Int. J. Aquat. Biol. (2023) 11(4): 321-327
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Effect of different light intensities on the hepatotoxin cylindrospermopsin production by Cyanobacteria algae, *Nostoc ellipsosporum*

Shaimaa Hassan Jassim, Haider A. Alghanmi*

Biology Department, College of Education, University of Al-Qadisiyah, Al Diwaniyah, Iraq.

Abstract: The cyanobacterial bloom leads to the deterioration of the aquatic environment because they release their secondary metabolic to the water, especially toxins. One of the important toxins is Cylindrospermopsin (CYN) which is one of the dangerous toxins that cause liver damage known as hepatic toxin, which poses great health risks to humans. Light plays an important role in the production of these toxins by cyanobacteria through its effect on the photosynthesis process and the gene regulator of these toxins. The current study tested the effect of different light intensities of 26, 52, 78, and 104 mol m^{-2} s^{-1} on hepatotoxic CYN production by cyanobacterium *Nostoc ellipsosporum*. The findings of this study showed that the highest inter and extracellular CYN reached 0.047 and 42.5 µg/ml, respectively with a total value of 42.547 µg/ml recorded at the light intensity of 78 µmol photons m^{-2} s^{-1}. The lowest production of intra and extracellular CYN was recorded at the light intensity of 26 µmol photons m^{-2} s^{-1}, which amounted to 0.0006 µg/mg and 7.73 µg/ml, respectively with a total value of 7.735 µg/ml. Also, the highest light intensity inhibited the CYN production which recorded 0.009 µg/mg and 26.39 µg/ml for intra and extracellular contents, respectively, and total production of 26.399 µg/ml. We conclude that light intensity has a vital role in CYN production especially in the optimal condition represented by moderate light, and this effect differs among different cyanobacterial species.

Article history:
Received 28 March 2023
Accepted 16 April 2023
Available online 25 August 2023

Keywords:
Light
Cyanobacteria
Nostoc ellipsosporum
Toxin

Introduction

The widespread cyanobacteria produce a variety of bioactive metabolites and, some of them are found to be highly toxic known as cyanotoxins (Bláha et al., 2009; Verma et al., 2022). Freshwater cyanobacterial blooms are a significant ecological and health concern for humans worldwide. Such blooms occur in aquatic habitats all over the world producing a variety of toxic bioactive metabolites, such as microcystins and cylindrospermopsin (Filatova et al., 2021). Cylindrospermopsin is a tiny alkaloid toxin produced by some cyanobacteria species such as *Aphanizomenon*, Anabaena, *Cylindrospermopsis*, *Lyngbya*, and *Raphidiopsis* (Beasley, 2020). Cylindrospermopsin is a hepatic toxin that accumulates over time in the liver, binds to DNA, induces DNA fragmentation, and prevents the synthesis of the protein. The toxin disrupts metabolism by causing oxidative damage linked to reduced glutathione concentrations. The liver, kidneys, and heart are among the organs that are the target of this toxin (Solter and Beasley, 2013).

Numerous environmental factors, including light intensity, nutrients, temperature, pH, salinity, and trace metals can affect the cyanobacterial species' growth and their cyanotoxin production. These factors also have an impact on the production of cylindrospermopsin by algal species. Thus, for the control of toxic cyanobacterial bloom, it is essential to know how environmental factors affect cyanobacterial bloom development and toxin production (Neilan et al., 2013; Boopathi and Ki, 2014).

An important environmental factor that controls the growth and production of toxins is light intensity. Since light intensity has a significant impact on growth and the production of the hepatotoxin

*Correspondence: Haider A. Alghanmi
E-mail: haider.alghanmi@qu.edu.iq

DOI: https://doi.org/10.22034/ijab.v11i4.1812
DOR: https://dorl.net/dor/20.1001.1.23830956.2023.11.4.7.3

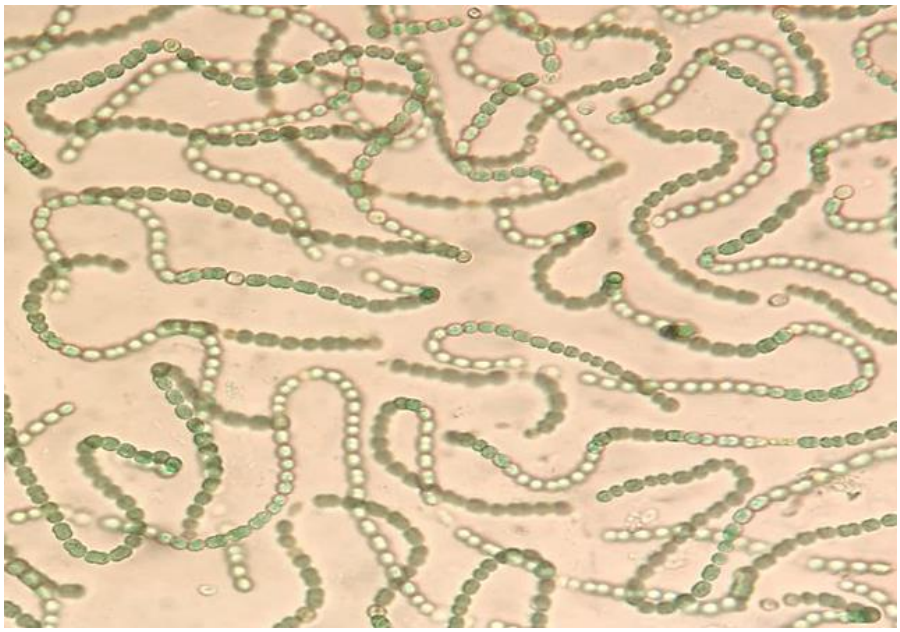

Figure 1. *Nostoc ellipsosporum* under the light microscope at magnification power (40X).

cylindrospermopsin, Dyble et al. (2006) found that the highest intracellular toxin concentrations occur in the cyanobacterium, *Cylindrospermopsis raciborskii* that have been actively growing at light intensities of 75-150 µmol photons m^{-2} s^{-1} for more than two weeks. Furthermore, Cirés et al. (2011) showed that light can be used as a prediction tool for the control of water bodies that may be impacted by the harmful cyanobacterium *Aphanizomenon ovalisporum*. They found that 60 µmol photons m^{-2} s^{-1} of light intensity induced maximum total CYN toxin production by *A. ovalisporum*. Whereas Bormans et al. (2014) found that at moderate light levels, µmol photons m^{-2} s^{-1} maximum production of CYN by *Oscillatoria* sp. PCC 6506. Hence, this study aimed to investigate how *Nostoc ellipsosporum* produces Cylindrospermopsin toxin in relation to light intensity.

Materials and Methods

Algal cultivation and biomass production: An axenic culture of cyanobacterium *N. ellipsosporum* (Fig. 1) was obtained from the Environmental Laboratory at the University of Al-Qadisiyah, Iraq. It was cultured on nutrient agar and incubated for 72 hours at 37°C to validate its purity and absence of bacteria and fungi (Tredici, 2004). The cyanobacterium was inoculated with 400 ml of BG-11 medium in a sterile glass flask of 500 ml and incubated at 28°C and 60 mol m^{-2} s^{-1} light intensity in a growth chamber to obtain mass culture (Andersen, 2005).

Light intensity experiments: To assess the impact of light intensity on the growth rate and Cylindrospermopsin production by *N. ellipsosporum*. It was cultivated in BG-11 medium, and grown under four light intensities of 26, 52, 78, and 104 µmol photons m^{-2} s^{-1} using LED lights, at 28°C, pH = 7.2, and a photoperiod of 16:8, Light: Dark.

Growth curve: For estimation of the growth curve, Chlorophyll a was extracted by centrifuging 5 ml of the culture at 5000 rpm for 5 minutes, collecting the algal cell precipitate, and discarding the supernatant. then placed in a shaking water bath for two hours at a temperature of 25°C with 5 ml of acetone 90%, followed by 10 minutes of centrifugation at 6000 rpm to take the supernatant then measured at 664 nm. Using a spectrophotometer, the chlorophyll a was estimated using the equation (Ritchie, 2006) of chl a [µg/mL] = 11.4062*A664.

Cylindrospermopsin measurement: Intra and extracellular Cylindrospermopsin was measured using an HPLC device (Knauer, Germany) with a separation column of C18 (250*4.6 mm i.d., 5 µm particle size, 80 Å pore size). The intracellular CYN extraction was as follows: 10 ml of cultured media from the stationary

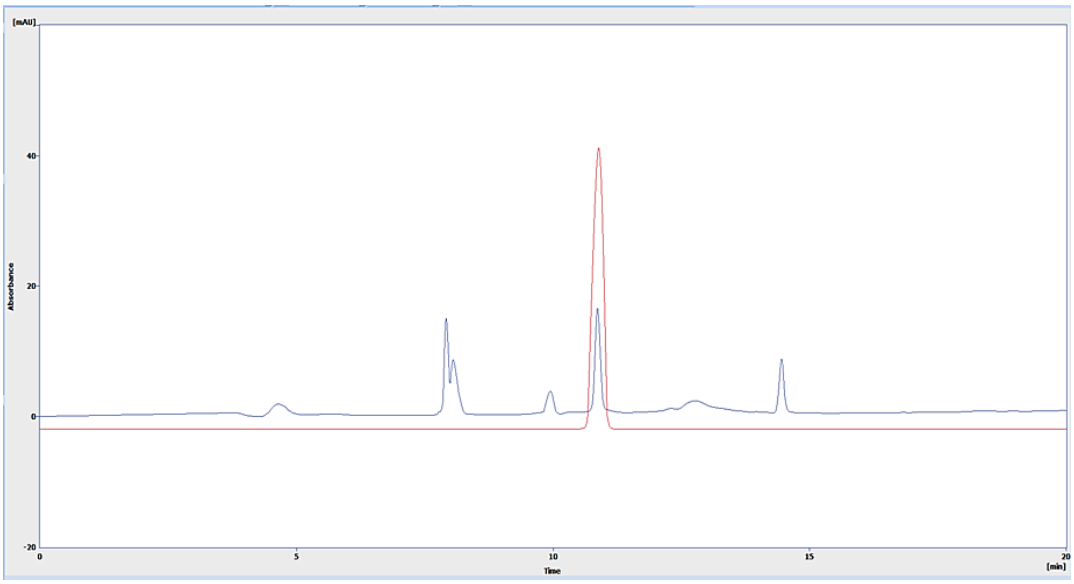

Figure 2. Identification of Cylindrospermopsin producing by *Nostoc ellipsosporum* with blue color matching with Cylindrospermopsin standard with red color at retention time 11 min by HPLC device.

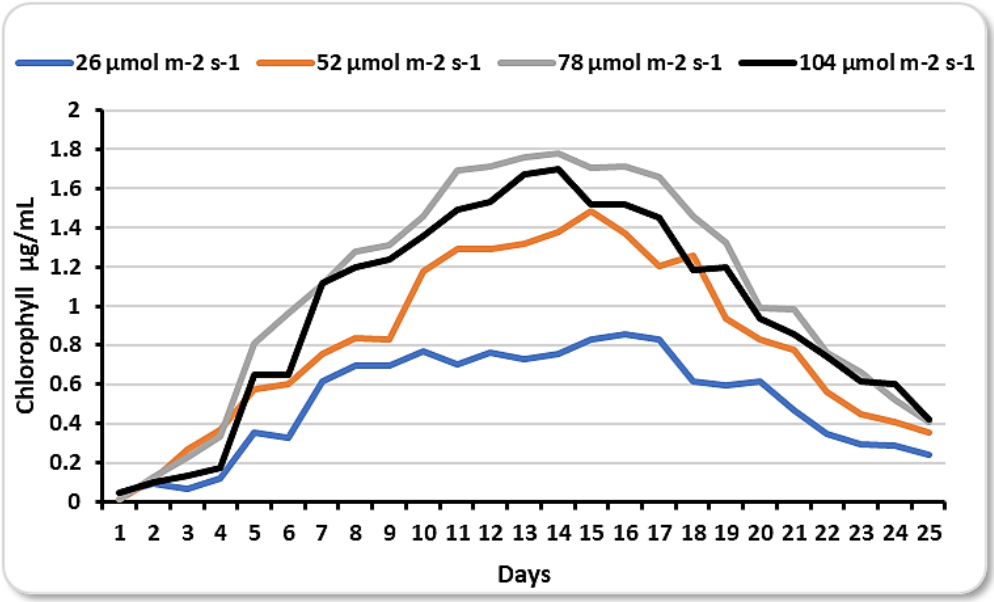

Figure 3. Growth curve of cyanobacterium *Nostoc ellipsosporum* according to the chlorophyll a concentration.

phase centrifuged at 7000 rpm for 15 minutes, the precipitated cells were dried on filter paper, weighed, and then suspended in 1 mL of distilled water. The suspension was then homogenized by a tissue homogenizer sonicator for 15 minutes in a water bath, shaken for 1 hour at room temperature, and then sonicated once more. Finally, the suspensions were centrifuged at 7000 rpm for 15 minutes, and 20 microliters of the supernatant were injected into the HPLC after its collection, and filtered through a 0.2 µm filter paper (Welker et al., 2002). For extracellular CYN, 100 of media were filtered throughout 0.2 µm filter paper, and then 20 microliters were injected into HPLC. The detection of CYN was performed by matching the retention time and absorbance spectrum of the sample with standards CYN which was purchased from Sigma Aldrich Company (32087-1ml) (Fig. 2)

Statistical analysis: Statistical analysis was carried out using the analysis of variance ANOVA one way and the least significant differences (LSD) for the comparison between the treatments represented by

Table 1. Effect of different light intensities on Cylindrospermopsin production by *Nostoc ellipsosporum*.

Light intensity µmol photons m^{-2} s^{-1}	Cylindrospermopsin toxin		
	Intercellular µg/mg Mean±SE	Extracellular µg/ml Mean±SE	Total µg/ml Mean±SE
26	0.005±0.0006B	7.73±0.0112D	7.735±0.0113D
52	0.0049±0.00003B	8.78±0.0043C	8.784±0.0044C
78	0.047±0.003A	42.5±0.031A	42.547±0.032A
104	0.009±0031B	26.39±0.4B	26.399±0.41B
LSD	0.019	0.4	0.41

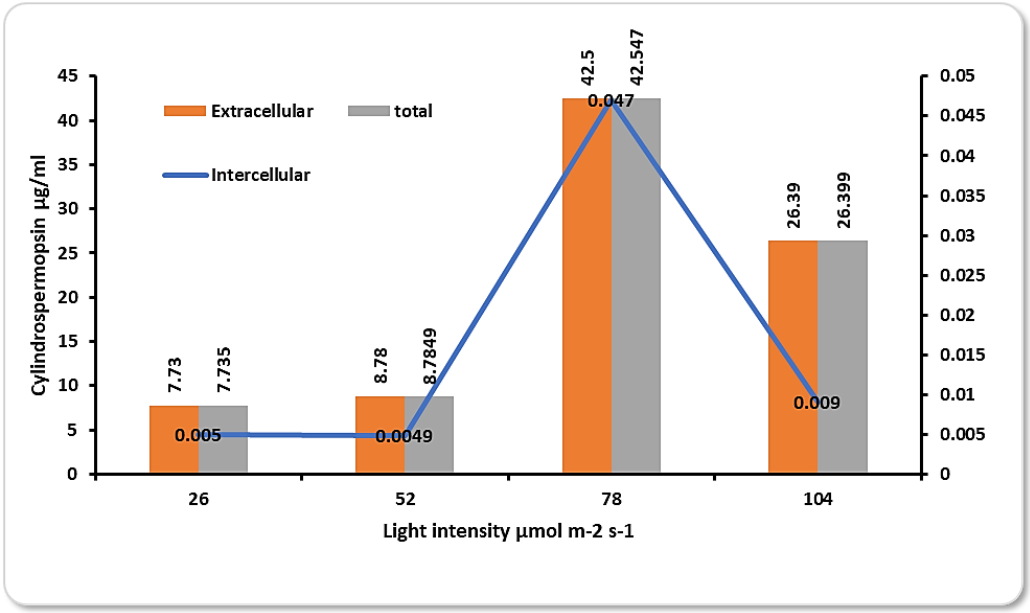

Figure 4. Effect of different Light Intensities on Cylindrospermopsin production by *Nostoc ellipsosporum*.

different light intensities and their effect on the CYN production by the cyanobacterium under study. all treatments were done in triplicate.

Results

Growth curve: Based on the results of chlorophyll a, the growth curve of the alga *N. ellipsosporum* was estimated (Fig. 3). A consistent increase in growth was detected after the fourth day, signaling the beginning of the exponential phase, which continued until the stationary phase was reached after the eleventh day. The results showed that the highest algal growth rate was at 87 µmol photons m^{-2} s^{-1} of light intensity, while the lowest growth rate was reported at 26 µmol photons m^{-2} s^{-1} of light intensity.

Cylindrospermopsin production: The results of the CYN production by *N. ellipsosporum* showed the highest inter and extracellular CYN reached 0.047 µg/mg and 42.5 µg/ml, respectively with a total value of 42.547 µg/ml recorded at the light intensity of 78 µmol photons m^{-2} s^{-1}. While the lowest production of intra and extracellular CYN was recorded at the light intensity of 26 µmol photons m^{-2} s^{-1}, which amounted to 0.0006 µg/mg and 7.73 µg/ml, respectively with a total value of 7.735 µg/ml (Table 1, Fig. 4).

Discussions

Light intensity is an important environmental factor that affects cyanobacterial growth and toxin production (Paerl and Otten, 2013). The community structure and dynamics of cyanobacteria and their toxins in the examined aquatic habitats are greatly affected by the availability of light in the water column (dos Santos Silva et al., 2020). One of the main constraints on the growth of microalgae is light intensity. Light duration and intensity have an impact

on the biochemical constitution of microalgae, biomass yield, and photosynthesis in microalgae (Krzemińska et al., 2014).

Based on the results of this study, the high and low light intensity registered the lowest CYN concentration agreeing with the findings of Hassan (2019), who studied the effect of light on CYN production by *C. raciborskii*. Hassan (2019) also pointed out that there was no cylindrospermopsin toxin in a sample throughout all treatments with both high and low light intensity, and this may indicate that light is not only the main factor affecting the production of toxin and may be due to the decrease in the concentrations of phosphate and nitrate. In the current work, the highest value of CYN was recorded at 78 µmol photons m^{-2} s^{-1}. Dyble et al. (2006) reported that the light environment will affect toxin production and may prove to be a key element in determining bloom toxicity. Dyble et al. (2006) also showed light intensity 18-75 µmol photons m^{-2} s^{-1} demonstrated a strong linear relationship between light intensity and both intra and extracellular CYN concentrations in *C. raciborskii*, and extracellular CYN concentrations increase significantly as the culture moved from log to stationary growth phase. Also, Briand et al. (2004) measured the growth rates of 10 different strains of *C. raciborskii* under various lighting conditions, showing that growth rates were positive over a wide range of lighting conditions (30-400 µmol photons m^{-2} s^{-1}), with the highest growth rates occurring at 80 µmol photons m^{-2} s^{-1}. Neilan et al. (2013) found that there may be a connection between the generation of toxins and photosynthesis given that the control of toxin genes and toxin production by light appears to be ubiquitous among cyanobacteria, so that may be the optimum condition i.e. moderate light led to the proper production of cyanotoxin.

Also, this study revealed that high or low light intensity affected the production of toxins by algae, and this may be because they are sensitive to light intensity, and cyanobacteria can get harmed by acute light conditions, whether they are high or low (Welkie et al., 2019). In natural ecosystems, the amount of sunshine normally rises, peaks about noon, and then falls until sunset. The cyanobacteria's preferred environment is impacted by this light cycle (Saha et al., 2016). The low toxin production by nostoc was due to the fact that the high light intensity caused oxidative stress that affected the photosynthesis process and inhibited it. Rahman et al. (2022) mentioned cyanobacteria undergo accelerated photosynthesis as a result of receiving more light, which is indicated by the increased H_2O_2 that results from this exposure and causes oxidative stress. Because of this, photoinhibition happens at greater light intensities to prevent photodamage when the photon energy surpasses what the photosystem can tolerate (Virtanen et al., 2019). In addition, the low light intensity can affect the production of toxins throughout impacting the efficiency of the photosynthesis process, as the low light is considered insufficient for some algae species to carry out their vital activity and inhibit physiological metabolic processes and biochemical components (Khan et al., 2018).

Another study examined the effects of light and temperature on the CYN production of two *Aphanizomenon flos-aquae* isolates at light intensities from 10 to 60 µmol photons m^{-2} s^{-1} at 16, 20, and 25°C (Preußel et al., 2009). Preußel et al. (2009) found that at 20°C, between 10 and 60 µmol photons m^{-2} s^{-1}, CYN concentrations considerably rise in both isolates. Likewise, the total CYN concentration of *Oscillatoria* sp. PCC was shown to be maximum during the stationary phase at lower and higher light intensity as well as during the exponential phase at moderate light intensity 10 µmol photons m^{-2} s^{-1} (Bormans et al., 2014). These results disagreed with the findings of this study and may be due to the variation of species in tolerance to the light intensity as Preußel et al. (2009) showed that different strains of the same species can produce different amounts of toxin in response to light.

The findings of this study revealed that extracellular CYN concentration at all light intensities is higher than intracellular CYN and this may be due to the sample collected in the stationary phase to

determine the CYN toxin. In this phase, many cyanobacterium cells become lysis in *C. raciborskii* cultures. Dyble et al. (2006) reported an enhanced intra-CYN and a consequent rise in extracellular CYN from 15-20% of the total CYN to 50%. Extracellular CYN stayed largely low during the exponential phase of the culture, however, after 1 month the cells had entered the stationary growth phase and the CYN content had dramatically increased (Saker and Griffiths, 2000). This demonstrates that the maximum cyanotoxin concentrations during the bloom may occur near its completion, perhaps as a result of cell lysis and CYN development during the bloom (Hawkins et al., 2001).

Conclusion

The results of the current study showed that light plays an important role in the production of cylindrospermopsin toxins by *N. ellipsosporum* and may have a thorough effect on the photosynthesis process of the gene regulator of these toxins. High production of CYN toxin occurred at moderate light 78 µmol photons m^{-2} s^{-1} which gave optimal growth, whereas the low and high light intensity led to inhibition production of CYN toxin.

Acknowledgment

We are very grateful to Ahmed J. Hassan for helping us in accomplishing the statistical analysis.

References

Andersen R.A. (2005). Algal culturing techniques. Elsevier. Academic Press. 592 p.

Beasley V.R. (2020). Harmful algal blooms (Phycotoxins). In: Reference Module in Earth Systems and Environmental Sciences. Elsevier: Amsterdam.

Bláha L., Babica P., Maršálek B. (2009). Toxins produced in cyanobacterial water blooms–toxicity and risks. Interdisciplinary Toxicology, 2(2): 36.

Boopathi T., Ki J.-S. (2014). Impact of environmental factors on the regulation of cyanotoxin production. Toxins, 6(7): 1951-1978.

Bormans M., Lengronne M., Brient L., Duval C. (2014). Cylindrospermopsin accumulation and release by the benthic cyanobacterium *Oscillatoria* sp. PCC 6506 under different light conditions and growth phases. Bulletin of Environmental Contamination and Toxicology, 92(2): 243-247.

Briand J.F., Leboulanger C., Humbert J.F., Bernard C., Dufour P. (2004). *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: Selection, wide physiological tolerance, orglobalwarming? Journal of Phycology, 40(2): 231-238.

Cirés S., Wörmer L., Timón J., Wiedner C., Quesada A. (2011). Cylindrospermopsin production and release by the potentially invasive cyanobacterium *Aphanizomenon ovalisporum* under temperature and light gradients. Harmful Algae, 10(6): 668-675.

dos Santos Silva R.D., dos Santos Severiano J., Aguiar de Oliveira D., Ferreira Mendes C., Virgínia Barbosa V., Ahii Chia M., de Lucena Barbosa J.E. (2020). Spatio-temporal variation of cyanobacteria and cyanotoxins in public supply reservoirs of the semi-arid region of Brazil. Journal of Limnology, 79(1).

Dyble J., Tester P., Litaker R. (2006). Effects of light intensity on cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsis raciborskii*. African Journal of Marine Science, 28(2): 309-312.

Filatova D., Jones M.R., Haley J.A., Núñez O., Farré M., Janssen E.M.-L. (2021). Cyanobacteria and their secondary metabolites in three freshwater reservoirs in the United Kingdom. Environmental Sciences Europe, 33(1): 1-11.

Hassan Z. (2019). Growth and Toxin production by *Cylindrospermopsis raciborskii* under different light intensities, Phosphate and Nitrates concentrations. University of South-Eastern Norway. Spring. 63 p.

Hawkins P.R., Putt E., Falconer I., Humpage A. (2001). Phenotypical variation in a toxic strain of the phytoplankter, *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) during batch culture. Environmental Toxicology, 16(6): 460-467.

Khan M.I., Shin J.H., Kim J.D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories, 17(1): 1-21.

Krzemińska I., Pawlik-Skowrońska B., Trzcińska M., Tys J. (2014). Influence of photoperiods on the growth rate and biomass productivity of green microalgae. Bioprocess and Biosystems Engineering, 37(4): 735-741.

Neilan B.A., Pearson L.A., Muenchhoff J., Moffitt M.C., Dittmann E. (2013). Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environmental Microbiology, 15(5): 1239-1253.

Paerl H.W., Otten T.G. (2013). Harmful cyanobacterial blooms: causes, consequences, and controls. Microbial Ecology, 65(4): 995-1010.

Preußel K., Wessel G., Fastner J., Chorus I. (2009). Response of cylindrospermopsin production and release in *Aphanizomenon flos-aquae* (Cyanobacteria) to varying light and temperature conditions. Harmful Algae, 8(5): 645-650.

Rahman M., Asaeda T., Abeynayaka H.D.L. (2022). Diurnal variations in light intensity and different temperatures play an important role in controlling cyanobacterial blooms. Research Square, https://doi.org/10.21203/rs.3.rs-2326349/v1

Ritchie R.J. (2006). Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynthesis Research, 89(1): 27-41.

Saha R., Liu D., Hoynes-O'Connor A., Liberton M., Yu J., Bhattacharyya-Pakrasi M., Balassy A., Zhang F., Moon T.S., Maranas C.D. (2016). Diurnal regulation of cellular processes in the cyanobacterium *Synechocystis* sp. strain PCC 6803: Insights from transcriptomic, fluxomic, and physiological analyses. MBio, 7(3): e00464-00416.

Saker M.L., Griffiths D.J. (2000). The effect of temperature on growth and cylindrospermopsin content of seven isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from water bodies in northern Australia. Phycologia, 39(4): 349-354.

Solter P., Beasley V. (2013). Safety assessment including current and emerging issues in toxicologic pathology. Haschek and Rousseaux's handbook of toxicologic pathology. 4th edition, Academic Press. Academic Press. 1152 p.

Tredici M.R. (2004). Mass production of microalgae: photobioreactors. Handbook of microalgal culture: Biotechnology and Applied Phycology, 1: 178-214.

Verma S., Thapa S., Siddiqui N., Chakdar H. (2022). Cyanobacterial secondary metabolites towards improved commercial significance through multiomics approaches. World Journal of Microbiology and Biotechnology, 38(6): 1-22.

Virtanen O., Valev D., Kruse O., Wobbe L., Tyystjarvi E. (2019). Photoinhibition and continuous growth of the wild-type and a high-light tolerant strain of *Chlamydomonas reinhardtii*. Photosynthetica, 57(2): 617-626.

Welker M., Bickel H., Fastner J. (2002). HPLC-PDA detection of cylindrospermopsin—opportunities and limits. Water Research, 36(18): 4659-4663.

Welkie D.G., Rubin B.E., Diamond S., Hood R.D., Savage D.F., Golden S.S. (2019). A hard day's night: Cyanobacteria in diel cycles. Trends in Microbiology, 27(3): 231-242.