

Original Research

IMPACT OF DIMETHYL PHTHALATE ON THE GROWTH AND STRUCTURE OF
GREEN ALGAE (*Chlorella* sp.)

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Abstract

The aim of this research was to examine the influence of dimethyl phthalate (DMP) on the growth and physiological response of a phytoplankton organism under laboratory conditions. A strain of the green alga *Chlorella* 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). Organism growth was monitored using the spectrophotometric determination of chlorophyll *a*, while potential morphological changes in cells and contamination were controlled using microscopy. Also, pH value monitoring was done. The results of this study show that the effect of DMP on the growth of the microalgae *Chlorella* sp. is not only concentration-dependent, but also largely depends on the availability of essential nutrients in the medium.

Keywords: dimethyl phthalate, green algae, chlorophyll *a*.

INTRODUCTION

Phytoplankton represents a diverse community of photosynthetic microorganisms, including prokaryotes and eukaryotes, that are adapted to a wide range of aquatic environments – from freshwater springs and streams, lakes, and lagoons, to coastal marine areas and the open ocean. These organisms play a fundamental role in global biogeochemical cycles and form the base of aquatic food webs [1].

Microalgae exhibit high growth and CO₂ fixation rates, and they are responsible for approximately 32% of global photosynthesis, producing more than 60% of the Earth's oxygen. They contain typical eukaryotic organelles, including plastids with chlorophyll, and inhabit both freshwater and marine ecosystems. Their size ranges from 3 to 30 µm [2].

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The genus *Chlorella* comprises unicellular green algae from the division Chlorophyta, commonly found in freshwater ecosystems. The cells are small, predominantly spherical, with a single chloroplast containing chlorophylls *a* and *b*, enabling efficient photosynthesis under varying light conditions. Members of this genus lack a motile stage in their life cycle, and reproduction occurs asexually [3].

Chlorella sp. most frequently occurs in mesotrophic and eutrophic aquatic ecosystems. Due to its simple structure, this genus thrives in conditions of limited nutrient availability and fluctuating physicochemical although their growth and metabolic activity remain sensitive to environmental stressors. Consequently, *Chlorella* sp. is widely used as model organisms in ecological and ecotoxicological studies to evaluate the effects of environmental pollutants. Among these, organic contaminants such as phthalates have been shown to influence algal growth, photosynthetic efficiency, and cellular structure, potentially disrupting energy flow and ecosystem stability [3].

MATERIALS AND METHODS

Phytoplankton cultivation

A culture of the green microalga genus *Chlorella*, obtained from the culture collection of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, was used for the experiment. 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 presented in Table 1.

Table 1 Composition of standard BG11 medium

Substance	Concentration (g/L)
NaNO ₃	1.5
K ₂ HPO ₄	0.04
MgSO ₄ ·7H ₂ O	0.075
CaCl ₂ ·2H ₂ O	0.036
C ₆ H ₈ O ₇	0.006
Ammonium ferric citrate green	0.006
EDTANa ₂	0.001
Na ₂ CO ₃	0.02
H ₃ BO ₃	0.00286
MnCl ₂ ·4H ₂ O	0.00181
ZnSO ₄ ·7H ₂ O	0.00022
Na ₂ MoO ₄ ·2H ₂ O	0.00039
CuSO ₄ ·5H ₂ O	0.00008
Co(NO ₃) ₂ ·6H ₂ O	0.00005

Experiment

The green algal strain used in the experiment was inoculated into sterile glass test tubes. A total of 48 test tubes were used, with 12 tubes per replicate. 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.

Phytoplankton samples, nutrient medium, and DMP were used to conduct the experiment. Four types of nutrient media were prepared: standard BG11 medium, nitrate-free BG11

(BG11-1), phosphate-free BG11 (BG11-2), and sulphate-free BG11 (BG11-3). The dimethyl phthalate (DMP) solution used during the experiment was prepared in the laboratory of the Department of Chemistry, Faculty of Sciences in Niš, by dissolving the pure substance (CAS Number: 131-11-3, Sigma Aldrich, USA).

Following sterilization, 10 mL of the corresponding medium and 1 mL (1000 µL) of the microalgal suspension were added to each test tube. Subsequently, various concentrations of DMP were added. All phthalate concentrations were tested in triplicate. Control tubes did not contain DMP. 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. An Agilent BioTek Epoch 2 Microplate Spectrophotometer was used.

In addition to spectrophotometric measurements, the test cultures were monitored via microscopic analysis (Leica Microsystems Leica DM1000 and Leica Microsystems DFC295 camera) to detect potential contamination and prevent irregularities that could affect the accuracy of the results.

Monitoring of pH value was done using SensIon Multimeter (Hach, Germany) during the experiment.

RESULTS AND DISCUSSION

Within experiment the production of chlorophyll *a* (Chl *a*) in strains of *Chlorella* sp. during two weeks of exposure to different concentrations of dimethyl phthalate (250, 500 and 1000 µg/L) was monitored. 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) [1]:

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

and presented in Figure 1.

In the first experiment, a typical hormetic response was observed in the standard BG11 medium, where the highest concentration (1000 mg/L) led to growth stimulation, while lower concentrations had an inhibitory effect. This type of response is well documented in the literature and represents an adaptive mechanism of microorganisms to the presence of stressors [4].

However, in the subsequent experiments (BG11-1 and BG11-2) different patterns were observed. In experiment BG11-1, where a modified nitrate-free medium was used as the medium, maximum growth was recorded at the lowest concentration (250 µg/L), while in the phosphate-free medium (BG11-2) the highest growth was observed at the middle concentration (500 µg/L).

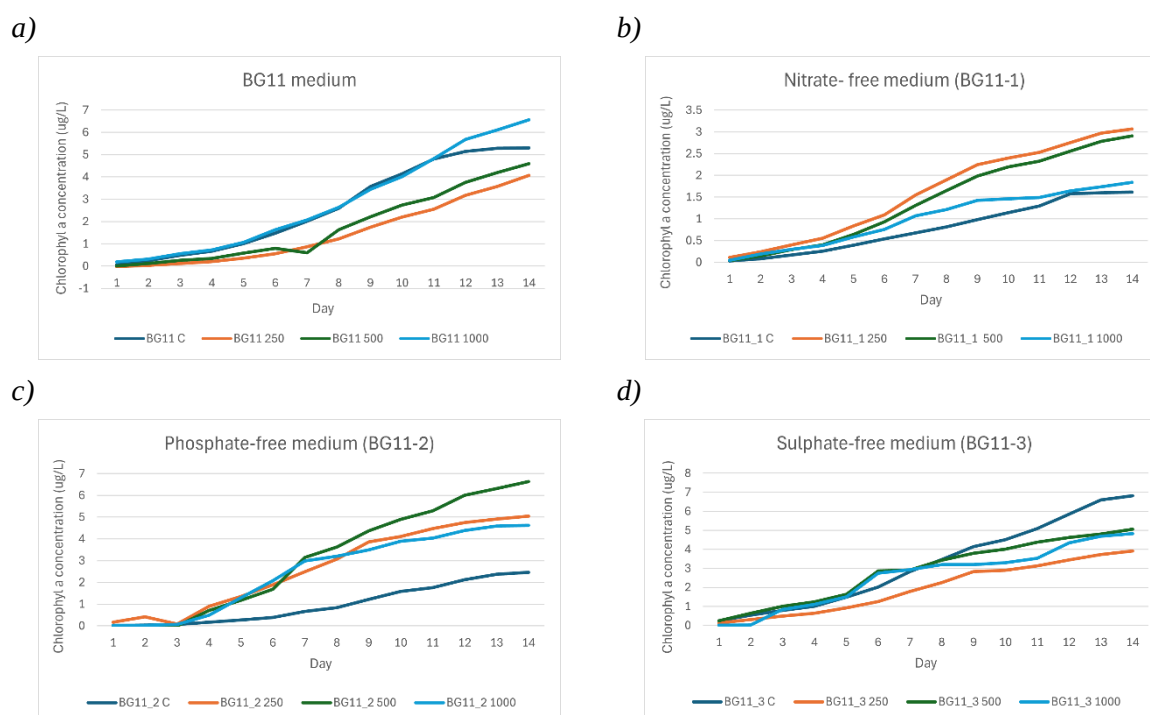


Figure 1 Chlorophyll *a* concentration (µg/L) during the growth period of *Chlorella* sp. in different media: a) standard BG11 medium; b) nitrate-free medium (BG11-1); c) phosphate-free medium (BG11-2); sulphate-free medium (BG11-3)

These results indicate the existence of an optimal concentration range, within which growth is stimulated, while outside this range there is a decrease in physiological activity.

In the fourth experiment, which was performed in a modified sulphate-free medium (BG11-3), an opposite trend was observed, where the control treatment had the highest chlorophyll *a* value, while increasing the DMP concentration led to a gradual inhibition of growth. Although statistical analysis did not show significant differences, the observed trend indicates a potential toxic effect at higher concentrations.

The results obtained in four experiments show that the effect of dimethyl phthalate on the growth of the microalgae *Chlorella* sp. is not linear, but highly variable and dependent on the experimental conditions. The observed patterns indicate a complex relationship between the concentration of the pollutant and the physiological response of the tested microalgae strain.

Results of pH measurements (Table 2) indicate that in the standard BG11 medium, and in the phosphate-free medium (BG11-2), a gradual decrease in pH values was observed with an increase in the concentration of DMP, which can be associated with growth inhibition and reduced photosynthesis. This effect can be explained by increased respiration, *i.e.*, by a higher production of CO₂, which leads to the formation of carbonic acid and a decrease in pH values.

Also, DMP degradation, *i.e.* the formation of phthalic acid, probably occurred, which also contributes to the decrease in pH value. A similar trend, although less pronounced, was observed in the nitrogen-free medium (BG11-1), where at higher concentrations there is a slight recovery of pH, which may be a consequence of reduced biomass and lower overall metabolic activity.

Table 2 pH values at the end of experiment in all investigated media and DMP concentrations

Medium	pH	Medium	pH	Medium	pH	Medium	pH
BG11 control	8.54	BG11-1 control	8.20	BG11-2 control	7.63	BG11-3 control	7.50
BG11 250 DMP	8.34	BG11-1 250 DMP	8.13	BG11-2 250 DMP	7.64	BG11-3 250 DMP	7.62
BG11 500 DMP	8.22	BG11-1 500 DMP	8.04	BG11-2 500 DMP	7.53	BG11-3 500 DMP	7.79
BG11 1000 DMP	8.05	BG11-1 1000 DMP	8.16	BG11-2 1000 DMP	7.48	BG11-3 1000 DMP	7.93

In contrast, in the sulphate-free medium (BG11-3), a continuous increase in pH was recorded with increasing DMP concentration, which is consistent with the pronounced growth inhibition. In this case, the limited availability of sulphate/sulphur further reduces the metabolic and photosynthetic activity of the cells, which leads to reduced gas exchange and a shift in the equilibrium of the carbonate system towards more alkaline environments.

CONCLUSION

The results of this study show that the effect of DMP on the growth of the microalgae *Chlorella* sp. is not only concentration-dependent, but also largely depends on the availability of essential nutrients in the medium. In the standard BG11 medium, a hormetic response was observed, where high concentration stimulates growth. In nitrate-free conditions, the maximum was achieved at the lowest concentration, indicating adaptation to nitrogen stress. Overall, pH variations follow changes in growth and indicate that photosynthetic activity is the main factor controlling the acid-base balance in the medium, with the inhibitory effect of dimethyl phthalate leading to a decrease in CO₂ fixation and pH changes.

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