patterns may explain the similar CCA trajectories of total phosphorus and precipitation (Figure 8). In addition, filamentous *Planktothrix agardhii* has been shown to positively respond to TP, similar behavior was observed by Dolman *et al*. (2012), confirming that importance.

According to Komárek and Anagnostidis (1998) and Sant'anna *et al*. (2004), the order Chroococcales is comprised of all unicellular or colonial prokaryotic cyanobacteria, which do not form true filaments with direct physiological interference between cells. *Microcystis aeruginosa*, a member of the order Chroococcales, is one of the dominant bloom-forming cyanobacterial species in freshwater environments. In addition to producing microcystin, *M. aeruginosa* is capable of producing taste and odor compounds including, geosmin and 2-methylisoborneol (Wang *et al*., 2016). Bartoli *et*

al., (2014) found seven microcystin variants (MC-RR, MC-LR, MC-YR, MC-LF, MC-LW, dm-MC-RR, and dm-MC-LR) in reservoirs from São Paulo – Brazil. Gkelis and Zautsos (2014) showed that the highest concentrations of MC were produced by *Microcystis viridis*, *Microcystis novacekii* and *Microcystis aeruginosa* compared to *Microcystis wesenbergii*. These studies show that most of the morphological types of *Microcystis* include toxic and non-toxic strains and that higher MC concentrations were found when *Microcystis aeruginosa* was the dominant species. *Microcystis aeruginosa* showed positive relationships with CYN; however, this species is not reported as a cylindrospermopsin-producer but is well known as a producer of MC (Bartoli *et al*., 2014; Gkelis and Zautsos, 2014). *Planktothrix agardhii* and TN had similar CCA trajectories while the cyanobacterium and MC had contrasting CCA trajectories (Figure 8). These findings contrast with past study that showed stronger relationships between *P. agardhii* and TP or MC (Dolman *et al*. 2012).

MC was positively correlated with temperature, irradiance, and temperature (Figure 8). Cyanobacterial maximum growth rate varies has been shown to vary within and across species (Wilson *et al*. 2006). For example, maximum growth rate for *Aphanizomenon flos-aquae* and *Planktothrix agardhii* is ~20ºC, while *Microcystis aeruginosa* prefers ~28ºC and some species of the genus, *Synechococcus*, can thrive in temperatures above 30ºC (Reynolds, 1989, 2006). According to Carey *et al*. (2011), the acceleration rate, commonly measured as Q_{10} (change in growth between 10°C temperature shifts; usually 10-20° C) was 2.6 for *Synechococcus sp*. but 3.7x higher for *Microcystis aeruginosa* (Q_{10} = 9.6). This value was the highest recorded among the cyanobacteria and eukaryotic phytoplankton; therefore, *Microcystis aeruginosa* species should have a physiological advantage when the water temperature is > 20ºC. Moreover, Merel *et al*. (2013) confirmed that cyanobacterial blooms are primarily regulated by three environmental factors; temperature, light exposure, and nutrients. In this study, duration of light exposure was more important for growth than light quality (Figure 8).

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

studies have shown that several *Aphanizomenon* species can produce CYN (Banker *et al*. 1997; Wormer *et al*. 2008; Preußel *et at*. 2009; Brient *et al*. 2009, Cires *et al*. 2011). In this study, there was no relationship between CR and CYN, however there was a positive correlation between APHAZ and CYN (Figure 8), which support findings in these past studies.

Secchi depth is a direct measure of waterbody transparency that may be influenced by organic (phytoplankton) and inorganic (suspended sediment) components (Gillion and Bortleson 1983). As expected, chlorophyll and Secchi depth were inversely correlated in this study (Figure 8). Saxitoxin-producing cyanobacteria were most associated with chlorophyll; while conductivity (CE) was inversely correlated with rain. Since 2012, according to the Foundation of Meteorological Studies of Ceará (FUNCEME), Ceará state has experienced below average precipitation that has influenced phytoplankton dynamics, including more frequent and intense cyanobacterial blooms. The weak correlation between rainfall and saxitoxin follow Barros *et al*. (2017) who studied reservoirs in the same region and noticed that precipitation below the historical mean favors the proliferation of cyanobacteria. Lastly, Reichwaldt and Ghadouani (2012) investigated the effect of rain patterns on toxic cyanobacterial blooms and determined that increased nutrient concentration associated with high evaporation (and concomitant less waterbody volume) and prolonged stratification processes favor the proliferation of toxic cyanobacteria.

4.5 Conclusion

Brazilian drinking water reservoirs in the Ceará state are dominated by cyanobacteria by a variety of factors, including nutrient pollution and a semi-arid climate. Throughout the study region, there is a predominance of colonial and filamentous cyanobacterial taxa, including representatives belonging to the order, Chroococcales, and the species, *Cylindrospermosis raciborskii*. Phosphorus and nitrogen directly influence the proliferation of cyanobacteria, which has been documented elsewhere around the world (Downing *et al*. 2001; Saker and Neilan 2001, Dolman *et al*. 2012, Chislock *et al*. 2014). Nitrogen influenced the development of STX producers, especially *C. raciborskii*, while phosphorus was weakly related to MC, STX and CYN producers (Figure 8). *Planktothrix agardhii* and *Merismopedia sp*. were associated with phosphorus and precipitation, which the latter can influence the former through runoff from watersheds. Water transparency was negatively correlated with STX-producing cyanobacteria (Figure 8), including *C. raciborskii* which has been shown to dominate water column phytoplankton communities instead of forming surface scums (Chislock *et al*. 2014). In this study, *C. raciborskii* dominated in eight reservoirs where STX and CHL were high. Furthermore, meteorological variables were shown to be associated with toxic cyanobacterial blooms. For example, the period for this project included a cycle of prolonged drought, high evaporation, and elevated temperature associated with water scarcity - factors that favor toxic cyanobacterial blooms in waterbodies around the world, including Ceará reservoirs.

Greater irradiation, and transparency, and elevated temperature favored the proliferation of toxigenic species, *Aphanizomenon sp.* and *Microcystis sp.,* which were strong correlated with CYN and MC. Since *Aphanizomenon* is not known to produce microcystin, *Microcystis sp.,* is likely responsible for its presence while *Aphanizomenon* is known to produce CYN and it is likely to be responsible for CYN in the study systems. Lastly, c*anonical correspondence analysis* (CCA) showed to be a valuable tool for identifying environmental factors associated with cyanobacterial blooms. We encourage future studies to use our findings to develop and conduct laboratory- and field-based experiments to determine if suggested environmental mechanisms mediate observed patterns related to cyanobacterial blooms across space and time.

5 INDEX CLASSIFICATION OF DRINKING WATER RESERVOIRS FOR IDENTIFYING VULNERABILITY TO CYANOHABS IN WATER TREATMENT PLANTS.

5.1 Introduction

Cyanobacterial blooms in freshwater environments have been increasing in frequency and intensity, thereby, affecting human water supplies. Managing water bodies has become a challenge for water companies worldwide as these select species of cyanobacteria can produce a wide variety of toxic secondary metabolites (e.g., microcystins, saxitoxins, nodularins, cylindrospermopsins; Carmichael *et al*., 1994, Mohamed *et al*., 2006, Van Apeldoorn *et al*., 2007), which are linked to a variety of deleterious effects. Furthermore, cyanobacteria may produce "*off-flavor*" compounds such as *2-Methylisoborneol* (MIB) and Geosmin, which are non-toxic to animals but produce unpleasant, taste and odors (Parinet *et al*., 2010; Pestana, 2012).

According to the United Nations (2011), drylands (otherwise known as Semi-arid regions) occupy about 41.3 % of the world's land surface and is populated by approximately 2 billion people. The Semi-arid region classification is mainly based on rainfall irregularity, in which annual rainfall ranges from 300 to 500 mm. Ceará State, in northern Brazil, is characterized by an annual rainfall average below 600 mm (FUNCEME, 2017), the majority of which is distributed in the rainy season between February and May; precipitation is irregular here, leading to prolonged droughts. Additionally, this region has a precipitation/evapotranspiration potential ratio (p / ETP) between 0.2 and 0.5 (FUNCEME, 2017), and is characterized by high temperature (23-27^{o}C average) and sunlight (2,800 h.year^{-1}; Neto *et al*., 2014). As elevated temperatures, high sunlight, and persistent drought conditions are linked to cyanobacterial bloom events (Moura *et al* 2017; Brasil *et al*., 2017), and the projected increases in the frequency, longevity and intensity of droughts in this region by climate change (Marego 2009), there is a serious threat of increased cyanobacterial bloom occurrences in the Ceará State (COGERH, 2018).

Cyanobacterial intoxication cases around the world have led to a number of legislations and guidelines that drives decision-making in freshwater systems around the world, especially decisions related to water used for human supply (Leigh *et al*., 2010).

However, conventional methods for evaluating the danger of cyanobacteria in water are based on the comparison of each experimental parameter value with the existing guidelines or legislation. Assessing water quality may be a complicated practice due to the numerous parameters involving quality of water (Bharti and Katyal, 2011). In this sense, water quality indices are such approaches that minimizes the data volume largely and simplifies the understanding of water quality status (Poonam *et al*., 2013)

Water quality indices involving multiple environmental parameters to evaluate the quality of water resources is a method used worldwide as a monitoring tool, mainly in areas that are subject to deterioration due to natural or anthropogenic impacts (Poonam *et al*., 2013). It consists of summarizing a set of interconnected parameters by a single numerical value situated on a fixed scale. This method allows users monitoring water quality to quickly evaluate and make fast decisions on the management of their resource, with a reduction in between-system inconsistencies. Index studies in water bodies were first proposed by Horton (1965), and has since been refined to improve accuracy and modified for targeted studies (Ewaid, Ali Abed 2017).

However, until today nothing has been published on indices that evaluate water quality regarding the potential hazards of cyanobacteria and their metabolites, creating a knowledge gap for decision-making in the water industry. Therefore, the goals of this work were propose an index (*Icyano*) for assessing cyanobacteria hazards in artificial reservoirs used for human suppl; as well as, confront the results of this index with the type of water treatment currently used, and identify which water treatment plant is the most susceptible to cyanobacteria hazards

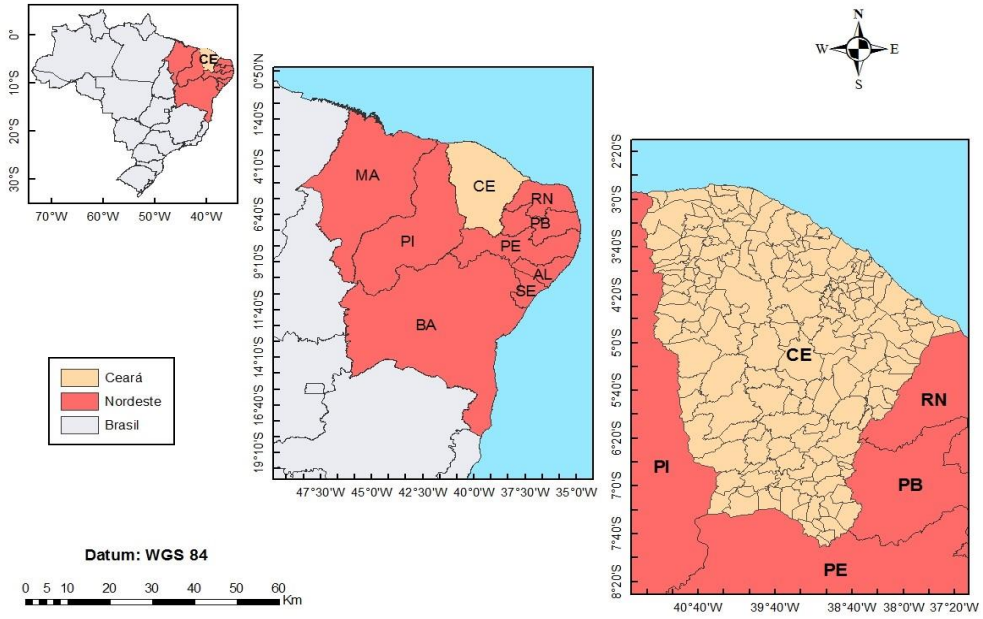
5.2 Materials and methods

5.2.1 Study region

The Ceará State is located in Northeast of Brazil between latitudes S2 º 30’ 00” and S8º 52’ 00” and longitudes W37 º 14’ 00” and W41º 30’ 00” (Figure 9). The study site belongs to the semi-arid region of Brazil that is characterized by low annual rainfall extending from early January to May (IPECE, 2017; NETO *et al.*, 2014). Besides low precipitation, the Ceará region has several conditions that are ideal for cyanobacterial growth, such as a high solar radiation (18 $Mj.m^{-2}.day^{-1}$) during approximately 8 $hours.day^{-}$

[1], surface water accumulation in reservoirs with high retention times, high average annual temperature, and intensive anthropogenic activity leading to the eutrophication of its reservoirs (Barros *et al*., 2017; COGERH 2017; FUNCEME 2017).

Figure 9 - Map of Ceará state, Brazil.



Source: Author (2017).

Table 8 - Characteristics of the studied reservoirs including trophic classification according to COGERH (2016).

Code	Reservoir	Trophic Classification	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	1506
BO	Boqueirão	Hypereutrophic	28,110,000	512
CN	Canafístula	Eutrophic	13,110,000	315
AT	Trussu	Eutrophic	301,000,000	5509
EQ	Edson Queiroz	Hypereutrophic	254,000,000	2660
E	Ema	Hypereutrophic	10,390,000	284
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
PT	Patú	Hypereutrophic	71,829,000	856
PS	Paulo Sarasate	Hypereutrophic	891,000,000	9600
PM	Pereira de Miranda	Hypereutrophic	395,638,000	5700
PU	Puiu (Parambú)	Eutrophic	8,530,000	159
SD	Serafim Dias	Hypereutrophic	43,000,000	688
SN	Sítios Novos	Hypereutrophic	126,000,000	2010
T	Trapia III	Eutrophic	5,510,000	130.2

Source: Author (2017).

5.2.2. Field sampling and analytical methods

Samples were collected at the Water Treatment Plants (WTP) by CAGECE intake of each reservoir, 30 cm below the surface. Each reservoir was sampled weekly or monthly, depending on the prevalence of cyanobacteria. Since Brazilian legislation (Brasil, 2017) dictates that when a sample contains cyanobacteria concentration above 20,000 cel.mL^{-1}, monitoring frequency must be weekly, otherwise it be monthly. For phytoplankton analyses, samples were preserved with Lugol's solution, while biological material used *in vivo* was kept under refrigeration to decrease organism metabolism and oxygen consumption in the absence of light. Cell counts (Cyano parameter) were carried out with a Sedgwick-Rafter cell according to Lawton (1999). Additionally, parameters including electrical conductivity (CE), total nitrogen (TN), total phosphorus (TP), chlorophyll (CHL), and Secchi depth (SD) were analyzed at the Water Company of Ceará (CAGECE) laboratory according to APHA, 2005. Toxicity analyzes were performed

using the Abraxis® kit ELISA for microcystins (MC), saxitoxins (STX), and cylinderspermopsin (CYN).

5.2.3 Calculation of the cyanobacteria index (Icyano)

The cyanobacteria index was calculated by using the dataset collected between January 2013 and January 2017. For the development of the cyanobacteria index (*Icyano*), a data-driven methodology proposed in this study was divided into three parts. First, a Pearson´s correlation coefficient measure was considered to determine the correlation between the parameters. Secondly, after determining the correlations between the variables, the KMO (Kaiser-Meyer-Olkin) adequacy test was performed in order to check whether the correlation structure of the dataset is suitable for the factorial analysis. The KMO value above 0.6 indicates that a factorial analysis is useful for the index determination (Hair, Anderson and Tatham 1987; Parinet *et al.* 2010). Therefore, since the KMO value turns out to be 0.62, we applied the factorial analysis to find out the latent variables i.e. common factors loads.

Factorial analysis is a widely used technique for the construction of indexes (Meireles *et al*., 2010). This multivariate statistical technique aims primarily to reduce the data dimension and to summarize the data, which analyzes the relationships between variables, and defines a set of common latent variables called factors. Through this technique, it is also possible to calculate the weight represented by each variable for the index composition (Wi) (Hair *et al* 1998; Johnson, Wichem 2007).Cyanobacteria index (*Icyano*) was calculated according following equation:

$$Icyano = \sum_{i=0}^{n} \mathrm{Qi}.\,\mathrm{Wi}$$

Where, *Icyano* is a dimensionless parameter ranging from 0 to 100; Qi represents a standardized variable (0 to 100), it is the quality of the i_{th} parameter and a function of its concentration or measurement; Wi is the normalized weight of the i_{th} parameter and a function of its importance in explaining the global variability in water quality. Wi is described according to Meirelles *et al*., (2010) by following equation:

$$\mathrm{Wi} = \sum_{i=1}^{n} \mathrm{Fj.\,Aij} / \sum_{j=1}^{k} \sum_{i=1}^{n} \mathrm{Fj.\,Aij}$$

Where, F_j is component 1 autovalue; A_{ij} is the explainability of parameter i by factor j; *i* is the number of parameter selected by the model, ranging from 1 to n; j is the number of factors selected in the model, varying from 1 to K.

All reservoirs were classified according to the index value presented in table 8. Statistical tests and modeling were performed in statistical program "*R"* using the packages "*corrplot*" and "*psych*". The result of index proposal was also compared with the type of treatment used in each water treatment plant. ANOVA followed by tukey test was also used in order to claim some affirmations in the results. Based on the calculated *Icyano* the category of water classification was done according Shweta *et al.*, (2013); and Ewaid, and Abed 2017 (table 8).

Table 9 - The *Icyano* classification categories.

Range	Quality
0-25	Good
26-50	Medium
51-75	Bad
76-100	Very Bad

Source: Author (2017)

5.3 Results

Appendix 1 provides the descriptive statistics of the analyzed parameters. It was observed little variability in the climatological variables ($\mu > \sigma$). High temperatures and low thermal amplitudes, especially in the coastal area (IPECE. 2017), characterize the region. Cyanobacteria concentration in AM, BO, E, G, MT, PU, SD and T reservoirs presented high variability, which may be explained by climatic conditions (De Senerpont Domis *et al*., 2013) and the high amount of nutrients available (Downing *et al*., 2001; Dolman *et al*., 2012). In addition, there are periods called "clear-water periods" that are characterized by the sudden drop in planktonic organisms due to zooplankton grazing (Ger *et al*., 2016).

Results from Pearson correlation matrix can be found in Table 9. A positive robust correlation was observed between total nitrogen (TN) and Chlorophyll (Chl) (0.811). TN was moderately associated with cyanobacteria concentration (Cyano) (0.549), and showed an inverse correlation with Secchi depth (-0.586). Chlorophyll concentration showed a moderate inverse correlation with Secchi depth (-0.574) and direct correlation with concentration of cyanobacteria (0.588). Besides that, strong positive correlation was observed between temperature (temp) and evaporation (Evap) (0.753).

Table 10 - 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	0.362	1						
TN	0.169	0.369	-0.115	1					
TP	-0.317	-0.158	-0.526	0.359	1				
Chl	0.248	0.426	-0.113	0.811	0.428	1			
CE	-0.109	0.251	0.036	0.444	0.526	0.391	1		
Sec	-0.313	-0.220	0.299	-0.586	-0.399	-0.574	-0.299	1	
Cyano	0.456	0.334	0.063	0.549	-0.163	0.588	-0.022	-0.394	1

Source: Author (2017).

The value of Kaiser-Meyer-Olkin (KMO) adequacy test result was 0.620, exceeding the value of 0.6 recommended by Te and Gin (2011). Bartlett's test (p-value = $2.2x10^{-16}$), suggesting that the variance is different for the treatment groups. Factorial analyses results can be observed in table 10.

In the proposed index model, temperature, evaporation, sunlight, electrical conductivity and phosphorus presented wi = 0.073, 0.061, 0.032, 0.039 and 0.042, respectively (Table 10). Chlorophyll, total nitrogen and cyanobacteria concentration presented Wi = 0.202, 0.191 and, 0.214, respectively, representing the most important parameters for index composition, approximately 60% of Wi. Appendix 2 shows the values of *Qi* calculed for the studied reservoirs as well as the value of the proposed

cyanobacteria index (*Icyano*). Figure 10 show the sites studied with their concentration average of cyanotoxins (MC and STX) and cyanobacteria indices.

Table 11 - Matrix for factorial loads, commonality estimated and Wi (weight of each variable).

Variables	**Factor 1**	**Factor 2**	**Factor 3**	**Wi**
Temperature (ºC)	0.289	0.755	-0.106	0.073
Evaporation (mm)	0.243	0.936	0.243	0.061
Sunlight (h)	-0.128	0.488	-0.263	0.032
Total Nitrogen ($mg.L^{-1}$)	0.759	*	0.392	0.191
Total phosphorus ($mg.L^{-1}$)	0.166	-0.418	0.793	0.042
Chlorophyll ($mg.L^{-1}$)	0.800	0.133	0.439	0.202
Electrical condutivity ($\mu S.cm^{-1}$)	0.155	*	0.655	0.039
Secchi Depth (m)	-0.577	*	-0.341	0.145
Cyanobacteria concentration ($cell.mL^{-1}$)	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	

Source: Author (2017) * Insignificant values

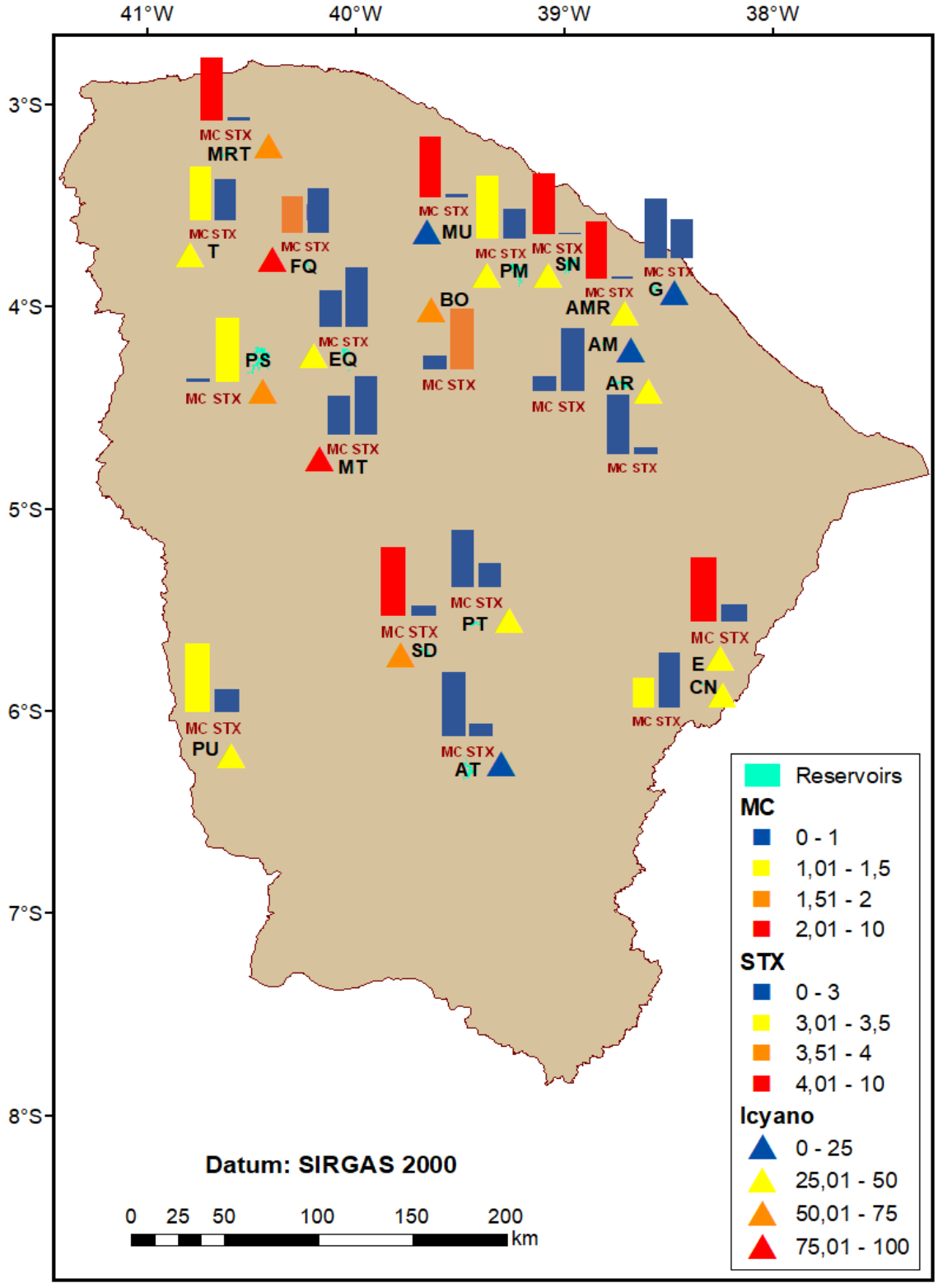
Table 11 shows the calculated index values in ascending order, the assigned *Icyano* classification, and the water treatment technology used. It can be observed that, as *Icyano* deteriorates from G to VB (water quality decreases) the treatment technology involved does not necessarily go from a less to a more robust technology (i.e. direct filtration to Pre Pressure filter + Direct Filtration to conventional treatment).

Table 12 - Type of treatment used in the WTP of each reservoir; calculated value of the cyanobacteria index (Icyano) and classification of each reservoir.

Reservoir	Water treatments used	Icyano	Classification
AT	Direct Filtration	6.36	G
G	Direct Filtration	22.13	G
MU	Direct Filtration	23.39	G
AM	Direct Filtration	24.02	G
AR	Direct Filtration	28.14	M
PT	Direct Filtration	32.63	M
AMR	Pre Pressure filter + Direct Filtration	36.55	M
PU	Conventional treatment	38.22	M
SN	Pre Pressure filter + Direct Filtration	38.42	M
CN	Direct Filtration	40.08	M
EQ	2 Pre Pressure filter + Direct Filtration	44.19	M
E	Direct Filtration	44.29	M
PM	Direct Filtration	45.94	M
SD	Conventional treatment	48.32	M
T	Pre Pressure filter + Direct Filtration	48.72	M
BO	Direct Filtration	50.47	B
MRT	Direct Filtration	52.65	B
PS	Pre filter + Direct Filtration	52.89	B
FQ	Conventional treatment	77.59	VB
MT	Direct Filtration	77.64	VB

G= Good, M = Medium, B = Bad and VB = Very Bad.

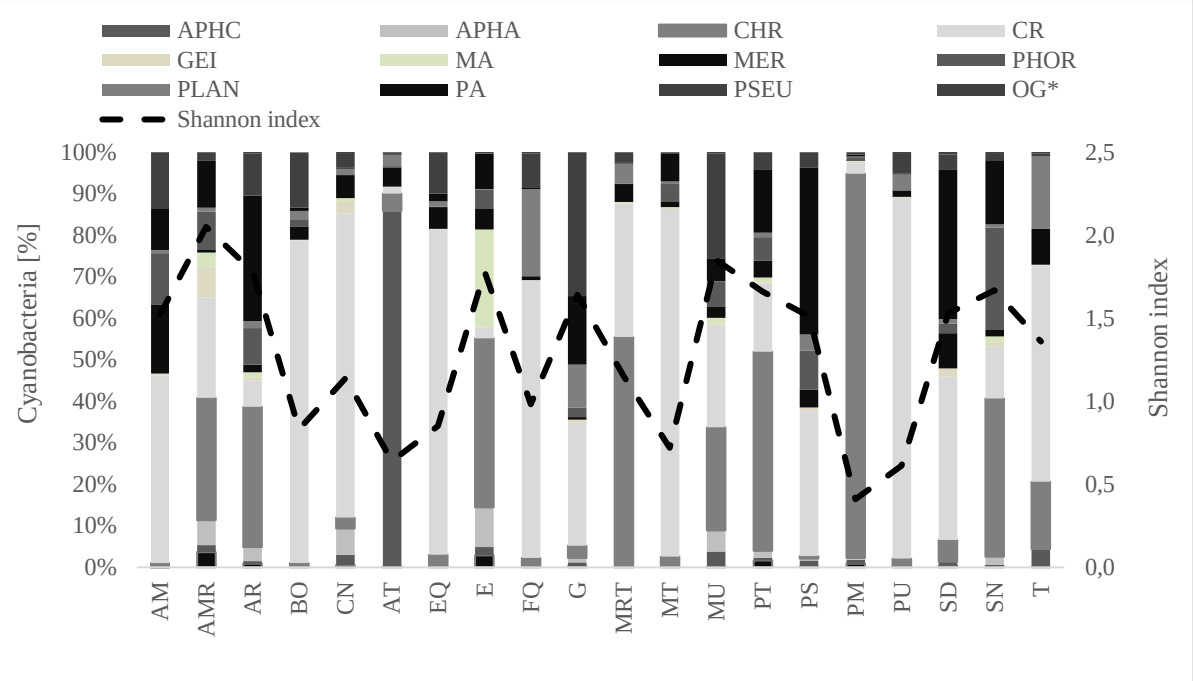
Figure 10 - Location of each reservoir studied, classification of the mean concentration of MC and STX and Icyano classification



Source: Author (2017).

The reservoirs AM, AT, G and MU were classified as good *Icyano*. Comparing them concerning microcystin concentration, only MU reservoir was statistical different (P > 0.05) from the others (Figure 12), exceeding the limits allowed in Brazilian drinking water legislation (PCR nº 5/2017), for microcystin (1 µg.L^{-1}). AM reservoir presented a statistical differences in relation to saxitoxin concentration (Figure 12) (P > 0.05), but did not exceed the threshold specified by Brazilian drinking water legislation (3 µg.L^{-1}). Although the reservoirs classified as good were dominanted by cyanobacteria, reservoirs AM, G, and MU showed a higher cyanobacterial diversity. It is worth mentioning that *Aphanocapsa sp*. represented more than 85% of the cyanobacterial composition of the AT reservoir (Figure 11).

Figure 11 - Representation of the percentage of cells found in Ceará reservoirs. (OG = other phytoplankton groups).

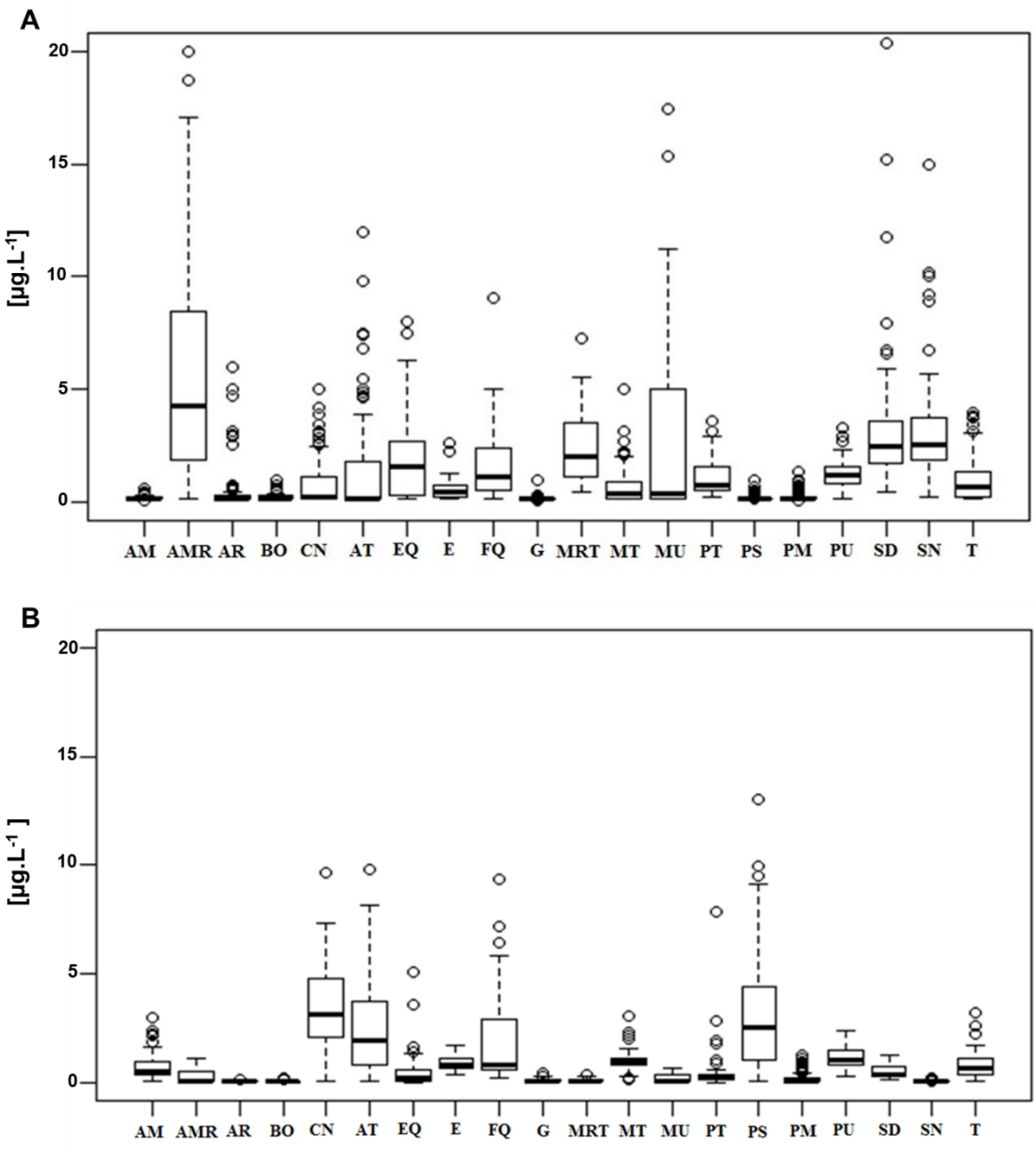


Source: Author (2017).

Reservoirs classified as bad (BO, MRT, PS) or very bad (FQ and MT) showed low cyanobacterial biodiversity (Figure 11). *Cylindrospermopsis raciborskii* was the predominant species in the FQ and MT reservoirs (P < 0.05) composing 67% and 84% of the phytoplankton cell count, respectively. Both reservoirs were classified as very bad and presented high cyanotoxin concentrations. Reservoirs FQ and MRT exceeded the threshold mandated by Brazilian legislation for microcystin (1 µg.L^{-1}), and BO and PS

reservoirs surpassed the concentrations determined for saxitoxin (3 µg.L^{-1}). Most reservoirs were classified as medium, of which only the reservoirs PT, EQ and AR were within the tolerance limit to both microcystin and saxitoxin (Figure 12).

Figure 12 - Boxplots (median; quartile 25% and 75%) of ELISA cyanotoxins concentration analyzed in the twenty reservoirs studied. a) Microcystins concentration (detection limit method = 0.15 µg.L^{-1}). b) Saxitoxins concentration (detection limit method = 0.11 µg. L^{-1}).



Source: Author (2017).

5.4 Discursion

According Huang *et al*. (2016), global warming trends are particularly intensified in semi-arid regions, with droughts becoming longer and more severe due to higher evaporation and lower precipitation volume. The most noticeable consequence of high temperatures and the other climatic forecasts proposed by Huang *et al*. (2016) in Ceará reservoirs are significant losses in water availability and possible water quality deterioration. Studies conducted by The Inter-American Institute for Cooperation on Agriculture in 2002 affirmed that evaporation in semi-arid environments may reach 1000 mm/year in coastal regions and more than 3000 m/year in inland areas. Banker and Hilt (2016) also concluded that decreases in reservoir levels during the summer causes an increase in water retention time, as well as an increase in the accumulation of nutrients in the water column and in the shallower layers, which may lead to cyanobacteria blooms.

Moreover, Brasil *et al*. (2017) affirmed that this phenomenon may increase in the electrical conductivity, increasing salinization issues. Low conductivity favors the presence of heterocysts in cyanobacteria, while high salinity typically favors growth of genera that did not present heterocysts (Srivastava et al., 2009). This may explain the dominance of the species *C. raciborskii* in 8 of the 20 reservoirs studied ($P < 0.05$).

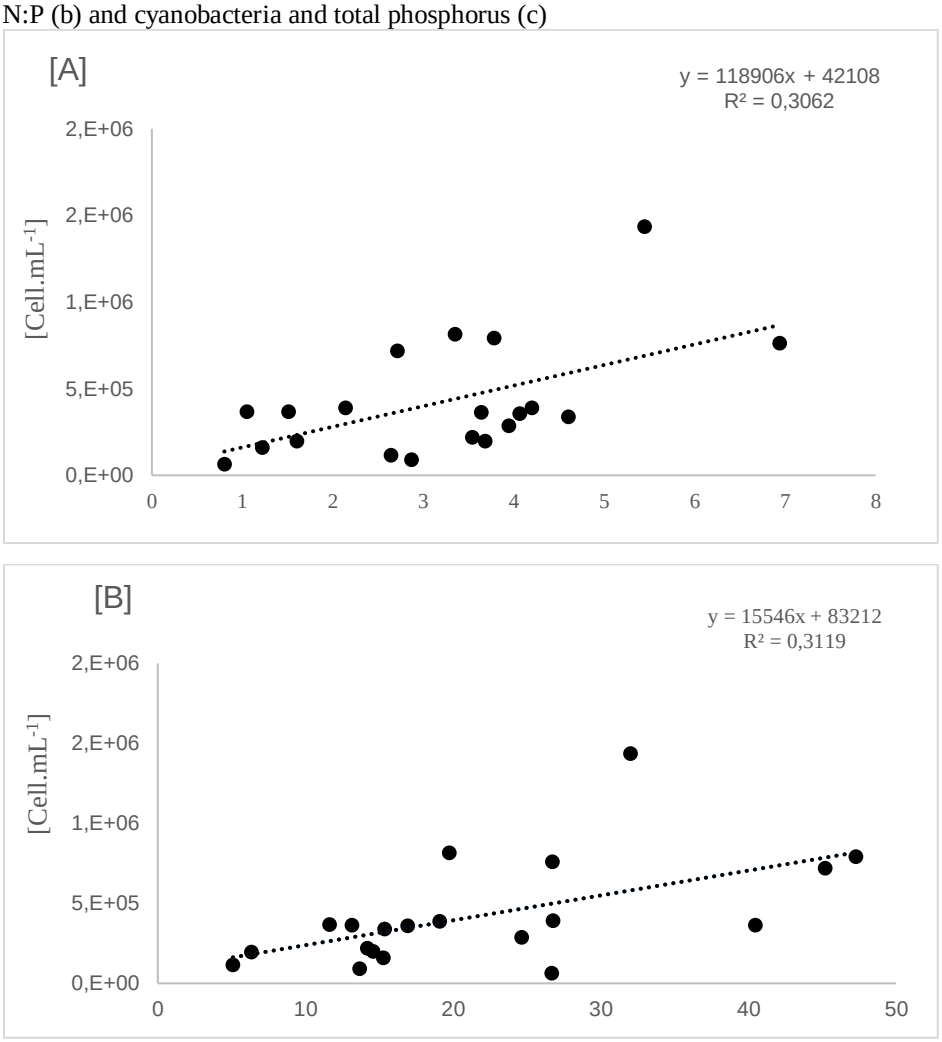
Walls *et al*. (2018) found that the best model to predict free microcystin included temperature, chlorophyll a, TP, TN, DOC, and the interaction between temperature and chlorophyll a. According to the authors, there was a significant positive relationship between microscystin and temperature, and a significant negative relationship between microcystin and chlorophyll a, indicating that the release rate of microcystin was a function a temperature increases and chlorophyll decreases. In this study, using the Pearson matrix, these relationships were found to be weak.

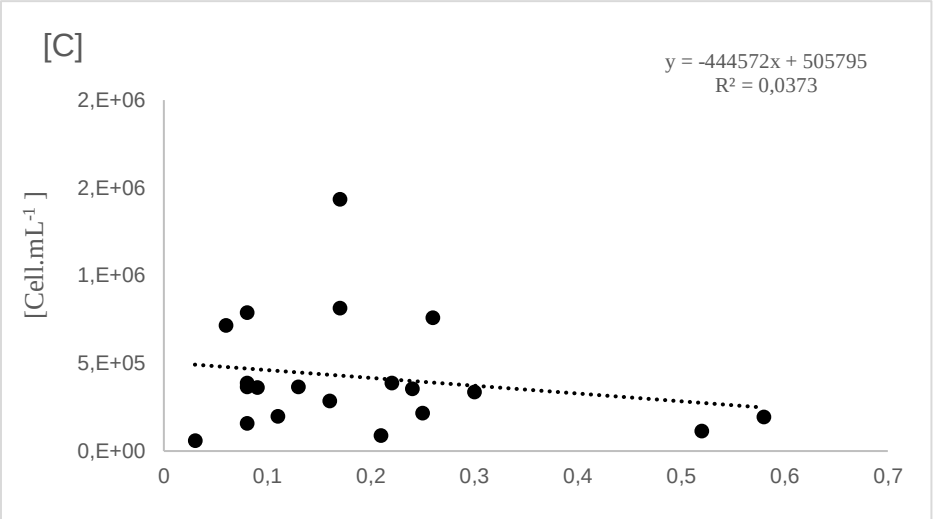
In this study, total nitrogen presented a Wi = 0.191. Beaulieu (2013) applied “empirical modeling approach” to quantify the impact of nutrients in the cyanobacteria concentration. They found that TN and temperature provided the best model of study, which explained 25% of the variance. In this study, similar value were found, the first factor of the factorial analysis explained 27.6 %.

According to Dolman *et al*. (2012) nitrogen may control eutrophication and consequently influence phytoplankton composition. According to the authors, the correlation between total cyanobacterial biovolume and TP became less significant at

high TP concentrations, but continue to increase when TN increased. These authors found a saturation between total phosphorus concentration and the cyanobacteria biovolume, which showed that other factors may be related to the proliferation of cyanobacteria in lakes enriched with phosphorus. This relation of saturation was not found in relation to total nitrogen. Scott and McCarthy (2011) said that reduction in nitrogen inputs can result in a decline of cyanobacteria abundance. The correlation between TN and cyanobacteria; TP and cyanobacteria; and N: P and cyanobacteria can be observed in Figure 13, in which the correlation between TN and cyanobacteria and N:P and cyanobacteria was higher than the correlation between TP and cyanobacteria.

Figure 13 - Relationships between cyanobacteria and total nitrogen (a), cyanobacteria and N:P (b) and cyanobacteria and total phosphorus (c)



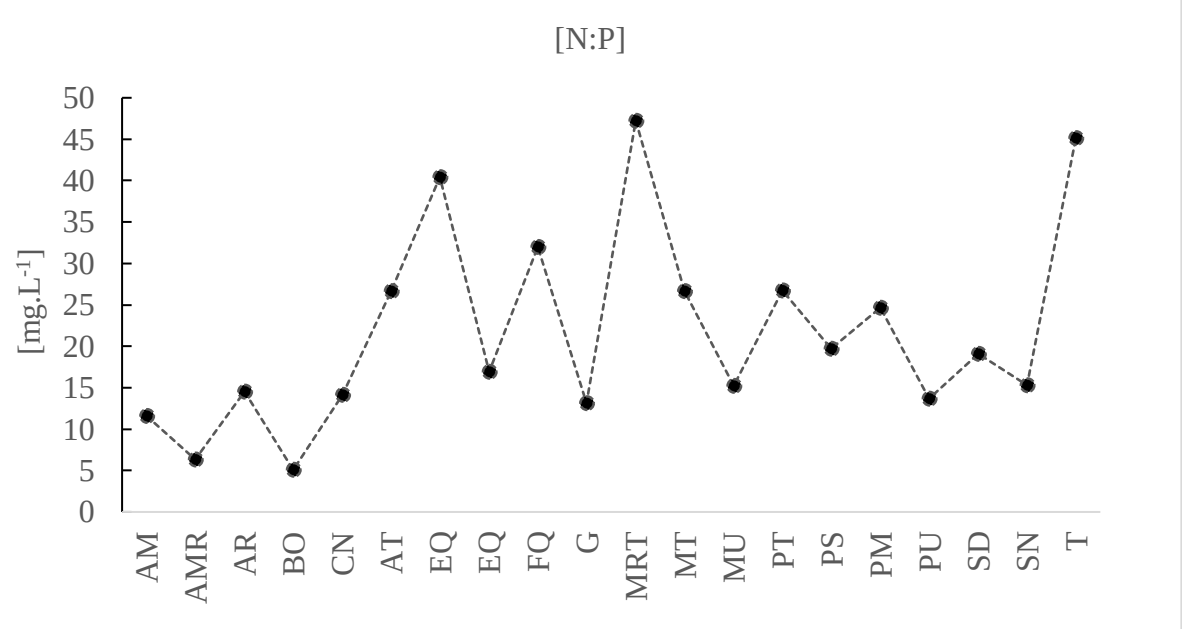


Source: Author (2018).

There is a saturation between phosphorus concentration and cyanobacteria biovolume (Dolman *et al* 2012) and between phosphorus and cyanobacteria biomass (Watson *et al* 1997) indicating that the limitation may occur by other factors in phosphorus-enriched lakes. According to Dolman *et al*. (2012), nitrogen plays a relevant role when cyanobacteria becomes dominant. A possible explanation for this association, and the fact that cyanobacteria may have higher requirements for nitrogen compared to eukaryotic algae, is that they have pigment structures called phycobiliproteins (Allen 1984). Lewis and Wurtsbaugh (2008), who stated that the nitrogen fixation is not always sufficient to overcome the nitrogen limitation due to the limitation of light or other microelements, proposed another possible explanation.

Smith (1983) compiled data from 17 lakes worldwide and found that cyanobacteria were dominant in lakes with low N:P ratios. Their results conclude that lakes having an epilimnetic N:P ratio higher than 29 by weight will exhibit a low proportion of cyanobacteria. Figure 14 shows that of N:P ratios observed, only four reservoirs were above the proportion suggested by Smith *et al*. (1983). When considering this explanation for our data, however, we find that the proportion of cyanobacteria was high in the four reservoirs that exceeded those proposed by these authors. This may suggest that, in addition to the N:P relationship, other factors, including parameters that compose the index, may influence the high cyanobacteria concentrations in these reservoirs.

Figure 14 - N: P ratio for the 20 analyzed reservoirs.



Source: Author (2018).

The use of WQI has been considered a criterion of classification, which takes into consideration the use of parameters that are monitored in water resources. The main purpose of index use is to reduce a large amount of information in a single value. (Chaturvedi and Bassin 2010). As for example, Misaghi *et al* (2017) developed index for water demand for irrigation; Husan, Jamil and Aimi 2015 studied a river in Malaysia.

The type of treatment and, consequently, the efficiency of water supply depends on several factors, mainly the quality of the raw water found in water sources. Cyanobacterial bloom presence in water sources used for water supply can change quality of the water treated and it has influenced in all the stages involved in the treatment. These problems increase the cost of water production and require more frequent monitoring (Di Bernardo *et al*., 2012; He *et al*., 2016). According Di Bernardo *et al*., (2012) direct filtration is widely used due to its low initial investments as well as a reduction in the area used in the construction of the water treatment plant.

When systems emit warning signals related to high cyanobacterial biomass and consequently high cyanotoxins concentration in the raw water supplying the water plants, measures are required that promote effective treatment to protect water within the system (He *et al*., 2016). Not very different from the direct filtration treatment,

conventional treatment consist of a variety of chemical and physical processes, such as coagulation, flocculation, sedimentation, filtration, disinfection and adsorption, more unitary operations are inserted into the process. Therefore, the fact that the WTPs classified as good present a direct filtration system, and with the increase of the index (worst quality), are necessary pre-treatment until culminating in the more robust treatment by complete cycle (MT reservoir), makes the use of the *Icyano* index as an effective tool in the management of water resources.

5.5 Conclusion

The parameters that best explained the proposed *Icyano* were temperature, evaporation, sunlight, electrical conductivity, chlorophyll, TN, TP, Secchi depth, and concentration of cyanobacteria. The high ratio N:P may be the best explanation for the dominance of cyanobacteria in studied reservoirs, however, it cannot be considered as the only factor favoring the dominance of cyanobacteria. In study case, the direct filtration technology used in WTPs, initially designed, it is not capable of providing the water with the characteristics required by the Brazilian law of potability, and this degradation is due to the presence of cyanobacteria, the stations initially designed to operate only with direct filtration, now use pretreatment technology to address this deficiency. The use of *Icyano* to manage reservoirs in the study area proved to be effective in assisting the decision making, in this sense, *Icyano* may help as a tool to in the changes of technologies in WTPs, as well as to increase the attention in sensitive points of the system, mostly, in raw water management, besides providing greater operational and financial attention to the stations considered bad.

5.6 Acknowledgments

We thank CAPES (Coordenação de Aperfeicoamento 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, as well as Thais Benevides for all the map support. 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. This project was partially

supported by the Alabama Agricultural Experiment Station and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

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Appendix 1 - Descriptive statistic (μ± σ) of water quality parameters analysed.

	Evaporation ($mm.day^{-1}$)	**Total Nitrogen ($mg.L^{-1}$)**	**Chlorophyll ($\mu g.L^{-1}$)**	**Sochi depth (m)**	**Electrical conductivity ($\mu S.cm^{-1}$)**	**Cyanobacteria concentration ($cell.mL^{-1}$)**
AM	3.68 ± 1.45	1.51 ± 0.59	36.48 ± 25.05	0.57 ± 0.20	393.9 ± 75.71	365,879.75 ± 550,745.87
AMR	3.64 ± 1.52	3.68 ± 2.43	131.97 ± 172.31	0.4 ± 0.13	1262.57 ± 477.10	196,279 ± 194,565.37
AR	7.09 ± 2.18	1.6 ± 0.57	38.31 ± 18.75	0.72 ± 0.12	850.29 ± 110.81	198,383.91 ± 147,076.30
BO	7.88 ± 4.28	2.64 ± 0.85	251.07 ± 348.51	0.15 ± 0.07	2150.3 ± 1760.72	117,220 ± 159,725.52
CN	7.49 ± 2.57	3.54 ± 3.47	101.56 ± 92.43	0.36 ± 0.19	917.43 ± 601.04	218,448.2 ± 211,806.81
AT	6.3 ± 3.56	0.8 ± 0.66	3.86 ± 1.16	2.5 ± 0.50	384.4 ± 34.65	62,338 ± 50,637.88
EQ	8.4 ± 4.39	3.64 ± 1.29	142.45 ± 67.73	0.41 ± 0.15	460.65 ± 40.90	363,158.57 ± 259,186.93
E	8.42 ± 1.76	4.06 ± 2.59	110.66 ± 95.74	0.63 ± 0.38	1273 ± 998.65	357,704.4 ± 441,140.43
FQ	9.38 ± 4.39	5.44 ± 3.72	264.17 ± 267.51	0.31 ± 0.07	1262.6 ± 435.53	1,436,346.73 ± 959,196.39
G	3.93 ± 1.43	1.05 ± 0.58	40.36 ± 24.21	0.84 ± 0.15	632.51 ± 113.49	365,371.00 ± 767.613.05
MRT	7.95 ± 4.76	3.78 ± 1.43	146.41 ± 167.83	0.18 ± 0.07	373.93 ± 181.07	790309.91 ± 601,961.13
MT	8.84 ± 6.34	6.94 ± 4.87	381.91 ± 234.15	0.17 ± 0.05	1657.2 ± 711.52	760,547.64 ± 1,114,748.15
MU	6.61 ± 3.92	1.22 ± 0.80	53.51 ± 37.15	0.73 ± 0.28	215.92 ± 40.30	158,391.14 ± 103,741.89
PT	5.63 ± 3.02	2.14 ± 1.27	81.12 ± 97.99	0.57 ± 0.22	571.85 ± 199.23	390,585.55 ± 371,078.86
PS	8.05 ± 4.52	3.35 ± 1.29	173.87 ± 36.14	0.28 ± 0.07	395.69 ± 99.62	815,846.14 ± 654,199.26
PM	8.87 ± 4.42	3.94 ± 2.91	90.42 ± 44.78	0.37 ± 0.19	3094.2 ± 2189.4	286,486.14 ± 228,191.53
PU	10.18 ± 4.06	2.87 ± 1.88	94.08 ± 118.88	0.48 ± 0.25	764.29 ± 455.28	89,918.4 ± 120,665.00
SD	5.9 ± 3.34	4.2 ± 0.73	191.29 ± 62.98	0.3 ± 0.01	790.6 ± 498.43	387,372.5 ± 458,230.90
SN	3.54 ± 1.52	4.6 ± 1.57	94.17 ± 54.75	0.39 ± 0.09	1029.2 ± 306.64	338,692.5 ± 332,389.83
T	8.68 ± 4.45	2.71 ± 0.76	104.7 ± 41.39	0.43 ± 0.17	324.52 ± 65.88	718,356.7 ± 829,344.00

Appendix 1 - Descriptive statistic (μ± σ) of water quality parameters analysed.

	Temperature (º C)	**Sunlight (h.day^{-1})**	**Total phosphorus (mg.L^{-1})**
AM	27.62 ± 0.97	7.45 ± 3.16	0.13 ± 0.08
AMR	27.34 ± 0.89	6.54 ± 3.7	0.58 ± 0.25
AR	28.28 ± 1.15	8.77 ± 2.4	0.11± 0.07
BO	28.22 ± 1.31	7.45 ± 2.78	0.52 ± 0.06
CN	28.08 ± 1.09	8.03 ± 3	0.25 ± 0.14
AT	27.61 ± 1.5	8.47 ± 2.68	0.03 ± 0.02
EQ	28.55 ± 1.36	7.51± 2.87	0.09 ± 0.03
E	27.96 ± 1.28	8.54 ± 2.61	0.24 ± 0.26
FQ	28.75 ± 1.46	8.03 ± 3.09	0.17 ± 0.08
G	27.56 ± 0.81	7.88 ± 2.81	0.08 ± 0.05
MRT	28.56 ± 1.4	7.76 ± 2.58	0.08 ± 0.05
MT	28.21 ± 1.64	7.81 ± 2.79	0.26 ± 0.12
MU	28.19 ± 1.18	7.35 ± 2.57	0.09 ± 0.03
PT	27.89 ± 1.58	8.45 ± 2.43	0.08 ± 0.05
PS	28.55 ± 1.32	7.88 ± 2.93	0.17 ± 0.4
PM	28.17 ± 1.29	8.31 ± 2.24	0.16 ± 0.06
PU	28.74 ± 1.25	7.66 ± 2.76	0.21 ± 0.15
SD	27.92 ± 1.55	8.55 ± 2.5	0.22 ± 0.06
SN	27.58 ± 0.94	6.88 ± 3.42	0.3 ± 0.12
T	28.33 ± 1.71	7.88 ± 2.79	0.06 ± 0.02

Appendix 2 - Calculated values of Qi and *Icyano* final value for each analyzed reservoir

	Temp	Evap	sunlight	TN	TP	Chl	CE	Secchi	Cyano	*Icyano*
AM	0.92	0.13	1.32	2.21	0.85	1.74	0.24	11.88	4.74	24.02
AMR	0.00	0.09	0.00	9.00	4.18	6.83	1.42	12.93	2.09	36.55
AR	2.78	3.27	3.23	2.46	0.62	1.84	0.86	10.95	2.12	28.14
BO	2.61	4.00	1.32	5.64	3.87	13.18	2.63	14.54	2.68	50.47
CN	2.19	3.64	2.16	8.54	1.70	5.22	0.95	13.24	2.44	40.08
AT	0.80	2.55	2.79	0.00	0.00	0.00	0.23	0.00	0.00	6.36
EQ	3.58	4.48	1.40	8.85	0.46	7.39	0.33	12.99	4.70	44.19
E	1.84	4.50	2.89	10.19	1.63	5.70	1.43	11.51	4.61	44.29
FQ	4.17	5.39	2.16	14.49	1.08	13.88	1.42	13.55	21.45	77.59
G	0.65	0.36	1.94	0.81	0.39	1.95	0.57	10.27	5.20	22.13
MRT	3.61	4.07	1.76	9.28	0.39	7.60	0.21	14.36	11.36	52.65
MT	2.58	4.89	1.84	19.13	1.78	20.16	1.96	14.42	10.90	77.64
MU	2.52	2.83	1.17	1.31	0.46	2.65	0.00	10.95	1.50	23.39
PT	1.63	1.93	2.76	4.17	0.46	4.12	0.48	11.94	5.12	32.63
PS	2.99	4.16	1.94	7.98	1.08	9.07	0.24	13.68	11.76	52.89
PM	2.46	4.92	2.56	9.78	1.08	4.62	3.91	13.12	3.50	45.94
PU	4.14	6.12	1.62	6.45	1.39	4.81	0.74	12.50	0.43	38.22
SD	1.72	2.18	2.91	10.59	1.47	9.99	0.78	13.61	5.07	48.32
SN	0.71	0.00	0.49	11.84	2.09	4.82	1.10	13.06	4.31	38.42
T	7.28	4.74	1.94	5.95	0.23	5.38	0.15	12.81	10.24	48.72