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RESEARCH ARTICLE

Effects of microplastics and cadmium on growth rate, photosynthetic pigment content and antioxidant enzymes of duckweed (*Lemma minor*)

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Abstract

Cadmium (Cd) and polyethylene (PE) seriously contaminate the aquatic environment and threaten human health. Many studies have reported the toxic effects of Cd and PE on plants, whereas few have reported the combined contamination of these two pollutants. In this study, duckweed (*Lemma minor*) was used as an indicator to explore the effect of PE microplastics (PE-MPs) at concentrations of 10, 50, 100, 200, and 500 mg/L on tolerance to 1 mg/L Cd. The results showed that different concentrations of PE-MPs inhibited the growth rate and chlorophyll content of duckweed to different degrees, both of which were minimal at 50 mg/L PE-MPs, 0.11 g/d, and 0.32 mg/g, respectively. The highest Cd enrichment (7.77 mg/kg) and bioaccumulation factors (94.22) of duckweed were detected when Cd was co-exposed with 50 mg/L of PE-MPs. Catalase and peroxidase activity first decreased and then increased with increasing PE-MPs concentrations, showing "hormesis effects", with minimum values of 11.47 U/g and 196.00 U/g, respectively. With increasing concentrations of PE-MPs, the effect on superoxide dismutase activity increased and then declined, peaking at 162.05 U/g, and displaying an "inverted V" trend. The amount of malondialdehyde rose with different PE-MPs concentrations. This research lay a foundation for using duckweed to purify water contaminated with MPs and heavy metals.

Keywords Polyethylene · Duckweed · Heavy metal · Antioxidase · Malondialdehyde · Cadmium

Introduction

Heavy metal and plastic pollution in terrestrial and aquatic environments have been widely reported, particularly concerning the potential risks associated with microscopic particles (Andrady 2011; Carpenter et al. 1972; Jambeck et al. 2015). Microplastics (MPs) are any synthetic solid particles or polymeric matrices with regular or irregular shapes and sizes ranging from 1 μm to 5 mm (Frias and Nash 2019). Polyethylene (PE), polypropylene, polystyrene (PS), polyvinyl chloride, polyamide, and polyoxymethylene are examples of MPs in general (Erni-Cassola et al. 2017; Khalid et al. 2020). Microscopic particle size, large specific surface area, complex surface functional groups, and high adsorption capability are the characteristics of MPs (Li et al. 2019). MPs can be absorbed and accumulated in the plant and may induce physiological, biochemical, and genetic toxic effects and then transfer through the food chain, eventually leading to human exposure, and presenting risks to the ecological environment (Jiang et al. 2019; Prata et al. 2020). For example, Li et al. (2020) found that PS could be enriched in the roots of wheat via a crack-entry mode and migrated to the aboveground parts, accumulated and distributed in the stems and leaves. Indeed, the uptake of MPs by different portions of the plant may be related to the surface charge of the plastic particles (Sun et al. 2020). Furthermore, transpiration pull is the dominant factor in the plant uptake and translocation of plastic particles (Azeem et al. 2021). MPs are pollutants, but they may also serve as carriers of contaminants, posing potential threats to aquatic organisms and human health (Liu et al. 2022; Yu et al. 2019). PE is one

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of the most commonly used plastics. PE-MPs account for approximately 90% of MP pollution in water environments (Ter Halle et al. 2016). Cadmium (Cd) is a very poisonous heavy metal that can cause significant illnesses when it enters the human body through the food chain and drinking water (Baudrimont et al. 2020; Elliott et al. 2017; Tchounwou et al. 2001). In addition to osteoporosis, emphysema, olfactory dysfunction loss, and abnormalities, research has linked Cd exposure to cancer (Davison et al. 1988; Mascagni et al. 2003; Schutte et al. 2008; Tchounwou et al. 2001).

The combined pollution of MPs and heavy metals is an emerging global environmental problem, and this co-occurrence poses a threat to the health of organisms (Khalid et al. 2021). Since MPs can act as carriers of heavy metals, they facilitate heavy metals' entry into the food chain and continue to accumulate toxicity through biomagnification (Bradney et al. 2019). The combined effects of MPs and heavy metals endanger organism health by interfering with aquatic species' normal physiological processes, causing a variety of adverse side effects, and impacting plant growth and development (Pinto-Poblete et al. 2022). The combined effect of MPs and Cd, for example, increases plant biomass, influences enzyme activity, and improves Cd bioavailability (Pinto-Poblete et al. 2022). MPs with adsorbed silver can decrease the root length and root cell viability of duckweed (Kalčíková et al. 2020). Wheat roots' ability to grow is impacted by polyethylene terephthalate (PET), which absorbs the heavy metals Pb, Cd, and Zn (Abbasi et al. 2020). The combination of MPs and heavy metals also affects the intestinal microbial community and intestinal cells of organisms, thereby affecting growth and development (Domenech et al. 2021; Li et al. 2021a). Through the consumption of foods like plants (vegetables and fruits) and specific marine species (fish and crustaceans), MPs are ingested by the human body (Pinto-Poblete et al. 2022; Smith et al. 2018). Once inside the body, the heavy metals the MPs have absorbed have cytotoxic effects on the intestine, brain, and epithelial cells (Hodson et al. 2017). Therefore, the remediation of MPs and Cd pollution in aquatic environments is essential.

As a low-cost, non-invasive complementary technology, plant-microbial combined remediation technology, which uses green plants and related microorganisms for environmental remediation, has become an important means of environmental remediation (Pilon-Smits 2005). The Lemnaceae family includes floating plants like duckweed (*Lemna minor*). One of the best plants for phytoremediation, it can bioaccumulate heavy metals as a hyperaccumulator. (Ekperusi et al. 2019; Zheng et al. 2022). Duckweed usually grows asexually and produces cotyledons asexually from the mother leaf. Its reproduction rate increases exponentially, and its biomass doubles in a short growth period of 1–2 d (Baek et al. 2021). Duckweed grows at temperatures of 10–40 °C and pH of 5.0–9.0, and it has strong adaptability to the natural environment (Feng et al. 2022; Stomp 2005). *L. minor* is an excellent material for environmental remediation research because of its rapid growth rate, high pollutant accumulation capacity, and adaptability.

L. minor was therefore utilized as the research subject in this work to examine the effects of various PE-MP concentrations on Cd stress in duckweed and to further study how PE-MPs affect the biomass and physiological and biochemical reactions of Cd in duckweed. We aimed to determine the concentration range at which PE-MPs promote the toxic effects of Cd on duckweed (synergistic effect), and the concentration range at which PE-MPs inhibit the toxic effects of Cd on duckweed (antagonistic effect). Knowing the right amount of PE-MPs to apply to stop the harmful effects of Cd on duckweed can help further enhance the repair effect when duckweed is used to treat water that is high in the heavy metal Cd. The results of this study will give the remediation of heavy metal-contaminated water their theoretical underpinnings.

Materials and methods

Materials and culture conditions

Duckweed used in this study was collected from Beijing, China (E116°42′47", N40°10′92"). After sterilization and sterile storage in the duckweed germplasm repository of the College of Life Sciences, Guizhou University. Before the experimental treatment, duckweed strains preserved in the germplasm bank were pre-cultured with a Hoagland culture solution (containing 1.5% sucrose) (Hoagland and Davis 1923). The culture conditions were as follows: temperature, 25 °C; photoperiod, 16 h/8 h; humidity, 75%; light intensity, 5,000 lx; and culture for approximately one week.

Experimental design

A $CdCl_2$ standard solution (Beijing Northern Weiye Metrology Technology Research Institute, Beijing, China) was added to a plastic box containing 250 mL of 1/5 Hoagland's solution to achieve a final Cd concentration of 1 mg/L. Subsequently, different concentrations of PE-MPs (Alfa Aesar, Ward Hill, MA, USA) were added to plastic boxes of 1/5 Hoagland's solution with a Cd concentration of 1 mg/L. The experiments were divided into the following six groups: control (0), 10 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, and 500 mg/L (Table 1). Duckweed samples, with a fresh weight of 5 g, were transplanted into 400 mL plastic boxes with different treatments and cultured for 7 d under the following conditions: temperature, 25 °C; light cycle, 16 h/8 h; and light intensity, 5,000 lx. After 7 d of treatment, the duckweed materials were collected for the subsequent measurement of

Table 1 Treatment conditions of duckweed

Group	0	10	50	100	200	500
Cd (mg/L)	1	1	1	1	1	1
PE-MPs (mg/L)	0	10	50	100	200	500

various indicators, data processing, and experimental analyses. No PE-MPs treatment was used as the blank control, and three replicates were used for each treatment group.

Determination of growth rate

After 7 d of culture, all of the treated duckweed samples were collected, and gently washed with flowing tap water and ultra-pure water, and the excess water on the surface was removed using filter paper. The fresh weight of the duckweed samples after each treatment was measured and recorded. The samples were then placed in an oven at 60 °C to dry overnight until the weight was constant. They were then ground into a powder with a mortar and placed in a dryer for testing. After the duckweed samples were treated for 7 d, the fresh weight was measured, and the growth rate (GR) at 7 d was calculated according to formula (1).

$$\mathrm{GR} = \frac{\mathrm{W}_7 - \mathrm{W}_0}{\mathrm{T}} \tag{1}$$

where *GR* is the growth rate in g/d, *T* is the treatment period (d), W_0 is the fresh weight on the first day (g), and W_7 is the fresh weight on day 7 (g).

Determination of chlorophyll content

After blotting the surface moisture of the duckweed samples with filter paper, they were weighed (0.5 g) in a 15 mL centrifuge tube and placed at -20 °C for 1 h. Ten milliliters of 95% ethanol preheated to 50 °C was then added and the samples were fully shaken. After standing in a dark cabinet for 3 h, the supernatant was collected from the samples. The amount of chlorophyll pigment in the supernatant was determined by measuring the absorbance at 663 and 645 nm using a full-wavelength microplate reader (Multiskan FC; Thermo Scientific, Shanghai, China). The chlorophyll content was calculated according to formulae (2) and (3).

$$C_a = 12.7A_{633} - 2.69A_{645} \tag{2}$$

$$C_a = 22.9A_{645} - 4.68A_{663} \tag{3}$$

In these formulae, C_a and C_b are the concentrations of chlorophyll a and b (mg/L), respectively and A_{663} and A_{645} are the absorbances of the chlorophyll solution at 663 nm and 645 nm, respectively. The concentration of chlorophyll in the extract was converted to chlorophyll content per gram of fresh leaves (mg/g).

Determination of Cd-related indices

Duckweed samples (0.1 g) were accurately weighed into a digestion tube, 2 mL of concentrated nitric acid was added, and the samples were left overnight. Added 4 ml of concentrated nitric acid, and the samples were digested at 180°C for 4 h and then overnight. Then, 2 mL of concentrated hydrochloric acid was added to drive the acid and samples were incubated at 280 °C for approximately 30 min. A blank control was prepared for each sample to control for errors caused by digestion. The digested samples were then filtered and placed in a centrifuge tube. Culture media (50 mL) from each treatment group were centrifuged at 3,500 xg for 10 min, and the supernatant was stored at 4 °C until testing. Cd content was measured using a flame atomic spectrophotometer (NovaAA 400P; Analytik Jena AG, Jena, Germany).

The rate of Cd removal from duckweed was calculated according to formula (4) and the Cd content of duckweed was calculated using formula (5).

$$\mathrm{R} = \left(\frac{\mathrm{C}_0 - \mathrm{C}_t}{\mathrm{C}_0}\right) \times 100\% \tag{4}$$

$$\mathrm{W} = \left(\mathrm{C}_1 - \mathrm{C}_2\right)\mathrm{V}/\mathrm{m} \tag{5}$$

In formula (4), R is the Cd removal rate, C_0 is the initial Cd concentration (mg/L), and C_t is the residual Cd concentration after treatment (mg/L). In formula (5), W is the Cd content per unit weight (mg/kg), C_1 is the Cd content of the sample digestion solution (mg/L), C_2 is the Cd content of the blank digestion solution (mg/L), V is the total volume of the sample digestion solution (mL), and m is the total amount of dry powder consumed during digestion (g).

The bioconcentration factor (BCF) refers to the concentration ratio of heavy metals in plant tissues and water environments, and it is commonly used to evaluate the accumulation of heavy metals in organisms (Chen et al. 2018). The BCF was calculated according to formula (6), where R_{BCF} denotes the bioconcentration factor, C_p is the Cd concentration in the plant tissue (mg/kg), and C_h is the final concentration of Cd in the culture solution (mg/L).

$$\mathrm{R_{BCF}} = \mathrm{C_p}/\mathrm{C_h} \tag{6}$$

Determination of antioxidative enzyme activity and malondialdehyde content

Duckweed samples (0.1 g) were frozen in liquid nitrogen to prevent enzyme inactivation, and 1 mL of extract underwent ice bath homogenization. The mixture was centrifuged at 8,000 xg and 4 °C for 10 min, and the supernatant was extracted and placed on ice for testing. Superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), and catalase (CAT, EC 1.11.1.6) activities were measured in 1 mL of supernatant using specific kits (Solarbio, Beijing, China).

Malondialdehyde (MDA) content was determined using the thiobarbituric acid method (Heath and Packer 1968). Four milliliters of 10% trichloroacetic acid were added to 0.5 g of duckweed, and the samples were ground in an ice bath. After centrifuging the samples at 12,000 xg for 10 min at 4 °C, the supernatant was diluted to 5 mL. The supernatant (2 mL) was mixed with 2 mL of distilled water, after which, 2 mL of 0.6% thiobarbituric acid was added and the samples were shaken thoroughly. After rapidly cooling the samples at 4 °C, placing them in a boiling water bath for 30 min, and centrifuging them at 3,000 xg for 10 min, the supernatant was collected. The absorbance of the supernatant was measured at 440 nm, 532 nm, and 600 nm.

Data analysis

SPSS 22.0 (IBM, Armonk, NY, USA) and Prism 6.02 (GraphPad, San Diego, CA, USA) were used for data processing and determining significant differences between the experimental group and the control group ($P < 0.05$).

Results and discussion

Effects of PE-MPs on the growth rate of duckweed under Cd stress

The growth rate of duckweed was reduced in the experimental group compared with the control group, indicating that the addition of PE-MPs inhibited the growth rate of duckweed to a certain extent (Fig. 1). The growth rate of duckweed was the lowest (only 0.11 g/d) at a PE-MPs concentration of 50 mg/L, indicating that its growth was markedly inhibited at this concentration. This agrees with the literature, which has shown an effect on plant biomass decreased in the treatments with Cd + PE-MPs added (Pinto-Poblete et al. 2022). This may be because PE-MPs could serve as vectors of heavy metals through sorption and desorption, resulting in a decreasing adsorption capacity of metals and an increasing desorption capacity of metals, which means higher bioavailability of metals increases the effect of Cd on the growth rate of duckweed (Hodson et al. 2017; Li et al. 2021b). Duckweed grew at 0.21, 0.25, 0.28, and 0.26 g/d when the concentration of PE-MPs was 10, 100, 200, and 500 mg/L, respectively. These results showed that higher PE-MPs concentrations (PE-MPs concentrations > 50 mg/L) did not affect the growth rate of duckweed. When the PE-MPs concentrations were 100, 200, and 500 mg/L, the growth rates of duckweed were not different from the growth rate of the control group (Fig. 1). Under the combined stress of a certain concentration of PE-MPs and Cd, the growth rate of duckweed was inhibited to a certain extent, which was consistent with the results of Wang et al. (2021a, b)

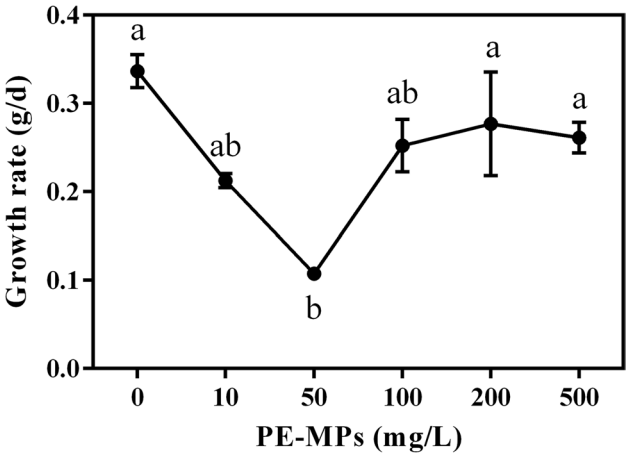

Fig. 1 Growth rate of duckweed after 7 d of different treatments

PE-MPs, polyethylene microplastics. Different letters indicate significant differences ($P < 0.05$) within each PE-MPs treatment level or between PE-MPs levels. The standard error of the mean ($n = 3$) is indicated by the vertical bars.

Effects of PE-MPs on chlorophyll content and apparent morphology in duckweed under Cd stress

The effects of PE-MPs and Cd co-stress on the apparent morphology of duckweed were evaluated. Compared with the 0 mg/L concentration of the control group, the 10, 50, 100, 200, and 500 mg/L treatment concentrations showed increased yellowing areas of duckweed fronds. These results showed that the addition of different concentrations of PE-MPs significantly increased the degree of damage to duckweed (Table 2).

Studies have shown that Cd strongly inhibits the synthesis of chlorophyll and directly affects photosystem II (FII) (Feng et al. 2018). The results of this study are shown in Table 2. The chlorophyll content was lower in the experimental group than in the control group. The chlorophyll content of the duckweed samples in the control group was 45.75 mg/L. When the PE-MPs concentration was 200 mg/L, the chlorophyll content was the lowest in the experimental group, at only 13.75 mg/L. When the PE-MPs concentrations were

Table 2 Chlorophyll content of duckweed treated with different concentrations of PE-MPs

PE-MPs[d] concentration	0 mg/L	10 mg/L	50 mg/L	100 mg/L	200 mg/L	500 mg/L
Apparent morphology						
Chl a[a]	0.3236a	0.1382b	0.1134b	0.1463b	0.0972b	0.1060b
Chl b[b]	0.5915a	0.2533b	0.2073b	0.2671b	0.1779b	0.1939b
Chl[c]	0.9151a	0.3915b	0.3206b	0.4134b	0.2751b	0.2999b

[a]chlorophyll a content of duckweed after 7 d of treatment; [b]chlorophyll b content of duckweed after 7 d of treatment; [c]Chl the chlorophyll content of duckweed after 7 d of treatment; [d]polyethylene microplastics. Different letters indicate significant differences ($P < 0.05$) within each PE-MPs treatment level or between PE-MPs levels

10, 50, 100, and 500 mg/L, the chlorophyll contents were 19.58, 16.03, 20.67, and 14.99 mg/L, respectively. The addition of PE-MPs increased the toxicity of Cd to duckweed and significantly inhibited chlorophyll synthesis. These results demonstrated that different concentrations of PE-MPs inhibited the chlorophyll synthesis of duckweed. These results are consistent with those shown in Fig. 1. Previous studies have shown that PE-MPs induced stress reduces the photosynthetic pigment content of aquatic plants, which may be because PE-MPs affect the enzymatic activity of chlorophyll-synthesis-related enzymes, thus affecting chlorophyll synthesis and reducing the chlorophyll content in duckweed (Tunali et al. 2020; Zhang et al. 2021). Our results confirmed these findings. Under the combined stress of PE-MPs and Cd, the chlorophyll content of duckweed also showed a sharp decrease.

Effects of PE-MPs on Cd enrichment and bioconcentration factors in duckweed under Cd stress

Cd enrichment increased after the addition of different concentrations of PE-MPs. When the concentration of PE-MPs was 10, 50, 100, and 200 mg/L, the Cd content was 5.87, 5.83, 3.77, and 5.16 mg/L, respectively. When the PE-MPs concentration was 500 mg/L, the Cd content was the highest, reaching 7.77 mg/kg. The results showed that the presence of PE-MPs increased the bioavailability of Cd and the accumulation of Cd in plants to some extent. This result is consistent with Wang et al. (2021), who reported that MPs significantly enhanced the Cd bioaccumulation in *Lactuca sativa* L. It has also been reported that MPs improve the bioavailability of Cd, and the Cd bioaccumulation in lettuce significantly increased as the MP levels grew (Huang et al. 2021; Wang et al. 2021).

The BCF is an important indicator used to measure the ability of plants to enrich pollutants (Fig. 2a). After the addition of different concentrations of PE-MPs, the BCF values increased, reaching their highest value (94.22) at a PE-MPs concentration of 100 mg/L. These results showed that the addition of PE-MPs significantly improved the Cd enrichment ability of duckweed. Thus, during the restoration of Cd-contaminated water, PE-MPs may be appropriately added to improve the Cd enrichment ability of duckweed (Fig. 2b).

Effects of PE-MPs on the Cd removal rate in duckweed under Cd stress

The Cd removal rate improved after treatment with the different concentrations of PE-MPs (Fig. 3). The Cd removal rate of duckweed was the highest (95.66%) at a PE-MPs concentration of 100 mg/L. This was a significant increase of 11.67% compared to the control group. When the concentration of PE-MPs was greater than 100 mg/L, the removal rate of Cd decreased with increasing concentration, to 82% and 83.33%. These results showed that the addition of a certain concentration of PE-MPs improved the Cd removal rate of duckweed. The PE-MPs may have adsorbed some of the Cd in the water, which significantly improved the Cd removal rate of duckweed (Abbasi et al. 2020).

Effects of PE-MPs on the activity of antioxidative enzymes in duckweed under Cd stress

One of the negative effects of Cd accumulation in plants is the increased generation of reactive oxygen species (ROS), which affects the growth and development of

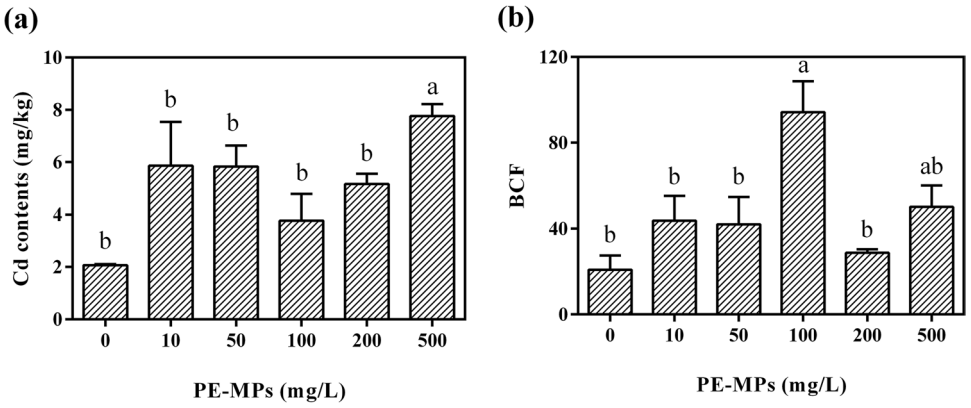

Fig. 2 Cd content and BCF values of duckweed treated with different concentrations of PE-MPs. **a** Cadmium (Cd) content after treatment with different PE-MPs concentrations; **b** Bioconcentration factor (BCF) after treatment with different polyethylene microplastic (PE-MPs) concentrations. Different letters indicate significant differences ($P < 0.05$) within each PE-MPs treatment level or between PE-MPs levels. The standard error of the means ($n = 3$) is indicated by vertical bars

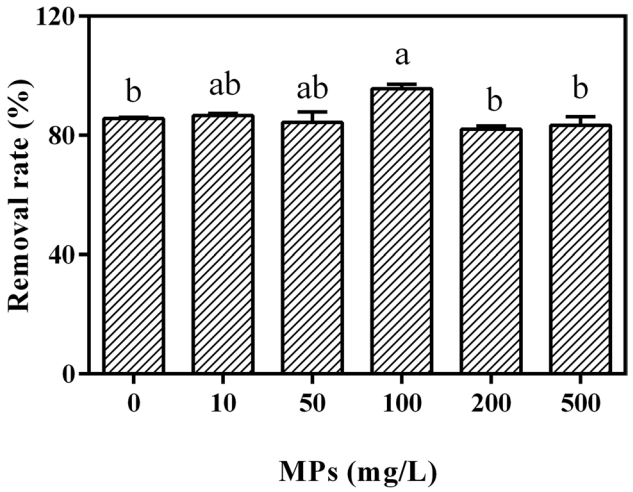

Fig. 3 Cadmium removal rate of duckweed treated with different concentrations of PE-MPs. PE-MPs, polyethylene microplastics. Different letters indicate significant differences ($P < 0.05$) within each PE-MPs treatment level or between PE-MPs levels. The standard error of the mean ($n = 3$) is indicated by the vertical bars

plants (Nahakpam and Shah 2011; Pallavi et al. 2012). CAT, POD, and SOD are important enzymes for various green plants to adapt to the environment and resist stress. They are key enzymes in scavenging ROS and are therefore known as antioxidant enzymes (Shah et al. 2001; Zornoza et al. 2010). PE-MPs and heavy metals greatly influence the antioxidant enzymes of aquatic plants (Wang et al. 2022; Wu et al. 2020).

Hydrogen peroxide is produced by plants during stress and senescence. Excessive production may lead to various types of oxidative damage, including the inactivation of enzymes and proteins, damage to DNA molecules, and the inhibition of growth and development (Nahakpam and Shah 2011; Pallavi et al. 2012). CAT further promotes the decomposition of hydrogen peroxide in plants, thereby reducing its toxic effects on plant cells. CAT activity is closely related to the stress resistance of plant growth (Wu et al. 2018). We detected significant differences in CAT activity in duckweed among the groups treated with the five PE-MPs concentrations (Fig. 4a). CAT activity was highest, reaching 45.87 U/g, when the PE-MPs concentration was 500 mg/L, and was lowest, at 11.47 U/g, when the PE-MPs concentration was 100 mg/L. These results showed that the addition of PE-MPs had a greater effect on CAT activity in duckweed. As the PE-MPs concentration increased, CAT activity showed a trend of first decreasing and then increasing. This finding was similar to the findings from related studies of the aquatic plant, wheat (Liao et al. 2019). These results showed that CAT activity increased and the toxicity of PR-MPs decreased in duckweed under stress caused by high concentrations of PE-MPs.

SOD is an important enzyme related to respiration in plants, and its activity is closely related to phenol metabolism and plant resistance (Grace 1990). As shown in Fig. 4b, under the combined stress of Cd and PE-MPs, the SOD activity of duckweed was inhibited to different degrees compared with its activity in the control group (124.13 U/g). When the concentration of PE-MPs was 100 mg/L, SOD activity increased to 162.05 U/g. When the concentrations of PE-MPs were 10 and 500 mg/L, SOD activity was significantly lower than its activity in the control group, at only 42.38 and 44.23 U/g, respectively. When the concentrations of PE-MPs were 50 and 200 mg/L, the SOD activities were 59.73 and 72.07 U/g, respectively. These results showed that PE-MPs had an inhibitory effect on SOD activity in duckweed. Under the combined stress of Cd and PE-MPs, SOD activity decreased, and the damage to the duckweed plants increased.

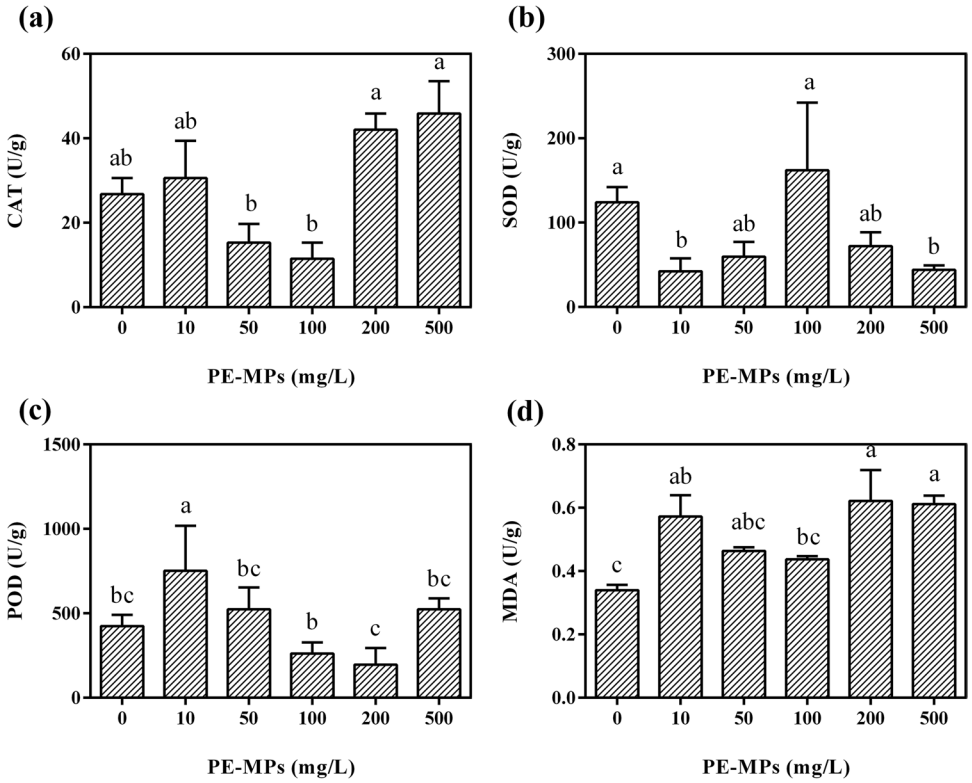

Fig. 4 Antioxidative enzyme activity of duckweed after 7 d of treatment. **a** Catalase (CAT) activity of duckweed after 7 d of treatment; **b** superoxide dismutase (SOD) activity of duckweed treated with different concentrations of polyethylene microplastics (PE-MPs); **c** peroxidase (POD) activity of duckweed treated with different concentrations of PE-MPs; **d** malondialdehyde (MDA) content of duckweed treated with different concentrations of PE-MPs. Different letters indicate significant differences ($P < 0.05$) within the PE-MPs treatment level or between PE-MPs levels. The standard error of the mean ($n = 3$) is indicated by the vertical bars

POD is a type of oxidoreductase produced by plants. It can effectively remove excess free radicals caused by damage, and it also eliminates the toxicity of hydrogen peroxide, phenols, amines, aldehydes, and benzene (Foyer et al. 2010). As seen in Fig. 4c, compared with the control group (424.67 U/g), the highest POD activity (881.00 U/g) was observed when the PE-MPs concentration was 10 mg/L. This indicated that the degree of damage to duckweed was lowest under the combined stress of Cd and 10 mg/L PE-MPs. POD activity reached 196.00 U/g when the PE-MPs concentration increased to 200 mg/L. These results showed that low concentrations of PE-MPs promoted POD activity in duckweed. When the PE-MPs concentration was 200 mg/L, the POD activity of duckweed decreased significantly, and the toxicity of PE-MPs to duckweed was at its highest. POD activity first decreased and then increased with increasing PE-MPs concentrations.

In organisms, free radicals act on lipids to produce peroxidation reactions, and the final product of these oxidation reactions is MDA, which causes the cross-linking polymerization of proteins, nucleic acids, and other macromolecules, and has strong cytotoxicity (Miller et al. 2010). A higher MDA content in plants indicates a greater degree of lipid peroxidation and more serious damage to the plant cell membrane. Therefore, MDA is a commonly used indicator of the degree of oxidative stress. Compared with the control group, the addition of PE-MPs induced lipid peroxidation and promoted the synthesis of MDA in duckweed, and increased the MDA content in duckweed cells. The MDA content was highest, reaching 0.62 μmol/g, when the PE-MPs concentration was 200 mg/L. When the PE-MPs concentration was 500 mg/L, the MDA content was 0.61 μmol/g, which was second only to the content at a PE-MPs concentration of 200 mg/L. When the concentrations of PE-MPs were 10, 50, and 100 mg/L, the content of MDA in duckweed was 0.57, 0.46, and 0.44 μmol/g, respectively (Fig. 4d). This may be due to the MPs adhering to the biomass of *Lemna minor* at low concentrations, which reduces the damage of MPs to duckweed (Rozman et al. 2022). These results showed that, compared with the control group, the combined stress of Cd and PE-MPs promoted the synthesis of MDA in duckweed to different degrees. A higher MDA content indicates a greater degree of damage to the cell membranes of duckweed (Miller et al. 2010). The addition of PE-MPs damaged duckweed to varying degrees. When the concentration of PE-MPs was higher, the MDA content of duckweed was also higher, indicating that the damaging effect was stronger.

Conclusions

In this study, under the combined stress of different concentrations of PE-MPs and Cd, the growth and chlorophyll synthesis of duckweed were inhibited to a certain extent. With the addition of PE-MPs, the Cd removal rate, Cd enrichment,

and Cd bioconcentration factor of duckweed significantly increased. When the concentration of PE-MPs was low (10 mg/L), the activity of antioxidant enzymes in duckweed increased, and as the concentration of PE-MPs increased, the activity of antioxidant enzymes was inhibited to a certain extent. PE-MPs may induce lipid peroxidation, as indicated by the strong promotion of MDA synthesis in duckweed. In general, PE-MPs aggravated the stress of the heavy metal, Cd, on duckweed to a certain extent, resulting in greater damage to the duckweed plants under the combined stress of the two pollutants. These results provide new ideas and directions for future studies on the effects of the combined stress of PE-MPs and the heavy metal Cd on aquatic plants and studies of water pollution control.

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Author Contributions **Xiao Yang:** Investigation, Methodology, Writing-original draft preparation. **Hai-Min Liao:** Investigation, Methodology. **Ai-Juan Tan:** Supervision. **Sheng-Xian Gan:** Investigation, Methodology, Writing-original draft preparation. **Gui-Li Yang:** Supervision, Methodology, Writing-reviewing & editing.

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Data availability The data are available by the corresponding author upon request.

Declarations

Ethical approval No prior ethical approval was necessary for the study.

Consent to publish Not applicable.

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