CARLOS YURE BARBOSA DE OLIVEIRA

MICROALGAS DO SEMIÁRIDO: FLORAÇÕES NOCIVAS, VARIABILIDADE SAZONAL E SUAS POSSÍVEIS APLICAÇÕES BIOTECNOLÓGICAS

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Microalga do Semiárido: florações nocivas, variabilidade sazonal e suas possíveis aplicações biotecnológicas

Carlos Yure Barbosa de Oliveira

Trabalho de conclusão de curso apresentado ao curso de Engenharia de Pesca da Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada como requisito para obtenção do título de Engenheiro de Pesca.

Profa. Dra.DANIELLI MATIAS DE MACEDO DANTAS
Orientadora

Prof. Dr. ALFREDO OLIVERA GALVEZ
Co-orientador

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Trabalho de conclusão de curso julgado adequado para obtenção do título de Engenheiro de Pesca. Defendido e aprovado em 18 de Julho de 2018 pela seguinte Banca Examinadora.

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Prof[a]. Dr[a]. Danielli Matias de Macedo Dantas - Orientadora
[Unidade Acadêmica de Serra Talhada/ Universidade Federal Rural de Pernambuco]

____

Prof[a]. Dr[a]. Girlene Fábia Segundo Viana – Membro Interno
[Unidade Acadêmica de Serra Talhada/ Universidade Federal Rural de Pernambuco]

____

Prof. M. Sc. Silvano Lima do Nascimento Filho – Membro Externo
[Unidade Acadêmica de Serra Talhada/ Universidade Federal Rural de Pernambuco]

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Prof. Dr. Dráusio Pinheiro Veras - Membro Interno (suplente)
[Unidade Acadêmica de Serra Talhada/ Universidade Federal Rural de Pernambuco]

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Resumo

Microalgas e cianobactérias, são organismos eucariontes e procariontes, respectivamente, fotossintetizantes e utilizam CO_2 atmosférico, luminosidade e compostos inorgânicos para o seu crescimento. Além disso, produzem compostos nutricionais, bioativos e algumas espécies podem produzir toxinas (como algumas espécies de cianobactérias e dinoflagelados). O presente estudo teve como objetivo identificar e isolar espécies da comunidade fitoplanctônica de reservatórios localizados no semiárido Pernambucano e posteriormente, avaliar o potencial biotecnológico de microrganismos previamente isolados do banco de cepas do Laboratório de Produção de Alimento Vivo (LAPAVI) – UFRPE (Sede). Para a coleta desses organismos foi utilizada uma rede com malha de 20µm, sendo parte do material coletado fixado em formol à 4% e o restante submetido à cultura. Após a triagem de todo o material coletado foi efetuada a análise de correspondência canônica, para avaliar possíveis interações entre os parâmetros abióticos e as espécies encontradas. A avaliação de potencial biotecnológico ocorreu pelo teste de capacidade de biorremediação da *Chlorella vulgaris* em efluente de um sistema bioflocos (BFT). Foram registrados 21 táxons, sendo a maioria destes da classe Chlorophyceae. Dentre as espécies encontrados, destaca-se a presença de *Ceratium furcoides*, um dinoflagelado exótico que encontra-se em uma crescente expansão geográfica no Brasil. Na avaliação do potencial de biorremediação, a presença da *Chlorella vulgaris* promoveu redução nos níveis de compostos nitrogenados e fosfatados do efluente BFT de aproximadamente, 79,2% e 48%, respectivamente. Além da capacidade de remoção destes compostos, efluente BFT apresentou como meio potencial para o cultivo desta microalga.

Palavras-chave: Fitoplâncton; isolamento; correspondência canônica; biorremediação.

Abstract

Microalgae and cyanobacteria are eukaryotes and prokaryotes, respectively, photosynthetic and use atmospheric CO2, light and inorganic compounds for their growth. In addition, they produce nutritional, bioactive compounds and some species may produce toxins (like some species of cyanobacteria and dinoflagellates). The present study aimed to identify and isolate species from the phytoplankton community of reservoirs located in the semiarid Pernambucano and later to evaluate the biotechnological potential of microorganisms previously isolated from the center of culture of the Laboratório de Produção de Alimento Vivo (LAPVI-UFRPE/Sede). For the collection of these organisms a network with a mesh of 20μm was used, being part of the collected material fixed in formaldehyde to 4% and the rest submitted to the culture. After the sorting of all collected material, the canonical correspondence analysis was performed to evaluate possible interactions between the abiotic parameters and the species found. The evaluation of biotechnological potential occurred by the bioremediation capacity test of *Chlorella vulgaris* in effluent from a biofloc system (BFT). Twenty-one taxa were recorded, most of them of the class Chlorophyceae. Among the species found, we highlight the presence of *Ceratium furcoides*, an exotic dinoflagellate that is found in a growing geographic expansion in Brazil. In the evaluation of bioremediation potential, the presence of Chlorella vulgaris promoted a reduction in the levels of nitrogenous and phosphate compounds of the BFT effluent of approximately, 79.2% and 48%, respectively. In addition to the removal capacity of these compounds, BFT effluent presented as potential medium for the cultivation of this microalgae

Keywords: phytoplankton; isolation; canonical correspondence; bioremediation.

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Sumário

Apresentação

O presente trabalho de conclusão de curso (TCC) de Engenharia de Pesca é composto por quatro artigos científicos visando contribuir para o enriquecimento do conhecimento sobre as microalgas do Semiárido. O primeiro artigo teve como objetivo descrever e isolar espécies da comunidade fitoplanctônica de quatro reservatórios da região Semiárida, este artigo, encontra-se em avaliação na Revista Brasileira de Engenharia de Pesca.

O segundo artigo objetivou descrever espécies de microalgas e cianobactérias e correlacionar a parâmetros abióticos de dois reservatórios ao longo de oito meses, este, será submetido a revista Algal research. O terceiro artigo trata pontualmente das florações do dinoflageado exótico *Ceratium furcoides* no reservatório Cachoeira II, pretende-se submetê-lo a revista International Journal of Aquatic Biology. Por fim, o quarto artigo, objetivou avaliar o potencial de biorremediação da microalga *Chlorella vulgaris* de um efluente de sistema biofloco, utilizado no cultivo da Tilápia-do-nilo, *Oreochromis niloticos*, que será submetido a revista Engenharia Sanitária e Ambiental.

2 Revisão de literatura

2.1 Corpos aquáticos na região semiárida

A construção de reservatórios no Brasil, iniciou por volta de 1900 para atender finalidades específicas, como abastecimento de água, geração de energia, propiciar fonte de renda através da introdução de organismos de valor comercial (Nogueira et al., 1999). Na região Sudeste, a maioria dos reservatórios está direcionada a geração de energia elétrica, devido à alta demanda por atividades industriais e densidade populacional, enquanto no Nordeste, a grande maioria é destinada ao combate das secas, devido aos longos períodos de estiagem (Maia, 1998).

A limnologia é uma ciência dentro da ecologia, destinada ao estudo dos corpos aquáticos continentais, que se subdivide em duas partes: biótica, voltada ao conhecimento dos organismos vivos, tais como o plâncton, macrófitas, peixes, dentre outros e; abiótica, direcionada as variações dos parâmetros físicos e químicos do meio, no qual os organismos vivos estão submetidos (Esteves, 2011).

No Brasil, a quantidade de estudos realizados em águas continentais tem aumentado gradativamente, entretanto, esses ainda podem ser considerados insuficientes para avaliar a dinâmica dos ecossistemas aquáticos dulciaquícolas, diante dos agravantes do aquecimento global, provocado pelos gases do efeito estufa (Pompêo, 2011).

A região Semiárida é caracterizada pela escassez hídrica, onde os reservatórios artificiais compõem uma importante fonte de recursos ecológicos e sociais (Machado, 2003; Brito et al., 2016). As externalidades provocadas pelas atividades antrópicas são um dos principais responsáveis pela degradação ambiental dos ecossistemas aquáticos (Callisto et al., 2002). Isso, implica negativamente na disponibilidade do recurso para o abastecimento doméstico, manutenção e diversidade aquática e outros (Lodi et al., 2011).

2.2 Diversidade fitoplanctônica

As microalgas são organismos eucariontes e fotossintéticos, que utilizam o CO_2 atmosférico, luminosidade e compostos inorgânicos para o seu crescimento. Além disso, produzem compostos nutricionais e bioativos como carotenóides, proteínas, ácidos graxos, antioxidantes naturais além de serem utilizadas como alimento para animais bem como potencial fonte de biocombustíveis de terceira geração (Borowitzka, 1992; Singh & Gu, 2010; Shuba & Kifle, 2018).

As cianobactérias, que antes integravam o termo não taxonômico supracitado, são bactérias similares às algas, mas com algumas adaptações que podem favorecer seu floramento, mesmo em condições adversas, como a capacidade de absorver o nitrogênio atmosférico, resistência a elevadas temperaturas e níveis de matéria orgânica dissolvida (Lezcano et al., 2018).

O monitoramento de mananciais utilizados para abastecimento e atividades recreativas também se faz necessário devido ao floramento das cianobactérias, atores da eutrofização, potenciais produtores de toxinas (Lezcano et al., 2018). As cianotoxinas são, em sua maioria, compostas por aminoácidos que resultam em alterações na biota aquática como também potentes hepatotóxicos, neurotóxicos e dermatotóxicos, podendo provocar ainda a inibição geral de síntese proteica em mamíferos (Sivonen & Jones, 1999; Galanti et al., 2013; Gibble et al., 2016). Adicionalmente, a possibilidade de bioacumulação das cianotoxinas, em especial às microcistinas (MC), em peixes (Deblois & Juneau, 2010; Amé et al., 2010; Ferrão-Filho & Kozlowsky-Suzuki, 2011) e outros organismos aquáticos cultiváveis, como crustáceos (Chen & Xie, 2005; Oberhaus et al., 2007) e moluscos (Svensen et al., 2005; Strogyloudi et al., 2006) podem ocasionar graves problemas de saúde pública por sua ingestão despercebida.

Além da disponibilidade de nutrientes, alguns parâmetros físico-químicos influenciam na dinâmica desses microorganismos, como temperatura, pH, condutividade e salinidade (Dokulil & Teubner 2000; Heisler et al., 2008) e a correlação desses parâmetros com tais florações é de suma importância para diminuir ou sanar as influencias causadores. Possivelmente o parâmetro físico de temperatura é o mais preocupante por estar ligado diretamente com a floração das cianobactérias, uma vez que diante do atual cenário de elevação da temperatura global e o aumento da produção de efluentes, a ocorrência de florações deve se tornar cada vez mais constante (Jacoby et al., 2000; Dolman et al., 2012; Harke et al., 2016).

2.3 Bioprospecção de cepas algais

Adicionalmente, a biotecnologia das microalgas vem sendo desenvolvida para elaboração de produtos com diversas aplicações comerciais (alimentos, nutracêuticos, medicamentos, biocombustíveis, dentre outros) (Singh & Gu, 2010, Concas et al., 2013 Shuba & Kifle, 2018). Entretanto, para as aplicações supracitadas, é necessário que essas microalgas, estejam isoladas, preferencialmente de águas não poluídas, a fim de evitar linhagens geneticamente adaptadas. (Pfleeger et al., 1991; Nascimento et al., 2002; Saranya et al., 2015).

A prospecção de cepas algais nativas contribui diretamente para essas demandas bem como para às mais diversas aplicações, dentre elas o tratamento de efluentes e a alimentação de animais com alto valor comercial (Bhatt et al., 2014).

Apesar de serem consideradas fontes energéticas promissoras, existem alguns entraves para a viabilidade da produção de biomassa microalgal, como a identificação de cepas nativas ricas em ácidos graxos, cepas que apresentem crescimento rápido e ainda, espécies que possuam alta resiliência ambiental para adaptação aos parâmetros de cultivos (Mendes et al., 2012).

Ainda é relativamente baixo o número de bancos de cepas algais dulcícolas na região Semiárida (Mendes et al., 2012). Adicionalmente, a formação de um banco de cepas nessa região, contribui para o conhecimento e manutenção da biodiversidade local, tendo em vista que se trata de um ecossistema diferenciado e ainda pouco estudado, sobretudo quando se refere a comunidade fitoplanctônica (Araújo et al., 2008).

3 Artigo científico

3.1 Artigo científico I

PROSPECTING THE FIRST CULTURE COLLECTION OF ALGAE OF THE PERNAMBUCO SEMIARID, BRAZIL

Carlos Yure Barbosa de OLIVEIRA[1]*, Ayanne Jamyres Gomes da Silva ALMEIDA[1], Marília de Viveiros e SILVA[1], Ivanilson de Lima SANTOS[1] & Danielli Matias de Macedo DANTAS[1]

[1] Laboratório de Biotecnologia de Microalgas (LABIM) Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada (UAST). Avenida Gregório Ferraz Nogueira, S/N, Bairro: José Tomé de Souza Ramos, CEP: 56909-535 Serra Talhada-PE, Brasil.

*E-mail: yureboliveira@gmail.com

Abstract - The growing concern about the shortage of fossil fuels drives the search for new sources of sustainable energy. Microalgae are considered promising in this area, since they are sources of third generation biofuels and protein. The present study aimed at make comented list and isolating species from the phytoplankton communities of four reservoirs in the Pernambuco semiarid, making them available for experiments that can evaluate the biotechnological potential and meet the demands of the region. The collections occurred in water bodies located in the Pernambuco semiarid and the identification of the taxa occurred based on morphological characters using specific literature. A total of twenty-one taxa were identified, eight of them Cyanophyceae (*Anabaena* sp., *Aphanocapsa elachista*, *Chroococcus dispersus*, *Dolichospermum* sp., *Merismopedia* sp., *Microcystis aeruginosa*, *Pseudanabaena limnetica*, *Synechococcus* sp.), ten Chlorophyceae (*Chlorella* sp., *Cosmarium bioculatum*, *Desmodesmus* spp., *Dictyosphaerium* sp., *Pediastrum duplex*, *Pediastrum simplex*, *Pseudokirchineriella subcapitata*, *Scenedesmus* sp., *Staurastrum leptocladum*, *Trochiscia* sp) and only one of the following classes Bacillariophyceae (*Aulacoseira granulata*), Euglenophyceae (*Trachelomonas volvocida*) and Dinophyceae (*Ceratium furcoides*). The bioprospection of microalgae in the semiarid region of Pernambuco has made possible the formation of a bank of strains, important for fictional studies and obtaining biomass of species with biotechnological potential.

Key-words: Biotechnology; microalgae; isolation.

PROSPECÇÃO DO PRIMEIRO BANCO DE CEPAS ALGAIS DO SEMIARIDO PERNAMBUCANO, BRASIL

Resumo - A crescente preocupação relacionada à escassez de combustíveis fósseis impulsiona a busca por novas fontes de energias sustentáveis. As microalgas são consideradas promissoras nessa vertente, pois são fontes de biocombustíveis de terceira geração e de proteína. O presente trabalho objetivou fazer uma lista comentada e isolar espécies das comunidades fitoplanctônicas de quatro reservatórios no semiárido Pernambucano, disponibilizando-os para experimentos que possam avaliar o potencial biotecnológico e suprir as demandas da região. As coletas ocorreram em corpos de água localizados no semiárido Pernambucano e a identificação dos táxons ocorreu com base em caracteres morfológicos utilizando literatura específica. Foram identificados 21 táxons, sendo oito da classe Cyanophyceae (*Anabaena* sp., *Aphanocapsa elachista*, *Chroococcus dispersus*, *Dolichospermum* sp., *Merismopedia* sp., *Microcystis aeruginosa*, *Pseudanabaena limnetica*, *Synechococcus* sp.), dez Chlorophyceae (*Chlorella* sp., *Cosmarium bioculatum*, *Desmodesmus* spp., *Dictyosphaerium* sp., *Pediastrum duplex*, *Pediastrum simplex*, *Pseudokirchineriella subcapitata*, *Scenedesmus* sp., *Staurastrum leptocladum*, *Trochiscia* sp) e apenas uma das seguintes classes Bacillariophyceae (*Aulacoseira granulata*), Euglenophyceae (*Trachelomonas volvocida*) e Dinophyceae (*Ceratium furcoides*). A bioprospecção de microalgas no semiárido pernambucano tem possibilitado a formação de um banco de cepas, importante para estudos ficológicos e obtenção de biomassa de espécies com potencial biotecnológico.

Palavras-chave: Biotecnologia; microalgas; isolamento

INTRODUCTION

The phytoplankton organisms play an important role in aquatic ecosystems, mainly by the primary production capacity in the food chain. Microalgae are also potentially used as bioindicators of water quality, mainly because they have a short life cycle and respond quickly to the dynamics of the ecosystem in which they are (Singh & Gu, 2010).

Additionally, microalgae biotechnology has been developed to produce products with various commercial applications (food, nutraceuticals, medicines, biofuels, etc.) (Concas et al., 2013 Shuba & Kifle, 2018). However, for the aforementioned applications, these microalgae must be isolated, preferably from unpolluted waters, in order to avoid genetically adapted lineages (Pfleeger et al., 1991; Nascimento et al., 2002; Saranya et al., 2015).

In Brazil, the number of studies carried out in continental aquatic environments, aiming at the prospection of algal strains, has increased considerably in the last years, however, the majority are directed towards the South and Southeast regions (Pompêo, 2011, Moura et al., 2012), with little literature on this subject in reservoirs in the Brazilian semiarid region.

The bioprospecting of native algal strains contributes directly to these demands as well as to the most diverse applications, among them the treatment of effluents and the feeding of animals with high commercial value (Mata et al., 2010; Bhatt et al., 2014). The formation of a bank of strains contributes to the knowledge and maintenance of local biodiversity, since in the semiarid region, due to the high evaporation rates and the long periods of drought, many of the reservoirs tend to dry up.

The present study aimed at make comented list and isolating species from the native phytoplankton communities of four reservoirs in the Pernambuco semiarid, making them available for experiments that can evaluate the biotechnological potential and meet the demands of the region.

MATERIAL AND METHODS

The collection of specimens occurred in the second half of 2017 in the reservoirs Cachoeira II (07° 58'23.1 "S 038° 19'24.0" W) (Figure 1-A) and Saco I (07° 56'53.1 "S 038° 16'58.6 "W) (Figure 1-B), both located in Serra Talhada, Pernambuco, Sítio dos Nunes Lake (08° 03'02.3" S 037° 50'15.4 "W) (Figure 1-C) and Varzinha Dam (08° 01'57.3"S 038° 07'11.4"W) (Figure 1-D). In these places, a trawl was carried out using a specific network for phytoplankton collection (20 µm). An aliquot of the samples was cultured in different mediums: Provasoli (McLachlan, 1973), F2 Guillard, Bolds Basal Medium (BBM).

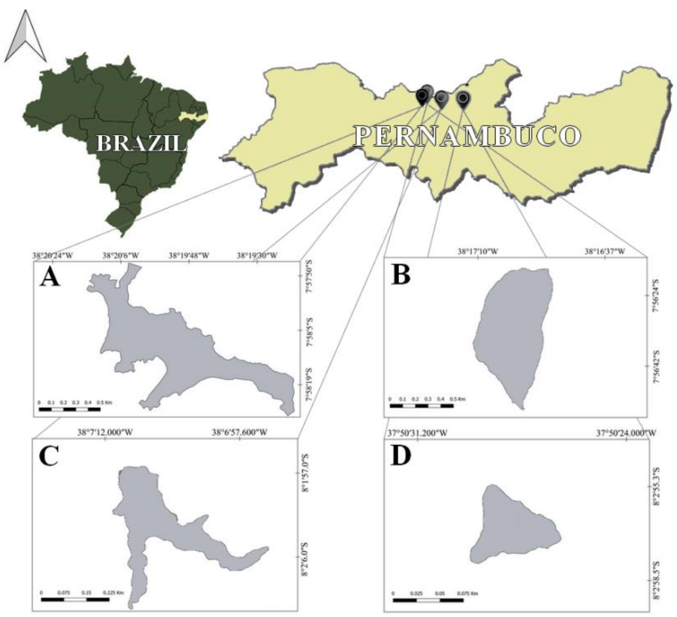


Figure 1. Collection place. A – Cachoeira II Reservoir; B – Saco I Reservoir; C – Sítio dos Nunes Lake, located in district of Sítio dos Nunes, belonging to the Flores city, and; D – Varzinha Dam, located in the Serra Talhada city both in Pernambuco, Brazil.

In order to identify the taxa, they were classified based on morphological characters, with the following references: Comas González (1996), Bicuco & Menezes

(2006), Cybis et al. (2006), Menezes & Bicudo (2008). For this, a Motic® Binocular Optical Microscope model BA300 was used, with a magnification of 100 or 400x.

Subsequently, attempts were initiated to isolate specimens that occurred by serial dilution, micropipation and plating, following the algal isolation protocol of the Microalgae Biotechnology Laboratory (LABIM), belonging to the Federal Rural University of Pernambuco, Unit Academic of Serra Talhada (UFRPE-UAST). These were kept in a refrigerated room at 20 ºC and an integral photoperiod of 270 $\mu E.m^2.s^{-1}$.

RESULTS

Following the chronological order of the study, 21 taxa were identified (Figure 1), eight Cyanophyceae (*Anabaena* sp., *Aphanocapsa elachista, Chroococcus dispersus, Dolichospermum* sp., *Merismopedia* sp., *Microcystis aeruginosa, Pseudanabaena limnetica, Synechococcus* sp.), ten Chlorophyceae (*Chlorella* sp., *Cosmarium bioculatum, Desmodesmus* spp., *Dictyosphaerium* sp., *Pediastrum duplex, Pediastrum simplex, Pseudokirchineriella subcapitata, Scenedesmus* sp., S*taurastrum leptocladum, Trochiscia* sp) and only one of the following classes Bacillariophyceae (*Aulacoseira granulata*), Euglenophyceae (*Trachelomonas volvocida*) and Dinophyceae (*Ceratium furcoides*).

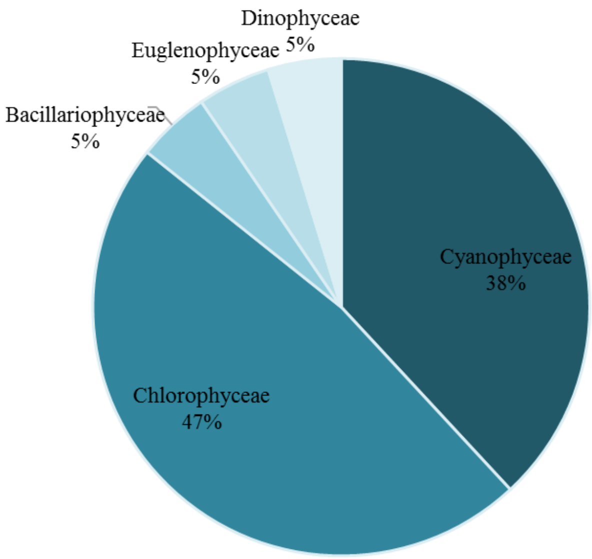


Figure 2. Percentage distribution of the planktonic classes identified in four reservoirs in the Pernambuco semiarid.

Classe Cyanophyceae

Family Nostocaceae

Anabaena sp. (Figure 3-A)

Solitary trichomes, spiral, wrapped in a narrow sheath. Spherical cells with approximate length and diameter of 9 µm and 10 µm, respectively.

Family Merismopediaceae

Aphanocapsa elachista West & G. S. West

Irregular and diffuse colonies surrounded by a mucilaginous sheath. Scattered and irregularly distributed, however, all with a spherical shape and a diameter of approximately 2 µm.

Merismopedia sp.

Tubular colonies consisting of at least 4 cells arranged in rows with a diameter between 1,5 and 3 µm.

Family Chroococcaceae

Chroococcus dispersus (Keissler) Lemmermann

Regular colonies and quadrangles individually surrounded by sheath and spherical shape. Approximate diameter of 4 µm.

Family Aphanizomenonaceae

Dolichospermum sp.

Solitary trichomes, twisted and with a notorious presence of akinetes.

Family Microcystaceae

Microcystis aeruginosa (Kützing) Kützing (Figure 3-B)

Microscopic or macroscopic colonies, elongated, and irregular with dark green cells. Mucilage type hyaline.

Family Pseudoanabaenaceae

Pseudanabaena limnetica (Lemmermann) Komárek

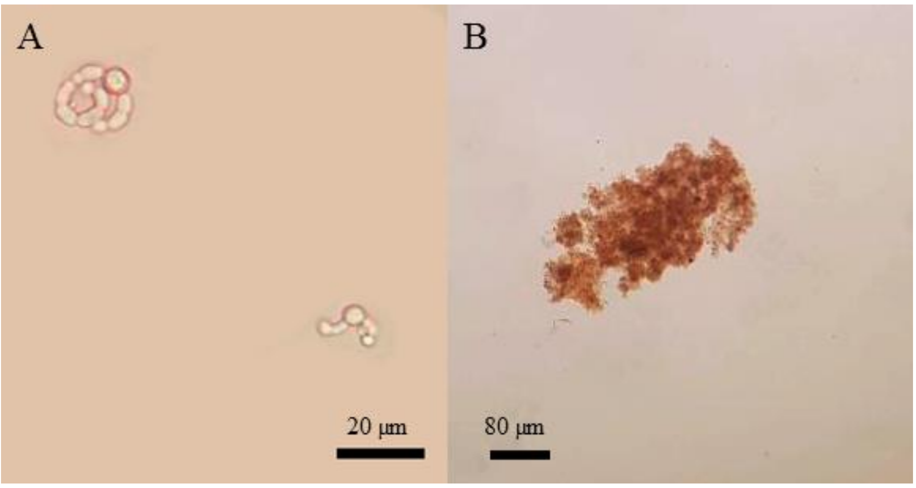
Solitary trichomes, straight, but slightly curved. Cylindrical shape cells with homogeneous cellular content.

Family Synechococcaceae

Synechococcus sp.

Isolated cells, oval or cylindrical without mucilage. With homogeneous cellular content, and approximate diameter of 2 μm.

Figure 3. Optical microscopy of microalgae species of the class Cyanophyceae, presents



in Pernambuco semiarid. A – *Anabaena* sp. and; B – *Microcystis aeruginosa.*

Class Chlorophyceae

Family Oocystaceae

Chlorella sp.

Solitary and free-living individuals. Spherical or ovoid cell with narrow cell wall. Diameter ranging from 1 to 10 μm.

Trochiscia sp. (Figure 4-A)

Solitary and free-living individuals. Spherical cell with relatively thick and ordered cell wall of pointed spines.

Family Desmidiaceae

Cosmarium bioculatum Brébisson ex Ralfs

Cells generally isolated, most often slightly elongated.

Staurastrum leptocladum Nordstedt (Figure 4-B)

Solitary cells with large variation in size. They have vertical and radial symmetry.

Family Scenedesmaceae

Desmodesmus spp.

Solitary individuals, but usually with habit to form colonies of 2, 4 or 8 cells arranged horizontally. The cells may be ellipsoids or ovoid.

Family Dictyosphaeriaceae

Dictyosphaerium sp.

Free-living colonies formed by four groups of four cells each. The cells have formed spherical or ellipsoidal.

Family Hydrodictyaceae

Pediastrum simplex Meyen (Figure 4-C)

Flat cenobios, hollow centered, with cell numbers of two multiple. Cells are triangle-shaped.

Pediastrum duplex Meyen (Figure 4-D)

Flat cenobios with cells numbers of two multiple. The cells are roughly shaped like a little flag.

Family Selenastraceae

Raphidocelis subcapitata (Korshikov) Nygaard, Komáred, J.Kristiansen & O.M.Skulberg (Figure 4-E)

Lone and half-moon shaped cells with one pointed and one rounded.

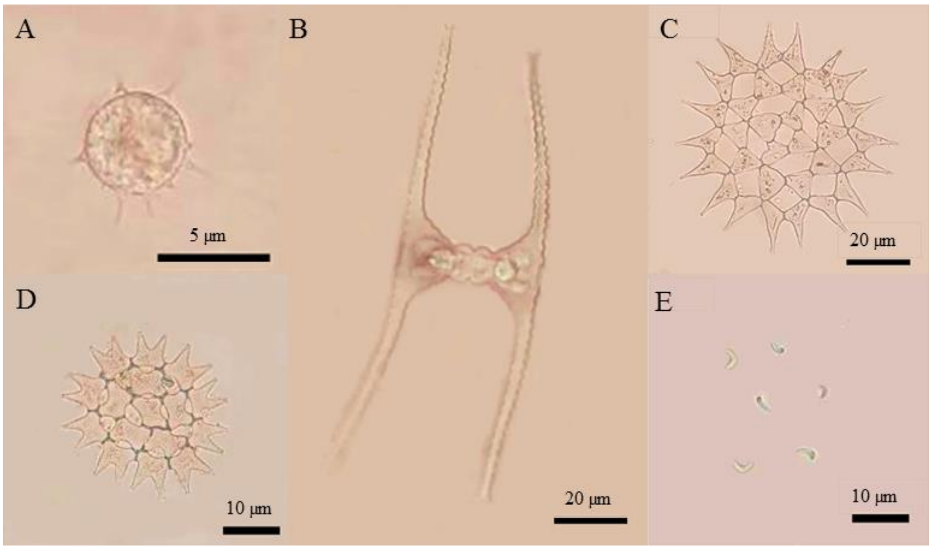


Figure 4. Optical microscopy of microalgae species of the class Chlorophyceae, presents in Pernambuco semiarid. A – *Trochiscia* sp.; B – S*taurastrum leptocladum;* C – *Pediastrum simplex*; D – *Pediastrum duplex* and; E – *Pseudokirchineriella subcapitata.*

Class Bacillariophyceae

Family Coscinodiscophyceae
Aulacoseira granulata (Ehrenberg) Simonsen (Figure 5)

Grouped elliptic and cylindrical cells. Ornate valves with a thorn at each end.

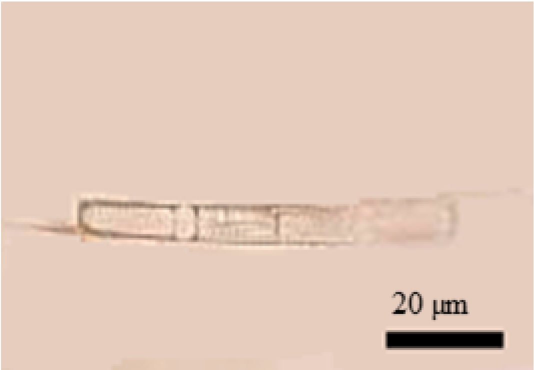


Figure 5. Optical microscopy of *Aulacoseira granulata*, presents in Pernambuco semiarid.

Class Euglenophyceae

Family Euglenaceae

Trachelomonas volvocina (Ehrenberg) Ehrenberg

Individual euglyzoid with a generally spherical or semi-spherical free life. It has a pore in its anterior part, which emerges the flagellum.

Classe Dinophyceae

Family Ceratiaceae
Ceratium furcoides (Levander) Langhans (Figure 6)

Solitary, free-naturing and asymmetric cells with tower format. Composed of 16 ornate plaques.

Figure 6. Optical microscopy of *Ceratium furcoides*, presents in Pernambuco semiarid.

Table 1 shows the taxa found and their respective places of occurrence**.**

Table 1. Distribution of species by place of occurrence in the Pernambuco semiarid. A1 – Cachoeira II Reservoir; A2 – Saco I Reservoir; A3 Sítio dos Nunes Lake – and; A4 – Varzinha Dam.

Taxa	Place of occurence			
	A1	A2	A3	A4
Anabaena sp.		X		
Aphanocapsa elechista	X	X		
Aulacoseira granulata		X		

Ceratium furcoides	X	X		
Chlorella sp.		X	X	X
Chroococcus dispersus		X		
Cosmarium sp.	X			
Desmodesmus spp.			X	X
Dictyosphaerium sp.	X			
Dolichospermum sp.		X		
Merismopedia sp.		X		
Microcystis aeruginosa	X	X		
Pediastrum duplex		X		
Pediastrum simplex		X		
Pseudanabena limnetica		X		
Pseudokirchineriella subcapitata				X
Scenedesmus sp.			X	X
Staurastrum leptocladum	X	X		
Synechococcus sp.		X		
Trachelomonas volvocida	X	X		
Trochiscia sp.		X		

DISCUSSION

The presence of some taxa, especially cyanobacteria, is worrying because they present biochemical risks to society, knowing that the four bodies are used by the population both for supply and for recreational activities. However, harmful algae were not recorded in the Vazinha Dam and Sítio dos Nunes Lake.

Microcystis aeruginosa is a potential producer of microcystin-LR (MC), the only cyanotoxin with water potability guidelines proposed by the World Health Organization (WHO), being tolerated up to 1 µg.L-1 (WHO, 2003).

The *Anabaena* is also potentially known to produce MC and also Anatoxin-a (ANTX), however, such a toxin is less likely to be found in the southern hemisphere, due to climatic factors. ANTX was the first cyanobacterial toxin with established toxicological effects, and is present in filamentous microalgae (Carmichael et al., 1975;

Devlin et al., 1977). Recent studies aim to identify the palatability of noxious algal species by zooplankton, in order to decrease the density of these cells by direct predation (dos Santos Severiano, et al., 2018).

Ceratium furcoides is an exotic dinoflagellate that was registered in Brazil in 2007 in the state of Minas Gerais, and only in 2016, its first occurrence record in the Northeast region, state of Bahia (Santos-Wisniewski et al., 2007; Oliveira et al. 2016). *C. furcoides* can produce unpleasant taste and odor in the water, resulting in serious problems for regions with supply problems, such as the present study (Jati et al., 2014; Meichtry de Zaburlín et al., 2016). This is the first time the species is recorded in reservoirs in the Pernambuco semiarid.

However, some algae have a biotechnological potential already described in the literature and do not present risks to society, such as the genus *Chlorella, Desmodesmus*, and *Pseudokirchineriella* (Matos, 2017). *Chlorella* is one of the most productive in the world, being used as a functional food, available in capsules, powder or length. *Chlorella* and *Scenedesmus* also present a great potential for the production of biofuels and several researches are done about increasing the lipid potential in order to obtain a higher yield.

Minhas et al., (2016) evaluating the production of lipids and carotenoids under stress conditions in the microalgae culture medium, considers the potential of microalgae to produce a variety of products and indicates that there is potential in future research for the development of appropriate criteria for species selection through bioprospecting of microalgae obtained from various habitats and climatic conditions.

CONCLUSIONS

In the present study, 21 taxa were identified, the highest and lowest diversity being found in Saco I Reservoir and Sítio dos Nunes Lake, respectively. The isolation of the sweet strains of the Pernambuco semiarid is in continuous process of tests in order to enable the conditioning of these species in the Laboratory of Biotechnology of Microalgae, UFRPE/UAST.

Future studies can be developed to evaluate the biotechnological application of the isolated species, as well as the use of these as an auxiliary tool in the fictional studies from the analysis of live samples.

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4.2 - Artigo científico II

Harmful algal blooms in the semiarid region of Brazil reservoirs

Carlos Yure Barbosa de Oliveira[1*], Ayanne Jamyres Gomes da Silva Almeida[1], Danielli Matias de Macedo Dantas[1]

[1] Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada(UAST), Laboratório de Biotecnologia de Microalgas (LABIM) Zip Code: 56909-535 Serra Talhada-PE, Brazil;

* Corresponding author: yureboliveira@gmail.com

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HARMFUL ALGAL BLOOMS IN THE SEMIARID REGION OF BRAZIL RESERVOIRS

ABSTRACT

Inland aquatic ecosystems are dynamic and particularly susceptible to invasion by harmful algal blooms (HAB), since their dispersion may be unnoticed and also favored by the flow of water. The present study aimed at evaluating and correlating the abiotic conditions to presence of cyanobacteria and other harmful groups present in two water reservoirs in the semiarid of Pernambuco, Brazil. The data were collected in Saco I and Cachoeira II reservoirs. During the eight months, 18 taxa were found, (*Anabaena* sp., *Aphanocapsa elachista, Chroococcus dispersus, Dolichospermum* sp., *Merismopedia* sp., *Microcystis aeruginosa*, *Pseudanabena limnetica*, *Synechococcus* sp., C*hlorella* sp., *Cosmarium bioculatum, Dictyosphaerium* sp., *Pediastrum duplex, Pediastrum simplex,* S*taurastrum leptocladum, Trochiscia* sp., *Aulacoseira granulata, Trachelomonas volvocida, Ceratium furcoides*. The calculation of regression coefficients in Canonical Correlation Analysis showed significant positive relationships between species and physicochemical conditions. Disposals of effluents, without previous treatment, and the culture of aquatic organisms in these reservoirs contribute to harmful algal blooms because they are usually more resistant to adverse environmental conditions, such as high levels of nutrients, which in the short to medium term lead to eutrophication.

Keywords: Ecology; Limnology; HAB; Seasonal dynamics.

1 INTRODUCTION

Inland aquatic ecosystems are dynamic and particularly susceptible to invasion by harmful algal blooms (HAB), since their dispersion may be unnoticed and also favored by the flow of water [1,2]. Such invasions may compromise local biodiversity, and their impacts on the environment are highly underestimated by many government authorities and entrepreneurs [3,4].

The monitoring of water sources used for water supply and recreational activities is also necessary due to the flowering of cyanobacteria, eutrophication agents, potential and producers of toxins [5]. The Cyanotoxins are mostly composed of amino acids that result in changes in the aquatic biota, as well potent hepatotoxic, neurotoxic and dermatotoxic agents, and may also lead to general inhibition of protein synthesis in mammals [6,7]. The possibility of cyanotoxins bioaccumulation, in particular microcystins-LR (MC), in fishes [8,9,10], and other aquatic organisms, as crustaceans [11, 12] and molluscs [13,14] may cause serious public health problems due to their unnoticed ingestion.

In addition to the availability of nutrients, some physicochemical conditions influence the dynamics of these algae, as temperature, pH and salinity [15,16], and the correlation of these parameters with HAB is important to understand the connection of these blooms with the exogenous influences. Possibly, the physical parameter of temperature is the most worrying because it is directly linked to the flowering of cyanobacteria, since, in view of the current state of global temperature rise and the increase in effluent production, the occurrence of blooms must become more and more constant [17,18,19,20].

Studies such as these contribute significantly to the knowledge and maintenance of local biodiversity in semiarid regions, where there are high evaporation rates and long periods of drought, and the most of the reservoirs are seasonal, as well as contribute the collective health and safety of individuals that performing activities in these water bodies.

In order to expand knowledge about harmful algal blooms and biodiversity in the Brazilian semiarid region, the present study aimed at evaluating and correlating presence of cyanobacteria and other harmful groups to abiotic conditions to present in in two water reservoirs in the semiarid of Pernambuco, Brazil.

2 MATERIALS AND METHODS

2.1 Study area

The data were collected in Saco I (038°17’35”W 07°56’53”S) and Cachoeira II (038°19’52”W 07°58’12”S) reservoirs, (Figure 1), both are located in the semiarid of Pernambuco, during February to September 2017. The data collect occurred in daytime, in the coastal area of the reservoirs. The horizontal hauls were carried out using a plankton net (20 µm), and after that the samples were conditioned and fixed in 4% formalin.

2.2 Sampling and laboratory studies

The identification occurred on the basis of morphological characters using a binocular optical microscope Motic® model BA300 and a Sedgewick-rafter camera. The following studies were used as reference: González [21], Bicudo and Menezes [22], Cybis et al. [23], for identification of taxa. They are considered as single-celled organisms, filamentous, trichomes, colonies and monastic.

Water temperature (°C), pH, salinity, and conductivity were measured *in situ* with a multiparameter probe (modelo YSI 6820-V2). The levels of Nitrate (N-NO3), Nitrite (N-NO2-) and Ammonia (N-NH3) were performed by a spectrometry with wavelength ranging from 530 to 630nm. The analysis of the soluble phosphate was performed following the ascorbic acid method described in American Public Health Association - American Water Works Association Standard Methods [24].

2.3 Controls performed

On data collection days, when visiting the second reservoir, all necessary care was taken in order to avoid crusted contaminations between the reservoirs studied. In addition, 70% alcohol was used to ensure contamination occurred during sample disposal.

2.4 Statistical analysis

The structural diversity was calculated using statistical methods recommended by Heywood [25] for taxonomic studies, the Shannon and Simpson index, to measure diversity and wealth, respectively. The statistical significance of abiotic variables was assessed using the test t-student, two paired samples for averages, with level significant of 5% [26]. The statistical analysis of the relationships between species diversity of microalgal communities and their environmental variables were studied by canonical correspondence analysis (CCA) with CANOCO for Windows 4.5 package.

3 RESULT AND DISCUSSION

3.1 Species diversity, richness and bio-indication

During the eight months, 18 taxa were found (Table 1), being eight of the class Cyanophyceae (*Anabaena* sp., *Aphanocapsa elachista* West & G.S.West, *Chroococcus dispersus* (Keissler) Lemmermann, *Dolichospermum* sp., *Merismopedia* sp., *Microcystis aeruginosa* (Kützing) Kützing, *Pseudanabaena limnetica* (Lemmermann) Komárek, *Synechococcus* sp.), five of Chlorophyceae (*Chlorella* sp., *Cosmarium bioculatum* Brébisson ex Ralfs, *Dictyosphaerium* sp., *Pediastrum duplex* Meyen, *Pediastrum simplex* Meyen, S*taurastrum leptocladum* Nordstedt, *Trochiscia* sp) and only one of the class Bacillariophyceae (*Aulacoseira granulata* (Ehrenberg) Simonsen), Euglenophyceae (*Trachelomonas volvocina* (Ehrenberg) Ehrenberg) and Dinophyceae (*Ceratium furcoides* (Levander) Langhans).

It was highlighted the presence of *Ceratium furcoides* in all month of the study in Cachoeira II reservoir, and it had being present in Saco I reservoir since June/2017. The *Microcystis aeruginosa* was also present in the eight months of analysis in Saco I reservoir. It is noteworthy that the bloom occurred in the two reservoir in April/2017 where densities reached, respectively, 120.000 and 180.000 ind.L-1 in Saco I and Cachoeira II reservoir, and it had being registered again in the month of June and September in the same year, however in lower densities.

The *Ceratium furcoides* was recorded for the first time in 2007 in Minas Gerais, Brazil [27]. Only in 2016, it was recorded for the first time in a semiarid region. [28]. Since then, the species is in continuous expansion in the country. The *C. furcoides* may produce harmful effects such as unpleasant taste and smell in water, resulting in a large impact on the use of the source for domestic supply [29,30].

The Shannon Index date explained that the diversity of the Cachoeira II (Table 2) is approximately three times smaller than the Saco I (Table 3), with the greatest diversities found in June and May. Similar results are observed in relation to the richness of the species of the reservoirs (Simpson index).

Due to a long drought period and low rainfall, both reservoirs dried up and had restricted access, in September, which prevented new data collect for the study.

3.2 Fluctuation of water parameters

The physical conditions of conductivity, salinity and pH presented significant differences ($p<0,05$) during the eight months of study between the reservoirs. The oscillations in salinity (Figure 2-A), pH (Figure 2-B) and conductivity (Figure 2-C) occurred due to the alternation between the ratio of precipitation volumes and evaporation. The temperature conditions (Figure 2-D), did not differ between the reservoirs ($p>0,05$), the maximum and minimum values for both reservoirs were measured in March and July, respectively.

The variations in chemical conditions, Nitrite, Nitrate and Phosphate (Figure 3), did not differ statistically ($p<0,05$) over the eight months between the reservoirs.

3.3 Species-environment relationships

The calculation of regression coefficients in CCA showed significant ($F = 14.349$; $p = 0.001$) positive relationships between species and physicochemical conditions. Six physicochemical variables has been chosen (N-NO3, P-PO4, N-NH3, Temperature, Salinity and pH) to verify a possible relationship with the species described in the reservoirs. The CCA ordination biplot showed the formation of six distinct groups as a function of nutrient concentration and the water physicochemical conditions.

The *C. furcoides* presented a direct correlation with the phosphate and the salinity parameters (Figure 4-A), and the abundance of this species is more linked to phosphate. The low correlation between the chemical variables and the species of Cachoeira II (Fig. 4-A), is probably related to the low reservoir diversity and the discontinuous presence of the taxa over the months.

The response of the phytoplankton community of Saco I to the anthropic activities (Figure 4-B) reveals two different sets of environmental variables; the first with the parameters of pH and temperature (Figure 4-B) and the second with the variables of salinity and nitrate. The first set indicates the abundance of nine of the fifteen species described: *Anabaena* sp., *Chlorella* sp., *Dolichospermum* sp., *Microcystis aeruginosa*, *Pediastrum duplex*, *Pediastrum simplex*, *Pseudanabena* sp., *Synechococcus* sp. and *Trochiscia* sp. The variables of the second group are relevant for species: *Chroococcus dispersus* and *Merismopedia* sp.

The pH and temperature parameters corresponded to the group with the highest diversity of species. The lack of significant association between physicochemical conditions and the most phytoplankton rate suggests that the organisms should not be limited In Cachoeira II reservoir the significant negative effect of increasing grazing pressure on *C. furcoides* may be closely related to the flagellated nature of these species. The *A. granulata* was the only species to present correlation with three parameters, ammonia, salinity and temperature.

3.4 Potential cyanotoxins

Like most mesotrophic lakes, the low diversity is directly related to the high temperatures of the region and the existence of effluent discharge in the water bodies that were studied. Then, the most resistant algal groups, the cyanobacteria, is predominant. The presence of *M. aeruginosa* is mainly worrisome because the toxin produced by this genus, the microcystina-LR (MC), is the only cyanotoxin with water potability guidelines proposed by the World Health Organization (WHO), tolerated until 1 $\mu g.L^{-1}$ of MC [31], and there are fishing and aquaculture activities in both reservoir. It is known about the potentiality of the genus over the production of other cyanotoxins, but the literature in this area is still scarce and it is not very concrete [32,33,20].

The *Anabaena* is known to produce MC and Anatoxin-a (ANTX), however, such a toxin is less likely to be found in the southern hemisphere. The ANTX was the first cyanobacterial toxin with established toxicological effects [34,35], and it is mainly present in filamentous cyanobacteria, such as *Anabaena* spp. [36], *Oscillatoria* sp. [37], *Arthrospira fusiformis* [38] among others.

3.5 Future perspectives

The anthropogenic processes on aquatic ecosystems, both the direct pollution and the culture of aquatic organisms stimulate the photosynthetic activity of phytoplankton, and consequently unwanted blooms [39]. For Vidotti & Rollemberg [40], the algae are directly affected by chemical and household effluents with potential sources of nitrogen and phosphorus. The awareness of the treatment of effluents before disposal is of paramount importance for HAB do not cause big problems in the short, medium or long term.

The advent of remote sensing in the most diverse areas may allow the monitoring of water sources, through measurements of chlorophyll-α, since this practice has proved to be very effective in studies already carried out, in the detection of HAB [41,42,43]

It was noted that the salinity of Saco I had higher values when compared to other inland water bodies. This was mainly due to the high evaporation rates of the semiarid region, and the long dry season. According to the Departamento Nacional de Obras Contra as Secas (DNOCS) this reservoir, since February/2017, is less than 1% of its maximum capacity. In the next rainy season in region, it is fundamental to make a correct and careful management for the maintenance of the biota and the guarantee of the use of these sources for the future generations.

The composition of the phytoplankton community of Cachoeira II and Saco I reservoirs may be classified as low diversity, with higher number of Cyanophyta. The abiotic variations are one of the main consequences of such standardization in the aquatic flora. The water scarcity, that is a characteristic of semiarid regions, may also be related to the absence of government monitoring in public aquatic supply sources, resulting in problems that cause silent harm to society and need to be treat.

The disposals of effluents, without previous treatment, and the culture of aquatic organisms in these reservoirs, contribute to harmful algal blooms, because they are usually more resistant to adverse environmental conditions, such as high levels of nutrients, which in the short to medium term cause eutrophication. In addition to the loss of biodiversity, eutrophication results in the impossibility of ecosystem services by numerous factors above mentioned.

Declaration of authors contributions

We reported that all co-authors participated directly in developing the manuscript.

Conflict of Interest Statement

We reported that all co-authors agree with the publication in Algal Research

Statement of Informed Consent, Human/Animal Rights

No conflicts, informed consent, human or animal rights applicable.

Declarations of interest

None.

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6 TABLES

Table 1. Occurence frequency of phytoplankton species in C – Cachoeira II reservoir and S – Saco I reservoir in February-September/2017. 0 represents the absence, and 1 the presence of the taxa.

CODE	SPECIES	FEB		MAR		APR		MAY		JUN		JUL		AUG		SEP	
		C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
SP1	*Anabaena* sp.	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	0
SP2	*Aphanocapsa* sp.	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1
SP3	*Aulacoseira granulata*	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1
SP4	*Ceratium furcoides*	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1
SP5	*Chlorella* sp.	0	1	0	1	0	0	0	1	0	1	0	1	0	1	0	1
SP6	*Chroococcus dispersus*	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
SP7	*Dolichospermum* sp.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
SP8	*Merismopedia* sp.	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1
SP9	*Microcystis aeruginosa*	0	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1
SP10	*Pediastrum duplex*	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0
SP11	*Pediastrum simplex*	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	1
SP12	*Pseudanabena* sp.	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
SP13	*Synechococcus* sp.	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
SP14	*Trachelomonas* sp.	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
SP15	*Trochiscia* sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
SP16	*Cosmarium bioculatum*	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
SP17	*Dictyosphaerium* sp.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
SP18	*Staurastrum leptocladum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Table 2. Dynamics of biological variables of phytoplankton assemblages in the Cachoeira II reservoir (s = species number)

Month	**S**	**Abundance (cells.L^{-1})**	**Shannon index**	**Simpson index**
FEB	1	370,000	0	0
MAR	2	408,000	0.097	0.038
APR	2	210,000	0.411	0.245
MAY	3	184,000	0.545	0.308
JUN	4	126,000	0.866	0.454
JUL	2	105,000	0.191	0.090
AUG	1	275,000	0	0
SEP	3	445,000	0.199	0.044
2017	7	2,133,000	0.487	0.229

Table 3. Dynamics of biological variables of phytoplankton assemblages in the Saco I reservoir (s = species number).

Month	S	Abundance (cells.L^{-1})	Shannon index	Simpson index
FEB	6	172,000	1.373	0.684
MAR	8	132,000	1.437	0.656
APR	3	264,000	1.735	0.489
MAY	10	115,000	1.870	0.789
JUN	6	106,000	1.486	0.724
JUL	6	141,000	1.502	0.720
AUG	7	176,000	1.381	0.681
SEP	8	150,000	1.771	0.752
2017	15	1,307,000	1.878	0.775

7 FIGURE LEGENDS

Figure 1. Study area located in the Serra Talhada city in semiarid of Pernambuco, Brazil. A - Cachoeira II reservoir with capacity of 21.031.000 m^3 of water. B – Saco I reservoir with capacity of 36.000.000 m^3 of water.
Figure 2. Montly variation: A - of salinity; B – pH; C – Conductivity ($\mu s.cm^{-1}$) and; D – temperature (ºC). For all images, the grey line with triangles represent the Cachoeira II reservoir, and the black line the Saco I reservoir.
Figure 3. Montly variation: A – N-NO3 ($mg.L^{-1}$); B – N-NO2 ($mg.L^{-1}$); C – N-NH3 ($mg.L^{-1}$) and; D – P-PO4- ($mg.L^{-1}$). For all images, the grey lines with triangles represent the Cachoeira II reservoir and the black line the Saco I reservoir.
Figure 4. Biplot of canonical corresponded analysis (CCA) for phytoplankton communities and environmental variables in the A – Cachoeira II reservoir and; B – Saco I reservoir. Abbreviated species names are given in Table 1.

Figure 1.

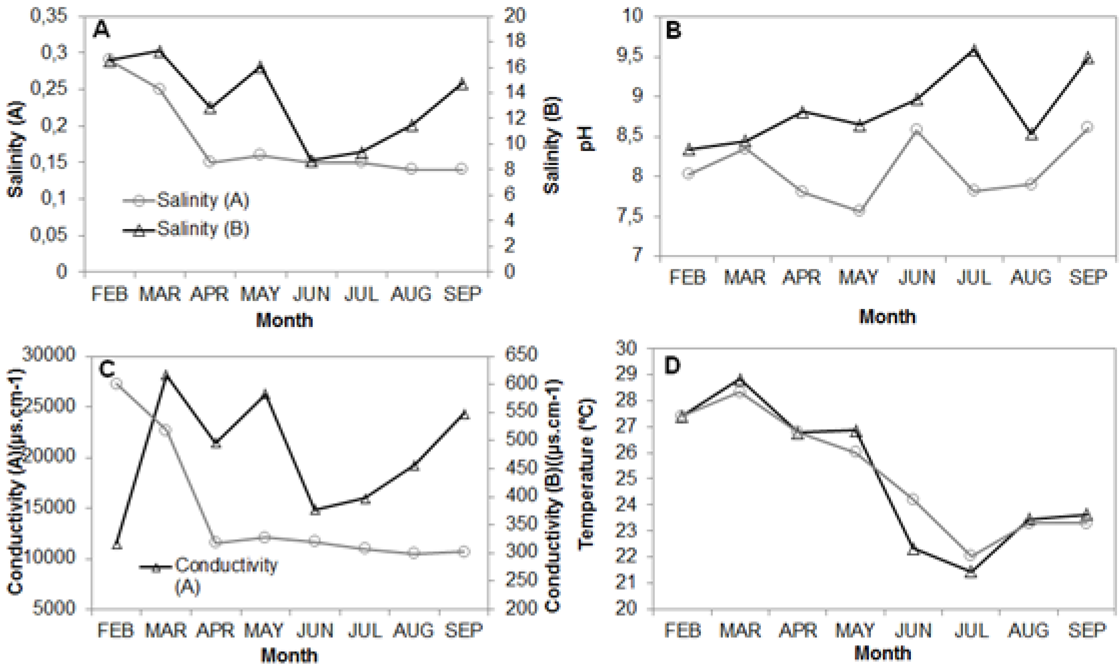

Figure 2.

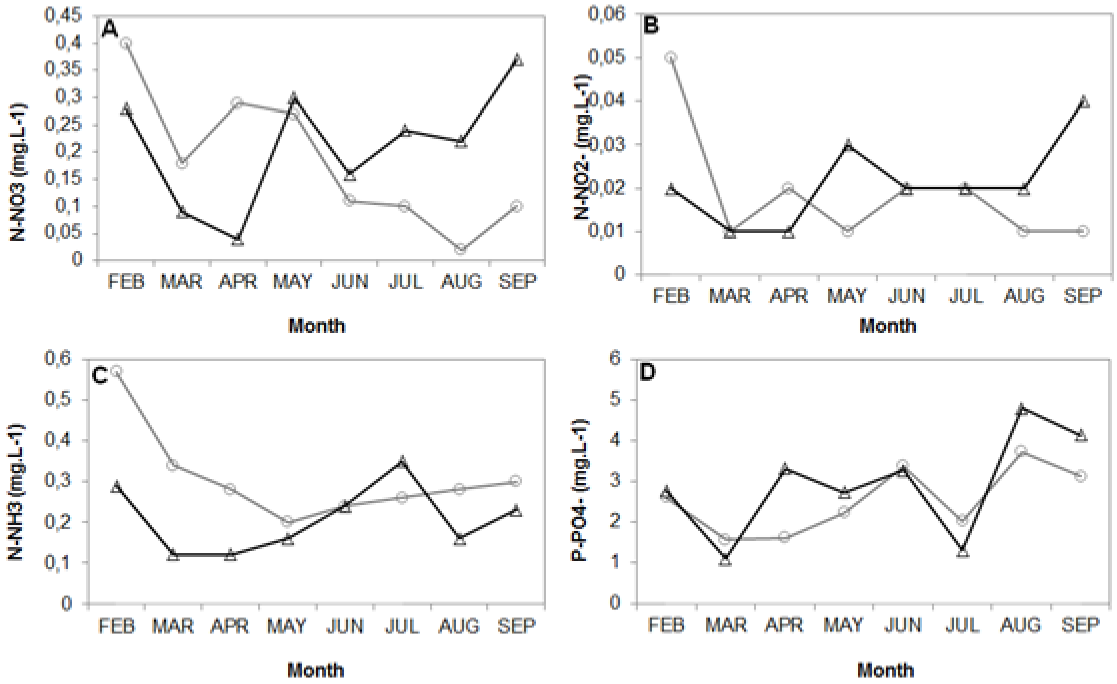

Figure 3.

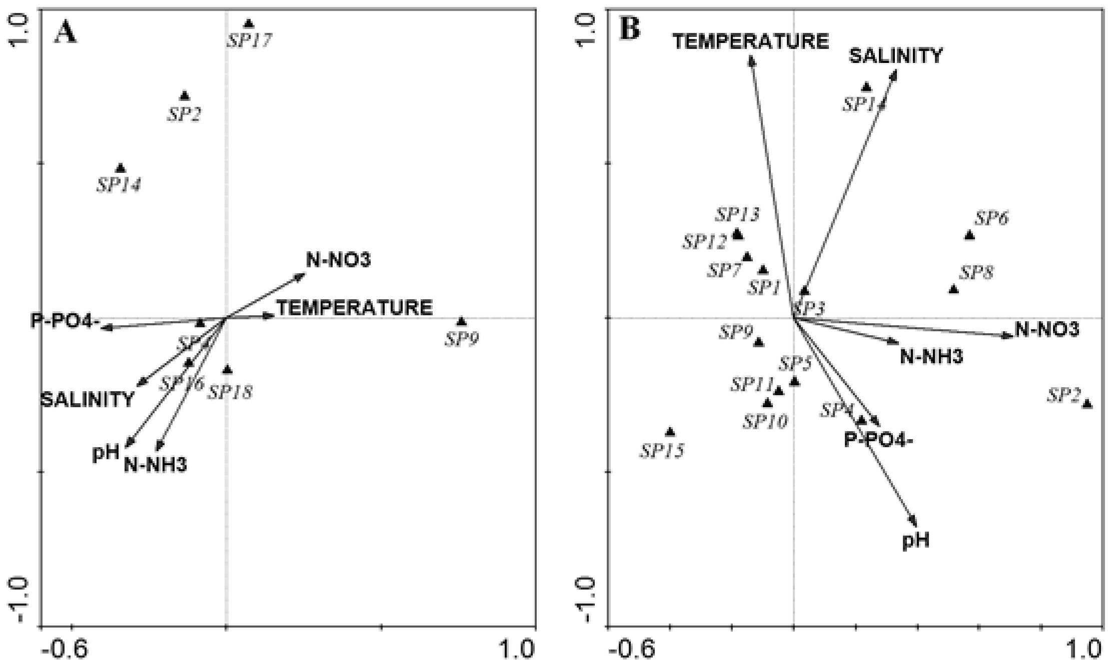

Figure 4.

4.3 - Artigo científico III

RELATION OF THE LIMNOLOGICAL CHARACTERISTICS AND THE DYNAMICS OF *Ceratium furcoides* (LEVANDER) LANGHANS (DINOPHYTA) IN A RESERVOIR IN THE BRAZILIAN SEMIARID

Carlos Yure Barbosa de Oliveira*, Ayanne Jamyres Gomes da Silva Almeida, and Danielli Matias de Macedo Dantas

Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada (UAST), Laboratório de Biotecnologia de Microalgas (LABIM). Ave. Gregório Ferraz Nogueira, Zip code: 56909-535 Serra Talhada-PE, Brazil;

*E-mail: yureboliveira@gmail.com

Running title: *Dynamic of Ceratium furcoides*

RELATION OF THE LIMNOLOGICAL CHARACTERISTICS AND THE DYNAMICS OF *Ceratium furcoides* (LEVANDER) LANGHANS (DINOPHYTA) IN A RESERVOIR IN THE BRAZILIAN SEMIARID

ABSTRACT

Biological invasions represent a serious risk to the planet's biodiversity. The knowledge about the behavior of alien species are fundamental to interpret the risks they pose. In this sense, the present study aimed at evaluating the *Ceratium furcoides* dynamics in Cachoeira II reservoir, in the semiarid region of Pernambuco, Brazil. Throughout the eight months of study, the cell density of *C. furcoides* ranged from 30 cells.L-1 to 435 cells.L^{-1}. The temperature and salinity physical parameters did not significant differences during the months of study. Already conductivity and pH parameters, nitrate levels, ammonia, and phosphate, presented statistical differences. The calculation of the regression coefficients in the Canonical Correlation Analysis expressed positive significant relationships between the species and the physicochemical parameters. Was observed relationship with *C. furcoides*, phosphate and salinity parameters showing a positive correlation, with higher for P-PO_{4^-}. Studies such as these need to be carried more out frequently, it does not only need to be in the Brazilian semiarid region, where the water issue is strongly impacted, so that new aquatic bodies do not become unviable for some anthropic services, but also in the throughout Brazil.

Keywords: Alien species; correlation; ecology; harmful algal blooms; limnology.

1 Introduction

The limnology is the branch of ecology that studies the freshwater. This area contributes to the knowledge and functioning of aquatic ecosystems (Esteves, 2011).

However, aquatic ecosystems show great variation in their biotic and abiotic conditions, which may favor the perceptible introduction of new species (Tilahun and Ahlgren, 2010).

The increase in the occurrence of biological invasions, linked to climate change, is becoming a prevailing situation all over the world, causing serious threats to biodiversity (Vitousek et al., 1997; Moro et al., 2012; Traveset and Richardson, 2014). Anthropogenic activities have harmed about 30% of the native flora of the most regions of the world in the last decades (Heywood, 1989). The success of introduced organisms depend on many factors, including their ability to survive under unfavorable conditions and their adaptability to new environments. (Baker and Stebbins, 1965). The dynamics of inland aquatic ecosystems contribute to the freshwater communities, due to the unobserved alien dispersion, resulting in irreparable problems in short to medium term (Nentwig, 2007; Meichtry de Zaburlín et al., 2016). The phytoplankton is a primary producer, and consequently energy source for the subsequent groups (Singh and Gu, 2010).

Some physical and chemical parameters of water, such as temperature, salinity, pH, and conductivity, may significantly influence the quantitative levels of the phytoplankton community, besides the herbivory pressure of zooplankton species. However, the availability of nutrients and luminosity are still the main requirements for cell survival and multiplication of the microorganisms (Heisler et al., 2008).

The dinoflagellates are biflagellated algae, although some are not, and relatively with low representativeness in freshwaters, about 220 species (Bellinger and Singee, 2010). *Ceratium* (Schrank, 1793) are asymmetrical and unicelular, and there are only six species in inland waters (Popovský and Pfiester, 1990) and only one species registered in Brazil (Santos-Wisniewski et al., 2007; Oliveira et al., 2016). *Ceratium furcoides*, can produce harmful effects such as unpleasant taste and bad smell in water. Eventually, this situation has an impact on local communities, particularly in regions with water supply problems (Matsumura-Tundisi et al., 2010; Cavalcante et al., 2016).

In order to contribute to the knowledge about the *Ceratium furcoides* blooms in tropical regions, the present study aimed at evaluating and correlating the dynamics of *C. furcoides* to abiotic parameters and their possible interactions in the Cachoeira II reservoir, located in a semiarid region of Pernambuco, Brazil.

2 Materials and method

2.1 Study area

The study occurred during February to September 2017 in the Cachoeira II reservoir (Figure 1), located in Serra Talhada city, semiarid region of Pernambuco, Brazil. The data collect occurred during daytime, in the coastal area of the reservoir (038°19'52"W and 07°58'12"S). The horizontal hauls were carried out using a plankton net (20 µm), and after that the samples were conditioned and fixed in 4% formalin. At the collect points, the temperature, pH, salinity and conductivity parameters were evaluated by multiparameter (model YSI 6820-V2).

Figure 1. Study area located in Serra Talhada city in the Pernambuco semiarid region of Brazil. Cachoeira II reservoir with capacity of 21.031.000 m^3.

2.2 Sampling and laboratory studies

The quantification of the specimens occurred on the basis of the morphological characters, using an optical microscope binocular Motic® modelo BA300 and a Sedgewick-rafter camara. For identification, it was used as reference Popovský e Pfiester (1990).

The levels of Nitrate (N-NO_3), Nitrite (N-NO_2-) and Ammonia (N-NH_3) were performed by a spectrometry with wavelength ranging from 530 to 630nm. The analysis of the soluble phosphate was performed, following the ascorbic acid method described in American Public Health Association - American Water Works Association Standard Methods (AWWA, 1992).

2.3 Statistical analysis

The statistical significance of abiotic variables was assessed using the Student's t-test, for sample data, with level significant of 5% (Zar, 2013). The statistical analysis of the relationships between *C. furcoides* and their environmental variables were studied by Canonical Correspondence Analysis (CCA) with CANOCO for Windows 4.5 package.

3 Results

Throughout the eight months of study, the cell density of *C. furcoides* ranged from 30 cells.mL^{-1} to 435 cells.mL^{-1}, being the lowest and highest value, recorded in April and September, respectively (Figure 2).

Figure 2. Buoyancy of cell density of *Ceratium furcoides* from February to September 2017 at Cachoeira II reservoir, Serra Talhada-PE, Brazil

In the same period evaluated, the presence of other taxa *Aphanocapsa elachista, Cosmarium* sp., *Dictyosphaerium* sp., *Microcytis aeruginosa,* S*taurastrum leptocladum, and Trachelomonas volvocida* were reported, and only in April, the density of *C. furcoides* was lower than that of the above-mentioned taxa (Table 1).

The highest and lowest temperature values were measured in April and July, while for salinity, February and September, respectively. The temperature (p=0.4964), salinity (p=0.3404) and pH (p=0.4894) physical parameters, the levels of nitrate (p=0.1538), nitrite (p=0.058), ammonia (p=0.4167), and phosphate (p=0.3268) did not significant differences during the study months. Only the conductivity parameter presented statistical differences ($p<0.0001$) (Table 2).

The calculation of regression coefficients in CCA showed significant positive relationships between species and physical chemical parameters. Six physic and chemical variables (N-NO_3, P-PO_4, N-NH_3, Temperature, Salinity and pH) has been chosen to verify a possible relationship with the species described in the reservoir. The *C. furcoides* presented a direct correlation with the phosphate and the salinity parameter with higher for P-PO_4 (Figure 3).

As a characteristic of the semiarid regions, due to a long drought period, the reservoir dried up, and it had its restricted access, which made continuity and periodicity of the study impossible.

Figure 3. Biplot of canonical correspondence analysis (CCA) and its correlations between cell density and physico-chemical parameters: sal = salinity; temperat = temperature; N-NH3 = ammonia; N-NO3 = nitrate; P-PO4 = phosphate; APHAN = *Aphanocapsa* sp.; C. FURC = *Ceratium furcoides*; COSMA = Cosmarium sp.; DICTY = *Dictyosphaerium* sp; M. AERU = Microcytis *aeruginosa;* S. LEPT = S*taurastrum leptocladum* and; TRACH = *Trachelomonas volvocida*

4 Discussion

Several factors are associated with the dispersion of phytoplankton species and may positively contribute to invasion success. Whether by the independent action of the winds, able to carrying spores out or by the very contamination of the anthropic action.

The *Ceratium furcoides* had its first occurrence in 2007, in Minas Gerais, Brazil (Santos-Wisniewski et al., 2007), and only in 2016, it was recorded for the first time in a Brazilian semiarid region (Oliveira et al., 2016), however, the species is in continuous expansion in the country. The invasion and appropriation of *Ceratium* in new areas may be linked to climate change and the construction of reservoirs, since it eventually develops better in lentic environments (Meichtry de Zaburlín et al., 2016; Cassol et al., 2014). The understanding of the causes and consequences that the species may cause are of extreme importance, since, when successful, they can make an unbalanced and atypical ecosystem.

The results of this study showed that *C. furcoides* was adapted to the environmental conditions in the reservoir. The density variations of specie demonstrated that although they are dominant in the environment, some factors may cause a relative decrease in the individual's number. The decrease, superior than 90%, in the density occurred between March and April did not prevent the species from developing again in the subsequent months and reached its maximum density in September (435 cells.mL^{-1}). Corroborating with the present study, Cavalcante et al., (2016) also recorded the highest densities of *C. furcoides* in spring-summer (Table 3).

The species that make up this genus rarely make up the dietary habits of copepados and other zooplankton (Santer, 1996). The low pressure the herbivory of the zooplankton species (Hargrave and Geen, 1970), its relative mobility (Santos-Wisniewski et al., 2007), low nutritional value (Williamson, 1984), and its size (Pollingher, 1987) making the species less subject to sedimentation, may have contributed to such blooms (Mac Donagh et al., 2005). According to Brandl and Fernando (1979), only copepods in advanced stages of life are able to prey on *C. furcoides*.

The temperature was not a limiting factor for the occurrence of *C. furcoides* in this study, taking into account that the two highest densities were recorded in seasons and different temperatures. It is important to highlight the area where this study was carried out. The Brazilian northeast region is characterized by high temperatures throughout the year (Figure 4), and studies that relate the occurrence of blooms to high thermal indexes (Canter and Heaney, 1984; Hickel, 1988; Jati *et al*. 2014; Cavalcante et al., 2016), in most cases, are still lower values than those recorded in the present study. However, some studies have also demonstrated the resistance of these organisms to low temperatures (Silva et al., 2012), ranging from 18 to 1 °C.

Figure 4. Temporal variation of temperature and salinity from February to September 2017 in Cachoeira II reservoir, Serra Talhada-PE, Brazil.

The density recorded in the present study, although relatively lower than the densities recorded in southern Brazil (Table 3), may pose a great risk to local biodiversity. The unique occurrence records of the *C. furcoides* species in three months of the eight month of study demonstrate the impossibility of other species to inhabit the environment.

In the canonical correspondence analysis, the species showed a positive interaction with salinity and phosphate parameters, with a higher interaction with the latter. Corroborating with this study, Rocha (2016) found positive relationships of high densities with total phosphorus and nitrogen concentrations, and transparency. Cavalcante et al. (2016) also emphasized the correlation between abiotic parameters, however, with the low pH values and high concentrations of nitrogen compounds. James et al. (1992) found a link between migrations of *C. hirundinela* related to the amount of phosphorus present in the environment, and also addressed that depending on the amount of this nutrient present in the environment, the cells may present morphological differences, as the amount of thorns. In the present study, no differences were observed in the morphology of *Ceratium furcoides*.

The presence of *Ceratium furcoides* was recorded in all months of study – February to September 2017, being the higher densities related to the higher levels of phosphate, evidencing the direct relation expressed in the Canonical Correspondence

Analysis (CCA). The apparent adaptation of the *Ceratium* to exotic environments was evident, corroborating with reports in other authors' literature throughout the world. Studies such as these need to be carried more out frequently, it does not only need to be in the Brazilian semiarid region, where the water issue is strongly impacted, so that new aquatic bodies do not become unviable for some anthropic services, but also in the throughout Brazil.

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TABLES

Table 1. Densities of the phytoplankton community (cells.mL^{-1}) in the Cachoeira II reservoir, located in a semiarid region of Pernambuco, Brazil.

TAXA	FEB	MAR	APR	MAY	JUN	JUL	AGO	SEP
Aphanocapsa sp.	0	0	0	4	4	0	0	0
Ceratium furcoides	370	400	30	150	90	100	285	435
Cosmarium sp.	0	8	0	0	0	0	0	0
Dictyosphaerium sp.	0	0	0	30	0	0	0	0
Microcystis sp.	0	0	180	0	20	5	0	8
Staurastrum leptocladum	0	0	0	0	0	0	0	2
Trachelomonas sp.	0	0	0	0	12	0	0	0

Table 2. Phosphate, Nitrate, Ammonia, pH, and Conductivity levels in Cachoeira II weir from February to September 2017.

Parameter	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
P-PO_{4-}(mg.L^{-1})	2.61	1.57	1.61	2.23	3.38	2.02	3.73	3.13
N-NO_3 (mg.L^{-1})	0,4	0,18	0,29	0,27	0,11	0,1	0,02	0,1
N-NH_3 (mg.L^{-1})	0,57	0,34	0,28	0,2	0,24	0,26	0,28	0,3
pH	8.03	8.35	7.81	7.57	8.58	7.82	7.9	8.61
Conductivity (µs.cm^{-1})	601	518	318	328	320	307	299	302

Table 3. Blooms-density of *Ceratium* spp. registered in the scientific literature.

Study área	Species	Maximum density (cells.mL^{-1})	Season	Reference
Cachoeira II weir, northeast of Brazil	*C. furcoides*	475	Spring	Present study
Faxinal and Maestra dams, south of Brazil	*C. furcoides*	2.819	Spring-summer	Cavalcante et al. (2016)
Blelham Tarn, north of England	*C. furcoides*	485	Summer	Canter & Heaney (1984)
Plubsee lake, north of Germany	*C. furcoides*	670	Summer	Hickel (1988)
Paso de las Piedras, central-eastern Argentina	*C. hirundinella*	2.000	Summer	Guerrero & Echenique (1997)
Tercero reservoir, Argentina	*C. hirundinella*	1.244	Summer	Mac Donagh et al. (2005)
Sumampa and Las Pirquitas dam, northwest of Argentina	*C. hirundinella*	5.634	Winter	Silverio et al. (2009)

Figure 1

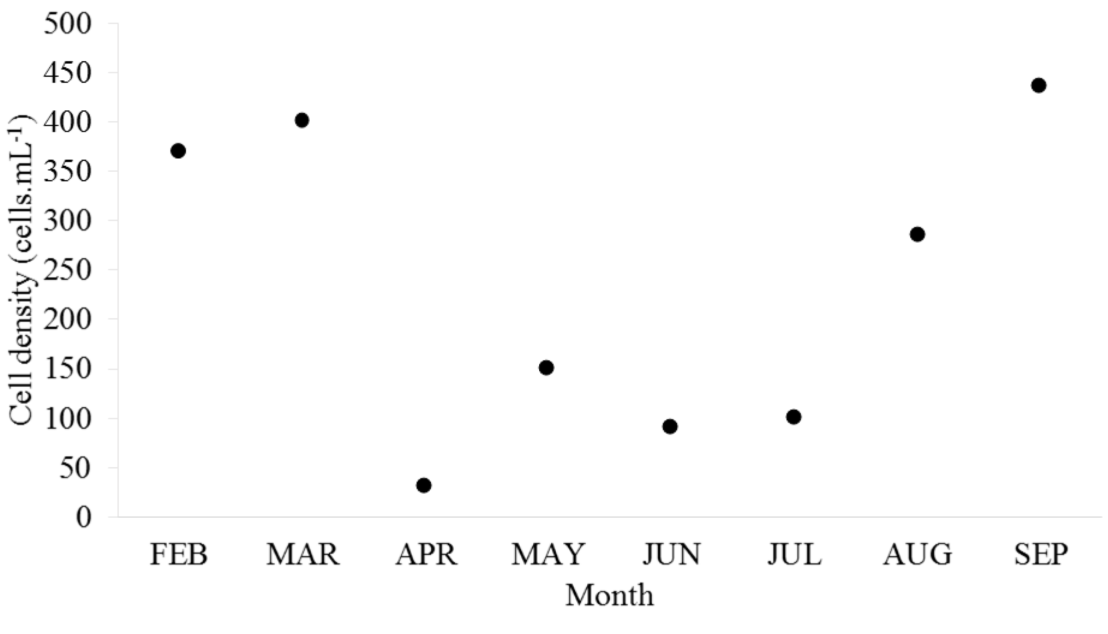


Figure 2

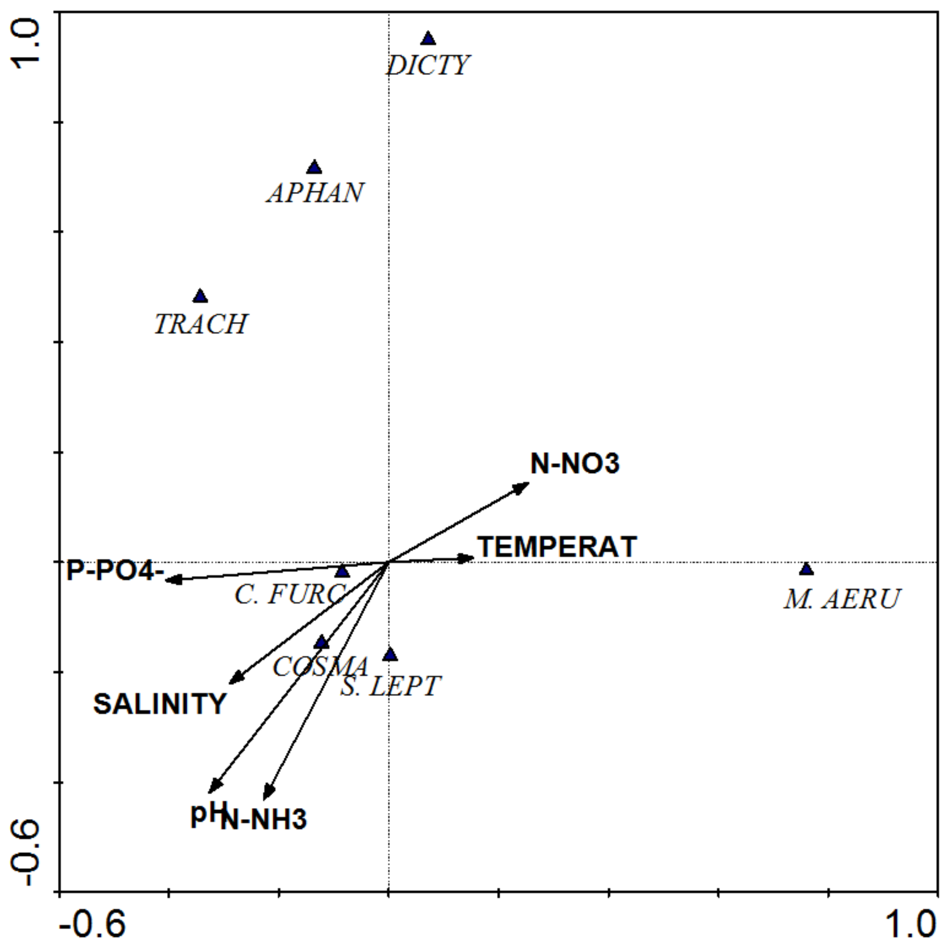


Figure 3

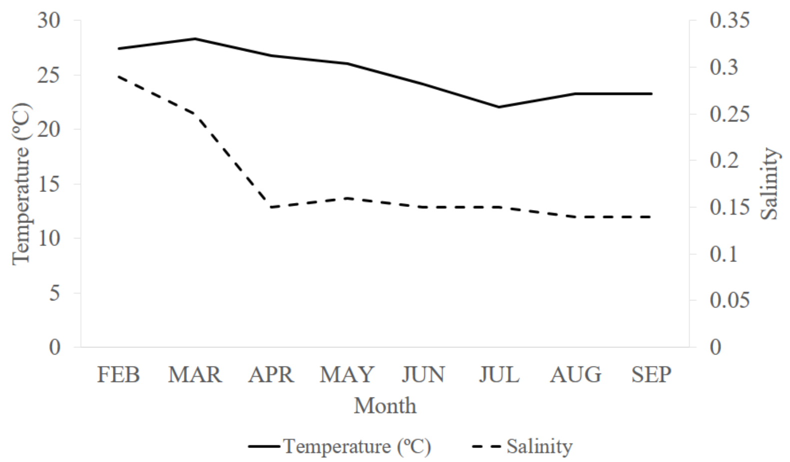


Figure 4

4.4 - Artigo científico IV

CRESCIMENTO DE *Chlorela vulgaris* BEYERINCK (BEIJERINCK) (TREBOUXIOPHYCEAE, CHLORELLACEAE) EM EFLUENTE DE UM SISTEMA DE BIOFLOCO

Carlos Yure Barbosa de Oliveira[1*]

Jéssika Lima de Abreu[2]

Alfredo Galvez Olivera[3]

Danielli Matias de Macedo Dantas[4]

[1] Graduando(a) em Engenharia de Pesca pela Universidade Federal Rural de Pernambuco (UFRPE), Unidade Acadêmica de Serra Talhada (UAST) - Serra Talhada-PE, Brasil;
[2] Doutoranda em Recursos pesqueiros e aquicultura pela Universidade Federal Rural de Pernambuco (UFRPE) – Recife-PE, Brasil;
[3] Doutor em Aquicultura pela Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP) – Recife-PE, Brasil;
[4] Doutora em Ciências Biológicas pela Universidade Federal de Pernambuco (UFPE), Serra Talhada-PE, Brasil;
*Autor correspondente: yureboliveira@gmail.com; +5587988547997

RESUMO

O presente estudo objetivou analisar o crescimento da microalga *Chlorella vulgaris* no efluente de um sistema de biofloco, utilizado no cultivo da Tilápia do nilo *Oreochromis niloticus.* As células foram submetidas ao cultivo por 10 dias com fotoperíodo integral de 90 µmol.$m^{-2}s^{-1}$ e aeração constante. Os tratamentos possuíam diferentes proporções de meio de cultura Provasoli e efluente do sistema de biofloco, sendo nomeadas de E0, E50 e E100, para as respectivas proporções 0%, 50% e 100% de efluente. Foram avaliados densidade celular máxima (DCM), velocidade de crescimento (K) e tempo de duplicação (TD). Os dados foram submetidos aos testes de normalidade e homogeneidade dos dados. Posteriormente, aplicou-se Análise de Variância (ANOVA), seguido do teste de Tukey ($P > 0,05$). Com relação ao parâmetro de densidade celular máxima, o tratamento E0 apresentou o melhor desempenho (1520 ± 75.10^4 cel.mL^{-1}), os tratamentos E50 e E100 foram estatisticamente iguais entre si. Entretanto, o início da fase exponencial no tratamento E100 ocorre entre o segundo e terceiro dia de cultivo, aumentaria consideravelmente a quantidade de efluente tratado em uma escala de tempo. Nesse sentido, o tratamento usando apenas efluente do BFT apresentou potencial no cultivo da microalga *Chlorella vulgaris*. Em adição, temos a eliminação dos custos referentes aos ingredientes utilizados na formulação do meio de cultura.

Palavras-chave: microalgas; biorremediação; aquicultura.

INTRODUÇÃO

O cultivo de organismos aquáticos em bioflocos (BFT) se mostra satisfatório diante do atual cenário da Pesca e Aquicultura, em especial para a Carcinocultura devido a disseminação de doenças, como a mancha branca (VINATEA et al., 2010; FAO, 2016). Entretanto, muito se discute sobre a quantidade de compostos nitrogenados e fosfatados acumulados durante o cultivo nestes sistemas, oriundos principalmente de resíduos de ração e das excretas dos organismos cultivados, uma vez que uma das características mais marcantes do sistema BFT é baixa renovação de água. (MISHRA et al. 2008; KRUMMENAUER et al., 2011).

As microalgas além de apresentar um papel importante na transferência de energia ao longo da teia alimentar, apresentam um grande potencial na biorremoção de compostos inorgânicos (GOUVEIA et al., 2006). Esses compostos servirão de nutrientes para as células que irão se desenvolver e multiplicar ao longo do tempo (BECKER & VENKATARAMAN, 1981; KIM & WIJESEKARA, 2010). A biomassa formada a partir desse processo, poderá ser utilizada em diversas finalidades, desde a nutrição de alguns animais aquáticos como para a produção de biodiesel. (DANTAS, 2013).

A depender da finalidade e da tecnologia empregada ao cultivo de microalgas o processo pode ser caro e até mesmo inviável, devido ao elevado custo dos nutrientes utilizados na produção do meio de cultura e da necessidade de mão de obra especializada (RODRIGUES, 2000; TEIXEIRA & MORALES, 2008). A capacidade de assimilar e converter compostos nitrogenados e fosfatados, e ainda alguns metais pesados, presentes no efluente do sistema BFT, em componentes celulares como lipídeos, proteínas e carboidratos, faz do uso desses efluentes uma alternativa não só econômica como também ecológica no cultivo, uma vez que na maioria das vezes, os efluentes são descartados sem nenhum tratamento prévio, causando danos incalculáveis aos ecossistemas (MIYAWAKI, 2014; SUALI & SATABATLY, 2012).

Chlorella vulgaris é uma Chlorophyceae, não móvel, de formato esférico e tamanho entre 2-10 μm de diâmetro (YAMAMOTO, 2004), sendo uma das mais conhecidas espécies de algas por ser utilizada como suplemento alimentar (HOEK et al., 1995; DANTAS, 2013). A capacidade da microalga *C. vulgaris* em biorremediar águas residuais já é comprovada na literatura (GONZÁLEZ, 1997; DE-BASHAN, 2002; BRENNAN, 2010), entretanto, não se tem registros da capacidade dessa microalga se desenvolver em efluentes de sistemas de biofloco, assim como sua resistência aos elevados níveis de nitrogênio e fósforo.

Com o intuito de expandir o conhecimento acerca do tratamento de efluentes da aquicultura, o presente estudo objetivou analisar o crescimento e a capacidade de biorremediação da microalga *Chlorella vulgaris* no efluente de um sistema de biofloco, utilizado no cultivo da Tilápia do nilo *Oreochromis niloticus.*

METODOLOGIA

Delineamento experimental

O delineamento experimental se deu inteiramente casualizado, com três tratamentos em tréplicas. Cada tratamento possuía diferentes proporções de meio de cultura Provasoli (McLACHLAN, 1973) (Tabela 1) e efluente do sistema de biofloco (Tabela 1), sendo nomeadas de E0, E50 e E100, para as respectivas proporções 1:0, 1:1 e 0:1. Antes do início do experimento, o efluente foi filtrado e a água foi clorada com 0,02 ppm de hipoclorito de sódio durante 1,5 horas e, em seguida, desclorada com solução de tiossulfato de sódio 0,025 ppm (MAGNOTTI et al., 2016). Ambos fluidos foram autoclavados à 121ºC por 20min.

A caracterização química quantitativa em relação às fontes de nitrogênio e fósforo (Tabela 1) do efluente foi realizada por espectrofometria com comprimento de onda entre 530 à 630nm. A análise do fosfato solúvel foi realizada seguindo o método do ácido ascórbico descrito na American Public Health Association - American Water Works Association Standard Methods (AWWA, 1992). Foram avaliados os dados de pH, temperatura, salinidade, turbidez e oxigênio dissolvido com o auxílio de equipamento multiparâmetro (YSI 100, Yellow Springs, Ohio, USA) no primeiro e último dia de cultivo.

Tabela 1. Composição do meio de cultura Provasoli e do efluente de biofloco utilizado no cultivo de *Oreochromis niloticus*

Provasoli		Efluente BFT	
Reagente	Quantidade	Composto	Quantidade
$NaNO_3$	105mg.L^{-1}	Amônia	0,45 ± 0,06 mg/L
Na_2Glicerofosfato	15mg.L^{-1}	Nitrito	0,39 ± 0,03 mg/L
Na_2EDTA	24,9mg.L^{-1}	Nitrato	0,95 ± 0,03 mg/L
H_3BO_3	3mg.L^{-1}	Ortofosfato	1,21 ± 0,07 mg/L
$Fe(NH_4)_2(SO_4)_2 . 6H_2O$	10,6mg.L^{-1}	-	-

$FeCl_3.6H_2O$	$0,15mg.L^{-1}$	-	-
$MnCl_2.4H_2O$	$0,6mg.L^{-1}$	-	-
$ZnCl_2$	$0,075mg.L^{-1}$	-	-
$CoCl_2.6H_2O$	$0,0015mg.L^{-1}$	-	-
Relação N:P	10:1	Relação N:P	1,78:1

Cultivo e crescimento da microalga

A cepa da microalga *C. vulgaris* foi cedida pelo Laboratório de Produção de Alimento Vivo (LAPAVI) da Universidade Federal Rural de Pernambuco. Realizou-se aclimatação em tubos de ensaio, apenas com fotoperíodo integral, nas diferentes porcentagens de meio de cultura e efluente, a fim de remover o privilégio do tratamento P100E0, visto que as cepas ficam acondicionadas nesse meio. Em seguida, foram submetidas ao cultivo por dez dias, em recipientes de 500mL, e submetidas a aeração constante e fotoperíodo integral de 90 $\mu mol.m^{-2}s^{-1}$, à uma concentração inicial de $10^5 cel.mL^{-1}$.

Durante o período de aclimatação e de todo o experimento, realizou-se a contagem das células diariamente, com o auxílio de uma câmara de Neubauer e microscópio óptico binocular Olympus® modelo BA300 com aumento de 400 vezes. Foram avaliados os seguintes parâmetros de crescimento: densidade celular máxima (DCM), tempo de duplicação (TD) e velocidade de crescimento (K). De acordo com a densidade celular diária média das três repetições foi obtida a curva de crescimento, através da equação descrita por Stein (1973), em que a velocidade de crescimento (K) é obtida através da fórmula:

$$K= [3,322.(T_2-T_1)^{-1}.(Log\ N_2.N_1^{-1})] \quad (2)$$

onde K = velocidade de crescimento; 3,322 = fator de conversão do logaritmo base 2 a base 10; (T_2-T_1) = intervalo de tempo em dias; Log = logaritmo em base 10; N_1 = densidade celular inicial; e N_2 = densidade celular final. Para o tempo de duplicação, que representa o tempo gasto para que ocorra a divisão de uma célula, foi utilizada a fórmula:

$$TD = 1.K^{-1} \quad (3)$$

em que TD = tempo de duplicação e K = velocidade de crescimento.

Análise estatística

Os resultados foram submetidos ao método estatístico descritivo. Em seguida, foram aplicados os testes de normalidade (Shapiro-Whilk) e homogeneidade (Cochran) dos dados. Posteriormente, aplicou-se Análise de Variância (ANOVA), seguido do teste de Tukey ($P > 0,05$), quando necessário, para comparação de médias entre os resultados obtidos (ZAR, 2013). Os dados foram analisados pelo *software RStudio*.

RESULTADOS

Durante a aclimatação foi observado o desenvolvimento e multiplicação das células em todas as unidades experimentais (Figura 1), a partir do segundo dia de cultivo, demonstrando que o efluente do sistema BFT utilizado no cultivo da Tilápia do Nilo foi propício para o cultivo da microalga *Chlorella vulgaris*.

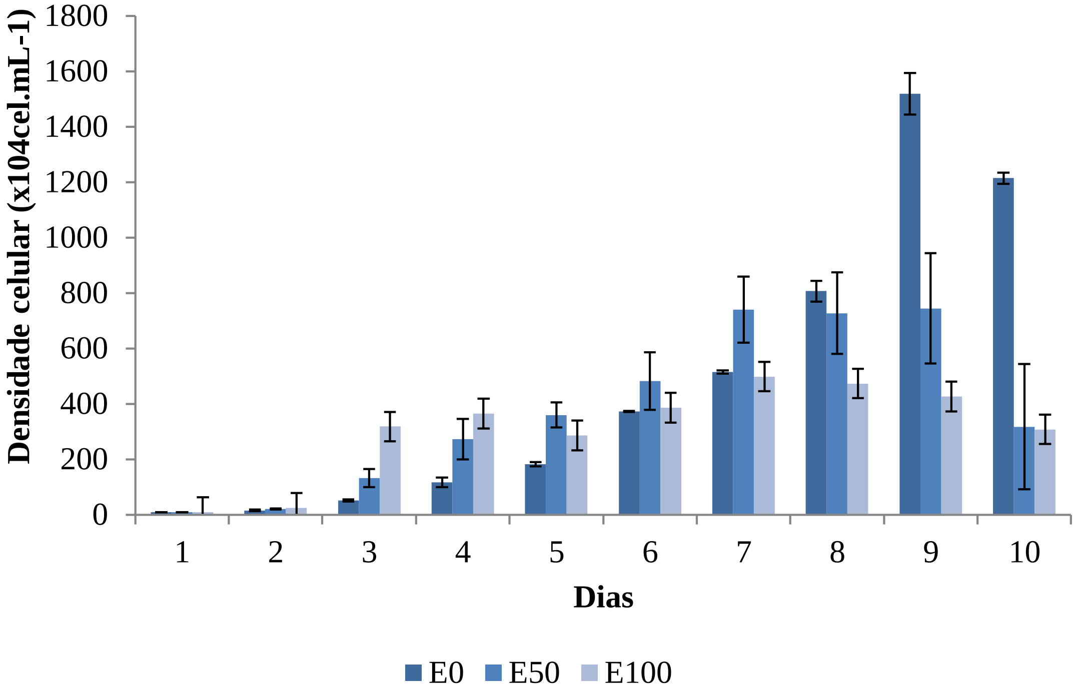


Figura 1. Densidade das células de *Chlorella vulgaris* ao longo dos dez dias de cultivo. Barras expressão os valores médios acompanhados de seus respectivos desvios padrão.

Com relação ao parâmetro de crescimento densidade celular máxima (Tabela 1), os tratamentos diferiram significativamente em dois grupos ($P = 0,0038$). O primeiro, formando pelo tratamento contendo somente o meio de cultura provasoli (E0), obteve o melhor desempenho, com média de 1520.10^4 cel.mL^{-1}. Os tratamentos E50 e E100 não apresentaram diferenças significativas entre si. Já com relação ao parâmetro de velocidade de crescimento (Tabela 1), não se observou diferença significativa (P =

0,0504) entre os tratamentos, fato que se repete para o parâmetro tempo de duplicação (*P* = 0,1164).

Tabela 2. Valores dos parâmetros de crescimento analisados: densidade celular máxima (DCM), velocidade de crescimento (K) e tempo de duplicação (TD). Letras iguais significaram diferenças significativas ($P < 0,05$).

Tratamento	DCM (x10^4 cel.mL^{-1})	K (div.dias^{-1})	TD (dias)
E0	1520 ± 75 ^{b}	0.77 ^{a}	1.30 ^{a}
E50	971 ± 302,38 ^{a}	0.63 ^{a}	1.66 ^{a}
E100	630 ± 236,4 ^{a}	0.53 ^{a}	1.93 ^{a}

Com relação aos parâmetros de pH e temperatura (Tabela 2) não foram observadas diferenças significativas entre os tratamentos bem como nas aferições iniciais e finais do cultivo (p=0,061).

Tabela 3. Média dos parâmetros físico-químicos analisados no início (MI) e final (MF) do experimento. Letras iguais significaram diferenças significativas ($P < 0,05$).

Parâmetros	E0		E50		E100	
	MI	MF	MI	MF	MI	MF
Temperatura (°C)	27,3	25,70	24,38	24,38	24,51	24,42
TDS	0,0009	0,0045	0,0047	0,0074	0,0081	0,01
Salinidade	0,72	1,44	5,21	5,21	7,15	5,86
O.D. (mg.L^{-1})	4,22	4,88	3,96	3,96	5,62	4,51
pH	7,95	7,62	6,95	6,95	6,52	6,95

As fases exponenciais dos tratamentos ocorreram em períodos distintos. Para os tratamentos E0 e E50 (Fig. 1 - A;B), essa fase têm-se início por volta do quarto terceiro dia de cultivo. Entretanto, no tratamento E100 (Fig. 1 - C), a fase exponencial se dá início já entre o segundo dia de cultivo.

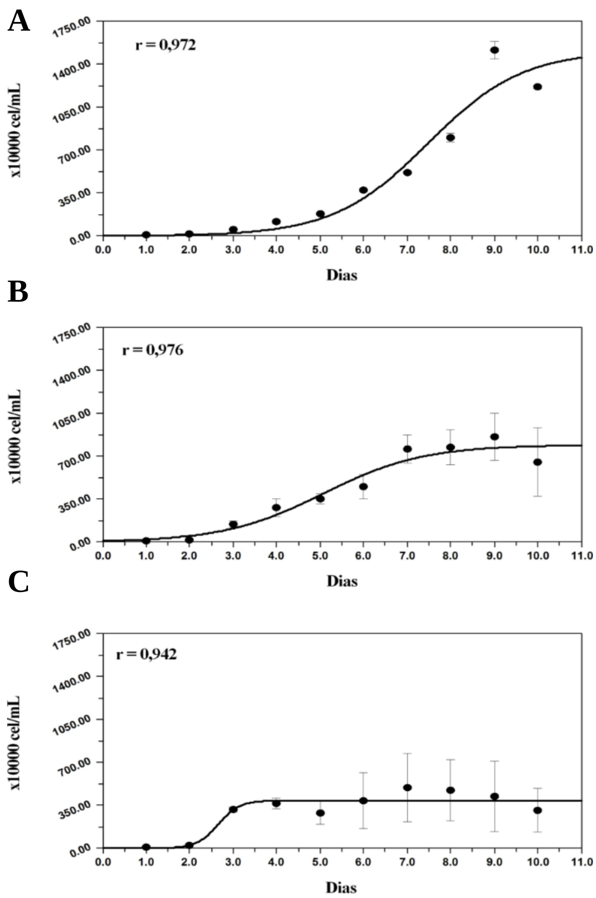
Tabela 4. Eficiência de biorremediação, do efluente BFT, de compostos nitrogenados e fosfatados por *Chlorella vulgaris.*

Composto	Quantidade inicial	Quantidade final	Eficiência de biorremediação (%)
Amônia	0,45 ± 0,06 mg.L^{-1}	0,09 ± 0,07 mg.L^{-1}	79,3

Nitrito	0,39 ± 0,03 mg.L^{-1}	0,08 ± 0,02 mg.L^{-1}	80,1
Nitrato	0,95 ± 0,03 mg.L^{-1}	0,21 ± 0,03 mg.L^{-1}	78,2
Ortofosfato	1,21 ± 0,07 mg.L^{-1}	0,63 ± 0,09 mg.L^{-1}	48,3

DISCUSSÃO

Os nutrientes utilizados para a formulação do meio Provasoli propiciam uma dieta equilibrada de macros e micronutrientes, que são fundamentais para as atividades celulares das microalgas, já os efluentes, além da presença e dos quantitativos serem altamente relativos, os nutrientes estão disponibilizados em formas diferentes, podendo



ocasionar deficiências metabólicas. Silva (2006), também encontrou diferenças estatísticas, relacionada ao parâmetro de densidade celular máxima no cultivo da mesma microalga comparando o rendimento de um efluente hidropônico e o meio de cultura Bold's Basal (BBM).

Figura 2. Curvas de crescimento logístico de *Chlorella vulgaris* para os diferentes tratamentos. Círculos representam valores médios diário com seus respectivos desvios padrões. A – E0, B – E50 e C – E100

A precocidade da fase exponencial obtida no tratamento contendo apenas o efluente do sistema BFT (Figura 2 – C) poderia reduzir quase que pela metade o tempo de repicagem (início de um novo ciclo de cultivo), aumentando consideravelmente a produtividade da biomassa, bem como um quantitativo maior tratamento de efluente.

Isso se comprova, após a análise dos mesmos parâmetros de crescimento, simulando o encerramento do experimento no quarto dia do cultivo (Tabela 5), onde com relação ao parâmetro de densidade celular máxima, o tratamento contendo apenas o efluente do sistema BFT, apresenta o melhor desempenho, numericamente, para todos os parâmetros analisados.

Tabela 5. Valores médios (±desvio padrão) dos parâmetros de crescimento analisados: densidade celular máxima (DCM), velocidade de crescimento (K) e tempo de duplicação (TD) analisados do primeiro ao quarto dia de cultivo. Letras diferentes significam diferenças significativas (P < 0,05)

Tratamento	DCM (x 10^4 cel.mL^{-1})	K (div.dias^{-1})	TD (dias)
E0	117,5 ± 17,5 ^{a}	1,18 ± 0,07 ^{a}	0,84 ± 0,05 ^{a}
E50	280 ± 73,5 ^{a}	1,59 ± 0,14 ^{b}	0,63 ± 0,06 ^{b}
E100	365 ± 45,69 ^{b}	1,73 ± 0,06 ^{b}	0,6 ± 0,02 ^{b}

A partir do quinto dia do cultivo, notou-se a presença de protozoários nos tratamentos que continham efluente BFT (E50 e E100), o que provavelmente possa ter afetado o desenvolvimento das células, resultando num impacto direto na densidade celular dessas unidades experimentais. No tratamento controle, protozoários estiveram presentes apenas a partir do oitavo dia de cultivo, provavelmente devido ao manejo. A facilidade das microalgas em assimilar compostos nitrogenados também foi perceptível e maior que a dos compostos fosfatados, quando analisadas as eficiências de biorremoção dos compostos químicos presentes no efluente.

Abreu et al. (2016), também não encontraram diferenças significativas ($p<0,05$) relacionada aos parâmetros de velocidade de crescimento e tempo de duplicação, no cultivo da diatomácea bentônica, *Navicula* sp., quando comparado o meio de cultura Conway com um resíduo sólido do efluente de um sistema de BFT. Ainda corroborando com os autores supracitados, com relação à densidade celular, o meio de cultura também sobressaiu o efluente. Provavelmente, esse fato está relacionado à composição não só das fontes de nitrogênio e fósforo (macronutrientes), como também dos micronutrientes, que possuem extrema importância para as vias mitocondriais das microalgas (SAFI et al., 2014)

Tais condições desfavoráveis sejam pela ausência ou excesso de algum nutriente, podem resultar em alterações na morfologia (MARTÍNEZ et al., 1991), composição primária (LIU et al., 2008; ANSILAGO et al., 2016) e na produção de pigmentos (YAMAGUCHI, 1996).

CONSIDERAÇÕES FINAIS

Nesse sentido, o tratamento usando apenas efluente do BFT apresentou potencial no cultivo da microalga *Chlorella vulgaris*. Em adição, temos a eliminação dos custos referentes aos ingredientes utilizados na formulação do meio de cultura. *C. vulgaris* biorremediou aproximadamente 80% dos compostos nitrogenados (amônia, nitrito e nitrato) e cerca de 50% do composto fosfatado analisado.

Pesquisas devem ser realizadas para avaliar a composição da biomassa algal, quanto ao percentual lipídico e proteico, bem como analisar a presença de compostos que possam estar aderidos na parede celular das células.

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4 Considerações finais

No presente trabalho foram identificados 21 taxons, sendo a maior e menor diversidade encontrada no Açude Saco e Lago Sítio dos Nunes, respectivamente. A composição da comunidade fitoplanctônica do açude Saco I é constituída quase que exclusivamente de cianobactérias, representando um sério risco a comunidade local. Sendo as variações abióticas uma das principais consequências de tal padronização na flora aquática.

O isolamento das cepas dulcícolas do semiárido Pernambuco encontra-se em processo contínuo de testes no intuito de viabilizar o condicionamento destas espécies no Laboratório de Biotecnologia de Microalgas, UFRPE/UAST. Futuros trabalhos podem ser desenvolvidos no intuito de avaliar a aplicação biotecnológica das espécies isoladas, assim como a utilização destas como ferramenta auxiliar nos estudos ficológicos a partir da análise de amostras vivas.

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ANEXOS

Revista Brasileira de Engenharia de Pesca
Diretrizes para autores

OBJETIVO - A Revista Brasileira de Engenharia de Pesca (REPesca) tem por objetivo publicar trabalhos que abordam temas de interesse na área de Recursos Pesqueiros e Engenharia de Pesca, do Brasil e de outros países do mundo.
INFORMAÇÕES GERAIS - Os originais devem ser redigidos em português, inglês ou espanhol, de forma concisa, com a exatidão e a clareza necessárias à sua fiel compreensão. Devem ser enviados ao site da REPESCA http://ppg.revistas.uema.br/index.php/REPESCA/index, de acordo com estas normas, donde serão enviados a dois consultores, especialistas no assunto e podem ser:Artigos, Trabalhos Técnicos, revisões e Resenhas, com os seguintes itens:
1. Artigos: contribuições destinadas a divulgar resultados de pesquisas científicas originais concluídas devem conter no máximo 15 páginas. Devem conter os seguintes itens: Resumo (+ Palavras-chave), Abstract (+ Keywords), Introdução, Material e Métodos, Resultados e Discussão (estes dois juntos ou separados), Conclusões (opcional), Agradecimentos (opcional) e Referências.
Trabalhos Técnicos: contribuições que relatam experiência ou trabalhos desenvolvidos por pessoas ou instituições da área e devem conter no máximo 15 páginas; Devem conter os seguintes itens: Resumo (+ Palavras-chave), Abstract (+ Key words), Introdução, Corpo (desenvolvimento do assunto) Conclusões (denominados de Comentários Conclusivos ou Finais, Considerações Finais), Agradecimentos (opcional) e Referências (quando houver citações no texto).
Resenhas: contribuições diversas, como relatos de experiências, estudo de casos, análises de fatos, reflexões, etc., com no máximo 10 páginas. Deve conter os seguintes itens: Resumo e/ou Abstract relato (+ Bibliografia, opcional - se houver citação).
Obs: Os nomes dos itens devem ser escritos em Versalete (caixa alta) em negrito, com apenas a primeira letra maiúscula. Os subitens também em Versalete, porém sem negrito.
APRESENTAÇÃO DO MANUSCRITO - As contribuições devem ser digitadas no Word, em papel “Carta/Letter”, com letra Times New Roman, 12, e em espaço 1,5 (entre linhas), com exceção do Resumo e Abstract (espaço simples), com margens de 2 cm em todos os lados, justificado e sem divisão de palavras no final da linha. Nomes científicos e palavras estrangeiras devem ser grafados em “itálico”.
Título: deve apresentar a idéia precisa do conteúdo, ser sucinto e explicativo escrito em letras maiúsculas, tamanho 12, centralizado e em negrito. Title: o título em inglês será da mesma forma, porém escrito com apenas a primeira letra maiúscula, em versalete (caixa alta), centralizado e em negrito.
Autor(es): devem constar sempre na sua ordem direta, sem inversões, com o sobrenome maiúsculo. Segue-se aos autores os endereços institucionais e após o e-mail do autor correspondente.
Ciro Mendes CASTOR1*; José Mário BRAGA2 & Maria da Penha PIRILO1
1Departamento de Educação, Universidade Federal de Carolina
2Instituto de Pesca de Carolina
*email: ciromc@ymail.com
Resumo: deve ser escrito em espaço simples inserto na primeira página (máximo 300 palavras), e sumariar o objetivo, os resultados e as conclusões.
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Abstract: versão fidedigna do resumo (máximo 300 palavras), deve ser precedido do título em inglês. Nunca utilize tradutores da Internet.
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Anais
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MATERIAL ILUSTRATIVO - As tabelas e figuras devem se restringir ao necessário para o entendimento do texto, numeradas em algarismos arábicos. As figuras devem ser “inseridas” no texto e nunca “recortadas” e “coladas”, devem ser de tamanho compatível, para não perder a nitidez quando reduzidas devem ser agrupadas, sempre que possível. As tabelas devem ser feitas com utilização da ferramenta Tabela do “Word”. As legendas devem ser auto-explicativas, em espaço simples, colocadas acima nas tabelas e abaixo nas figuras. Símbolos e abreviaturas devem ser definidos nas legendas.
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Algal research

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Types of articles
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International Journal of Aquatic Biology

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Agusa T., Kunito T., Tanabe S., Pourkazemi M., Aubrey D.G. (2004). Concentrations of trace elements in muscle of sturgeons in the Caspian Sea. Marine Pollution Bulletin, 49: 789-800.
<ins>Book</ins>
Townsend C.R., Begon M., Harper J.L. (2003). Essentials of ecology, 2nd. Blackwell Science publishing. 530 p.
Cooke G.D., Welch E.B., Peterson S.A., Nichols S.A. (2005). Restoration and management of lakes and reservoirs. CRC Press. Florida, 591 p.

Book Section
Coutteau P. (1996). Micro-algae. In: P. Lavens, P. Sorgeloos (Ed.). Manual on the production and use of live food for aquaculture, FAO Fisheries Technical Paper. No. 361. Rome, FAO. pp: 7-47.
Conference Paper
Cellario C., George S. (1990). Second generation of *Paracentrotus lividus* reared in the laboratory: Egg quality tested. In: C.d. Ridder, P. Dubois, M.C. Lahaye, M. Jangoux (Ed.). Echinoderm research: proceedings of the second European conference on Echinoderms. Brussels, Belgium, Balkema, Rotterdam. pp: 65-70.
Thesis
Clarke M. (2002). The effect of salinity on distribution, reproduction and feeding of the starfish *Coscinasterias muricata*(Echinodermata: Asteroidea) in a rocky subtidal community of a New Zealand fiord. M.Sc. thesis, Department of Marine Science, University of Otago. 84 p.
Webpage
Wray G.A. (1994). Echinodermata. Available from: www.tolweb.org. Retrieved 3/19/2004.

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Tables should be numbered and cited in the text, for example: Table 1. All tables should have a caption above the table ending to a “.”. All tables should be inserted at the end on main text. The authors can indicate the insertion place of tables in the text by putting the table's number in the bracket, for example [Table 1].

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11. New description of fish species

Since the **International Journal of Aquatic Biology** is published by the Iranian Society of Ichthyology, therefore based on its scopes, description of new fish species has been provided. For this purpose, all new descriptions must follow the guidelines provided by the International Commission on Zoological Nomenclature (ICZN).

Latin abbreviations such as gen. nov., sp. nov., ssp. nov., syn. nov., and comb. nov. must follow new taxa, synonymies or new combinations.

The complete data of the types, name bearing (e.g., holotype, lectotype, syntype) or otherwise (e.g., paratypes, topotypes), and the name of the depository should be provided in the original description.

Type material should contain the following details: registration number, sex, date of collection, exact name of the site of collection, broader area name, District/Province/State, Country, latitude longitude

information of the site of collection, and collector’s name. Manuscript will not be accepted if the registration numbers and name of the depository are not mentioned.
For example: Holotype: GUIC CC1462MA; 68.4 mm SL, Iran, Hamedan prov., Gamasiab River at Dehno, a tributary to Karkheh, 34°10’15"N, 48°21'19"E, altitude 1610 m, 20 September 2011, S. Vatandoust, H. Mousavi-Sabet. Paratypes: GUIC CC1462M; 6, 65.2–98.2 mm SL; same data as holotype; FSJF 3225; 18, 26–37 mm SL, Iran, Hamedan prov., Gamasiab River south of Habibabad, a tributary to Karkheh, 34°16'54" N, 48°09'26"E.

Engenharia Sanitária e Ambiental

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- a) publication of more than 70% of scientific articles - At the moment the Magazine presents at least 5 original articles of scientific nature (article) and technical nature (technical summary) in each issue. In addition, topics of extreme interest to scientific circles are disseminated, which originate the "Books", "Technical News" and opinion articles' sections.

- b) the selection of articles - is based on the "Regulations for the Publication of Articles" with opinions presented by at least four referees, who are members of the Scientific Editorial Counsel (see attached regulations). The referees, based on the attached form, indicate the need for revision of the text, the inclusion of aspects, or the non-acceptance of the article. The suggestions are forwarded to the author for consideration and revision.

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- d) dedication to the thematic area: Papers in the Sanitary and Environmental Engineering Magazine are dedicated to the area, which is indicated in the title of the publication.

-.e) number of articles published: The Scientific Editorial Counsel opted to publish at least 12 articles per issue, as of the second semester of 1997.

- The articles must include the date of receipt by the Editorial Counsel of the version which has been revised by them, the authors' addresses for correspondence, as well as the title of the article and the name of the author in the heading of the page

- Titles, the abstract and key words are published in English and the text is normally edited in Portuguese.

- The summary of each author's *curriculum* is published together with the article showing education details and the institution with whom the author is affiliated.

- f) scope of activity - The magazine is not to be seen as a departmental institutional or regional publication, which receives articles from all over the Country, from researchers affiliated to different institutions.

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