African Journal of Biotechnology Vol. 10(72), pp. 16202-16208, 16 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1406
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effects of different photoperiods and concentrations of phosphate on the growth of the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska)

Sabahi asl Mitra[1]*, Nejatkhah Parisa[1], Ramezanpour Zohreh[2], Heidary Negin[1]

[1]Islamic Azad University, North Tehran Branch, Faculty of Marine Science and Technology, Marine Biology Group, Tehran, Iran.
[2]International Sturgeon Research Institute, P.O Box: 41635-3464, Rasht, Iran.

Accepted 5 October, 2011

The freshwater cyanobacterium, *Cylindrospermopsis raciborskii* is potentially toxic and widely distributed in tropical and sub-tropical regions. This species is highly abundant throughout the Anzali Lagoon during the months of July and August. The effects of four different photoperiods (L/D 12:12, 10:14, 14:10, 8:16) as well as three different concentrations of phosphate (2 fold, 3 fold and half of the main culture) on growth rates of C*. *raciborskii* were studied. *C. raciborskii,* isolated from water samples collected from the Anzali Lagoon, was cultured in Zehnder 8 media (using Z_8 without a nitrogen source) under light intensity of 3000 Lux at 25±2°C. A control group cultured with the main culture medium was also used. This experiment was carried out for 25 days. Inoculants and C*. *raciborskii* cells were counted every other day throughout the experiment period using a Neubauer chamber and specific growth rates (SGR) and cell division rates (G) were estimated. The maximum growth rate ($8x10^6 \pm 5$ cells ml^{-1}) was observed at 12 L:12 D photoperiod followed by 14 L:10 D, 10 L:14 D and 8 L:16 D. The results obtained in the present study especially with 14 L:10 D and 12 L:12 D were in complete agreement with general growth pattern of *C. raciborskii* observed in warmer months with rather longer daytime in tropical and subtropical areas. The maximum growth rates were observed in the group treated with 2 fold PO_4 concentration. The strategy of growth in this species may depend on nitrogen fixation, when other algae were nitrogen limited and fast PO_4 uptake even if very low concentration is available.

Key words: Cyanobacteria, *Cylindrospermopsis raciborskii*, Anzali Lagoon, photoperiod, phosphate, growth rate.

INTRODUCTION

Dehiscence of Cyanobacteria produces dangerous poisons in native water which leads to serious problems worldwide. Besides, loss of many species in lakes, rivers, estuaries, oceans and storage water systems are among the crucial effects of this phenomenon (Wilson et al., 1999). Physiological capacities of Cyanobacteria are highly dominant in competence with other phytoplankton species (Duval et al., 2005). *Cylindrospermopsis raciborskii* belongs to *Nostocaceae* family and Oscillatoriales order from Cyanobacteria.

C. raciborskii (Woloszynska, 1972) is kind of Cyanobacteria which can produce poison. *C. raciborskii* is identified by the presence of gas vacuoles and by the shape and dimensions of terminal heterocysts, vegetation cells and trichomes (Wilson et al., 1999). In this species, one or two heterocysts are observable at both ends of the trichome as a candle flame (Figure 1). Akinetes are normally oval form placed inactively near heterocysts and include spherical cells. Note that growth cell walls are covered by nuclear cells, including clear gas vacuoles (Hawkins et al., 1985). Recent studies have explored that this species has a permanent effect on mice and also infect and destroy liver tissue. Note t hat the produced

*Corresponding author. E-mail: Mitra.sabahi2@gmail.com.

Abbreviations: SGR, Specific growth rates; **ANOVA,** one-way analysis of variance.

Figure 1.*Cylindrospermopsis raciborskii* sampled from Anzali Lagoon (x400).

poison damage kidneys, adrenal glands, lungs and intestines. Probably, this complication occurs in humans and other mammalian (Hawkins et al., 1985). There are various factors that affect upon the combination and succession of phytoplankton and cyanobacteria including light, temperature, concentration of nutritional material, simulative and preventive material which influence upon growth and elective feeding (Ahelgern, 1977).

Blooms of cyanobacteria threaten aquatic life. The cyanobacter *C.raciborskii* blooms lead to animal fatalities and human poisoning in various countries such as Iceland, 1999, Florida, 1997, Japan, 1999, Africa, 2004, and Australia, 1998-2001(Chapman and Schelske, 1997; Zakaria and Al-Shehri, 2004). In tropical regions, the algae growth is the same in all months of the year because of uniform environmental conditions. However, in temperate zones, seasonal change cause physic-chemical changes and thus, in some months, high growth of blue-green algae creates blooms in water (Bernard et al., 2004). Many different researchers reported about daily periodical influence of light and darkness upon phytoplankton and freshwater species like *Oscillatoria agardhii*. These reports state the importance of light intensity and quality on the occurrence of phenomena in lakes and sea. Though, according to Foy et al. (1993), light duration is an effective factor on the growth of phytoplankton that has different effects upon every species of algae. Phosphate plays a basic role on growth in all plants. Thus, decline in phosphate during the growth season is a limiting factor for algal growth. Also, phosphate is critical element in controlling the growth of cyanobacteria. This study attempts to investigate the influence different phosphate concentrations (2 fold, 3 fold and half of the main culture) on growth of *C. raciborskii* and estimate the optimum growth conditions in different photoperiods (L/D 12:12, 10:14, 14:10, 8:16).

MATERIALS AND METHODS

Sampling from the Anzali Lagoon was carried out in the month of August of 2007 using plankton net (25 µm).Isolation and preparation of pure cultures of algae was carried out at the Ecology Department of the international Sturgeon Research Institute. The algal cells were cultured in Z8 medium (without nitrogen resources) (Kotai, 1972). All treatments were run in three replicates. Four different photoperiods (8 L:16 D, 14 L:10 D, 12 L:12 D, and 10 L:14 D) were considered. To examine the influence of different concentrations of phosphate (PO_4), three various treatments were studied using 6.2 gl^{-1} (two-fold), 9.3 gl^{-1} (three-fold) and 1.53 gl^{-1} (half the amount of Z_8 batch culture) (without nitrogen resources). A control group 3.1 gl^{-1} of Z_8 (main culture) was also used. One milliliter of pure culture cells were inoculated to each treatment using a syringe pipette. The cellular concentration after injection at t_0 time for each replicate was 10^4 cells ml^{-1}. Finally, cultivated tubes were placed in culture shelves for 25 days. The culture room temperature was adjusted to 25±2°C. Sample counting was performed daily and three repetitions were done for every sample by Neubauer chambers. SGR (µ) and G were calculated by the equations (Fogg and Thake, 1987):

$\mu = \ln x_1 - \ln x_0 \ (t_1 - t_0)$.

$G = \ln 2 \mu_1$

Where, X_0 is the mean cellular number at t_0 time; X_1 is the mean cellular number at t_1 time; µ is the specific growth rate (d^{-1}); G is the generation time (d)

Data analysis was performed by one-way analysis of variance (ANOVA) and Duncan's separator test associated with Excel (2003) and SPSS (16.0) statistical software.

RESULTS

Result of different photoperiods

Statistical analysis of different photoperiods results show significant differences on algal growth (Figure 2).

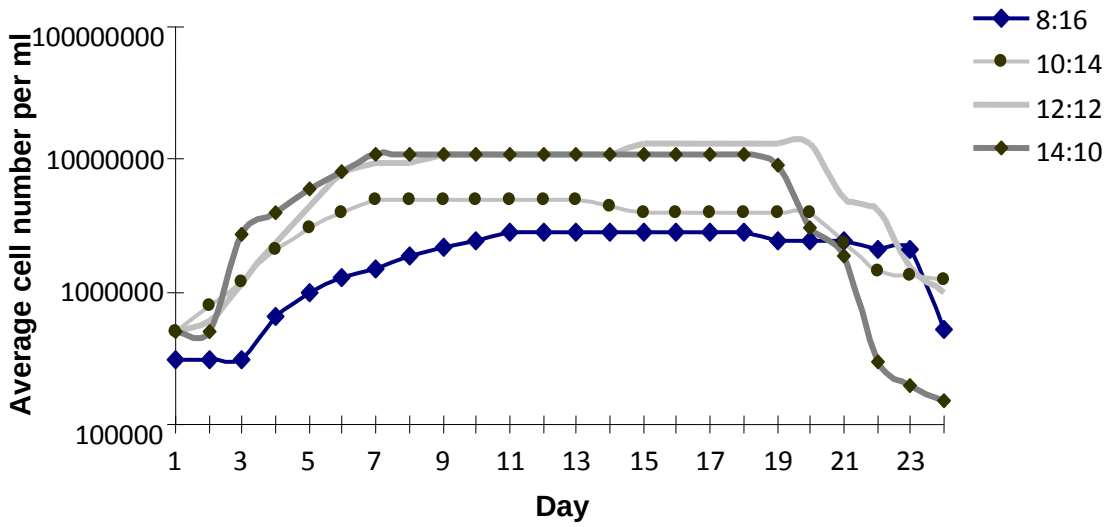

Figure 2. Mean cell number of *Cylindrospermopsis raciborskii* under different photoperiods.

Photoperiods 12 L:12 D and 14 L:10 D with mean cell numbers 8×10^6 ± 5cells ml^{-1} and 7×10^6 ± 4 cells ml^{-1}, respectively were higher than the other two treatments($P<0.05$) (Figure 2).

C. raciborskii growth follows growth curves like other phytoplankton. As shown in Figure 2, delay phase growth observed between first and third day was very short in 10 L:14 D photoperiod. In all treatments, exponential phase were between fourth and seventh day. Average of cell number was approximately same from day eight to day nineteen when the algae was in the stationary phase of growth. Algal cells enter death phase from twentieth day and showed negative growth.

According to Figure 3, the highest SGR (µ: 0.6 d^{-1}) was recorded in treatment under photoperiod 12 L:12 D, while the lowest growth rate (µ: 0.4 d^{-1}) was recorded in 14 L:10 D treatments. Highest generation of cellular division (G=0.9 d) was recorded for the 12 L:12 D treatments, and the shortest time was (G=0.6 d) in the 14 L:10 D treatments. Obtained results from one way ANOVA showed significant differences ($p<0.05$) between mean cellular concentration of photoperiods 14 L:10 D and 12 L:12 D with the other treatments (Figure 2). However, no significant difference were detected in specific growth rate and generation of cellular division in various photoperiods studied (Figures 3 and 4).

Results of different phosphate concentrations

No significant effect of treatment was observed in SGR of *C. raciborskii* ($p > 0.05$) exposed to different concentrations of phosphate (Figure 6). In all groups studied, the cells growth started with three day delay. Exponential phase was recorded between seventh day and ninth day. It is worthy to note that control group entered this phase earlier than other groups. In all groups studied, with the starting of cellular division, the cells number increased progressively. Mean cellular growth per day was in the same level between tenth day and twentieth day when algae were in the stationary phase. The cells entered to death phase from twentieth day and showed negative growth. The highest mean cells numbers were recorded in the control group (3.1 gl^{-1}) (Figure 5).

Highest specific growth rate (µ: 0.2 d^{-1}) and highest generation of cellular generation per day (G=0.32 d) belonged to the group treated with (1.53 gl^{-1}) phosphate (Figures 6 and 7). Although, no significant differences ($p>0.05$) were detected for generation time of cellular division in day in the different groups studied.

DISCUSSION

In this study, the cells used in different treatments were selected from stationary phase of pure culture cells. Such cells reached to ultimate growth phase in initial environment and practically, they would be lost if were not transferred to new environment similar to their common conditions. Growth was observed after three days lag in all treatments (Figures 2 and 5). Lag (delay) time in phytoplankton response to suitable growth can influence bloom of phytoplankton in natural environments and experimental culture (Smith et al., 1992). Examination of lag time in initial growth of different treatments indicated that light does not influence upon lag phase. However, existence of lag time in growth phase is highly attributed to the age of pure culture cells (Fogg and Take, 1987).

According to obtained results, there is delay in duration of growth phase. In treatments under 10 L:14 D

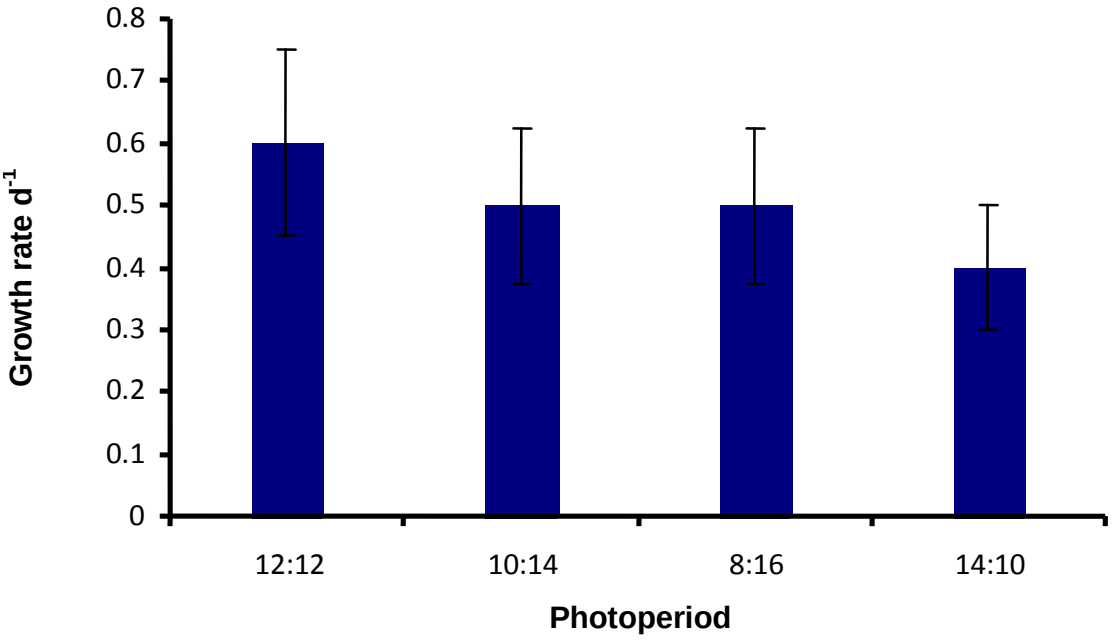

Figure 3. Growth rate of *Cylindrospermopsis raciborskii* under different photoperiods (±SE)

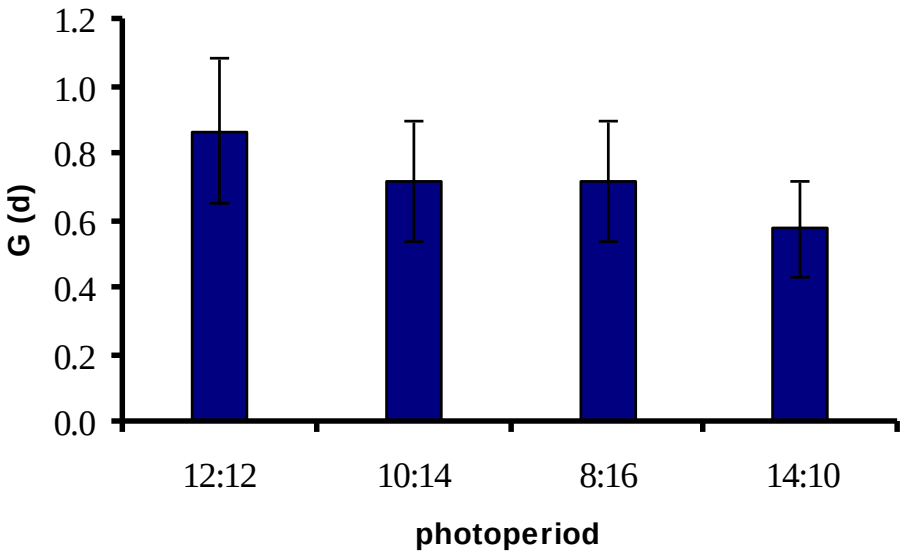

Figure 4. Generation of cellular division of *Cylindrospermopsis raciborskii* under different photoperiods (±SE)

photoperiod, delay in growth phase probably depends on young pure culture cells. With an increase in light time duration, the cellular concentration increases, whereas under this kind of light the growth rate is minimum as compared to that in other treatments. Also generation time of cellular division is short with increasing photoperiod. Hence, highest cellular division was recorded in treatments under 14 L:10 D and 12 L:12 D photoperiod.

Increasing growth rates (μ) with increasing photoperiod was also observed. The influence of different photoperiods upon growth of *C.raciborskii* algae was studied by Shafik et al. (2001) and found that under 12 L:12 D photoperiod, highest growth rates recorded were between (0.8 d^{-1}) and (1 d^{-1}). They also reported that photoperiods longer than 12 L:12 D reduced growth. These results are in agreement with those obtained from this study where reduced growth rates (μ) were recorded

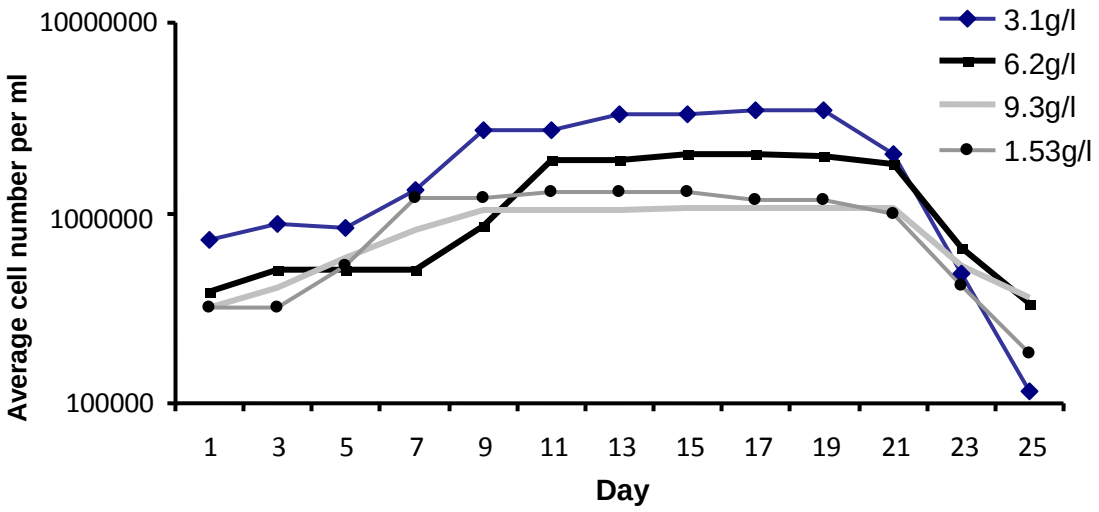

Figure 5. Mean cell numbers of *Cylindrospermopsis raciborskii* in different concentrations of phosphate

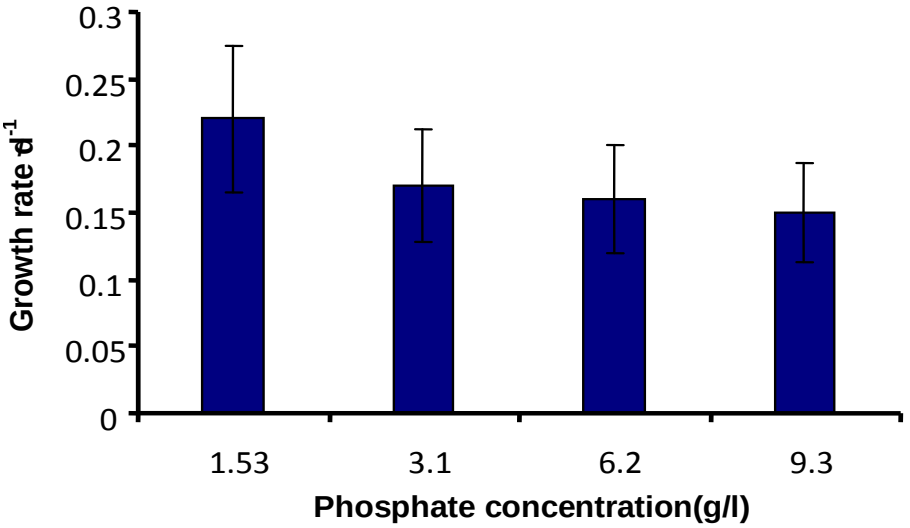

Figure 6. Growth rates of *Cylindrospermopsis raciborskii* in different concentration of phosphate (±SE).

under the 14 L:10 D photoperiod. Continuous and high photoperiods such as 14 L:10 D, stimulate bio-production of Tillacoied and biosynthesis of chlorophyll in cells (Ibrahim, 1993). Increased synthesis of chlorophyll leads to increased photosynthesis and consequently results in increased production of photosynthesis in cell.

If exposed to continuous light, there will not be enough time for consumption of these products. Investigations demonstrated that accumulation of materials obtained from photosynthesis such as glucose and carbohydrates can reduce photosynthesis intensity (Ibrahim, 1995). Thus, reducing photosynthesis will decrease cellular growth efficiency. In longer photoperiods, despite high cellular division speed and high cellular concentration, the cellular growth rate (μ) was low as compared to other photoperiods. On the other hand, shorter day length decreased growth rates of cyanobacteria and diatoms, and this decrease was greater in cyanobacteria (Foy and Gibson, 1993).

Foy and Gibson (1993) observed that in comparison of cyanobacteria with Diatoms, there is severe reduction in growth process that resulted from photoperiod reduction. On this basis, minimum cellular concentration (accumulation) as well as SGR was observed in photo duration 8 L:16 D.

Growth of most freshwater phytoplankton is regulated by the availability of phosphate Rhee (1980) and Thompson et al. (1994) suggested that under P limitation conditions, the growth is related to the P cell quota due mostly to a large storage capacity for P by these

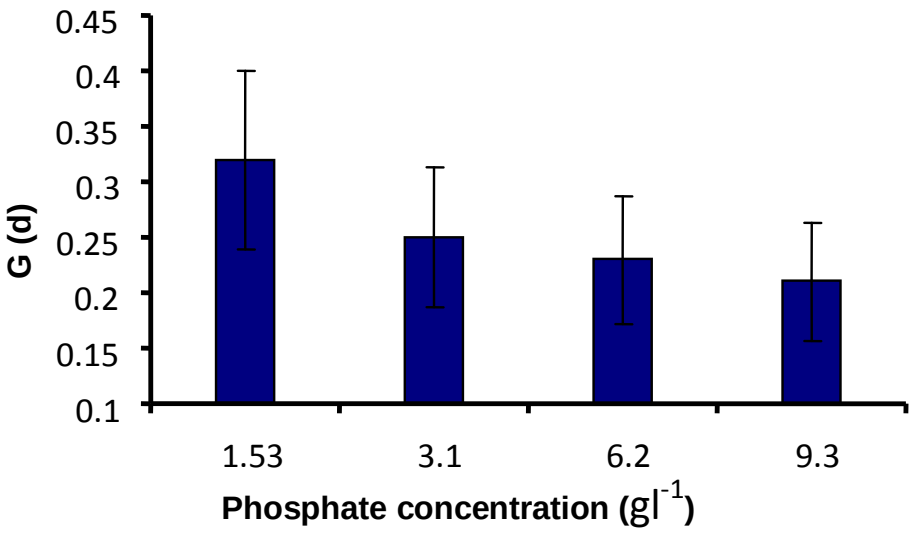

Figure 7. Generation of cellular division of *Cylidrospermopsis raciborskii* in different concentrations of phosphate (±SE)

organisms. Phosphate storage in cyanoprokaryotes appears to be much larger than other species, and this capacity was reported to give them a competitive advantage over diatoms and chlorophytes (Shafink et al., 2001). Highest mean cellular concentration recorded in the control group and in the group exposed to 6.2 gl^{-1} phosphate indicates that limitation of phosphate even under desirable photo conditions reduces the rate of cellular chlorophyll, growth and photochemical capacity (Cullen and MacInyre, 1998).

Therefore, reducing or increasing the amount of phosphate in the environment resulted in the reduction of cellular division and cyanobacteria population. Desirable levels of phosphate in the culture environment and also increasing cellular division in this treatment could be the possible reason for increase in growth rate in the control group. Since cyanobacteria have high ability for absorption of phosphate even in case of the scarcity, reduction of environmental phosphate reduces photosynthesis rate and the effect of this reduced photosynthesis on cellular division and increased cellular division was significant. In the treatments exposed to (1.53 gl^{-1}) phosphate, it appears that low concentration of phosphate in the initial stages of culture does not create limitation for growth process. Hence, cells absorb phosphate quickly and grow rapidly, however, as culture progresses further reduction in phosphate results in decreased cellular division and as well as reduction in mean cellular number.

Previous investigations have demonstrated the relationship between phosphate intake and photoperiod. This explains increased cyanobactria growth during long days of the year (Litchman, 2003). It is evident from the results of the present study that in different treatments studied; *C. raciborskii* was compatible with photoperiod changes and different amounts of phosphate. Otherwise these algae would have to face a lot of problems in its natural life environment. In all treatments used in this study, cellular division and growth increase was observed. Ecological conditions are various ways which allow growth and dehiscence to occur in both tropical and sub-tropical regions. Tang and Vincent (2000) showed that growth and photosynthesis of pole cyanobacteria during day time depend on temperature. Finally, it was found that the growth of *C. racibroskii* algae is influenced by photo duration. The specific physiological traits possessed by this species explain its global distribution in various geographical regions. Three hypothesis may be considered in this regard; (1) colonies of this species are compatible with temperate climate and it may be suitable for advancement in the north latitude, (2) this species has wide range of tolerance from physiological perspective, (3) Climate changes resulting from global warming has resulted in greater dispersion and spreading of this species (Bernard and Dufour, 2004).

This kind of cyanobacteria has high potential in storing phosphate and also it can be grown in various tropical lakes. Increased growth of *C. raciborskii* species in France-bech pool in France has been reported because of its compatibility and wide tolerance towards undissolved materials (Bernard and Dufour, 2004). On the other hand, Moisandr et al. (2002) reported that high salinity in estuary of Neuse Lake prevented the dominance of *C. raciborskii* species in that region. Reports about global dispersion of these algae indicate that *C. raciborskii* has high compatible ability and can also enter mid geographical latitudes. However, a global analysis of these results suggests that *C. raciborskii* has good adaptability, but poor ability to compete with micro

algae. This adaptability was demonstrated by the fact that *C. raciborskii*, which is described as a tropical cyanobacteria, is able to grow under different light and temperature conditions. In addition, *C. raciborskii* seems to be very tolerant of nutrient concentrations (Briand et al., 2001). This study probably shows that the growth strategy of cyanobacteria is under the effect of three factors: (1) Nitrogen fixation ability intermediate to existence of heterocysts when other algae groups face reduction in nitrate, (2) Ability of high phosphate absorption even when the amount of phosphate is low, (3) Growth ability lies in low light conditions and high temperature (Shafink et al., 2001). Thus, reduction and increasing of phosphate concentration in the environment is important as a preventive factor of growth. Eventually, it must be mentioned that colonies of *C.raciborskii* are different from the physiological and genetic perspectives. Hence, it must be emphasized that results from other studies indicate that special colony have considerable compatibility and other colonies show different behavior in this conditions.

ACKNOWLEDGEMENTS

The authors wish to appreciate Mr. Parandavar, the Head of the Ecology Department as well as Mrs. Marjan Sadeghi and Uma Arshad of the Hydrology laboratory for their valuable assistance in executing this investigation. Authors also want to show their gratitude to Mr. Hamid Reza Pourali, the Head of Nutrition Section of the International Sturgeon Research Institute.

REFERENCES

Ahlegern G (1977). Growth of *Oscillatoria agardhii* in chemostate culture nitrogen and phosphorus reqirement. Oikos, 29: 209-224.

Bernard C, Dufour PH (2004). *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: Selection, wide physiological tolerance, Phycol. Soc. Am. 40: 231-238.

Briand JF, Robillot C, Quiblier-Lloberas C, Humbert JF (2001). Environmental context of *Cylindrospermopsis raciborskii* (Cyanobacteria) blooms in a shallow pond in france. Water Res. 36(2002): 3183-3192.

Chapman AD, Schelske CL (1997). Recent appearance of *Cylindros-permopsis* (Cyanobacteria) in five hypertrophic Florida Lakes. J. Phycol. 33: 191-195.

Cullen JJ, MacIntyer JG (1998). Behavior,physiology and the niche of depth_regulating Phytoplankton in : Physiol. Ecol. Harmful Algal Blooms, (ed . DM Andrerson,AD Cembella , GM Hallegraff). Springer. Berlin. 41: 559-579.

Duval E, Gragnon B, Varliner D (2005). Effects of two cyanotoxins,Microcystin-LR and Cylindrospermopsin,on *Euglena gracilis*, Earth. Environ. Sci. pp. 659-671.

Fogg GE, Thake B (1987). Algal culture and phytoplankton ecology (3rd edition), the University of Wisconsin press.

Foy RH, Gibson CE (1993). The influence of irradiance photoperiod and temperature on thegrowth kinetics of three planktonic diatims. E. J. Phycol. 28: 203-212.

Hawkins PR, Runngar MTC, Gackson ARB (1985). Several hepatotoxicity caused by the tropical, cyanobacterium *Cylindrospermopsis raciborskii*. Dept. Bot. James Cook University, Queensland 4811, Australia.

Ibrahim ZH (1993). Photosynthesis: Botanic Physiology, Tehran University Publication. 4: p. 690.

Ibrahim ZH (1995). Metabolism: Botanic Physiology, Tehran University Publication. 3: p. 514.

Kotai J (1972).Instruction for preparation of modified nutrient solution Z8 for algae. Publication B-Norwegian institute for water research, Blindern, Oslo. 5: 11-69.

Litchman E (2003). photosynthetic and growth responses of three freshwater algae to phosphorus limitation and day length, Limnological Research. Centre, EAWAG, kastanienbaum, Switzerland. 48: 2141-2148.

Moisander PH, McClinton E, Paerl, HW (2002). Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cynobacteia. microb. Ecol. 43: 432-442.

Rhee GY (1980). Continuous culture in phytoplankton ecology. in Droop MR, Janasch HW (Eds). Adv. Aquat. Microbiol. 2: 151-203.

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

Smith GJ, Zammerman RC, Alberte R (1992). Molecular and physiological responses of diatoms to variable levels of Irradiance and nitrogen availability growth of costatumin simulated up welling conditions., limnol. Oceanogr. 37(5): 989-107.

Tang EPY, Vincent WF (2000). Effects of daylength and temperature on the growth and photosynthesis of an Arctic Cyanobacterium, *Schizothrix calcicola* (Oscillatoriaceae), J. Phycol. 35: 263-272.

Thompson PA, Hee-mock OH, Ree GY (1994).Storage of phosphorus in nitrogen-fixing *Anabaena flos-quae* (Cyanophyceae). J. Phycol. 30: 267-273.

Wilson K, Mark SM, Baker P (1999). Molecular characterization of the toxic Cyanobacterium *Cylindrospermopsis raciborskii* and Design of a Species-Specific PCR. The Cooperative Research center for Water Quality and Treatment, Australian Water Quality Center. SA Water Corporation, Australia.

Zakaria A, Al-Shehri MAM (2004). Cyanobacteria and their toxins in treated-water storage reservoirs in Abha city, Saudi Arabia.Dept. Biol. Sci. Faculty Sci. King Khalid University, Saudi Arabia.