Int J Health Life Sci. 2019 January; 5(1):e88281. doi: 10.5812/ijhls.88281.

Published online 2019 January 27. **Research Article**

Impacts of Different Phosphorus Contents on the Growth of *Cylindrospermopsis raciborskii* in the Aquatic Environment

Seyede Parvin Moussavi [1], Naghmeh Ashena [2], Mohammad Mahdi Soori [3], Milad Mousazadeh [4, *], Reza Ghanbari [5, 6], Reza Rostami [7] and Zohreh Naghdali [4]

[1]Environmental Health Research Center, International Branch of Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran
[2]Chemical Engineering Department, Amir Kabir University of Technology, Tehran, Iran
[3]Department of Environmental Health Engineering, School of Health, Jiroft University of Medical Sciences, Jiroft, Iran
[4]Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran
[5]Social Determinants of Health Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
[6]Department of Environmental Health Engineering, School of Health, Qazvin University of Medical Sciences, Qazvin, Iran
[7]Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran

[*]*Corresponding author*: Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran. Email: m.milad199393@gmail.com

Received 2018 December 25; **Revised** 2019 January 20; **Accepted** 2019 January 20.

Abstract

Background: Cyanobacteria are the most important group of algae. Many factors are involved in the rapid growth of algae such as phosphorus and nitrogen contents.
Objectives: The current study aimed at evaluating the effect different phosphorus concentrations on the growth rate of *Cylindrospermopsis raciborskii* during the aquatic life.
Methods: The experiment was carried out for 12 days at three phosphorus concentrations and one control group in Zehnder 8 and Blue-Green 11 media. The rates of cell division per day (G) and specific growth rate (μ) were separately estimated for each group. Cyanobacterium was tested at three phosphorus concentrations; 150, 300, and 600 μg/L.
Results: The current study results showed that increasing phosphorus concentration had a significant effect on the growth of *C. raciborskii* in some particular days of cultivation. The maximum growth rate (0.9 per day) was observed at 7 g/L phosphorus concentration.
Conclusions: Phosphorus was a limiting factor and contributed to the removal of toxins from the alga and its blooming. The maximum growth rate was observed in the group treated on the day 8 of cultivation with phosphorus concentration of 300 μg/L.

Keywords: Cyanobacteria, *Cylindrospermopsis raciborskii*, Phosphorus, Growth Rate

1. Background

The overgrowth of aquatic plants causes problems for the function of water treatment plants. However, the aquatic plants play important roles in the maintenance of the ecological balance in lakes, ponds, and rivers. Algae are primary plants without factual leaves, stems, and roots (1). They classify into green algae, cyanobacteria, red algae, yellow-green algae, Chrysophyceae, etc. Cyanobacteria constitute the most important group of algae. They can cause many problems such as toxin release, which can be detrimental to the quality of drinking water and lead to diseases in humans and animals. Cyanobacteria can form colonies and grow quickly in large masses (2, 3). *Cylindrospermopsis raciborskii* belongs to *Nostocaceae* family and *Oscillatoriales* order from cyanobacteria. Echinite and growing cells make them distinguishable. Humans poisoning and animal death due to *C. raciborskii* bloom are reported in different countries such as the USA, Iceland, the Netherlands, and Australia (4, 5). Physical and chemical variations due to seasonal changes can cause an algal bloom. Algal overgrowth can cause operational problems in water systems such as changing taste, odor and color of the water, causing toxicity, sludge accumulation, and corrosion, as well as trihalomethanes (THMs) release. Consequently, it probably affects the ecological systems and causes the domination of cyanobacteria. Zooplanktons do not use cyanobacteria as food sources. They consume algae that compete with cyanobacteria. In this process, necessary nutrients are not consumed and they improve cyanobacteria growth (6, 7). Nutrients such as nitrogen and phosphorus are necessary for the growth of cyanobacteria. One of the major pollutants found in aquatic environments is phosphorus. The average amount of phosphorus in water resources is < 1 mg/L; exceeding the amounts permitted in water causes a serious threat to the environment, animals, and aquatic life (8-10). Phosphorus is a limiting factor in lakes. A little rise in the content of this nutrient influences the toxin production, since it increases the growth of the alga. According to previous reports, reducing phosphorus content results in microcystin and anatoxin reduction (11-13). Produced toxins are structurally different with various complications from liver cancer to neurotoxicity (14). Some of these toxins accumulate biologically in the food chain and can enter human and animal life cycle. Recent studies to control and prevent diseases by the American institutions show that harmful algal bloom (HAB) is transferred by air through wind and wave motion. Humans are likely to inhale HAB even in a far distance of polluted waters (15). Phosphorus content is an influential factor in algal growth. Hence, decline in phosphate during the growth season is a limiting factor for algal growth.

2. Objectives

The current study aimed at investigating the influence of different phosphate contents on growth of *C. raciborskii*.

3. Methods

A stock of *C. raciborskii* was provided which tested with a Neubauer slide and optical microscope (Nikon) at a 100× magnification. All experiments were performed at three phosphorus concentration and one control group was cultured in Zehnder 8 (Z8) (using Z8 without a nitrogen source) and Blue-Green 11 (BG11) media. First, the alga was purified and the experiments were performed under laboratory conditions (temperature 25°C ± 1 and light intensity of 2200 lux). Thalli were counted daily for 12 days. After 72 hours, a wide variety of phosphorus colors from green (in Z8) to yellow (in BG11) was observed. Finally, sample counting was performed daily in triplicate for every sample by Neubauer chambers. The rates of cell division one day (G) and specific growth (μ) were calculated using Equations 1 and 2 (16):

$$\mu = lnx_1 - lnx_0\,(t_1 - t_0) \tag{1}$$

$$G = ln2\mu_1 \tag{2}$$

x_0: The average number of cells at t_0
x_1: The average number of cells at t_1
μ: The rate of specific growth (daily)
G: Necessary time for per cell division (day)

To analyze the data, an accidental plan was used in MSTAT-C software on the days 2, 4, 6, 8, 10, and 12 in three media containing phosphorus 150, 300, and 600 μg/L. To determine the effect of phosphorus and compare the average values of parameters, one-way analysis of variance and Duncan post hoc test (P value < 0.05) were independently and simultaneously used for each phosphorus concentration. Furthermore, SPSS and Excel were applied to analyze the data.

4. Results and Discussion

The dried mass of *C. raciborskii* on different days of cultivation showed no significant difference in the produced biomass among the three phosphorus concentration groups by the day 8 of cultivation, according to One-way ANOVA and Duncan test results.

Nevertheless, it increased significantly on the day 10 at 300 μg/L of phosphorus, when compared to those of the two other groups. According to Figure 1, there was an increasing trend in growth from the beginning to the day 8 in all the three groups. However, on the day 10, it was close to the death phase.

According to Figure 2, the growth was much higher at a phosphorus concentration of 7 g/L compared with those of the triple contents of phosphorus and the control group. No significant effect of treatment was observed in growth rate of *C. raciborskii* (P> 0.05) exposed to different contents of phosphorus. The highest specific growth rate (μ: 0.9 daily) belonged to the group treated with 7 g/L phosphorus (Figure 2).

The influence of different phosphorus concentrations on the growth of *C. raciborskii* was studied by Shafik et al. and it was found that under different phosphorus concentrations, the highest recorded growth rates were 0.8 - 1 daily that was in agreement with those of the current study. Shorter day length decreased growth rates of cyanobacteria and diatoms, and this decrease was greater in cyanobacteria (17); this finding also consistent with that of the current study. Previous investigations demonstrated the increased cyanobacteria growth during long days of the year (18). The results of the current study showed that *C. raciborskii* growth was compatible with the phosphorus concentration changes. Cells used for cultivation in different groups were picked from the growth phase of stock cells. These cells continued growing in the new cultivation environment. That is why a tangible delay was not observed in the growth phase. *C. raciborskii* strains have striking compatibility with different conditions. According to the results, the growth of this cyanobacterium was probably under the influence of factors such as nitrogen saving capacity (due to heterocyst), while other algal groups faced nitrate scarcity (19, 20).

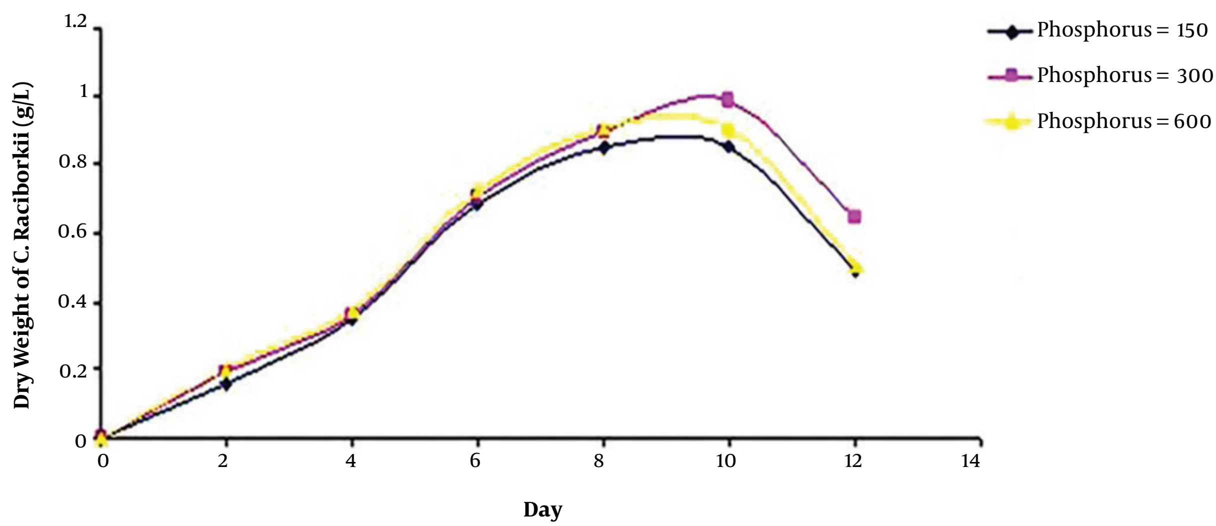

Figure 1. Comparison of the dry weight of *Cylindrospermopsis raciborskii* in different cultivation days under different phosphorus concentrations

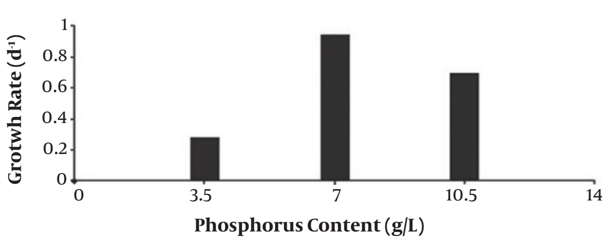

Figure 2. The specific growth rate at different phosphorus concentrations

4.1. Conclusions

Cyanobacteria save phosphate in the growing phase that improves their growth. Thus, reduction and increase of phosphate content in the environment is important as a preventive factor of growth. Eventually, it should be noted that colonies of *C. raciborskii* are different in terms of physiological and genetic perspectives. Hence, it should be highlighted that results of other studies indicated that special colonies had considerable compatibility and other colonies showed different behaviors under such conditions. Overall, studying *C. raciborskii* and the effect of different phosphorus concentrations on the growth of these cyanobacteria were the reasons to conduct the current study. For future work, more influential factors such as temperature, light, nitrogen content, etc., on the dominant algae species will be investigated.

Acknowledgments

This paper was elaborated with the support of Shahid Sadoughi University of Medical Sciences and Health Services in Yazd, Iran. Therefore, the authors express their gratitude to them.

Footnotes

Conflict of Interests: The authors declared no conflict of interests.

Funding/Support: No funding.

References

1. James KJ, Carey B, O'Halloran J, van Pelt FN, Skrabakova Z. Shellfish toxicity: Human health implications of marine algal toxins. *Epidemiol Infect*. 2010;**138**(7):927–40. doi: 10.1017/S0950268810000853. [PubMed: 20412612].
2. Deeds JR, Wiles K, Heideman GB 6th, White KD, Abraham A. First U.S. report of shellfish harvesting closures due to confirmed okadaic acid in Texas Gulf coast oysters. *Toxicon*. 2010;**55**(6):1138–46. doi: 10.1016/j.toxicon.2010.01.003. [PubMed: 20060850].
3. Farabegoli F, Blanco L, Rodriguez LP, Vieites JM, Cabado AG. Phycotoxins in marine shellfish: Origin, occurrence and effects on humans. *Mar Drugs*. 2018;**16**(6). doi: 10.3390/md16060188. [PubMed: 29844286]. [PubMed Central: PMC6025170].
4. Pitcher GC, Krock B, Cembella AD. Accumulation of diarrhetic shellfish poisoning toxins in the oyster Crassostrea gigasand the mussel Choromytilus meridionalisin the southern Benguela ecosystem. *Afr J Mar Sci*. 2011;**33**(2):273–81. doi: 10.2989/1814232x.2011.600372.
5. Terseleer N, Gypens N, Lancelot C. Factors controlling the production of domoic acid by Pseudo-nitzschia (Bacillariophyceae): A model study. *Harmful Algae*. 2013;**24**:45–53. doi: 10.1016/j.hal.2013.01.004.

6. Okumura M, Tsuzuki H, Tomita B. A rapid detection method for paralytic shellfish poisoning toxins by cell bioassay. *Toxicon*. 2005;**46**(1):93–8. doi: 10.1016/j.toxicon.2005.03.018. [PubMed: 15922387].
7. Agriculture Organization. *The state of food insecurity in the world 2014: Strengthening the enabling environment for food security and nutrition*. Food and Agriculture Organization; 2014.
8. Ahmad N, Sereshti H, Mousazadeh M, Rashidi Nodeh H, Kamboh MA, Mohamad S. New magnetic silica-based hybrid organic-inorganic nanocomposite for the removal of lead(II) and nickel(II) ions from aqueous solutions. *Mater Chem Phys*. 2019;**226**:73–81. doi: 10.1016/j.matchemphys.2019.01.002.
9. Mohammadi P, Lotfi S, Moussavi SP, Mousazadeh M, Rostami R. Studying quality of drinking water and determining sustainable indicators for water resources of villages of Harsin town of Iran. *Int J Health Life Sci*. 2018;**4**(2). e83508. doi: 10.5812/ijhls.83508.
10. Moussavi SP, Hallaji SM, Mohebbi S, Hosseinabadi MK, Mousazadeh M, Rostami R. Removal of phosphorus from aqueous solution using multi-wall carbon nanotube as adsorbent: Kinetics and isotherms. *Desalin Water Treat*. 2019;**Forthcoming**.
11. Garcia-Villada L, Rico M, Altamirano MM, Sanchez-Martin L, Lopez-Rodas V, Costas E. Occurrence of copper resistant mutants in the toxic cyanobacteria Microcystis aeruginosa: Characterisation and future implications in the use of copper sulphate as algaecide. *Water Res*. 2004;**38**(8):2207–13. doi: 10.1016/j.watres.2004.01.036. [PubMed: 15087203].
12. Ray S, Bagchi SN. Nutrients and pH regulate algicide accumulation in cultures of the cyanobacterium Oscillatoria laetevirens. *New Phytol*. 2002;**149**(3):455–60. doi: 10.1046/j.1469-8137.2001.00061.x.
13. Sharp JH, Underhill PA, Hughes DJ. Interaction (allelopathy) between marine diatoms: Thalassiosira pseudonana and phaeodactylum tricornutum. *J Phycol*. 1979;**15**(3):353–62. doi: 10.1111/j.1529-8817.1979.tb04396.x.
14. Aune T, Sorby R, Yasumoto T, Ramstad H, Landsverk T. Comparison of oral and intraperitoneal toxicity of yessotoxin towards mice. *Toxicon*. 2002;**40**(1):77–82. doi: 10.1016/S0041-0101(01)00192-1. [PubMed: 11602282].
15. Rechcigl JE, Rechcigl NA. *Insect pest management: Techniques for environmental protection*. CRC Press; 2016.
16. Fogg GE, Thake B. *Algal cultures and phytoplankton ecology*. Univ of Wisconsin Press; 1987.
17. Foy RH, Gibson CE. The influence of irradiance, photoperiod and temperature on the growth kinetics of three planktonic diatoms. *Eur J Phycol*. 1993;**28**(4):203–12. doi: 10.1080/09670269300650311.
18. Litchman E, Steiner D, Bossard P. Photosynthetic and growth responses of three freshwater algae to phosphorus limitation and daylength. *Freshw Biol*. 2003;**48**(12):2141–8. doi: 10.1046/j.1365-2427.2003.01157.x.
19. Kruskopf M, Plessis SD. Growth and filament length of the bloom forming oscillatoria simplicissima (oscillatoriales, cyanophyta) in varying N and P concentrations. *Hydrobiologia*. 2006;**556**(1):357–62. doi: 10.1007/s10750-005-1061-0.
20. Shafik H, Herodek S, Presing M, Vˆrˆs L. Factors effecting growth and cell composition of cyanoprokaryote Cylindrospermopsis raciborskii (Woloszynska) Seenayya et Subba Raju. *Int J Psychol Res*. 2001;**Supplement**(3):75–93.