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Article

Differential physiological response of microalgae exposed to petroleum- and bio-based microplastics

Luming Jin[1*], Yihe Xu[1*], Jia Fu[1*], Jiaqi Sun[1*], Xuanming Zhang[1*], Shuhan Hu[1*], Jingwen Wu[1*], Xiaoming Wang[1]
[1] Hefei Thomas School, Hefei, China
[*]These authors contributed equally to this work

SUMMARY

Petroleum-based plastic pollution has become a severe problem all over the world. Bio-based plastics have garnered the attention of researchers since their appearance because of their simple molecular structure and easier degradation compared to petroleum-based plastics. However, almost no studies have clearly compared the biological toxicity and environmental hazards of petroleum-based plastics and bio-based plastics to the organisms in the ecosystem. To fill this research gap, two different microalgae species (*Chlorella pyrenoidosa* and *Tetradesmus obliquus*) were selected and exposed to two types of microplastics. We compared polystyrene (PS), a petroleum-based plastic, and polylactic acid (PLA), a bio-based plastic. We hypothesized that microalgae would exhibit higher stress levels when exposed to PS than PLA, which means lower growth rates and photosynthesis activity levels and more antioxidant responses. The results indicated that the growth rates and photosynthetic activities of both microalgae decreased with increasing microplastic concentration when exposed to PS. Comparatively, the negative effect on microalgae growth in the PS-treated group is more significant than in the PLA-treated group. In addition, in both microplastic groups, the negative impacts were more profound on *C. pyrenoidosa* than on *T. obliquus*. The findings indicate that petroleum-based plastics like PS have a more detrimental effect on microalgae compared to bio-based plastics, likely due to differences in obstructing light absorption and causing oxidative damage and aggregation. Our study provides reliable data on the different impacts caused by two types of plastics and gives a potential way to reduce hazards caused by plastics by replacing petroleum-based plastics with bio-based plastics.

INTRODUCTION

The production of plastic was estimated to be 2 million tons in 1950, and it rapidly increased to 368 million tons by 2019 (1). Although the effects of plastic waste on terrestrial and aquatic ecosystems are well-documented, including the entrapment of plant and animal species, the often-neglected concern of microplastic contamination arising from the production and use of plastics deserves further scrutiny (2). Microplastics refer to tiny particles that result from the fragmentation and weathering of plastics, typically with a size of less than 5 mm (3). Microplastics are extensively disseminated in marine and freshwater ecosystems due to their durability against degradation (4). The global dispersion of microplastics is facilitated by hydrodynamic processes, such as tides, turbulence, and ocean currents, resulting in their concentrated distribution throughout aquatic ecosystems worldwide, encompassing both freshwater and marine environments (5).

There are two main kinds of plastics produced in recent years, which are petroleum-based plastics and bio-based plastics. They are classified by their natural composition: petroleum-based plastics are primarily derived from petroleum, whereas bio-based plastics mainly include biomass materials, like cellulose and starch (6). Since their introduction, petroleum-based plastics such as polystyrene (PS), polyethylene, and polyvinyl chloride have been extensively manufactured and employed (7). PS, in particular, is widely utilized across various sectors, including the light industry and light market, for applications such as foam plastics, packaging containers, and instrument casings (8). Petroleum-based plastics could have adverse effects on organisms' growth by causing endocrine disruption and oxidative stress (9, 10). Petroleum-based microplastics and bio-based microplastics are small fragments derived from their respective parent plastics, with their properties and characteristics exhibiting consistent differences to those of petroleum-based and bio-based plastics themselves. Microplastics have the potential to accumulate and migrate within the food chain, which will threaten the survival of certain species, thereby disrupting the ecological balance (11–14).

Increased awareness of environmental concerns associated with plastics has led to a heightened interest in bio-based plastics, which may reduce petroleum consumption and promote easier degradation due to their simpler molecular structure. Bio-based plastics, primarily composed of polyester plastics like polylactic acid (PLA), polyhydroxyalkanoates, polycaprolactone, and polybutylene succinate, originate from organic materials like cellulose in plant materials (15). The materials demonstrate physical, chemical, and mechanical properties that are either comparable to or superior to those of petroleum-based plastics after processing or modification (13). PLA has gained recognition as a viable alternative to traditional plastics within the category of biodegradable materials (14). PLA plastics degrade faster in terrestrial and marine environments (16, 17). The toxicological and environmental impacts of PLA plastics in comparison to petroleum-based plastics remain uncertain and necessitate further research.

To measure the impacts of plastics on the environment, we used microalgae to reflect the effects of microplastics since microalgae utilize photosynthesis to generate oxygen and trap carbon, which is an important step in the global carbon cycle (18). Examining the interaction mechanisms between microalgae and toxins is essential for evaluating

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the risks associated with particular pollutants. *Chlorella pyrenoidosa*, a species within the Chlorophyta phylum, is frequently detected in marine ecosystems, both in coastal areas and in open oceans (18). Previous research has utilized *C. pyrenoidosa* to examine the toxicological impacts of PS and various petroleum-derived plastics (19, 20). Additionally, *Tetradesmus obliquus*, a species of *Chlorophyta* phylum found in freshwater environments, is endorsed by the Organization for Economic Co-operation and Development for use in environmental monitoring (21, 22). Xu et al. indicated that PS exposure suppressed photosynthesis in *T. obliquus* and stimulated its antioxidant system, while Liu et al. evaluated the toxicological impacts of PS and other petroleum-derived plastics on *T. obliquus* (22, 23). Few research studies focused on the interaction between PLA and microalgae. Therefore, we chose these two species of microalgae as representatives from both freshwater and marine environments that are impacted by plastic pollution.

In this study, we hypothesized that the negative effect of petroleum-based PS on microalgae growth would be more pronounced compared to bio-based PLA. Our study examined the biological responses of *T. obliquus* and *C. pyrenoidosa* to varying concentrations of petroleum-based PS plastic and bio-based PLA, including photosynthesis, oxidative stress, and aggregation. We used Scanning Electron Microscopy (SEM) to examine the physical properties of microplastics and their interactions with microalgae, and measured superoxide dismutase (SOD) activity to assess the antioxidant response. Because reactive oxygen species (ROS) can induce lipid peroxidation and generate malondialdehyde (MDA), these indicators together reflect oxidative stress and cellular damage (24). We found that petroleum-based PS microplastics exert significantly stronger inhibitory effects on microalgae than bio-based PLA. Across both *T. obliquus* and *C. pyrenoidosa*, PS exposure led to greater reductions in growth rate and chlorophyll content, stronger impairment of photosynthetic activity, and more pronounced oxidative stress responses, as indicated by elevated SOD and MDA levels. SEM imaging further revealed that PS induced more extensive aggregation and surface damage compared with PLA. In contrast, PLA caused only mild physiological disturbances at comparable concentrations. Together, these results demonstrate that PS poses substantially higher ecological risk to microalgae than PLA, suggesting that bio-based plastics may represent a less harmful alternative in aquatic environments (25, 26). This study provides insights into the environmental impact of bio-based plastic PLA for future evaluations.

RESULTS

To test our hypothesis that petroleum-based PS microplastics have a stronger negative effect on microalgae than bio-based PLA microplastics, we exposed two microalgae species, *T. obliquus* and *C. pyrenoidosa*, to PS and PLA at concentrations of 100 mg/L and 500 mg/L in a 72-hour experiment. We used Blue-Green 11 Medium (BG11) medium with no microplastics as a control condition.

Characteristics of microplastics

We used SEM to examine the physical properties of microplastics. These properties can affect how microplastics interact with microalgae. SEM images showed that PS microspheres have a smooth, spherical shape with a particle

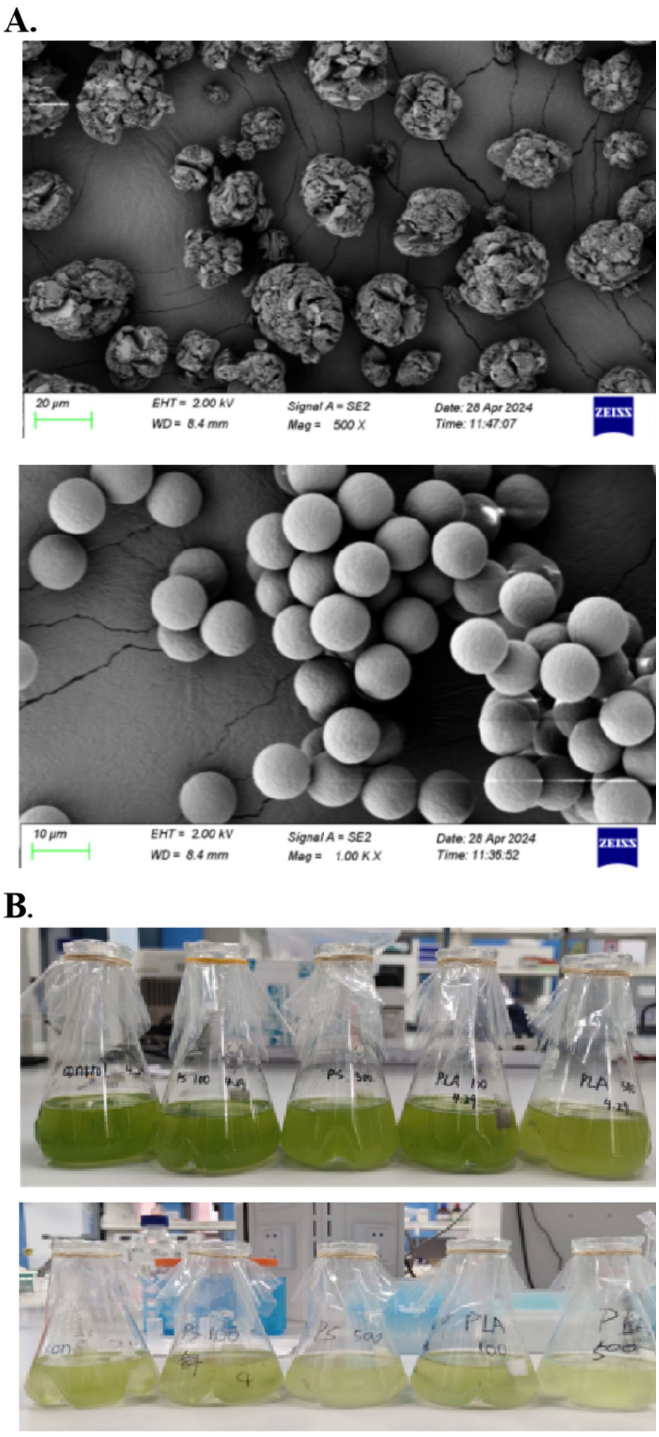

Figure 1: Characterization results of microplastics and microalgae employed in the experiment. (A) SEM images reveal the surface morphology of microplastics, top: polylactic acid, bottom: polystyrene. **(B)** The growth of two microalgal species cultured in flasks, with the culture medium alone serving as the control group and microplastic-added media as the experimental groups. Top row: *T. obliquus*. Bottom row: *C. pyrenoidosa*. From left to right in both rows, the culture conditions are as follows: Control group: Microalgae cultured in pure culture medium (no microplastics added). Microalgae exposed to 100 mg/L polystyrene, 500 mg/L polystyrene, 100 mg/L polylactic acid, and 500 mg/L polylactic acid. Microalgae were cultured in BG11 medium at 25°C, 140 rpm, with a 12-hour photoperiod and 3000 Lux light intensity.

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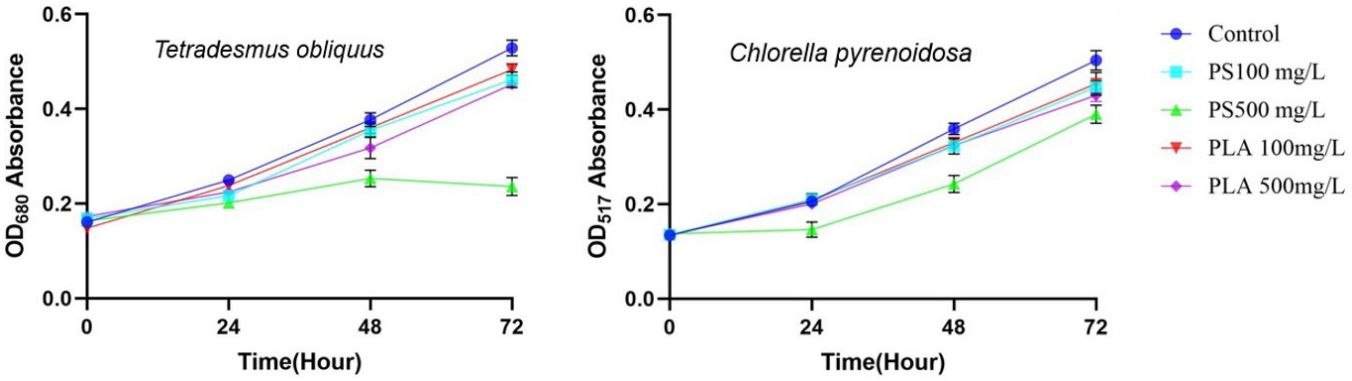

Figure 2: The effects on the growth of microalgae in response to exposure to different microplastic concentrations. Cell growth of *T. obliquus* (left) and *C. pyrenoidosa* (right) was measured in terms of optical density (OD) across five treatments, without microplastics (control) and with (100 mg/L PS, 500 mg/L PS, 100 mg/L PLA, and 500 mg/L PLA), respectively. Error bars indicate the standard deviation of the mean from three biological replicates (n=3).

size of 10.3±0.12 μm (n=5). PLA microspheres have a rougher, less uniform surface with a particle size of 12.3±0.15 μm (n=5) (**Figure 1a**). The particle sizes of PS and PLA are similar. However, their surface textures show a notable difference, which may influence their biological interactions.

Effects of microplastics on the growth of microalgae

The cellular density of microalgae serves as a crucial indicator for assessing the growth progression of microalgae. Each species of microalgae was divided into five groups: a control group cultured in a microplastic-free environment, and four experimental groups exposed to varying concentrations of microplastics (**Figure 1b**). We conducted growth inhibition experiments to assess how PS and PLA microplastics affect *T. obliquus* and *C. pyrenoidosa*. We measured optical density at 680 nm for *T. obliquus* and 517 nm for *C. pyrenoidosa*, as these wavelengths correspond to their respective maximum absorbance peaks between PS and PLA treatments in growth inhibition for *T. obliquus* ($F(4,10)=6.32$, $p=0.007$, one-way ANOVA test). For *C. pyrenoidosa*, the growth curves were similar to the control across all treatments, with optical density between 0.4 and 0.5 after 72 hours (**Figure 2**).

Effects of microplastics on the photosynthesis of microalgae

Photosynthesis is essential for the physiological functions of microalgae, serving as a fundamental activity for these primary producers. The light reaction phase entails the capture of light energy by chlorophyll, which is transformed into chemical energy. This transformation produces high-energy electrons via an electron transport chain, subsequently facilitating additional reactions in the process of photosynthesis (22). We measured chlorophyll a and b contents to evaluate photosynthetic activity. These are key indicators to reflect the process of photosynthesis (22). We used ethanol extraction and spectrophotometry after 72 hours of microplastics exposure. For *T. obliquus*, chlorophyll a and b show significant progressive reductions in the

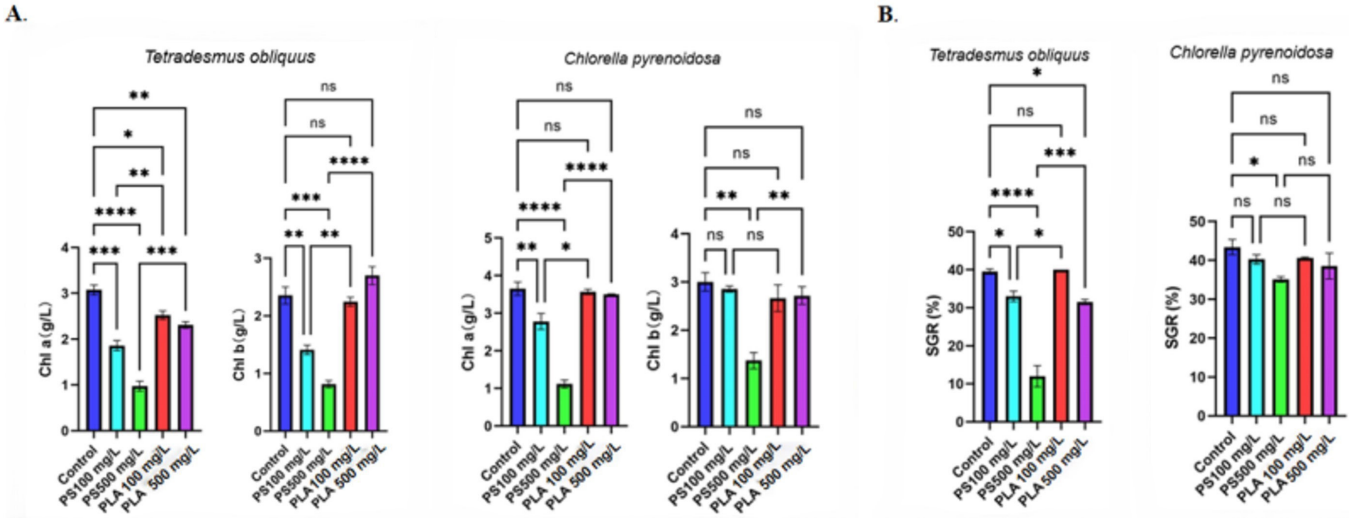

Figure 3: Chlorophyll a/b content and specific growth rate of *T. obliquus* and *C. pyrenoidosa* after 72-hour exposure to different concentrations of microplastics. Chlorophyll a/b (Chl a/b) content **(A)** and specific growth rate **(B)** of two microalgal species—T. obliquus (left subpanels of A and B) and C. pyrenoidosa (right subpanels of A and B)—following a 72-hour exposure to microplastics at four different concentrations: 100 mg/L PS, 500 mg/L PS, 100 mg/L PLA, and 500 mg/L PLA. Error bars represent the standard deviation of the mean derived from three biological replicates (n=3). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test; significance levels are indicated as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

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presence of microplastic PS at particle concentrations of 100 mg/L and 500 mg/L compared to controls (one-way ANOVA with Tukey HSD test, p=0.0016). At 500 mg/L PS, reductions were approximately 66.7% for chlorophyll a and 65.8% for chlorophyll b. PLA exposure caused smaller reductions of 21.9% and 28.2% at 100 mg/L and 500 mg/L, respectively. PLA had no significant effect on chlorophyll b. There are significant differences in chlorophyll a content for *T. obliquus* between PS and PLA treatments (F(4, 10) = 8.45, p = 0.002, one-way ANOVA test). For *C. pyrenoidosa*, PS significantly lowered chlorophyll a (P<0.0001) and chlorophyll b content (p<0.01), while PLA had no notable effect on chlorophyll a or b levels (**Figure 3a**).

Specific growth rates (SGR) were measured to reflect the concentration of microalgae. *T. obliquus* at 500 mg/L PS was significantly lower than the control group (one-way ANOVA with Tukey HSD test, p<0.0001), while the specific growth rates with PLA were consistently higher than those with the same concentration of PS (**Figure 3b**), indicating a lesser impact of PLA on microalgae. The specific growth rates of *C. pyrenoidosa* were consistent across varying concentrations of PS and PLA except for microalgae at 500 mg/L PS. The concentrations of chlorophyll a and b are consistent with the value of SGR, indicating that chlorophyll concentrations may correlate with the growth status of microalgae.

Effects of microplastics on the antioxidant system of microalgae

We used the xanthine oxidase (XO, an enzyme that generates superoxide radicals during purine catabolism) method after 72 hours of microplastics exposure (27). SOD (superoxide dismutase, a key antioxidant enzyme that catalyzes the transformation of superoxide radicals into oxygen and hydrogen peroxide) activity in *T. obliquus* and *C. pyrenoidosa* increased significantly with PS exposure (one-way ANOVA with Dunnett's test: p=0.0458 for *T. obliquus*, p=0.0009 for *C. pyrenoidosa*) (28). Higher PS concentrations caused greater increases. PLA exposure caused only slight increases compared to the control (**Figure 4a**).

We also measured MDA (malondialdehyde, a major end-product of lipid peroxidation and a common biomarker of oxidative damage) content, an indicator of lipid peroxidation and cellular damage, using the thiobarbituric acid method (22). MDA levels increased with higher PS concentrations, indicating cellular damage. PLA-treated groups showed no significant differences from the control (**Figure 4b**). There are significant effects of PS on SOD activity in *T. obliquus* (F(4, 10) = 5.89, p = 0.009, One-way ANOVA test).

SEM analysis for surface morphology changes in microalgae and microplastics

We used SEM imaging to study physical interactions between microplastics and microalgae. This provided insights into aggregation and surface attachment. SEM images showed that *T. obliquus* had more hetero-aggregation (microplastics with algal cells) and homo-aggregation (algal cells with each other) with PS than with PLA at 100 mg/L and 500 mg/L (**Figure 5a, b**). This led to greater cellular damage, such as shrinkage and detachment. Similar patterns, including attachment and aggregation, appeared in *C. pyrenoidosa* at 100 mg/L and 500 mg/L (**Figure 5c, d**). *C. pyrenoidosa* showed higher aggregation with tetrads (clusters of four cells). These clusters indicate a stronger stress response and cell grouping under microplastic exposure.

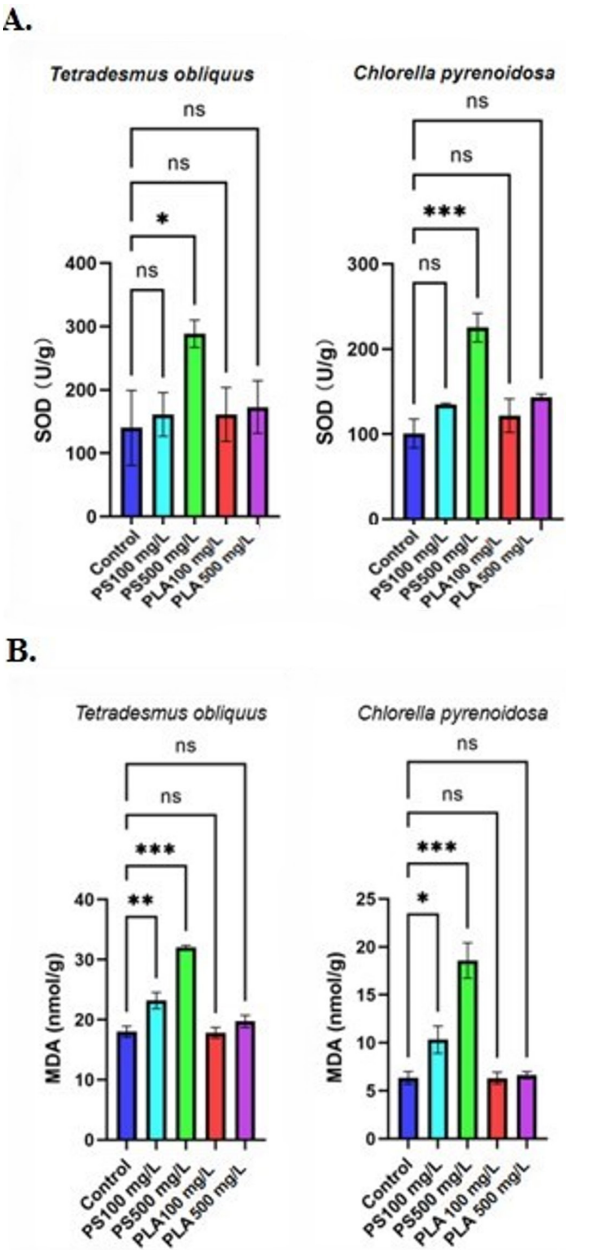

Figure 4: Effects of microplastics exposure on the antioxidant system of *T. obliquus* and *C. pyrenoidosa* for 72 h. (A) Superoxide dismutase (SOD) activity. **(B)** Malondialdehyde (MDA) content. Exposure concentrations: 100 mg/L PS, 500 mg/L PS, 100 mg/L PLA, 500 mg/L PLA. Error bars represent the standard deviation of the mean from three biological replicates (n=3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

DISCUSSION

This study investigated the potential impact of bio-based plastic PLA and petroleum-based plastic PS on freshwater and marine environments by using *T. obliquus* and *C. pyrenoidosa* as environmental indicators to evaluate the biological impacts of these microplastics on microalgae. The SEM results revealed that the microplastic particle size

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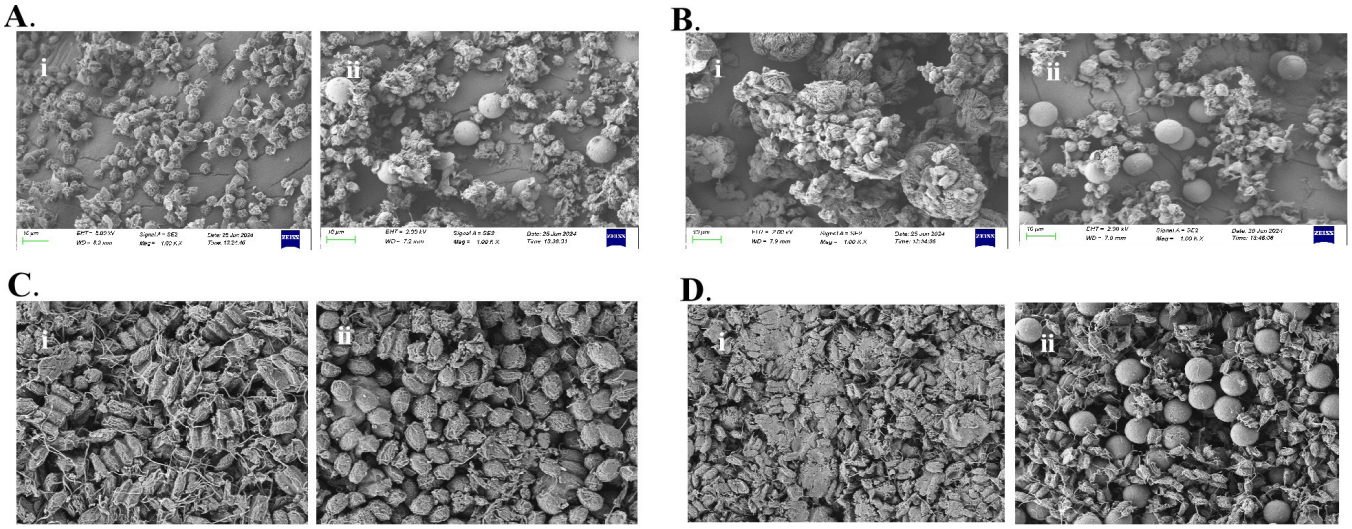

Figure 5: Scanning electron microscope (SEM) images of microalgae incubated with microplastics. SEM was used to visualize the morphological characteristics and interactions between microalgae and microplastics at high resolution. **(A)** *T. obliquus* exposed to 100 mg/L PLA (left) and 100 mg/L PS (right). **(B)** *T. obliquus* exposed to 500 mg/L PLA (left) and 500 mg/L PS (right). **(C)** *C. pyrenoidosa* exposed to 100 mg/L PLA (left) and 100 mg/L PS (right). **(D)** *C. pyrenoidosa* exposed to 500 mg/L PLA (left) and 500 mg/L PS (right).

in our experiment was larger than that of the microalgae. Large-sized microplastics present a flat or low-curvature surface, which substantially enhances the contact area with microalgae. This enables microalgae to adhere fully to the surface of the microplastics. Thus, some microalgae adhered to the surface of the microplastics, and due to the presence of numerous folds on the PLA surface, certain microalgae even became embedded within these folds **(Figure 5)**. Our SEM images showed microalgae cells attached to PS and PLA particles. This attachment enables microplastics to transport sediments, causing algae precipitation or reduced nutrient availability through adsorption, which imposes nutritional constraints on algae and affects their growth. Moreover, the adhesion of smaller microalgae onto larger microplastics surfaces also hampers light energy absorption by algae, resulting in negative effects on photosynthetic efficiency and mass transfer that ultimately inhibit cell growth. In addition, PS exhibited a higher propensity than PLA to induce the aggregation of *T. obliquus*, while both types of microplastics demonstrated negligible disparities in their impact on *C. pyrenoidosa* aggregation.

The current literature lacks comprehensive studies on the impact of bio-based plastics on microalgae. In our study, PLA, a bio-based plastic, exhibited differential effects on microalgae compared to the traditional plastic PS. The same concentration of PLA demonstrated reduced inhibition on chlorophyll production in microalgae and correspondingly accelerated their growth rate. The observed stronger growth inhibition by PS relative to PLA in our study aligns with prior findings on its pronounced inhibitory effects (8). This investigation demonstrated that the optical density of microalgae decreased as the concentration of PS rose, indicating an intensified negative impact on microalgae growth with higher concentrations of microplastics, which was in line with numerous research findings (22, 29–32). Contrary to Li et al., our findings showed that microalgae density did not increase with higher PS concentrations. This may be due to the physiological features of *Scenedesmus quadricauda* in the cited study being more adaptive to nanoplastics. Specifically, the chosen nanoplastics promoted cellular proliferation of this algal species, exhibiting a stimulatory effect. Consequently, subsequent adaptation to nanoplastic-induced physiological stimuli likely contributed to improved metabolic performance (33). Growth suppression occurred in both microalgal species upon microplastic exposure in the experiment. However, *C. pyrenoidosa* exhibited greater resistance to lower concentrations of PS and PLA compared to *T. obliquus*, with no significant disparity in growth inhibition rates between the plastics and the control group without plastics (**Figure 2)**. Mao et al. found that *C. pyrenoidosa* has the ability to cope with microplastic stress by utilizing both homophase and heterophase aggregation, which may promote its growth when exposed to microplastics (19). At the same time, we found that the high concentration of microplastics had a more substantial growth inhibition effect on microalgae than that of the low concentration. These results are in accordance with the finding that PS exhibits a more pronounced inhibitory effect on microalgae growth.

The growth of algal cells is likely hindered by microplastics due to their shading effect, which can interfere with photosynthesis (34). With microplastics uniformly dispersed in the medium, there was an increased frequency of interactions between high-concentration plastics and microalgae, leading to a more pronounced shading effect. Additionally, microplastics can adhere to algal surfaces through extracellular polymeric substances, hindering nutrient and gas exchange and accelerating the accumulation of toxic metabolites within algal cells (19).

In our study, the contents of chlorophyll decreased with increasing microplastic concentration and were concentration-dependent. The reduction in pigment levels indicates a potential inhibition of light reactions, likely resulting from shading caused by microplastics and the buildup of reactive oxygen species within cells, which lead to cell structural damage and hinder chlorophyll production during photosynthesis (29). Zhao et al. have also observed a

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decline in pigment concentration, indicating that the presence of 100 mg/L mPVC resulted in reduced levels of chlorophyll a in *Karenia mikimotoi* (35). Furthermore, *Tunali et al.* observed that elevated concentrations (50, 100, 1000 mg/L) of microplastics notably decreased the growth and chlorophyll a content in *Chlorella vulgaris* (36), which was in line with our findings that the growth rate of microalgae strongly correlated with the concentration of photosynthetic pigment (**Figure 3**). Significant differences were observed in the growth rate and chlorophyll content of *T. obliquus* when comparing the effects of PS and PLA at the same concentration. Earlier studies have suggested that microplastics derived from petroleum have the potential to interfere with the redox process occurring in microalgae's photosynthetic reaction centers. This interference can impede intracellular electron transfer, leading to a decline in photosynthetic efficiency and inhibition of cell growth (25, 36).

The impact of varying PS and PLA MP concentrations on the antioxidant balance in microalgae was evaluated by measuring SOD activity and MDA levels. High concentrations of PS in the medium significantly increased SOD and MDA activity in both microalgae, indicating a disruption of the antioxidant balance and induction of oxidative stress in algal cells (**Figure 4**). No significant difference in SOD content was observed under low concentrations of PS or varying concentrations of PLA compared to the control group. These findings once again validate that PLA has a less negative impact on microalgae compared to PS. *C. pyrenoidosa* produced fewer antioxidant enzymes compared to *T. obliquus*, indicating a stronger self-adaptation capability to counteract the negative effects of PS nanoplastics. Wu et al. and Xu et al. also suggested similar phenomena (22, 25). Compared to freshwater species, marine species demonstrate a notable variation in the genetic sequences accountable for diverse membrane functions (33). These functions are essential for marine microalgae to adapt to changing environmental conditions. This may be explained by the marine environment's complexity, especially regarding salinity, which fluctuates continuously due to river and ocean currents (37). Consequently, the observed variations in the expression levels of MDA and SOD between freshwater and marine species can be attributed to the distinct adaptive capacities of these organisms to withstand fluctuating environmental conditions, which have evolved as a result of the disparities in their respective growth habitats.

Two types of microalgae were utilized as model organisms in our study to elucidate the biological impacts of bio-based PLA and petroleum-based PS in aquatic systems. Our results validate prior research regarding the detrimental impact of petroleum microplastics on microalgae, specifically by hindering microalgae growth, increasing inhibition rate, decreasing the content of chlorophyll, and negatively affecting the antioxidant system (38–40). Bio-derived plastics are polymers made from renewable biomass sources that have the ability to break down over time, thus minimizing negative impacts on living organisms and preventing pollution of waterways (41). Nevertheless, the influence of bio-based plastics to organisms in ecosystems have not yet been thoroughly investigated. Our research indicates that bio-based PLA demonstrates distinct effects from petroleum-based PS to the microalgae used in this study. Compared to petroleum-based polymers, bio-based plastics possess favorable properties and a lower carbon footprint (42). Despite being observed at environmentally unrealistic concentrations of uniform-sized microplastics, the reduced harm from bio-based microplastics may offer significant environmental benefits at the population level. The highest microplastic concentration observed in the surface water of the Yangtze Estuary was 10.2 particles/L, with concentrations in Chinese coastal waters varying between 0.01 and 12.5 particles/L (43, 44). Thus, the microplastic concentration gradient set in our experiment far exceeds real-world levels, which also provides experimental data for the future severity of microplastic pollution (45). Further research into the interactions and impacts of various concentrations of bio-based microplastics on representative marine and freshwater microalgae is warranted due to potential implications for ecosystem services.

MATERIALS AND METHODS

C. pyrenoidosa was supplied by Hefei University of Technology in Anhui Province, China. *T. obliquus* (FACHB-12) was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, National Aquatic Biological Resource Center in Wuhan Province, China. Polystyrene (PS) are smooth spheres with a particle size of 10.3±0.12 μm and polylactic acid (PLA) microplastics, with a particle size of 12.3±0.15 μm, both were ground up when received and sterilized by ultraviolet irradiation and obtained from the University of Science and Technology of China. Pure microalgae were cultured separately in BG11 medium, an enriched medium often used for the cultivation of freshwater and marine microalgae (46). Microalgae were cultured at 25°C, 140 revolutions per minute, with a 12-hour photoperiod and 3000 Lux light intensity for 3 days. The BG11 was prepared by dissolving BG11 medium powder from Qingdao Haibo Biology Company in distilled, sterilized water and buffered to avoid pH changes during microalgal growth with a pH of 7.0.

Experimental setup

Three-day exposures of *C. pyrenoidosa* and *T. obliquus* in BG11 medium were placed in a series of 250 mL Erlenmeyer flasks in a shaker under the conditions above. Then, microalgae were diluted to a value of 0.4 at OD_{600} to start the experiment. Each type of microalgae was subjected to three independent biological replicates in five different environments, containing five groups of a control (no microplastics), PS microplastics of different concentrations (100 mg L^{-1} PS and 500 mg L^{-1} PS), and PLA microplastics with the same concentrations. PS and PLA were analyzed using SEM (Hitachi Regulus8100) to confirm uniform size. PS and PLA microplastics were first dried, sputter-coated with a conductive material, and then imaged under high vacuum to analyze particle size using SEM.

The growth of microalgae and inhibition rate

An ultraviolet visible spectrophotometer was employed to measure the inhibition ratio (IR) of microplastics on microalgae. The spectrophotometer was blanked with BG11 medium containing the corresponding microplastic concentrations. During the measurement, 3 mL algal liquid was sampled in the colorimetric dish after shaking, and the absorbance was adjusted to 680 nm for *T. obliquus* and 517 nm for *C. pyrenoidosa* (22, 23). Different microalgae have

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distinct pigment compositions, leading to unique absorption peaks. Three replicates were generated per experimental group.

Photosynthesis of microalgae and specific growth rate

After 72h, the content of chlorophyll of algae was measured by centrifuging 5 mL of algal suspension (47). The pellet was combined with an equal volume of 95% ethanol, suspended in a centrifuge tube, and stored in the dark at 4°C for 24 hours. Subsequently, the samples were centrifuged at 4,500 g for 5 minutes. Following centrifugation, the supernatant was carefully discarded, retaining the microalgae, and absorbance values were measured at 649 nm and 665 nm. Finally, chlorophyll (Chl) a and b contents were calculated, as follows.

$$\text{Chl a(g/L)} = 13.95\times A665 - 6.88\times A649 \qquad \text{(Equation 1)}$$

$$\text{Chl b(g/L)} = 24.96\times A649 - 7.32\times A665 \qquad \text{(Equation 2)}$$

The SGR was determined utilizing an equation that factors in the concentration of microalgae (22).

$$\text{SGR}\ (\%) = \frac{\ln N_n - \ln N_0}{t_n - t_0}\times 100\% \qquad \text{(Equation 3)}$$

N_n (cells/mL) represents the microalgae concentration at a specific time, N_0 (cells/mL) denotes the initial microalgae concentration, t_n (h) is the specific time, and t_0 (h) is the initial time. Since the N value is positively correlated with the optic density value, the OD value was used instead of the N value in the formula.

The measurement of specific antioxidant enzyme and molecule activity

The SOD activity and MDA contents in microalgae, treated with or without microplastics, were measured using Xanthine Oxidase and Thibabituric Acid methods, respectively (48, 49). The microalgae were weighed and then added to the homogenizing medium in the ratio of weight (g): volume (mL) of 1:4. Cells were disrupted using the mechanical homogenate method. The solution was centrifuged at 4000 g for 10 minutes, after which the supernatant was extracted and combined with reagents to create a reaction solution (test sample, distilled water and 10 nmol/mL Tetraethoxypropane, anhydrous ethanol). Subsequently, the sample's absorbance was measured at 560 nm and 532 nm to determine SOD activity and MDA content. All reagents (SOD and MDA) utilized in the aforementioned measurements were sourced from commercial kits (SOD assay kit, #A001–1–1 for SOD and MDA assay kit, #A003-1, Nanjing Jiancheng Bioengineering Institute, China).

Scanning electron microscopy analyses

A Hitachi S-4800 cold field emission scanning electron microscope (SEM) was used to refine the interaction between microplastics and microalgae (50). Algal cells were collected by centrifugation at 3000 rpm for 10 minutes after a 72-hour exposure to PS and PLA at concentrations of 100 mg/L and 500 mg/L. Microalgae and microplastics were collected together by centrifugation. The cells were preserved overnight at 4°C in 2.5% glutaraldehyde. The samples were rinsed three times with 0.1 M phosphate buffer solution (PBS, pH 7.4) by centrifugation at 3000 rpm for 10 minutes. The algal cells were fixed with 1% osmium tetroxide at 4°C for 1 hour, then rinsed three times with 0.1 M PBS (pH 7.4) by centrifugation at 3000 rpm for 10 minutes. The samples were sequentially dehydrated in a gradient of alcohol solutions (30%, 50%, 70%, 80%, 90%, 95%, and 100%) for 20 minutes each. After dehydration, the samples were treated with tert-butyl alcohol and then fixed by freeze-drying for final observation.

Statistical analysis

Each experiment was repeated three times and the results were reported as means ± standard error of the mean. Analysis of variance was performed by one-way ANOVA, following Tukey's HSD Post Hoc multiple comparison analysis for all pairwise comparisons between group means, or Dunnett's test for multiple comparisons against a single control group, using Graphpad Prism 9.

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