

Original Research

IMPACT OF DIMETHYL PHTHALATE ON THE GROWTH AND STRUCTURE OF
CYANOBACTERIA (*Microcystis* sp.)

DOI: 10.5937/EcoTER26222K

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Abstract

This research was conducted to examine the influence of dimethyl phthalate (DMP) on the growth and physiological response of a cyanobacteria under laboratory conditions. A strain of the cyanobacteria Microcystis sp., isolated from freshwater ecosystems, was used in the experiment. The organisms were cultivated in a standard BG11 medium and its three modifications (nitrate-free BG11-1, phosphate-free BG11-2, and sulphate-free BG11-3) in the presence of three dimethyl phthalate concentrations (250, 500, and 1000 µg/L). Spectrophotometric determination of chlorophyll a was used for monitoring of organism growth and potential morphological changes in cells and contamination were controlled using microscopy. Also, pH value monitoring was done. Results demonstrate that the growth and physiological responses of Microcystis sp. are primarily governed by nutrient availability, while the effect of dimethyl phthalate is secondary and generally weak.

Keywords: dimethyl phthalate, cyanobacteria, chlorophyll a.

INTRODUCTION

Aquatic ecosystems are exposed to increasing pressure from anthropogenic activities in the modern era [1]. Industrialization, urbanization, and the extensive use of synthetic materials have led to a significant rise in the input of organic pollutants into the aquatic environment. A particular category is represented by the so-called “new” or emerging contaminants, which are increasingly being detected in surface and groundwater, but whose long-term effects on aquatic organisms and ecosystems are still not fully understood.

Phytoplankton communities are highly sensitive to environmental changes that influence their growth, composition, and dynamics. Therefore, monitoring variations in phytoplankton structure and dynamics is important for predicting future ecological changes. The ecological dynamics of phytoplankton are influenced by various factors: physical (temperature, salinity,

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light radiation), chemical (availability of nutrients and metals) and biological (predators, viral infections, and toxic effects of bacteria).

Due to these factors, the biomass density of each local phytoplankton population is highly variable and temporally unpredictable. The basic requirements for phytoplankton growth are water, light, and certain inorganic substances. Prokaryotic forms of phytoplankton are referred to cyanobacteria [2].

Cyanobacteria comprise a large, morphologically and ecologically heterogeneous group of phototrophic bacteria. These autotrophic microorganisms have a long evolutionary history, a wide range of metabolic pathways, and numerous adaptive mechanisms. They are photosynthetic prokaryotes that use chlorophyll *a* and phycobiliproteins to harness sunlight as an energy source, and they do not reproduce sexually. Certain species are capable of nitrogen fixation and play important roles in oxygen production, CO₂ fixation, and biomass production [3].

Research shows that representatives of the genus *Microcystis* are sensitive to the presence of various pollutants, including organic micropollutants such as phthalates. These substances can affect growth, photosynthetic activity, and physiological processes, and may also indirectly alter the structure of phytoplankton communities. Due to its frequent dominance, ecological importance, and potential toxicity, the genus *Microcystis* is often used as a model organism in studies examining the impact of pollutants on phytoplankton and the stability of aquatic ecosystems [4].

MATERIALS AND METHODS

Phytoplankton cultivation

The strain *Microcystis* sp. was isolated from a marsh ecosystem. The strain was cultivated under controlled laboratory conditions, using the BG11 nutrient medium for cultivation and maintenance. The chemical composition of the BG11 medium is following (per liter): 1.5 g NaNO₃, 0.04 g K₂HPO₄, 0.075 g MgSO₄·7H₂O, 0.036 g CaCl₂·2H₂O, 0.006 g C₆H₈O₇, 0.006 g ammonium ferric citrate green, 0.001 g EDTANa₂, 0.02 g Na₂CO₃, 0.00286 g H₃BO₃, 0.00181 g MnCl₂·4H₂O, 0.00022 g ZnSO₄·7H₂O, 0.00039 g Na₂MoO₄·2H₂O, 0.00008 g CuSO₄·5H₂O and 0.00005 g Co(NO₃)₂·6H₂O.

Experiment

The cyanobacteria strain used in the experiment was inoculated into sterile glass test tubes (a total of 48 test tubes). The impact of three dimethyl phthalate (DMP) concentrations (250 µg/L, 500 µg/L, and 1000 µg/L) on the growth and physiological parameters of the test organism was examined. All phthalate concentrations were tested in triplicate.

Impact of DMP were conducted in four types of nutrient media: standard BG11 medium, nitrate-free BG11 (BG11-1), phosphate-free BG11 (BG11-2), and sulphate-free BG11 (BG11-3).

Following sterilization, 10 mL of the corresponding medium and 1 mL (1000 µL) of the cyanobacterial suspension were added to each test tube and various concentrations of DMP were added. Control tubes did not contain DMP. The pure substance of DMP (CAS Number: 131-11-3, Sigma Aldrich, USA) was used for preparing a stock and working solutions. All DMP solutions used during the experiment were prepared at the laboratory of the Department

of Chemistry, Faculty of Sciences in Niš. The tubes were sealed with medical cotton plugs and placed under laboratory cultivation conditions. After setting up the experiment, the chlorophyll *a* concentration was monitored using the spectrophotometric method by an Agilent BioTek Epoch 2 Microplate Spectrophotometer.

In order to detect potential contamination and prevent irregularities that could affect the accuracy of the results, the test cultures were monitored via microscopic analysis (Leica Microsystems Leica DM1000 and Leica Microsystems DFC295 camera). Also, monitoring of pH value was done SensIon Multimeter (Hach, Germany) during the experiment.

RESULTS AND DISCUSSION

The experiment monitored the production of chlorophyll *a* (Chl *a*) in strains of *Microcystis* sp. during two weeks of exposure to different concentrations of DMP (250, 500 and 1000 µg/L). Measurements were made spectrophotometrically at wavelengths of 678 nm, 720 nm and 750 nm, and the concentration of Chl *a* was calculated according to the formula (1) [2]:

$$\text{Chl } (\mu\text{g} \cdot \text{mL}^{-1}) = 14.96 \cdot (\text{OD}_{678} - \text{OD}_{750}) - 0.616 \cdot (\text{OD}_{720} - \text{OD}_{750}) \quad (1)$$

The results, presented in Figure 1, show that the response of *Microcystis* sp. varies depending on nutrient conditions and the concentration of dimethyl phthalate, indicating a complex interaction between nutrients and pollutants.

Considering that BG11 is a standard medium for the cultivation of cyanobacteria and microalgae, while BG11-1, BG11-2, and BG11-3 are media with selective deficiencies of nitrate, phosphate, and sulfate, respectively, the obtained results allow for a detailed analysis of the effects of dimethyl phthalate under different nutrient conditions.

In the standard BG11 medium, which provides optimal conditions for growth, *Microcystis* sp. showed a continuous increase in chlorophyll *a* throughout the experiment. The highest values were recorded in the control treatment, while all treatments with dimethyl phthalate (DMP) exhibited slightly lower values. This indicates that under conditions of full nutrient availability, dimethyl phthalate did not have a stimulatory effect but rather acted as a mild growth inhibitor. *Microcystis* sp. did not demonstrate the ability to utilize the presence of the stressor to enhance its growth. Although the differences were not statistically significant ($p > 0.05$), the observed trend suggests that under optimal conditions, the control outperforms the pollutant-treated samples.

Under nitrate-deficient conditions (BG11-1), the overall growth of *Microcystis* sp. was significantly reduced compared to the standard medium, which is expected since nitrogen is essential for the synthesis of proteins, pigments, and nucleic acids. In these conditions, differences between treatments were minimal, and a slight increase in growth was observed at lower concentrations of dimethyl phthalate. However, this effect was not pronounced enough to be considered significant. This suggests that under nitrogen stress, nutrient limitation is the dominant factor, while the influence of dimethyl phthalate is of secondary importance.

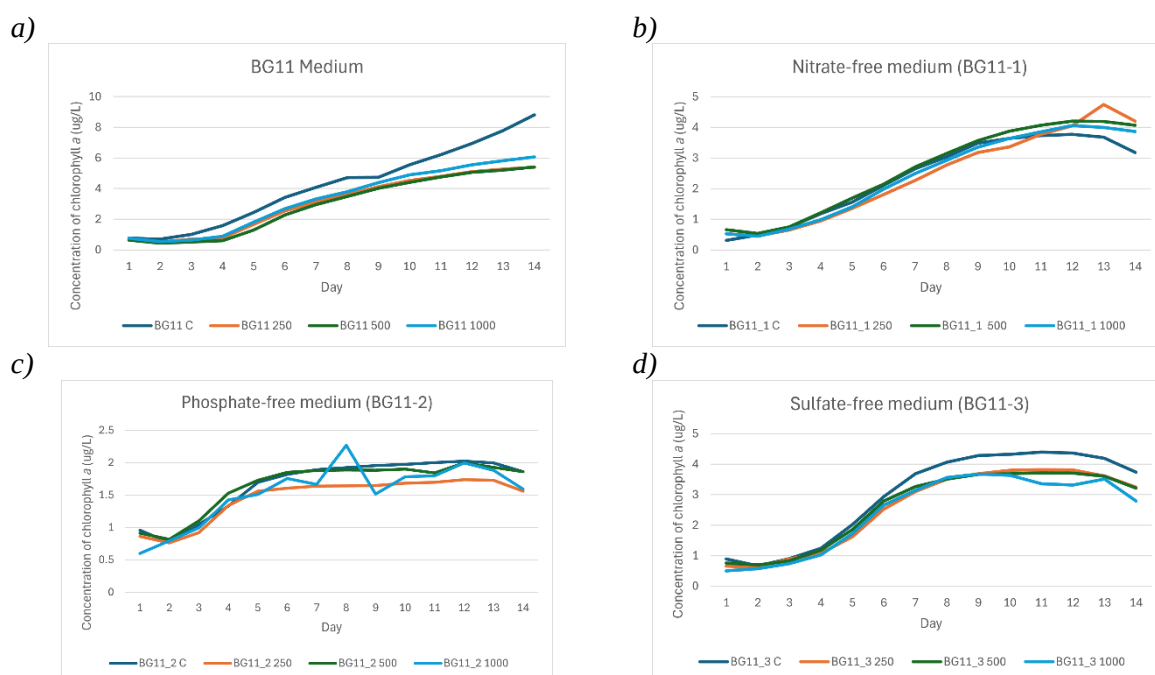


Figure 1 Chlorophyll *a* concentration ($\mu\text{g/L}$) during the *Microcystis* sp. growth period in different media a) standard BG11 medium; b) nitrate-free medium (BG11-1); c) phosphate-free medium (BG11-2); d) sulphate-free medium (BG11-3)

In the phosphate-deficient medium (BG11-2), the most pronounced decrease in the growth of *Microcystis* sp. was observed compared to all other conditions. This confirms that phosphorus is critical for energy metabolism, DNA synthesis, and overall cellular function. The treatments showed an uneven response, with some concentrations (e.g., 500 $\mu\text{g/L}$) yielding values similar to the control, while others were lower. However, no statistically significant differences were found, indicating that phosphate limitation is again the primary factor determining growth, while the effect of dimethyl phthalate is relatively weak and variable.

In the sulfate-deficient medium (BG11-3), the growth of *Microcystis* sp. was higher than under phosphate-deficient conditions but lower than in the standard medium. The highest values were again recorded in the control treatment, while the highest concentration of DMP (1000 $\mu\text{g/L}$) showed the strongest inhibitory effect. This result is consistent with the role of sulfur in the synthesis of antioxidant molecules such as glutathione. Under sulfate-deficient conditions, the cells likely have a reduced capacity to cope with stress caused by the pollutant. Changes in pH values during the experiment (Table 1) show a clear relationship with the growth dynamics of the cyanobacterium *Microcystis* and its photosynthetic activity. In the standard BG11 medium, where a continuous increase in chlorophyll *a* was recorded, pH values remained high and relatively stable (around 10.4–10.5), which is characteristic of intensive photosynthesis and efficient CO_2 uptake from the medium. Slight variations in the presence of dimethyl phthalate indicate that, under conditions of optimal nutrient availability, this pollutant has a limited effect on photosynthetic activity.

Table 1 pH values at the end of experiment in all investigated media and DMP concentrations

Medium	pH	Medium	pH	Medium	pH	Medium	pH
BG11 control	10.54	BG11-1 control	10.18	BG11-2 control	9.73	BG11-3 control	10.51
BG11 250 DMP	10.42	BG11-1 250 DMP	10.33	BG11-2 250 DMP	10.21	BG11-3 250 DMP	10.53
BG11 500 DMP	10.51	BG11-1 500 DMP	10.41	BG11-2 500 DMP	9.44	BG11-3 500 DMP	10.42
BG11 1000 DMP	10.53	BG11-1 1000 DMP	10.30	BG11-2 1000 DMP	9.51	BG11-3 1000 DMP	10.37

Under nitrogen-limited conditions (BG11-1), where growth was significantly reduced, a gradual increase in pH values was observed with increasing concentrations of dimethyl phthalate (from 10.18 to 10.41). This may indicate a reduction in biomass and a shift in metabolic balance, where lower culture density and reduced carbon assimilation lead to variations in the carbonate system. In the phosphate-deficient medium (BG11-2), where growth was most strongly inhibited, pH showed more pronounced oscillations (9.73; 10.21; 9.44; 9.51), reflecting unstable photosynthetic activity and a variable metabolic response of cells under strong nutrient stress, as well as the possible degradation of dimethyl phthalate into phthalic acid in some cases. The initial increase in pH may result from short-term adaptation, while the subsequent decrease indicates the dominance of inhibitory processes and reduced CO₂ fixation.

Under sulfate-limited conditions (BG11-3), a slight decrease in pH values was observed with increasing concentrations of dimethyl phthalate (from 10.51 to 10.37), which is consistent with gradual growth inhibition and reduced photosynthetic activity. This trend may be related to the limited ability of cells to maintain redox balance and efficient carbon fixation under sulfate/sulfur deficiency. Overall, the obtained results indicate that changes in pH values follow the intensity of growth and photosynthesis, with nutrient limitations modifying the response to dimethyl phthalate and leading to stable, variable, or declining pH trends depending on the dominant stress factor.

CONCLUSION

Results demonstrate that the growth and physiological responses of *Microcystis* sp. are primarily governed by nutrient availability, while the effect of dimethyl phthalate is secondary and generally weak. Under optimal conditions, DMP acted as a mild inhibitor of growth and photosynthetic activity, whereas nutrient limitations—particularly of nitrogen and phosphorus—were the dominant factors restricting biomass development and stabilizing or altering the response to the pollutant. Variations in pH further supported these findings, reflecting changes in photosynthetic intensity and carbon assimilation under different nutritional and stress conditions. In general, the study indicates that nutrient stress strongly modulates the response of *Microcystis* sp. to dimethyl phthalate, highlighting the importance of environmental context in assessing the ecological impact of organic pollutants.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Science, Technological Development and Innovation of the Republic of Serbia for financial support according to the contract with registration No. 451-03-33/2026-03/200124 and 451-03-34/2026-03/200124.

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