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EFFECTS OF NUTRIENT DEPLETION ON THE GROWTH AND CELL CONCENTRATION OF *Cylindrospermopsis Raciborskii*

*Mário Ubirajara Gonçalves Barros[1]
Ismael Keslley Carloto Lopes[1]
Wladimir Ronald Lobo Farias[2]
José Capelo Neto[1]

EFEITOS DA DEPLEÇÃO DE NUTRIENTES NO CRESCIMENTO E NA DENSIDADE DE CÉLULAS DA ESPÉCIE Cylindrospermopsis Raciborskii

Abstract

Eutrophication damages water supply by promoting the proliferation of potentially toxin-producing cyanobacteria. In Ceara State, the abundance of these algae in artificial reservoirs has been reaching up to 95% phytoplankton density of cells. The knowledge of the species growth dynamics depending on the availability of nutrients can promote the understanding of a recurring natural phenomenon, the cyanobacteria blooms. This study aimed to evaluate the influence of macronutrient depletion on the development of *C. raciborskii* T3 cultures. Experiments were conducted in ASM-1 medium and variations with the removal of 75 and 50% phosphorus and nitrogen from its original composition. Cultures were grown in non-axenic conditions, under constant light of 6.75 $\mu.mol.m^{-2}.s^{-1}$, 12: 12 photoperiod, and at temperature of 24 ± 2°C. Nitrogen-depleted cultures clearly showed lower growth in comparison with other experiments, reaching the stationary phase earlier, besides lower cell concentrations. Whereas, phosphorus-depleted cultures presented steeper growth curves, similar to the growth registered in the regular ASM-1 medium, and thereby demonstrating that under these experimental conditions, nitrogen was the limiting nutrient for the growth of *C. raciborskii*.

Key words: cyanobacteria, limiting nutrients, reservoirs.

[1] Centro de Tecnologia, Universidade Federal do Ceará, Brasil.
[2] Centro de Ciências Agrárias, Universidade Federal do Ceará, Brasil.
Autor corresponsal: Departamento de Engenharia Hidráulica e Ambiental, Bloco 710, Centro de Tecnologia, Universidade Federal do Ceará, Av.Mister Hull S/N, Bairro: Campus do pici, Fortaleza, Ceará, Cep: 60455-900, Brasil.
Email: mariobarros86@hotmail.com

Resumo

A eutrofização da água prejudica o abastecimento público pelo fato de favorecer a proliferação de cianobactérias potencialmente produtora de toxinas. No estado do Ceará, a concentração de cianobactérias, em reservatórios artificiais, tem atingido até 95 % da concentração celular do fitoplâncton. O conhecimento da dinâmica de cresimento das espécies em função da disponibilidade de nutriente pode favorecer a compreenção do um fenômeno natural recorrente, as florações de cianobactérias. O presente trabalho teve como objetivo realizar uma avaliação sobre a influência da depleção de macronutrientes no desenvolvimento de culturas da *C. raciborskii* T3. Os experimentos foram realizados utilizando-se como meio o ASM-1 e variações do mesmo com a retirada de 75 e 50% de fósforo e nitrogênio da sua composição inicial. As culturas foram desenvolvidas em condições não axênicas, mantidas sob intensidade luminosa constante de 6,75 $\mu.mol.m^{-2}.s^{-1}$ submetidas à fotoperíodo de 12h:12h e temperatura de 24±2 °C. Os resultados mostraram que as culturas com depleção de nitrogênio apresentaram nitidamente menor crescimento com relação aos outros experimentos, atingindo a fase estacionária mais cedo e com menores concentrações celulares. Já as culturas com depleção de fósforo apresentaram curvas de crescimento mais acentuadas similares ao crescimento utilizando o meio ASM-1, demonstrando assim que, nas condições experimentais adotadas, o nitrogênio foi limitante para o crescimento da *C. raciborskii*.

Palavras chave: cianobactérias, nutrientes limitantes, reservatórios.

Introduction

Water quality is influenced by numerous natural factors (biological, geological, hydrological, meteorological and topographical). These factors interact in the watersheds of lakes, rivers and estuaries and may vary seasonally according to different conditions of weather, volumes of flows and water levels. Eutrophication of water supply reservoirs is a remarkable problem in the management of these water resources. The causes of anthropic eutrophication are closely related to nutrient concentration, mainly phosphorus and nitrogen, which reach the water bodies from activities like discharge of sewage, agriculture, fish farming and other activities in the watershed. In this sense, eutrophication can result in the degradation of water quality, with occurrence of algal blooms, oxygen deficits, unpleasant odors and excessive growth of macrophytes. (Cogerh, 2008).

Cyanobacteria blooms have been a serious concern for the Brazilian authorities. In a hemodialysis center in Caruaru, Pernambuco State, 110 patients showed symptoms of poisoning related to hepatoxin after routine hemodialysis. After, 100 patients developed acute liver failure and 70 died (Azevedo *et al.*, 1996; Carmichael, 1996). Studies have indicated that the toxins produced were the cause of death.

According to Ferreira (2008), on account of the climate and the proximity of Equator, Ceará holds a set of optimal conditions for the development of phytoplankton, such as high light incidence, with approximately 12 hours of light per day throughout the year; high temperatures, which accelerate the absorption of nutrients by algae and high nutrient assimilation capacity, associated with high rates of recycling, besides being shallows aquatic systems.

Several reservoirs were constructed in the south and southeastern regions of Brazil for power generation. Whereas in the northeastern region, the main purposes were distribution of drinking water, irrigation and fishing. In freshwater ecosystems in the Brazilian semiarid, water level fluctuations are pronounced because of the low rainfall and high evaporation levels. Thus, the concentrations of nutrients available for the growth and survival of phytoplankton species are increased, especially for cyanobacteria (Chellappa; Medeiros Costa, 2003).

The dominance of some species in a phytoplankton community depends on a complex of physical, chemical and biological factors. Cyanobacteria are commonly found in many eutrophic reservoirs and the blooming of these microorganisms is also responsible for the deterioration of the aquatic environment. The identification of factors that promote the rapid growth of these species is a crucial issue for the effective management of reservoirs (COGERH, 2008).

The occurrence of cyanobacterial blooms in human water supply sources is a serious problem both ecological as public health, because of the potential production of toxic compounds. Therefore, research is needed to expand the knowledge concerning its physiological control mechanisms, especially those related to its population growth and toxicity (Cogerh, 2008).

In Brazil, cyanobacteria strains isolated from blooms have been described in different states: Rio Grande do Sul (Yunes *et al.*, 1994), São Paulo (Azevedo *et al.*, 1996; Zagatto, 1995), Distrito Federal (Branco; Senna, 1994), Minas Gerais (Jardim *et al.*, 1999), Pernambuco (Bouvy *et al.*, 1999), Rio de Janeiro (Magalhães; Soares; Azevedo, 2001), Para (Vieira *et al.*, 2005) and Ceará (Carloto, 2013).

Cylindrospermopsis raciborskii is a filamentous cyanobacteria species that contains gas vesicles, being capable of performing migrations to the deeper layers of the water column, thus having the ability to develop at low light intensities, around μmol.photons m^{-2}.s^{-1} (CARNEIRO *et al.*, 2009). It is a highly successful competitor in aquatic systems, and various features support this ability: high affinity for phosphorus and storage capacity of this nutrient, fixation of atmospheric nitrogen, high affinity for ammonia, ability to form akinets, enabling easy dispersion and environmental resistance (Briand *et al.*, 2004). According to the same author, it has a wide thermal tolerance and allelopathic interference.

C. raciborskii has occurred in Australia (Mcgregor; Fabbro, 2000), Thailand (Li *et al.*, 2001), South America (Figueredo; Giani, 2009) and Africa (Haande *et al.*, 2008). There is growing evidence of increase in toxic *C. raciborskii* in temperate waters. According to Briand *et al.* (2004), this spread of cyanobacteria can be attributed to climate change resulting in increased water temperature, which favors their proliferation. In this way, many countries of the northern hemisphere, such as Hungary (Neilan *et al.*, 2003), France (Gugger *et al.*, 2005), Portugal (Neilan *et al.*, 2003), Austria (Dokulil; Mayer, 1996), Serbia (Sanja, 2011), Poland (Kokocinski *et al.*, 2010) and Italy

(Messineo *et al.*, 2010) have reported constant blooms of *C. raciborskii*. In Brazil, specifically in the Ceará State, Barros (2013) recorded the occurrence of *C. raciborskii* in all reservoirs investigated, with dominance in three reservoirs: Acarape do Meio Reservoir (64%), Serafim Dias Reservoir (60 %) and Coronel Reservoir (73%). In this context, the present study aimed to assess the influence of macronutrient depletion on cell growth of *C. raciborskii* cultures in order to increase the knowledge of the dynamics of this species according to the availability of nutrients in inland freshwater ecosystems.

Material and methods

The strain cultivated was *Cylindrospermopsis raciborskii* T3 (Lagos *et al.*, 1999) which was isolated from Billings Reservoir, in Taquacetuba, São Paulo State, in 1996, during a bloom. It is part of the collection of the Laboratory of Ecophysiology and Toxicology of Cyanobacteria, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, kindly donated by Dr. Sandra Maria Feliciano de Oliveira Azevedo. LC-MS chromatographic analysis showed that this strain can produce STX, NeoSTX, decarbamoil-STX and decarbamoil-NSTX (Carneiro *et al.*, 2009).

Experiments were conducted with the ASM-1 medium as a control culture. This method was proposed by Gorham *et al.*, 1964 and modified to encompass a variability of macronutrient composition, with pH adjusted to 8.0. Strains were maintained under a light intensity of approximately 6,75 $\mu.mol.m^{-2}.s^{-1}$ for white light at 470 nm, measured with a light meter (DIGITAL LUX TESTER YF-1065), at 24 ± 2°C without aeration and with a 12:12 light/dark photoperiod.

The amounts described in Table 1 were used in preparing of 1 liter of ASM-1 medium. The stock solutions were made according to the composition shown in the same table, with four different stocks solutions: A, B, C, and D.

Variations of nutrients were calculated to simulate the different conditions of eutrophication, through the reduction in nitrogen or phosphorus concentrations by 50 or 75% compared to those originally contained in the ASM-1, in order to observe the influence on cell growth (Table 2).

According to Carneiro *et al.*, 2009, *C. raciborskii* requires a period of five generations to be adapted to the new experimental conditions. The adaptation consisted of transferring 5 mL of the original strain grown at cell concentration of 10^7 cel.mL^{-1} to five 25 mm test tubes, each containing 1 mL of the original culture and 10 mL of ASM-1 culture medium modified in various levels of nutrient depletion (Castro *et al.*, 2004). At the end of the adaptation, cultures were inoculated at an initial concentration of 10^5 cel.mL^{-1}, in triplicate.

Tabela 1. Composition and volume needed of each stock solutions that make up the ASM-1 medium

	Weight(g)	Make up to	Amount used to 1L of ASM- 1 medium
Stock solution A			
NaNO$_3$	1.7		
MgCl$_2$.6H$_2$O	0.41	200 mL	20 mL
MgSO4.7H$_2$O	0.49		
CaCl$_2$.2H$_2$O	0.29		
Stock solution B			
K$_2$HPO$_4$ ou	0.87		
K$_2$HPO$_4$ .3H$_2$O	1.14	100 mL	2.0 mL
Na$_2$.HPO$_4$.12H$_2$O	1.78		
Stock solution C			
H$_3$BO$_3$	2.48		
MnCl$_2$. 4 H$_2$O	1.39		
FeCl$_3$.6H$_2$O	1.08	100 mL	0.1 mL
ZnCl$_2$	0.335		
CoCl$_2$.6H$_2$O	0.019		
CuCl$_2$	0.0014		
Stock solution D			
EDTA Na$_2$	1.86	100 mL	0.4 mL

Table 2. Modifications made in the original concentration of the limiting nutrients (N and P)

Stock solution A	Weight (g)	Make up to	↓50% P	↓75% P	↓50% N	↓75% N
NaNO$_3$	1.7	200 mL			0.85	0.425
Stock solution B	Weight (g)	Complete to				
K$_2$HPO$_4$	0.87	100mL	0.435	0.2175		
Na$_2$.HPO$_4$	0.63		0.315	0.1575		
Ratio N:P			13.8:1	28.8:1	3.4:1	1.6:1

The use of absorbance or optical density to evaluate the phytoplankton growth is based on physical obstruction of light by the cells. The more cells present in the sample, the greater the light absorption (absorbance) and the lower the light passing through the sample (transmittance). These two variables, absorbance at 680 nm and cell counts were subjected to a linear correlation to obtain a linear regression equation by the formula $Y = a \cdot X + b$ (Figure 1), where **Y** is the cell Concentration (cel mL^{-1}), **X** is the optical density (OD$_{680nm}$); **b** is the slope and **a** is the linear coefficient (XU *et al.*, 2006). For this analysis, we used the spectrophotometer Hach DR-2000.
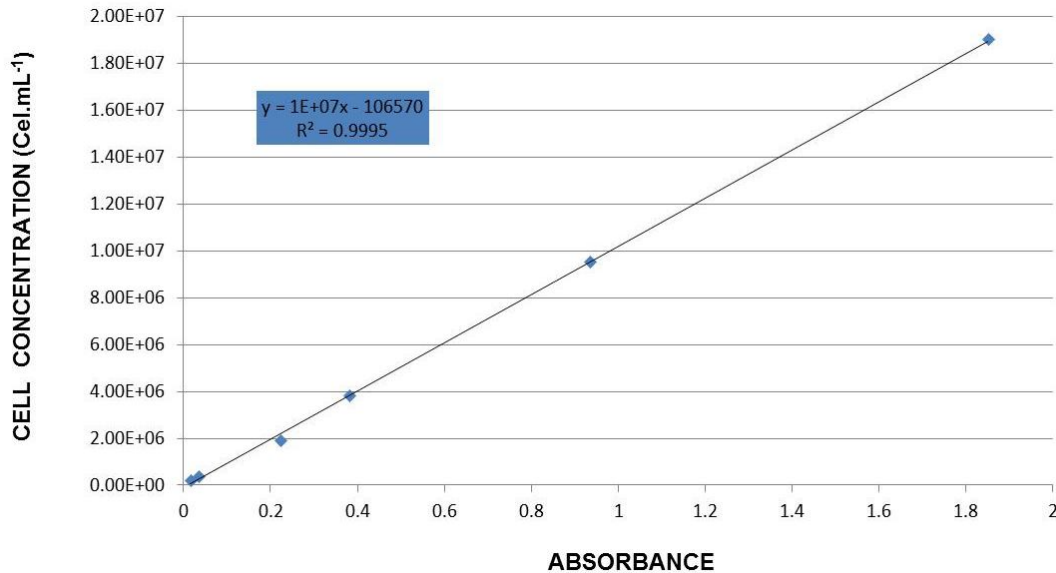
Figure 1. Correlation between absorbance and cell concentration at 680 nm

Cell counts for this correlation were determined by optical microscopy with the aid of a micrometer eyepiece and the Improved Neubauer hemocytometer, following the methodology proposed by Pollard and Young (2010). Counts were performed in the central, lower and upper areas of the Neubauer improved chamber and if the average for the three counts did not present a greater deviation than 10%, the results would be accepted. For this procedure the strain was fixed with 1:1 Lugol, , approximately 0.05 mL. After observing a strong correlation an amount of 10 ml were used for spectrophotometer readings, thus determining the abundance of cells.

The determination of optical density for the cultures was carried out according to Sipaúba Tavares and Rocha, (2001), using the equation:

$$V_I = V_{meio}.D_f/(D_I - D_f)$$
<div align="right">**Equation (1)**</div>

Where:
V_I - volume of the inoculum
V_{meio} – volume of the medium
D_I – optical density of the inoculum
D_f – initial optical density desired.

The cultures were performed in triplicate and timeless. First, 45 ml of a pre-adapted culture, with known density of cells, were transferred into 500 ml Erlenmeyer flasks containing 450 ml of original ASM-1 and the same procedure was performed for all ASM-1 modified media. After that, aliquots were taken for checking the cell abundance. The optical density was approximately 10 ml. (Raynouds 1978 and Castro *et al.*, 2004).

Mean values of cell counts, obtained by optical absorbance, were used to draw growth curves. From these curves the growth phases were identified and the maximum cell concentrations (MCC) and growth rates (K) were obtained from the log phase (Ohse *et al.*, 2008). Growth rates (K) were calculated according to equations described in hereafter (Fogg; Thake 1987).

$$K = (\ln N_2 - \ln N_1)/(t_2 - t_1) \qquad \text{Equation (2)}$$

Where:
K – Growth rate
N_2 and N_1 – number of cells/mL at times t_2 and t_1.

For testing the differences between maximum cell concentrations and growth rates in the different concentrations of limiting nutrients, data were subjected to Student's t-test in Microsoft Excel. In all analyses, the significance level was set at 5%.

Results and Discussion

Regarding the growth rate, the control ASM-1 showed a significant difference from the treatment with 75% nitrogen depletion ($p < 0.05$), with mean growth rate of 0.107 and 0.049 day^{-1}, respectively (Table 3). The control (ASM-1) also differed ($p < 0.05$), from the 50% nitrogen depletion treatment, with growth rates of 0.107 and 0.077 day^{-1}, respectively

Table 3. Kinetic parameters of *C. raciborskii* T3 cultures

Culture	Growth rate	MCC*
ASM-1	0.107	2.32×10^7
75%N	0.049	7.58×10^6
50%N	0.077	1.27×10^7
75%P	0.081	2.27×10^7
50%P	0.080	2.26×10^7

MCC- Maximum cell concentration

Unlike that observed in the present study, Smith and Haney (2006) concluded that the stratification in the water column, low concentrations of nitrogen and low water transparency favor the dominance of *Cylindrospermopsis raciborskii*. Morever, Mischke, 2003 noted that the growth of *Microcystis aeruginosa* is inhibited with increasing concentration of nitrate, which was not observed for *C. raciboskii* studied here. In the same way, Kosten *et al.* (2009) reported that the dominance of *Planktothrix agardii* in reservoirs may be related to the thermal stratification of the water column, affinity for nitrogen and low phosphate concentrations.

In agreement with Vasconcelos *et al.* (2011), in most of the reservoirs, after the period of dominance, *Planktothrix agardii* is replaced by *C. raciborskii*. This alternation is due to decreased availability of nitrogen. These authors also emphasized that the presence of heterocysts in *C. raciborskii* and its ability to fix nitrogen favor the dominance of this species. Menéndez, 2005 asserted that moderate levels of nitrate can increase the photosynthesis rate of the green algae *Chaetomorpha linum*. Likewise Leong *et al.* (2004) found a positive correlation between nitrate concentration and *Alexandrium tamarense* cell density, indicating the peculiarity of this species in relation to concentration of limiting nutrients.

The control ASM-1 was significantly different ($p < 0.05$) from the experiment with 75% phosphorus depletion, with mean growth rates of 0.107 and 0.081 day^{-1}, respectively (Table 3). Also, the control was significantly different ($p < 0.05$) from the experiment using 50% phosphorus depletion, with growth rates of 0.107 and 0.080 day^{-1}, respectively.

Wu *et al.* (2012) cultivated *C. raciborskii* with five different levels of phosphorus (0.00; 0.02; 0.05; 0.50 and 1.00 mgL^{-1}) and observed significantly higher growth rates under high concentrations of phosphorus. With phosphorus concentration of 0.05 mgL^{-1}, the abovementioned authors registered growth rates similar to those verified in the experiments with phosphorus depletion (75 and 50%).

Furthermore, Jacobs (1995) found that phosphorus deficiency leads to a lower electron transport rate and ineffective functioning of photosynthesis. For Gillor *et al.* (2002), under phosphorus shortage, phytoplankton can produce alkaline phosphatase to hydrolyze organic phosphorus, compensating the deficiency in the environment. According to Wu *et al.*, 2012, *C. raciborskii* is able to regulate its physiology to acclimate to an environment with phosphorus concentration below 0.05 mg.L^{-1}, through a decrease in the growth rate and photosynthetic activity and compensated by increased activity of alkaline phosphatase and catalase.

The experiments with 50 and 75% nitrogen depletion were significantly different ($p < 0.05$), with growth rates of 0.077 and 0.049 day^{-1}, respectively. In the same way, experiments with 75% nitrogen depletion and 75% phosphorus depletion were also significantly different ($p < 0.05$),

with growth rates of 0.049 and 0.081 day^{-1}, respectively. However, the experiments performed with 75 and 50% phosphorus were not statistically different ($p>0.05$), with growth rates of 0.081 and 0.080 day^{-1}, respectively.

When compared the maximum cell concentrations (MCC) of the culture ASM-1 with those of the cultures with 75 and 50% nitrogen depletion, it has been detected a significant difference between them and the control culture ($p<0.05$), with MCC of 2.32 x 10^7; 7.58 x 10^6 and 1.27 x 10^7 cel.mL^{-1}, respectively. Comparing the maximum cell concentrations (MCC) of the control ASM-1 with those of the cultures with 75 and 50% phosphorus depletion, no significant difference was found ($p>0.05$), with MCC of 2.32 x 10^7; 2.27 x 10^7; 2.26 x 10^7 cel.mL^{-1}, respectively. With a less steep growth curve, the culture with 75% nitrogen depletion reached earlier the stationary phase, compared with the other cultures (Figure 2). The culture with phosphorus depletion exhibited lower growth rates compared with the control. But the maximum cell concentrations showed no statistical differences ($p>0.05$) compared with the ASM-1 control.
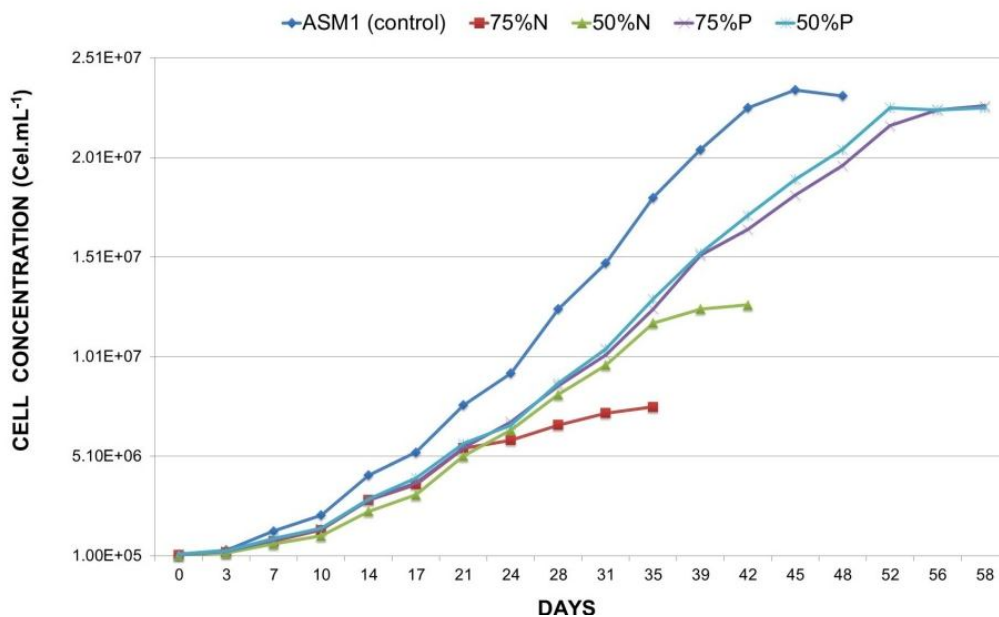

Figure 2. Growth curve of *Cylindrospermopsis raciborskii* subjected to five different N: P ratios

The growth curves of the cultures (ASM-1, 75% P, 50%P, 75%N and 50%N) showed quite similar induction phases, especially until the tenth day of growth. The log or exponential phase was different among the different culture media. The control culture exhibited a steeper growth curve, while the culture grown under 50% nitrogen depletion showed a less steep growth curve.

From the day ten to the twenty-one, differences between the growth curves were more notable with greater emphasis for the culture performed with the ASM-1 medium. The culture with 75% nitrogen depletion presented a marked reduction in growth, differing from the other curves with a slower growth. This latter culture reached the stationary phase on the 35th growth day with a maximum cell concentration of 7.58 x 10^6 cel.mL^{-1}.

Briand *et al.* (2002) stated that *C. raciborskii* has heterocysts, which are used for fixing atmospheric nitrogen. Nevertheless, this species has few heterocysts and low capacity for nitrogen storage and thus is less dependent on these structures to obtain nitrogen. This observation may explain the limitations to growth imposed by nitrogen deficiency, mainly in the culture with 75% nitrogen depletion. In other words, in closed culture systems, this species grows faster using ammonium ion or nitrate than the source of atmospheric nitrogen (Saker; Griffiths; 2000) and Hawkins *et al.* 2001).

The culture with 50% nitrogen depletion showed a growth curve slightly different from that of the cultures under phosphorus depletion, seen from the seventh to the thirty-fifth day. From that day on, this dissimilarity became more pronounced, and was clearly perceived by the less steep growth curve. On the forty-second day of growth, the culture with 50% nitrogen depletion reached the stationary phase with maximum cell concentration of 1.27 x 10^7 cel.mL^{-1}.

Cultures with phosphorus depletion (75 and 50% P) demonstrated similarities from the late induction phase to the 39th day. Thereafter, until the fifty-second day, there was a minimal distinction with higher concentrations for the curve with 50% phosphorus depletion relative to 75 % phosphorus depletion. On the day 56, the cultures reached approximately the same cell concentration, reaching stationary phase of growth with the respective concentrations of 2.27 x 10^7 and 2.26 x 10^7 cel.mL^{-1}. According to Briand *et al.* (2002), *C. raciborskii* is able to grow under low concentrations of phosphorus, due to adaptations in its cellular structure. These authors argued that owing to this mechanism of phosphorus storage, this species is well adapted to the temperate climate. In turn, Istvánovics *et al.* (2000) and Shafik *et al.* (2001) affirmed that *C. raciborskii* has high affinity and capacity of storage of phosphorus compared with other cyanobacteria, which may explain the low impact of limiting this nutrient when compared to cultures under nitrogen limitation.

From the late induction phase, the control culture with the ASM-1 culture medium showed a clear difference with respect to other cultures (75% N, 50% N, 75% P and 50% N), with higher cell concentrations in most of the period. This culture reached its stationary phase of growth in the forty-fifth day, with a maximum cell concentration of 10^7 cel.mL^{-1}. From that day on, the culture reached the senescence stage. A rather unique feature of this culture was the short stationary phase which was not observed in the other cultures.

Conclusions

In summary, the cultures grown with 50% and 75% nitrogen depletion demonstrated a growth rate and MCC significantly lower than the control ASM-1. Thus, it is inferred that a N:P ratio lower than 7:1 may impose a strong limitation to the *Cylindrospermopsis raciborskii* growth. Cultures made with 75 (N:P = 28.8:1) and 50% (N:P = 13.8:1) phosphorus depletion showed a growth rate significantly different from the control culture (ASM-1) reaching later the stationary phase. Meantime, the maximum cell concentrations were statistically similar compared with the control. Our results point out to the idea that *Cylindrospermopsis raciborskii* might be more susceptible to nitrogen than to phosphorus limitation, leading us to a change in the interpretation of environmental monitoring data using physico-chemical parameters in artificial reservoirs of the Brazilian semi-arid region.

References

Azevedo, S.M.F.O., Evans, W.R., Carmichael, W.W., Namikoshi, M. (1996) First report of microcystins from a Brazilian isolate of the cyanobacterium *Microcystis aeruginosa*, *J. Appl. Phycol.* **6**, 261–265.

Branco, C.W.C., Senna, P.A.C. (1994) Factors influencing the development of *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* in the Paranoá Reservoir, Brasília, Brazil, *Algological Studies*, **75**, 85-96.

Barros, M.U.G. (2013) *Prospecção de Cylindrospermopsis raciborskii em reservatórios no Ceará e efeitos da depleção de nutrientes na sua concentração celular*, Tese de mestrado, Universidade Federal do Ceará, Programa de Pós-graduação em Engenharia Civil (Recursos Hídricos), Departamento de Engenharia Hidráulica e Ambiental, 100 pp.

Briand, J.F., Robillot, C., Quiblier-Lloberas, C., Humbert, J.F., Coute, A., Bernard, C. (2002) Environmental context of *Cylindrospermopsis raciborskii* (Cyanobacteria) blooms in a shallow pond in France, *Water Research*, **36**(13), 3183-3192.

Briand, J.F., Leboulanger, C., Humbert, J.F., Bernard, C., Dufour, P. (2004), *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: Selection, wide physiological tolerance, or global warming?, *J. Phycol*, **40**, 231–238.

Bouvy, M., Molica, R., Oliveira, S., Marinho M., Beker, B. (1999) Dynamics of a toxic cyanobacterial bloom (*Cylindrospermopsis raciborskii*) in a shallow reservoir in the semi-arid region of northeast Brazil, *Aquatic Microbial Ecology*, **20**, 285-297.

Carmichael, W.W. (1996) Liver failure and human deaths at a hemodialysis center in Brazil: microcystins as a major contributing factor, *Harmful Algae News*, **15**, 11.

Carneiro, R.L., Santos, M.E.V., Pacheco, A.B.F., Azevedo, S.M.F.O. (2009) Effects of light intensity and light quality on growth and circadian rhythm of saxitoxins production in *Cylindrospermopsis raciborskii* (Cyanobacteria), *Jornal of plankton research*, **1**(1), 1-8.

Carloto, I.K.C.L (2013*) Identificação de cianobactérias produtoras de satoxinas em reservatorios de usos múltiplos no semi-arido cearense*, Tese de mestrado, Universidade Federal do Ceará, Programa de Pós graduação em Engenharia Civil (Recursos Hídricos), Departamento de Engenharia Hidráulica e Ambiental, 85 pp.

Castro, D., Vera, D., Lagos, N., Gárcia, C., Vásquez, M. (2004). The effect of temperature on growth and production of paralytic shellfish poisoning toxins by the cyanobacterium *Cylindrospermopsis raciborskii C10*, *Toxicon*, **44**, 483-489.

Chellappa, N.T., Medeiros Costa, M.A. (2003) Dominant and co-existing species of cyanobacteria from a eutrophicated reservoir of Rio Grande do Norte State, Brazil, *Acta Oecologica*, **24**, 3-10.

COGERH, Companhia de Gestão de Recursos Hídricos, (2008) *Rede de monitoramento da qualidade de água,* Governo do Estado do Ceará, Fortaleza, 1-6.

Dokulil, M.T., Mayer, J. (1996) Population dynamics and photosynthetic rates of a *Cylindrospermopsise Limnothrix* association in a highly eutrophic urban lake, Alte Donau, Vienna, Austria, *Algological Studies*, **83**(1), 79 - 185.

Figueredo, C.C., Giani, (2009) A. Phytoplankton community in thetropical lake of Lagoa Santa (Brazil): conditions favoring a persistent bloom of *Cylindrospermopsis raciborskii, Limnologica e Ecology and Management of Inland Waters,* **39**(4), 264-272.

Foog, G.E. Thake. (1987) *Algae cultures and phytoplankton ecology.* The University of Wisconsin Press, Ltd., London. Third Ed. 269 pp.

Gillor, O., Hadas, O., Post, A.F., Belkin, S. (2002) Phosphorus bioavailability monitoring by a bioluminescent cyanobacterial sensor strain, *J. Phycol.*, **38**, 107-115.

Gorham, P.R., Maclachlav, J.R., Hammer, V.T., Kim, W.K. (1964) Isolation and culture of toxic srains of *Anabaena flos-aquae* (Lyngb.) de Bréb, *Verh. Int. Vereinigung für Theoretische und Angewandte Limnologie*, **15**, 796-804.

Gugger, M., Molica, R., Le Berre, B., Dufour, P., Bernard, C., Humbert, J.F. (2005) Genetic diversity of *Cylindrospermopsis* strains (Cyanobacteria) isolated from Four continents, *Environmental Microbiology*, **71**(2), 1097-1100.

Haande, S., Rohrlack, T., Ballot, A.R., Berg, K., Skulberg, R., Beck, M., Wiedner, C. (2008) Genetic characterisation of *Cylindrospermopsis raciborskii* (Nostocales, cyanobacteria) isolates from Africa and Europe, *Harmful Algae,* **7**(5), 692-701.

Hawkins, P.R.; Putt, E.; Falconer, I.; Humpage, A. (2001) Phenotypical variation in atoxic strain of the phytoplankter, *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) during batch culture, *Environ. Toxicol,* **16**, 460-467.

Istvanovics, V., Shafik, H.M., Presing, M., Juhos, S.Z. (2000) Growth and phosphate up take kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in through flow cultures, *Freshwater Biology*, **43**, 257-275.

Jacobs, J. (1995) Phosphate deficiency increases the rate constant of thermal dissipation of excitation energy by photosystem II in intact leaves of sunflower and maize, *Aust. J. Plant Physiol.* **22**, 417-424.

Jardim, F.A., Fonseca, Y.M.F, Azevedo, S.M.F.O. (1999) A ocorrência de *Microcystis viridis* e *Cylindrospermopsis raciborskii* tóxicas em um manancial da COPASA MG, *Anais da VIII Reunião Brasileira de Ficologia*, Porto de Galinhas – PE.

Kokocinski, M., Stefaniak, K., Mankiewicz-Boczek, J., Izydorczyk, K., Soininen, J. (2010) The ecology of the invasive cyanobacterium *Cylindrospermopsis raciborskii* (Nostocales,Cyanophyta) in two hypereutrophic lakes dominated by *Planktothrix agardhii* (Oscillatoriales, Cyanophyta), *European Journal of Phycology*, **45**(4), 365-374.

Kosten, S., Huzsar, V. L. M., Mazzeo, N., Scheffer, M., Jeppesen. E. (2009) Lake and watershed characteristics rather than climate influence nutrients limitation in shallow lakes, *Ecological applications,* **19**, 1791-1804.

LagoS, N., Onodera, H., Zagatto, P.A., Andrinolo, D., Azevedo, S.M.F.O., Oshima, Y. (1999) The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil, *Toxicon*, **37**, 1359-1373.

Leong, S.C.Y., Murata, A., Nagashima, Y., Taguchi, S. (2004) Variability in toxicity of the dinoflagellate *Alexandrium tamarense* in response to different nitrogen sources and concentrations, *Toxicon*, **43**, 407-415.

Li, R., Carmichael W.W., Brittain, S., Eaglesham, G.K., Shaw, G.R., Mahakhant, A., Noparatnaraporn, N., Yongmanitchai, W., Kaya, K.; Watanabe, M.M. (2001) Isolation and identification of the cyanotoxin cylindrospermopsin and deoxycylindrospermopsin from a Thailand strain of *Cylindrospermopsis raciborskii* (Cyanobacteria), *Toxicon,* **39**, 973-980.

Magalhães, V.F.; Soares, R.M.; Azevedo, S.M.O. (2001) Microcystin contamination in fish from the Jacarepagua Lagoon (Rio de Janeiro, Brazil): ecological implication and health risk, *Toxicon*, **39,** 1077-1085

Mcgregor, G.B.; Fabbro, L.D. (2000) Dominance of *Cylindrospermopsis raciborskii* (Nostocales, Cyanoprokaryota) in Queensland tropical and subtropical reservoirs: implications for monitoring and management. Lakes and Reservoirs*: Research and Management*, **5**(3), 195-205.

Menéndez, M. (2005) Effect of nutrient pulses on photosynthesis of *Chaetomorpha linum* from a shallow Mediterranean coastal lagoon, *Aquat Bot,* **82**, 181-192.

Messineo, V.; Melchiorre, S.; Di Corcia, A.; Gallo, P.; Bruno, M. (2010) Seasonal succession of *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* blooms with cylindrospermopsin occurrence in the volcanic Lake Albano, central Italy, *Environmental Toxicology*, **25**(1), 18-27.

Mischke, U. (2003) Cyanobacteria associations in shallow polytrophic lakes: influence of environmental factors, *Acta Oecologica*, **24**, 11-23.

Neilan, B.A., Saker, M.L.; Fastner, J.; Torokne, A., Burns, B.P. (2003) Phylogeography of the invasive cyanobacterium *Cylindrospermopsis raciborskii, Molecular Ecology*, **12**(1), 133-140.

Ohse, S.; Derner, R. B.; Ozório, R. A.; Braga, M. V. C.; Cunha, P.; Lamarca, C. P.; Santos, M. E. (2008) Crescimento de microalgas em sistema autotrófico estacionário, *Revista Biotemas, Florianópolis*, **21**(2), 7-18.

Pollard, P.C.; Young, L.M. (2010) Lake viruses lyse cianobactéria, *Cylindrospermopsis raciborskii*, enhances filamentous-host dispersal in Australia, *Acta oecologica*, **36**, 114-119.

Reynolds, C. S. (1978) Phosphorus and the eutrophication of lakes - a personal view. *Found Symp.* **57,** 201-228.

Saker, M.L.; Griffiths, D.J. (2000) The effect of temperature on growth and cylindrospermopsin content of seven isolates of the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszyinska) Seenayya and Subba Raju from water bodies in northern Australia, *Phycologia*, **39**(4), 349-354.

Sanja, C.M.F. The first finding of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju,(Cyanoprokaryota) in Serbia. *Archives of Biological Sciences*, **63**, 507-510.

Shafik, H.M., S. Herodek, M., Presing.L., Voros (2001) Factors effecting growth and cell composition of cyanoprokaryote *Cylindrospermopsis raciborskii*, *Algological Studies*, **103**, 75-93.

Sipaúba-Tavares, L.; Rocha, H. (2001*) Produção de plâncton (fitoplâncton e zooplâncton) para alimentação de organismos aquáticos*, São Carlos: Rima, 106 pp.

Smith, J.L., Haney, J.F. (2006) Foodweb transfer, accumulation, and depuration of microcystins, a cyanobacterial toxin, in pump kinseed sunfish (Lepomis gibbosus), *Toxicon,* **48**, 580–589.

Vasconcelos, J.F.; Barbosa, J.E.L.; Diniz, C.R.; Ceballos, B.S.O. (2011) Cianobactérias em reservatórios do Estado da Paraíba: ocorrência, toxicidade e fatores reguladores, *Boletim da Sociedade Brasileira de Limnologia*, **39**(2), 1-20.

Vieira, J.M.S., Azevedo, M.T.P., Azevedo, S.M.F.O., Honda, R.Y., Corrêa, B. (2005) Toxic cyanobacteria and microcystin concentrations in a public water supply reservoir in the Brazilian Amazonia region, *Toxicon,* **45**, 901-905.

Wu, Z., Zeng, B., Li, R., Song, L. (2012) Physiological regulation of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) in response to inorganic phosphorus limitation, *Harmful Algae*, **15**, 53-58.

Xu, H., Miao, X.L., Wu, Q.Y. (2006) High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters, *Journal of Biotechnology, Amsterdam*, **126**(4), 499-507

Yunes, J.S.; Niencheski, F.H.; Salomon, P.S.; Parise, M. (1994) *Development and toxicity of cyanobacteria in the Patos Lagoon estuary, Southern Brazil*, Workshop Report No. 101, (COI/UNESCO), Annex III, Montevideo, Uruguay, 15-17 June, 480 pp.

Zagatto, P.A. (1995) *Évaluation écotoxicologique du reservoir Guarapiranga, SP-Brésil, en relation avec le probleme des algues toxiques et des algicides.* These Docteur, Université de Metz, Centre dês Sciences de l'Enviroment, France, 87 pp.