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Research Article

Unraveling the Complexities of *Microcystis aeruginosa* Species Growth: The Role of Cell Density and Phosphorus

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Abstract

Microcystis aeruginosa (M. aeruginosa) is a common cyanobacterial species that can form harmful algal blooms (HABs) that negatively affect freshwater ecosystems and water quality. Environmental factors, particularly phosphorus availability and initial cell density, strongly influence its growth and bloom-forming potential. However, the response *of M. aeruginosa* to different phosphorus sources under varying cell densities remains insufficiently understood. This study investigated the effects of high (2×10^5 cells mL^{-1}) and low (2×10^4 cells mL^{-1}) initial cell densities and different organic and inorganic phosphorus sources on the growth and alkaline phosphatase activity of *M. aeruginosa* under laboratory conditions. Cultures were exposed to *dissolved organic phosphorus* (DOP), dissolved inorganic phosphorus (DIP), and phosphorus-free control media. Changes in cell abundance were monitored, while intracellular alkaline phosphatase activity (IAPA) and extracellular alkaline phosphatase activity (EAPA) were measured to assess phosphorus-acquisition responses. Initial cell density significantly influenced *M. aeruginosa* growth, with cultures established at higher cell densities showing a 108.41% greater growth rate than those at lower densities. A similar relationship between growth and phosphorus availability was observed at both initial cell densities. Cell abundance was generally higher in DOP- and DIP-containing media than in the phosphorus-free control. The highest recorded cell abundance was $0.755 \pm 0.005 \times 10^6$ cells mL^{-1}, while other recorded values were $0.320 \pm 0.005 \times 10^6$ cells mL^{-1} for DOP, $0.270 \pm 0.005 \times 10^6$ cells mL^{-1} for DIP, and $0.269 \pm 0.005 \times 10^6$ cells mL^{-1} for the control. *M. aeruginosa* also demonstrated the potential to develop into blooms even at the relatively low initial density of 2×10^4 cells mL^{-1}. Furthermore, IAPA was 25% higher than EAPA, highlighting the importance of intracellular phosphorus-acquisition mechanisms. Overall, both initial cell density and phosphorus availability play important roles in regulating *M. aeruginosa* growth. These findings highlight the importance of monitoring phosphorus sources and cyanobacterial populations to improve the prediction and control of harmful algal blooms.

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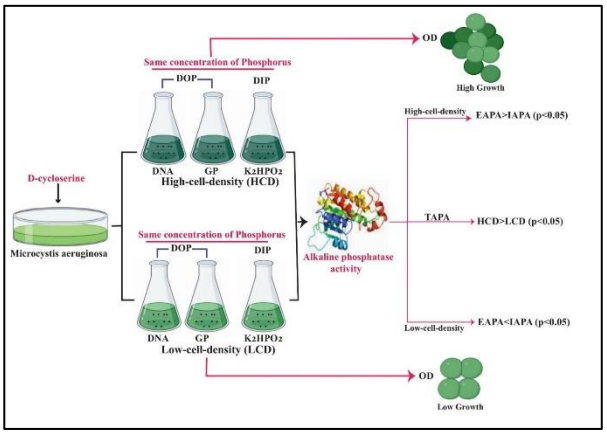

Graphical abstract

Introduction

Cyanobacteria, also known as blue-green algae, are aquatic microorganisms that are found in a wide range of aquatic ecosystems including lakes, ponds, rivers, and oceans. These organisms play an important role in the ecosystem as primary producers and are involved in the cycling of nutrients [1]. However, under certain environmental conditions, cyanobacteria can grow rapidly and form harmful algal blooms (HABs), which can have negative impacts on human health and aquatic ecosystems [2]. One of the most commonly observed cyanobacteria in freshwater ecosystems is *Microcystis aeruginosa*. This species is known for its ability to produce toxic compounds, including microcystin, which can cause a range of health problems in humans and animals [3]. *Microcystis aeruginosa* is also capable of rapidly reproducing under favorable conditions, which can lead to the formation of dense populations or blooms [4].

The growth of *Microcystis aeruginosa* is influenced by a range of environmental factors, including temperature, light, nutrients, and cell density [5]. Phosphorus is an essential nutrient for the growth of cyanobacteria, and it is often the limiting factor for the growth of these organisms in freshwater ecosystems. Cyanobacteria can utilize both inorganic and organic forms of phosphorus, and the availability of these forms can influence the growth rate and biomass production of these organisms [5,6]. Inorganic phosphorus is often found in the form of phosphate and is the most commonly available form of phosphorus in aquatic ecosystems [7]. The uptake of inorganic phosphorus can be influenced by a range of factors, including the concentration of phosphate in the water and the pH of the water [8]. Organic phosphorus is often found in the form of *dissolved organic phosphorus* (DOP) and particulate organic phosphorus (POP). DOP can be taken up by cyanobacteria through the activity of phosphatases, which hydrolyze DOP into inorganic phosphorus that can be taken up by the cells. POP can be taken up by cyanobacteria through the activity of extracellular enzymes that break down the particles and release the phosphorus [9].

Moreover, previous studies have shown that the growth of *Microcystis aeruginosa* is influenced by the cell density of the population. Low cell density has been found to be associated with slower growth rates, while high cell density can lead to increased growth rates and biomass production. However, some studies have shown that increasing cell density can lead to a decrease in growth rate due to resource competition [9,10]. For instance, [11] found that high cell density reduced the photosynthetic efficiency of *Microcystis aeruginosa*, which resulted in a decrease in growth rate. In contrast, other studies have suggested that high cell density can enhance growth rate through various mechanisms; e.g., some studies have found that high cell density can increase nutrient uptake efficiency, leading to faster growth (Lee, 1996). Additionally, high cell density can promote the formation of colonies, which can provide protection against grazers and enhance nutrient uptake [11]. The inconsistency in results among studies may be due to differences in experimental conditions, such as nutrient availability and light intensity, which can affect the impact of cell density on growth rate. Likewise, [11,12] found that increasing cell density had a negative effect on growth rate under nutrient-limited conditions but

not under nutrient-replete conditions. Overall, the impact of cell density on growth rate in *Microcystis aeruginosa* appears to be complex and dependent on various factors. Hence, more research is needed to understand the mechanisms underlying the relationship between cell density and growth rate in this species and to determine the conditions under which high cell density is beneficial or detrimental to growth. Cell densities improved cell-cell communication (quorum sensing). Enhanced nutrient utilization efficiency. Metabolic cooperation and environmental stabilization.

Additionally, the effect of cell density on the growth of *Microcystis aeruginosa* under different types of inorganic and organic phosphorus culture media is not well understood. Previous study have investigated the effect of different types of phosphorus sources on the growth of *M. aeruginosa* [11,13,14]. *M. aeruginosa* grew better on organic phosphorus sources than on inorganic phosphorus sources [13]. However, other studies have found that *M. aeruginosa* can use both inorganic and organic phosphorus sources, but with different uptake rates and efficiencies [11]. Hence, investigation of the combined effects of cell density and phosphorus sources on the growth of *M. aeruginosa* becomes vital. This is an important knowledge gap since the interaction between cell density and phosphorus sources can have a significant impact on the growth and toxicity of *M. aeruginosa* in natural systems. Similarly, under high cell density conditions, *M. aeruginosa* can release more extracellular organic compounds that can affect [14]. However, the effect of cell density on *M. aeruginosa* growth under different phosphorus sources remains unclear.

The primary aim of this study is to investigate the influence of cell density and phosphorus sources on the growth dynamics, nutrient utilization, and biochemical activities of *Microcystis aeruginosa*, with a specific focus on its potential for harmful algal bloom (HAB) formation in freshwater ecosystems. The study will explore the following objectives:

1. To examine the effect of low and high cell densities on the growth rate and biomass production of *Microcystis aeruginosa*. This objective will assess whether increased cell density promotes growth through enhanced nutrient uptake or reduces it due to resource competition.

2. To evaluate how different phosphorus sources (inorganic and organic) affect the growth of *Microcystis aeruginosa*, focusing on phosphorus uptake efficiency and growth rates. This will investigate whether *Microcystis aeruginosa* grows better on inorganic or organic phosphorus and how these nutrient sources influence its proliferation under different environmental conditions.

3. To assess the interaction between cell density and phosphorus type and determine its influence on the overall growth and productivity of *Microcystis aeruginosa*. Understanding whether high cell density and phosphorus type interact synergistically or antagonistically will offer deeper insights into the bloom-forming potential of *Microcystis aeruginosa*.

4. To measure and compare the concentrations of dissolved total phosphorus (DTP) and dissolved inorganic phosphorus (DIP) under low and high cell density conditions in both inorganic and organic phosphorus media. This objective will examine how the availability of different forms of phosphorus, in combination with cell density, influences nutrient cycling in the system.

5. To investigate the intra- and extracellular alkaline phosphatase activity (APA) of *Microcystis aeruginosa* at low and high cell densities, in relation to phosphorus acquisition and metabolic adaptations. By assessing APA, this study will explore how *Microcystis aeruginosa* adapts its phosphorus acquisition strategies under varying nutrient availability and cell densities.

Materials and Methods

Preparation of *M. aeruginosa* PCC7806

The species used in this study was *M. aeruginosa* PCC7806, provided by the Plant Biotechnology Laboratory of the School of Life Sciences. Cultures were generated in BG-11 at 27±1°C and illuminated with cool-white fluorescent light at 3000lux (48hrs

light/24hrs dark). Cultures were gently shaken three times a day by hand.

The specific growth rate (μ) of the algae cultures was determined using the following equation: $\mu = \ln(Nt) - \ln(N0)/t$

Where:

- μ = specific growth rate (1/day)
- Nt= cell concentration at time (cells/mL)
- N_0 = initial cell concentration (cells/mL)
- t = time period of the observation (days)

This equation allows for the estimation of the rate at which the algae cultures grew during the light and dark cycles.

Experimental design

Harvesting of *M. aeruginosa* medium PCC7806BG-11 required a standard procedure. First, we cultured *M. aeruginosa* PCC7806BG-11 in a light incubator at an intensity of 3000lux and at 27±1℃ temperature for 48 hrs. Second, an antibiotic 10 mol L^{-1}D-cycloserine was added and incubated in the dark for 24 hrs. Third, cultures were centrifuged at 7300rpm for 5 minutes in the exponential phase. In addition, a sterile phosphorus-free (P-free) medium was used to clean the pelleted cells three times [15]. Afterward, the cells were nurtured in a P-free medium for 72 hrs to diminish the intercellular phosphorus concentration. After phosphorus malnourishment, the cells were re-inoculated into 100ml beaker packed with 100 ml BG11 medium, holding different kinds of phosphorus compounds, e.g., deoxyribonucleic acid (DNA), Sodium Glycerophosphate (GP), and Orthophosphate (Pi). Particularly, DNA and GP represent organic phosphorus (DOP), while Pi represents inorganic phosphorus (DIP). The examination also included Phosphorus-free (P) medium. The study utilized the same phosphorus concentration for each medium, i.e., 0.05mg/L. Consequently, the examination employed 80 beakers in this experiment. Each analysis was conducted three times. The autoclaving technique was used to sterilize the material before operations. The study used the growth of *M. aeruginosa* at two different algae concentration levels, i.e., High Cell Density (HCD) 2×10^5 cells mL^{-1} and Low Cell Density (LCD) 2×10^4 cell mL^{-1}, to examine the ecological implications of DOP and DIP for algal blooms. The samples were collected at different time intervals, i.e., 0 hrs, 3hrs, 6hrs, 12hrs, 24hrs, 48hrs, and 100hrs.

Cell density examination, DTP, DIP, and APA

An algal sample of 3ml was analyzed at specific time intervals defined above for each category, i.e., DNA, GP, Pi, and P. The algal sample absorbance was examined at 680 nm in a spectrophotometer. The study followed the standard curve method to compute the cell densities [16]. The study used the malachite green chromogenic method to analyze the Dissolved Total Phosphorus (DTP) concentration of the medium (25 mL) [17] by using 2 mL of total phosphorus oxidant and autoclaved at 123 ℃ for 30 min. Subsequently, 2 mL malachite green chromogenic agent and 1 mL polyvinyl alcohol solution were added and shaken well. Finally, the absorbance was measured after 1 hr at 620 nm. The study employed the same technique for DIP; however, it was not autoclaved. To analyze the Alkaline Phosphates Activity (APA), a mixture of 1) 0.7 mL 1M-Tris-Buffer solution (pH=8.4), 2) 1 mL 10^{-3}pNPP solution, and 3) 30 μL 0.1M $MgCl_2$ solution was added to a sample of algae (1.3 mL) and placed at 37 ℃ for 1 hr in a dark incubator. Later, 0.3 mL 1M NaOH was added, centrifuged at 5000rpm for 5 min, and measured spectrophotometrically at 410 nm [18]. Extracellular APA sampling needs to be filtered through a 0.45 0.45μm membrane and does not need to be centrifuged. However, the mixture of solution remains the same. To examine the intercellular APA, the sample size of algae depends on its density; if the density is weak, then the sample size should be 30 mL to 40 mL. Nonetheless, if the density is strong, then the sample size can be reduced to 5 mL to 10 mL, centrifuged at 8000 rpm for 10 min, and the supernatant is poured. However, be careful not to discard the algae cells and then clean three times with a P-free medium. Next, 1 mL 1 M Tris-buffer solution was added; the algae solution was suspended, transferred to a 2mL lysis tube, and physically crushed for 40 s with a crusher. The remaining steps are the same as above.

Statistical analysis

The data were analyzed through Microsoft Excel 2023 and Origin Pro 2022. The results and data from the experiments were presented as the means ± standard deviation (SD), and figures and tables are included to summarize the results, and p values less than 0.05 were considered statistically significant.

Results

Figure 1 nominates the findings of the growth of *M. aeruginosa* at low cell density (2×10^4 cells-mL^{-1}) at different phosphorus levels, i.e., DNA, GP, Pi, and P-free medium. The results indicate that the continuous slow growth of M. aeruginosa was observed throughout the incubation period on all phosphorus sources. During the Initial low cell density adaptation period of 12 hours in culture medium, the growth curves of *M. aeruginosa* in different phosphorus experimental groups showed less significant differences in algal concentration. When the algae density was increased after 24 hrs, the acclimation period of *M. aeruginosa* entered the logarithmic growth period. However, the DNA organic compound grew rapidly throughout the incubation, and it was higher than in other phosphorus media. The highest value of DNA was recorded at $0.755 \pm 0.005\times10^6$cells. mL^{-1}; whereas GP, Pi, and P were $0.320 \pm 0.005\times10^6$cells. mL^{-1}, $0.270 \pm 0.005\times10^6$cells. mL^{-1}, $0.269 \pm 0.005\times10^6$cells. mL^{-1}, respectively. Hence, the outcomes revealed that LCD is a vital factor to enhance the growth of *M. aeruginosa* in organic, inorganic, and P-free media. Moreover, the outcomes of HCD showed that overall growth of *M. aeruginosa* was high throughout the incubation period.

The examination found no significant difference ($P>0.05$) in *M. aeruginosa* growth at 48 hrs in all phosphorus compounds. However, at 144 h, DNA exhibited the highest cell density ($0.575 \pm 0.005 \times 10^6$ cells mL^{-1}), followed by Pi ($0.490 \pm 0.005 \times 10^6$ cells mL^{-1}), GP ($0.437 \pm 0.005 \times 10^6$ cells mL^{-1}), and P ($0.402 \pm 0.005 \times 10^6$ cells mL^{-1}). Consequently, the findings revealed that high cell density was faster at promoting the population of *M. aeruginosa* at initial incubation period in comparison to low cell density. The examination specified that *M. aeruginosa* utilized the DNA at a high rate in comparison to other organic and inorganic phosphorus compounds at both LCD and HCD. The key reason behind this action can be that DNA is considered a vital source of phosphorus, carbon, and energy. The investigation also revealed that *M. aeruginosa* species are the toxin-producing algal blooms even at low cell density. Conclusively, the initial cell density and adaptation of *M. aeruginosa* showed significant differences in the phosphorus culture medium. Both low and high cell densities are responsible for increasing the growth of *M. aeruginosa*.

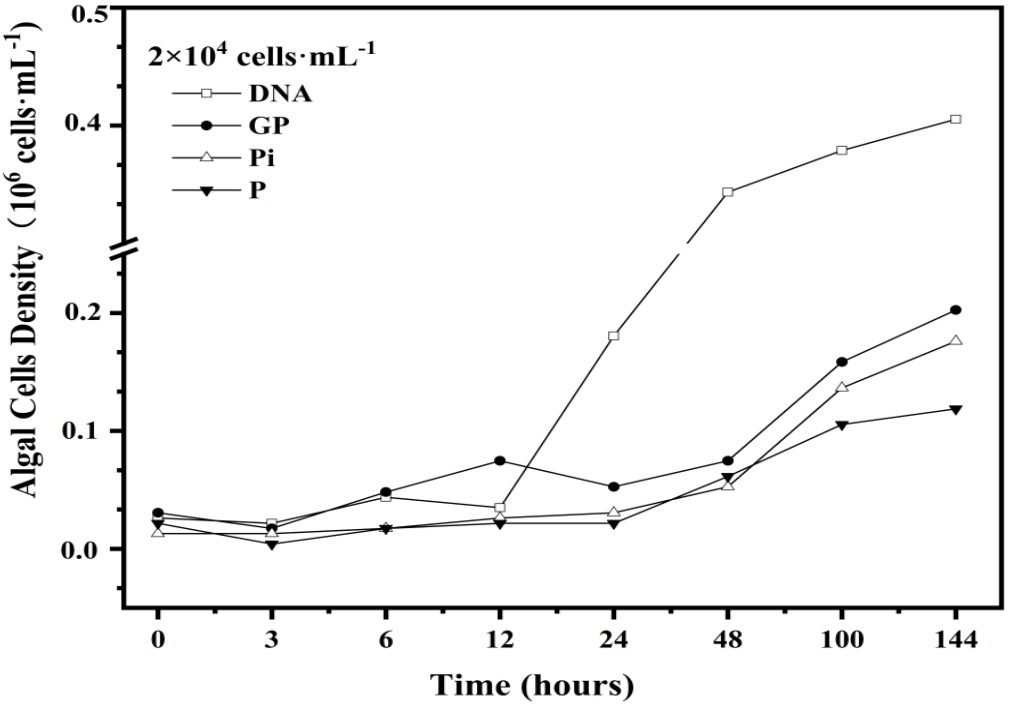

Figure 1: *M. aeruginosa* growth at LCD with different phosphorus compounds. Note: here DNA, GP, Pi, and P denote the deoxyribonucleic acid, sodium glycerophosphate, orthophosphate, and phosphorus-free media, respectively.

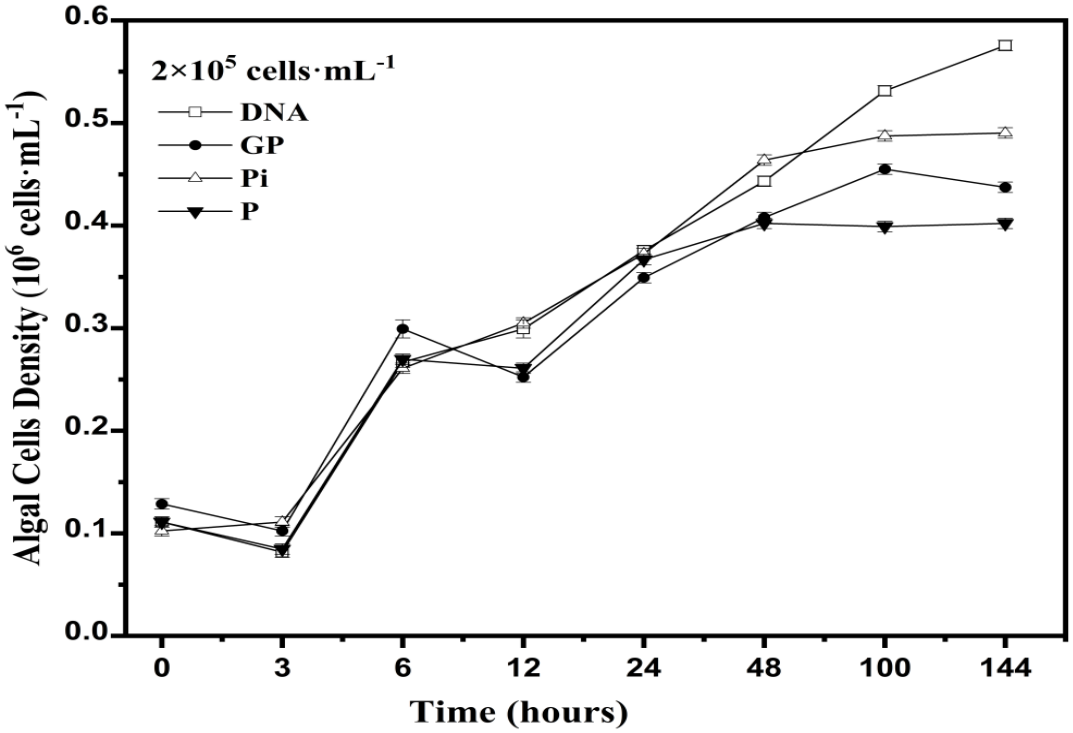

Figure 2: *M. aeruginosa* growth at HCD with different phosphorus compounds. Note: here DNA, GP, Pi, and P denote deoxyribonucleic acid, sodium glycerophosphate, orthophosphate, and phosphorus-free media, respectively.

Table 1: High and Low cell concentrations of DNA, GP, Pi, and P across the different time points with standard Deviations

Time	DNA (High)	SD (High)	DNA (Low)	SD (Low)	GP (High)	SD (High)	GP (Low)	SD (Low)	Pi (High)	SD (High)	Pi (Low)	SD (Low)	P (High)	SD (High)	P (Low)	SD (Low)
0h	0.11	0.01	0.06	0.01	0.13	0.01	0.06	0.01	0.1	0.01	0.02	0.01	0.11	0.01	0.05	0.01
3h	0.08	0.01	0.05	0.01	0.1	0.01	0.04	0.01	0.11	0.01	0.02	0.01	0.08	0.01	0.01	0.01
6h	0.27	0.01	0.09	0.01	0.3	0.01	0.1	0.01	0.26	0.01	0.04	0.01	0.27	0.01	0.04	0.01
12h	0.3	0.01	0.07	0.01	0.25	0.01	0.15	0.01	0.31	0.01	0.05	0.01	0.26	0.01	0.05	0.01
24h	0.38	0.01	0.36	0.01	0.35	0.01	0.11	0.01	0.37	0.01	0.06	0.01	0.37	0.01	0.05	0.01
48h	0.44	0.01	0.16	0.01	0.41	0.01	0.32	0.01	0.46	0.01	0.11	0.01	0.4	0.01	0.14	0.01
100h	0.53	0.01	0.68*	0.01	0.46	0.01	0.15	0.01	0.49	0.01	0.11	0.01	0.4	0.01	0.13	0.01
144h	0.58	0.01	0.75	0.01	0.44	0.01	0.32	0.01	0.49	0.01	0.27	0.01	0.4	0.01	0.21	0.01

Note: Values are presented as mean ± standard deviation (SD). High and Low refer to high and low initial cell density conditions, respectively. Measurements were taken at different time intervals from 0 to 144 h. DNA, GP, Pi, and P represent the parameters measured during the experiment. An asterisk (*) indicates a statistically significant difference ($p < 0.05$).

The findings in Figure 3A indicate that the concentration of dissolved total phosphorus (DTP) in low cell density showed a rapid decreasing curve after the first 3 hrs and a steep decline after 24 hrs of observation. Consequently, it revealed that the growth of *M. aeruginosa* is inversely proportional to the DTP concentration. Moreover, DIP level in the solution was initially high at 12 hrs

of observation for Pi and GP, but suddenly it went down at 24th hrs (Figure 3b). Also, DIP concentration in DNA and P-free medium showed a declining behavior at 48th hrs. Nevertheless, after 48th hrs all compounds showed upward behavior in DIP concentration. The study indicated that DTP and DIP have inverse affiliation with each other at LCD. The results of Figure 4A indicated that DTP concentration at HCD is continuously decreasing within 24 hrs for all phosphorus compounds and P-free media. However, it increases at 48th hrs but decreases again till 100th hrs. In addition, Pi and GP exposed the highest values in DIP concentration at 12th hrs. Nonetheless, it diminishes at 24th hrs but Pi and GP reach their maximum value again till 100th hrs. Additionally, utilization of DNA and GP in DIP concentration showed smooth behavior during the whole incubation period.

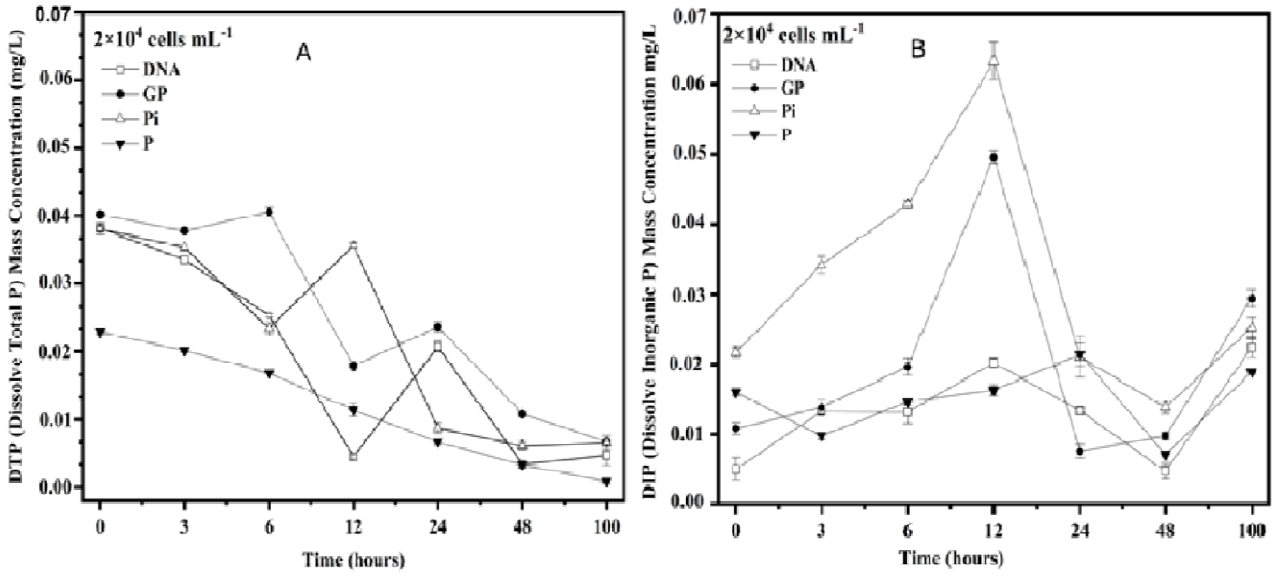

Figure 3A and 3B: Dissolved Total and Inorganic phosphorus concentration at low cell density. Note: here DNA, GP, Pi, and P nominate the deoxyribonucleic acid, sodium glycerophosphate, orthophosphate, and phosphorus-free media, respectively.

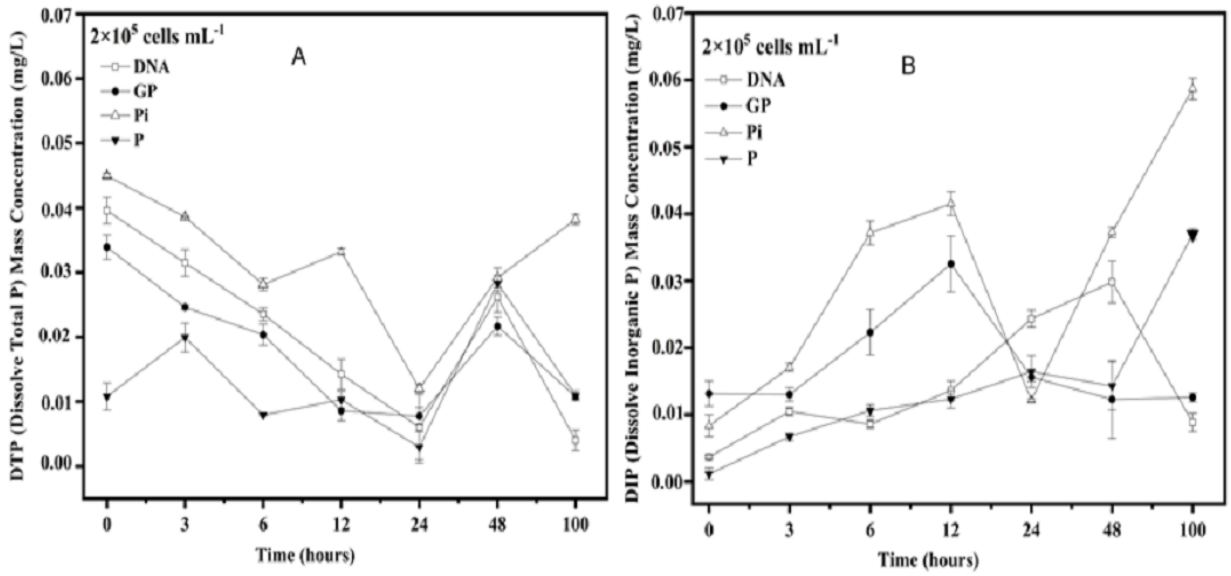

Figure 4A and 4B: Dissolved Total and Inorganic phosphorus concentration at high cell density (HCD). Note: here DNA, GP, Pi, and P denote deoxyribonucleic acid, sodium glycerophosphate, orthophosphate, and phosphorus-free media, respectively.

The study investigated per–cell extracellular and intracellular alkaline phosphatase activity at low and high cell density. The findings of Figure 5A indicate that per-cell extracellular alkaline

phosphatase activity (EAPA) is continuously increasing throughout the incubation period in LCD. Nonetheless, EAPA of *M. aeruginosa* was highest during the whole incubation period in the DNA and GP phosphorus compounds and achieved the value of 0.045± 0.0013×10^6 mg. L^{-1}per cell.h^{-1} and 0.040 ± 0.0005×10^6 mg. L^{-1}per cell.h^{-1}, respectively. Likewise, the findings of Figure 5B indicated that intracellular APA (IAPA) remains high during the overall incubation period in all media. The analysis concluded that intracellular activity was high at initial hours in DNA, GP, and Pi solutions, but it was low in P-free media. However, after passing the time, IAPA began to increase even in P-free media, implying that IAPA plays a vital role in the survival of *M. aeruginosa* in P-free media. In other words, P-free media can also boost the IAPA of *M. aeruginosa*. Similarly, IAPA showed the highest increase at in DNA compound. The maximum growth of IAPA at 100 hrs. for DNA, GP, Pi, and P was 0.0074 ± 0.00048×10^6 mg. L^{-1}per cell.h^{-1}, 0.004 ± 0.00019×10^6 mg. L^{-1}per cell.h^{-1}, 0.0044 ± 0.00062×10^6 mg. L^{-1}per cell.h^{-1}, 0.005 ± 0.00026×10^6 mg. L^{-1}per cell.h^{-1}, respectively. The result of low cell density indicated that *M. aeruginosa* could utilize DOP and hydrolyze it to DIP, passing through APA.

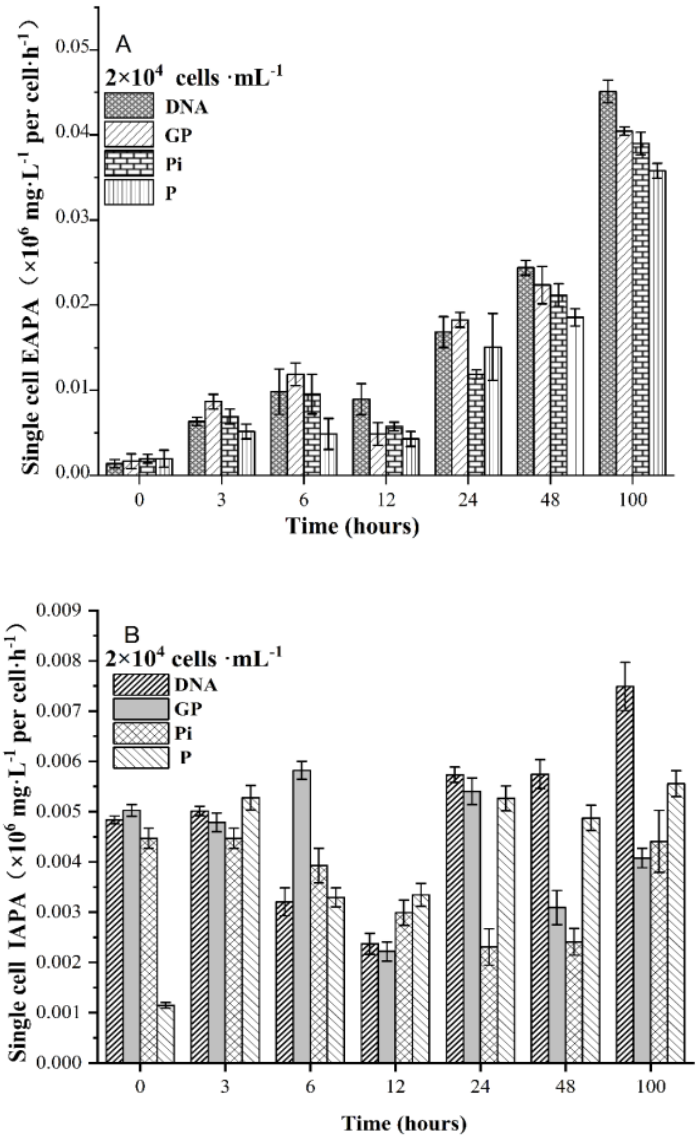

Figure 5A and 5B: Single-cell extracellular and Intracellular alkaline phosphatase activity at LCD. Note: here DNA, GP, Pi, and P denote deoxyribonucleic acid, sodium glycerophosphate, orthophosphate, and phosphorus-free media, respectively.

The results demonstrated in Figure 6A showed that the single-cell EAPA population increased significantly in all medium groups during the initial period. The maximum growth of per-cell

EAPA in HCD at 100 hrs remains 0.142 ± 0.0021×10⁶ mg. L^{-1} per cell.h^{-1}, 0.177 ± 0.0008×10⁶ mg. L^{-1} per cell.h^{-1}, 0.175 ± 0.002×10⁶ mg. L^{-1} per cell.h^{-1}, and 0.291 ± 0.0017×10⁶ mg.L^{-1} per cell.h^{-1} for DNA, GP, Pi, and P, respectively. Conversely, EAPA indicated maximum values at 6th and 48th hrs in Pi phosphorus medium. The findings of IAPA for high cell density are presented in Figure 6B. The outcomes stated that the enzyme activity of the single-cell IAPA was also increasing gradually. Whereas IAPA remained high in GP, P, DNA, and Pi medium at 3 hrs, 6 hrs, 48 hrs, and 100 hrs, respectively.

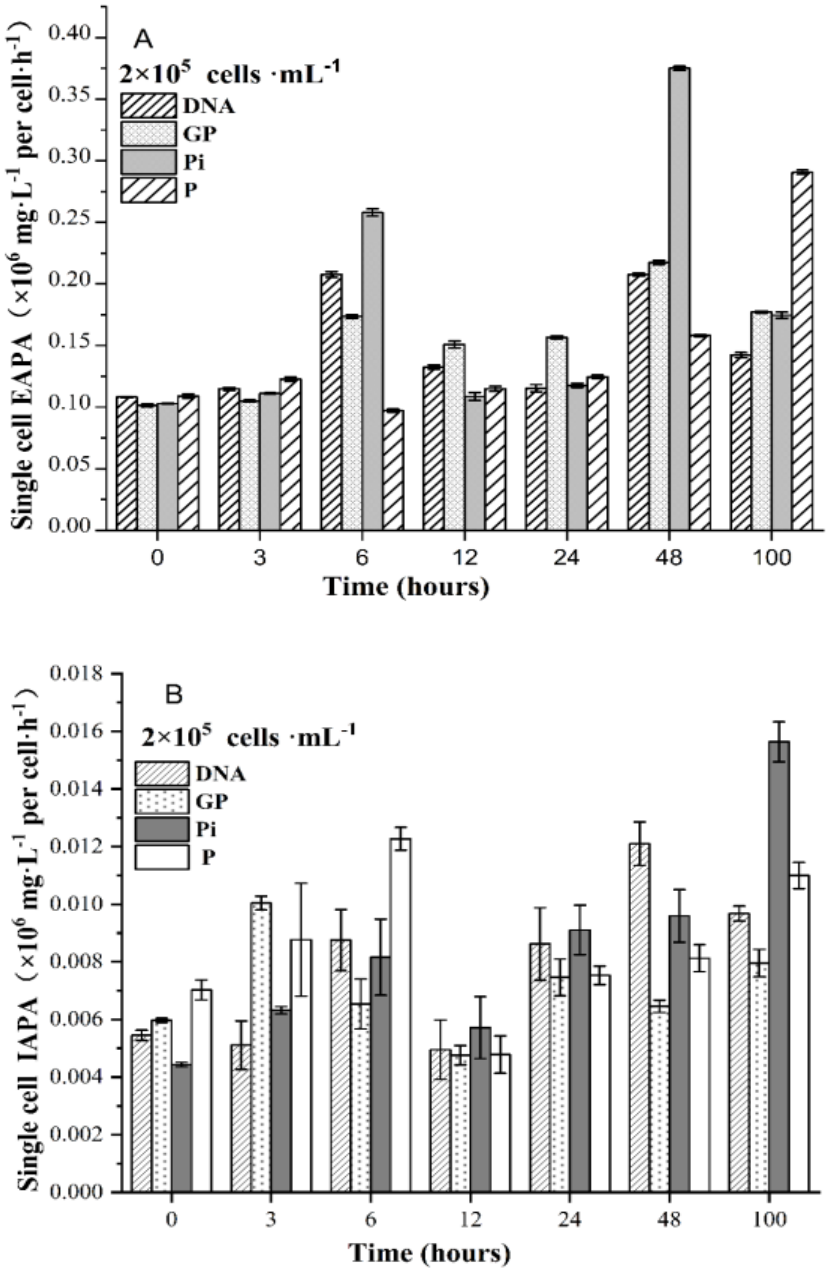

Figure 6A and 6B: Single-cell of Extra –and intracellular APA in HCD. Note: here DNA, GP, Pi, and P nominate the deoxyribonucleic acid, sodium glycerophosphate, orthophosphate, and phosphorus-free media, respectively.

Figure 7A nominates the findings of total cell Alkaline Phosphates Activity at LCD. The results revealed that the level of TAPA increases continuously with the passage of time. The initial low cell density of *M. aeruginosa* showed a higher growth curve when we tested the single cell of Total APA and when *M. aeruginosa* was treated with organic phosphorus during the incubation period. However, it showed the highest level at 100th hrs in DNA medium. The maximum growth of single-cell total APA with LCD in whole incubation for 100th hrs of medium culturing

period was 0.164 ± 0.0018×10⁶ mg.L⁻¹·per cell.h⁻¹ for DNA, 0.0235 ± 0.0017×10⁶ mg.L⁻¹·per cell.h⁻¹ for GP, 0.019 ± 0.0026×10⁶ mg.L⁻¹·per cell.h⁻¹ for Pi, 0.052 ± 0.0009×10⁶ mg.L⁻¹·per cell.h⁻¹ for P. On the other hand, Figure 7B showed that TAPA (HCD) was high even at the initial stage from 0 h to 3 hrs than the growth of low cell density at the same period and concentration $p<0.05$. The maximum growth of single-cell total APA with HCD in whole incubation for 100 hrs period DNA (0.3916 ± 0.0250×10⁶ mg.L⁻¹·per cell.h⁻¹), GP (0.2214 ± 0.0070×10⁶ mg.L⁻¹·per cell.h⁻¹), Pi (0.304 ± 0.0038×10⁶ mg.L⁻¹·per cell.h⁻¹), P (0.208 ± 0.008×10⁶ mg.L⁻¹·per cell.h⁻¹). This result also indicates that, combined with the analysis of intracellular and extracellular solubilized enzyme activity, there was no significant difference in TAPA in the DNA group EAPA compared to both initial cell densities in the organ phosphorus DNA groups; in both groups, the DNA gradually increased, whereas compared to IAPA, both cell densities were significantly increased.

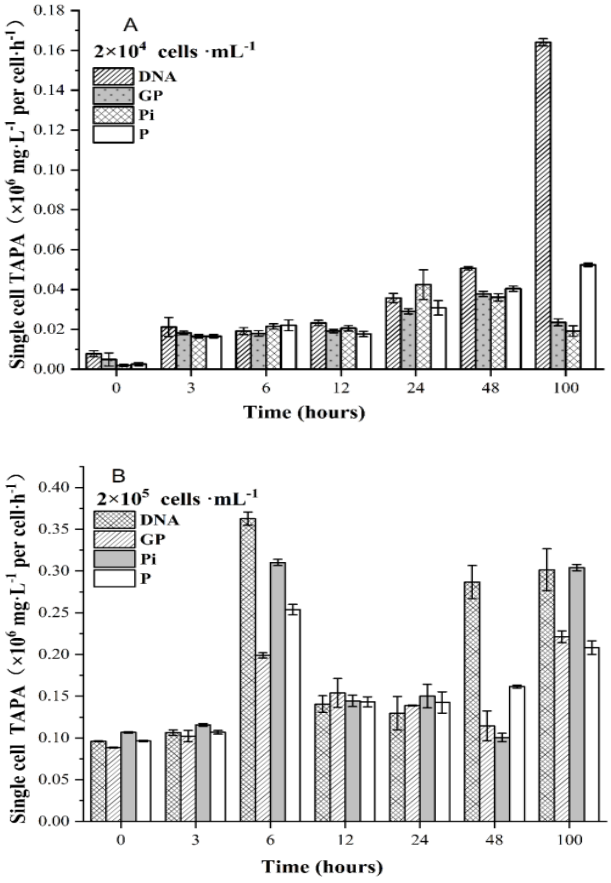

Figure 7A and 7B Single-cell of TAPA curve with low cell density (LCD) and high cell density (HCD). Note: here DNA, GP, Pi, and P nominate the deoxyribonucleic acid, sodium glycerophosphate, orthophosphate, and phosphorus-free media, respectively.

Discussion

The present study demonstrated that the initial cell density of *Microcystis aeruginosa* played an important role in determining its growth and phosphorus-acquisition capacity under different phosphorus conditions. Both low- and high-cell-density cultures were able to maintain growth in DNA, sodium glycerophosphate (GP), orthophosphate (Pi), and phosphorus-free media, indicating considerable physiological flexibility in phosphorus acquisition. Growth was initially slow during the adaptation phase, particularly at low cell density, but subsequently increased as cultures entered the logarithmic phase. Notably, DNA supported the greatest increase in cell density at both initial cell densities, suggesting that *M. aeruginosa* can efficiently exploit organic phosphorus sources when readily available inorganic phosphorus is limited. Previous studies have similarly shown that cyanobacteria can persist and proliferate under low-phosphorus conditions through physiological adaptations that improve nutrient acquisition [20–23]. The ability of *M. aeruginosa* to maintain growth even in phosphorus-free medium further highlights its capacity to survive under severe phosphorus limitation and may contribute to bloom development when phosphorus availability fluctuates in aquatic ecosystems.

The observed changes in dissolved total phosphorus (DTP) and dissolved inorganic phosphorus (DIP) further supported active phosphorus utilization by *M. aeruginosa*. The progressive decline in DTP during the early incubation period, particularly at low cell density, was consistent with active phosphorus uptake associated with increasing algal biomass. Differences among DNA, GP, and Pi treatments indicated that the availability and chemical form of phosphorus influenced nutrient utilization. The transient increases in DIP observed in several treatments may reflect enzymatic hydrolysis of dissolved organic phosphorus followed by uptake or temporary release of inorganic phosphorus during cellular metabolism. The contrasting patterns between DTP and DIP suggest that phosphorus was continuously being transformed between dissolved organic and inorganic pools rather than simply removed from the medium. These observations agree with previous reports showing that *M. aeruginosa* can utilize a range of organic phosphorus compounds and that

phosphorus availability is closely linked to cyanobacterial growth and bloom formation [24–26]. Thus, the ability to access both inorganic and organic phosphorus may provide *M. aeruginosa* with a competitive advantage in phosphorus-variable aquatic environments.

Alkaline phosphatase activity provided further evidence of the physiological mechanisms underlying phosphorus acquisition. Extracellular alkaline phosphatase activity increased progressively at low cell density, with particularly high activity in the DNA and GP treatments, indicating enhanced extracellular hydrolysis of organic phosphorus compounds. Intracellular alkaline phosphatase activity also increased over time and was especially pronounced in the DNA treatment. Importantly, enzyme activity increased even under phosphorus-free conditions, suggesting that phosphorus limitation stimulated the cellular mechanisms required to scavenge alternative phosphorus sources. These findings indicate that *M. aeruginosa* can enzymatically hydrolyze dissolved organic phosphorus to generate bioavailable inorganic phosphorus when environmental DIP becomes limiting. Similar phosphorus-starvation responses have been reported in cyanobacteria, where alkaline phosphatase is induced to improve the utilization of otherwise unavailable organic phosphorus [13,27–29]. The relatively greater enzyme activity associated with DNA further suggests that DNA-derived phosphorus may represent an important alternative nutrient source for *M. aeruginosa*, potentially supplying phosphorus while also providing carbon and energy.

The higher total alkaline phosphatase activity observed under high-cell-density conditions further demonstrated the importance of population density in phosphorus acquisition. High-cell-density cultures exhibited greater early enzyme activity than low-cell-density cultures, indicating that established populations may respond more rapidly to phosphorus limitation and activate nutrient-acquisition mechanisms more efficiently. The particularly high total activity observed in the DNA treatment suggests that organic phosphorus availability and enzymatic capacity acted together to support cellular proliferation. The increase in total activity under phosphorus-free conditions also demonstrates that phosphorus deprivation itself can stimulate extracellular and intracellular enzymatic processes. Such physiological plasticity may allow *M. aeruginosa* to maintain growth when inorganic phosphorus is depleted and to exploit organic phosphorus pools that are unavailable to less adaptable phytoplankton. Consequently, the interaction between initial cell density, phosphorus source, and alkaline phosphatase activity may be an important determinant of cyanobacterial persistence and expansion in nutrient-stressed waters.

Overall, the findings indicate that *M. aeruginosa* possesses substantial flexibility in phosphorus acquisition and can sustain growth across contrasting phosphorus conditions. High initial cell density promoted faster population development during the early incubation period, whereas low-density cultures demonstrated progressive physiological adaptation through enhanced alkaline phosphatase activity and utilization of alternative phosphorus sources. The consistently strong response to DNA suggests that dissolved organic phosphorus, particularly phosphorus-containing biomolecules, may contribute substantially to *M. aeruginosa* growth when inorganic phosphorus is scarce. These adaptations are ecologically important because they may enable *M. aeruginosa* to persist at low abundance and subsequently expand when environmental conditions become favorable, thereby contributing to the development and persistence of potentially harmful cyanobacterial blooms.

Conclusion

In the last decade, cyanobacterial blooms have become a matter of growing concern and can form dense algal blooms in freshwater and marine environments. There is growing evidence that common cyanobacterial species are produced at low and high cell densities, which contribute to the survival of the cyanobacterial population, such as *M. aeruginosa*. The present study analyzed and compared the initial cell growth of M. aeruginosa in different phosphorus-containing culture media. We investigated the effects of the nutrient compounds DOP and DIP on the growth of *M. aeruginosa* at low cell density (LCD) and high cell density (HCD). The findings of the study showed that *M. aeruginosa* species were toxin-producing algal blooms even at LCD. The results suggested that *M. aeruginosa* could respond to the LCD and

HCD environment by turning DOP into DIP and enhancing the transport of DIP. Hence, it might be an important reason behind the rapid growth of *M. aeruginosa* even from water blooms. In addition, because the growth of algal blooms, high and low cell densities depend on the component of the phosphorus source, algal cell assemblage could be largely determined by the composition of the phosphorus source in the phosphorus-limiting water ecosystem. The results showed that the mechanism of initial cell density and phosphorus source is a very important factor influencing the growth of *M. aeruginosa*. The relatively strong ability of *M. aeruginosa* to utilize organic compounds can be one of the reasons that *M. aeruginosa* is the dominant species in eutrophic Lakes. The changes in APA were similar in LCD and HCD medium.

This study addresses significant knowledge gaps concerning the combined effects of cell density and phosphorus sources on the growth and toxicity of *Microcystis aeruginosa*. While individual studies have investigated the role of cell density or phosphorus sources in cyanobacterial growth, the interaction between these two factors remains poorly understood, especially under different phosphorus regimes (inorganic vs. organic). The novelty of this study lies in exploring the combined impact of cell density and phosphorus types on *Microcystis aeruginosa*, a factor that has been largely overlooked in previous research; investigating nutrient dynamics (DTP and DIP concentrations) at varying cell densities, providing insights into phosphorus cycling and how it affects bloom dynamics; and evaluating enzymatic activities (APA) at different cell densities, offering a deeper understanding of metabolic cooperation and phosphorus acquisition strategies in *Microcystis aeruginosa*. The findings of this study will contribute to more effective management strategies for controlling harmful algal blooms, providing critical information on the environmental factors that regulate cyanobacterial growth in freshwater ecosystems. Future research should focus on providing preparation to control algal blooms in order to maintain the integrity of biological ecosystems for conservation and management.

Abbreviation

M. aeruginosa	*Microcystis aeruginosa*
DOP	Dissolved organic phosphorus
DIP	Dissolved Inorganic phosphorus
APA	Alkaline phosphatase activity
IAPA	Intracellular alkaline phosphatase activity
EAPA	Extracellular alkaline phosphatase activity
LCD	Low Cell Density
HCD	High Cell Density
HABs	Harmful Algal Blooms
DNA	Deoxyribonucleic acid
GP	Glycerophosphate
Pi	Orthophosphate
P	phosphorus-free

Credit authorship contribution statement

Musharraf Ahmed Ghori: Conceptualization, methodology, investigation, data collection, writing – original draft, and writing – review and editing, under the supervision of Mansoor Akram.
Raza Ahmed: Methodology, investigation, data collection, and writing – review and editing.
Muhammad Zakir Khan: Investigation, data collection, data curation, and writing – review and editing.
Hammad Ahsan: Investigation, data collection, formal analysis, and writing – review and editing.
Adnan Altaf: Data curation, investigation, visualization, and writing – review and editing.
Abbasi Hoosh Muhammad: Investigation, literature review, data interpretation, and writing – review and editing.
Amir Tanveer: Formal analysis, data interpretation, visualization, and writing – review and editing.
Mansoor Akram: Supervision, conceptualization, methodology, validation, project administration, writing – review and editing, and overall oversight of the study.

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Availability of data and materials

The data are available from the corresponding author upon reasonable request.

Conflict of interest

The authors reported no conflict of interest

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