Environment, Resource and Ecology Journal (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/erej.2025.090201
ISSN 2616-3756 Vol. 9 Num. 2

The Impact of Microplastics on the Growth of Skeletonema Costatum

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Keywords: Microplastics, *Skeletonema Costatum*, Superoxide Dismutase, Dose-Dependent Effects, Marine Phytoplankton

Abstract: This study investigated the effects of microplastics (MPs) on *Skeletonema costatum* growth by monitoring algal responses to polystyrene MPs (0.1 μm diameter) at concentrations of 0.10 mg/L and 10.00 mg/L. Results demonstrate that the addition of MPs plays an important role in algal growth, chlorophyll and oxidative stress. After three days of MP exposure, both low and high concentration exposures significantly increased cell growth and chlorophyll content, with pronounced effects observed under the low concentration exposure. Additionally, MP exposure markedly elevated superoxide dismutase (SOD) activity, indicating a physiological stress response in algal cells. Collectively, this work establishes critical data for assessing the impacts of MPs on marine phytoplankton in marine ecosystems.

1. Introduction

Plastics have been widely used in our daily life since their large-scale commercial production in the middle of 19th century. Owing to their low cost, durability, and light weight, these materials have been widely utilized in packaging, construction, electronics and agriculture, etc. Once released into the environments, plastics are fragmented into microplastics (MPs; < 5 mm) and nanoplastics (NPs; < 1 μm) via photodegradation, mechanical abrasion and biodegradation. Notably, it has been found that a large number of MPs were found in seawater and biological samples (Alfaro-Núñez et al., 2021; Mao et al., 2018; Yu et al., 2020; Zhao et al., 2019)[1,3,6,7].

Phytoplankton, including microalgae, are fundamental primary producers in marine ecosystems, driving critical energy flows and biogeochemical cycles (e.g., carbon, nitrogen and phosphorus). Recent research demonstrates that MPs inhibit phytoplankton growth and photosynthesis while altering community structure (Mao et al., 2018; Yu et al., 2020)[3,6]. For instance, polystyrene MPs suppress *Chaetoceros curvisetus* growth due to the adsorption and aggregation mechanisms between MPs and *Chaetoceros curvisetus* (Wang et al., 2022)[4].

This study investigates the dose-response relationship between MPs and phytoplankton (*Skeletonema costatum*) through quantitative measurements of three parameters: cell density, chlorophyll *a* (Chl *a*) fluorescence, and superoxide dismutase (SOD) activity.

2. Materials and Methods

2.1 Algae Cultivation

The *Skeletonema costatum* strain isolated from Jiaozhou Bay, was cultivated in sterilized f/2 medium. Cultures (500 mL) with an initial cell density of 2×10^5 cells/mL were maintained in an illuminated incubator (GXZ, Ningbo Jiangnan Instrument Factory) at 20 °C under a 12 h:12 h light-dark cycle (8,000 lx light intensity). Three replicates were prepared for each treatment group.

2.2 Experimental Design

Polystyrene MPs (0.1 μm diameter; Jiangsu Zhichuan Technology Co., Ltd.) were added to the f/2 medium at concentrations of 0 mg/L (control), 0.10 mg/L (low), and 10.00 mg/L (high). The concentration levels were selected based on established microplastic toxicity thresholds from prior literature.

2.3 Sample collection

Samples were collected every 24 hours. For cell density analysis, 2 mL aliquots were fixed with Lugol's reagent (Beijing Solibo Technology Co., Ltd.) and stored at room temperature. For biochemical analyses, 15 mL aliquots were centrifuged at 10,000 × g for 10 min (4 °C). After that, the supernatant was discarded, and cell pellets were stored at -20 °C for subsequent SOD activity and Chl *a* measurement.

2.4 Experimental Parameter Determination

2.4.1 Cell Density Quantification

Fixed samples (100 μL) were loaded onto a hemocytometer, covered with a coverslip, and counted microscopically (BX 51, Olympus). Results were converted to cell density (units: 10^5 cells/mL).

2.4.2 SOD Activity Assay

SOD activity was measured using a commercial kit (Nanjing Jiancheng Bioengineering Institute). Cell pellets containing 5×10^6 cells were resuspended in 1 mL extraction buffer and homogenized on ice using a 200 W ultrasonic probe (3 s pulse; 10 s interval; 30 cycles). The homogenates were centrifuged at 8,000 × g for 10 min at 4 °C, and the supernatant was analyzed spectrophotometrically at 450 nm. SOD activity (U/10^4 cells) was calculated using Equation (1) and (2):

$$\text{Inhibition (\%)} = (\Delta A_{blank} - \Delta A_{measurement})/\Delta A_{blank} \times 100\% \quad (1)$$

$$\text{SOD activity} = [10 \times \text{Inhibition} / (100 - \text{Inhibition})] / N \times F \quad (2)$$

Where N is the total cell count (cells) and F equals dilution factor (5).

2.4.3 Measurement of Chlorophyll Fluorescence Intensity

Dark-adapted samples were analyzed using a Phyto-PAM chlorophyll fluorometer (Shanghai Zecquan Technology Co., Ltd.).

2.5 Data Processing

Data are presented as mean ± standard deviation (SD) of three biological replicates. Significant

differences ($P < 0.05$) between treatments were determined using the Student's *t*-test.

3. Results

3.1 Algal Cell Density Dynamics

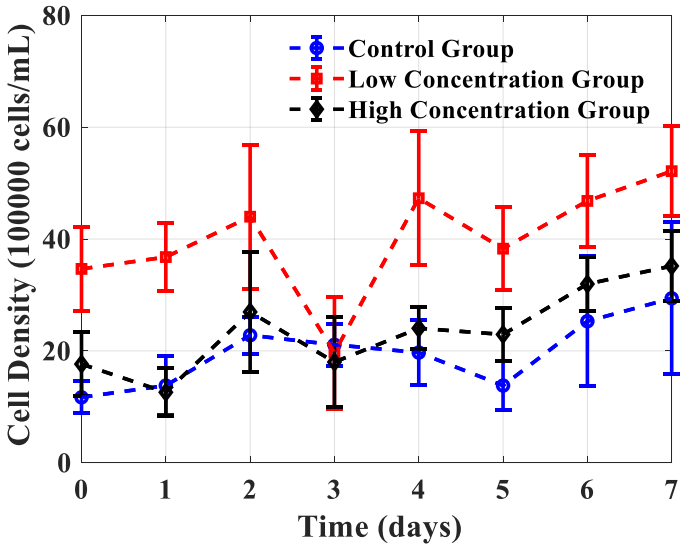

Figure 1 Changes in algal cell density under the exposure of microplastics

Algae growth curves revealed concentration-dependent responses to MP exposure (Figure 1). The *t*-test showed that there were significant differences in the changes of algae cell density among the different treatment groups and the control group ($P < 0.05$), indicating that the concentration of MPs has significant effects on cell growth.

Overall, cell growth in the control group showed a gradual upward trend. The average cell density increased from 11×10^5 cells/mL to 29×10^5 cells/mL from day 1 to 7, with an exception between 3 and 7 days of incubation. The low concentration treatment (0.10 mg/L) initially enhanced *Skeletonema costatum* growth in the first two days, compared to the control group. However, a significant decrease was observed on day 3, followed by a significant increase in the following days. The high concentration treatment consistently suppressed the growth of *Skeletonema costatum*. Although a slight increase in cell density was observed in the early stage (day 1 and day 2), the growth rate remained lower than that of the control group. From day 4 onward, the inhibitory effect disappeared but the growth trend remained relatively flat.

3.2 Chlorophyll a Response

In this study, observations and analyses were conducted to investigate the impact of MPs on the Chl *a* fluorescence intensity (Figure 2).

The overall trend of the Chl *a* fluorescence intensity in control treatment remained a gradual upward increase, although there were some fluctuations during the cultivation. In the control group during the experiment, a certain upward fluctuating trend was observed. Specifically, the value of Chl a fluorescence intensity was increased from 40.82 (day 1) to 51.46 (day 3), decreased to 20.01 on day 5. Afterwards, Chl *a* fluorescence intensity in the control group significantly increased reaching 163.69 on day 7, exceeded that of the other two treatments.

In the low concentration MP (0.10 mg/L) treatment group, the Chl *a* fluorescence intensity was

3.8 on day 0, and increased to 45.95 on day 1. A significant decrease was observed on day 3. After day 4, the Chl *a* fluorescence intensity of algae in the low-concentration group was higher than that of in the control group, indicating that this concentration of MPs had a promoting effect on the increase of Chl *a* fluorescence intensity of algal cells. However, after day 6, Chl *a* fluorescence intensity of algae in the low-concentration group was significantly lower than that of in the control group.

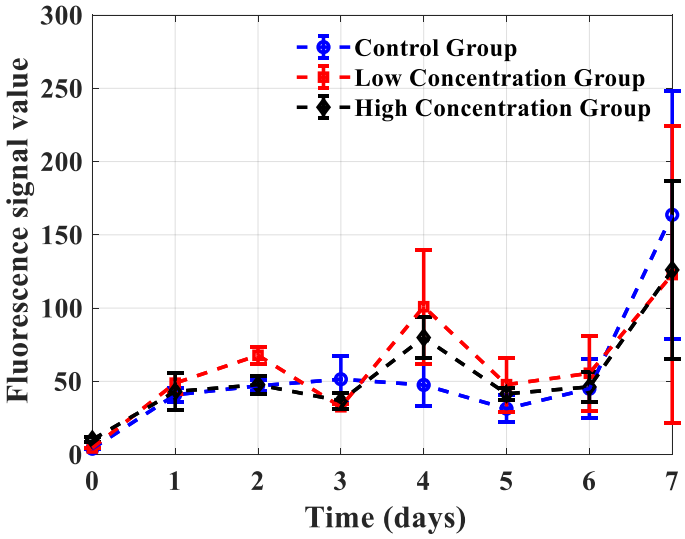

Figure 2 Changes in chlorophyll a fluorescence intensity of algal cells under the exposure of microplastics

In the high-concentration MP (10.00 mg/L) treatment group, Chl *a* fluorescence intensity remained largely similar to the control group. on day 0, the chlorophyll fluorescence intensity was 4.54, increased to 41.55 on day 1. A pronounced inhibition occurred on day 3, followed by a transient increase on day 4. After day 6, the Chl *a* fluorescence intensity in the high concentration treatment was significantly lower than that of in the control. These findings indicate that MPs had a temporary stimulatory effect on Chl *a* during the early cultivation phase, but as growth stabilized, the inhibitory effect of MPs began to appear, and the Chl *a* fluorescence intensity was significantly lower than that of the control group.

3.3 Oxidative Stress Indicators

As shown in Figure 3, the control group algal cells initially produced a small amount of reactive oxygen species (ROS) while adapting to the new environment. This activated the antioxidant defense system, leading to an increase in SOD activity. Subsequently, the SOD declined and stabilized after day 5, indicating *Skeletonema costatum* had adapted to the environment.

In the low-concentration treatment, SOD activity of the algae increased from day 2 to day 4. This suggests that low concentration MP exposure induced a non-significant oxidative stress response to algae, leading to an elevation of intracellular ROS levels. To mitigate the excess ROS and maintain redox homeostasis, the algae enhanced its antioxidant defenses by increasing SOD activity. After day 4, SOD activity gradually declined, potentially due to algal adaptation to the stressor, regulation of antioxidant system and/or a decline in energy depletion from a sustained stress response.

In the high concentration group, SOD activity reached a peak on day 1 and then declined rapidly. The high MP concentration caused severe oxidative stress, resulting in an immediate and massive ROS generation and a rapid SOD synthesis response. However, this intense stress likely caused

cellular damage, impairing SOD synthesis and general metabolic functions, which led to a sharp decrease in SOD-resulting in the sharp decrease in SOD activity on day 2. From day 2 to day 7, SOD activity remained consistently low and stable. This indicates significant cellular damage: although the antioxidant system was still active, it was impaired and unable to maintain high SOD activity.

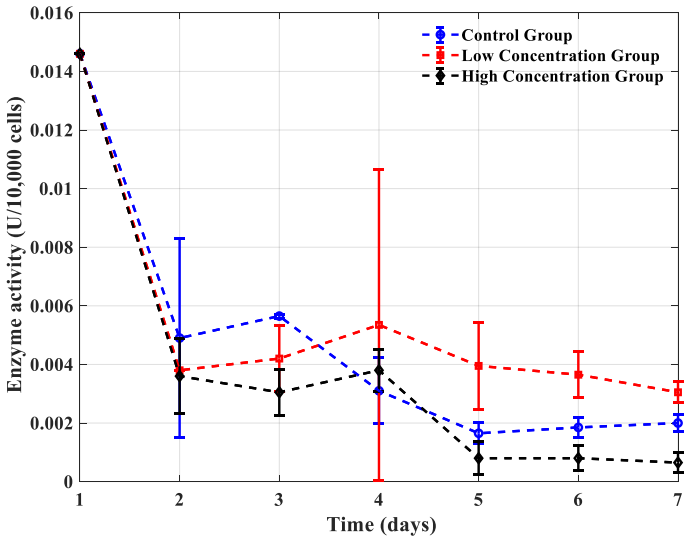

Figure 3 Changes in the SOD activity of algal cells under the microplastic exposures

4. Discussion

4.1 Comparison with Other Studies

The experimental results demonstrated that different concentrations of MPs had distinct effects on *S. costatum* growth. The low concentration of MPs (0.10 mg/L) initially stimulated algal cell growth during the early cultivation period, showed an inhibition on day 3, and then subsequently recovered. In contrast, high concentration MP exposure (10.00 mg/L) consistently suppressed algal growth throughout the entire cultivation period.

These findings showed both similarities and differences compared with previous studies on the effect of MPs on algae. For instance, Wu et al. (2021)[5] observed that of 1 μm polystyrene MPs at 5 mg/L enhanced *Microcystis aeruginosa* growth over 96 hours (increase rate: 12.42% ± 0.94%), which is consistent with the early-stage stimulatory effects of low MP concentrations observed in the present study. Similarly, Lang et al. (2022)[2] reported that 10 mg/L PS concentrations (0.5 μm) exerted relatively weak growth inhibition on *Phaeodactylum tricornutum*, whereas 100 mg/L MP exposure significantly suppressed cell growth, chl *a* content, and photosynthetic efficiency, with maximum inhibition rates of 31.75%, 10.38%, and 8.82%, respectively. However, this concentration was not included in our study.

4.2 Potential Mechanisms Underlying Concentration-Dependent Effects

The observed low concentration stimulation and inhibition at high-concentration may arise from algal adaptation mechanisms and the dose-dependent effects of MPs. At low concentrations, MPs may act as mild environmental stressors, triggering adaptive stress responses and growth-promoting signaling pathways. Additionally, MP surfaces may adsorb nitrogen, phosphorus, and other nutrients, potentially providing supplementary nutrients that enhance algal growth.

In contrast, at elevated concentrations, extensive adhesion of MPs to algal cell surfaces likely

impedes CO_2 and/or O_2 diffusion and nutrient and/or waste transport. Supporting this, Zhao et al. (2019)[7] demonstrated that high polyvinyl chloride MP concentrations significantly increased culture medium turbidity in *Karenia mikimotoi*, substantially reducing light availability and impairing photosynthesis. Concurrently, intense oxidative stress responses may deplete cellular energy reserves, further contributing to growth inhibition.

5. Conclusion and outlooks

This study investigated the effects of polystyrene MPs (0.10 mg/L and 10 mg/L) on the growth and physiological responses of *S. costatum*. Results showed a significant dose-dependent response: low concentration of MPs transiently stimulated algal cell density and chlorophyll fluorescence intensity during early growth, shifting to inhibition after three days, while their high concentration consistently suppressed algal growth and significantly reduced chlorophyll fluorescence. Microplastic exposure also induced oxidative stress. In the low concentration group, SOD activity initially increased and then declined, however, in the high-concentration group it sharply decreased and remained at low levels, indicating impairment of the antioxidant system.

We hypothesize that low MP concentrations may provide transient growth stimulation by supplying adsorbed nutrients on particle surfaces, whereas their high concentrations inhibit growth by impeding substance exchange, increasing turbidity, and inducing energy depletion. Overall, these findings demonstrate a complex and concentration-dependent effects of MPs on algae, which are also influenced by species-specific characteristics. Future studies should incorporate molecular level analyses to elucidate signaling pathways and gene expression mechanisms involved in algal responses to microplastic stress, and establish environmental relevant MP concentration thresholds to comprehensively assess their potential risks to marine ecosystems.

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