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Growth inhibition, toxin production and oxidative stress caused by three microplastics in *Microcystis aeruginosa*

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ABSTRACT

Microplastics (MPs) have aroused widespread concern due to their extensive distribution in aquatic environments and adverse effects on aquatic organisms. However, the underlying toxicity of different kinds of MPs on freshwater microalgae has not been examined in detail. In this study, we investigated the effects of polyvinyl chloride (PVC), polystyrene (PS) and polyethylene (PE) MPs on the growth of *Microcystis aeruginosa*, as well as on its toxin production and oxidative stress. We found that all three kinds of MPs had an obvious inhibition effect on the growth of *M. aeruginosa*. Considering the results of antioxidant-related indicators, the activity of superoxide dismutase (SOD) and catalase (CAT), and cell membrane integrity were greatly affected with exposure to PVC, PS and PE MPs. Moreover, the content of intracellular (intra-) and extracellular (extra-) microcystins (MCs) had a noticeable increase due to the presence of PVC, PS, and PE MPs. Finally, according to the comprehensive stress resistance indicators, the resistance of *M. aeruginosa* to three MPs followed the order: PE (3.701) > PS (3.607) > PVC (2.901). Our results provide insights into the effects of different kinds of MPs on freshwater algae and provide valuable data for risk assessment of different types of MPs.

1. Introduction

Microplastics (MPs) are commonly defined as plastic particles with the size or aerodynamic diameters less than 5 mm (Peng et al., 2019). This concept of MPs was first put forward in a previous study (Thompson et al., 2004). Current research has found that MPs are widely distributed in freshwater environments. A study reported that the abundance of MPs in Taihu Lake, China, reached 0.01×10^6–6.8×10^6 particles/km^2 in plankton net samples, 3.4–25.8 particles/L in surface water, and 11.0–234.6 items/kg dry weight in sediments (Su et al., 2016). Another study reported that the abundance of MPs in Poyang lake, China, was 5–34 items/L for surface water, and 54–506 items/kg for sediment (Yuan et al., 2019). In another recent work it was observed that 92% of water samples included MPs ranging from 3.52 to 32.05 particles/m^3, and 69% of sediment samples contained MPs ranging from 0.46 to 1.62 particles/kg in Central and Eastern European surface waters (Bordós et al., 2019). It has also been shown that the abundance of MPs in the surface water of Dongting Lake, China, and Hong Lake, China was 900–2800 particles/m^3 and 1250–4650 particles/m^3, respectively (Wang et al., 2018). The high abundance of MPs is widely found not only in marine environments but also in freshwater ecosystems (Li et al., 2020). Thus, such large number of MPs in the freshwater environment can cause varying degrees of damage to aquatic organisms. It was found that the plain polystyrene (PS) induced the reactive oxygen species (ROS) production and activated the mitogen-active protein kinases, thereby resulting in higher acute toxicity towards *Daphnia magna* and affecting their physiological behaviors (Lin et al., 2019). Another study showed that highly concentrated PS microbeads caused 78 proteins of zebra mussel (*Dreissena polymorpha*) to be differentially modulated, which related to the response against the oxidative stress (Magni et al., 2019). It was also observed that both amidine and carboxyl PS nanoplastics were ingested by the zooplankton and concentrated mainly in the gut of water flea *D. magna* and larvae *Thamnocephalus platyurus*, and in the stomach of rotifer *Brachionus calyciflorus* (Saavedra et al., 2019).

Nano-PS were also reported to reduce population growth and chlorophyll concentrations in the green alga *Scenedesmus obliquus* (Besseling et al., 2014). Moreover, PS MPs exhibited a size-dependent inhibitory effect on *Chlorella pyrenoidosa,* with the 96 h-IC_{50} at 6.90 mg/L and 7.19 mg/L for 0.1 μm and 0.55 μm PS MPs respectively, but little toxicity was observed with 5 μm PS MPs (Li et al., 2019). However, environmentally

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relevant concentrations of PS MPs (0.1 μm and 1.0 μm in size) caused a dose-dependent negative effect on *C. pyrenoidosa* growth and photosynthetic activity at first, but then stimulated algal growth and photosynthetic activity (Mao et al., 2018). However, the impact of MPs on *M. aeruginosa*, the dominant species causing cyanobacterial blooms, has received little attention. Meanwhile, some researches have reported that the presence of MPs promote an increase in the growth of algae (Canniff and Hoang, 2018; Prata et al., 2019). Moreover, a previous study has confirmed that the microcystins (MCs) in *M. aeruginosa* cells play the role of protectant against oxidative stress, stabilizes the photosynthetic apparatus and regulates the protein metabolites (Zhang et al., 2018a). The population of *M. aeruginosa* and MCs production can be affected by some environmental stresses, including pollutants (Wang et al., 2016; Zhang et al., 2018a). Therefore, whether MPs can promote the growth of *M. aeruginosa* and promote production and release of more MCs by the algae has not been fully elucidated. Meanwhile, most of the current studies are only centralized on the influence of MPs of the same type but of different particle sizes, on algae. However, only a few scholars are comparing the effects of the same particle size MPs which are of various types on algae. Therefore, it is necessary to explore the underlying mechanism of algal responses to different types of MPs. Moreover, current research mainly focuses on the effects of MPs on the growth and photosynthetic activity of algae. The impact of different MPs types on the oxidative stress of algae has not been investigated till date.

The present study, thus, attempted to investigate the effects of different kinds of MPs on *M. aeruginosa* growth, MCs production, and its antioxidant system. For the model MPs, the most common PE, PS, and PVC in water were chosen. The concentration of the tested MPs ranged from 10 mg/L to 200 mg/L, covering and far exceeding the actual environmental levels detected (Su et al., 2016). The particle size of 3 μm was chosen, which was similar to that of *M. aeruginosa.* More specifically, the objectives of the present work were: (1) To evaluate the toxicity of PVC, PE, and PS MPs on *M. aeruginosa* growth, and antioxidant system, (2) To determine the relationship between the toxicity of MPs and the particles type, and (3) To explore the potential mechanisms of the effects of PVC, PE, and PS MPs on *M. aeruginosa.* The data obtained from this study could be useful for the evaluation of potential risks of MPs to freshwater ecosystem.

2. Materials and methods

2.1. Chemicals preparation and M. aeruginosa culture

PVC, PS and PE microplastics (diameter 3 μm) were purchased from Dongguan Jing tian raw material of plastics Co. Ltd., China. The stock solutions of 1 g/L PVC, PS, and PE were each prepared in ultrapure water, and the suspension was well dispersed through sonication for 30 min using an ultrasound device before use.

The axenic strain *M. aeruginosa* FACHB905, Which can only produce MC-LR, was isolated from Lake Dianchi in China (Dai et al., 2019). *M. aeruginosa* was purchased from the Institute of Hydrobiology, Chinese Academy of Science (FACHB-Collection), Wuhan city, China. All apparatuses used in culturing *M. aeruginosa* were sterilized by autoclaving at 121 °C for 30 min before use. *M. aeruginosa* was grown in 1000 mL Erlenmeyer flasks containing 500 mL of BG-11 medium and cultivated in an illumination incubator with 12 h light: 12 h dark cycle (light intensity: 2500 lx) at 25 ± 1 °C.

2.2. Acute microalgae growth inhibition tests

The acute toxicity effects of PVC, PS, and PE MPs on *M. aeruginosa* were investigated by 96 h standard microalgae growth inhibition tests based on the OECD (Organization for Economic Cooperation and Development) guidelines 201 (OECD, 2011). The initial algal density was maintained at 3.74×10^5 cells/mL and was taken into flasks added MPs until the algae reached the logarithmic growth phase. Microplastics of three types (PVC, PS, and PE) were added to individual algae cultures to obtain MPs concentration of 10, 25, 50, 100, and 200 mg/L. Algal culture with no MPs was used as a control. During 96 h of algal exposure to MPs, sampling was done aseptically every 24 h to measure the content of Chlorophyll a (Chla). Chlorophyll a was measured spectrophotometrically according to a published method (Wang et al., 2013). Briefly, 10 mL algal culture was centrifuged at 8000×*g* for 5 min, and the overnight extraction with 95% ethanol was done in the dark at 4 °C. Finally, the samples were centrifuged at 4000×*g* for 5 min and OD_{665} and OD_{649} of the supernatant was measured using a spectrophotometer (L6S, Lengguang, China). The content of Chla was obtained according to the following equation: $Chla = 13.7 \times OD_{665} - 5.76 \times OD_{649}$. Each test concentration was replicated three times, and all operations were carried out under sterile conditions to avoid any bacterial contamination.

2.3. Flow cytometric measurements

To investigate the damage to *M. aeruginosa*, the effects of PVC, PS, and PE MPs on cell membrane integrity and the esterase activity were ascertained using a flow cytometry (BD caliber, USA). In brief, *M. aeruginosa* cells were stained for 15 min with 65.2 mg/L propidium iodide (PI) (Sigma, Saint-Louis, USA) and 10 mg/L fluorescein diacetate (FDA) (Sigma, Saint-Louis, USA). Thereafter the integrity of the cell membrane was detected using the FL2 detector with the excitation wavelength of 488 nm and 585/40 nm, and using the FL1 detector with the excitation wavelength of 488 nm and 533/30 nm. The flow cytometry operational flow rate was 12 μL/min. At least 20,000 events were counted for each sample and analyzed using CellQuest software (BD Biosciences, Franklin Lakes, NJ, USA). The detailed methodology is described in a previous report (Zhao et al., 2019).

2.4. Antioxidant measurements

Algal cells exposed to PVC, PS, and PE MPs for 96 h 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, catalase (CAT) activity, glutathione oxidized (GSSH), and superoxide dismutase (SOD) activity was measured using the respective assay kits (Nanjing Jiancheng Bioengineering Institute, China), according to the manufacturer's instructions.

2.5. Measurement of microcystin

Algal cells were collected after 96 h exposure to MPs for MC-LR measurement. For this, 20 mL of culture was centrifuged at 10,000×*g* for 10 min at 4 °C and the supernatant was filtered with a 13 mm diameter nylon syringe filter (0.22 μm pore size) to obtain the solution containing the extracellular (Extra-) MC-LR. Subsequently, the centrifugal sediment was resuspended in 5 mL of 50% methanol/water (vol/vol) containing 1% acetic acid (vol/vol), and the suspension was ultrasonicated on an ice bath for 5 min at 350 W (Yang et al., 2020). Thereafter, the suspension was centrifuged at 10,000×*g* for 10 min at 4 °C and the supernatant was filtered with a 13 mm diameter nylon syringe filter (0.22 μm pore size) to obtain the solution containing the intracellular (Intra-) MC-LR. Both Extra- and Intra-MC-LR were determined with the Microcystin ELISA Kit (Shanghai Enzyme-linked Biotechnology Co., Ltd, China), according to the manufacturer's instructions.

2.6. Comprehensive evaluation of M. aeruginosa

The membership function method of fuzzy mathematics was used to determine the membership values corresponding to different physiological indicators of *M. aeruginosa* (Zhang et al., 2021). Positive

correlation with stress resistance was calculated using Formula (1) and negative correlation using Formula (2). Combining these factors, a comprehensive index of stress resistance was obtained, and the larger the value, the stronger the corresponding stress resistance.

$$X(\mu) = \frac{X_0 - X_{min}}{X_{max} - X_{min}} \quad (1)$$

$$X(\mu) = 1 - \frac{X_0 - X_{min}}{X_{max} - X_{min}} \quad (2)$$

In the equations, $X(\mu)$ is the membership value, X_0 is the average measurement value in this study, X_{min} is the minimum measurement value, and X_{max} is the maximum measurement value. According to previous studies, SOD, CAT, GSSH, FDA (representative esterase activity), Chla and cell membrane integrity (CMI) are positively related to *M. aeruginosa* resistance, whereas MDA is negatively related to *M. aeruginosa* resistance (Zhang et al., 2021).

2.7. Statistical analyses

All the experiments were performed in triplicates and the data is presented as mean ± standard deviation. Significant differences at $p < 0.05$ were analyzed by IBM SPSS v20 (SPSS Inc. Chicago, USA) using Duncan's post-hoc test.

3. Results and discussion

3.1. Effect of different microplastics on algal growth inhibition

Chlorophyll a, as the primary pigment in algae, can act as a standard to reflect the growth and proliferation of algae (Fan et al., 2018). Given that the size of the MPs used in this experiment is similar to the size of an algal cell, and the suspension of MPs interfered with algal number calculation through absorbance detection in our pre-experiment. Therefore, Chla was used as an *M. aeruginosa* growth indicator in PVC, PS, and PE MPs containing experiments. As illustrated in Fig. 1, the Chla content was not statistically affected by the PVC and PE treatment after 24 h of exposure and by the PS treatment after 48 h of exposure, which indicated that the effect of PS MPs on *M. aeruginosa* growth inhibition was lower than that of PVC and PE MPs at the beginning of the experiment. However, the content of Chla decreased significantly after 72 h and 96 h of treatment at all concentrations of PVC, PS, and PE MPs ($p < 0.05$). A previous study has also reported that the Chla content of *M. aeruginosa* decreased significantly after 96 h of treatment with nPS-NH_2 MPs (Zhang et al., 2018b). Similar to the above results, the content of Chla in *C. pyrenoidosa* and *Microcystis flos-aquae* was significantly inhibited after exposure to polypropylene (PP) and PVC MPs (Wu et al., 2019). In the current study, the effective quantum yield (Y(II)) and the maximal quantum yield (F_v/F_m) on photosystem II of *M. aeruginosa* showed an obvious decrease after 96 h treatment with MPs (Fig. S3). This revealed that MPs affect the light energy conversion on PS II of *M. aeruginosa*. Moreover, the photosynthetic efficiency (Alpha), the maximal electron transport rates (rETRmax) and the light saturation

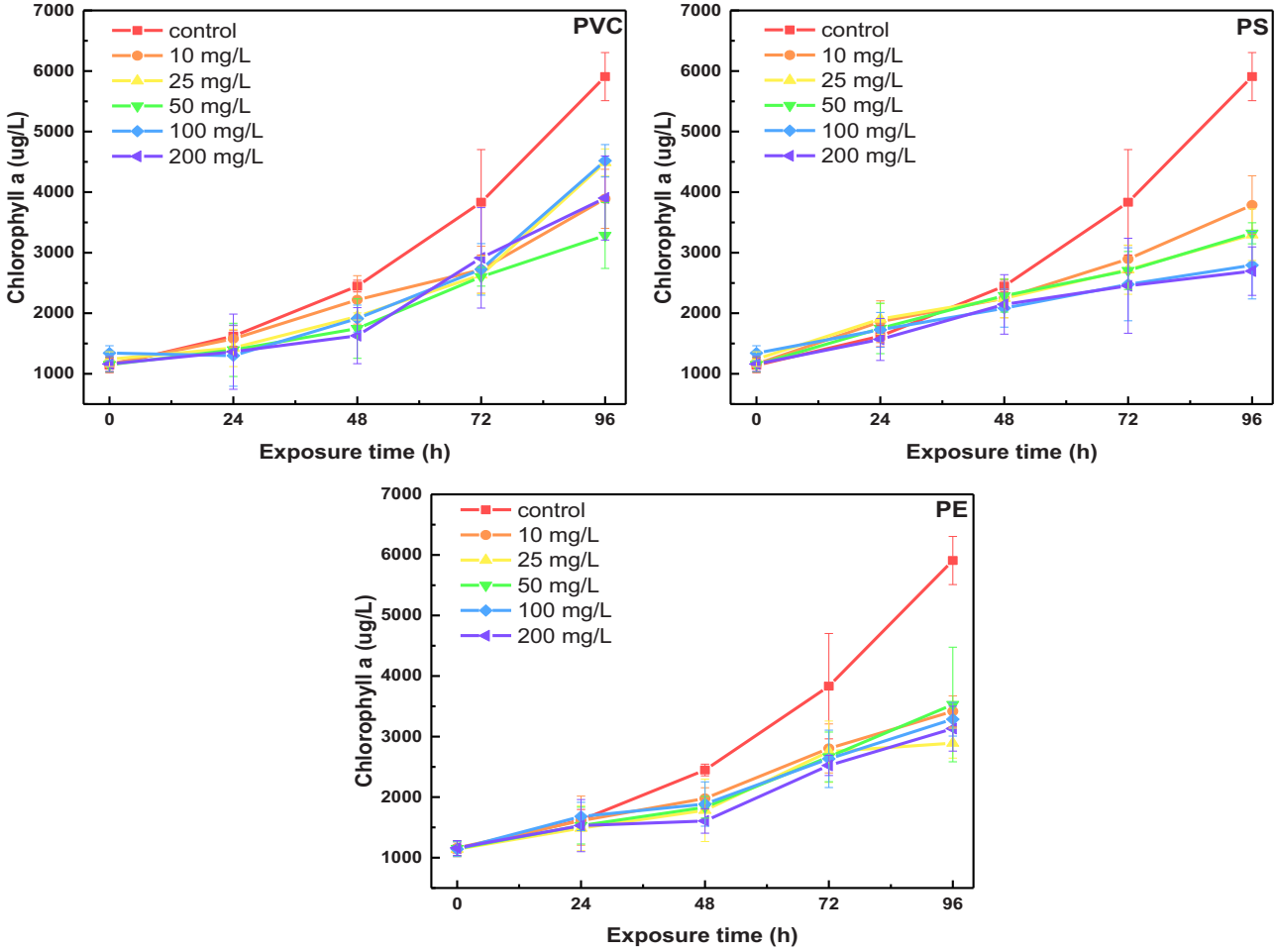

Fig. 1. Effect of PVC, PS and PE on the Chla content of *M. aeruginosa*. Vertical bars represent mean ± SD.

coefficient (I_k) also showed an obvious decrease after 96 h treatment with MPs (Fig. S3). The decreased fluorescence parameters suggested an inhibition of the photosynthetic system of *M. aeruginosa*. Therefore, it is reasonable to believe that the presence of MPs affect the photosynthesis of *M. aeruginosa,* thereby inhibiting its growth. Moreover, we can also observe from Fig. 1 that between 48 h and 96 h of exposure, the effect of PS MPs on algae was significantly greater than that of PVC and PE microplastics. The change in inhibition rate from 48 h to 96 h exposure also confirms the above conclusion (Fig. S1).

Based on the inhibition rate curve calculated from the Chla content, it can be found that with the increase in exposure time, the growth inhibition rate of *M. aeruginosa* under PS MPs exposure continues to increase (Fig. S1). When the exposure time was 96 h, and the PS MPs concentration was 200 mg/L, the inhibition rate of *M. aeruginosa* was maximum, reaching 54.4%. However, the maximum inhibition rate of *M. aeruginosa* growth under PVC and PE MPs after 96 h treatment with respective concentrations of 50 mg/L and 25 mg/L, reached 44.4% and 51.0%, respectively. A previous study also found that the inhibition rate of *Skeletonema costatum* reached 39.7% under 50 mg/L of PVC (1 μm) at 96 h exposure time, and the algal photosynthetic efficiency and Chla content decreased (Zhang et al., 2017). The current study observed that the growth inhibition effect of the three MPs followed the order: PS > PE > PVC, which demonstrated that the toxicity of MPs was related to their types. Although, a previous work reported that the toxicity of MPs was related to their chemical components, but the growth inhibition of *Skeletonema costatum* under the exposure of four types of MPs followed the order: PVC800 (1 μm) > PVC (74 μm) > PE (74 μm) > PS (74 μm) (Zhu et al., 2019). This is contrary to the results of the current study, which might be due to the use of different MPs size and algal type since the effect of MPs on algae is reported to vary with the characteristics of MPs, like size and shape (Sjollema et al., 2016). Additionally, it was also observed that under the exposure of PVC and PE MPs, there was no correlation between the algal growth inhibition rate and the concentration of MPs. However, when exposed for 72 h and 96 h, the growth inhibition of *M. aeruginosa* by PS MPs increased with increasing PS concentration.

As shown in Fig. S2, the specific growth rate (SGR) of *M. aeruginosa* under PVC, PS and PE MPs exposure illustrated that different types of MPs and different exposure times have different effects on the growth of *M. aeruginosa.* Firstly, $SGR_{(48–72)}$ of algae with PVC at 200 mg/L and $SGR_{(72–96)}$ of algae with PVC at 25 mg/L and 100 mg/L was significantly higher than that of control ($p < 0.05$), which revealed that PVC MPs might promote the growth of *M. aeruginosa* during the later period of exposure. A previous study has also reported increased growth of *Raphidocelis subcapitata* following exposure to PE MPs within 5 days (Canniff and Hoang, 2018). Secondly, $SGR_{(0–24)}$ of algae with PS < 50 mg/L was higher than that of control. While, $SGR_{(48–72)}$ and $SGR_{(72–96)}$ of the control groups were significantly higher than that of the PS MPs test groups. This illustrated that lower exposure concentrations of PS MPs promote the growth of *M. aeruginosa* during the initial period of exposure. Thereafter, the presence of PS MPs significantly inhibits the growth of *M. aeruginosa* ($p < 0.05$). This phenomenon was similar to that reported by a previous study (Zhu et al., 2019), in which the algae density was higher with triclosan at 0.1, 0.2, and 0.3 mg/L for 24 h exposure, as compared to control. This indicates that the low dose of PS MPs might also cause hormesis. However, the low-toxic stimulus disappeared after 48 h of PS MPs exposure which indicated that the phenomenon was temporary. This indicated that different types of MPs affect *M. aeruginosa* in different ways. The possible reason is that the three MPs have different densities, PVC > PS ≈ 1 g/cm^3 > PE, which causes them to contact the algae differently in the water, thus causing different effects on the growth of the algae.

3.2. Effect of different microplastics on oxidative stress

3.2.1. Antioxidant enzyme activities

The activities of GSSH, CAT, and SOD were measured to evaluate the effect of different concentrations of PVC, PS, and PE MPs on antioxidant enzymes of *M. aeruginosa* (Fig. 2). The antioxidant enzyme SOD is considered to be the first barrier in the intracellular antioxidant system and responsible for the elimination of superoxide radicals in cells (Lu et al., 2018). This enzyme can transform the radical superoxide into H_2O_2. The enzymatic activity of SOD was enhanced to 1.50–3.57-fold, 1.68–2.85-fold and 1.95–3.00-fold as compared to the control treatment, upon exposure to PE, PVC, and PS MPs, respectively, for 96 h ($p < 0.05$). The results were similar to a previously reported study which showed that the SOD activity of *Euglena gracilis* significantly increased following treatment with 0.1 μm and 5 μm PS MPs (Xiao et al., 2020). Increased SOD activity was observed in all MPs treatments at 96 h exposure, suggesting that ROS were produced excessively in the algae cells of the experimental group. Moreover, the increased SOD activity may result from the overproduction of superoxide, a core component of signal transduction, which activates existing enzyme pools, destroys critical cellular macromolecules, or increases the expression of genes encoding SOD (Chen et al., 2020). Catalase (CAT) is another important antioxidant enzyme that can significantly catalyze H_2O_2 generated in peroxisomes into H_2O, thus protecting algae from damage by excessive ROS (Hu et al., 2015). In the present study, the enzymatic activity of CAT was enhanced by 1.76–3.47-fold, 1.77–2.61-fold, and 1.47–2.59-fold compared to the control treatment, upon exposure to PE, PVC and PS MPs, respectively, for 96 h ($p < 0.05$). The enhancement of CAT activity is considered to be an adaptive characteristic of antioxidative stress damage (Fan et al., 2019), which suggests that MPs induce excessive production of ROS and cause damage to the oxidative system of algae (Zhang et al., 2019). Glutathione (GSH) is important intracellular non-protein thiol (Dong et al., 2020; Wang et al., 2020), which can be used as a substrate for glutathione peroxidase to scavenge oxygen free radicals or lipid peroxides in cells and convert it into fatty acids and water, while GSH is oxidized to GSSH. Content of GSSH serves as an indicator to assess the extent of pollutant stress in phytoplankton and to investigate the underlying toxic mechanisms. Compared with the control, the content of GSSH upon exposure to PE, PVC, and PS MPs for 96 h was increased by 1.04–1.63-fold, 1.21–1.33-fold, and 1.02–1.46-fold, respectively. The above results show that not only GSSH content but also SOD and CAT enzyme activities increased significantly with the increasing concentration of MPs. Besides, the activities of SOD and CAT peaked when exposed to 10 mg/L PE MPs, and the content of GSSH reached the maximum when exposed to 200 mg/L PE MPs. The effect on algal oxidative system when exposed to MPs followed the order: PE > PS > PVC. However, a previous work reported that different types of MPs have different effects on marine microalgae, and when exposed to 50 mg/L MPs, the effect on SOD activity followed the trend: PVC = PS > PE (Zhu et al., 2019). This illustrated that different types and different exposure concentrations of MPs had significant differences in the impact on algae.

3.2.2. Cell membrane damage

The influence of PVC, PS, and PE MPs on the cell membrane of *M. aeruginosa* was studied by measuring the MDA content and cell membrane integrity. MDA is indicative of lipid peroxidation, and is formed during fatty acid degradation. The higher the MDA content, the deeper the degree of lipid peroxidation of the cell membrane, which also indicates that the respective organism is subjected to greater environmental stress and damage (Ni et al., 2018). The results of the current study show that all three MPs increased the content of MDA in *M. aeruginosa* (Fig. S4). This indicated substantial damage to the antioxidant system of *M. aeruginosa,* which caused oxidative stress in the cyanobacterial cells. A similar phenomenon was also triggered by PS-NH_2 MPs (Zhang et al., 2018b). Furthermore, it can also find a

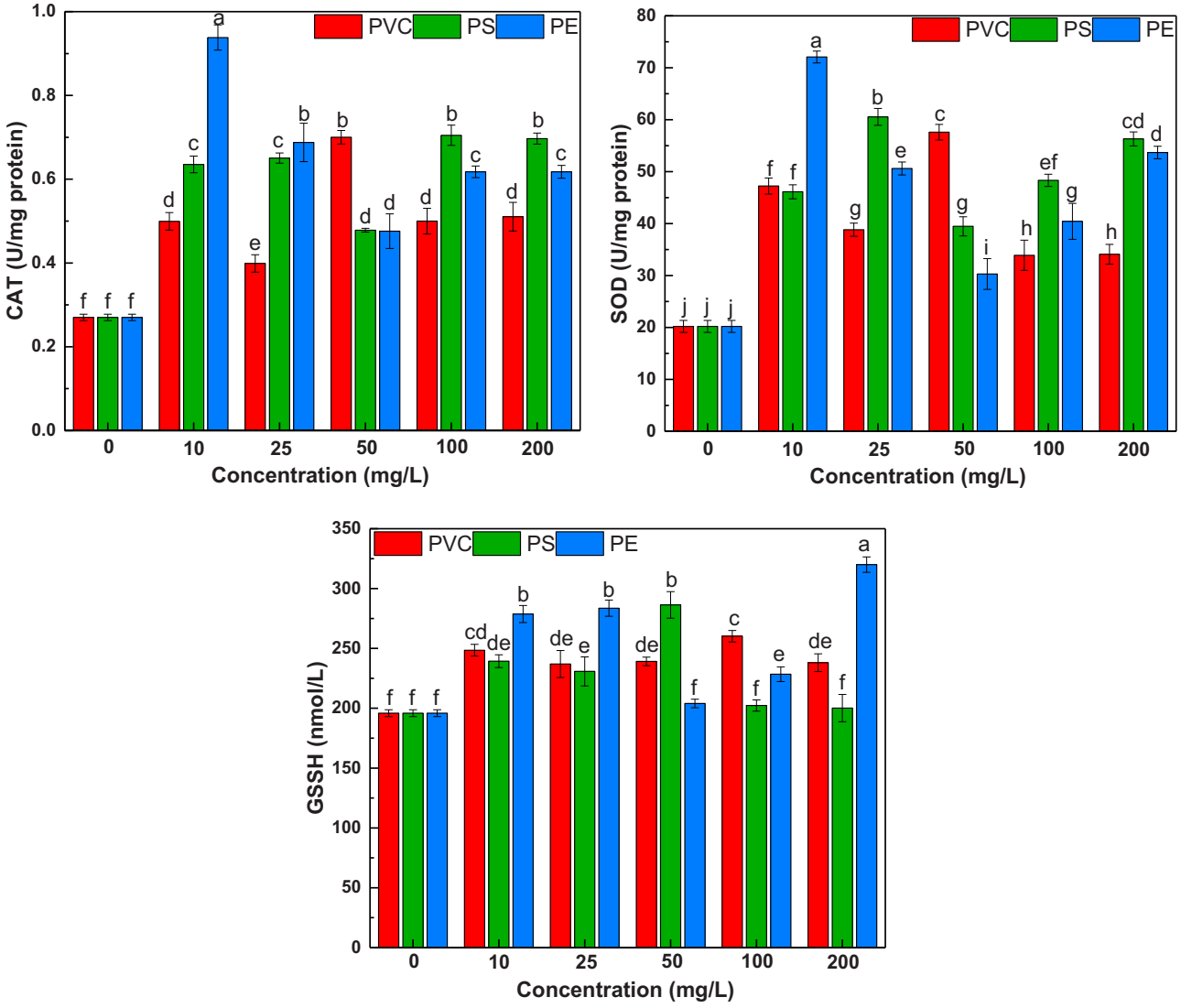

Fig. 2. Effect of PVC, PS and PE on the GSSH content, SOD and CAT activity of *M. aeruginosa.* Vertical bars represent mean ± SD, and different letters are significantly different at $p \leq 0.05$.

positive correlation between the PS concentrations and MDA content. This indicated that with higher PS MPs concentrations, the membrane injury increased. Thus, affecting the membrane fluidity and permeability, accompanied by the release of more intracellular material into the water (Liu et al., 2014). This can be further confirmed from another research which observed that the MDA content of *C. pyrenoidosa* showed a positive response to PS MPs exposure (Mao et al., 2018). However, the content of MDA did not increase with the concentration of PE and PVC MPs. In the present study, the effect on MDA content of *M. aeruginosa* when exposed to MPs followed the order: PS > PVC > PE. This illustrated that the influence of PVC and PE MPs on the algal cell membrane was weaker than that of PS MPs.

To further study the effect of PVC, PS, and PE MPs on cell membranes, the current research investigated cell membrane integrity (Fig. 3). The stain PI is unable to cross the membrane and intercalate with nucleic acids inside the cell if the membrane is intact. However, under damaged membrane conditions, PI can penetrate the cell membrane and stains intracellular nucleic acids, thereby producing a bright red fluorescence light indicating cell membrane depolarization. It was observed that with an increase in PS MPs dosage, the percentage of cells with disrupted cell membranes showed a significant increase from 0.85% in case of control to 2.78% when treated with 200 mg/L PS MPs. Previous results also documented that the membrane-damaged cells of *C. pyrenoidosa* increased in the presence of PS MPs (Mao et al., 2018). Another research observed the damage in microalgal cytomembrane with MPs treatment as compared to control, and the MPs were aggregated on the surface of the cells, which could limit the transfer of energy and nutrient, resulting in the restrained microalgae growth (Zhu et al., 2019). By comparison, in the current work, the exposure to PVC and PE MPs resulted in the maximum, cell membrane damage rate of 2.68% and 2.36% following 100 mg/L and 200 mg/L dosage, respectively. It illustrated that the effect on cell membrane integrity of *M. aeruginosa* under PVC, PS, and PE MPs exposure followed the order: PS > PVC > PE. On comparing these results with Fig. 2, it can be concluded that the inhibitory effect of PS MPs on *M. aeruginosa* might be caused by the combination of cell membrane damage and the effect on the antioxidant system. This was supported by a previous study which revealed that the mechanisms of the MPs toxicity to *C. pyrenoidosa* was attributed to the physical damage (cell membrane damage) and oxidative stress (Mao et al., 2018).

3.2.3. Comprehensive resistance

In order to compare the resistance of *M. aeruginosa* to PVC, PS, and PE MPs in this study, we evaluated the resistance using comprehensive

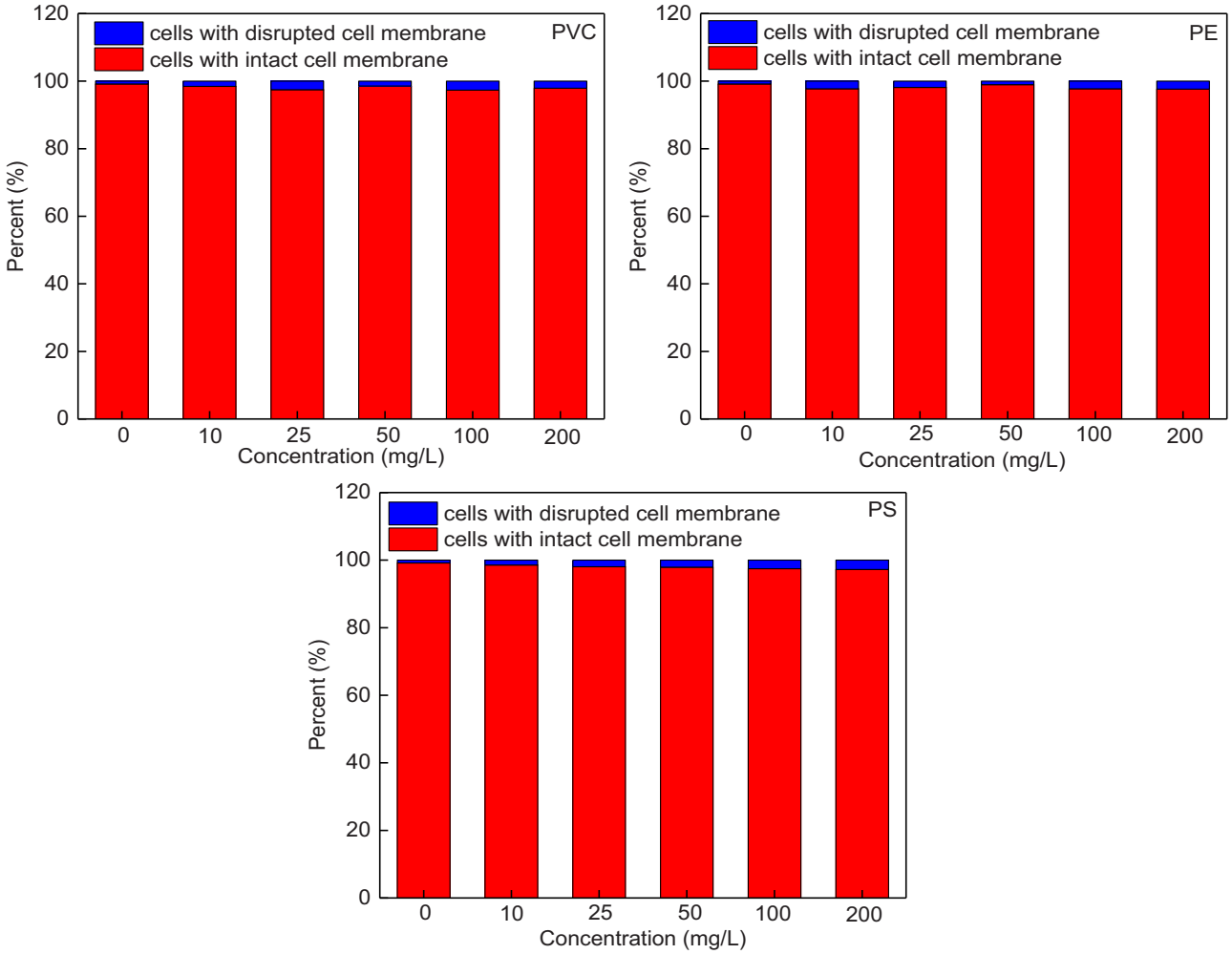

Fig. 3. Cell membrane integrity of M. aeruginosa after 96 h of exposure to different concentrations of PVC, PS, and PE MPs.

stress resistance indicators. The comprehensive stress resistance indicators were calculated via the membership function method of fuzzy mathematics. Among them, SOD reduces the intracellular damage caused by oxygen free radicals (Ni et al., 2018); CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen (Zhang et al., 2018a); MDA reveals the algal cell membrane lipid peroxidation and reflected the degree of algal cell damage indirectly (Tang et al., 2018); and CMI indicates the degree of cell membrane damage. Therefore, SOD, CAT, GSSH, FDA, Chla, and CMI are positively related to *M. aeruginosa* resistance, while MDA is negatively related to *M. aeruginosa* resistance. The results obtained using the experimental data from 96 h exposure to MPs are shown in Table 1. The results illustrated that the resistance of *M. aeruginosa* to three MPs followed the order: PE (3.701) > PS (3.607) > PVC (2.901). In conclusion, the impact of PVC MPs on *M. aeruginosa* was more severe than the other two MPs.

3.3. Effect of different microplastics on esterase activity

To determine whether the significant decrease recorded in the growth of *M. aeruginosa* after 96 h exposure to MPs was associated with the loss of esterase activity, an FDA-based method was assessed. FDA has been commonly used as a probe for cell viability. Esterase activity has been proved to relate well with the general metabolic status of the algal cell (Esperanza et al., 2020). In addition, enzyme inhibition measurements are popular indicators of environmental stress in algal cells. The activity of esterase in algal cells exposed to PVC, PE, and PS MPs was observed to decrease as compared to control cultures (Fig. 4). In addition, with an increase in PS MPs dosage, the esterase activity of algal cells showed a significant decrease from 254 (FDA-related fluorescence), in case of control, to 216 (FDA-related fluorescence) when treated with 200 mg/L PS MPs ($p < 0.05$). It has been previously reported that the inhibitory effect of the extracts of eucalyptus leaves on *M. aeruginosa* may be caused by the significant inhibition of the algal esterase activity (Zhao et al., 2019). Therefore, the above results can explain the *M. aeruginosa* growth inhibition rate, which reached a maximum value when exposed to 200 mg/L PS MPs for 96 h. Meanwhile, when the concentration of MPs was in the range of 0–50 mg/L, PVC MPs showed the maximum inhibitory effect on the algal cell esterase activity. Thus, in the present study, the inhibitory effect on esterase activity of *M. aeruginosa* when exposed to PVC, PS, and PE MPs followed the order: PS > PVC > PE.

3.4. Effect of different microplastics on intra- and extra-microcystins

To determine whether MPs can promote the production and release of MC-LR from *M. aeruginosa*, the content of intra- and extra-MC-LR in response to PVC, PE, and PS MPs exposure at different concentrations after 96 h exposure were measured (Fig. 5). After the addition of PVC, PE, and PS MPs (10, 25, 50, 100, and 200 mg/L), intra- and extra-MC-LR content were observed to be higher than those in the control group. In addition, when the concentration of PS MPs increased, the extra-MC-LR content also increased. From Fig. 3 and Fig. S4, it can be observed that PS MPs promote the release of more MC-LR by affecting cell membrane integrity and cell membrane lipid peroxidation. A previous study also

Table 1
Comprehensive stress resistance evaluation of *M. aeruginosa*.

Algae	Algae	Treatments	Treatments	Single index						Single index						Comprehensive index	Comprehensive index
				SOD	SOD	CAT	CAT	GSSH	FDA	FDA	Chla	CMI	CMI	MDA	MDA		
M. aeruginosa	*M. aeruginosa*	PVC	PVC	0.599	0.599	0.523	0.523	0.327	0.343	0.343	0.202	0.499	0.499	0.408	0.408	2.901	2.901
		PS	PS	0.486	0.486	0.540	0.540	0.367	0.460	0.460	0.549	0.613	0.613	0.592	0.592	3.607	3.607
		PE	PE	0.593	0.593	0.532	0.532	0.508	0.536	0.536	0.588	0.306	0.306	0.638	0.638	3.701	3.701

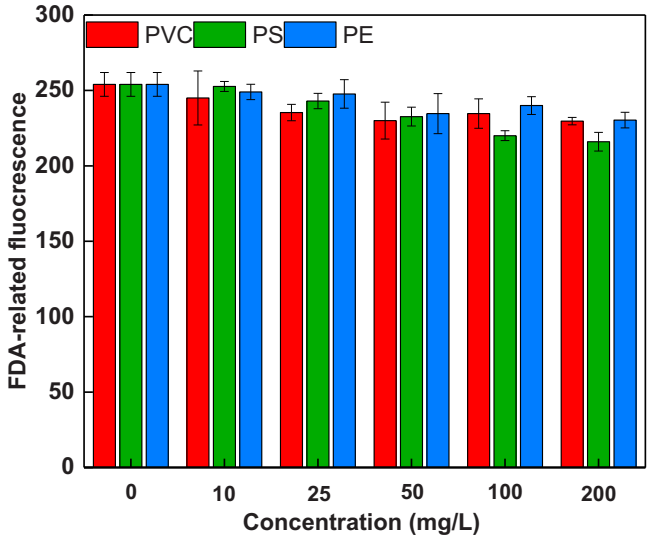

Fig. 4. Effect of PVC, PS, and PE on the esterase activity of *M. aeruginosa*. Vertical bars represent mean ± SD.

found that when the cell membrane becomes weak and damaged due to environmental stress, it promotes the release of MCs to the surrounding environment (Merel et al., 2013). Meanwhile, when the exposure concentration range was 0–100 mg/L, the intra-MC-LR content increased with the increase in PS MPs concentration. However, when the PS MPs concentration continued to increase to 200 mg/L, intra-MC-LR decreased significantly ($p < 0.05$). This observation could be attributed to the highest algal growth inhibition rate and the lowest esterase activity at this concentration.

However, different from the PS MPs group, the content of intra-MC-LR did not increase significantly with the increase in the exposure concentration of PVC and PE MPs. When the concentration of PVC MPs was 10 mg/L, the intra- and extra-MC-LR reached maximum concentrations of 1424.2 ng/L and 1003.0 ng/L, respectively, after 96 h exposure. In addition, the content of intra- and extra-MC-LR reached maximum values of up to 1353.6 ng/L and 918.1 ng/L, respectively, when the PE MPs concentration was 25 mg/L, with 96 h exposure. These observations might be attributed to the oxidative stress. The MCs from *M. aeruginosa* cells might play an important role against ROS when some enzyme activities are inhibited.

4. Conclusion

In this study, we investigated the effects of PVC, PS, and PE MPs on *M. aeruginosa* growth, as well as its MCs production and oxidative stress. The results showed that all the three MPs caused the growth inhibition of *M. aeruginosa* and promoted the production of MCs while causing *M. aeruginosa* to be in an oxidative stress state. Furthermore, the main mechanism of the effect of MPs on the growth inhibition of *M. aeruginosa* was the oxidation stress, and cell membrane disruption caused by MPs. Finally, the resistance of *M. aeruginosa* to three MPs indicated that the impact of PVC MPs on *M. aeruginosa* was more severe than the other two MPs. The finding of this work contributed to improve the understanding of the toxic effects of MPs on natural phytoplankton species which will be helpful for further evaluation of the real ecological risks of MPs in freshwater environment.

CRediT authorship contribution statement

Xiaowei Zheng: Methodology, Software, Investigation, Writing - original draft. **Weizhen Zhang**: Investigation, Formal analysis, Visualization, Software. **Yuan Yuan**: Formal analysis, Visualization, Data

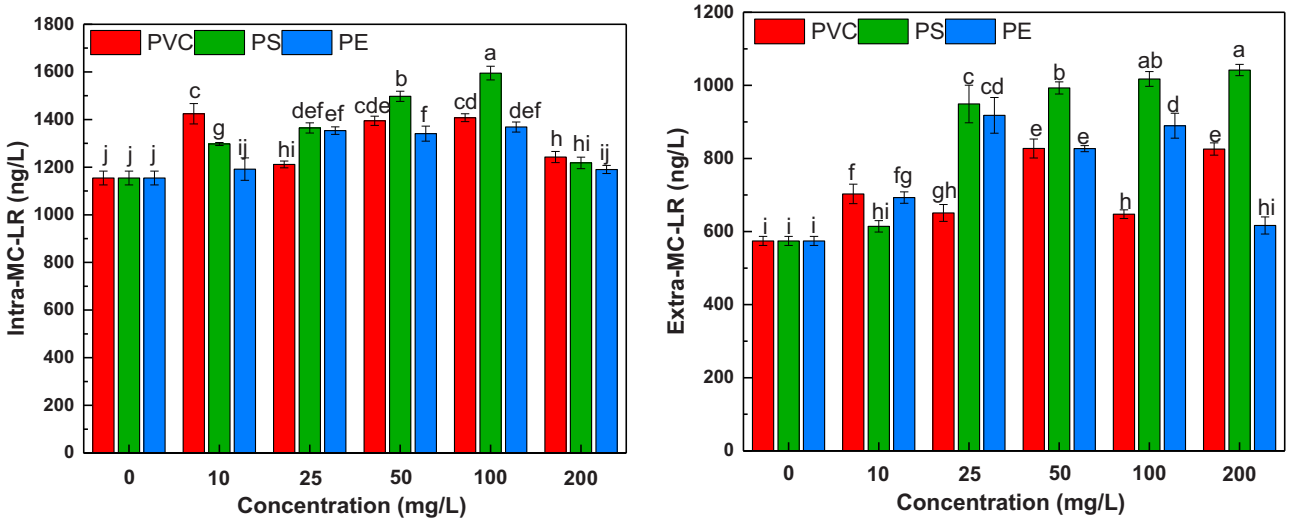

Fig. 5. Effect of PVC, PS and PE on the intra-MC-LR content and extra-MC-LR content of *M. aeruginosa* at 96 h exposure. Vertical bars represent mean ± SD, and different letters are significantly different at $p \leq 0.05$.

curation. **Yanyao Li**: Writing - review & editing, Data curation. **Xianglin Liu**: Software, Writing - review & editing. **Xiangrong Wang**: Conceptualization, Supervision, Writing - review & editing. **Zhengqiu Fan:** Conceptualization, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of interest statement

We declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111575.

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