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ECOLOGICAL PHYSIOLOGY AND BIOCHEMISTRY OF AQUATIC ORGANISMS

Cyanotoxin Production Dynamics: a Comprehensive Study of the Growth Stage of Selected Cyanobacteria, H_2O_2-triggered Apoptosis, and Light Conditions

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Abstract—Understanding the link between cyanobacterial growth and cyanotoxin production is key to controlling their proliferation in freshwater reservoirs. This study examined microcystin production in five cyanobacterial isolates, *Microcystis aeruginosa, Fischerella* sp., *Nostoc* sp., *Pseudanabaena* sp., and *Leptolyngbya* sp., across growth stages and stress conditions, using batch cultures in BG11 medium. Chlorophyll-a levels and Microcystin content were continuously monitored within cyanobacteria samples every eight days for two months. Under apoptosis-inducing conditions, H_2O_2 concentrations: 10, 60, 125, 250 mg L^{-1}, and exposure duration: 3, 6, 9, 12 h were applied to assess the cytotoxicity, MC content, and morphological activities. Samples were exposed to two light intensities: 0 and 50 μmol m^{-2} s^{-1}. Analyses were conducted on total soluble protein content, MC content, and ascorbate peroxidase activity (APX). The research shows cyanotoxin production varies between lag and exponential phases, especially in filamentous cyanobacteria (>150 and 60–150 nm colonies). *Pseudanabaena* sp. (2.541 ± 0.57 mg L^{-1}) and *Fischerella* sp. (1.5152 ± 0.14 mg L^{-1}) showed higher microcystin toxicity than unicellular *Microcystis aeruginosa*. Intracellular toxin concentrations surpassed extracellular levels. The MC-LR microcystin variant showed significant differences in toxin levels compared to other variants ($p < 0.05$, ANOVA). Specifically, MC-LR levels were about 8.15 times higher than MC-RR and 2.16 times higher than MC-YR in treated cultures. Higher H_2O_2 levels and longer incubation times affected cyanobacterial toxicity, morphology, and microcystin output. Under intense light, APX activity rose by 25.77%, and microcystin production increased by 35.8%. However, toxin production in these organisms can fluctuate due to various environmental and molecular factors.

Keywords: artificial apoptosis, cyanobacteria, growth stages, light intensity, microcystin

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INTRODUCTION

Cyanobacteria Harmful Algal Blooms (cyano-HABs) pose a significant threat to human health and aquatic ecosystems. Cyanobacterial proliferations have acquired considerable interest in recent decades due to their frequent and extreme occurrence in freshwater systems worldwide (Polyak et al., 2023). A significant concern with cyanobacterial blooms is their ability to produce microcystin, a hepatotoxic peptide with adverse effects on mammals, including humans (Dittmann et al., 2013a; Liyanage et al., 2016; Vehovszky et al., 2012; Zhou et al., 2021). Microcystins (MCs) are cyclic heptapeptides synthesized through a non-ribosomal pathway by multifunctional enzymes known as peptide synthetase and polyketide synthase (Kaebernick et al., 2002; Nishizawa et al., 2000). These cyanotoxins are present across most cyanobacterial orders, but research has predominantly focused on *Microcystis* species(Heck et al., 2018; Kurmayer et al., 2002; Nishizawa et al., 2007; Rouhiainen et al., 2004; Tillett et al., 2000). Among microcystin, more than 279 MC variants are known (Bouaïcha et al., 2019). Each shares the typical cyclic structure: D-alanine-X-D-MeAsp-Z-Adda-D-glutamate-Mdha. Here, Adda represents 3-amino-9-methoxy-2,6,8,-trimethyl-10-phenyldeca-4,6-dienoic acid, D-MeAsp corresponds to D-erythro-β-methyl-aspartic acid, and Mdha denotes N-methyldehydroalanine. Nota-

bly, modifications in the Adda region or acylation of the glutamate significantly influence toxicity levels (Nishizawa et al., 2007; Rantala et al., 2003; Tillett et al., 2000).

This toxicity presents a substantial threat to global ecosystem services. Cyanobacteria encompass both microcystin-producing (toxic genotypes) and non-producing (non-toxic genotypes) populations (Magonono et al., 2018; Mikalsen et al., 2003). The genetic makeup of the toxic genotypes includes the *mcy* gene cluster responsible for microcystin synthesis, including ten genes spanning 55 kb, denoted from *mcy A* to *mcy J* (Farkas et al., 2014; Kurmayer et al., 2002; Tillett et al., 2000). MC production in cyanobacterial blooms is also responsive to a variety of environmental factors (Jacinavicius et al., 2018; Werner et al., 2018). However, the exact mechanisms by which these environmental factors regulate microcystin production are still being investigated. (He et al., 2020; Long et al., 2001; Martin et al., 2020).In understanding cyanobacterial blooms, the relationship between morphological traits, physiology, and autecology assumes a pivotal role (Lewis, 1977); factors such as size, shape of the organism, whether unicellular or colonial remarkably influence the growth rates, resource uptake, and light interception properties (Álvarez et al., 2020a). Among cyanobacteria, they can take a wide range of shapes and forms, from individual cells with gas vesicles to groups covered in slimy material. These different forms help control and forecast blooms (Mantzouki et al., 2016). These variations in size in cyanobacterial colonies are subject to influences from physiological, ontogenetic, and environmental factors (Dmitrieva et al., 2024). Moreover, the morphology of colonies has demonstrated links with microcystin concentrations in ecosystems. Notably, larger colonies have exhibited a correlation with elevated microcystin levels compared to their smaller counterparts (Gan et al., 2012a; Kurmayer et al., 2003). Existing evidence implies that microcystin production develops in communities dominated by large colonies.

Emergency methods to rapidly remove toxic cyanobacteria from water bodies may be necessary to avoid drinking water contamination and bathing prohibitions. However, many existing emergency methods have undesirable side effects, such as the addition of harmful chemicals or the killing of non-target species, which can severely damage the ecosystem. Hydrogen peroxide (HP) has been used as an emergency method to control cyanobacterial blooms for several years. HP is a ROS that can damage cells at high concentrations. Cyanobacteria are more sensitive to oxidative stress than eukaryotic phytoplankton, so HP selectively suppresses them (Barrington and Ghadouani, 2008; Matthijs et al., 2012). A key advantage of HP treatments is that they degrade to water and oxygen within a few days, so they do not persist in the freshwater reservoirs. However, to avoid damaging non-target species, it is essential to improve the effectiveness of HP treatments against cyanobacteria. One of the environmental variables that is likely to influence the effectiveness of HP is light (Drábková et al., 2007). So, the stress encountered in the toxin production of cyanobacteria is an exciting area, but the specific drivers of this relationship still need to be clarified.

Planktonic species such as *Microcystis aeruginosa* are known for forming toxic blooms in eutrophic water bodies (Mohan et al., 2020). Benthic cyanobacteria are increasingly recognized as important contributors to toxin contamination in different water sources and thermally influenced aquatic systems (Wood et al., 2020). Although benthic cyanobacteria do not form large-scale surface blooms, their ability to colonize sediments, form biofilms, and release toxins into surrounding water makes them highly relevant in toxin contamination studies (Noffke et al., 2003). Therefore, this study examines microcystin production across both planktonic and benthic cyanobacteria. Thus, it aims to provide a more comprehensive understanding of their role in water quality and public health risks. *Microcystis aeruginosa* is one of the most well-known producers of microcystins, with numerous studies reporting its ability to produce a range of microcystin variants under different environmental conditions (Álvarez et al., 2020a; Magana-Arachchi and Liyanage, 2012; Liyanage et al., 2024). Similarly, *Fischerella* sp. (Heck et al., 2018b), *Nostoc* sp. (Bajpai et al., 2009; Liyanage et al., 2016.), *Pseudanabaena* sp. (Jayatissa et al., 2006; Mustapha et al., 2021), and *Leptolyngbya* sp. (Genuário et al., 2010; Ramya et al., 2024) have also been identified as microcystin-producing species in various studies. Research has shown that *Nostoc* sp. can produce microcystins under conditions of nutrient enrichment, particularly in eutrophic water bodies (Kurmayer, 2011).

This study investigates the relationship between the growth, morphology, and stress factors of five cyanobacteria species, namely *Microcystis aeruginosa*, *Fischerella* sp., *Nostoc* sp., *Pseudanabaena* sp., and *Leptolyngbya* sp., to elucidate their impact on microcystin production. The selection of cyanobacterial strains for this study was based on their ecological significance, environmental adaptability, and potential microcystin production across diverse aquatic environments in Sri Lanka. The isolates analyzed in this study were obtained from three environmentally and epidemiologically significant water bodies. The *Microcystis aeruginosa* was isolated from Beira Lake, Colombo (Urban Eutrophic Freshwater) highly urbanized and nutrient-rich lake that frequently experiences cyanobacterial blooms due to excessive nutrient input from industrial and domestic sources. The *Fischerella* and *Nostoc* species were isolated from CKDu-endemic well Water in Girandurukotte areas with a high prevalence of Chronic Kidney Disease of Unknown Etiology (CKDu). These filamentous cyanobacteria are capable of nitrogen fixation and have been reported to

produce secondary metabolites, including toxins. The species *Pseudanabaena* and *Leptolyngbya* were isolated from a natural Mahapelessa Hot Spring (Thermally Influenced Aquatic System), a site frequently visited for recreational purposes. These thermotolerant, benthic cyanobacteria thrive in extreme conditions and have been associated with toxin production, potentially contributing to long-term toxin accumulation in aquatic sediments and surrounding waters. Therefore, this study aimed to achieve the following specific objectives: (1) to discover the correlation between cell growth and microcystin production in the selected cyanobacterial species; (2) to assess the impact of both artificial and natural stressors on microcystin production.

MATERIALS AND METHODS

Cyanobacterial Cultures Identification and Incubation

Five cyanobacterial strains were selected for this research, including *Microcystis aeruginosa* (EF051239), *Fischerella* sp. (OR727806), *Nostoc* sp. (OR727808), *Pseudaanabaena* sp. (ON870364), and *Leptolyngbya* sp. (ON870366), with the numbers in parentheses representing the GenBank accession numbers assigned to the 16S rRNA gene sequences of each respective strain. These strains were taken from the Molecular Microbiology and Human Diseases Project (MM&HD) culture collection. They were previously isolated during studies conducted by the MM&HD, National Institute for Fundamental Studies (NIFS), Kandy, Sri Lanka. To cultivate the samples, BG11 medium was employed (Rippka et al., 1979). The samples were exposed to a 25 μmol m^{-2} s^{-1} photon flux to ensure controlled growth conditions at a temperature of 25 ± 2°C, which is optimal for the growth of the selected cyanobacterial strains.

Measuring Chlorophyll-a (Chl-a) Content

Chlorophyll-a was quantified in the cyanobacteria samples following the methodology outlined by Muhetaer et al., (2020) and Zavřel et al., (2015). Initially, 1 mL culture suspension of each cyanobacterial species, including the mat-forming benthic cultures, was gently disrupted by vortexing for 30 s to break up the mats and ensure a uniform suspension. The suspension was then centrifuged at 10000 *g* for 10 min at room temperature (25°C) to separate the cyanobacterial cells from any remaining debris. The pellet containing the cells were used for further analysis. After centrifugation, the supernatant was carefully removed, and each cell pellet was washed once with distilled water. Subsequently, the cell pellets were extracted with 1 mL of 80% ethanol. To ensure thorough mixing, the mixture was vigorously shaken and then incubated in darkness for 5 h at room temperature (25 ± 2°C). After the incubation period, each sample underwent another round of centrifugation at 10000 *g*. The resulting supernatant was measured using a UV-Vis spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at specific absorption wavelengths of 665 nm and 720 nm. It was required to be done within eight days after culturing and continuously for two months. The content of chlorophyll-a was later calculated using equation [1] (Ritchie, 2006)

$$\text{Chl-a } [\mu g/mL] = 12.9447(A_{665} - A_{720}). \quad (1)$$

In Eq. (1), Chl-a signifies the content of chlorophyll a, quantified in micrograms per milliliter (μg/mL), 12.9447 is the absorption coefficient for Chl-a in methanol whereas A_{665} and A_{720} correspondingly indicate the absorbance values at 665 and 720 nm.

Cyanobacterial Size Estimation

The size assessment involved a comprehensive morphological characterization of individual organisms encompassing single colonies and filaments. Measurements of length and width (Maximum linear dimension of unattached colonies, referred to as MLD, in μm) of each organism were precisely conducted using ImageJ software (Alcántara et al., 2018; Álvarez et al., 2020; Brzozowska et al., 2019). For the filamentous cyanobacteria, the measurements were based on single filaments. To investigate if the filaments were homogeneously distributed within the image, results were obtained by comparing differently oriented picture grids. Each image was analyzed four times, with 4 different fields of view per sample selected along varying grids. That ensured the comprehensive coverage and accurate assessment of the filament distribution (Ernst et al., 2006). The size (in μm) was derived by calculating the mean of six distinct MLD measurements taken from single colonies of each cyanobacterium. This size estimation procedure was performed in triplicate for each species. The size measurement dataset was then categorized into three classes: organisms larger than 150 μm, organisms within the 60–150 μm range, and organisms smaller than 50 μm.

Artificial Apoptosis Using H_2O_2 Treatments

In this study, cultures in the exponential phase were harvested by centrifugation at 3000 *g*. The resulting pellet underwent triple washes with distilled water before being inoculated into 50 mL flasks containing BG11 media. The initial cell concentration was maintained at an average of 1×10^8 cells mL^{-1}. The investigation into the impact of H_2O_2 included concentrations of 10 mg L^{-1} (0.29 mM), 60 mg L^{-1} (1.76 mM), 125 mg L^{-1} (3.67 mM), and 250 mg L^{-1} (7.35 mM) for time durations of 3, 6, 9, and 24 h (Ding et al., 2012). The treatments were conducted in duplicate, and cultures without HP addition served as the control. The control and treated cultures were maintained at 26°C,

illuminated by a 25 μmol m^{-2} s^{-1} light intensity, following a 14:10 h light: dark photocycle. The initial cell counts were determined by measuring absorbance at OD_{750} utilizing a UV-Vis spectrophotometer (UV-2450) and software (UV Probe 2.35).

Cytotoxicity Assay

The assessment of cytotoxicity for the extracts was conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, following the methodologies outlined in references (Bittner et al., 2021; Ding et al., 2012; Giannuzzi et al., 2021). This assay measures the reduction of MTT into an insoluble formazan product within metabolically active cells. For this analysis, cyanobacterial cells exposed to various concentrations of HP over 24 h were utilized. Subsequently, the cells were incubated at 37°C for 3 h after adding MTT at a final concentration of 0.5 mg mL^{-1}. After incubation, the medium was removed, and the resulting formazan crystals were dissolved in DMSO. The MTT assay was conducted three times for each concentration as part of the experiment. The samples' optical density (OD) was quantified at 595 nm using a microplate reader (FLUOstar Omega). Cell viability was determined by comparing the measured absorbance values of the test samples to the absorbance values of the control.

Light and Fluorescence Microscopy

The prepared slides were observed under an inverted fluorescence microscope (Olympus CKX-41SF, Philippines) equipped with an Olympus DP73 universal camera (Olympus DP73, Japan) at magnifications of ×10 and ×40. Morphological identification was performed on the control and treated samples after 24 h of exposure to each HP concentration.

Experimental Setup for Light Treatments

Cyanobacterial cells were subjected to two distinct photon flux levels (0 and 50 μmol m^{-2} s^{-1}) to investigate the growth response under different light conditions. The saturation light intensities were determined at a constant temperature of 25°C. Cold white fluorescent lamps provided light for the cultures. The illumination cycle followed a 14 h light and 10 h dark cycle (14L:10D). A light intensity of 0 μmol m^{-2} s^{-1} indicated complete darkness, where no external light source was employed. Cultures in the exponential growth phase, starting with initial average cell concentration 1×10^8 cells mL^{-1}, were utilized for this treatment. Harvesting of the cultures was performed through centrifugation at 3000 *g*. The resultant pellet underwent a series of three washes with distilled water before being introduced into 50 mL flasks containing BG11 media. The treated cell cultures under distinct light levels at twenty days were subjected to subsequent microcystin, protein, and enzyme analyses. Each treatment was carried out in triplicate to ensure reliable results and reduce experimental variability.

Total Soluble Protein Content Analysis

A 5 mL of each light-treated cell culture was homogenized and centrifuged at 4°C for 20 min at 10000 *g* to separate the cell pellet. Once the supernatant was removed, the cell pellet was thoroughly washed with autoclaved distilled water. Afterwards, it was dried in a vacuum chamber and ground using a motor. The total protein was extracted using a 0.5 M NaOH solution and centrifuged at 4°C for 20 min at 10000 rpm. The liquid portion obtained after centrifugation was used as a crude protein extract. For determining protein concentration, the Bradford method was employed (Bradford, 1976). A crude protein extract was initially treated with Coomassie (G-250) to induce colour development. This mixture was then incubated at room temperature (25 ± 2°C) for 10 min. After incubation, the solution's absorbance was measured at 595 nm using a microplate reader (FLUOstar Omega). A standard curve was created using Bovine Serum Albumin (BSA) standards ranging from 100 to 1000 μg mL^{-1}. (Asaeda et al., 2022b; Muhetaer et al., 2020).

Ascorbate Peroxidase Activity (APX) Assay

For the APX assay, 1mL of cell culture was thoroughly mixed with ice-cold phosphate buffer (50 mM, pH 6.0) containing polyvinylpyrrolidone (PVP). The PVP is used in this solution to mask the influence of phenolic compounds on the enzyme activity. Subsequently, the cultures were centrifuged at 5000 *g* at 4°C for 15 min; then, the supernatant (enzyme extract) was separated. The reaction mixture consisted of 100 μL of enzyme extract, 200 μL of 0.5 mM ascorbic acid in 50 mM potassium phosphate buffer (pH 7.0), and 2 mL of 50 mM potassium phosphate buffer (pH 7.0). This mixture was then mixed with 60 μL of 1 mM H_2O_2. The decreasing absorbance at 290 nm was measured every 20S for 3 min (Asaeda et al., 2022a, 2022b). The APX activity was calculated using the molar extinction coefficient of ascorbate 2.8 mM^{-1} cm^{-1}.

Microcystin Analysis

The MC concentrations were assessed four times throughout the experimental procedure: for preliminary cytotoxicity assay, for toxin-producing variability in different growth stages, for toxin-producing variability in artificial apoptosis conditions, and for toxin-producing variability in different light treatment conditions.

To extract microcystin, 10 mm of cyanobacterial culture were used. The cells were harvested during the exponential stage and centrifuged for 15 min at 3000 *g*.

The MC was extracted using 1 mL of 80% methanol (MeOH) and evaporated until dry. Finally, the MC was reconstituted in 1 mL of methanol (Bakr et al., 2022).

The extracted MC was further analyzed using high-performance liquid chromatography (HPLC) (Lawton et al., 1994). For the HPLC analysis, an HPLC system was equipped with an ultimate 3000 HPLC system, VWD detector, and C_{18} column. Detections were performed with reference to the MC standard (SIGMA ALDRICH 33578). The final measurement of microcystin was expressed in milligrams per liter (mg L^{-1}).

Preliminary Bioassay

The brine shrimp bioassay was performed for the extracts from cyanobacterial pellets and supernatants. The assay was conducted according to the procedure by Meyeret et al., (1982). Subsequently, the intracellular and extracellular toxins extracted from each cyanobacterial species were introduced into pre-marked 12-well plates. The plate wells contained ten live brine shrimp nauplii suspended in 2 mL of artificial seawater media. After incubating for 24 h at room temperature (25°C), the wells were inspected using a magnifying glass to count the number of surviving nauplii in each well. The endpoint for mortality in this bioassay was defined as nauplii's lack of controlled forward motion for 30 s during observation. After the incubation, the number of dead larvae in each well was recorded. From this data, the percentage of lethality of the brine shrimp nauplii for each concentration and control was calculated.

$$\% \text{ Mortality} = [(\text{Number of dead larvae in the well after 24 h})/(\text{Number of larvae initially placed in the well})] \times 100$$

$$\% \text{ of Final mortality} = \% \text{ of mortality} - \% \text{ of mortality in controls}$$

Toxin Extraction in Different Growth Stages

The microcystin was extracted within eight days after culturing, which was then maintained continuously for two months. To evaluate the cellular microcystin (MC) contents, the cells retained on the filters (referred to as intracellular MC) were used. In the concentrations of MC in the water phase, particular extractions were applied for the filtrates (referred to as extracellular MC).

Toxin Extraction in Artificial Apoptosis Conditions

The MC content was quantified at two distinct time points: at the initiation of the experiment (time zero) and after 24 h of exposure to HP in the H_2O_2 toxicity test. Specifically, only cellular microcystin (MC) contents were analyzed in this experiment.

Toxin Extraction in the Dark and under Intense Illumination Conditions

After twenty days of light treatments, at 0 μmol m^{-2} s^{-1} and 50 μmol m^{-2} s^{-1} light intensities, cellular MC content was analyzed from all samples.

Statistical Analysis

The chl-a and MC toxin analysis tests were summarized using calculated means and standard deviations. Before the analysis, the homogeneity and normality of the data were evaluated. The statistical analysis was carried out using Analysis of Variance (ANOVA). To determine the significance of differences in cytotoxicity, which were expressed as mean ± standard error (SE) at 24 h. Comparison tests, based on the Fisher significant differences table, were applied with a significance level of 0.05 for microcystin (MC) analysis; ANOVA and regression analysis were used. The results presented regarding light intensity in this study are reported as the mean ± standard error (SE) with the sample size (n) of 5. The statistical analysis was done using IBM SPSS Statistics for Windows and Origin Pro 18.

RESULTS

Cyanobacterial Species Confirmation

The cyanobacterial strains selected for this study were identified using a combination of molecular and morphological methods. Taxonomic identification was primarily based on 16S rRNA gene sequencing, with the obtained sequences being compared to those available in the GenBank database. The strains used in this study include *Microcystis aeruginosa* (EF051239), *Fischerella* sp. (OR727806), *Nostoc* sp. (OR727808), *Pseudanabaena* sp. (ON870364), *Leptolyngbya* sp. (ON870366), and the respective GenBank accession numbers are listed for reference. Morphologically, *Microcystis aeruginosa* is characterized by spherical or ovoid cells (2–5 μm in diameter) with gas vacuoles, while *Fischerella* sp. is recognized for its straight, unbranched filamentous trichomes. *Nostoc* sp. forms colonial aggregates with heterocytes and akinetes, which are indicative of its nitrogen-fixing capabilities. *Pseudanabaena* sp. displays a filamentous morphology with distinct trichomes, and *Leptolyngbya* sp. is another filamentous cyanobacterium often distinguished by its slight trichome curvature. These strains were selected based on their relevance to the study of microcystin production, and their identification was further confirmed through the analysis of their molecular and morphological characteristics.

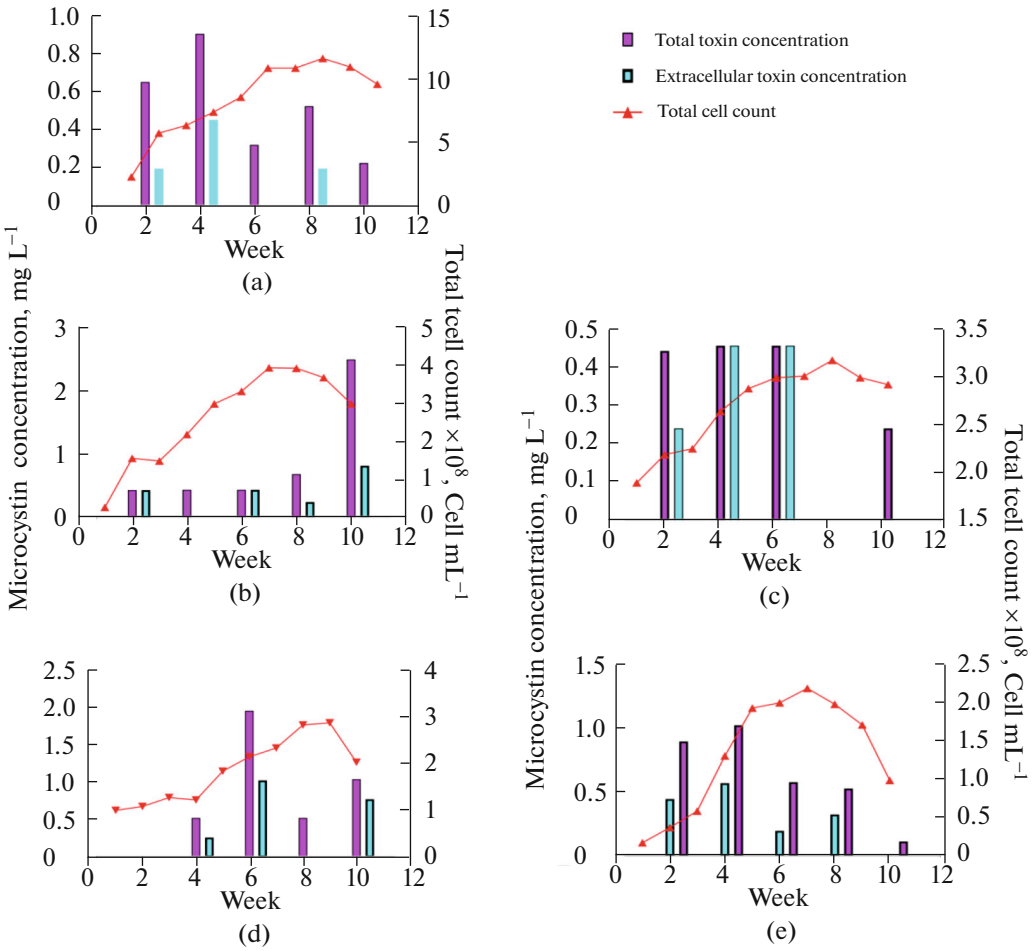

Fig. 1. Total Microcystin concentration and cyanobacteria cell count (secondary *y*-axis) measured in 2–10 weeks of growth in cyanobacterial isolates. Total extracellular toxin concentration is plotted for comparison (additional *y*-axis on the left). (a) *M. aeruginosa.,* (b) *Pseudanabaena* sp., (c) *Leptolyngbya* sp., (d) *Fischerella* sp., (e) *Nostoc* sp.

Growth Curve Analysis and Toxicity of the Different Size Fractions

Analysis of the growth curves revealed three distinct phases in cyanobacterial growth: lag, exponential, and stationary (Fig. 1). The average final cell concentration was 3.96×10^8 cells mL^{-1}. The results indicate a significant difference in cyanotoxin production between the lag phase and exponential phase of growth ($P < 0.05$). However, no significant difference was observed in the stationary phase ($P = 0.083$). *Pseudanabaena* sp. (2.541 ± 0.57 mg L^{-1}) and *Fischerella* sp. (1.5152 ± 0.14 mg L^{-1}) exhibited the highest toxin concentrations in stationary and exponential phases.

Figure (2a) illustrates the significant difference in cyanotoxin types between selected cyanobacterial species. The highest intracellular toxin was recorded from *Pseudanabaena* sp. and the lowest from *Leptolyngbya* sp. The intracellular toxin concentration is 11.78% higher than the extracellular concentration. The highest intracellular concentration was recorded during the stationary phase. Figure (2b) illustrates the cyanotoxin production between different size class colonies. The colonies in >150 and 60–150 µm size ranges showed significant differences in MC production. In this comparison, *Microcystis aeruginosa* was considered a colonial-type cyanobacterium with <50 µm range colonies, while the others were classified as filamentous cyanobacteria. In filamentous type cyanobacteria, *Fischerella* sp. and *Nostoc* sp. are in the 60–150 µm size range, and *Pseudanabaena* sp. and *Leptolyngbya* sp. belong to the >150 µm size range. In colonial-type cyanobacteria, particularly *Microcystis aeruginosa*, a significant negative correlation ($P < 0.05$, $r = -0.7135$) was observed between cyanotoxin production and its growth over time. In contrast, all selected filamentous cyanobacteria showed a positive correlation ($P > 0.05$, $r = 0.3324$).

This study detected the main microcystin variants MC-LR (Microcystin-LR), MC-YR (Microcystin-YR), and MC-RR (Microcystin-RR) in different growth conditions. The MC-LR microcystin variant exhibited a statistically significant difference in intra-

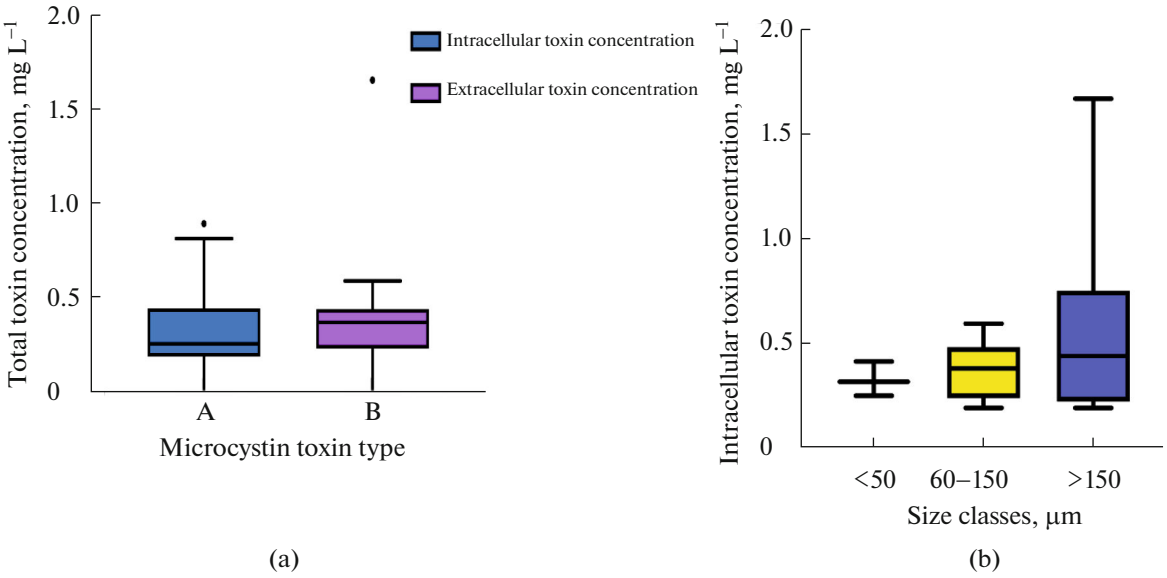

Fig. 2. (a) Boxplot illustrating the variation in cyanotoxin types relative to the total cyanotoxin concentration. (b): Distribution patterns of cyanotoxin-producing performance by cyanobacteria based on size fractions of the colonies (<50, 60–150, >150 μm).

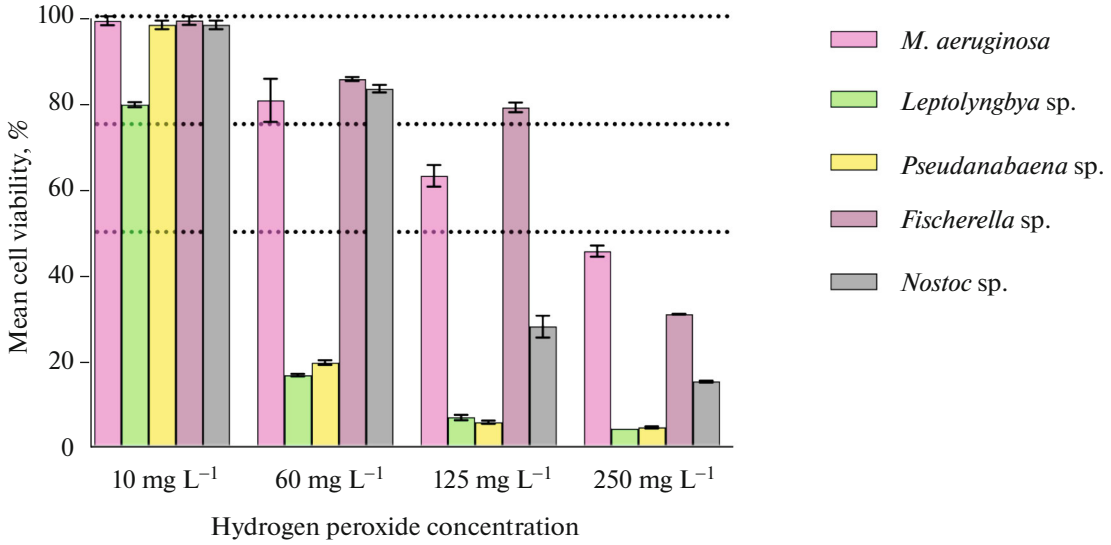

Fig. 3. The effect of graded H_2O_2 concentrations on selected cyanobacterial cultures with the MTT assay. Data are represented here as compared to the percentage of average OD_{595} of the control. The dashed lines represent the 100, 75, and 50% viability thresholds.

cellular and extracellular toxin concentration compared to the other variants ($p < 0.05$, ANOVA). Specifically, MC-LR levels were approximately 8.15-fold higher than MC-RR and 2.16-fold higher than MC-YR in treated cyanobacterial cultures. All the selected species produced MC-LR and MC-YR toxin variants, and MC-RR was not detected from *Pseudanabaena* sp. and *Leptolyngbya* sp. The MC-LR, MC-YR, and MC-RR were detected in 58.31, 37.34, and 4.34% of selected samples, respectively (Online Resource 01).

When considering the *Artemia salina* lethality assay, the supernatant represents the extracellular toxin production of cyanobacteria. The *Pseudanabaena* sp. exhibited a mortality rate of 45% in the extracellular toxin concentration. In contrast, the pellets, representing the intracellular toxin production of cyanobacteria, from *Fischerella* sp. and *Nostoc* sp. recorded a higher mortality rate of 85% in intracellular toxin concentration. These results indicate that *Pseudanabaena* sp. had the highest toxicity in the superna-

Table 1. Concentration of Microcystin in cyanobacterial pellets

Organism	Microcystin, mg L^{-1}			Total Microcystin content, mg L^{-1}
	MC-YR	MC-LR	MC-RR	
Microcystis aeruginosa	0.295	0.5623	0.1247	0.982
Pseudanabaena sp.	0.2048	<0.10	0.126	0.3308
Leptolyngbya sp.	<0.3	0.5562	<0.03	0.5562
Fischerella sp.	0.5927	0.2592	<0.03	0.8519
Nostoc sp.	<0.3	<0.10	<0.03	<0.03

tant, whereas *Fischerella* sp. and Nostoc sp. had the highest toxicity in the pellets. Notably, all three cultures with the highest toxicity are filamentous cyanobacteria. (Online Resource 02).

Artificial Apoptosis Analysis Using H_2O_2 Treatments

The cell suspensions from *Microcystis aeruginosa* had no significant influence on cell viability at 10 and 125 mg L^{-1} concentrations, while others showed a substantial reduction in cell survivability after 10 mg L^{-1}. The highest cytotoxicity occurred within *Pseudanabaena* sp. and *Leptolyngbya* sp. The cell viability of these species was reduced by 78.27 and 62.79%, respectively, compared with the cell viability % of the first lowest H_2O_2 concentration (10 mg L^{-1}) ($p < 0.05$). After 6 h of incubation, most species' cell viability significantly reduced ($p < 0.05$) (Fig. 3).

The microcystin detection ranged from the limit of detection (LOD) to 1 mg L^{-1} (LOD of MC-YR, MC-LR, MC-RR <0.30, <0.10, <0.03 respectively). The

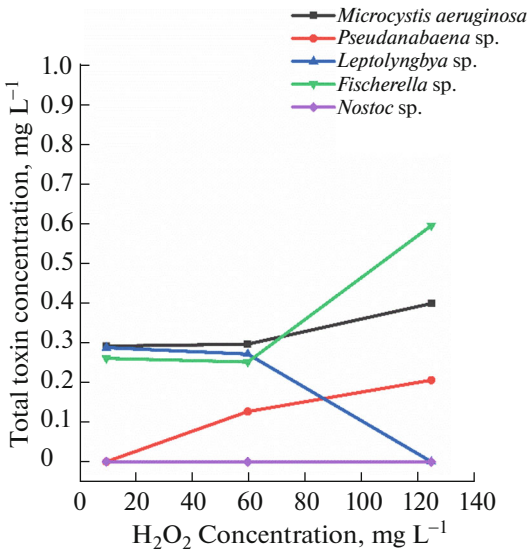

Fig. 4. Effect of different H_2O_2 concentration levels on total intracellular MC concentration in cyanobacterial extracts (mg L^{-1}).

MCs were not detected in *Nostoc* sp. compared with others. *Microcystis aeruginosa* and *Fischerella* sp. showed the highest intracellular MC content (Table 1).

The association between cellular MC content and cytotoxicity was calculated using linear regression ($R^2 = 0.1085$). There is an association that could be found in cellular total MC content ($r = 0.329$, $p > 0.05$) and cellular MC-LR content ($r = 0.518$, $p > 0.05$) with cytotoxicity (Average cell viability %) (Fig. 4). These show a strong correlation between total intracellular MC concentration with different H_2O_2 concentration levels compared with cyanobacterial species: *Microcystis aeruginosa* ($r = 0.918$), *Pseudanabaena* sp. ($r = 0.978$), *Leptolyngbya* sp. ($r = -0.921$), *Fischerella* sp. ($r = 0.890$), *Nostoc* sp. ($r = 0$) (Fig. 5).

Light Treatment Test

The TPC level did not significantly differ between the two light intensities ($p > 0.05$). The utmost protein content was recorded from *Microcystis aeruginosa* (509.27 ± 32.25 µg mL^{-1}) in high-light conditions. The lowest was recorded from *Leptolyngbya* sp. (362.06 ± 87.65 µg mL^{-1}). In low light conditions, the highest TPC was recorded from *Microcystis aeruginosa* and the lowermost from *Nostoc* sp. (Online Resource 3(A)).

The APX activity was significantly increased in high-light intensities ($p < 0.05$) compared to lower intensities. In high-light intensity conditions, the highest APX activity was recorded from *Fischerella* sp. (11.213 ± 3.44 nmol min^{-1} mg^{-1}). The lowest was recorded from *Microcystis aeruginosa* (7.103 ± 1.78 nmol min^{-1} mg^{-1}). Higher APX activity was recorded in low light conditions from *Microcystis aeruginosa* and the lowest from *Pseudanabaena* sp. The APX activity was increased by 25.77% in 50 µmol m^{-2} s^{-1} compared to the 0 µmol m^{-2} s^{-1} intensity. (Online Resource 3(B)) The total MC concentration shows a significant 35.8% rise in high-light intensities than the lower intensities ($p < 0.05$). In high-light intensity, the highest concentration was recorded from *Fischerella* sp. (0.9206 ± 0.08 mg L^{-1}) and the lowest from *Microcystis aeruginosa* (0.2563 ± 0.12 mg L^{-1}). In low-light

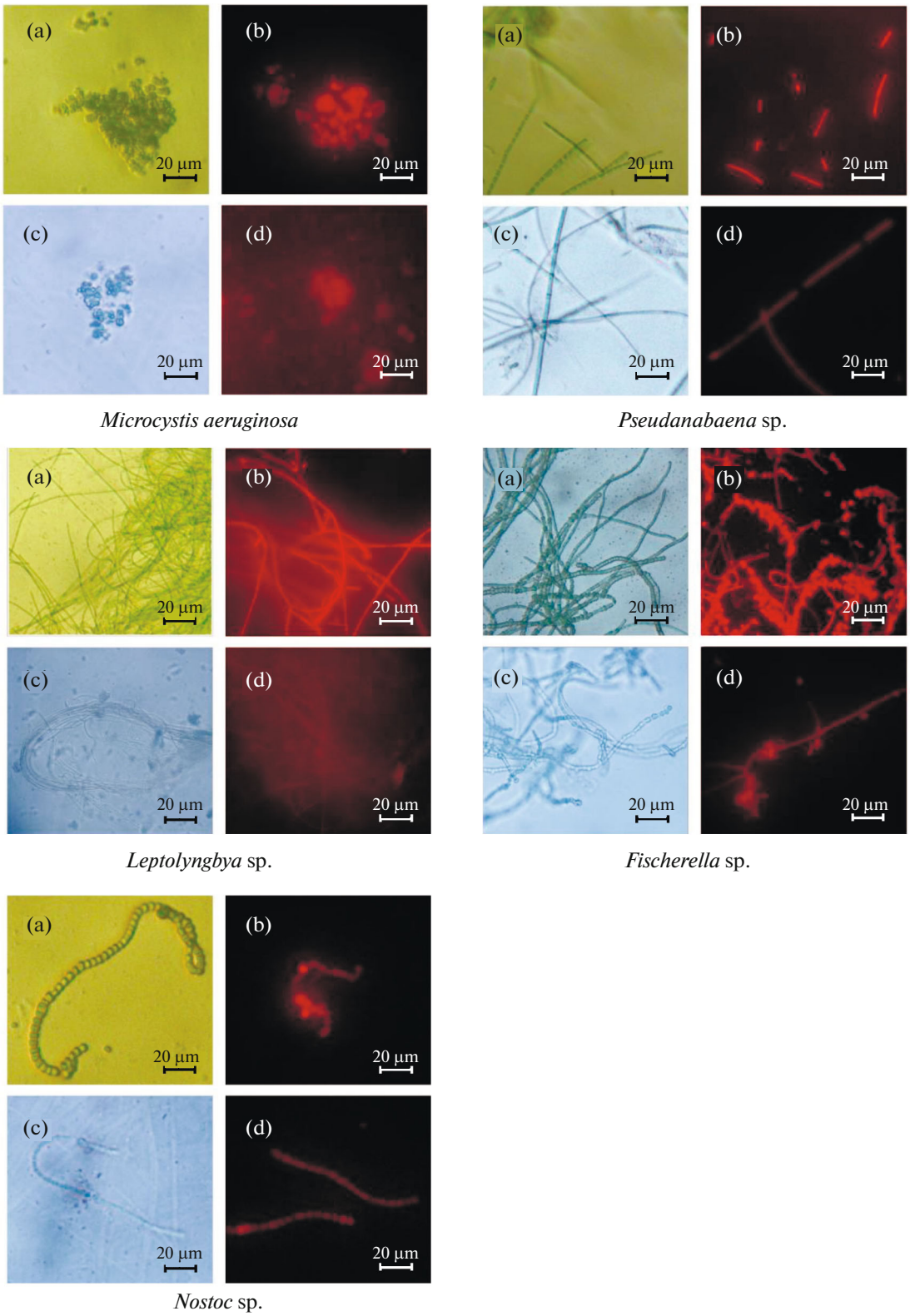

Fig. 5. Untreated sample and control samples of each cyanobacterial isolate before and after being treated with 250 mg L^{-1} H_2O_2 at 24 h observed with light and fluorescent microscope (Captured using Olympus CKX41 inverted microscope at 400× total magnification (10× eyepiece × 40× objective lens). Scale bar = 20 μm). (a) Microscopic view of an untreated culture displaying its typical appearance. (b) Fluorescence image showcasing cyanobacterial chlorophyll red fluorescence in untreated cultures. (c) The cultures after exposure to 250 mg L^{-1} H_2O_2 for 24 h. (d) Chlorophyll red fluorescence intensity in treated cultures after exposure to 250 mg L^{-1} H_2O_2 for 24 h.

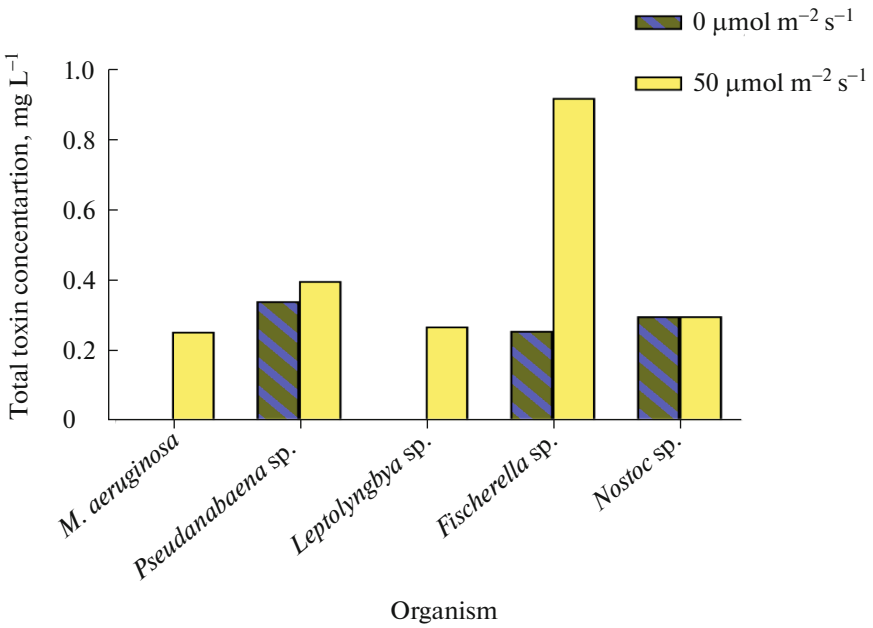

Fig. 6. The relationship between total toxin concentration in different light intensities after 20 days for each cyanobacterial isolate.

intensity, only the MC-LR variant was detected from *Fischerella* sp. (0.2591 ± 0.09 mg L^{-1}) and *Pseudanabaena* sp. (0.3430 ± 0.16 mg L^{-1}). Both MC-YR and MC-LR toxin variants are present in high-light-intensity conditions. The toxin amount of *Nostoc* sp. does not change in both light intensities (0.30 ± 0.07 mg L^{-1}) (Fig. 6).

DISCUSSION

Cyanobacterial Growth Curve Analysis

Our study highlighted several factors influencing cyanobacterial toxin production under average growth and stress conditions. In the context of their growth, two prominent phases have been distinguished: the lag and the exponential phases, covering approximately 1–8 weeks of growth, which hold utmost importance in cyanobacterial development. When introduced to a new environment or growth medium, cyanobacteria undergo a period of adaptation before beginning cell development. Cyanobacteria may have to adjust to new nutrient availability, temperature, pH, and other environmental factors. The cells might need to synthesize new enzymes or mechanisms to utilize the available nutrients effectively (Giannuzzi, 2019). If they experience stress or damage during acclimatization to a new medium, the lag phase allows them to repair and recover before they begin active replication. Cyanobacteria may produce specific enzymes or regulatory proteins to utilize nutrients in a new environment (Baracho and Lombardi, 2023; Muhetaer et al., 2020).

During the lag phase, individual cells within a population may exhibit variations in growth due to differences in new physiological conditions. Their physiological systems function optimally within the exponential phase, as they adapt to their environment. In this phase, the population undergoes rapid and balanced cell division. The exponential phase is characterized by the highest metabolic activity of the cyanobacterial cells, wherein nutrient uptake, energy production, and synthesis of cellular components such as DNA, RNA, proteins, and lipids occur at their maximum rates. In this study, all cyanobacterial species enter the exponential stage approximately within 3–8 weeks (Clark et al., 2018; Giannuzzi, 2019).

All cyanobacterial species except *Pseudanabaena* sp. displayed high microcystin production at the exponential stage. The enhanced metabolic activity can increase the production of secondary metabolites, including toxins (Smirnova et al., 2023). Cyanobacteria require various nutrients to grow and reproduce, such as nitrogen and phosphorus (Dittmann et al., 2013; Long et al., 2001; Zhou et al., 2021). During the exponential phase, nutrient availability is generally high as the cells efficiently utilize nutrients from the growth medium. An abundance of nutrients can stimulate the biosynthesis of toxins. The exponential phase can be a period of physiological stress as cells rapidly divide and compete for resources. So, that might be produced as part of the cellular defence mechanism or due to metabolic byproducts (Holland and Kinnear, 2013). These mechanisms could include switching between metabolic pathways, repairing damage to cellular structures, and adjusting their physiological processes to adapt to changing conditions (Li et al., 2020; Luo et al., 2017).

Some cyanobacteria produce toxins in response to high cell densities, a phenomenon known as quorum sensing.

In quorum Sensing, cyanobacteria release some chemicals called autoinducers into their environment. As the population of cyanobacteria grows, the concentration of autoinducers increases. When the concentration of autoinducers reaches a certain level, the cyanobacterial population can detect it and coordinate certain gene expressions within the population, like secondary metabolite production, pathogenicity, and competence (Kokarakis et al., 2023). As the population density increases during the exponential phase, signalling molecules produced by the cyanobacteria can trigger toxin production to communicate and coordinate responses to changing environmental conditions (Herrera and Echeverri, 2021). Some cyanobacteria exhibit cellular differentiation, producing specialized cells such as heterocysts (involved in nitrogen fixation) or akinetes (resistant cells). These specialized cells might also be involved in toxin production, and their formation can be more prevalent during the exponential phase (Legrand et al., 2016; Wood et al., 2021).

It is important to note that the reasons for increased toxin production during the exponential phase can vary among different cyanobacterial species and environmental conditions (Fernández et al., 2021). Past studies proposed that during exponential growth (the active phase), cells produce more microcystin to replace losses due to division into daughter cells. Most of the cell's constituents are doubled during mitosis and then equally divided between the two daughter cells, including the secondary metabolites, proteins, carbohydrates, chlorophyll, etc. The equal distribution of cell constituents between the two daughter cells is essential for ensuring each daughter cell has the resources it needs to survive and grow (Lyck, 2004). That could be a reason for maintaining higher toxin dosage in the exponential growth phase of cyanobacteria. With the energy-intensive nature of microcystin synthesis, toxic cyanobacterial populations should exhibit high growth rates, which can be attained mainly under sufficient resource availability.

Cyanobacteria frequently exhibit complex physiological responses to shifting environmental circumstances, which can impact their patterns of toxin production. During the stationary phase, the growth of cyanobacteria slows down due to the exhaustion of nutrients and the accumulation of waste products in the growth medium. In reaction to these conditions, certain cyanobacteria may increase the production of secondary metabolites, including toxins (Jacinavicius et al., 2019). Most prior studies have indicated that *Pseudanabaena* sp. reveals superior tolerance to low light conditions, pH variations of approximately 7 to 9, and phosphorus deficiency. These factors likely contribute to its dominance in reservoirs (Gao et al., 2018). The high tolerance of *Pseudanabaena* sp. to environmental changes could influence its ability to maintain toxin production during adverse conditions, such as the stationary phase, thereby contributing to high toxin production by *Pseudanabaena* sp. in the stationary phase. Furthermore, *Fischerella* sp. and *Pseudanabaena* sp. produced the peak toxin amount correspondingly in their exponential and stationary growth periods (Fig. 1). It is respectively (1.5152 ± 0.14 mg L^{-1}) and (2.541 ± 0.57 mg L^{-1}).

Past studies revealed that morphology is a good representative of microcystin production of cyanobacterial blooms. The fraction of medium-sized colonies (60 to 150 μm) showed high toxin production (Álvarez et al., 2020). Similarly, in the present study, the colonies between 60–150 μm and above 150 μm showed higher toxin production than the other classes. It is nearly an 85% increment than the <50 μm size class cyanobacterial species' toxin production. There could be several causative reasons. Previous research indicates that the cyanobacterial production of extracellular polysaccharides (EPS) contributes to increased colony size and the upregulation of genes associated with toxin synthesis (Gan et al., 2012). EPS production has increased colony size in several cyanobacterial species, including *Microcystis aeruginosa*. EPS production has also been shown to up-regulate genes related to toxin synthesis in some cyanobacteria. In the natural environment, large-sized cell colonies dominate (>150 μm); thus, small-sized colonies become stressed due to nutrient depletion. It caused the reduction of the toxin production. This size class indicated higher transcript abundance and microcystin concentration per cell (Jungmann et al., 1996; Wang et al., 2013). In this scenario, based on the present results and the available literature, it can be predicted that medium and large-sized cyanobacterial colonies produce the highest amount of microcystin during the active growth phase of a bloom (represented here as the 60–150 μm and >150 μm size-classes).

Furthermore, our study has obtained a significant difference in toxin quantities throughout distinct growth stages of the selected cyanobacterial colonies. The cumulative concentration of microcystin (MCs) was calculated as the sum of extracellular and intracellular toxin levels. According to the results, the intracellular toxin concentration showed an 11.78% increment than the extracellular toxin portion. These results are similar to previous literature records because cyanobacteria produce toxins within their cells. At standard conditions, the rate of toxin production exceeds the release rate so that intracellular concentrations can accumulate. The cell membrane's permeability can control toxins' movement between the intracellular and extracellular spaces. If the membrane becomes less permeable to toxins, they may remain trapped within the cell. Cell permeability can change when

cyanobacterial cells undergo stress conditions like high light intensities, chemical treatments, and radiation (Piel et al., 2019). Cyanobacteria might retain toxins within their cells during growth and division, increasing intracellular concentrations as the cell population expands. Some cyanobacteria can sequester toxins in specialized cell compartments, preventing their release into the extracellular environment (Georgieva and Vankova et al., 2019). The most important fact of the increment of intracellular toxin concentration is that extracellular toxins can sometimes be degraded by environmental factors and microorganisms in the medium, thus reducing their concentration outside the cells. Intracellular toxins might not be as quickly degraded (Schmidt et al., 2014). Different mechanisms can degrade the extracellular toxins. Microorganisms in the water, such as bacteria and fungi, can utilize cyanotoxins as a carbon or energy source, breaking them into more superficial and less toxic compounds (He et al., 2022). Sunlight, especially ultraviolet (UV) radiation, can initiate the degradation of cyanotoxins by breaking chemical bonds within the toxin molecules. This process is known as photolysis. In aquatic environments, Oxygen and reactive oxygen species (ROS) can react with cyanotoxins, causing their degradation. Processes like ozonation, which involves treating water with ozone, can also facilitate toxin breakdown. Cyanotoxins can adhere to the medium's particles and organic matter surfaces, leading to their physical removal from the water column. These processes can decrease their concentration and availability. Cyanotoxins can undergo hydrolysis in the presence of water, leading to the cleavage of chemical bonds and the formation of less toxic byproducts. Cyanotoxins can experience chemical reactions with other substances in the medium, transforming into less harmful compounds (Sidelev et al., 2024). The effectiveness of these degradation methods can depend on various factors, including the specific type of cyanotoxin, environmental conditions, water chemistry, and the presence of degrading microorganisms (Schmidt et al., 2014). So, this phenomenon indicates that part of the extracellular MC was lost or not recovered by analysis. Some of these reasons could be a possibility for reducing the extracellular toxin portion of this analysis.

Upon considering the microcystin toxin variants, the prevalent variants, namely MC-LR, MC-YR, and MC-RR, are all observable within this study. Furthermore, MC-LR is the most common toxin variant across all cyanobacterial species, constituting 58.31% of the total toxin content. The MC-YR variant is also identified across all samples, accounting for 37.34% of the total toxin content. In contrast, MC-RR was not detected in *Pseudanabaena* sp. and *Leptolyngbya* sp., making up only 4.34% of the entire toxin content. The most significant portion was recorded for MC-LR, widely considered a potent toxin characterized by its chemical stability in water. Acute exposure to MC-LR results in significant intra-hepatic haemorrhage, liver enlargement, and fatality, whereas chronic exposure has been linked to genotoxic and carcinogenic effects (Dittmann and Wiegand, 2006). Furthermore, the World Health Organization has categorized MC-LR as "probably carcinogenic for humans" (Grosse et al., 2006; Máthé et al., 2013). Conventional treatment systems face challenges in effectively breaking down the robust cyclic structure of MC-LR (Hitzfeld et al., 2000). Even after algaecide treatment, MC-LR exhibits notable persistence within the aquatic environment. A past study mentioned that the presence of MC-LR endured up to 21 days following the treatment of a *Microcystis aeruginosa* bloom with algaecides (Mokoan et al., 1995). The increment of the MC-LR portion in the present study also could happen due to its persistence in the medium.

Artificial Apoptosis Analysis Using H_2O_2 Treatments

The cytotoxicity results highlight the impact of various classic parameters on the apoptosis of selected cyanobacterial cells when exposed to four different HP concentrations. All concentrations, 60, 125, and 250 mg L^{-1}, except for 10 mg L^{-1}, significantly affected cell viability. Specifically, cells exposed to an HP concentration of 250 mg L^{-1} exhibited a substantial disruption in their intracellular microenvironment after 24 h, as evidenced by morphological indicators, including direct cellular damage (Fig. 4). The imagery revealed a reduction in green colour after treatments. This event may be caused by HP, which can cause oxidative stress in cells. Oxidative stress can potentially harm proteins, lipids, and nucleic acids within cells. In the case of chlorophyll, oxidative stress can lead to the breakdown of chlorophyll molecules, which in turn can result in a loss of the characteristic green colour of the cells (Zhou et al., 2018).

Furthermore, a substantial increase of nearly 66% in cellular toxin production, compared to the control value, was observed in *Microcystis aeruginosa, Pseudanabaena,* and *Fischerella* species during the treatments. However, the toxin production exhibited an increase only up to a concentration of 125 mg L^{-1}; beyond this threshold, the toxin concentration dropped to zero, leading to the further disintegration of the cells (Fig. 5). Many factors contribute to this occurrence, such as the diversity of species and strains, different stress responses, various mechanisms that control the production of toxins, oxidative stress, and cellular reactions. These factors depend mainly on the specific treatment conditions and the exposure length (Giannuzzi et al., 2021; Zhou et al., 2018). Nonetheless, the response from *Leptolyngbya* and *Nostoc* species deviated from these patterns. Compared to their respective control values, these species experienced a reduction in their toxin concentration. This phenomenon may be caused by the gradual breakdown of cyanobacterial toxins during regulated cell death, espe-

cially at high HP doses (Zhou et al., 2020). MC within the cell occasionally becomes bound to cellular proteins, preventing its extraction through methanol. After the occurrence of stress conditions induced by HP, MC bound to proteins can gradually be released into BG11 mineral media as the cells further disintegrate. The cytotoxicity of these species exhibited a significant reduction under the 250 mg L^{-1} HP condition, suggesting a potential explanation for this observation. The fate of these released microcystin can be elucidated through several paths. None of the species showed the presence of MC in their pellets after 24-h treatment at 250 mg L^{-1}. Because the cells naturally get degraded and release their intracellular toxins into the surrounding medium (Piel et al., 2019).

The fluorescence and light microscope images provide compelling evidence supporting the above Concepts. Following 250 mg L^{-1} HP treatment for 24 h, all species exhibited a reduction of the intensity of green colour and chlorophyll red fluorescence from their colonies. The reason could be that cyanobacterial cells underwent morphological disintegration. In natural settings, this scenario could occur during the application of HP for the cytotoxicity assessment of cyanobacteria (Giannuzzi et al., 2021).

Recent studies have demonstrated that complete MC degradation in field conditions can emerge within days (Schmidt et al., 2014). The findings from the lake treatment study conducted by Matthijs et al. (2012) align with the fact that MC vanished within 1–2 days after adding HP. Whether interactions between H_2O_2 and high light intensities influence the microbial degradation of MC remains an unclear question that could warrant further exploration in upcoming environmental management studies.

Light Triggering Stress

The growth of cyanobacteria heavily relies on the availability and quality of light. Cyanobacteria employ an oxidative stress response mechanism to shield themselves from extreme environmental conditions and to initiate antioxidant defense system reactions. In the present study, neither species displayed any noticeable effects on oxidative stress response mechanisms, such as toxin production and APX activity, under low-light intensity (0 μmol m^{-2} s^{-1}) for twenty days, especially when compared to the intense light treatment. However, when exposed to prolonged periods of high-light intensity (50 μmol m^{-2} s^{-1}), all species survived but experienced enhanced stress levels. Most species can handle short-term light-induced stress, but prolonged exposure reduces tolerance.

Interestingly, as light intensities increased, all species exhibited higher APX activity than those with lower light intensities. After 20 days of treatment, all species displayed elevated APX activity. This finding confirms that the APX activity of all cyanobacterial species played a more distinct role in antioxidant activity. Under increased stress conditions, the balance of antioxidants was also disturbed. These findings yielded similar results in the case of *Microcystis* and *Anabaena* species, where an increase in light intensity led to a decrease in their growth (Ashraful Islam and Beardall, 2017; Muhetaer et al., 2020; Venugopal et al., 2006).

The average protein content of selected cyanobacteria is relatively insensitive to changes in light intensity. It could be due to various reasons. Many cyanobacteria adjust to light changes through acclimation and adaptation. Higher light encourages adjustments in photosynthesis, photooxidative damage, and other cellular activities (Sukharevich et al., 2020). Cyanobacteria use pigments (e.g., carotenoids) to disperse excess energy. Such safeguards support cellular integrity and protein content under high light (Muhetaer et al., 2020). This enhanced light intensity might also be within the tolerance range for these selected species (Muhetaer et al., 2020; Rahman et al., 2023). Various cyanobacterial species and strains can display varying reactions to high-light conditions. Certain species could manifest more noticeable alterations in protein content compared to others.

The overall MC concentration was notably influenced by light intensity. After exposure to different light levels for twenty days, *Microcystis aeruginosa* and *Leptolyngbya* sp. showed an increase in intracellular MC content at 50 μmol m^{-2} s^{-1} despite not producing toxins under low light conditions (Fig. 6). Oxidative stress due to high light likely boosted cell growth, causing MC levels to rise over time(Boopathi and Ki, 2014). Some cyanobacteria can enhance toxin production in bright sunlight, known as light-induced toxin production. This is part of their stress response. High light can damage photosynthetic pigments, causing imbalanced energy capture and reactive oxygen species (ROS) production, producing toxins as a defensive response against oxidative damage (Polyzois et al., 2020; Wiedner et al., 2003).

Toxin production in cyanobacteria involves intricate genetic, biochemical, and ecological interactions. The natural intensity of sunlight is greater than the intensity of light in a laboratory setting; therefore, understanding cyanobacterial behaviour in high light is vital for maintaining water quality and ecosystem health. Researchers seek to find the link between light and cyanotoxin production by examining gene expression, tracking toxin levels, and delving into the driving molecular mechanisms.

CONCLUSIONS

Eutrophication in aquatic ecosystems has intensified, increasing the global possibility of cyano-HAB events. According to our findings and existing literature, it is proposed that medium and large-sized

microcystin-producing cyanobacterial species will generate more microcystin during a bloom's exponential growth phase (represented here as the 60–150 μm size class and >150 μm within the first eight weeks of the growth). Apoptosis causes changes in cyanobacterial cells, such as decreased chlorophyll levels, alterations in cellular morphology, and cyanotoxin production. At 250 mg L^{-1} HP, cells experience intracellular disruption. The cyanotoxin production initially increases, then dramatically decreases. 50 μmol m^{-2} s^{-1} light is unfavorable for these cyanobacterial species. *Fischerella* sp. and *Pseudanabaena* sp. could tolerate excessive light. Elevated light levels can govern the increased production of toxins. Thus, Assessing the growth patterns and size structure of cyanobacteria can be used to predict potential toxicity. Examining the toxin-producing behaviour of cyanobacteria under apoptosis and various light conditions can provide valuable information on these selected cyanobacteria. These findings offer insights for monitoring strategies and management approaches.

SUPPLEMENTARY INFORMATION

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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