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RESEARCH ARTICLE

Polystyrene nanoplastics affect growth and microcystin production of *Microcystis aeruginosa*

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Abstract

Nanoplastics are widely distributed in freshwater environments, but few studies have addressed their effects on freshwater algae, especially on harmful algae. In this study, the effects of polystyrene (PS) nanoplastics on *Microcystis aeruginosa* (*M. aeruginosa*) growth, as well as microcystin (MC) production and release, were investigated over the whole growth period. The results show that PS nanoplastics caused a dose-dependent inhibitory effect on *M. aeruginosa* growth and a dose-dependent increase in the aggregation rate peaking at 60.16% and 46.34%, respectively, when the PS nanoplastic concentration was 100 mg/L. This caused significant growth of *M. aeruginosa* with a specific growth rate up to 0.41 d^{-1} (50 mg/L PS nanoplastics). After a brief period of rapid growth, the tested algal cells steadily grew. In addition, the increase in PS nanoplastics concentration promoted the production and release of MC. When the PS nanoplastic concentration was 100 mg/L, the content of the intracellular (intra-) and extracellular (extra-) MC increased to 199.1 and 166.5 μg/L, respectively, on day 26, which was 31.4% and 31.1% higher, respectively, than the control. Our results provide insights into the action mechanism of nanoplastics on harmful algae and the potential risks to freshwater environments.

Keywords Polystyrene nanoplastic · *Microcystis aeruginosa* · Microcystin · Aggregation rate · Photosynthetic activity · Dose-dependent toxicity

Introduction

In recent decades, in-depth research has been conducted into the problem of plastic pollution in aquatic environments, which has aroused widespread concern (Wang et al. 2019). Due to the lightweight nature of plastic, it is easily spread by wind and ocean currents across vast distances. Additionally, these plastic particles can break down into smaller sizes due to exposure to sunlight, wind, water, and other environmental conditions (Peng et al. 2018). Nanoplastics are commonly defined as plastics with at least one dimension less than 1 μm (Lin et al. 2019a). Some reports have claimed that nanoplastics have more significant harm because they can spread widely with water and air currents or be ingested by organisms causing potential toxicity to biota (Besseling et al. 2014; Liu et al. 2020; Zhang et al. 2018b).

Polystyrene (PS) nanoplastics have been frequently employed as an example of nanoplastics to detect the accumulation and the toxicity of nanoplastics in organisms (Qiu et al. 2019; Wang et al. 2020). Some reports have demonstrated that PS nanoplastics exposure can cause adverse effects on both freshwater and marine organisms by affecting growth, reproduction, movement, activities of antioxidants, and mitogen active protein kinases (Fadare et al. 2019; Lin et al. 2019b; Saavedra et al. 2019). For example, Zhao et al. (2019b) found that PS nanoplastics can induce more reactive oxygen species (ROS) and activate mitochondrial unfolded protein of *Caenorhabditis elegans*. Saavedra et al. (2019) showed that PS nanoplastics were ingested by the zooplankton and mainly concentrated in the gut of *Daphnia magna*, larvae *Thamnocephalus platyurus*, and the stomach of *Brachionus*

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calyciflorus. In addition, Jiang et al. (2019) reported that PS nanoplastics can significantly inhibit growth and induce higher genotoxic and oxidative damage to *Vicia faba*. Moreover, algae is an important primary producer at the bottom of the trophic chain that plays an important role in the oxygen, nitrogen, and phosphorus biogeochemical cycles and is widely studied for its sensitivity to environmental hazards (Prata et al. 2019). Besseling et al. (2014) showed that PS nanoplastics reduce population growth and chlorophyll concentrations in green algae, and when their concentration reached 1 g/L, there was approximately 2.5% growth inhibition of *Scenedesmus obliquus*. Casado et al. (2013) showed that polyethyleneimine PS inhibited the growth of *Pseudokirchneriella subcapitata* and that the concentration for 50% of maximal effect (EC_{50}) was 0.58 mg/L and 0.54 mg/L for particle sizes of 50 and 100 nm, respectively, at 72 h. Zhang et al. (2017a) demonstrated that microplastics had obvious inhibition on the growth of *Skeletonema costatum*, with an inhibition ratio up to 39.7% after 96 h.

Harmful algal blooms (HABs) have become a notorious and serious environmental phenomenon, threatening water resources on a global scale (Huo et al. 2015; Zhang et al. 2018a; Zhao et al. 2016). HABs may lead to increased turbidity of the water, reduced diversity of phytoplankton and other biological species, and formation and potential accumulation of various toxins in aquatic environments (Fan et al. 2018). *Microcystis aeruginosa* one of the most widely reported cosmopolitan and common toxic cyanobacterial species in freshwater can grow stably and rapidly under suitable conditions (Ni et al. 2018). *Microcystis aeruginosa* produces and releases cyclic heptapeptide toxins called microcystins (MCs) that are responsible for the death of fish, birds, wild animals, and agricultural livestock in many regions, and they are known to be detrimental to human health (Omori et al. 2019). To date, few studies have studied the effects of microplastics on harmful algal species. Moreover, some reports have indicated that the presence of microplastics promotes the growth of algae (Canniff and Hoang 2018; Mao et al. 2018; Yokota et al. 2017). However, no relevant research currently exists to indicate if the presence of microplastics will increase the growth of *M. aeruginosa* and the release of MCs.

As such, this work investigated the effects of nanoplastics on *M. aeruginosa* growth and release of MCs over its whole growth period. The concentration of tested nanoplastics ranged from 25 to 100 mg/L, covering and exceeding environmentally relevant levels (Mao et al. 2018). Polystyrene (PS), which is the most common in water, was chosen as the model nanoplastic. The particle size was chosen on the nanoscale (60 nm) because it is more harmful to algal. More specifically, the objectives of this work were to (1) evaluate the effects of PS nanoplastics on the growth of *M. aeruginosa* and the subsequent MC production and release by the algae; (2) identify the damage caused by PS nanoplastics to the photosynthetic system, antioxidant system, and cell morphology of *M. aeruginosa*; and (3) explore the potential mechanisms by which PS nanoplastics induce physiological and biochemical changes to *M. aeruginosa*. Data provided from this work will be helpful to understand the potential risks of microplastics on the freshwater ecosystems.

Materials and methods

Microcystis aeruginosa culture

The MC-producing strain *M. aeruginosa* FACHB905 was purchased from the Institute of Hydrobiology, Chinese Academy of Science (FACHB-Collection, Wuhan, China). The PS nanoplastic (60 nm, 5% *w*/*v*) was purchased from Xi'an Ruixi Biological Technology Company (Xi'an, China). All reagents used in this study were analytical reagent.

Pre-cultured *M. aeruginosa* in the logarithmic growth phase were separated by centrifugation at 6000×*g* for 10 min at 4 °C, washed three times with sterilized distilled water, and finally added into 1000-mL Erlenmeyer flasks containing 600 mL of BG-11 medium. The initial algal density was maintained at 6.73×10^5 cells/mL. The PS nanoplastics were individually added to the cultures to reach concentrations of 0, 25, 50, and 100 mg/L. Each treatment was performed in triplicate. Cultivation was performed under controlled laboratory conditions that remained constant throughout the experiment (25 °C, 2500 lx, 12:12 h light/dark cycle).

Algal growth tests

Microcystis aeruginosa was cultured for its whole growth period (30 days) and aseptically sampled every 2 days to measure the cell density and aggregation rate using a Countstar® Algae system (ALIT Life Science, Shanghai, China). Each sample was measured five times, and then the average value was taken after removing the maximum and minimum values. The algal growth inhibition efficiency was calculated according to the published method (Mao et al. 2018). The *M. aeruginosa* specific growth rate was calculated by regression analysis according to the following equation (Zhang et al. 2018a):

$$\mu = \frac{\ln N_n - \ln N_0}{t_n - t_0}$$

where μ represents the algal specific growth rate (d^{-1}) and N_n and N_0 (cells/mL) represent the algal density at time t_n and t_0, respectively.

Photosynthetic activity fluorescence measurement

The photosynthetic activity of *M. aeruginosa* was measured using a pulse-amplitude-modulated fluorescence monitoring system (Walz, Effectnich, Germany). *Microcystis aeruginosa* was harvested every 2 days and then used to measure the chlorophyll fluorescence. Briefly, each sample was incubated in the dark for 15 min, and then both the maximum fluorescence (F_m) and the minimum fluorescence (F_o) were measured according to a previous study (Wang et al. 2010). The maximum quantum yield of photosystem II (PSII) (F_v/F_m) was then calculated following the equation $F_v/F_m = (F_m - F_o)/F_m$. The maximal relative electron transport rate ($rETR_{max}$) and Alpha were obtained directly from the relative value of the plateau phase in the rapid light curve (RLC) (Yang and Wang 2019).

Phycobiliprotein measurement

In order to investigate damage to the phycobiliproteins of *M. aeruginosa*, algal cells were collected on days 6, 12, 18, 24, and 30. Algal cell suspensions were centrifuged at 10,000×*g* for 15 min at 4 °C, and then the supernatant was discarded. Next, algal cells were resuspended with phosphate-buffered saline (PBS) and repeatedly frozen 3 times in liquid N_2. Finally, the samples were centrifuged at 10,000×*g* for 10 min, and the absorbance of the supernatant was measured at 565 nm, 620 nm, and 650 nm using a spectrophotometer (L6S, Lengguang, China). The concentrations of phycocyanin (PC), phycoerythrin (PE), and allophycocyanin (APC) were calculated as previously reported (Fan et al. 2018).

Antioxidant measurements

Algal cells exposed to the PS nanoplastics for 6, 12, 18, 24, and 30 days were collected for enzyme activity measurement. The collected algal cells were centrifuged at 6000×*g* for 10 min at 4 °C and then resuspended in PBS. Subsequently, the suspension was ultrasonicated on an ice bath for 5 min at 350 W. The malondialdehyde (MDA) content and the superoxide dismutase (SOD) activity were measured using MDA and SOD assay kits, respectively (Nanjing Jiancheng Bioengineering Institute, China), according to the manufacturer's instructions.

Morphologic properties measurement

Algal cells exposed to the PS nanoplastics for 1 and 10 days were collected for morphologic properties measurement. The collected algal cells were centrifuged at 8000×*g* for 10 min at 4 °C and then fixed with 2.5% glutaraldehyde for 24 h at 4 °C. The algal cells were imaged with a scanning electron microscope (SEM, Hitachi Model TM-1000, Japan).

Measurement of microcystin

Microcystin was determined with the Microcystin ELISA Kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., China), according to the manufacturer's instructions.

Statistical analyses

All the experiments were performed in triplicate, and the data are presented as the mean ± standard deviation. Significant differences at $p < 0.05$ were analyzed by IBM SPSS v24.0 (SPSS Inc., Chicago, USA) using Duncan's post hoc test.

Results and discussion

Growth of *M. aeruginosa*

Two indicators of algal density and aggregation rate were chosen to investigate the potential effects of PS nanoplastics on the growth of *M. aeruginosa*. Algal density is generally regarded as the most intuitive indicator for *M. aeruginosa* growth. Figure 1 shows the algal density and aggregation rate of *M. aeruginosa* after exposure to different concentrations of PS nanoplastics. For the first 6 days of exposure to 25 mg/L PS nanoplastics, algal growth showed inhibition, and the aggregation rate was significantly higher than that of the control group ($p < 0.05$). Thereafter, a promoting effect was observed on algal cell growth. From day 8 until the end of the experiment, the algal density of the experimental group was higher than that of the control group. The algal density after exposure to 50 mg/L PS nanoplastics showed an inhibitory trend for the first 8 days with the inhibition rate reaching 51.09% on day 8 and then exhibited a significant increase with the specific growth rate reaching 0.41 d^{-1} on day 10. Similarly, it was

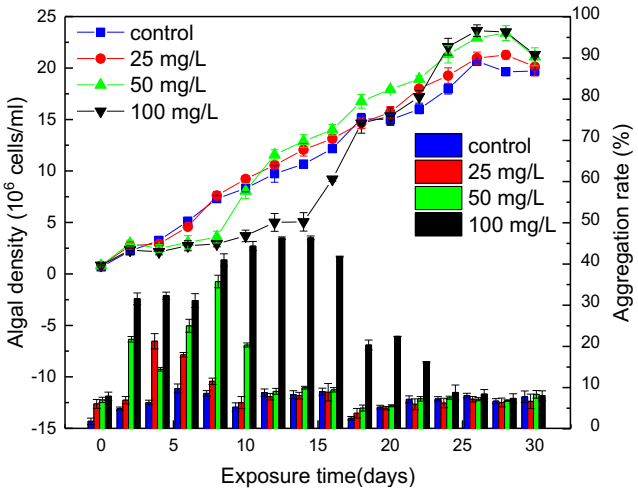

Fig. 1 Growth dynamics and aggregation rate of *M. aeruginosa* under PS nanoplastic exposure

observed that when the inhibition rate was the highest, the aggregation rate was also the largest, reaching 35.75% on day 8. However, the aggregation rate dropped significantly on day 10 ($p < 0.05$), while the specific growth rate had peaked. Hence, this suggests that aggregation may affect the growth of *M. aeruginosa*. The profile of algal growth exposed to 100 mg/L PS nanoplastics showed a pattern similar to the 50 mg/L PS nanoplastic treatment. The maximum inhibition rate of *M. aeruginosa* growth under 100 mg/L PS nanoplastic exposure reached 60.16% on day 8. On day 14, the aggregation rate of the algal cells peaked at 46.34%. Thereafter, the aggregation rate significantly decreased ($p < 0.05$), whereas the specific growth rate reached the maximum (0.30 d^{-1}). Moreover, it was also found that the aggregation rate first increased with increasing exposure time, and then decreased, finally approaching that of the control group. Figure S1 (Appendix Fig. S1) clearly shows the change in *M. aeruginosa* aggregation on days 0, 10, 20, and 30.

The growth of *M. aeruginosa* can be mainly divided into three phases under PS nanoplastic stress (Fig. 1). Phase I (the "lag phase"), which is generally the only research phase considered in other studies, is the main stage in which algal cells adapt to their new environment, as demonstrated by the fact that *M. aeruginosa* growth was inhibited and the aggregation rate increased. Moreover, this phase was prolonged at higher PS nanoplastic concentrations. Phase II (the "stimulation phase") showed a significant increase in the algal density and specific growth rate, while the aggregation rate decreased significantly ($p < 0.05$). Although the exact mechanisms by which PS nanoplastics stimulate the growth of *M. aeruginosa* have not yet been proven, it may be due to the following reasons: (1) after adapting to the stress caused by PS nanoplastics, the physiological state of the algae is enhanced to a certain extent, which is conducive to the future growth of cells (Mao et al. 2018); (2) nanoplastics stimulate the algal cells to release more extracellular polymeric substances (EPS), which could be used as a nutrient by the algal cells; (3) nanoplastics might create better growth conditions for algae and contribute to cell growth as the substrates for algal growth (Canniff and Hoang 2018); and (4) cellular aggregation inhibits cell division, and when the aggregation rate decreases, cell division is significantly stimulated. In phase III (the "normal phase"), in which growth tends to be stable, *M. aeruginosa* growth was similar to that of the control group. These phenomena indicate that *M. aeruginosa* adapted to the new environment and that the damage caused by PS nanoplastics was only temporary. This agrees with previous studies demonstrating that the damage to algal cells caused by microplastics is only temporary because, after the initial vulnerable period, algal cells exhibit adaptive reactions that lead to recovery (Prata et al. 2019; Yokota et al. 2017; Zhang et al. 2017a).

Photosynthetic activity of *Microcystis aeruginosa*

It has been reported that the photosynthetic activity measurement (PAM) fluorescence method can effectively measure the activity of PSII in cyanobacteria, thereby better identifying the physiological mechanism in vivo (Zhao et al. 2019a). In this study, the photosynthetic activity of *M. aeruginosa* was determined using three key photosynthetic parameters, namely, F_v/F_m, $rETR_{max}$, and Alpha. F_v/F_m, an important indicator of PSII activity, is used for the reduction of the primary electron acceptor and could signal the inhibition of PSII (Wang et al. 2016). In addition, the F_v/F_m ratio is relatively stable under normal conditions and is not affected by species or growth conditions; however, reduced values indicate that plants are inhibited by light (Wu et al. 2019). Throughout the whole experimental process, the variation trends of F_v/F_m values in the experimental group were as follows: first falling, next rising, then falling, and finally rising. Furthermore, it can be found that the inhibition of the F_v/F_m ratio of the microplastic does not increase with the increase of the microplastic concentration nor does it increase with the increase of the exposure time. After incubation with 25, 50, and 100 mg/L PS nanoplastics, the maximum inhibition was reached on days 20 (72.6% of control), 4 (80.2% of control), and 24 (77.5% of control), respectively (Fig. 2a). In addition, due to the response of the algae under nanoplastic exposure, the F_v/F_m ratio was higher than that of the control group in some days (days 8, 10, and 12), which is similar to previous report (Wu et al. 2019). A higher F_v/F_m ratio may mean that the presence of PS nanoplastics can promote photosynthetic electron transport in algal cells. It was found that the $rETR_{max}$ value of *M. aeruginosa* exposure to PS nanoplastics during the experimental period was lower than that of the control group, with the most severe impairment observed on day 4 at 72.6%, 74.7%, and 76.4% of control when exposed to 25, 50, and 100 mg/L PS nanoplastics, respectively (Fig. 2b). The Alpha parameter is responsive to the utilization efficiency of photosynthetic organs in light energy (Ralph and Gademann 2005). In this study, the trend of Alpha was similar to that of F_v/F_m (Fig. 2c).

PS nanoplastics have a negative impact on the photosynthetic activity of algal cells, which may be because they (1) interrupt photosynthetic electron transport between Q_a and Q_h (Deng et al. 2014), (2) promote the reduction of chlorophyll content in algal cells (Fig. S1), and (3) cause Q_a to be in a reduced state, which hinders the transfer of electrons from PSI to PSII (Msilini et al. 2011).

Phycobiliprotein content of *Microcystis aeruginosa*

To investigate the changes in PC, APC, and PE content, different concentrations of PS nanoplastics were used to treat *M. aeruginosa*. The content of PC, APC, and PE in the control

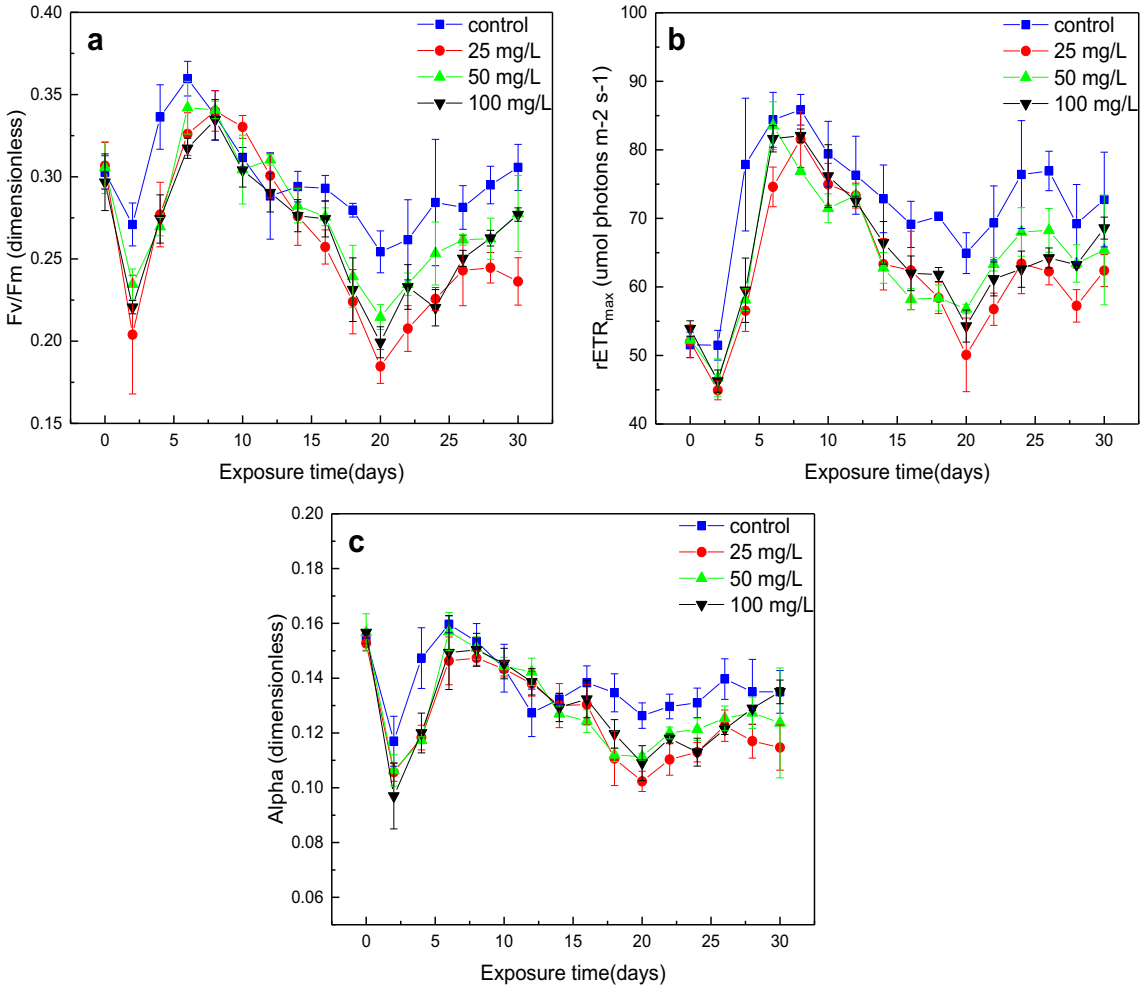

Fig. 2 Response of *M. aeruginosa* photosynthetic parameters when exposed to different concentrations of PS nanoplastics. (**a**) F_v/F_m, (**b**) $rETR_{max}$, and (**c**) Alpha

group increased over time (Fig. 3). When *M. aeruginosa* was exposed to 25 mg/L PS nanoplastics, the PC, APC, and PE content showed a similar trend as the control. However, as the concentration of PS nanoplastics increased, the content of PC, APC, and PE changed significantly, with the contents first increasing and then decreasing. In particular, when the PS nanoplastic concentration reached 100 mg/L, the APC and PE contents peaked on day 18 and were significantly higher than that of the control and other experimental groups ($p < 0.05$). Furthermore, the APC and PE contents on day 30 were significantly lower than that of the control. The presence of PS nanoplastics significantly affected the content of phycobiliproteins, which may be due to the oxidative stress protection mechanism of the algal cells. It has been reported that phycobiliproteins act as good antioxidants under oxidative stress owing to their nucleophilic ability to neutralize active oxidants (Cano-Europa et al. 2010). Moreover, the light energy captured by PE is sequentially transmitted to PC, APC, and the fat-soluble pigment chlorophyll and finally to the D1 protein for a photochemical reaction (Fan et al. 2018). Compared with Fig. 1, the significantly increased

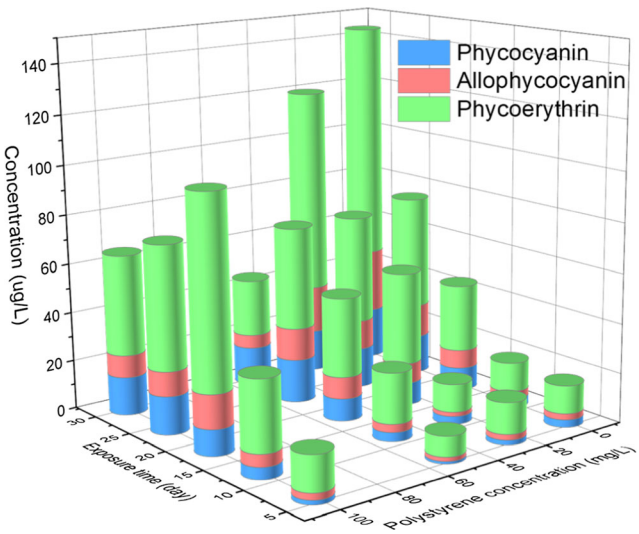

Fig. 3 Phycobiliprotein content of *M. aeruginosa* exposed to different concentrations of PS nanoplastics

phycobiliprotein content on day 18 may be the reason for the rapid growth of *M. aeruginosa* when exposed to 100 mg/L PS nanoplastics.

Morphology change of *Microcystis aeruginosa*

The surface changes of algal cells on the days 1 and 10 after treatment with PS nanoplastics are shown in Fig. 4. When comparing Fig. 4a with 4c, it can be found that after 10 days of cultivation, most of the algal cells in the control group were round and had a smoother exterior. As shown in Fig. 4b, some of the PS nanoplastics agglomerated with each other and were adsorbed on the surface of the algal cells. Furthermore, the cells were deformed and obvious disruptions in the cellular membrane could be observed after treatment. These results confirm that PS nanoplastics can directly cause physical damage to algal cells. Similarly, when studying the effects of microplastics on the growth of *Chlorella pyrenoidosa* and *M. aeruginosa*, researchers found that the presence of microplastics caused algal cell deformation and membrane rupture (Mao et al. 2018; Zhang et al. 2018b). After 10 days of exposure to PS nanoplastics, some algal cells were covered with an unknown substance (Fig. 4d). This substance, which might be cell constituents released by the algal cells or the added PS nanoplastics, can inhibit algae from capturing light and then affect the transfer of electrons, thereby inhibiting photosynthesis. This agrees with finding of previous studies on allelopathic inhibition of juglone on the growth and physiological properties of *M. aeruginosa* (Hou et al. 2019). This indicates that PS nanoplastics can not only directly cause cell membrane damage to *M. aeruginosa* but also covers the surface of the cell membrane to obstruct photosynthesis, inhibiting the growth of *M. aeruginosa*.

Oxidative damage to *Microcystis aeruginosa*

Microcystis aeruginosa was treated with different PS nanoplastic concentrations to determine the changes in MDA content and SOD activity. The MDA content of *M. aeruginosa* under PS nanoplastic stress increased significantly on day 6 ($p < 0.05$) and significantly increased with the increase of PS nanoplastic concentration ($p < 0.05$) (Fig. 5). MDA is an indicator of lipid peroxidation of the cell membrane. The higher the MDA content, the deeper the degree of lipid peroxidation of the cell membrane, which also indicates that organisms are subjected to greater environmental stress and more serious damage (Ni et al. 2018). The increased MDA content also illustrates the occurrence of membrane lipid peroxidation, a change in membrane fluidity and permeability accompanied by membrane injury (Liu et al. 2014).

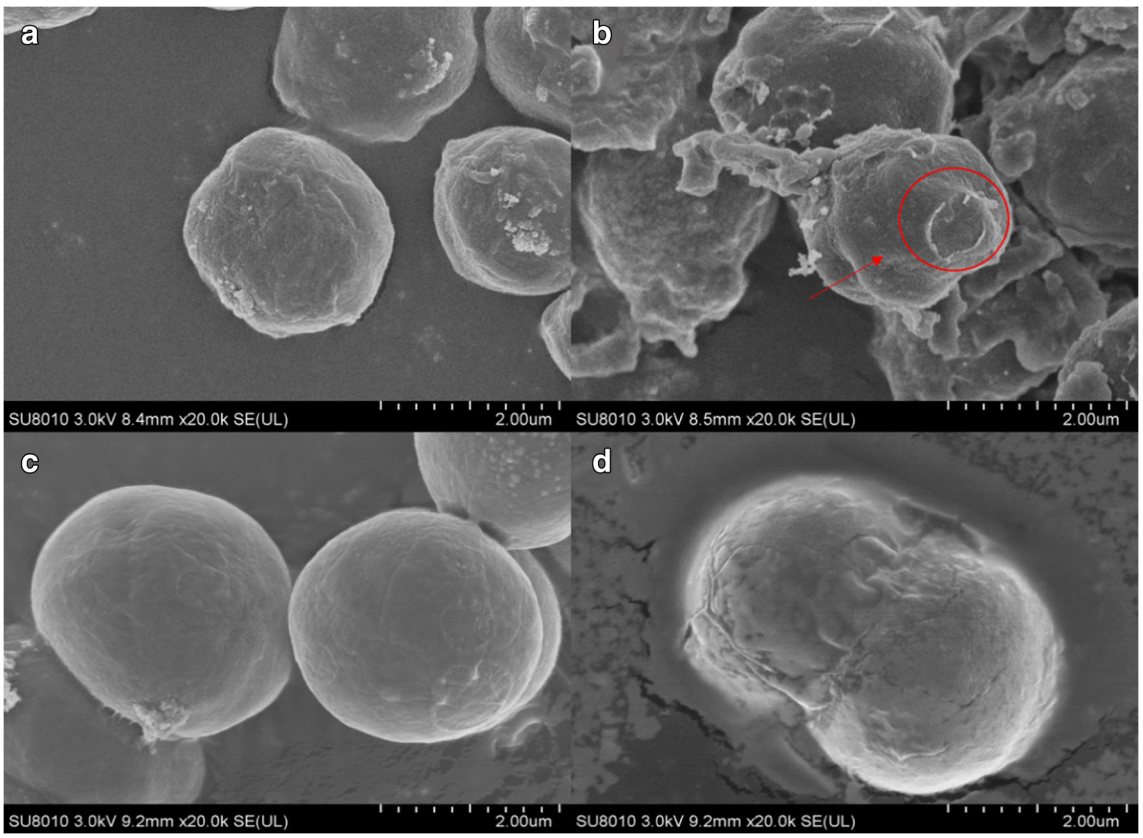

Fig. 4 SEM images of *M. aeruginosa* treatments: (**a**) control, day 1; (**b**) 100 mg/L PS nanoplastics day 1; (**c**) control, day 10; and (**d**) 100 mg/L PS nanoplastics day 10

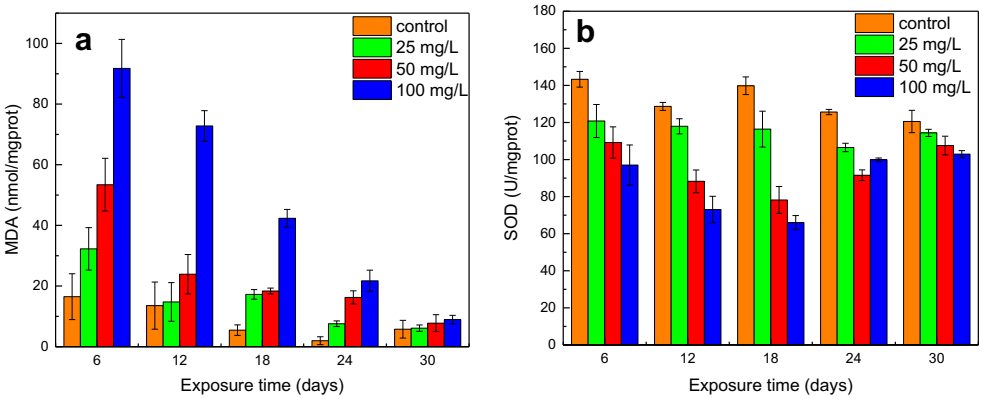

Fig. 5 Effect of PS nanoplastics on MDA content (**a**) and SOD activity (**b**) of *M. aeruginosa* after 30 days of exposure

Meanwhile, with increasing exposure time to PS nanoplastics, the MDA content showed a significant decreasing trend. On day 30, there was no significant difference in MDA content between the experimental and control groups ($p < 0.05$). This phenomenon suggests that damage to the *M. aeruginosa* membrane caused by PS nanoplastics weakened as the exposure time increased, indicating that *M. aeruginosa* has a good ability to recover from pollutant stress, which agrees with a similar results obtained by Mao et al. (2018). This may be because the presence of PS nanoplastics stimulates the secretion of EPS by *M. aeruginosa*, which may reduce contact with algal cells by encapsulating the nanoplastic particles.

SOD is considered the key antioxidant enzyme because it is the first line of defense against reactive oxygen species (ROS) (Lu et al. 2018). A decrease in SOD activity was observed in all PS nanoplastic treatments on day 6 (Fig. 5), suggesting significant inhibition of antioxidant enzyme activity in *M. aeruginosa*. This inhibitory effect was more obvious as the PS nanoplastic concentration increased. The inhibition of SOD activity may be attributed to an excess of ROS, which is similar to the finding of Fan et al. (2019) that antioxidant enzyme activities were inhibited at a high ROS level. Thus, the effect of antioxidative stress was diminished, which explains why higher PS nanoplastic concentrations are associated with greater toxicity. At the same time, the activity of SOD was significantly inhibited under PS nanoplastic stress ($p < 0.05$) on days 6, 12, and 18, and the inhibition of SOD activity decreased with increasing exposure time. These results illustrate that the oxidative stress induced by PS nanoplastics exceeds the antioxidant capacity of the algae and may only lead to temporary damage.

Intra- and extra-microcystins of *Microcystis aeruginosa*

On days 11, 16, 21, and 26, the content of MCs secreted by *M. aeruginosa* in response to PS nanoplastic exposure at different concentrations was measured (Fig. 6). After the addition of PS nanoplastics (25, 50, and 100 mg/L), intra- and extra-MC contents were significantly higher than those of the control group ($p < 0.05$). In addition, as the concentration of PS nanoplastics increased, both intra- and extra-MCs increased significantly ($p < 0.05$). On day 11, the intra-MC content of the 25, 50, and 100 mg/L PS nanoplastic groups was 172.0, 185.2, and 198.9 μg/L respectively, which was 8.5%, 16.8%, and 25.4% higher, respectively, than the control. Additionally, the extra-MC content of the 25, 50, and 100 mg/L PS nanoplastic groups was also significantly increased to 67.0, 72.4, and 77.6 μg/L, which was 10.1%, 18.8%, and 27.4% higher, respectively, than the control. Furthermore, as *M. aeruginosa* continued to be exposed to 100 mg/L PS nanoplastic, the content of extra-MCs was significantly increased from 77.60 on day 11 to 166.46 μg/L on day 26, which was 27.4% and 31.1% higher, respectively, than the control. Similarly, from days 11 to 26, when the PS nanoplastic concentration was 25 and 50 mg/L, the extra-MC content increased from 67.04 to 136.79 μg/L and 72.36 to 146.68 μg/L, respectively, which was 10.1 to 7.7% and 18.8 to 15.5% higher, respectively, than the control group. These results indicate that PS nanoplastics markedly stimulated the release of MCs and that the content of extra-MCs increased with exposure time to PS nanoplastics.

Compared with Fig. 1, it can be clearly found that on day 11, the density of algal cells exposed to 100 mg/L PS nanoplastics was significantly lower than that of the control group, while both the intra- and extra-MC contents were significantly higher than that of the control group. This demonstrates that the presence of PS nanoplastics significantly promoted MC production and release. The enhancing effect of PS nanoplastics on MC production and release might be partly explained in three ways. First, PS nanoplastics can affect the permeability of cell membranes and promote the release of more intracellular substances, thereby promoting the release of MCs. In this study, damaged cell membranes (Fig. 4), elevated MDA levels (Fig. 5), and increased EPS (Fig. S3) support this hypothesis. When oxidative damage is severe, the *M. aeruginosa* membrane may leak or senescence, thereby promoting the release of MCs into the surrounding environment (Merel et al. 2013). Second, the MCs from *M. aeruginosa* cells might play a protective role against

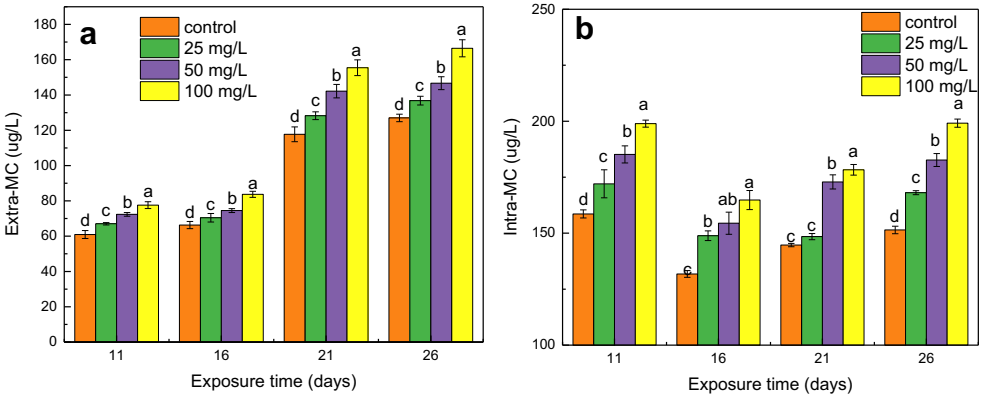

Fig. 6 Effect of PS nanoplastics on the extra-MC content (A) and intra-MC content (B) on days 11, 16, 21 and 26. The mean and standard deviation of three replicates are shown; different letters indicate a significant difference in the means among groups at each PS nanoplastic level at $p < 0.05$ according to one-way ANOVA

oxidative stress. When SOD activity is inhibited, algal cells require other mechanisms to withstand oxidative stress. As such, MCs may play a role in stabilizing the photosynthetic apparatus and protein-modulating metabolites (Yang et al. 2015). Third, the presence of nanoplastics may cause upregulation of microcystin synthetase genes. The microcystin synthetase (*mcy*) gene cluster consists of 10 genes (*mcyA* to *mcyJ*), each of which affects the synthesis and transport of MCs (Tillett et al. 2000). According to a previous study, the transcriptional levels of MC synthesis-related genes (*mcy*A and *mcyD*) in algae were significantly increased when exposed to environmentally relevant concentrations of glufosinate (Zhang et al. 2017b). Indeed, detailed approaches involved in the stimulatory effect of nanoplastics on MCs require further and multidisciplinary investigation. Nevertheless, this study is the first report of this phenomenon.

Conclusions

In this study, we investigated the effects of PS nanoplastics on *M. aeruginosa* growth, as well as MC production and release throughout the whole algal growth period. In a 30-day exposure experiment, the growth of *M. aeruginosa* was divided into three stages in response to PS nanoplastics. The first "lag" phase, which mainly involved *M. aeruginosa* adaptation to the new environment, showed that algal growth, chlorophyll content, and photosynthetic efficiency were significantly inhibited and that the aggregation rate increased. Furthermore, the length of this phase was prolonged with increasing PS nanoplastic concentrations. The second "stimulation" phase showed a sharp increase in algal density, specific growth rate, and chlorophyll content, as well as a significant decrease in the aggregation rate ($p < 0.05$). Finally, during "normal" phase, in which the growth of *M. aeruginosa* was similar to that of the control group, the growth tended to be stable. In addition, the increase of PS nanoplastic concentration promoted the production and release of MC, which could have potentially adverse effects on the ecological environment. Herein, our work not only shows the effect of nanoplastics on *M. aeruginosa* but also helps to better assess the ecological risks of nanoplastics in aquatic environments.

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