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MARIANNA CORREIA ARAGÃO GOES

DYNAMICS OF METABOLITES PRODUCTION AND APPLICATION OF HYDROGEN PEROXIDE FOR THE REMOVAL OF NOXIOUS CYANOBACTERIA FROM ISOLATED CULTURES AND WASTEWATER

FORTALEZA
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Tese apresentada a Coordenação do Programa de Pós-Graduação em Engenharia Civil da Universidade Federal do Ceará, como requisito para obtenção do título de doutora em Engenharia Civil. Área de concentração: Saneamento Ambiental.

Orientador: Prof. Dr. José Capelo Neto

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DYNAMICS OF METABOLITES PRODUCTION AND APPLICATION OF HYDROGEN PEROXIDE FOR THE REMOVAL OF NOXIOUS CYANOBACTERIA FROM ISOLATED CULTURES AND WASTEWATER

Thesis presented to the Coordination of 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.

Approved on: ___/___/_____.

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To God,

To my parents, my husband, and my son.

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“Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível.”

Charles Chaplin

ABSTRACT

The problems caused by the flowering of cyanobacteria in the environment are a worldwide concern. In the case of drinking water, the treatment process to remove cyanobacteria and their intracellular metabolites must be carried out without compromising cell integrity, to avoid exposing consumers to toxins and disinfection by-products. One of these methods is oxidation in situ using hydrogen peroxide (H_2O_2). The application of H_2O_2 is a viable alternative, in reservoirs and the effluents of Wastewater Treatment Plants (WTPs), as it is effective in removing cyanobacteria and metabolites and is ecologically correct, as it decomposes quickly into oxygen and water and does not accumulate in the environment. To understand the kinetics of metabolites production and help to predict the best time for application of the oxidant, the concentrations of the metabolites can be predicted by mathematical models from easily measured parameters. Also, it is essential to investigate the occurrence of potentially toxic cyanobacteria in WTPs to assess the potential impact of their effluent on the receiving water bodies. Therefore, this thesis aimed to develop mathematical models to estimate the concentration of metabolites in cyanobacterial species of isolated cultures using easily measurable pigments, to evaluate the effects of H_2O_2 in these cyanobacterial species and their metabolites, and to investigate the occurrence of toxic cyanobacteria in stabilization ponds in the Brazilian semi-arid region. The equations obtained of multiple regression models were able to predict the concentration of microcystin and geosmin and explained 82.4% and 89.8% of these metabolites in cultures of *Microcystis aeruginosa* and *Dolichospermum circinale*, respectively. The application of H_2O_2 on a bench scale was efficient in suppressing isolated cultures of *Microcystis aeruginosa* and *Dolichospermum circinale* cyanobacteria. *Dolichospermum circinale* cells were visibly more sensitive to H_2O_2 than *Microcystis aeruginosa*, since, after the application of 2 $mg.L^{-1}$, no whole cells were detected. As for the occurrence of toxic cyanobacteria in the stabilization ponds of the Brazilian semi-arid region, the dominance of the species *Plankthothrix agardhii*, *Sphaerocavum brasiliensis* and *Microcystis aeruginosa* was evident, demonstrating that the monitoring of these organisms must be permanent. Based on these results, mathematical modeling can be an important tool to alert the presence of toxins in the water and the need for complementary treatments such as the application of hydrogen peroxide or, in extreme cases, the total stop of water distribution. In addition to this, monitoring in reservoirs before water treatment plants and in effluents from

waste stabilization ponds in WTPs is necessary to accurately quantify the potential impact of cyanotoxins on the environment.

Keywords: Hydrogen peroxide, Cyanobacteria, Metabolites, Stabilization ponds.

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SUMMARY

INTRODUCTION

Cyanobacteria or blue-green algae are prokaryote photosynthetic organisms and feature among the pioneering organisms of planet Earth. They have been existing for at least 2.7 billion years, and are considered to be the main primary producers and the first organisms to have released oxygen into the primitive atmosphere (ZANCHETT; OLIVEIRA-FILHO, 2013). Cyanobacteria blooms have been reported throughout history worldwide (SVIRČEV et al., 2017).

Cyanobacteria are common in aquatic systems, where they grow in the water column, planktonic (CATHERINE et al., 2013). Besides being found in limnetic and marine environments, many species are capable of living in terrestrial habitats, where they are important to ecosystem processes and nutrient cycling (ZANCHETT; OLIVEIRA-FILHO, 2013). They can be found in the extreme environments of Antarctica to thermal springs, even surviving for long periods in complete darkness (ZANCHETT; OLIVEIRA-FILHO, 2013). They can be found in colonial form or filaments and planktonic or benthonic species are classified into four different orders: Chroococcales, Oscillatoriales, Nostocales, and Stigonematales (SANT'ANNA et al., 2006).

Due to intense eutrophication and climatic changes, cyanobacterial blooms are increasing in freshwater ecosystems all around the world (SCHOLZ; ESTERHUIZEN-LONDT; PFLUGMACHER, 2017). They can multiply quickly in surface waters and form blooms when favorable conditions prevail, such as high temperature, intense light, high pH, and high nutrients availability, especially phosphorous and nitrogen released by anthropogenic activities (ZANCHETT; OLIVEIRA-FILHO, 2013). These blooms formed by cyanobacterial species can produce a variety of toxic secondary metabolites, known as cyanotoxins, which are harmful to both humans and animals, (SUKENIK et al., 2017; SVIRČEV et al., 2017).

The impacts of toxic cyanobacteria bloom to reservoirs used for drinking water supply depend on many factors, such as the extent and nature of the bloom, specific toxins, and climatic conditions (HE et al., 2016). Great distresses caused by cyanobacterial blooms and their cyanotoxins have been reported in some of the world's largest water bodies, including Taihu Lake (China), Okeechobee (USA), and Kasumigaura (Japan) (WANG et al., 2017).

Cyanotoxins are considered an ecotoxicology and toxicology important group of chemical compounds in environmental chemistry (ZANCHETT; OLIVEIRA-FILHO, 2013). These cyanotoxins can cause acute, chronic and subchronic toxicity in wild/domestic animals

and humans (WANG et al., 2017), produce chronic diseases such as cancer and even lead to death (ZANCHETT; OLIVEIRA-FILHO, 2013). Cyanotoxins can be divided based on two main criteria: (1) their action mechanism in three principal classes: hepatotoxins, neurotoxins, dermatotoxins; and (2) their chemical structure: cyclic peptides, alkaloids, or lipopolysaccharides (LPS) (ZANCHETT; OLIVEIRA-FILHO, 2013).

Hepatotoxins and neurotoxins are the cyanotoxins most frequently produced by cyanobacteria (YUNES, 2019). The hepatotoxins, in particular the Microcystin, are the most frequent and can be released from the genera *Microcystis, Planktothrix, Anabaena (Dolichospermum), Aphanizomenon, Nodularia, Nostoc, Cylindrospermopsis*, and *Umezakia* (SORLINI; COLLIVIGNARELLI; ABBA, 2018). Among the microcystin variants, the most frequent, toxic, and present in greater concentration is the Microcystin-LR which is generally contained in the algal cell but can be easily released following cell lysis (SORLINI; COLLIVIGNARELLI; ABBA, 2018; SVIRČEV et al., 2017).

The Neurotoxins, in particular the saxitoxins, have been responsible for several animal poisonings around the world (SANSEVERINO et al., 2017). This molecule binds to the sodium channel in neuronal cells with great intensity, blocking the nervous transmission, and causing nerve dysfunction and death from paralysis of respiratory muscles (SANSEVERINO et al., 2017). These toxins are also produced by freshwater cyanobacteria (the genera *Anabaena (Dolichospermum), Lyngbya, Cylindrospermopsis,* and *Planktothrix*) and marine dinoflagellates (KAEBERNICK; NEILAN, 2001; SANSEVERINO et al., 2017).

Some metabolites of cyanobacterial, especially 2-methylisoborneol (2-MIB) and geosmin (GSM), although not toxic, can produce the taste and odor of drinking water and increase water treatment costs. In this context, the removal of harmful cyanobacteria from lakes and reservoirs is essential for environmental and human health (WANG et al., 2017).

According to Sorlini et al. (2018), the adaptable alternatives to reduce the risk of cyanobacteria and their metabolite in drinking water for humans: (1) to use an appropriate choice of the water source, avoiding raw water from contaminated sources; (2) to reduce nutrient supply, in particular phosphorus, to the source of supply; (3) the use of adequate treatments for intact algae removal and (4) to use adequate treatments for the removal of algal toxins.

Water treatment operations must remove cyanobacteria without compromising cell integrity to remove, at the same time, cells and intracellular metabolites (MEREL et al., 2013). However, during the conventional water treatment process, the release of cyanotoxins and the formation of toxic by-products can occur and make water treatment more laborious, time-

consuming, and expensive (GER et al., 2010; ZI et al., 2018). To overcome difficulties, considering that the productivity of metabolites can be related to different stages of the growth phase of cells, the development of mathematical models that estimate the concentration of metabolites using easily measurable parameters, such as pigments, can be used as an auxiliary tool in the application of water pretreatment methods (MCQUAID et al., 2011).

Some methods of pretreatment in water reservoirs are suggested before water treatment plants, but most chemical methods of removal result in the production of harmful by-products, or the accumulation of heavy metals in ecosystems (BARRINGTON; REICHWALDT; GHADOUANI, 2013). The application of H_2O_2 is a viable alternative as it is effective in removing cyanobacteria and cyanotoxins during large-scale application and is environmentally friendly since it does not accumulate in the environment and decomposes rapidly by biological, chemical, and photochemical mechanisms (BURSON et al., 2014; CHANG; HUO; LIN, 2018). Although several kinds of research have studied the effects of H_2O_2 on the control of cyanobacteria (CHEN et al., 2016; WANG et al., 2019; WEN et al., 2017; YANG et al., 2018), there is a knowledge gap on how H_2O_2 will affect some species of cyanobacteria, their metabolites degradation, and cells regrowth potential. This type of research is of great relevance to guarantee water quality since the results obtained on a laboratory scale can guide large-scale applications.

One of the remarkable characteristics of stabilization ponds, a type of wastewater treatment plant, is the frequent proliferation of cyanobacteria (FLORENTINO et al., 2019). When toxic cyanobacteria are present in stabilization ponds, it imposes a substantial threat to ecological, human, and animal systems, and can affect its treatment efficiency (BARRINGTON; REICHWALDT; GHADOUANI, 2013). These organisms can suppress the growth of phytoplankton species necessary for the treatment of wastewater (BARRINGTON; GHADOUANI; IVEY, 2011). Additionally, the treated effluent, which is generally discarded for irrigation or environmental flow, can act as a cyanobacteria inoculum for the receiving water body and contaminate the environment with cyanotoxins (BARRINGTON; GHADOUANI; IVEY, 2011). Reports on toxic cyanobacteria in stabilization ponds have been published in several studies (KOTUT et al., 2010; PASTICH et al., 2016; SHANTHALA; HOSMANI; HOSETTI, 2009). The Brazilian semi-arid region presents a set of optimal conditions for the development of cyanobacteria, such as high light incidence and temperatures throughout the year, restricted rainy season, and surface waters mostly confined by dams (BITTENCOURT-OLIVEIRA et al., 2012). Due to the importance of the stabilization ponds as a cheap treatment unit in the region, the release of treated wastewater into local aquatic bodies becomes

concerning, making it essential to investigate the occurrence of toxic cyanobacteria and their relationship with the limnologic and climatic parameters.

This thesis is divided into four chapters; Chapter 1, an article published in the Journal of Water Supply: Research and Technology – AQUA in 2020, investigated the intra- and extracellular microcystins throughout the life cycle of *Microcystis Aeruginosa* culture under controlled conditions and developed a mathematical model to estimate microcystin concentration based on pigments concentration (chlorophyll-a, phycocyanin, and pheophytin), parameters of easy and quick analysis. Chapter 2, similar to Chapter 1, article that was submitted to the Annals of the Brazilian Academy of Sciences, determined the generation of intra and extracellular geosmin throughout the life cycle of *Dolichospermum circinale* culture under controlled conditions and develop a mathematical model to estimate geosmin concentration from pigments concentration (chlorophyll, phycocyanin, and pheophytin). Chapters 1 and 2 are necessary to understand the dynamics of metabolite production by each species of cyanobacteria in isolated cultures. Chapter 3, an article published in Aquatic Toxicology Journal in 2021, investigated the effects of H_2O_2 application on *Microcystis aeruginosa* and *Dolichospermum circinale* cell integrity, density, and re-growth potential after H_2O_2 treatment, the fate of microcystins and geosmin, and the impact on pigments concentration, organic matter, and true color. This chapter is necessary to understand the effects of the application of peroxide in isolated cultures of cyanobacteria. Chapter 4, an article that was submitted to the Journal of Arid Environments, highlighted the species of cyanobacteria with the highest occurrence in stabilization ponds located in the Brazilian semi-arid region and determined a better understanding of the occurrence of toxic cyanobacteria and their relationship with environmental factors. This chapter shows the importance of applying hydrogen peroxide to remove cyanobacteria and cyanotoxins from stabilization pond effluents and thus avoid contamination of the environment where this effluent is released.

1 MODELING TOTAL MICROCYSTIN PRODUCTION BY *MICROCYSTIS AERUGINOSA* USING MULTIPLE REGRESSION

1.1 Introduction

Cyanobacteria that produce toxic or taste and odor metabolites have become a widespread and critical problem in aquatic environments around the world (SARAF et al., 2018; ZHANG et al., 2018). Intensive agricultural practices, the release of untreated domestic and industrial wastewater into water bodies, and climate change have boosted the growth rates of cyanobacteria biomass (JACINAVICIUS, 2015). The annual global estimates of the socioeconomic impacts of cyanobacteria metabolites can reach billions of US dollars when considering water monitoring, treatment, and analysis as well as adverse impacts on recreational use and fishing (WILTSIE et al., 2018).

One of the most studied species of cyanobacteria in lakes is *Microcystis aeruginosa* – MA (CHAFFIN et al., 2018). It can produce a group of toxic monocyclic heptapeptides called Microcystins – MCs (MINASYAN et al., 2018), the most common toxin found in natural waters (WALLS et al., 2018). Also, these cyanobacteria are problematic because it rapidly increases biomass on the water surface, limiting sunlight penetration, reducing dissolved oxygen concentration and making water treatment more laborious and costly (GER et al., 2010). MCs can accumulate in the trophic network and cause harmful effects to mammalian health, especially to the liver, inhibiting essential enzymatic functions of the hepatic tissue (MASSEY et al., 2018; MOHAMED; BAKR; SOLIMAN, 2018; YOSHIZAWA et al., 1990). As a consequence, the World Health Organization proposed a maximum concentration of 1 $\mu g.L^{-1}$ in finished water (WHO, 2003, 2011), a limit adopted by Brazil and Uruguay. Countries such as Australia and Canada have adopted MCs limit concentrations of 1.3 and 1.5 $\mu g.L^{-1}$, respectively (CANADA, 2002; NHMRC; NRMMC, 2011).

The release of MCs occurs preferentially due to cellular senescence, when membrane lysis occurs naturally, rather than continuous excretion (ZI et al., 2018). Lysis may also occur during the application of chemicals for the treatment or control of cyanobacteria, especially algaecides (CHORUS; BARTRAN, 1999). After cyanobacteria lysis, the intracellular contents are dissolved into water, making them available to organisms (SCHMIDT et al., 2013) and accumulating in the aquatic systems (PEREIRA et al., 2018).

Dissolved MCs are more difficult and costly to remove in conventional water treatment plants (VAN APELDOORN et al., 2007) since extra treatment steps must be

incorporated into the treatment train (HAMILTON et al., 2013). Also, to minimize risks, frequent analysis of MCs and cyanobacteria cell density is required, further increasing the costs and time consumed. Frequently, by the time analysis confirms the presence of toxins, the finished water has already been delivered to consumers.

To overcome the high costs and time required for cell count and cyanotoxin analysis, some attempts to develop predictive methods were carried out to evaluate how environmental variables are connected to the growth of toxic cyanobacteria and toxin production (BORTOLI et al., 2014). Harris and Graham (2017) observed that pigments, iron, and dissolved oxygen presented a high correlation with MCs concentration. Marion et al. (2012) developed a multivariate logistic regression model using real-time measurements of phycocyanin in vivo and Secchi depth to evaluate the risks of MCs concentration exceeding 4 $\mu g.L^{-1}$ in waters of a recreation pond. The final model reached high statistical significance as well as a good calibration, proving to be effective in identifying the risks involved with bathing. (MCQUAID et al., 2011) used in vivo analyses of phycocyanin in an attempt to evaluate the abundance of cyanobacteria and the associated MCs concentration in water sources. The results of this study demonstrated that there is a potential for the prediction of cyanobacteria biovolume and the concentration of MCs using phycocyanin probes.

Phycocyanin is a water-soluble and strongly fluorescent compound, making it viable to detect it with portable instruments such as probes (MCQUAID et al., 2011). Probes based on phycocyanin fluorescence are applied for the rapid assessment of cyanobacteria in drinking water sources (BASTIEN et al., 2011; MCQUAID et al., 2011), offering an alternative to monitoring cyanobacteria and hence cyanotoxins. However, most probes still have limitations when transforming pigment concentration signals into other cyanobacterial parameters, such as biomass and cell densities, and only perform accurately within very limited concentration ranges (GLIBERT et al., 2018). In this sense, the development of an efficient model that precisely correlates pigment concentration and cyanotoxins would be beneficial to the water industry.

Although it has been demonstrated that the concentration of MCs is related to the number and the growth phase of cells (MCQUAID et al., 2011), studies comparing the productivity of metabolites at different stages of growth and with different MCs variants are still limited. Crettaz Minaglia et al. (2017), for example, mathematically modeled *Microcystis aeruginosa* growth and microcystin-LR production in BG11 media at different temperatures. The authors established that there is a relationship between some environmental variables and one variant of MC. However, a mathematical model that estimates the concentration of total

microcystin (McT) using easily measurable parameters, such as pigments, has not yet been found in the literature.

In this context, the main objective of this work was to evaluate the production of intra- and extracellular MCs throughout the life cycle of *Microcystis aeruginosa* culture under controlled conditions and to correlate McT concentration and cell age, cell density, concentrations of chlorophyll-a, phycocyanin, and pheophytin to develop a mathematical model to estimate the concentration of McT using few parameters with relatively easy and rapid analysis.

1.2 Materials and methods

The suspension using *Microcystis aeruginosa* culture and an ASM-1 medium was prepared in one batch and, after that, it was subdivided into five individual flasks of 1 L each. Each flask was completely used for that week's analysis, so no changes in the initial volume occurred. The flasks were submitted to the same conditions. The *Microcystis aeruginosa* strain (CIAR 03) was cultured in 1 L flasks in an ASM-1 medium (GORHAM et al., 1964) using 12 : 12 h (L : D) photoperiod at 22 $\mu.E.m^{-2}.s^{-1}$ (Digital Lux Tester YF-1065), pH 8, 25 ºC (±1 ºC) and under continuous aeration. In each of the subsequent five weeks (cell age – CA), one flask was used for the analysis of the following parameters: MA cell density (Dens), chlorophyll-a (Chlo.a), phycocyanin (Phyc) and pheophytin (Pheo), called the explanatory variables, and Total Microscystin – McT, the response variable. The experiment was carried out in triplicate for 5 weeks, totaling 15 samples for each analysis. Aliquots of 1 mL were used for cell density analysis. For the determination of chlorophyll-a (Chlo. a) and pheophytin (Pheo), an aliquot of 100 mL was used; for phycocyanin (Phyc), an aliquot of 100 mL was used and for total microcystin – McT, an aliquot of 100 mL was used.

For microcystin analysis, the aliquot of 100 mL was filtered through 110 mm GF/C discs (Whatman, Maidstone, Kent, UK). The GF/C discs with the cell-bound or intracellular microcystin were freeze-thawed and placed in glass beakers containing 10 mL of methanol (100%) (J.T. Baker – Grade UV-HPLC) to extract the intracellular content for 1 h at room temperature (25 ºC). The extract was removed and evaporated at 50 ºC in a vacuum until dry. The residue was resuspended in 1 mL of methanol (80%) before analysis by HPLC. For the extracellular microcystin, the filtered samples (100 mL) were passed through C18 Cartridges (SupelClean LC-18 – SUPELCO 1 g and 3 mL) using a vacuum manifold system (Varian Analytical Instruments, San Fernando, CA, USA). The cartridges were conditioned before

sample concentration using 10 mL of methanol (100%) (J.T. Baker – Grade UV-HPLC) followed by 10 mL of water. Samples were applied to the cartridges at a flow rate of 1 drop.min^{-1}. After the sample was passed through, the cartridge was washed with 10 mL of 10, 20, and 30% v/v aqueous methanol in sequence. The air was drawn through the cartridge for about 10 min to minimize the amount of aqueous methanol eluted from the previous step. Then, the cartridge was eluted with 0.8 mL of methanol (80%).

The analysis was performed using high-performance liquid chromatography - HPLC (Agilent Technologies, model 1260 Infinity), with a reverse-phase column (Waters Symmetry C18, 5 μm, 2.1 × 150 mm) at 40 ^{o}C with a DAD detector set at wavelengths 238 and 222 nm. The mobile phases used were (i) 0.05% Trifluoroacetic acid – TFA acid (Dynamic – Grade UV-HPLC) in water and (ii) 0.05% TFA in acetonitrile (J.T. Baker – Grade UVHPLC) with a flow rate of 0.5 mL.min^{-1} and a total run time of 35 min. Gradient elution was applied using the following program: linear increase from 25 to 100% B within 22 min, hold at 100% B for 2 min, and subsequently decrease to 25% B within 11 min. A calibration curve for each variant (Mc-LR, Mc-LA, and Mc-LY) was produced using standards provided by the Cyanosol Laboratory (UK). The equipment's limit of detection (LOD) and quantification (LQD) was 0.25 and 0.5 mg.L^{-1}, respectively, but the method's limit of detection (LOD) and quantification (LQD) was based on the degree of sample concentration detailed previously. Therefore, the method's limit of detection (LOD) and quantification (LQD) will be 100 times smaller than the equipment's limit of detection (LOD) and quantification (LQD). The method used was from Lawton et al. (1994).

Total microcystin concentration (McT) comprised of the sum of the Mc-LR, Mc-LA, and Mc-LY concentrations (intra and extracellular). For the cell quota calculation, the total intracellular microcystin concentration (sum of the intracellular Mc-LR, Mc-LA, and Mc-LY) was divided by the number of cells or biomass, in the same base volume.

After preserving *Microcystis aeruginosa* samples with Lugol, cell density (Dens) at different growth stages was measured using a Sedgewick-rafter chamber and an inverted optical microscope with a magnification of 40x (Zeiss, Model Vert. A1; APHA, 2012). For the determination of cell density, each unicellular organism was considered as an individual (YOSHIDA et al., 2006).

The spectrophotometric method (10200H), proposed in APHA (2012), was used for the determination of chlorophyll-a (Chlo.a) and pheophytin (Pheo). Samples were analyzed at wavelengths of 664, 665 and 750 nm, respectively. Phycocyanin (Phyc) was analyzed using the

spectrophotometric method at 665 and 750 nm wavelengths proposed by Bennett and Bogorad (2011), with adaptations (Genesys 10-S, Thermo-Scientific, USA).

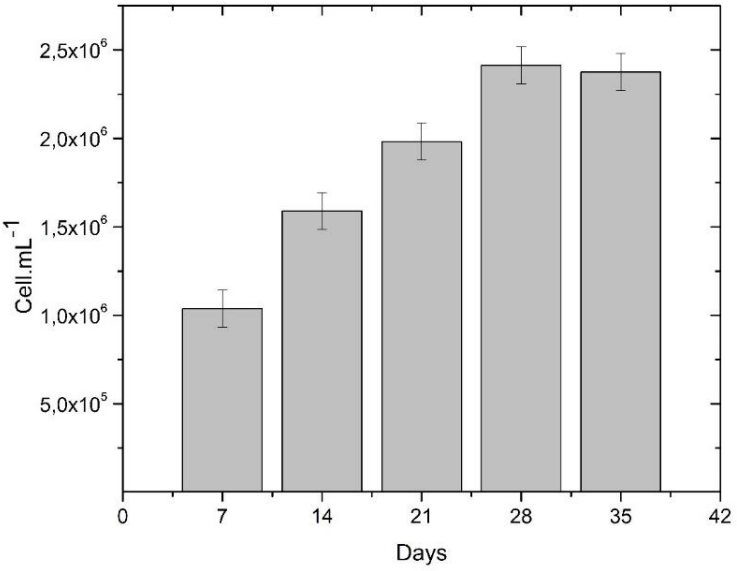
The hypothesis of data normality and homogeneity of variance was verified using the Shapiro–Wilk (PS-W) test and the Levene test. For comparisons between the analyzed values at each week after using the ANOVA test, the Tukey test was performed for samples with normal distribution, and pairwise comparisons using the Wilcoxon signed-rank test with Bonferroni adjustment for samples without normal distribution. The Spearman correlation coefficient (R_S), as well as its intensity (weak, moderate and strong) and significance, was determined to verify the degree of association between all variables. For this study, it was considered a strong association when $|R_S| \geq 0.7$ and moderate when $0.7 > |R_S| \geq 0.5$ (ZAR, 1996).

The explanatory variables (CA, Dens, Chlo.a, Phyc and Pheo) that correlated significantly with the response variable (McT) were incorporated into the model using the Stepwise method. The regression model that contained easily determined explanatory variables was preferred among the models with the highest Adjusted R^2. The selected regression model was then evaluated by both normality and homoscedasticity of the residues (evaluated graphically – Figure 4) and coefficient significance. All the statistical tests were performed using a significance level of 5% ($\alpha = 5\%$) and the RStudio program (R CORE TEAM, 2018). Details of the data analyses mentioned are available in Table 4 in Appendix A.

1.3 Results and Discussion

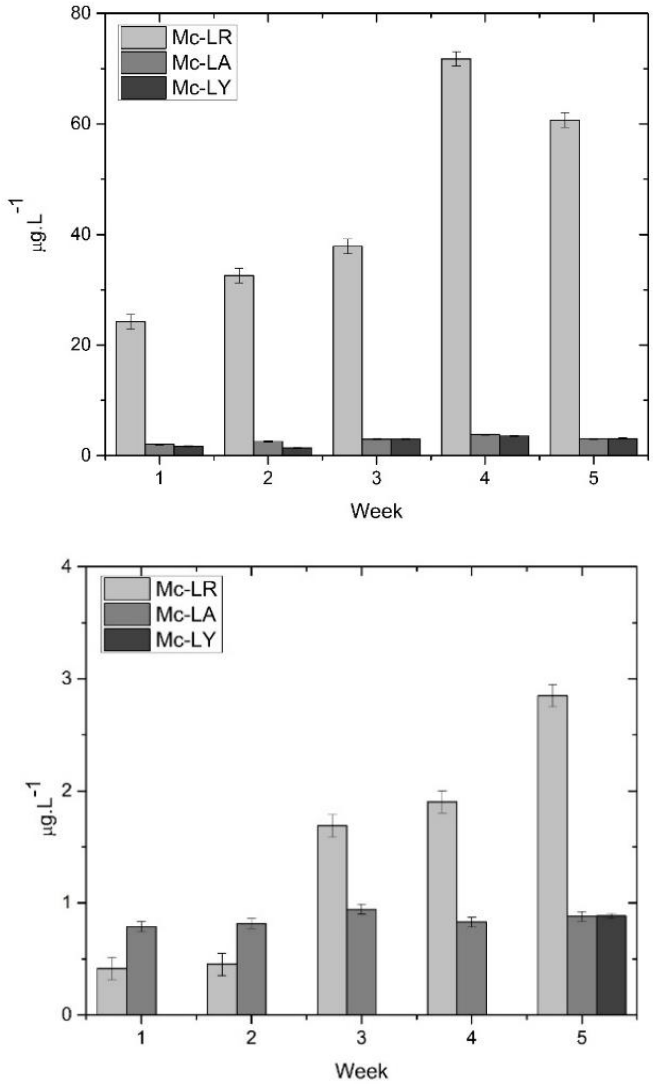
Until the fourth week of the experiment (28 days), there was a significant ($p < 0.05$) increase in cell density (Figure 1). However, no significant difference ($p > 0.05$) was observed between the fourth and fifth weeks indicating that the culture reached the stationary phase. There was no need for additional points after that since they would not improve the model precision. Bortoli et al. (2014) studied the growth of a *Microcystis aeruginosa* strain isolated from the Billings Reservoir (Brazil) under different nutritional conditions and observed that the stationary phase was reached after 21 days, differing from this experiment probably because of the different experimental conditions and *Microcystis aeruginosa* strain. According to Bittencourt-Oliveira et al. (2005), *Microcystis aeruginosa* growth and microcystin production vary according to a biochemical activity regulator, which is different for each strain.

Figure 1 – Average MA cell density values during the growth cycle under controlled conditions.



The MA strain cultivated produced Mc-LR, Mc-LA, and Mc-LY which were detected in the chromatograms at 4.7, 16.1, and 19.7 min, respectively. The intracellular Mc-LR variant was predominant during the whole duration of the experiment (Figure 2). In the fourth week, intracellular Mc-LR concentration was 70 $\mu g.L^{-1}$, while extracellular concentration was 1.25 $\mu g.L^{-1}$, close to the concentrations observed by Turner et al. (2018). On the other hand, Almuhtaram et al. (2018) studying a reservoir used for water supply, observed that intra- and extracellular MC concentrations were approximately 4 and 0.5 $\mu g.L^{-1}$, respectively. This difference between intra and extracellular proportions may be explained by the fact that extracellular MC modifies the intracellular MC synthesis (SCHATZ et al., 2007). The authors suggested that Microcystis cell lysis enhances MC concentration in the medium, inducing accumulation of mcyB (genetic subunits involved in the synthesis of MCs) and increasing the intracellular production of MC in the remaining intact cells. With the release of MCs and other oligopeptides, microgynin, and micropeptin, the intact population responded by increasing the intracellular production of MC (SCHATZ et al., 2007), a feature more easily identified in axenic cultures.

Figure 2 - Concentrations of Intracellular (a) and Extracellular (b) Mc-LR, Mc-LA, and Mc-LY variants.



In natural waters, MCs are preferentially found inside the *Microcystis aeruginosa* cells (MEREL et al., 2013; WESTRICK et al., 2010) and released in greater amounts during the cell senescence or death phase (MEREL et al., 2013; YE et al., 2017). This behavior may also be observed in MA cells submitted to controlled conditions (NEILAN et al., 2013). The concentration of MC may be correlated with the cell growth rate, with the highest concentrations of MCs observed at the end of the exponential phase of growth. In the stationary phase, concentrations of MCs tend to stabilize and then decrease in the cell senescence phase (REPKA et al., 2004). This reduction of MC concentration can be explained by the reduced activity of enzymes complex NRPS/PKS, responsible for the biosynthesis of secondary metabolites (NIKOLOULI; MOSSIALOS, 2012).

According to Crettaz Minaglia et al. (2017), the Mc-LR cell quota is an important parameter in determining the cellular physiology of MC production. The authors also observed average cell quota values (20 ± 10 fg.cel^{-1}) during *Microcystis aeruginosa* growth similar to the ones found in our study (20 ± 10 fg.cel^{-1}).

The cell quota, a relation between total intracellular MC concentration and cell density or biomass, showed no significant difference ($p > 0.05$) along all growth periods, suggesting that the cell quota was constant throughout this experiment (Table 1). Long et al. (2001) found that to be a common behavior during the growth phase of MA. This linear correlation between MC production and the cyanobacterial growth rate has also been reported by Orr and Jones (1998) and Bortoli et al. (2014). Puddick et al. (2019) evaluated 187 MC quotas in New Zealand and found that the highest value detected was 13 pg.cel^{-1} (equivalent to 13,000 fg.cel^{-1}), much higher than the quota found in our study (32 fg.cel^{-1}). This may be because Microcystis strains found in natural environments have shown the capacity to produce higher cell quota than strains cultivated under controlled conditions (WOOD et al., 2012).

Table 1- Cell quota for intracellular microcystin throughout the experiment weeks.

Time (Day)	**Cell Quota (fg.cell^{-1}) ± SD***
7	26.62 ±1.55E-03
14	23.81 ± 1.56E-03
21	23.56 ± 1.25E-03
28	33.94 ± 1.33E-03
35	30.77 ± 1.86E-03

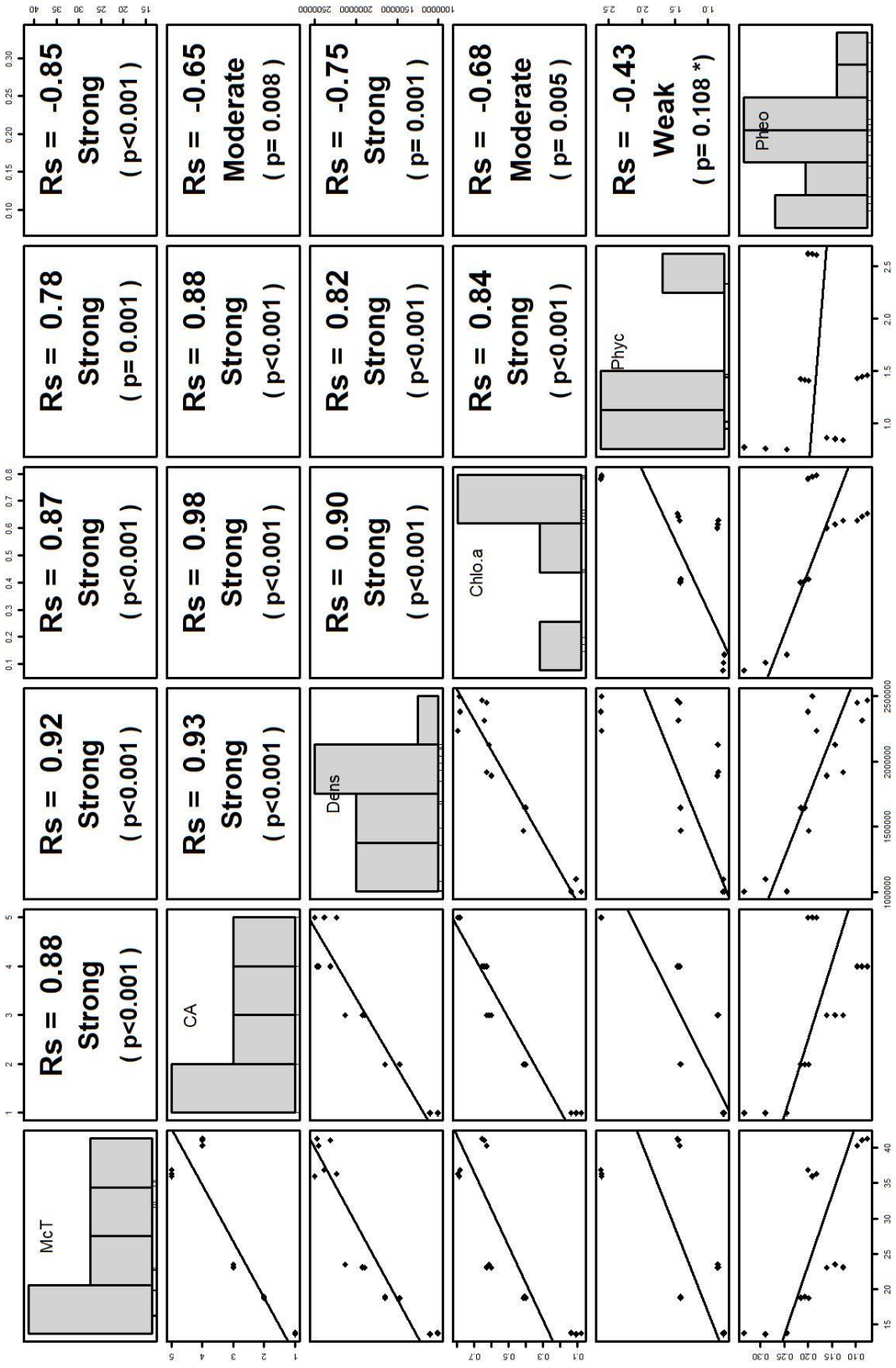
*SD: Standard Deviation

Therefore, it is feasible to consider that the number of microcystins cells is an indication of the concentration of MCs in both cultured and natural waters. It is important to remember, however, that the cellular quota can vary between different microcystins taxa, environmental conditions, and in different natural reservoirs (SALMASO et al., 2014).

The first line of Figure 3 shows that all explanatory variables were strongly correlated ($p < 0.001$) to the response variable (McT). In the left column, the scatter plots of the McT values versus the covariates (explanatory variables) are presented with their respective linear regression lines. The visual verification of the scatter plots can be used to estimate the McT values from each explanatory variable and to evaluate how closely the experimental

results are to the modeled values. Looking at the linear regression adjustment lines (graphs below the main diagonal of Figure 3), it is noted that the explanatory variables alone do not explain the McT production adequately because the vertical distances between the points and the line are large. However, a model with more variables together makes the prediction of microcystin concentration more relevant. This justifies the search for a more elaborate model to estimate McT concentration. Thus, according to Figure 3, the most relevant variables are CA, Dens, Chlo.a, Phyc and Pheo with Rs > 0.7, indicating that these variables could be used to estimate McT.

Figure 3 - Visualization matrix of cell age (CA) and density (Dens), chlorophyll-a (Chlo.a), phycocyanin (Phyc), pheophytin (Pheo) and total microcystin (McT).



In the search for a mathematical model capable of estimating McT concentration based on an explanatory variable or a set of them, a multiple regression of the data was performed. Multiple regression models were generated, with their respective correlation coefficients (Adjusted R^2), involving all possible combinations (Table 2).

Models containing only pigments presented Adjusted R^2 values lower than those containing Dens and CA (Table 2). Beversdorf et al. (2018, 2015) showed that there is a strong correlation between *Microcystis aeruginosa* cell density and MCs production in isolated *Microcystis aeruginosa* cultures, indicating that it is possible to estimate MCs concentration using the number cells. Pereira et al. (2018) demonstrated that increased cell density of *Microcystis aeruginosa* induced an increase in chlorophyll and MCs.

On the other hand, although the model selected to describe the response variable (McT) should have a high Adjusted R^2, for simplicity's sake, it is preferable that it requires the smallest number of variables and that these variables are easily analyzed. According to Table 2, the presence of a single variable in the mathematical model does not explain the production of McT satisfactorily. This can also be observed by the small Adjusted R^2 of single-variable models (Models 12, 19, 28, 30, and 31 – Table 2). Determining cell age (CA) and density (Dens) in environmental samples is not an easy task, and therefore, models based only on pigments were selected since they can be analyzed by simple multiparametric probes. On this basis, the models selected for determining McT were models 25 and 26 with an Adjusted $R^2 = 0.824$ and 0.814, respectively. The adjusted coefficient of determination, also called Adjusted R^2, is a criterion used for the selection of an optimal model with the largest Adjusted R^2 with a reduced set of explanatory variables. This choice is corroborated by studies that have shown a strong correlation between pigments and McT concentrations in environmental samples (DOWNING et al., 2005; FRANCY et al., 2016; LEE et al., 2000).

Table 2 - Multiple regression models between the response variable (McT) and the explanatory variables (CA, Dens, Chlo.a, Phyc and Pheo).

Model	**Variables**	**Adjusted R^2**	**Model**	**Variables**	**Adjusted R^2**
1	CA + Dens + Chlo.a + Phyc + Pheo	0.988	17	Dens + Chlo.a + Pheo	0.854
2	CA + Dens + Chlo.a + Pheo	0.986	18	CA + Chlo.a	0.842
3	CA + Chlo.a + Phyc + Pheo	0.985	**19**	**CA**	**0.832**
4	Dens + Chlo.a + Pheo	0.984	20	Dens + Pheo	0.841
5	CA + Dens + Chlo.a	0.935	21	Dens + Phyc + Pheo	0.848
6	CA + Dens + Chlo.a + Phyc	0.934	22	CA + Dens + Phyc	0.847
7	Dens + Chlo.a + Phyc + Pheo	0.899	23	CA + Phyc	0.838
8	Dens + Chlo.a + Phyc	0.881	24	CA + Dens + Phyc + Pheo	0.848
9	CA + Chlo.a + Phyc	0.880	***25***	***Phyc + Pheo***	***0.824***
10	CA + Pheo	0.870	***26***	***Chlo.a + Phyc + Pheo***	***0.814***
11	Dens + Chlo.a	0.866	27	Chlo.a + Pheo	0.693
12	**Dens**	**0.853**	**28**	**Chlo.a**	**0.674**
13	CA + Dens	0.859	29	Chlo.a + Phyc	0.661
14	CA + Phyc + Pheo	0.860	**30**	**Pheo**	**0.529**
15	CA + Dens + Pheo	0.860	**31**	**Phyc**	**0.387**
16	Dens + Phyc	0.849			

Table 3 shows the coefficients of the selected multiple regression models, as well as p-values and upper and lower limits for each estimated coefficient. Models 25 and 26 were then evaluated using the linear regression method to choose the most appropriate one. From Table 3, it is possible to observe that model 26 is not the most appropriate because Chlo.a *p*-value is higher (*p*-value = 0.546) than the adopted significance level (α = 5%) and the confidence interval for each coefficient goes through zero. On the other hand, model 25, which includes the concentration of Phyc and Pheo pigments, is a reasonable choice since its *p*-values are below the adopted significance level (α = 5%) and the confidence interval for each coefficient does not go through zero.

In this work, chlorophyll rapidly degraded to pheophytin, due to the greater sensitivity of chlorophyll to the incidence of light. Therefore, the pigment pheophytin, in greater quantity about chlorophyll, was better correlated with the production of microcystin.

Table 3 - Coefficients of equations obtained from the correlation between parameters.

Estimated Parameter	Related Parameter	Coefficients	*p*-Value	Confidence Interval 95% Inferior Limit	Confidence Interval 95% Upper Limit
Model 25 (R^2 = 0.849/Adjusted R^2 = 0.824)					
McT	Intercept	64.99	1.00E-5	45.44	84.54
	Pheo	-194.65	9.01E-5	-268.29	-121.02
	Phyc	17.01	4.60E-4	9.23	24.79
Model 26 (R^2 = 0.854/Adjusted R^2 = 0.814)					
McT	Intercept	75.82	2.7E-3	32.46	119.17
	Chlo.a	-16.64	0.546	-80.08	44.79
	Pheo	-232.77	7E-3	-387.78	-77.76
	Phyc	20.70	0.012	5.36	36.04

Arranging the variables and their respective coefficients, Equation (1) is obtained. Our results could be an improvement to the state-of-the-art since so far researches have suggested that monitoring chlorophyll could be used as a warning sign for the presence of toxins in water as the correlation between pigments and cyanobacterial cyanotoxins was observed (FRANCY et al., 2015; RINTA-KANTO et al., 2009; WICKS; THIEL, 1990).

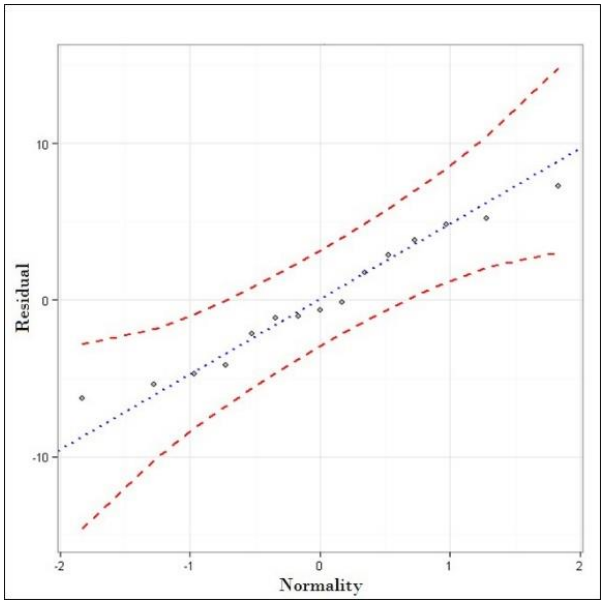
$$\boldsymbol{McT = -194.65Pheo + 17.01Phyc + 64.99} \quad \textbf{(1)}$$

Figure 4 shows the analysis of the residues obtained from the model selected (25) for the prediction of McT. According to Figure 4a, the residues were found to be normally distributed, a required condition for the regression model validity (MANN, 2010; ZAR, 2010). Figure 4b shows the distribution of the residues, indicating a good fit since the residues are randomly scattered around zero. This graph is important for verifying the assumption that residues are randomly distributed and have constant variance (MANN, 2010; ZAR, 2010). Therefore, there are no indications of a violation of assumptions to certify the quality of the model according to Cook and Weisberg (1982).

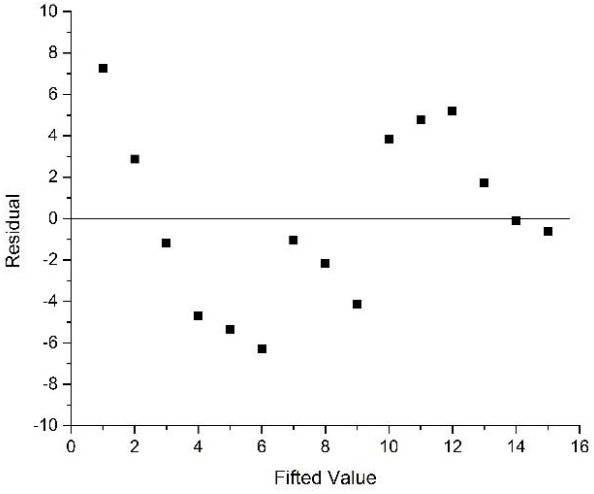
A comparison between the actual values obtained in the experiment and those predicted by the model selected for the McT variable (Eq. 1) is presented in Figure 4(c). Based on this figure, it is possible to state that the evidence of normality and homoscedasticity of the residues was confirmed by adopting an Adjusted R^2 of 0.824. Thus, the multiple regression model proved to be valid and was able to explain 82.4% of the McT variable.

Figure 4 - (a) Normal probability for residues obtained from prediction results; (b) residual dispersion provided by the selected model and (c) comparison between the values predicted by the model and the actual values obtained experimentally with confidence intervals.

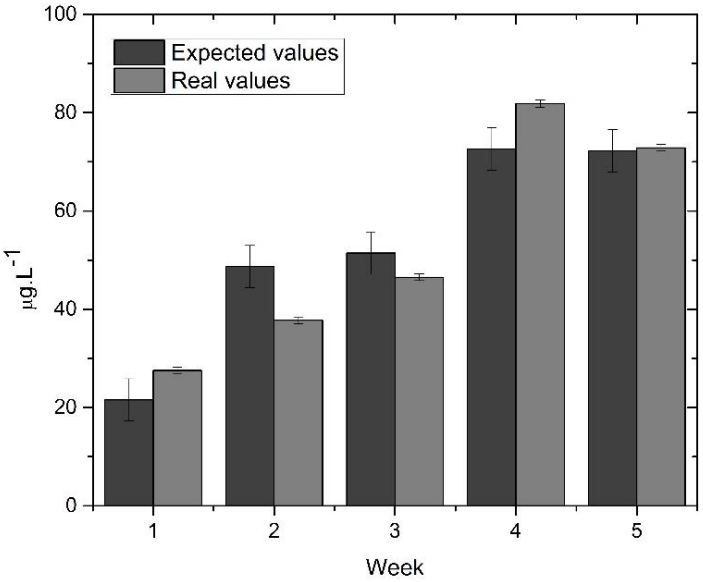
a)



b)



c)



1.4 Conclusions

The multiple regression model was able to predict McT concentration in cultured *Microcystis aeruginosa* using easily measured variables such as pheophytin and phycocyanin. Although the model containing only pigments presented an Adjusted R2 value lower than the one with all the studied variables, it was considered a more practical solution and showed good precision.

Although differences between calculated and measured McT concentrations were less than 5%, demonstrating that the model can be applied for our cultured *Microcystis aeruginosa*, its use for environmental samples still depends on further investigations. The combination of sensor technology and accurate mathematical modeling can be an important tool to alert for toxins in water and to the need for complementary treatments such as activated carbon application or, in extreme cases, the complete stop of the water distribution.

2 PREDICTING GEOSMIN PRODUCTION BY *DOLICHOSPERMUM CIRCINALE* BASED ON PIGMENTS CONCENTRATION.

2.1 Introduction

Cyanobacteria are photosynthetic prokaryotes and one of the most important organisms on earth because they release a large amount of oxygen into the earth's atmosphere. Besides chlorophyll, cyanobacteria produce phycocyanin which gives the cells a bluish color and is responsible for their popular name, blue-green algae (WHITTON; POTTS, 2012). Cyanobacterial blooms are considered a widespread problem in the aquatic environment because of the production of a vast array of compounds that have negative effects, are toxic, or adversely affect mammals, including humans (WATSON et al., 2016).

One of the noxious consequences of cyanobacterial blooms is the release of odorous compounds (MA et al., 2012). Volatile organic compounds (VOCs) cause unpleasant taste and odor (T&O) in drinking and recreational water, fish, shellfish, and other seafood. Although not toxic to humans, these T&O compounds severely impair public trust in this commodity, resulting in a substantial increase in treatment costs, loss of revenue to drinking water, aquaculture, food and beverage, and tourist/hospitality industries (WATSON et al., 2016). The problems associated with T&O compounds have been frequently reported worldwide (GIGLIO et al., 2008; JIANG; HE; CANE, 2007; KUTOVAYA; WATSON, 2014; WANG et al., 2011b).

The three main groups of VOCs are terpenoids, nor-carotenoids, and sulfides. These compounds are not produced by all species or strains of cyanobacteria and are also not exclusive to these taxa, but they are produced by other organisms, such as actinomycetes and fungi, which confused many attempts to trace their sources. The two most problematic T&O compounds are geosmin and 2-methylisoborneol (MIB) (WATSON et al., 2016). MIB and geosmin are potent T&O metabolites that can be detected by the human's olfactory senses at very low concentrations (at parts per trillion levels or ng L^{-1}) (CLERCIN; DRUSCHEL, 2019; JOHN et al., 2018).

Various species of *Dolichospermum* (*Anabaena*) such as *D. flos-aquae*, *D. laxum*, *D. lemmermannii*, *D. planktonicum*, and *D. ucrainicum* have been reported to produce MIB and Geosmin (LI; DREHER; LI, 2016). However, the species *Dolichospermum circinale* is the most known producer of these compounds in freshwater (JOHN et al., 2018; KIM et al., 2016; LI; DREHER; LI, 2016).

The removal of cyanobacteria cells from water is a challenge because, in the process, cells can be lysed and intracellular metabolites released, not only toxins and T&O compounds but other natural organic matter contributing to the formation of harmful disinfection by-products (KIM et al., 2016; WERT et al., 2014). MIB and Geosmin are biodegradable but extremely resilient to chemical oxidation, thereby escaping conventional water treatment processes with short detention times (JOHN et al., 2018). Because of this, the removal or reduction of these compounds from drinking water becomes a hard task and requires expensive and time-consuming procedures, for example, particulate activated carbon treatment or advanced oxidation processes (JØRGENSEN; PODDUTURI; BURFORD, 2016). Thus, alternative monitoring and treatment approaches are necessary to ensure lower levels of T&O compounds in drinking water (JØRGENSEN; PODDUTURI; BURFORD, 2016).

For monitoring T&O compounds in drinking water reservoirs, for example, the concentration of geosmin can be estimated from the number of cyanobacteria at regular intervals during periods of expected high concentrations of geosmin. This allows operators of water treatment plants to switch to alternative water sources (JØRGENSEN; PODDUTURI; BURFORD, 2016). However, this method can be inaccurate, laborious, and may not identify or differentiate geosmin-producing cyanobacteria, as several species or genera are morphologically similar and can coexist in a complex population (GAGET et al., 2017). Besides that, there is limited knowledge about the processes that control the production of T&O compounds, for example, the influence of specific environmental conditions (light, inorganic and organic matter, and temperature) on the abundance of T&O producing organisms has only been studied in some cases (JØRGENSEN; PODDUTURI; BURFORD, 2016).

Direct determination of the concentration of MIB and Geosmin by gas chromatography coupled with mass spectrometry (GC-MS) is an effective tool for quantifying cyanobacterial metabolites; however, this approach can be expensive, time-consuming, and requires technical knowledge, complex extraction procedures, and high-resolution instruments (JØRGENSEN; PODDUTURI; BURFORD, 2016).

To overcome the high costs and time required for cell count and cyanobacteria metabolites analysis, some attempts to develop predictive methods were carried out to evaluate how environmental variables are connected to the growth of toxic cyanobacteria and metabolites production (BORTOLI et al., 2014). Environmental characteristics such as nutrients concentration, light penetration, temperature, and interactions between bacteria have an impact on the production of T&O compounds. Some correlations were observed between Geosmin / MIB and pigments (PARINET; RODRIGUEZ; SÉRODES, 2010). Naes et al. (1985)

showed a direct positive correlation between chlorophyll-*a* and geosmin produced by *Oscillatoria brevis* at a constant temperature of 20°C under different light regimes. Zimba et al. (1999) showed a consistent relationship between MIB production and photopigment accumulation under varying irradiance. Zhang et al. (2009) reported maximal geosmin production decreased chlorophyll-*a* concentration at a lower temperature in *Lyngbya* culture. Kakimoto et al. (2014) provided similarly important insight into MIB gene expression, using a combination of molecular and analytical measures on cultures of *Pseudanabaena galeata* grown under constant light at different temperatures. All of the aforementioned studies used different organisms under various incubation conditions, and a consistent relationship between chlorophyll-*a* and geosmin synthesis cannot be reliably established from these data alone (WATSON et al., 2016). Aragão et al. (2020) showed that the differences between calculated and measured microcystin concentrations were less than 5%, demonstrating that the mathematical model can be applied for cultured *Microcystis aeruginosa* and can be used for the prediction of microcystin production.

Pigments, for example, chlorophyll and phycocyanin, can be detected with portable instruments such as probes (MCQUAID et al., 2011). Probes that are based on phycocyanin fluorescence are applied for the rapid assessment of cyanobacteria in drinking water sources (BASTIEN et al., 2011; MCQUAID et al., 2011), offering an alternative to monitoring cyanobacteria and hence cyanotoxins. However, most probes still have limitations when transforming pigment concentration signals into other cyanobacterial parameters, such as biomass and cell densities, and only perform accurately within very limited concentration ranges (GLIBERT et al., 2018). In this sense, the development of an efficient model that precisely correlates pigment concentration and T&O compounds (MIB and geosmin) would be beneficial to the water industry.

In this context, the main objective of this article was to develop a mathematical model to correlate cell density, concentrations of pigments, and the concentration of geosmin, to try to predict the production of this metabolite throughout the life cycle of a *Dolichospermum circinale* culture under controlled conditions.

2.2 Materials and Methods

The *Dolichospermum circinale* strain was cultured in 1 L flasks in ASM-1 medium (GORHAM et al., 1964) using 12:12h (L:D) photoperiod at 22 $\mu.E.m^{-2}.s^{-1}$ (Digital Lux Tester YF-1065), pH 8, 25 ºC (± 1 ºC), and under continuous aeration. Each flask contained one liter

and the volume didn't significantly change during the experiment. The diluted suspension of *Dolichospermum circinale* culture was prepared from the cultures and after that, it was subdivided into five individual flasks of one litter each. Each flask was completely used for each week's analyses so no changes in volume occurred. Each flask was submitted to the same conditions of light and temperature. In each of the subsequent five weeks (cell age – CA), aliquots were withdrawn from each flask for analysis of the following parameters: *Dolichospermum circinale* cell density (Dens.), chlorophyll-a (Chlo.a), phycocyanin (Phyc), and pheophytin (Pheo), called the explanatory variables, and geosmin (G), the response variable. The experiment was carried out in triplicate for five weeks, totaling 15 samples for each analysis. Aliquots of 1mL were used for cell density analysis. For the analysis of chlorophyll-a (Chlo.a), pheophytin (Pheo), and phycocyanin (Phyc), aliquots of 100 mL for each analysis were used, and for the determination of the geosmin concentration, aliquots of 40 mL were used.

Geosmin quantification was performed by pre-concentrating the samples using the solid-phase microextraction (SPME) technique. The SPME technique involves the use of a fiber coated with an extracting phase, which extracts different types of analytes from different kinds of media, according to its adsorptive specificity (SPIETELUN et al., 2010). In this work, the fiber used (SPME Fiber Assembly 75µm CAR/PDMS, Fused silica, 24 Ga, Manual holder, 3pk black - SUPELCO) did not have an adsorptive affinity with MIB, so this metabolite was not counted. The SPME technique was followed by analysis on a Thermo Scientific gas chromatography (TRACE 1300 Series, splitless injection, injector at 220 ºC and helium flow rate of 1 $mL.min^{-1}$ coupled to a Thermo Scientific Single Quadrupole, mass spectrometer at 150 °C and Electronic Impact Ionization) according to Graham and Hayes (1998). Calibration curves were prepared using standard geosmin solution (Sigma Aldrich) diluted in ethyl acetate (Dynamic - UV-HPLC Grade).

Cell density (Dens.) of *Dolichospermum circinale* samples preserved in Lugol's iodine at different growth stages was measured using a Sedgewick-Rafter chamber and inverted optical microscope with a magnification of 40X (Zeiss, Model Vert. A1) (APHA, 2012). For the determination of cell density, each unicellular organism was considered as an individual (YOSHIDA et al., 2006).

The spectrophotometric method (10200H), was used for the determination of chlorophyll-a (Chlo.a) and pheophytin (Pheo) according to APHA (2012). Samples were analyzed at wavelengths of 664, 665, and 750 nm. Phycocyanin (Phyc) was analyzed using the

spectrophotometric method at 665 and 750 nm wavelengths proposed by Bennett and Bogorad (2011) (Genesys 10-S, Thermo-Scientific - USA).

Statistical tests, Shapiro-Wilk (P_{S-W}), and Levene tests were used to verify the hypothesis of data normality and homogeneity. ANOVA test was used to compare the results of analyzed values at each week, the Tukey test, to perform the results with normal distribution, and the Wilcoxon signed-rank test with Bonferroni adjustment, to pairwise comparisons using for samples without normal distribution. The Spearman correlation coefficient (R_s) was used to verify the degree of association between all variables, and it was considered $|Rs| \geq 0.7$, to a strong association and $0.7 > |Rs| \geq 0.5$ to a moderate association (ZAR, 2010).

The variables CA, Dens, Chlo.a, Phyc, and Pheo, were considered explanatory variables and correlated with the response variable (G) using the Stepwise method. The selected regression model was chosen amongst the models with high Adjusted R^2. The selected regression model was then evaluated by both normality, homoscedasticity of the residues (evaluated graphically), and coefficient significance. All the statistical tests were performed using the RStudio program (R CORE TEAM, 2018) with a significance level of 5% ($\alpha = 5\%$).

2.3 Results and discussion

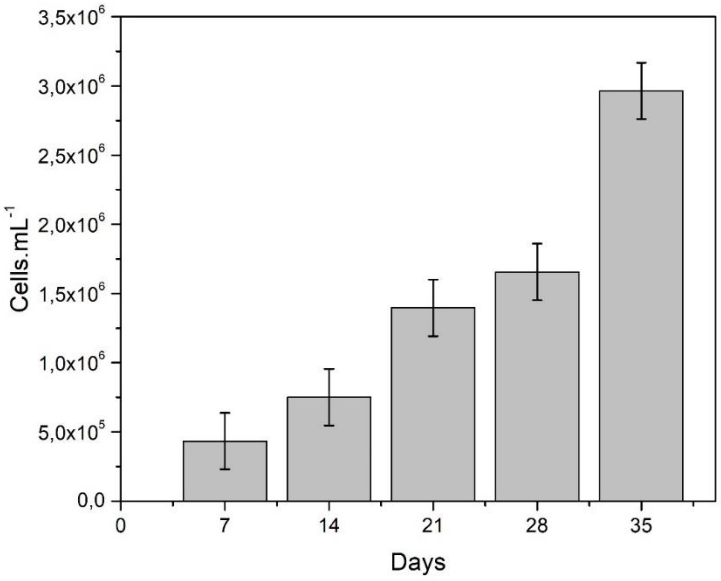
The evaluation of the cyanobacteria metabolites production throughout the life cycle of a *Dolichospermum circinale* culture under controlled conditions enabled the development of a mathematical model that correlates cell density, concentrations of pigments, and concentration of geosmin.

There was a significant ($p < 0.05$) increase of *Dolichospermum circinale* cell density (Figure 5) until the fifth week of the experiment (35 days), indicating that the culture had not reached the stationary phase yet. The cell density depends on several environmental factors, such as light, rainfall, water temperature, and nutrients (YOUN et al., 2020). In particular, the cell densities of *Dolichospermum sp.* are favored at temperatures between 14 and 23 °C and its abundance often increases in the summer when water temperatures exceed 15 °C (YOUN et al., 2020). Youn et al. (2018) studied the growth of *Dolichospermum circinale* isolated from the Bukhan river and observed that the maximum cell density was reached in approximately 24 days. Subsequently, the cell density was unstable with alternating maximums (2.10^4 cells.mL^{-1}) and minimums (5.10^3 cells.mL^{-1}) values.

Dolichospermum has been identified as one of the geosmin-producing species. (JOHN et al., 2018; KIM et al., 2016; LI; DREHER; LI, 2016). High geosmin concentrations

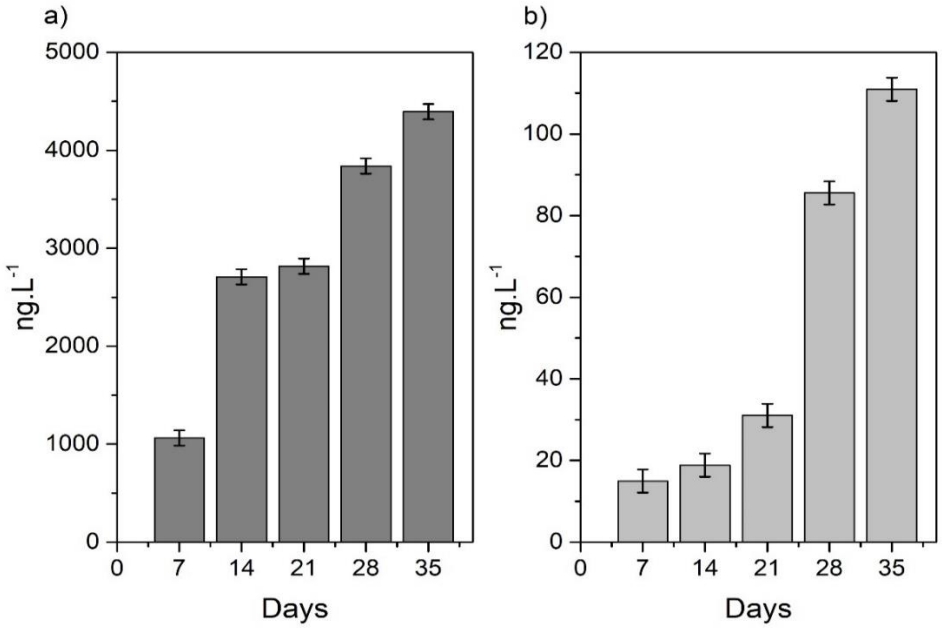
have been reported to be highly correlated with increases in the number of cells of *Dolichospermum sp.* (YOUN et al., 2020). *Dolichospermum circinale* strain cultivated for this work produced geosmin and MIB but this work studied just the production of geosmin which were detected in the chromatograms at 12.56 minutes.

Figure 5 - *Dolichospermum circinale* cell density during the growth cycle under controlled conditions.



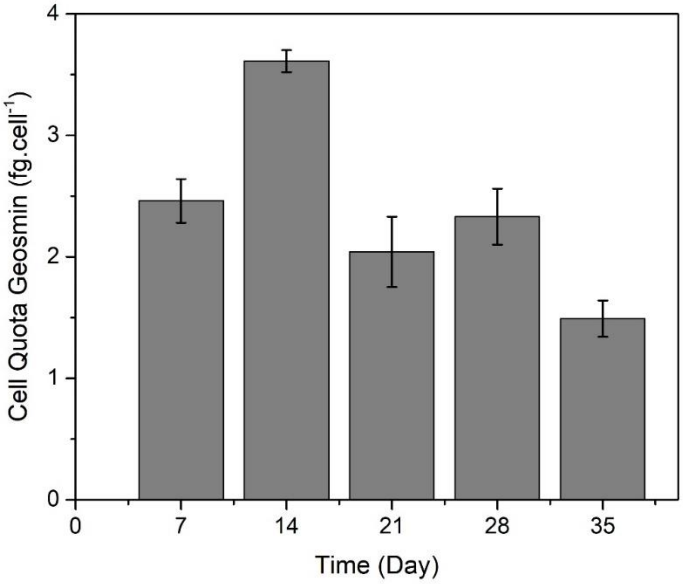
In the fifth week (Figure 6), intracellular geosmin concentration was 4500 ng.L^{-1} while extracellular concentration was 110 ng.L^{-1}, different from the concentrations observed by Youn et al. (2018) where extracellular geosmin measured 750 ng.L^{-1}. Zhang et al. (2009) studied geosmin produced by *Lyngbya kuetzingii* (500 ng.L^{-1} for intracellular geosmin and 50 ng.L^{-1} for extracellular geosmin) and suggested that the majority of geosmin produced was cell-bound. Similar to what happened in our study, this difference between intra and extracellular proportions may be explained by the fact that even though extracellular geosmin was released along the growth stage, the great majority of geosmin was still retained in thes cells during their growth.

Figure 6 - Concentrations of a) Intracellular and b) Extracellular Geosmin.



The cell quota, a relation between intracellular geosmin concentration and cell density, showed a significant difference ($p < 0.05$) along the growth period, suggesting that the quantity of geosmin inside each cell was not constant throughout the experiment (Figure 7). Li et al. (2010) showed that, contrary to what we found, the concentration of geosmin was strongly correlated with the number of *Dolichospermum sp.* cells. Li et al. (2010) also analyzed samples of *Anabaena spiroides* in Yanghe reservoir, North China, and found the average geosmin cell quota was about 0.1 pg.cell^{-1} (equivalent to 100 fg.cell^{-1}), much higher than the quota found in our study (2.38 fg.cell^{-1}). Jones and Korth (1995) reported the average geosmin cell quota 0.01 pg.cell^{-1} (10 fg.cell^{-1}) in an Australian bloom associated with *Dolichospermum sp*. This may be because cyanobacteria strains found in natural environments have shown the capacity to produce higher cell quotas than strains cultivated under controlled conditions (WOOD et al., 2012).

Figure 7 - Cell quota for intracellular microcystin throughout the experiment weeks.



The first line of Figure 8 shows that all explanatory variables were strongly correlated ($p < 0.001$) to the response variable (geosmin - G). In the left column, the scatter plots of the geosmin values versus the covariates (explanatory variables) are presented with their respective linear regression lines. The verification of the scatter plots can be used to estimate the geosmin values from each explanatory variable and to evaluate how closely the experimental results are to the modeled values. Looking at the linear regression adjustment lines, graphs below the main diagonal of Figure 8, it is noted that the explanatory variables alone do not explain the geosmin production adequately because the vertical distances between the points and the line are large. This justifies the search for a more elaborate model to estimate geosmin concentration. Thus, according to Figure 4, the most relevant variables are CA, Dens, Chlo.a, Phyc and Pheo with Rs > 0.7, indicating that these variables could be used to estimate geosmin concentrations.

In the search for a mathematical model capable of estimating geosmin concentration based on an explanatory variable or a set of them, a multiple regression of the data was performed. Multiple regression models were generated, with their respective correlation coefficients (Adjusted R^2), involving all possible combinations (Table 5).

Models containing only pigments presented Adjusted R^2 values lower than that containing CA and higher than those containing Dens, Chlo.a, Pheo and Phyc (Table 5). This is corroborated by studies that have shown a strong correlation between pigments and geosmin concentrations in environmental samples. Smith et al. (2002) showed that the geosmin

concentration is correlated with Chlo.a concentration in the water column, indicating that it is linearly related to Chlo.a.

On the other hand, although the model selected to describe the response variable (geosmin - G) should have a high Adjusted R^2, it is preferable that it uses the smallest number of variables and that these variables have easy analytical procedures. According to Table 5, the presence of a single variable in the mathematical model does not explain the production of Geosmin satisfactorily. This can also be observed by the small Adjusted R^2 of single-variable models (Models 1, 25, 27, 30, and 31 – Table 5). Determining cell age (CA) and density (Dens) in environmental samples is not an easy task, and therefore, models based only on pigments were selected since they can be analyzed by simple multiparametric probes. On this basis, the models selected for determining geosmin concentration were models 21 and 22 with the same Adjusted R^2 = 0.881 for each model. The adjusted coefficient of determination, also called Adjusted R^2, is a criterion for the selection of an optimal model with a maximum Adjusted R^2 with a reduced set of explanatory variables. As the models have the same value for the Adjusted R^2, the choice of the best method was made by analyzing the explanatory variables and their respective p-value.

Figure 8 - Visualization matrix of cell age (CA) and density (Dens.), chlorophyll-a (Chlo.a), phycocyanin (Phyc) and pheophytin (Pheo) and total geosmin (G).

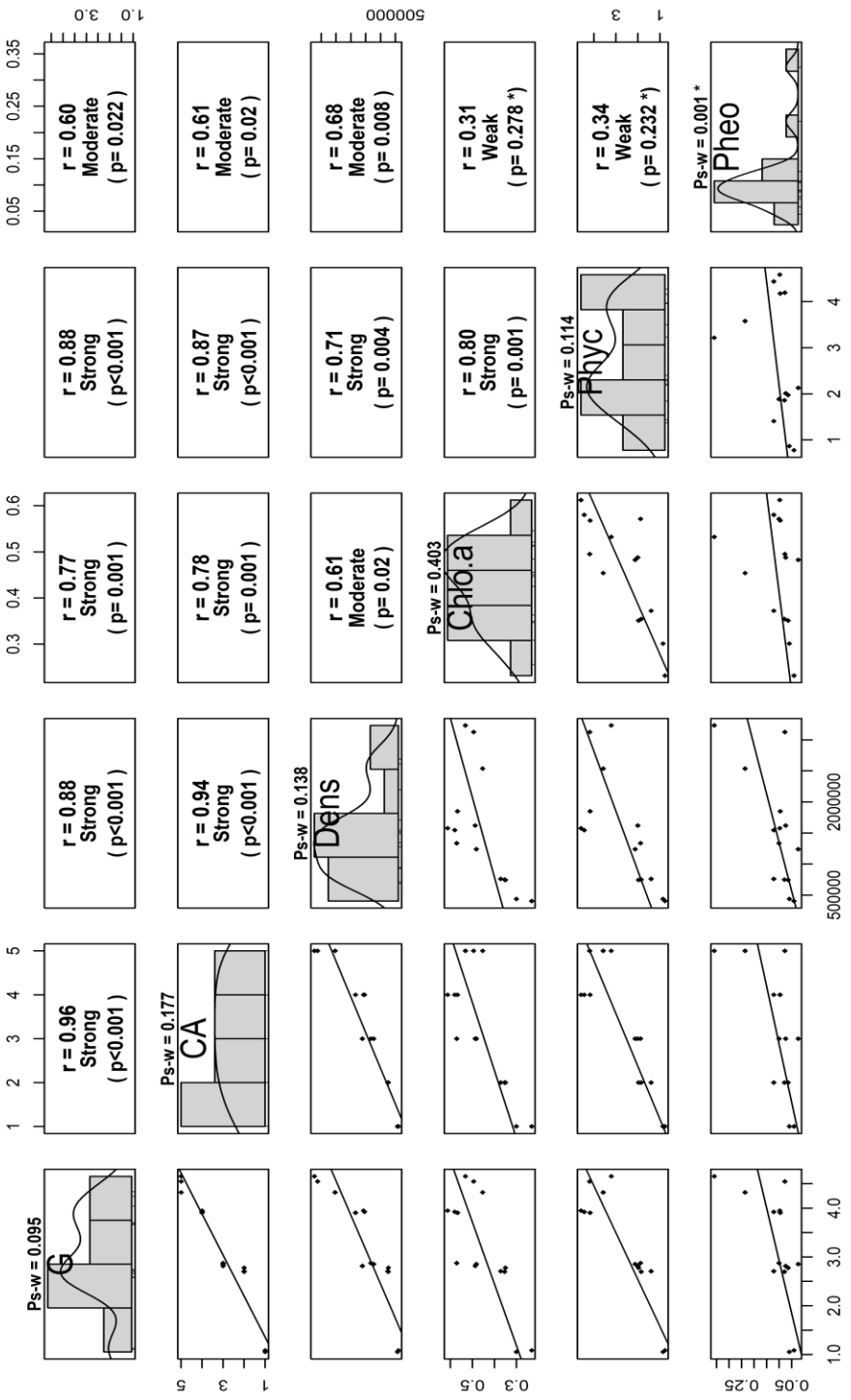


Table 4 - Multiple regression models between the response variable (G) and the explanatory variables (CA, Dens, Chlo.a, Phyc, and Pheo).

Model	**Variables**	**Adjusted R^2**	**Model**	**Variables**	**Adjusted R^2**
1	**CA**	**0.934**	17	Dens + Phyc + Pheo	0.909
2	CA + Phyc	0.933	18	Dens + Phyc	0.902
3	CA + Dens	0.933	19	Dens + Chlo_a + Phyc + Pheo	0.909
4	CA + Phyc + Pheo	0.933	20	Dens + Chlo_a + Phyc	0.902
5	CA + Chlo_a	0.927	***21***	***Phyc + Pheo***	***0.881***
6	CA + Dens + Pheo	0.930	***22***	***Chlo_a + Phyc + Pheo***	***0.881***
7	CA + Dens + Phyc	0.929	23	Dens + Chlo_a	0.857
8	CA + Pheo	0.925	24	Dens + Chlo_a + Pheo	0.849
9	CA + Chlo_a + Phyc	0.928	**25**	**Phyc**	**0.794**
10	CA + Dens + Chlo_a	0.927	26	Chlo_a + Phyc	0.793
11	CA + Dens + Phyc + Pheo	0.929	**27**	**Dens**	**0.766**
12	CA + Chlo_a + Phyc + Pheo	0.927	28	Dens + Pheo	0.747
13	CA + Chlo_a + Pheo	0.921	29	Chlo_a + Pheo	0.747
14	CA + Dens + Chlo_a + Pheo	0.923	**30**	**Chlo_a**	**0.633**
15	CA + Dens + Chlo_a + Phyc	0.922	**31**	**Pheo**	**0.344**
16	CA + Dens + Chlo_a + Phyc + Pheo	0.921			

Table 6 shows the coefficients of the selected multiple regression models, as well as p-values and upper and lower limits for each estimated coefficient. Models 21 and 22 were then evaluated using the linear regression method to choose the most appropriate one. Although the models have the same adjusted R^2, in Table 6, it was possible to observe that model 22 is not the most appropriate because Chlo.a *p*-value is higher (*p*-value = 0.333) than the adopted significance level ($\alpha = 5\%$) and the confidence interval for each coefficient goes through zero. On the other hand, model 21, which includes the concentration of Phyc and Pheo pigments, is a reasonable choice since its *p*-values are below the adopted significance level ($\alpha = 5\%$) and the confidence interval for each coefficient does not go through zero. Arranging the variables and their respective coefficients, Equation 2 was obtained.

Table 5 - Coefficients of equations obtained from the correlation between parameters.

Estimated Parameter	Related Parameter	Coefficients	p-Value	Confidence Interval 95% Inferior Limit	Confidence Interval 95% Upper Limit
Model 21 (R^2 = 0.898/Adjusted R^2 = 0.881)					
G	Intercept	0.833	0.004	0.312	1.353
	Phyc	0.675	5.42E-06	0.484	0.866
	Pheo	4.584	0.007	1.489	7.679
Model 22 (R^2 = 0.907/Adjusted R^2 = 0.881)					
G	Intercept	0.413	0.405	-0.639	1.466
	Chlo.a	1.646	0.333	-1.935	5.227
	Phyc	0.557	0.002	0.235	0.878
	Pheo	4.464	0.009	1.330	7.598

$$\boldsymbol{G = 4.584Pheo + 0.675Phyc + 0.833} \quad (2)$$

The results obtained in this research can be considered an advance in determining the correlation between different pigments and geosmin since researches so far have suggested that only chlorophyll monitoring could be used as a warning sign for the presence of noxious metabolites in the water (FRANCY et al., 2015; RINTA-KANTO et al., 2009).

In this work, chlorophyll rapidly degraded to pheophytin, due to the greater sensitivity of chlorophyll to the incidence of light. Therefore, the pigment pheophytin, in greater quantity about chlorophyll, was better correlated with the production of microcystin.

Figure 9 shows the results of statistical analysis of the residues obtained from model 21 selected for the prediction of geosmin. Figures 9a and 9b show that the residues were normally distributed and randomly scattered around zero, respectively. This indicates that the required conditions for the regression model validity were met (MANN, 2010; ZAR, 2010). Additionally, according to Cook and Weisberg (1982), there are no indications of violations of the assumptions used to certify the quality of the model.

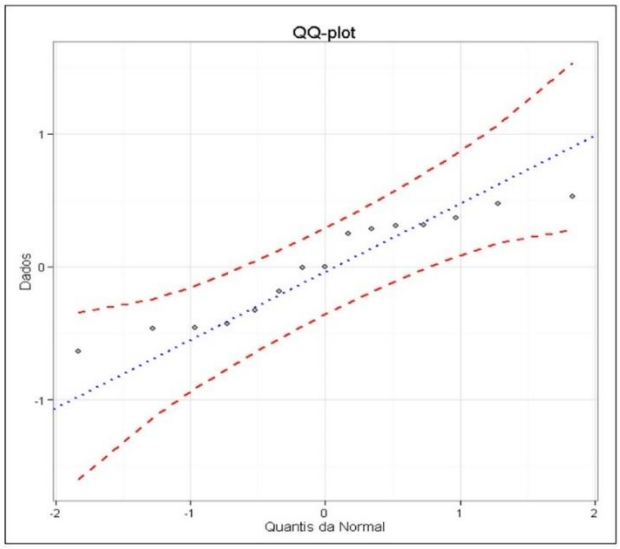
Figure 9c shows the values obtained in the experiment and those predicted by the selected model for the geosmin (Eq. 1). These results indicate that the assumption of normality

and homoscedasticity of the residues was confirmed by adopting an Adjusted R^2 of 0.898. Thus, the multiple regression model proved to be valid and was able to explain 89.8% of the G variable.

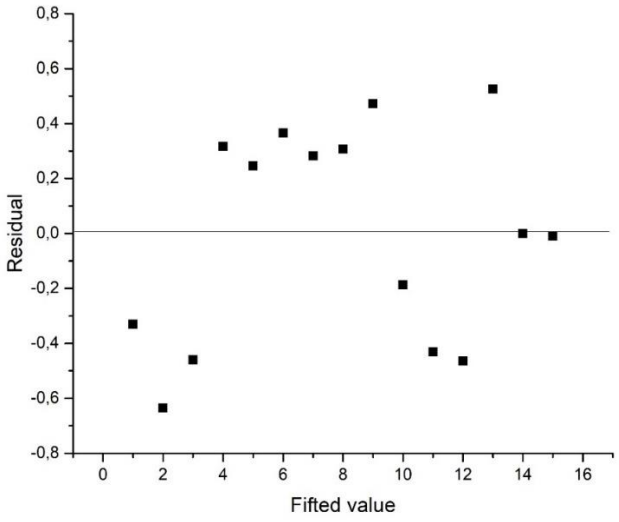
Otten et al. (2016) also used linear regression models to predict cyanobacterial secondary metabolites and found that a model with 4 explanatory variables was able to explain only 51% of the variation of geosmin concentrations, much lower than the value found in our study.

Figure 9 - (a) Normal probability from prediction results; (b) Residual dispersion provided by the selected model; (c) Comparison between the values predicted by the model and the actual values obtained experimentally with confidence intervals.

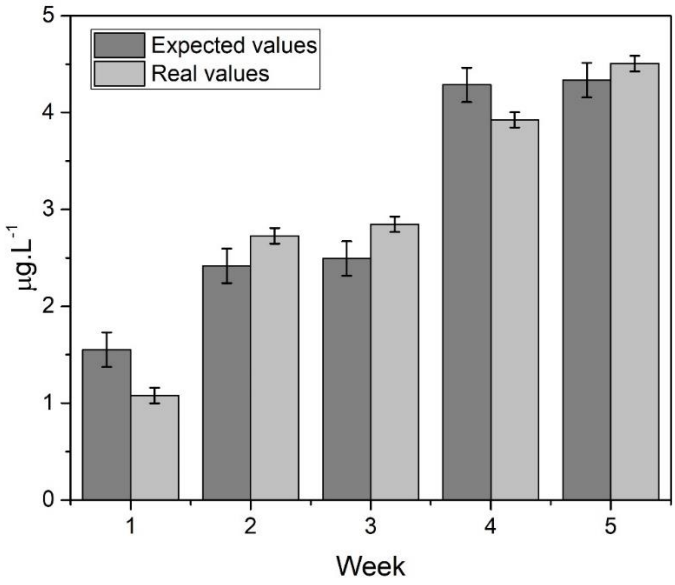
a)



b)



c)



2.4 Conclusion

The concentration of geosmin from isolated *Dolichospermum circinale* cultures could be efficiently predicted by the multiple regression model containing the explanatory variables pheophytin and phycocyanin. Although the model presented an Adjusted R^2 value lower than the model containing all the variables evaluated, it was considered a more practical solution and presented good precision, since the variables pheophytin and phycocyanin are easy to measure. The differences between the theoretical and experimental geosmin concentrations were on average 15%, demonstrating that the model can be applied to isolated cultures of *Dolichospermum circinale*. However, the application of this model for environmental samples depends on further investigations as it needs to take into account other environmental variables. Thus, we believe that the combination of sensor technology and precise mathematical modeling could be an important tool to assist in water treatment to remove cyanobacteria metabolites and to formulate new mitigation strategies.

3 REMOVAL OF DOLICHOSPERMUM CIRCINALE, MICROCYSTIS AERUGINOSA, AND THEIR METABOLITES USING HYDROGEN PEROXIDE AND VISIBLE LIGHT.

3.1 Introduction

Cyanobacteria are a highly adaptive and diverse group of microorganisms that can exist in a wide range of habitats (LI et al., 2014). Their blooms are increasingly becoming more intense and frequent in continental freshwater worldwide (HO et al., 2010; LI et al., 2014) imposing a serious threat to water companies. These organisms are considered a problem primarily because they can produce toxic metabolites, which can negatively impact human health, aquatic biota, and local wildlife, or substances that are not harmful to health but can give a musty or earthy taste and odor (T&O) to water (CHANG; HUO; LIN, 2018).

Microcystis is a genus of freshwater cyanobacteria which includes *Microcystis aeruginosa,* one of the most frequently studied organism and a source of great concern when present in reservoirs used for human or animal supply (BITTENCOURT-OLIVEIRA et al., 2014; FAN et al., 2020) because they can produce toxic metabolites known as microcystins (MCs) (WANG et al., 2018; ZHOU et al., 2018). The World Health Organization (WHO) has established a guideline value of 1.0 $\mu.L^{-1}$ microcystin-LR equivalent (WHO, 2011) to ensure the safe use of drinking water. Exposure to microcystins is known to cause cellular damage and genotoxicity within the livers, kidney, heart, reproductive systems, and lungs of mammalians (ROEGNER et al., 2014). One variant of microcystins, MC-LR, was chosen as a reference because it is one of the most toxic and prevalent cyanotoxins found in freshwater (MERILUOTO; SPOOF; CODD, 2017; MIAO et al., 2010; ZONG; SUN; SUN, 2013).

In addition to MCs, 2-methylisoborneol (MIB) and geosmin are a major problem in water distribution systems since they can cause unpleasant Taste and Odor (T&O) in drinking water (XIE et al., 2015). Although these T&O compounds are not toxic, their unpleasant organoleptic characteristics contribute to a negative water quality perception and impel consumers to alternatives and riskier water sources (DOEDERER; GALE; KELLER, 2019; LI; DREHER; LI, 2016). MIB and Geosmin can be produced by actinomycetes, proteobacteria, myxobacteria, and some fungi. However, cyanobacteria, especially the genera *Dolichospermum* and more specifically *Dolichospermum circinale,* are the major producers of those substances in freshwater (JOHN et al., 2018; KIM et al., 2016; LI; DREHER; LI, 2016).

The removal of intact cyanobacterial cells from water is challenging because, in the process, cells can be damaged and the intracellular released. Cell lysis contributes to increasing the dissolved toxin and T&O compounds fraction, making it more difficult to remove them in a conventional water treatment plant (WTP) (BARRINGTON; REICHWALDT; GHADOUANI, 2013).

Mathematical models can be important tools to estimate the concentration of these noxious cyanobacterial compounds when using easily measurable parameters, such as pigments, to alert for the presence of toxins in water and the application of complementary treatments (ARAGÃO et al., 2020). Aragão et al. (2020) showed, for example, that the differences between calculated and measured MC concentrations were less than 5%, demonstrating that the models can be applied for cultured *Microcystis aeruginosa* and, in the future, for environmental samples depending on further investigations.

Although dissolved MCs can be removed by environmental mechanisms, such as biodegradation, photolysis, and adsorption, they can take days or weeks (GAN et al., 2012). T&O compounds are biodegradable but extremely resilient to chemical oxidation, making it also hard to remove them in conventional WTP with short detention times (KIM et al., 2016). Furthermore, cell disruption may expose not only toxins and T&O compounds but other natural organic matter contributing to the formation of harmful disinfection by-products (KIM et al., 2016; WERT et al., 2014). Therefore, the water treatment plants should remove cyanobacteria cells without breaking them, so the intracellular content, such as toxins, T&O compounds, and other organic matter are not released into the water (FAN et al., 2013). Despite the risks of damaging cyanobacteria, pre-oxidation, especially pre chlorination, is commonly used to optimize phytoplankton coagulation and removal (CHEN; YEH; TSENG, 2009; STEYNBERG; PIETERSE; GELDENHUYS, 1996). Other options of pro-oxidants are ozone, potassium permanganate, and chlorine dioxide. However, besides being expensive, these technologies are limited by the potential formation of harmful by-products or staining water at the customer's tap (CHANG; HUO; LIN, 2018; LI et al., 2014).

Advanced Oxidation Processes (AOP), which has a reaction that involves highly reactive hydroxyl radicals, is an alternative method to control cyanobacteria in water (CHANG; HUO; LIN, 2018). The advantage of this reaction is, besides the ability to disrupt cyanobacterial cells, it can destroy the toxins and T&O compounds produced (CHANG; HUO; LIN, 2018). Hydrogen Peroxide (H2O2) is used in AOP and it is a naturally occurring reactive oxygen species produced by the photolysis of dissolved organic matter exposed to ultraviolet (UV) radiation and by phytoplankton metabolic processes (BURSON et al., 2014). It is considered

an environmentally benign algaecide because when associated with light, it quickly decomposes into water and oxygen, therefore not accumulating in the environment (BARRINGTON; REICHWALDT; GHADOUANI, 2013; CHANG; HUO; LIN, 2018).

UV light applied during the application of H_2O_2 functions as a catalyst to produce hydroxyl and hydroperoxyl radicals which are the main reactive species responsible for the effective destruction of cyanobacterial cells and metabolites (BARRINGTON; REICHWALDT; GHADOUANI, 2013). As UV radiation intensity is increased, the required dose of H_2O_2 may be reduced (BARRINGTON; REICHWALDT; GHADOUANI, 2013). However, the most abundant kind of light spectrum in the sunlight is visible light, therefore it is important to study a way to enhance the production of hydroxyl radicals from H_2O_2 under visible light (CHANG; HUO; LIN, 2018). Previous studies have used low-intensity fluorescent light to simulate the effects of visible light on the activity of H_2O_2 on cyanobacteria and its metabolites (BARRINGTON; REICHWALDT; GHADOUANI, 2013; CHEN et al., 2016; MATTHIJS et al., 2012; WANG et al., 2019).

Several types of research used different conditions to observe the effects of H_2O_2 on the degradation of cyanobacteria, mainly *Microcystis aeruginosa*, (CHEN et al., 2016; WANG et al., 2019; WEN et al., 2017; YANG et al., 2018). On the other hand, researchers that studied the effect of H_2O_2 under visible light on *Dolichospermum circinale* cells and its regrowth potential, geosmin, organic matter, and pigment degradation have not been observed in the literature. Therefore, this work aimed to evaluate the effects of H_2O_2 on cultures of *Microcystis aeruginosa* and *Dolichospermum circinale* and their metabolites under visible light. To meet this goal we (1) investigated the effects of H_2O_2 on *Microcystis aeruginosa* and *Dolichospermum circinale* cell integrity and density after H_2O_2 treatment and their re-growth potential, (2) determined the fate of microcystins and geosmin, (3) investigated the impact on chlorophyll-a, pheophytin, phycocyanin, organic matter, and true color following the addition of H_2O_2.

3.2 Materials and methods

Axenic cultures of *Microcystis aeruginosa* and *Dolichospermum circinale* used in this experiment were isolated from reservoirs in the state of Ceará (Brazil) and are part of Selaqua/Federal University of Ceará collection. Both cultures were grown in ASM-1 medium (GORHAM et al., 1964), a photoperiod of 12/12 h (L:D) at 22 μmol photons.m^{-2}.s^{-1} (Digital Lux Tester YF-1065), pH 8, 25 ºC (± 1 ºC), and under continuous aeration. *Microcystis*

aeruginosa used in this study mainly produced MC-LR and grew in single cells. *Dolichospermum circinale* mostly produced Geosmin. Cell suspensions were prepared by diluting the cultures into ultrapure water to densities of 10^4 cells.mL^{-1} for *Dolichospermum circinale* and 10^5 cells.mL^{-1} for *Microcystis aeruginosa*, concentrations normally found in reservoirs in the state of Ceará (COGERH, 2019).

The experiment was performed in continuous stirred-reactors (Total volume of 2 L), called from now on microcosms. The microcosms were filled with the cyanobacterial suspension (1.5 L) and dosed with H_2O_2 at the concentrations of 2 and 5 mg.L^{-1}, under constant low levels of luminous flux (14 μmol.photons.m^{-2}.s^{-1}) using fluorescent light (Sylvania 65 W) with no or undetectable amounts of UV radiation. Fluorescent light was used in the experiment to verify, under the same culture conditions, what would be the reaction of applying hydrogen peroxide under visible light (fluorescent light). These concentrations of H_2O_2 were used since the efficacy of low concentrations (< 2 mg.L^{-1}) on cyanobacterial suppression in lakes was already reported by Matthijs et al. (2012). After the proper dilution and before subdividing it into the microcosms, the culture suspension was analyzed in triplicate (Control BP – Before application of Hydrogen Peroxide) to identify its initial characteristics. Three microcosms were run without the application of H_2O_2 but using visible light (Control AP – After application of Hydrogen Peroxide) to evaluate the effect of the new environment on the organisms. Finally, three microcosms were dosed with 2 mg.L^{-1} and the other three, with 5 mg.L^{-1} of H_2O_2, all with the same incidence of visible light. Samples were collected from these microcosms after the H_2O_2 degradation. Thus, the experiments were comprised of 12 samples in total for each analysis.

A Hydrogen peroxide solution, 30 wt. % in H_2O, (Merck, Germany), was dosed by adding the appropriate volumes to the microcosms containing the specific cyanobacterial suspension and kept under gentle and continuous agitation to avoid cell sedimentation. The concentration of H_2O_2 solution was verified using the iodometric method proposed by Skellon and Wills (1948). During the experiment, the concentration of H_2O_2 was monitored by Quantofix Peroxide Test Strips (Macherey-Nagel Company) until the H_2O_2 completely degraded.

The re-growth experiment was performed right after the H_2O_2 complete degradation. Aliquots of 50 ml of the treated samples were incubated into flasks containing 100 mL of ASM-1 medium (GORHAM et al., 1964), using the same conditions adopted for growing the cultures explained previously. After 15 and 30 days of incubation, samples were removed from the flask, and cells were counted and measured.

Analysis of intra and extracellular microcystin, total geosmin, total chlorophyll-*a*, total pheophytin, and total phycocyanin concentrations cyanobacterial cell density and size, true-color, and organic matter were performed. To determine cell density, aliquots of 1 mL were used. For the determination of chlorophyll-a (Chlo.a), phycocyanin (Phyc), intra and extracellular microcystin, and geosmin, 100 mL was used for each analysis. For true color and organic matter determination, aliquots of 50 mL were used for each analysis.

Extraction and analysis of intra and extracellular MC-LR were performed by a modified method initially proposed by Lawton et al. (1994). For intracellular MC-LR, an aliquot of 100 mL was filtered through 110 mm GF/C discs (Whatman, Maidstone, Kent, UK). The GF/C discs with the cell-bound or intracellular microcystin were freeze-thawed and placed in glass beakers containing 10 mL of methanol (100 %) (J.T. Baker – Grade UV-HPLC) to extract the intracellular content for 1h at room temperature (25ºC). The extract was filtered, evaporated, and the residue was resuspended in 1 mL of methanol (80 %) before analysis in high-performance liquid chromatography (HPLC). For the extracellular microcystin, the filtered samples (100 mL) were passed through C18 Cartridges (SupelClean LC-18 – SUPELCO 1 g and 3 mL) in a sample concentration system using a vacuum manifold (Varian Analytical Instruments, San Fernando, CA, USA) and after this, analyzed. Both analyses were performed using Agilent Model 1260 Infinity HPLC, with reverse-phase column (Waters Symmetry C18, 5 μm, 2.1 × 150 mm) at 40ºC and a DAD detector adjusted to wavelengths 238 and 222 nm. The mobile phases used consisted of 0.05 % TFA in water and 0.05 % TFA in acetonitrile with a flow rate of 0.5 mL min- 1 and a total running time of 30 min. MC-LR, MC-LA, and MC-LY standards were provided by the Cyanosol Laboratory (Robert Gordon University, UK). The other reagents used were: Methanol, Acetonitrile (J. T. Baker - UV-HPLC Grade), and Trifluoroacetic Acid (TFA, Dynamic - UV-HPLC Grade).

Total geosmin quantification was performed by pre-concentrating the samples using the solid-phase microextraction technique (SPME) in the headspace, followed by analysis on a Thermo Scientific GC–MS (Gas chromatograph TRACE 1300 Series, splitless injection, injector at 220ºC and helium flow rate of 1 mL min- 1 coupled to a Thermo Scientific Single Quadrupole, mass spectrometer at 150 ◦C and Electronic Impact Ionization) according to Graham and Hayes (1998). Calibration curves were prepared using standard Geosmin solution (Sigma Aldrich) diluted in Ethyl Acetate (Dynamic - UV-HPLC Grade).

The samples used for cyanobacterial cell density were preserved with Lugol's Iodine. *Microcystis aeruginosa* and *Dolichospermum circinale* cell densities were measured using a Sedgewick-rafter chamber and an inverted optical microscope (Zeiss, Model Vert. A1)

with a magnification of 40X adapted from APHA (2012). For the cell density count, each unicellular organism was considered as an individual. To determine the size of the cells, the scale overlay method (HRYCIK; SHAMBAUGH; STOCKWELL, 2019; SUN, 2003) was used in which the microscope eyepiece contained a graduated scale and the sample slide contains another engraved scale.

Organic Matter is defined as carbon-based compounds that may be found in various sources of natural waters (POURZAMANI et al., 2015). True color results primarily from the presence of natural organic matter and, therefore, we used it as indicative of organic matter. True color and organic matter parameters were analyzed using the spectrophotometric method proposed by (ALBREKTIENĖ et al., 2012) and APHA (2012), respectively. Organic matter, measured by absorbance at a wavelength of 254 nm, was used as an alternative method for estimating the total organic carbon concentration in a water sample (EDZWALD; BECKER; WATTIER, 1985).

For the determination of chlorophyll-*a* and pheophytin, the spectrophotometric method (10200 H) proposed by APHA (2012) was used. Briefly, the method consisted of filtering (110 mm GF/C discs, Whatman, Maidstone, Kent, UK) the sample to retain the cyanobacterial cels, macerating the filter, extracting the pigments with acetone (90 %), filtering the extract, and analyzing it in a spectrophotometer (Genesys 10-S from Thermo-Scientific - USA) with wavelengths 664, 665, and 750 nm. Phycocyanin was analyzed using a spectrophotometric method adapted from Bennett and Bogorad (2011). Briefly, the method consisted of filtering the sample to retain the cyanobacterial cells, freeze-thawing the filters three times, extracting phycocyanin with sodium azide (0,02 $g.mL^{-1}$), filtering the extract, and analyzing it in a spectrophotometer (Genesys 10-S from Thermo-Scientific - USA) with wavelengths 280, 615, and 652 nm.

Data normality and homogeneity of data variance were verified using the Shapiro-Wilk (PS-W) and Levene test, respectively. For comparisons between the different conditions, the ANOVA test was used. Tukey test was performed for samples with normal distribution, while pairwise comparisons using the Wilcoxon signed-rank test were used for samples not normally distributed (ZAR, 1996). All the statistical tests were performed using a significance level of 5 % (α = 5 %) and the RStudio software (R CORE TEAM, 2018).

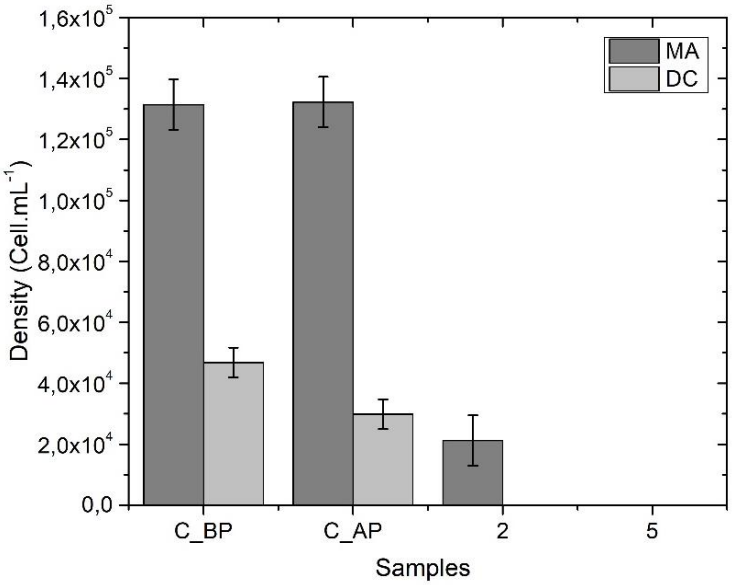
3.3 Results

To evaluate the full impact of the treatment, samples were collected after the complete degradation of H_2O_2, which happened after 24 and 48h for the reactors dosed with 2 and 5 $mg.L^{-1}$, respectively. The control AP (C_AP) was collected after 24h. After the degradation of 2 $mg.L^{-1}$ (24h), a decrease of 84 % in *Microcystis aeruginosa* cell density was observed, and after the extinction of 5 $mg.L^{-1}$ (48h), no intact *Microcystis aeruginosa* cells were found (Figure 10). After 15 days of the application of 2 $mg.L^{-1}$ (regrowth experiment), the number of cells did not show a significant difference ($p < 0.05$) when compared to the number just after the H_2O_2 degradation. After 30 days of the regrowth experiment, just cell fragments were present, indicating that the remaining cells were not viable, without the ability to reproduce.

Microcystis aeruginosa cells showed a remarkable reduction in size from 8.658 ± 0.001 μm in the Controls BP and AP, to 6.197 ± 0.001 μm after the H_2O_2 degradation (2 $mg.L^{-1}$), and then to 4.329 ± 0.002 μm after 15 days in the regrowth experiment, probably indicating the cell's response to the stress of the oxidative environment. A reduction of *Microcystis aeruginosa* biovolume was also observed, from 44.49 ± 0.01 $mm^3.L^{-1}$ in the Controls BP and AP to 2.64 ± 0.01 $mm^3.L^{-1}$ after the H_2O_2 degradation (2 and 5 $mg.L^{-1}$), and then to 0.63 ± 0.01 $mm^3.L^{-1}$ after 15 days in the re-growth experiment, probably indicating a cell response to oxidative stress.

Dolichospermum. circinale biovolume was also observed, 8.78 ± 0.01 $mm^3.L^{-1}$ in the Controls BP and AP at the concentration used, *Dolichospermum. circinale* was noticeably more sensitive to H_2O_2 than *Microcystis aeruginosa*, since, after the application of 2 and 5 $mg.L^{-1}$, no cells were detected (Figure 10) and therefore no biovolume measurement was possible. Comparing the Control BP to the Control AP showed that the experimental conditions did not cause significant variation ($p < 0.05$) of cell densities of either *Microcystis aeruginosa* or *Dolichospermum circinale*.

Figure 10 - Cellular densities of *Microcystis aeruginosa* (MA) and *Dolichospermum circinale* (DC) suspensions. Where: C_BP = C_BP = Control sample collected at time T=0; C_AP = Control sample (with no H_2O_2) collected after 24 hours from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 $mg.L^{-1}$ of H_2O_2 (24h); 5 = Sample collected after the compete degradation of 5 $mg.L^{-1}$ of H_2O_2 (48h).

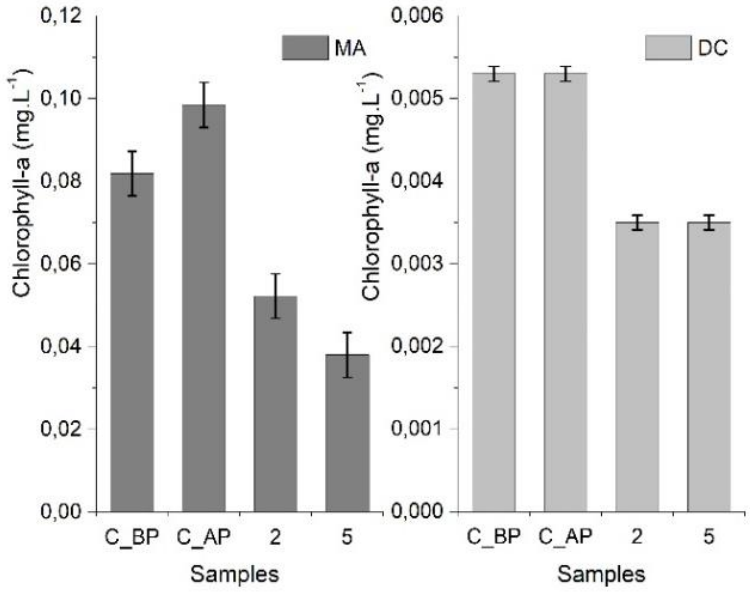


After the application of 2 and 5 $mg.L^{-1}$ H_2O_2, the concentrations of chlorophyll-a decreased significantly ($p < 0.05$) after 24 and 48h for both species (Figure 11a). The chlorophyll-a concentration did not show significant variation ($p < 0.05$) between the Control AP to the Control BP (Figure 11a) also for both species, the same behavior was observed for the cell densities. No pheophytin (Limit of Quantification <0.001 $mg.L^{-1}$) was detected during the experiment.

Phycocyanin concentration varied between 0.6 and 0.9 $mg.L^{-1}$ for both H_2O_2 concentrations and both species (Figure 11b), showing no significant difference ($p < 0.05$) except for the sample Control AP for culture *Dolichospermum circinale* which presented a significant increase to approximately 2 $mg.L^{-1}$. This value may have been generated from an error in the execution of the analysis and should, therefore, be disregarded.

Figure 11 – Chlorophyll-a (a) and Phycocyanin (b) concentrations of *Microcystis aeruginosa* (MA) and *Dolichospermum circinale* (DC) suspensions. C_BP = Control sample collected at time T=0; C_AP = Control sample (with no H_2O_2) collected after 24 hours from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 $mg.L^{-1}$ of H_2O_2 (24 hours); 5 = Sample collected after the compete degradation of 5 $mg.L^{-1}$ of H_2O_2 (48 hours).

a)



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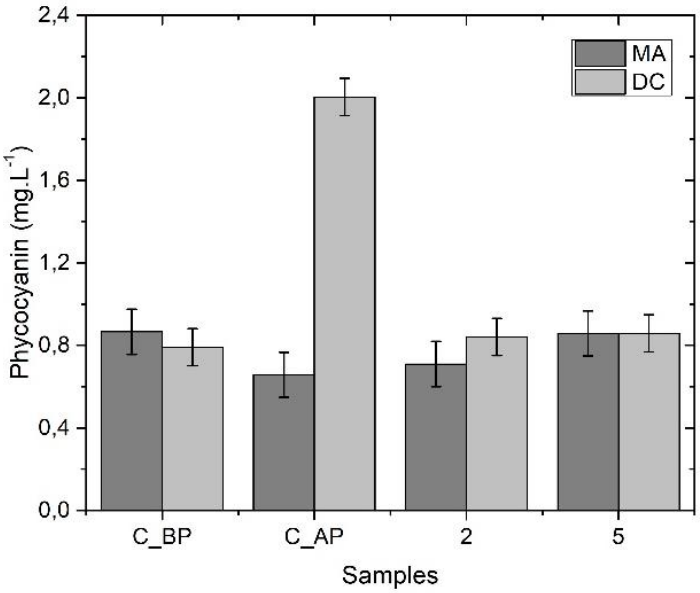
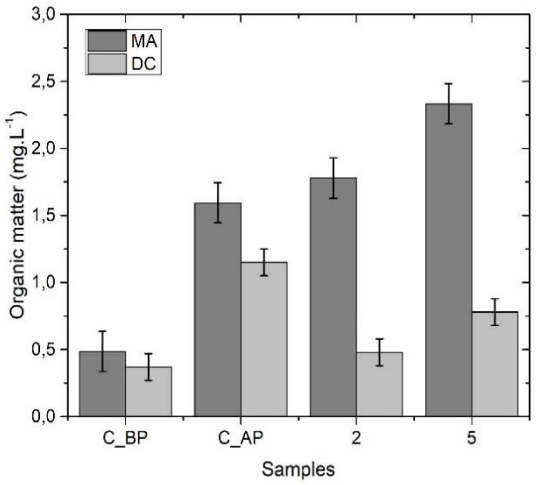


Microcystis aeruginosa culture showed a higher increase in dissolved organic material (organic matter 254 nm and true color) than *Dolichospermum circinale* (Figures 12a and 12b), probably due to its higher concentrations of cells at the start of the experiment; 10^5 $cells.mL^{-1}$ for *Microcystis aeruginosa* as opposed to the 10^4 $cells.mL^{-1}$ for *Dolichospermum circinale.* A significant increase ($p < 0.05$) in dissolved organic material was observed in the *Microcystis aeruginosa* culture from the Control BP to the other samples (Control AP, 2 and 5

mg.L^{-1}) indicating that both, the environmental conditions and the H_2O_2 application, may have interfered in the release of intracellular content. This statement is only partially true for *Dolichospermum circinale* where there was a significant increase ($p < 0.05$) in dissolved organic material from the Control BP to the Control AP but not to the treatment microcosms (2 and 5 mg.L^{-1}). This indicates that the environmental conditions may have interfered in the release of intracellular content but the H_2O_2 application did not. Another possibility is that the amount of oxidant was sufficient to cause the cell lysis, the release, and the degradation of intracellular *Dolichospermum circinale* content but not enough to degrade all the released intracellular content of *Microcystis aeruginosa*.

Figure 12 - Organic matter - 254 nm (a) and true color (b) of *Microcystis aeruginosa* (MA) and *Dolichospermum circinale* (DC) suspensions. C_BP = Control sample collected at time T=0; C_AP = Control sample (with no H_2O_2) collected after 24h from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 mg.L^{-1} of H_2O_2 (24h); 5 = Sample collected after the compete degradation of 5 mg.L^{-1} of H_2O_2 (48h).

a)



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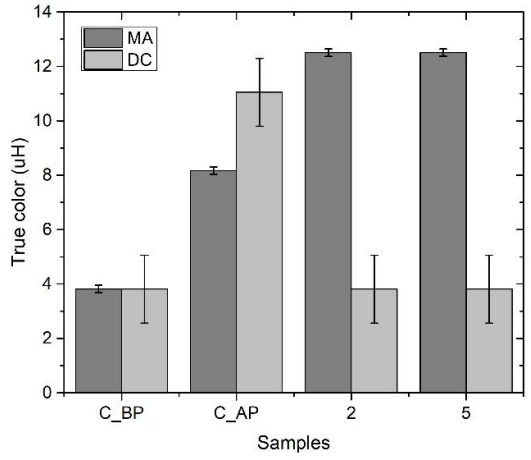
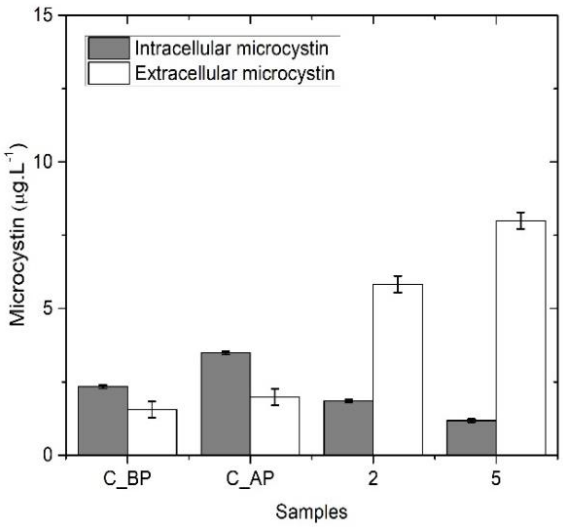


Intracellular microcystin decreased while the extracellular fraction increased, both significantly ($p < 0.05$), from the Control BP to the treatment with 5 mg,L^{-1} (Figure 13a). This behavior was expected since the integrity of *Microcystis aeruginosa* cells was impacted by the oxidant. What was not expected, though, was the increase in the total microcystin concentration from around 4 – 9 μg.L^{-1}. The amount of H_2O_2 seemed not to be sufficient to degrade dissolved microcystin, similar to what happened to the dissolved organic material, even with 5 mg.L^{-1} being applied.

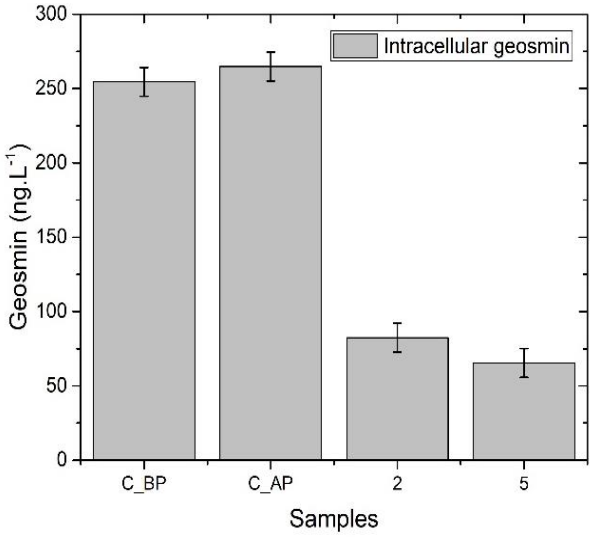
On the other hand, geosmin did not vary significantly ($p < 0.05$) from the Control BP to the Control AP, but decreased significantly ($p < 0.05$), from 264.82 ng.L^{-1} in the Control AP to 72.02 ng.L^{-1} in the microcosm with 5 mg.L^{-1} of H_2O_2 (Figure 13b). This concentration decrease, after the application of oxidant, was also observed for the dissolved organic material of the *D. circinale* suspension (Figure 12).

Figure 13 – Intra and extracellular Microcystin from *Microcystis aeruginosa* (a) and Total Geosmin from *Dolichospermum circinale* (b) suspensions. C_BP = Control sample collected at time T=0; C_AP = Control sample (with no H_2O_2) collected after 24h from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 mg.L^{-1} of H_2O_2 (24h); 5 = Sample collected after the compete degradation of 5 mg.L^{-1} of H_2O_2 (48h).

a)



b)



3.4 Discussion

The main objective of this research was to evaluate the effects of H_2O_2 on cultures of *Microcystis aeruginosa* and *Dolichospermum circinale* and their metabolites under visible light. The application of H_2O_2 was effective in inducing cyanobacterial death and releasing intracellular content. The reduction of biovolume and cell numbers was evident for both strains studied.

In freshwater environments, *Microcystis aeruginosa* cell size is reported to vary between 4 and 6 μm (OLENINA et al., 2006), which differs from the initial cell size of our experiment (~8.66 μm). Cultured *Microcystis aeruginosa* responds differently to different environmental conditions. According to Doers and Parker (1988), when exposed to different experimental conditions (1st condition: 30ºC, 30 $\mu E.m^{-2}.s^{-1}$ from white fluorescent lamps, and 2nd condition: 25ºC, 10 $\mu E.m^{-2}.s^{-1}$ from white fluorescent lamps), *Microcystis aeruginosa* presented morphological differences in terms of cell size, pigmentation, colony disintegration, mucilage sheath, the distance between cells, among others.

In addition to the reduction in cell size and biovolumes, the application of H_2O_2 reduced *Microcystis aeruginosa* cell numbers. Zhou et al. (2018) observed *Microcystis aeruginosa* lysis, reduction in growth, and a reduction of cell size from 2.1 to 1.7 μm on the 3rd day of the application of 24–51 $mg.L^{-1}$ H_2O_2. Bsrrington (2013) also observed a selective reduction of cyanobacteria cells (up to 96 %) using 0.37 $g.L^{-1}$ of H_2O_2 in a lake containing high concentrations of cyanobacteria, mainly *Microcystis aeruginosa*, *Microcystis flos-aquae*, and

Planktothrix. isothrix. Matthijs et al. (2012) showed that the application of 2 $mg.L^{-1}$ of H_2O_2 in a lake in the Netherlands (Lake Koetshuis), decreased the population of *Planktothrix agardhii* from 6.105 $cells.mL^{-1}$ to 1.104 $cells.mL^{-1}$.

Yang et al. (2018) observed the effect of six different concentrations of H_2O_2 (0, 0.3, 0.9, 2.7, 8.1, and 24.3 $mg.L^{-1}$) on 4 cyanobacteria (*Dolichospermum* sp., *Cylindrospermopsis* sp., *Planktothrix* sp., *Microcystis* sp.). After the treatment, *Dolichospermum sp*. was not detected even with low H_2O_2 concentrations (0.9 mg. L^{-1}), showing to be significantly more sensitive than *Microcystis sp*, behavior also observed in our study. Lin et al. (2009) compared the cultures of *Microcystis aeruginosa* and *Dolichospermum circinale* after the addition of another oxidant, sodium hypochlorite (NaClO), and also observed a higher rate of disruption of *Dolichospermum circinale* cells, by a factor of 1.3–5.0 when compared to *Microcystis aeruginosa* cells. According to Yang et al. (2018), *Microcystis aeruginosa* cells are more resistant to changes in the external environment because they have greater fluctuating regulation, colonial morphology, and resistance to high radiation, giving the species greater competitive dominance. Also, when microcystis cells occur in colonies, which is normally the case of strains encountered in natural waters, they are more resistant to H_2O_2 probably because the mucus formed in the colonies has antioxidant properties (ZHANG; BENOIT, 2019). This difference between cultivated and environmental strains should be considered when evaluating the concentration to be used in natural waters to controls cyanobacteria blooms.

The decrease in the chlorophyll-a concentrations after the application of H_2O_2, for both species, suggests that after the cell lysis chlorophyll-a is released and oxidized preferentially over the other organic material. Some authors verified the same effect after the application of H_2O_2. Liu et al. (2017) applied H_2O_2 (5 and 20 $mg.L^{-1}$) to colonies of *Microcystis* sp. and observed a significant reduction of chlorophyll-a concentration after 12h, from 60 $\mu g.L^{-1}$ to 39 $\mu g.L^{-1}$. Yang et al. (2018) also found a significant reduction in chlorophyll-a (150 to 25 $\mu g.L^{-1}$) in *Microcystis aeruginosa* cultures due to the addition of 1, 3, 6.7, and 20 $mg.L^{-1}$ of H_2O_2. Barrington and Ghadouani (2008) studied the effects of H_2O_2 on the decay of chlorophytes and diatoms from environmental samples and observed that, under laboratory conditions, the application of 3 mg $H_2O_2.\mu gCl\text{-}a^{-1}$ reduced *in vivo* photosynthetic activity of phytoplankton. According to this author, concentrations of chlorophyll-a were reduced to negligible values.

The determination of phycocyanin is also important because it relates closely to the cyanobacteria biomass and, therefore, it's generally used to estimate the density of

cyanobacteria (YANG et al., 2018). This pigment is a conjugated biliprotein and an accessory photosynthetic pigment. The chromospheres present in this pigment are the phycocyanobilin known to display a wide range of photochemical activity (ROBERTSON; LAWTON; CORNISH, 1999). The oxidative stress after the application of H_2O_2 did not significantly affect the phycocyanobilins. Thus, phycocyanin has potential as an antioxidant agent due to its stability under oxidative stress (SONANI et al., 2014). This argument reinforces the idea that peroxide did not affect phycocyanin concentration, as observed in our study.

The increase in organic matter 254 nm and true color may be related to the higher cell density of *Microcystis aeruginosa* (10^5 cells.mL^{-1}), as opposed to what happened to the *Dolichospermum circinale* suspension (10^4 cells.mL^{-1}). According to Jarusutthirak and Amy (2007), if there is a greater number of cells in the medium when H_2O_2 is added, there will be a greater level of organic matter to be released when the cells lyse. Additionally, the aromatic fraction present in some organic matter can not only consume hydroxyl radicals but also produce them. These new radicals react with other remaining particulate organic compounds, increasing the concentration of dissolved organic matter in the medium (HO; NEWCOMBE; CROUÉ, 2002; PARK et al., 2017). Thus, there is an optimum H_2O_2 dosage for a given system at which maximum removal of the target substance is achieved. Wang et al. (2000) showed that the degradation rate of humic acid during the UV/ H_2O_2 process increased with the increase of hydrogen peroxide concentration until 0.01 % (2.94 mmol.L^{-1}) and then decreased with further increase in H_2O_2 concentration.

An increase in extracellular microcystin and geosmin was expected because of the cell disruption caused by H_2O_2. Instead, a decrease of geosmin and an increase of total microcystin were observed. In the case of geosmin, some authors have found that when enough H_2O_2 is applied, apart from breaking the cells, it can further degrade secondary metabolites. Other studies have shown that if H_2O_2 is applied in low concentrations, it can initially increase extracellular toxins and other metabolites and, after some time, these metabolites are significantly reduced after they are released to the surrounding waters. This may suggest that bacterial degradation can assist in the breakdown of dissolved toxins and other metabolites when treating waters using H_2O_2 in natural environments such as lakes and ponds (MIAO et al., 2010; PAPADIMITRIOU et al., 2016). A possible explanation for the increase of total microcystin might be that this toxin was initially bound to internal proteins before the H_2O_2 addition, and therefore not visible to the HPLC method used. However, after cell death and internal content release, the toxin may have detached from the proteins and became visible to the analytical method (SCHUURMANS et al., 2018).

3.5 Conclusion

Hydrogen peroxide (H_2O_2) was used to control *M. aeruginosa* and *D. circinale* and their metabolites in the water. A low concentration of H_2O_2 (2 $mg.L^{-1}$) was enough to reduce the concentrations of chlorophyll-a and geosmin, destroy cyanobacterial cells, or render them unviable, preventing their re-growth. Phycocyanin, however, was resilient to oxidation indicating that it may not be a good indicator of cyanobacterium density in natural waters when H_2O_2 is applied. This oxidant caused the release of intracellular content but was not enough to degrade it, increasing the dissolved organic material in the water, decreasing water quality, and increasing the potential formation of disinfection by-products during the conventional water treatment process. Total microcystin concentration doubled during the treatment, probably because it was detached from the proteins after cell lysis. Most of this microcystin was extracellular, representing an additional risk to conventional water treatment plants. Our findings suggest that, if the application of H_2O_2 *in situ* is to be considered for a reservoir used for drinking water purposes, it should be done at a distant point from the water treatment plant, allowing natural degradation processes of organic and toxic compounds, such as biological or photolysis degradation, to occur before the water reaches the water treatment plant intake.

4 TOXIC CYANOBACTERIA IN WASTE STABILIZATION PONDS IN THE BRAZILIAN NORTHEASTERN SEMIARID REGION

4.1 Introduction

Waste Stabilization Ponds (WSPs) have traditionally been regarded as a treatment system suitable for hot climate areas, normally found in developing countries (MARA et al., 1992). WSPs are artificial aquatic environments that use natural processes to treat domestic, animal, or industrial wastewater before it is released into the environment (ABDEL-RAOUF; AL-HOMAIDAN; IBRAHEEM, 2012). WSPs are capable of removing organic matter with high efficiency and different kinds of pathogens. Besides, they are easy to design, operate, and maintain but require long retention times, normally several weeks, and though need extensive areas to be constructed (MARA et al., 1992).

One of the common characteristics of these ponds is the proliferation of phytoplankton (D'ALESSANDRO; NOGUEIRA; HOFFMANN, 2020). Although about 15.000 species have been recorded in nature, only a few of them are functionally useful in the waste stabilization ponds (FLORENTINO et al., 2019; SHANTHALA; HOSMANI; HOSETTI, 2009). Phytoplanktons are the primary producers and constitute the first level of trophic status in the aquatic food chain (SHANTHALA; HOSMANI; HOSETTI, 2009). High temperatures, wind velocity, and concentrations of nutrients and organic matter, conditions normally found in tropical areas, are adequate factors for blooms. A common group of phytoplankton that thrives in these conditions is cyanobacteria, which include opportunistic and often toxic microorganisms that adversely affect human health and the quality of the water resources (KOTUT et al., 2010).

Some potentially toxin-producer cyanobacteria may be present in WSPs. The presence of these toxic cyanobacteria in stabilization ponds can affect its treatment efficiency and imposes a substantial threat to ecological, human, and animal systems (BARRINGTON; REICHWALDT; GHADOUANI, 2013). These organisms can suppress the growth of phytoplankton species necessary for the treatment of wastewater. Additionally, the treated effluent, which is generally discarded for irrigation or environmental flow (BARRINGTON; REICHWALDT; GHADOUANI, 2013), can act as a cyanobacteria inoculum for the receiving water body, contaminate the environment with cyanotoxins, and hamper water treatment operations (BARRINGTON; GHADOUANI; IVEY, 2011). Reports on toxic cyanobacteria in

stabilization ponds have been published in several studies (KOTUT et al., 2010; PASTICH et al., 2016; SHANTHALA; HOSMANI; HOSETTI, 2009).

The Brazilian semi-arid region presents a set of optimal conditions for the development of cyanobacteria, such as high light incidence and temperatures throughout the year, restricted rainy season, and surface waters mostly confined by dams in reservoirs and stabilization ponds (BITTENCOURT-OLIVEIRA et al., 2012). For this reason, the release of treated wastewater into local aquatic bodies is a concern of water and sewage treatment companies in the region (BARRINGTON; REICHWALDT; GHADOUANI, 2013), because it is essential that an effective treatment is added, in addition to WSPs, to ensure the removal of cyanotoxin before their insertion into the environmental flow (BARRINGTON; GHADOUANI; IVEY, 2011; FLORENTINO et al., 2019).

The application of oxidants such as ozonation, potassium permanganate, and chlorine dioxide, in addition to being expensive, is limited by the formation of environmentally harmful by-products (CHANG; HUO; LIN, 2018; LI et al., 2014). On the other hand, advanced oxidation processes using hydrogen peroxide (H_2O_2) have shown potential in reducing cyanobacteria concentrations in reservoirs and stabilization ponds (CHANG; HUO; LIN, 2018).

Since WSPs are an integral part of the wastewater treatment strategy in many developing countries, a better understanding of the occurrence of toxic cyanobacteria and their relationship with environmental factors is essential for selecting the appropriate treatment to complement the activity of WSPs.

In this sense, considering the presence of toxic cyanobacteria in WSPs, the importance of this technology in developing countries, and the impacts caused by these organisms on human health and the environment, this study aimed to identify the species of cyanobacteria most prevalent in WSPs in the Brazilian semi-arid region of the State of Ceará, to understand the impact caused by these cyanobacteria on the treated effluent and propose the most appropriate treatment to complement the WSPs treatment.

4.2 Materials and Methods

4.2.1 Studied WSP's

The WSP´s in this study (WTP-Itapipoca, WTP-Russas, WTP- Catarina, WTP-Campos Sales, and WTP-Revoltosos) are used to treat domestic wastewater and are located in different cities in the State of Ceará, in the Brazilian Northeastern semi-arid region (Figure 14). The city, pond configuration and river receiving treated effluent are shown in Table 7.

Figure 14 – Location of the WTP´s in the state of Ceará, Brazil.

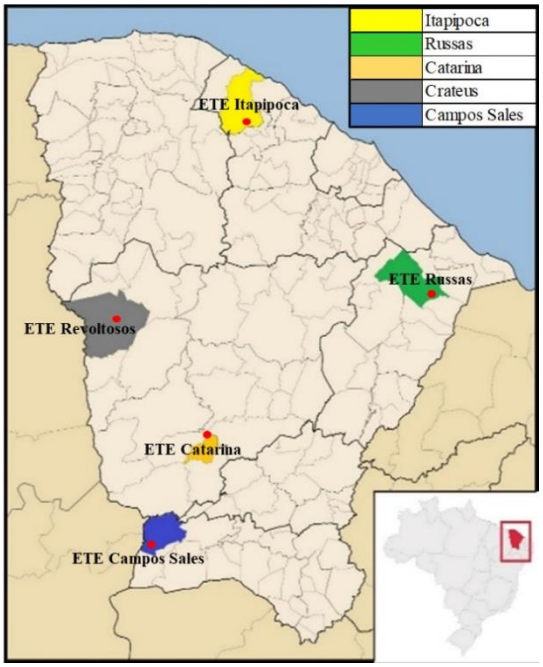


Table 6 - Wastewater Treatment Plants (WTP), the cities where they are located, the pond configuration, and the river that receives the treated effluent.

WTP	City	Pond Arrangement	River
WTP-Itapipoca	Itapipoca	Anaerobic followed by Facultative	Macaco
WTP-Russas	Russas	Anaerobic followed by Facultative	Arahibu
WTP-Catarina	Catarina	Facultative followed by Maturation	Condado
WTP-Campos Sales	Campos Sales	Facultative followed by Maturation	NA
WTP-Revoltosos	Crateús	Facultative followed by Maturation	Poti

4.2.2 Studied data

The physical-chemical and biological data were provided by the state water companies (Companhia de Água e Esgoto do Ceará - CAGECE and Companhia de Gestão dos Recursos Hídricos - COGERH). The monthly averages for each parameter were considered. The data were obtained from samplings carried out over eight months, covering four months of each season (dry and rainy): from September to December of 2014, corresponding to the dry season, and from January to April of 2015, corresponding to the rainy period. Samples were collected and measurements were taken at the effluent outlet.

4.2.3 Analysis methods

Conductivity and Temperature were measured using a multiparametric probe (ORION Model 105 A+). Total phosphorus, nitrate, and ammonia concentrations were measured using the methods shown in Table 8. For the analysis of seasonality dynamics, average monthly rainfall, temperature, and wind velocity data were obtained from (FUNCEME, 2014, 2015).

Table 7 – Methods used for the determination of nutrients (APHA, 2012).

Abiotic parameters	**Methods**
Total phosphorus	4500P
Nitrate	4110C
Ammonia	4500 NH3 C

After preserving samples with Lugol, cell density was measured using the method proposed in APHA (2012) with each unicellular organism considered as an individual (YOSHIDA et al., 2006). For taxa identification in the samples, specialized bibliography was consulted (BICUDO; MENEZES, 2017; KOMÁREK; ANAGNOSTIDIS, 2005, 2008; SANT'ANNA et al., 2006).

4.2.4 Diversity and equitability indexes

Species diversity (H') (Eq. 3) was calculated using the Shannon Index taking into account the number of species and the dominant species (SHANNON, 1948). Equitability (E)

(Eq. 4), derived from the Shannon diversity index, was calculated using the Pielou Index (PIELOU, 1977).

$$H' = -\sum_{i=1}^{S} p_i . ln\ p_i \qquad \text{Eq. 3}$$

$$E = \frac{H'}{H'_{max}} \qquad \text{Eq. 4}$$

$$H'_{max} = \ln S$$

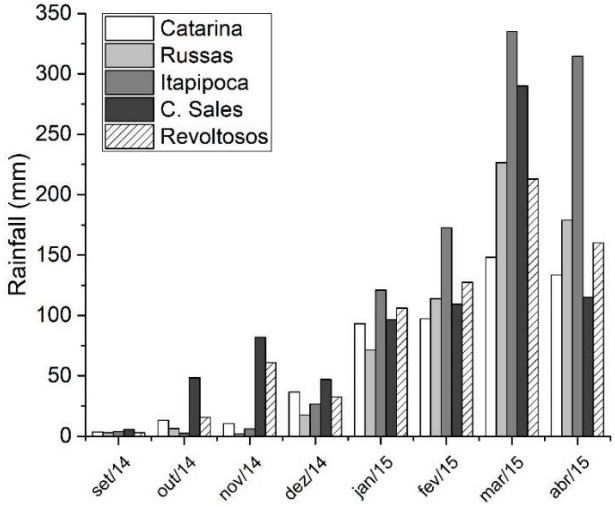
4.2.5 Statistical analysis

To verify the relationship between environmental factors associated with the cyanobacteria present in the WTP´s, Principal Components Analysis (PCA) was applied to the data. The PCA was performed to verify the relationships between the 8 variables (represented by vectors) studied in the plane defined by the first two components and to group similar WSPs by the variance of the data. The Clustering method was also performed using the k-means algorithm. Statistical analysis was performed in the RStudio software (R CORE TEAM, 2018).

4.3 Results and discussion

In the state of Ceará, in 2014 and 2015, the monthly rainfall recorded indicated that the most intensive precipitation began in December and lasted until April; therefore, the dry period was considered from September to December (Figure 15).

Figure 15 - Average monthly rainfall of the municipalities under study corresponding to the collection period Sep / 2014 to Apr / 2015.



The phytoplankton community of WTP Catarina, Russas, Itapipoca, Campos Sales, and Revoltosos was represented by 16, 23, 13, 22, and 12 taxa, respectively, distributed in Cyanobacteria, Euglenophyta, Bacillariophyta, and Chlorophyta phylum (see Table A and B in Appendix B). *Planktothrix agardhii* was present in Russas, Itapipoca, Campos Sales, and Revoltosos WTP in both years 2014 and 2015 (Figure 16a and 16b) and was the most frequent species with approximately 100% occurrence, both in the dry and rainy periods. In 2014, *Sphaerocavum brasiliensis* and *Microcystis aeruginosa* showed high frequency respectively in Catarina and Campus Sales` WTP. Several studies point to *Microcystis aeruginosa* and *Planktothrix agardhii* as being potentially toxic cyanobacteria (LONE; KOIRI; BHIDE, 2015), widely distributed worldwide, and bloom-forming (BLACK; YILMAZ; PHLIPS, 2011; MULLER; CYBIS; RODRIGUEZ, 2012; WANG et al., 2011a).

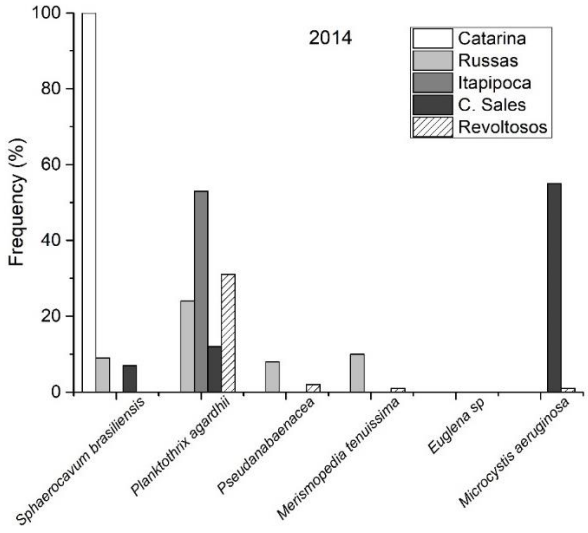
Other taxa, such as *Pseudoanabaenaceae, Merismopedia tenuissima, Microcystis aeruginosa,* and *Euglena sp*. were also present in the WSP´s under this study. These taxa have also been found in other WSP´s (AMENGUAL-MORRO; MOYÀ NIELL; MARTÍNEZ-TABERNER, 2012; KOTUT et al., 2010; PASTICH et al., 2016; SHANTHALA; HOSMANI; HOSETTI, 2009) as well as in artificial reservoirs used for human supply (DELAZARI-BARROSO et al., 2009; OLANO et al., 2019).

In reservoirs located in the northeastern semi-arid region, with the intense evaporation, there is a greater concentration of nutrients and also deposition of sediments impacting the trophic state of the water (BEZERRA et al., 2014; LIRA; MEDEIROS; NETO, 2020). These conditions increase the occurrence of eutrophication of water bodies and,

consequently, the increase in cyanobacterial blooms (ESTEVES, 2011). This represents a greater risk for the qualitative and quantitative conservation of water, which assesses water availability even more challenging for water supply companies (MAMEDE et al., 2018; MOURA et al., 2020).

Figure 16 - Frequency of occurrence of the cyanobacteria present in the studied WTPs during the study period.

a)



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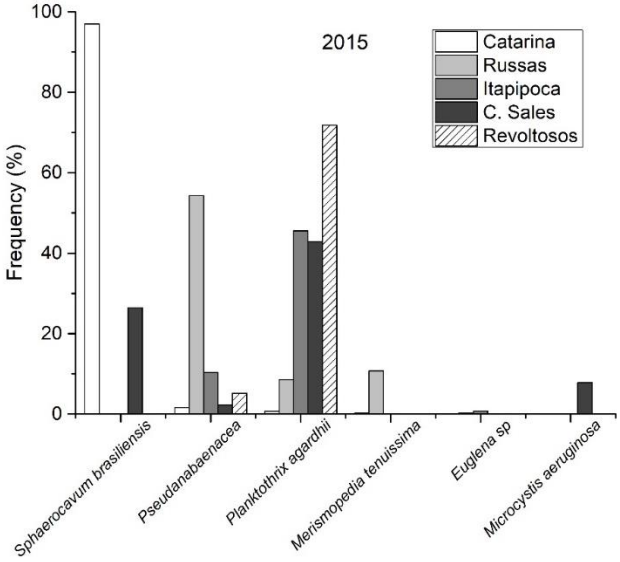


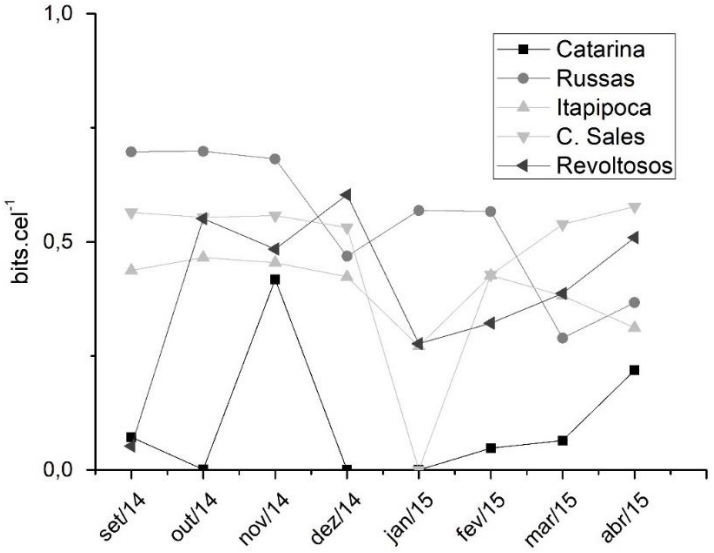
Diversity, which normally decreases with increasing eutrophication, is an important tool to understand the structure of the local community (SHANTHALA; HOSMANI; HOSETTI, 2009). The diversity of the studied WTP showed values ranging from 0.698 bits.cel^{-}

[1] to zero (Figure 17a). When the values, calculated using the Shannon index, are lower, the degree of uncertainty is lower and, therefore, the diversity of the sample is low (URAMOTO; WALDER; ZUCCHI, 2005). Due to the predominance of *S. brasiliensis* in both dry and rainy, the WTP Catarina had the lowest diversity among the other WTPs. This extremely low diversity can be explained by the high eutrophication state present in wastewater stabilization ponds, which increases the dominance of one or two species in the environment to detriment of the other species, reducing the phytoplankton diversity, and increasing the frequency and intensity of blooms (TONETTA et al., 2015).

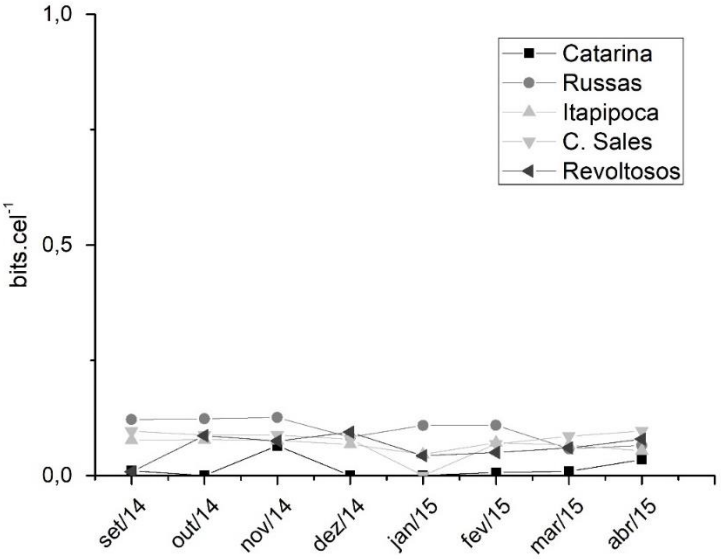
The equitability index reflects the degree of organization of the community, the percentage variation of its components, and the distancing of that community from another with species equitably represented. Therefore, when species are evenly distributed, equitability is close to 1 (PIELOU, 1977). In this work, the equitability index values ranged from 0.125 to zero (Figure 17b). When all species in a sample are equally abundant, the equitability index must assume the maximum value and decrease, tending to zero, as the relative abundances of species diverge from this equality (URAMOTO; WALDER; ZUCCHI, 2005). In this last case, low values indicate that the phytoplankton community is not uniformly distributed, characterizing the dominance of a few species in most of the period evaluated. Other studies have reported low levels of diversity and equitability in environments where cyanobacterial blooms are frequent and intense (ADLOFF et al., 2018; BORGES; TRAIN; RODRIGUES, 2008; GENTIL; TUCCI; SANT'ANNA, 2008). Low values of diversity and equitability of cyanobacteria are a negative consequence of eutrophication and may increase the potential of toxin production and pose a constant threat to the water body where the effluent is discharged (COSTA et al., 2009).

Figure 17 – (a) Diversity and (b) Equitability of the phytoplankton in the WTPs, during the study period.

a)



b)

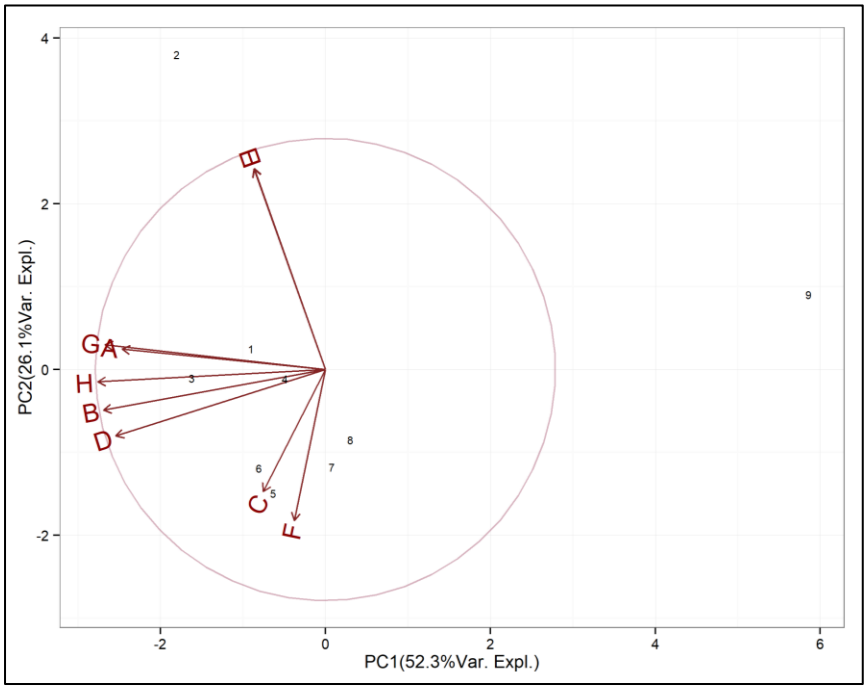


It has been reported that environmental factors such as temperature, pH, dissolved oxygen, and nutrient availability (see Table C in Appendix B) play an important role in regulating the structure and distribution of phytoplankton communities in lakes or rivers (DUONG et al., 2013). In this sense, Figure 18 shows the relationships between the 8 variables (represented by vectors) studied in the plane defined by the two main components (PC1 and PC2) and, through this plane, similar WSPs are grouped by the data variance. PC1 and PC2 are just the two main components of the new coordinate system which, in the graphs of Figures 5a, 5b, 5c, 5d, and 5e represent, respectively, 63.8%, 77.7%, 70.2%, 69%, and 60.9% of the original

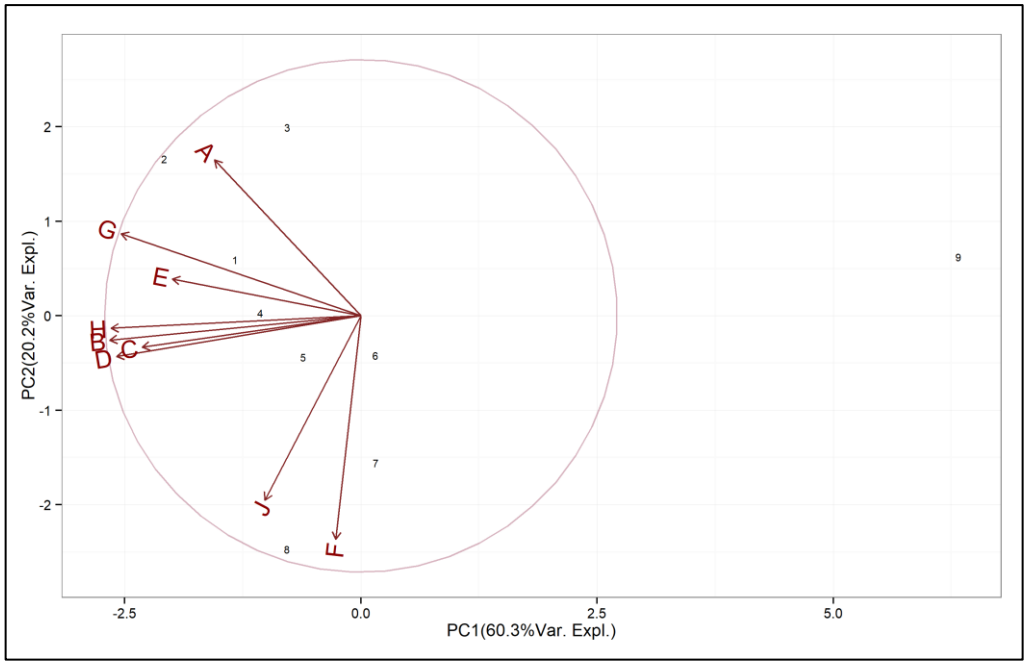
data variance. In this new space, the variables (represented by the arrows), present or not correlation according to the internal product of vectors. Thus, vectors that form 90º do not correlate, and vectors that form 0° or 180° have a greater positive or negative correlation, respectively.

Figure 18 – Principal Component Analysis (PCA) of biotic and abiotic factors, through the biplotting of the first two factors (PC1 and PC2) of the WTPs a) Catarina, b) Russas, c) Itapipoca, d) C. Sales, and e) Revoltosos. Where: A = Total Phosphorus (mg P-PO_4^{3-}/L), B = Conductivity (µS/cm), C = Nitrate (mg N-NO^{3-}/L), D = Ammonia (mg N-NH_3/L), E = Density (cells/mL), F = Rainfall (mm), G = Wind (m/s), H = Temperature (ºC), I = Sphaerocavum brasiliensis (cells/mL), J = Plankthothrix agardhii (cells/mL), K = Microcystis aeruginosa (cells/mL), M1 = September/2014, M2 = October/2014, M3 = November/2014, M4 = December/2014, M5 = January/2015, M6 = February/2015, M7 = March/2015 and M8 = April/2015.

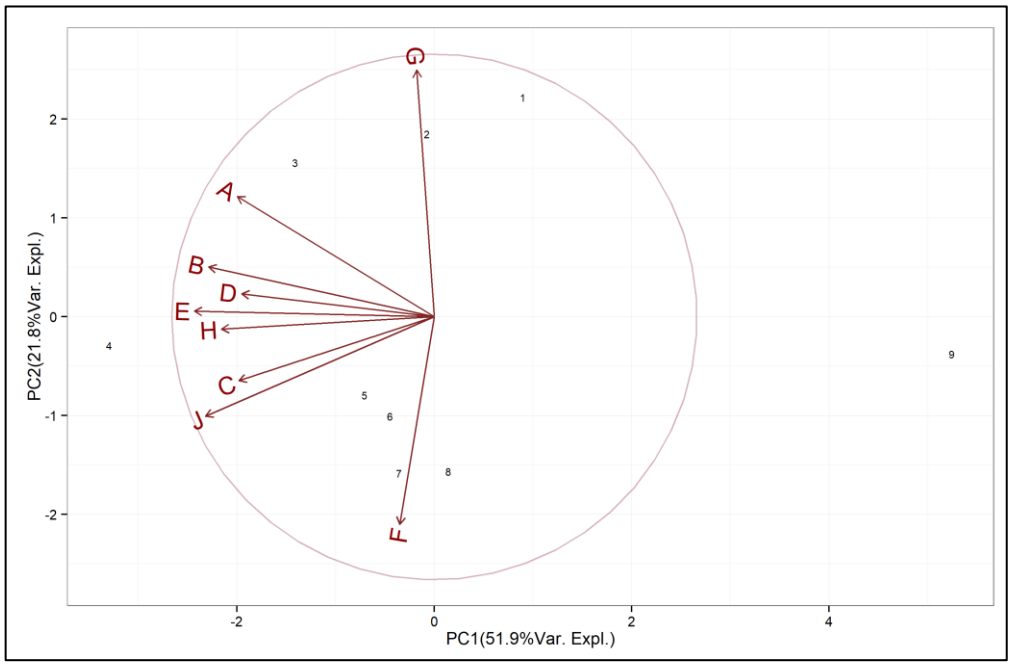
a)



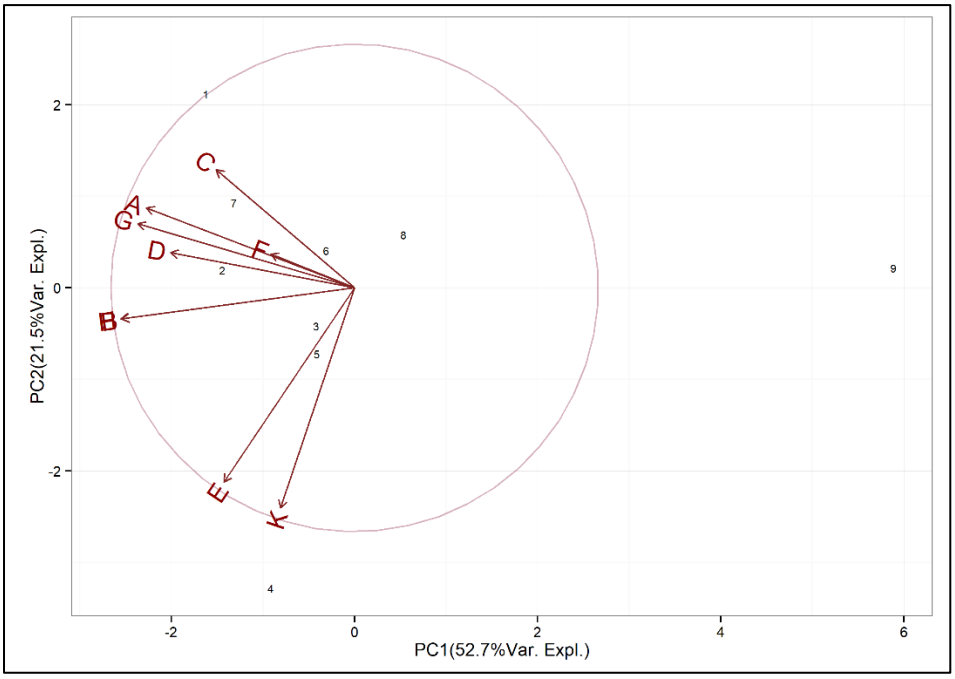
b)


A
G
E
H
B
C
D
J
F
1
2
3
4
5
6
7
8
9
PC2(20.2%Var. Expl.)
PC1(60.3%Var. Expl.)
2
1
0
-1
-2
-2.5
0.0
2.5
5.0

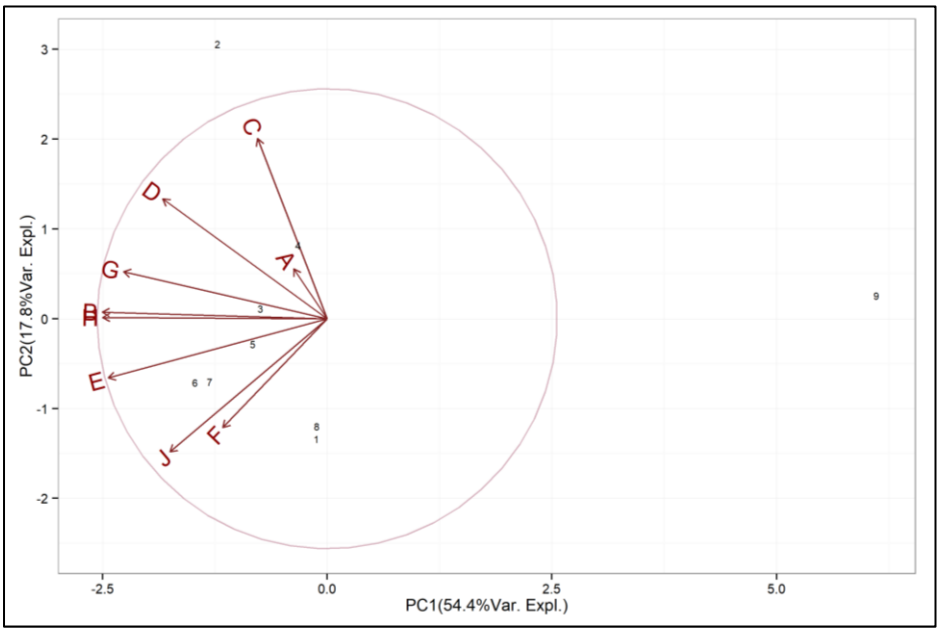
c)


G
A
B
D
E
H
C
J
F
1
2
3
4
5
6
7
8
9
PC2(21.8%Var. Expl.)
PC1(51.9%Var. Expl.)
2
1
0
-1
-2
-2
0
2
4

d)



e)



The predominance of practically isolated species was observed in the studied WTPs: *P. agardhii* in the Russas, Itapipoca, and Revoltosos WTPs, the species *S. brasiliensis* in the WTP Catarina, and the species *M. aeruginosa* in the WTP C. Sales. Due to observation, it is clear that in the stabilization ponds that presented this behavior (Figures 18a, 18b, 18c, 18d e 18e), the density of species of cyanobacteria in each WSP is correlated positively with the total density of cyanobacteria and negatively with rainfall. This correlation together with the

values of diversity and equitability and the semi-arid high temperatures corroborates with the dominance of a single species in the WTPs.

According to Huszar et al. (2000) e Costa et al. (2009), among the factors that control the dominance of cyanobacteria are low turbulence and luminosity, high temperatures and pH, and low CO_2 concentration, characteristic of stabilization ponds and quite favorable to the growth of cyanobacteria. In general, cyanobacteria have a higher optimal growth temperature compared to other groups of phytoplankton (PAERL; HUISMAN, 2008). In the Nui Coc reservoir, located in northern Vietnam, both cyanobacteria and *Microcystis aeruginosa*, in particular, were positively correlated with elevated temperature during the studied period. (DUONG et al., 2013).

Cyanobacteria dominance in every WTP was negatively correlated with rainfall intensity. According to Hubble and Harper (2001), the reduction in volume during droughts and the shallow depth of reservoirs seemed to promote high turbidity, decreasing the availability of light, favoring the prevalence and increase of the biomass of some cyanobacteria. Also, the difficulty of the water column mixing allows for the bloom of species such as *Planktothrix agardhii, Sphaerocavum brasiliensis,* and *Microcystis aeruginosa*, mainly in reservoirs with longer water residence times in the Brazilian semiarid region (COSTA et al., 2009). The increase in the biomass of cyanobacteria in stabilization ponds results from an increase in the organic matter load to the system (HOEK; MANN; JAHNS, 1995).

Biotic and abiotic factors are important indicators of water quality, which directly influence the dynamics of processes within the water bodies (ADLOFF et al., 2018). In most stabilization ponds in Kenya, plant management programs generally focus on nutrient concentration to assess microbial water quality (KOTUT et al., 2010). According to Dantas et al. (2012), nutrients also interfere with the composition and biomass of phytoplankton inside the reservoirs. Several studies have also shown some correlations between cyanobacteria and environmental factors, but these relationships have not always been consistent (GRAHAM et al., 2006; JACOBY et al., 2000; WU et al., 2006). Albay et al. (2005) observed that in stabilization ponds, the lack of correlation between cyanobacteria biomass and toxin levels, even when dominant cyanobacteria are known as toxin-producer, suggests that toxin production may also be influenced by prevailing environmental conditions, such as temperature (between 24 and 28, 5 ºC), high concentration of dissolved nutrients, high light intensity and pH. In this study, total cyanobacteria density was correlated with water temperature but poorly correlated with other parameters, such as nutrient concentrations. Amé et al. (2003) and Dai et al. (2008) suggested that among the physical-chemical characteristics, the water temperature was an

important factor related to cyanobacteria. Naselli-Flores (2000) showed that reservoirs of different trophic states are characterized by constant fluctuations in the water level and this influences the phytoplankton composition more strongly than the concentration of nutrients in the medium.

In a watercourse, the predominant form of nitrogen indicates the stage of the pollution or the distance between the release and the analysis point. If the pollution is recent, the nitrogen is in the form of organic nitrogen or ammonia and if the pollution is older, the nitrogen is basically in the form of nitrate (HU et al., 2013). Some nutrients, especially ammonia when present in high concentrations in aquatic environments, can cause damage to biota (HU et al., 2013). Moreover, with the presence of potentially toxin-producing cyanobacteria at the wastewater treatment plant in the city of Nakuru, the presence of toxins, albeit at low levels, indicated that animals using lake water were exposed to toxin poisoning, affecting the local fauna.

The method of clustering was used to promote the grouping of similarities (Figure 19). Because the nature of the algorithm cluster analysis consists of an iterative procedure, a set of trial processes were performed before reaching a final grouping (LEMENKOVA, 2019). According to figure 19, the optimal number of clusters was two, grouped according to their climatic and hydrological similarities (Figure 20). According to Figure 20, the clusters obtained indicate no significant variations in the climatic and hydrobiological data, since they are in the same climatic region. They also showed a dominance of cyanobacterial but with different species in each WTP (*Planktothrix agardhii* in Russas, Itapipoca, and Revoltosos WTP, *Sphaerocavum brasiliensis* in the WTP Catarina, and *Microcystis aeruginosa* in the WTP C. Sales).

Figure 19 - Results of the K-means clustering with different k-values

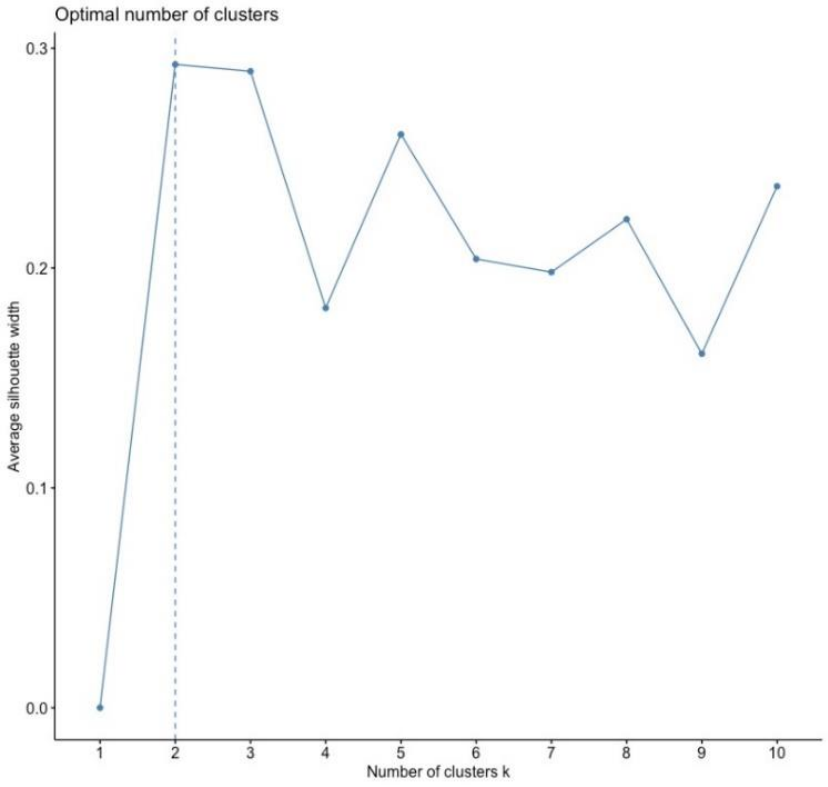
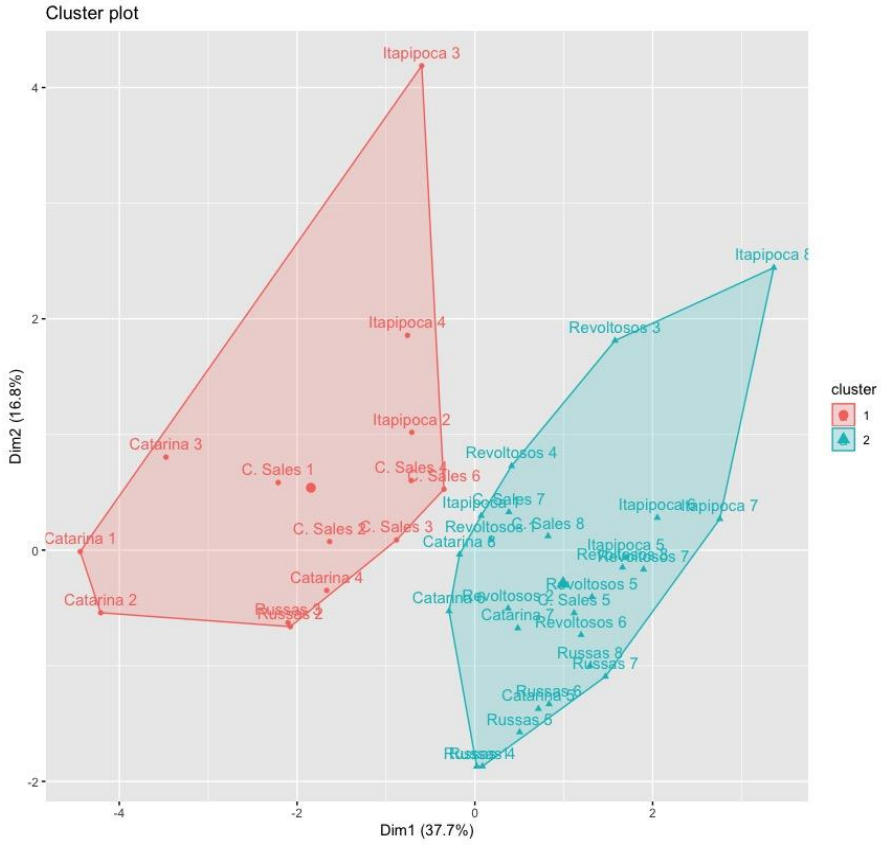


Figure 20 - Cluster groups according to the similarities of the studied WTPs.



Cyanobacteria bloom prevention methods have been suggested, such as the de-stratification of the hydraulic body through aeration or the use of mechanical mixers. These physical remediation techniques may offer alternative control over the proliferation of cyanobacteria, but they have limited results (BARRINGTON; GHADOUANI, 2008b). Therefore, cyanobacteria removal technologies have been widely studied including adsorption, flocculation, bacterial degradation, chemical degradation, and photolysis. Many of these technologies are potentially harmful to the environment and often destroy cyanobacterial cells, but not all harmful metabolites, which are released into the surrounding water after cell lysis (BARRINGTON; GHADOUANI, 2008b). Therefore, the application of H_2O_2 in treated effluents from WSPs can be a viable alternative as it is effective in removing cyanobacteria and cyanotoxins during large-scale application and is environmentally friendly since it does not accumulate in the environment and decomposes rapidly into oxygen and water by biological, chemical, and photochemical mechanisms (BURSON et al., 2014; CHANG; HUO; LIN, 2018).

4.4 Conclusion

In the present study, the phytoplanktonic community was dominated by cyanobacteria, presented very low diversity and equitability, and a high potential for toxin production. This may indicate an environmental impact on the rivers and reservoirs receiving the treated effluent. It may also increase the risk to public health, water body eutrophication state, and decreases the economic value of the water resources. The dominance of cyanobacterial species *(Planktothrix agardhii*, *Sphaerocavum brasiliensis*, and *Microcystis aeruginosa*) in the studied WTPs is concerning since they may act as inoculum to the recipient water bodies, especially in the artificial reservoir. As a result, we propose to intervene with some kind of treatment in the effluent of these WTPs, aiming to protect human health and water resources. Besides, the use of Principal Component Analysis and the K-means cluster can contribute to the development of more complex multidimensional modeling for monitoring cyanobacteria and toxins in reservoirs.

CONCLUSIONS

The presence of cyanobacteria in water supply sources is a reality all over the world. in the semi-arid region of Brazil. it is even more remarkable. The understanding of their relationships within the local water system and ways to control their proliferation is extremely necessary.

The development of a mathematical model using pheophytin and phycocyanin concentration to estimate the concentration of metabolites (microcystin and geosmin) from the life cycle of the isolated cyanobacteria cultures (*Microcystis aeruginosa* and *Dolichospermum circinale*). was considered a more practical solution and showed good precision. The combination of sensor technology and accurate mathematical modeling can be an important tool to warn water companies of high levels of toxins in the water to activate complementary treatments for the control of cyanobacteria and the removal of noxious metabolites. The differences between calculated and measured metabolites concentrations using the model were less than 5%. demonstrating that it can be applied for isolated cyanobacteria cultures. and open the possibility for the use in environmental samples. though more investigation is necessary.

The effectiveness of H_2O_2 under visible light on *Microcystis aeruginosa* and *Dolichospermum circinale* cells. its regrowth potential. microcystin and geosmin degradation. organic matter. and pigments were investigated and demonstrated that low concentrations of H_2O_2 (2 mg/l) were sufficient to reduce the concentrations of chlorophyll-a. geosmin. destroy cyanobacterial cells or render them unviable. preventing their re-growth but increased dissolved organic matter. These findings indicate that if the application of H_2O_2 *in situ* could be considered for a reservoir used for drinking water purposes but using extreme caution. at a distant enough point from the water treatment plant intake. However. further research on the possible effects of low concentration dosing should be investigated.

Identifying the presence of the phytoplankton group and relating it to effluent quality parameters can help to understand the ecological dynamics of the system. This study of the phytoplanktonic community in the Brazilian semi-arid region showed that is composed of low diversity / high biomass of potential cyanotoxins producer cyanobacteria and indicating a high environmental impact on the receiving water bodies and a threat to reuse of this treated effluent. Thus, the knowledge of the dominance of cyanobacteria, H_2O_2 could assist in controlling these organisms in stabilization ponds and helping with the management and maintenance operations.

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APPENDIX A: DATA OF EXPLANATORY VARIABLES

Table 8 - Data of explanatory variables (Average of CA, Dens, Chlo.a, Phyc and Pheo).

CA (Week)	McT ($\mu g.L^{-1}$)	Dens ($Cells.mL^{-1}$)	Chlo.a ($mg.L^{-1}$)	Phyc ($mg.L^{-1}$)	Pheo ($mg.L^{-1}$)
1	27,58	1,00E+06	0,08	0,77	0,33
1	27,31	1,10E+06	0,11	0,76	0,29
1	27,74	1,01E+06	0,14	0,75	0,25
2	37,67	1,65E+06	0,40	1,42	0,22
2	37,93	1,65E+06	0,41	1,42	0,21
2	37,64	1,47E+06	0,41	1,41	0,20
3	46,17	1,89E+06	0,60	0,86	0,16
3	47,12	2,13E+06	0,62	0,85	0,14
3	46,30	1,92E+06	0,63	0,84	0,13
4	82,67	2,47E+06	0,66	1,46	0,08
4	82,23	2,32E+06	0,64	1,45	0,09
4	80,63	2,45E+06	0,63	1,43	0,10
5	73,92	2,38E+06	0,78	2,62	0,20
5	71,97	2,50E+06	0,79	2,62	0,19
5	72,74	2,24E+06	0,80	2,61	0,18

- CA: Cell Age (Week)
- McT: Total Microcystin ($\mu g.L^{-1}$)
- Dens: Density ($Cells.mL^{-1}$)
- Chlo.a: Chlorophyll ($mg.L^{-1}$)
- Phyc: Phycocyanin ($mg.L^{-1}$)
- Pheo: Pheophytin ($mg.L^{-1}$)

APPENDIX B: DATA OF CYANOBACTERIA AND PARAMETERS OF THE STUDIES WTPs

Table A: List of cyanobacteria taxa identified in studied WTPs in 2014

2014				
- WTP Catarina				
Táxons	Pi	Pi*log(Pi,10)	Diversity	Equitability
Sphaerocavum brasiliensis	0.99715	-0.00124	0.00124	0.00015
Pseudanabaenacea	0.00096	-0.00290		
Radiococcaceae	0.00070	-0.00220		
Merismopedia tenuissima	0.00048	-0.00159		
Micractinium pusillum	0.00046	-0.00154		
Chlorococales 1	0.00005	-0.00022		
Merismopedia cf. punctata	0.00004	-0.00020		
Closteriopsis longissima	0.00003	-0.00013		
Monoraphidium griffithii	0.00003	-0.00012		
Monoraphidium sp	0.00003	-0.00012		
Pseudanabaena sp1	0.00001	-0.00007		
Chlorococales 2	0.00001	-0.00007		
Scenedesmus dimorphus	0.00001	-0.00007		
Chlorogonium cf. fusiforme	0.00001	-0.00006		
Bacilariophyta ND	0.00001	-0.00005		
Pennales	0.00001	-0.00004		
- WTP Russas				
Táxons	Pi	Pi*log(Pi,10)	Diversity	Equitability
Planktothrix agardhii	0.24373	-0.14943	0.14943	0.02658
Planktothrix sp	0.22418	-0.14558		
Planktothrix isothrix	0.13425	-0.11708		
Merismopedia tenuissima	0.10079	-0.10045		
Sphaerocavum brasiliensis	0.09124	-0.09487		
Pseudanabaenacea	0.08263	-0.08948		
Merismopedia sp	0.07056	-0.08124		
Monoraphidium griffithii	0.01605	-0.02881		
Crococales	0.00632	-0.01390		
Pseudanabaena sp	0.00478	-0.01109		
Nostocales	0.00434	-0.01026		
Planktolyngbya sp	0.00391	-0.00941		
Monoraphidium sp	0.00365	-0.00890		
Actinastrum sp	0.00339	-0.00837		
Chlorococales	0.00311	-0.00779		
Romeria sp	0.00261	-0.00674		
Phacus sp	0.00202	-0.00544		

Naviculaceae.1	0.00061	-0.00196		
Monoraphidium contortum	0.00054	-0.00177		
Tetrastrum sp	0.00030	-0.00107		
Closteriopsis acicularis	0.00022	-0.00080		
Naviculaceae.2	0.00022	-0.00080		
Chlorophyta ND	0.00022	-0.00080		
Euglena texta	0.00013	-0.00051		
Phacus tortus	0.00009	-0.00035		
Nitzschia cf palea	0.00009	-0.00035		
Euglena sp	0.00004	-0.00019		
- WTP Itapipoca				
Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Planktothrix agardhii	0.52743	-0.14654	0.14654	0.02441
Planktothrix sp	0.30301	-0.15712		
Planktothrix isothrix	0.15473	-0.12540		
Chlorococcales 1	0.00578	-0.01294		
Oscillatoriales	0.00390	-0.00939		
Crococales	0.00185	-0.00505		
Ankistrodesmus cf acicularis	0.00084	-0.00258		
Monoraphidium sp	0.00071	-0.00223		
Monoraphidium griffithii	0.00061	-0.00197		
Micractinium sp	0.00051	-0.00168		
Tetrastrum sp	0.00035	-0.00120		
Closteriopsis longissima	0.00025	-0.00089		
Monoraphidium contortum	0.00004	-0.00019		
- WTP C. Sales				
Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Microcystis aeruginosa	0.54652	-0.14340	0.14340	0.02253
Planktothrix isothrix	0.12815	-0.11435		
Planktothrix sp	0.12564	-0.11319		
Planktothrix agardhii	0.11513	-0.10809		
Sphaerocavum brasiliensis	0.06683	-0.07853		
Arthrospira platensis	0.00909	-0.01856		
Merismopedia tenuissima	0.00378	-0.00916		
Pseudanabaenacea	0.00189	-0.00515		
Dictyosphaerium pulchellum	0.00101	-0.00302		
Pseudanabaena cf catenata	0.00095	-0.00286		
Tetrastrum sp	0.00019	-0.00070		
Pseudanabaena sp	0.00019	-0.00070		
Nostocales	0.00019	-0.00070		
Closteriopsis longissima	0.00018	-0.00068		
Cyclotella cf meneghiniana	0.00006	-0.00026		

Monoraphidium griffithii	0.00006	-0.00026		
Monoraphidium sp	0.00005	-0.00021		
Chlorococcales 1	0.00003	-0.00014		
Gomphonema parvulum	0.00002	-0.00009		
Cyclotella sp.	0.00001	-0.00005		
Naviculaceae	0.00001	-0.00005		
Cryptophyta ND	0.00001	-0.00005		
- WTP Revoltosos				
Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Planktothrix sp	0.49127	-0.15165	0.15165	0.02367
Planktothrix agardhii	0.31169	-0.15780		
Planktothrix isothrix	0.14623	-0.12210		
Pseudanabaenacea	0.02024	-0.03428		
Merismopedia tenuissima	0.01254	-0.02385		
Microcystis aeruginosa	0.00966	-0.01947		
Spirulina sp.	0.00342	-0.00843		
Pseudanabaena sp	0.00234	-0.00615		
Monoraphidium griffithii	0.00183	-0.00501		
Eunotiaceae	0.00061	-0.00195		
Aulacoseira granulata	0.00013	-0.00050		
Monoraphidium contortum	0.00004	-0.00019		

Table B: List of cyanobacteria taxa identified in studied ETEs in 2015

2015				
- WTP Catarina				
Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Sphaerocavum brasiliensis	0.97016	-0.01276	0.01276	0.00190
Pseudanabaenacea	0.01649	-0.02940		
Planktothrix agardhii	0.00661	-0.01440		
Merismopedia tenuissima	0.00276	-0.00707		
Geitlerinema cf. amphibium	0.00130	-0.00374		
Closteriopsis longissima	0.00090	-0.00274		
Monoraphidium griffithii	0.00073	-0.00230		
Micractinium pusillum	0.00026	-0.00093		
Chlorococcales 1	0.00017	-0.00065		
Monoraphidium contortum	0.00017	-0.00065		
Desmodesmus sp	0.00014	-0.00055		
Naviculaceae	0.00012	-0.00045		
Nitzschia palea	0.00011	-0.00043		
Chlorococcales 2	0.00004	-0.00019		
Gomphonema parvulum	0.00004	-0.00016		
- WTP Russas				

Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Pseudanabaenacea	0.54310	-0.14399	0.14399	0.02706
Merismopedia tenuissima	0.10732	-0.10403		
Planktothrix agardhii	0.08619	-0.09175		
Monoraphidium griffithii	0.08480	-0.09087		
Planktothrix isothrix	0.05315	-0.06774		
Monoraphidium sp	0.04266	-0.05845		
Oscillatoriales	0.03752	-0.05349		
Planktothrix sp	0.02027	-0.03432		
Didymocystis sp	0.00568	-0.01276		
Romeria sp	0.00313	-0.00784		
Phacus longicauda	0.00284	-0.00724		
Euglena sp	0.00284	-0.00724		
Lepocinclis texta	0.00284	-0.00724		
Naviculaceae.1	0.00278	-0.00710		
Actinastrum sp	0.00261	-0.00673		
Geitlerinema cf. amphibium	0.00150	-0.00424		
Actinastrum hantzschii	0.00047	-0.00156		
Chlorococales	0.00014	-0.00055		
Closteriopsis longissima	0.00014	-0.00055		
Nitzschia cf palea	0.00001	-0.00005		
- WTP Itapipoca				
Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Planktothrix agardhii	0.45526	-0.15558	0.15558	0.02650
Chlorococales 1	0.33382	-0.15906		
Pseudanabaenacea	0.10328	-0.10183		
Planktothrix sp	0.06103	-0.07412		
Planktothrix isothrix	0.02440	-0.03935		
Euglena sp	0.00810	-0.01694		
Pseudanabaena sp	0.00638	-0.01401		
Monoraphidium griffithii	0.00497	-0.01145		
Tetrastrum sp	0.00098	-0.00295		
Closteriopsis longissima	0.00089	-0.00270		
Monoraphidium sp	0.00089	-0.00270		
Dictyosphaerium sp.	0.00000	-0.00002		
- WTP C. Sales				
Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Planktothrix agardhii	0.42917	-0.15766	0.15766	0.02597
Sphaerocavum brasiliensis	0.26442	-0.15276		
Planktothrix sp	0.11127	-0.10611		
Planktothrix isothrix	0.09009	-0.09417		
Microcystis aeruginosa	0.07826	-0.08659		

Pseudanabaenacea	0.02300	-0.03768		
Actinastrum sp	0.00112	-0.00331		
Merismopedia tenuissima	0.00075	-0.00234		
Radiococcaceae	0.00056	-0.00182		
Chlorococales 1	0.00049	-0.00162		
Closteriopsis longissima	0.00033	-0.00114		
Monoraphidium griffithii	0.00021	-0.00077		
Monoraphidium sp	0.00009	-0.00038		
Chryptophyta ND	0.00009	-0.00038		
Chlorococales 2	0.00007	-0.00029		
Euglena sp	0.00007	-0.00029		
- WTP Revoltosos				
Táxons	Pi	Pi*log(Pi.10)	Diversity	Equitability
Planktothrix agardhii	0.71782	-0.10336	0.10336	0.01603
Planktothrix isothrix	0.12423	-0.11252		
Planktothrix sp	0.10400	-0.10223		
Pseudanabaenacea	0.05200	-0.06677		
Merismopedia tenuissima	0.00078	-0.00243		
Monoraphidium griffithii	0.00054	-0.00176		
Pseudanabaena sp	0.00029	-0.00104		
Dictyosphaerium sp	0.00020	-0.00073		
Closteriopsis longissima	0.00005	-0.00021		
Phacus tortus	0.00005	-0.00021		
Euglena sp	0.00005	-0.00021		

Table C: List of parameter data of the studied WTPs

- WTP Catarina								
Parameters	Setember/ 2014	October/ 2014	November/ 2014	December/ 2014	January/ 2015	February/ 2015	March/ 2015	April/ 2015
Total Phosphorus (mg P-PO_4^{3-}/L)	10.64	11.82	14.34	7.00	7.00	12.34	7.00	8.04
Conductivity (µS/cm)	2203.00	2048.00	2306.00	2383.00	2204.00	2091.00	1821	1791
Nitrate (mg N-NO^{3-}/L)	0.09	0.11	0.09	0.07	0.81	0.65	0.28	0.08
Ammonia (mg N-NH_3/L)	10.75	10.30	16.08	11.27	12.10	11.01	11.92	11.14
Density (cells/mL)	7212576.42	378279475.99	2972342.91	7863202.32	10061070.26	8513828.23	10250655.01	1465502.19
Rainfall (mm)	3.50	13.20	10.50	36.50	93.20	97.30	148.30	133.50
Wind (m/s)	7.58	7.42	6.25	6.61	5.89	5.53	5.00	4.89
Temperature (ºC)	37.00	38.00	37.00	36.00	34.00	33.00	32.00	30.00
Sphaerocavum brasiliensis (cells/mL)	6977030.57	378198689.96	2181950.51	7563202.32	8061070.26	8335516.74	9984279.48	1306550.22
- WTP Russas								
Parameters	Setember/ 2014	October/ 2014	November/ 2014	December/ 2014	January/ 2015	February/ 2015	March/ 2015	April/ 2015
Total Phosphorus (mg P-PO_4^{3-}/L)	3.00	9.75	10.28	3.00	3.00	3.00	3.00	3.00
Conductivity (µS/cm)	1889	1832	1639	1712	1689	1671	1658	1645
Nitrate (mg N-NO^{3-}/L)	0.28	0.42	0.15	0.28	0.35	0.22	0.27	0.27
Ammonia (mg N-NH_3/L)	5.53	5.31	5.74	5.53	5.63	5.42	5.52	5.52

Density (cells/mL)	541994.18	470524.01	259825.33	399563.32	169432.31	154844.80	94494.94	419213.99
Rainfall (mm)	2.90	6.20	2.00	17.50	71.50	114.00	226.60	179.00
Wind (m/s)	7.56	8.14	8.08	7.56	6.50	5.69	4.94	4.83
Temperature (ºC)	36.00	36.00	36.00	36.00	35.00	34.00	33.00	32.00
Plankthothrix agardhii (cells/mL)	113537.1179	65502	32751.1	196506.55	140432.31	72229.43	90494.94	410213.99
- WTP Itapipoca								
Parameters	Setember/ 2014	October/ 2014	November/ 2014	December/ 2014	January/ 2015	February/ 2015	March/ 2015	April/ 2015
Total Phosphorus (mg P-PO_4^{3-}/L)	3.00	7.89	9.12	9.24	3.00	3.00	3.00	3.00
Conductivity (µS/cm)	1822	1818	1708	1802	1689	1671	1658	1645
Nitrate (mg N-NO^{3-}/L)	0.11	0.27	0.37	1.59	0.59	0.32	0.68	0.23
Ammonia (mg N-NH_3/L)	7.37	3.69	11.79	6.93	7.45	7.15	8.48	6.12
Density (cells/mL)	499272.20	841048.03	947423.57	1747717.34	797935.69	944819.36	594614.27	627373.36
Rainfall (mm)	4.00	2.60	6.00	26.70	121.00	172.50	335.20	314.60
Wind (m/s)	3.80	2.80	2.10	0.50	0.30	0.10	0.30	0.10
Temperature (ºC)	24.90	25.20	24.90	24.50	31.70	27.80	27.60	0.00
Plankthothrix agardhii (cells/mL)	97611.10	393013.10	565938.86	1071853.91	674870.98	674870.98	494614.27	607373.36
- WTP C. Sales								
Parameters	Setember/ 2014	October/ 2014	November/ 2014	December/ 2014	January/ 2015	February/ 2015	March/ 2015	April/ 2015
Total Phosphorus (mg P-PO_4^{3-}/L)	6.03	5.70	4.14	4.00	4.00	6.24	6.18	6.04

Conductivity (µS/cm)	2750.00	3070.00	3196.00	2853.00	2848.00	2843.00	2685.00	2179.00
Nitrate (mg N-NO^{3-}/L)	4.57	3.02	2.03	0.37	1.77	0.09	2.28	0.07
Ammonia (mg N-NH_3/L)	8.95	4.29	1.58	6.49	5.33	5.33	6.49	4.29
Density (cells/mL)	718670.61	2044541.48	1934788.94	4541484.71	1707666.21	1737461.97	2066516.69	913501.82
Rainfall (mm)	5.40	48.50	82.00	47.00	96.60	109.40	290.00	114.90
Wind (m/s)	7.33	7.33	6.00	4.00	4.33	5.00	5.00	3.67
Temperature (ºC)	25.30	27.96	27.82	27.08	27.36	23.82	26.16	24.96
Microcystis aeruginosa (cells/mL)	71152.82	1044541.48	1092867.54	2841048.03	1657666.21	39698.29	157028.80	172467.03
- WTP Revoltosos								
Parameters	Setember/ 2014	October/ 2014	November/ 2014	December/ 2014	January/ 2015	February/ 2015	March/ 2015	April/ 2015
Total Phosphorus (mg P-PO_4^{3-}/L)	1.00	1.00	1.00	11.14	1.00	1.00	1.00	1.00
Conductivity (µS/cm)	2061.00	2166.00	2090.00	2014.00	2095.00	2073.00	1938.00	1741.00
Nitrate (mg N-NO^{3-}/L)	0.07	4.85	0.16	0.02	1.27	0.12	1.33	0.06
Ammonia (mg N-NH_3/L)	0.17	2.10	1.31	0.99	1.19	1.31	1.71	0.74
Density (cells/mL)	2734497.82	2320087.34	2777292.58	2381368.26	2564956.33	2771288.21	2993995.63	2838973.80
Rainfall (mm)	2.90	15.60	61.00	33.00	106.00	127.40	213.00	160.10
Wind (m/s)	2.60	3.70	3.60	3.6	2.80	4.20	2.40	2.50
Temperature (ºC)	29.14	30.52	0.00	30.32	29.52	30.14	27.60	27.06

Plankthothrix agardhii (cells/mL)	2704497.82	1021834.06	1244541.48	917030.57	2087882.1	2171397.38	2171397.38	1586790.39