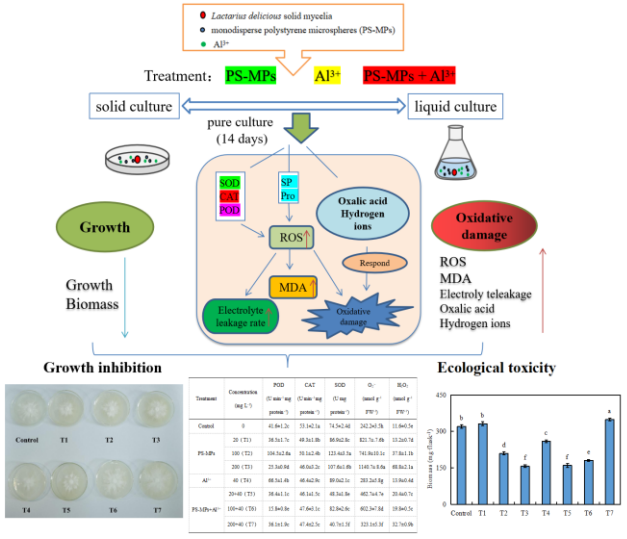
Global NEST Journal, Vol 25, No 8, pp 156-166

Ecological toxicity of microplastics, aluminum and their combination to ectomycorrhizal fungi (*Lactarius delicious*)

Jia Liu†, Zhengming Cheng†, Yuting Xie, Yunling Zhang, Wenwen Jiang, Zhiwen Jiang, Liang Zhang*
School of Chemistry and Bioengineering, Taizhou College, Nanjing Normal University, Taizhou 225300, China
†The authors contributed equally to this article.
Received: 02/06/2023, Accepted: 22/08/2023, Available online: 06/09/2023
*to whom all correspondence should be addressed: e-mail: liangzai0061@126.com
https://doi.org/10.30955/gnj.005172

Graphical abstract



Abstract

To investigate the combined toxic effects of microplastics and aluminum (Al) on ectomycorrhizal fungi, *Lactobacillus delicious* (Ld) was selected as the experimental subject to conduct research on monodisperse polystyrene microplastics (PS-MPs) (0, 20, 100, 200 mg L^{-1}) and Al^{3+} (40 mg L^{-1}) single pollution on Ld growth, the antioxidant system and organic acid secretion, followed by their combined effect. The results showed that the growth of Ld was inhibited and the biomass decreased significantly (P < 0.05) under single exposure to PS-MPs or Al^{3+}, and the contents of superoxide anion radical ($O_2^{\cdot-}$) and H_2O_2 in mycelia increased significantly (P < 0.05). Meanwhile, superoxide dismutase (SOD) and peroxidase (POD) activities were also significantly increased (P < 0.05), while catalase (CAT) activity was significantly decreased (P < 0.05), indicating that PS-MPs or Al^{3+} stress had different effects on antioxidant enzyme activities in Ld mycelia, which used highly active antioxidant enzymes to eliminate excess reactive oxygen species (ROS) and maintain their dynamic balance. In addition, the electrolyte leakage rate, proline and malondialdehyde (MDA) contents in Ld mycelia were significantly increased (P < 0.05). Under the combined exposure of PS-MPs and Al^{3+}, compared with the control group, the combined exposure of high concentrations of PS-MPs and Al^{3+} (200+40 mg L^{-1}) had a certain promoting effect on the biomass of Ld. In addition, the $O_2^{\cdot-}$, H_2O_2, electrolyte leakage rate and malondialdehyde content in Ld mycelia increased significantly (P < 0.05), but these indices were significantly lower than those in the single PS-MPs exposure group at the same concentration. This indicates that the combined effect of PS-MPs and Al^{3+} could reduce the damage of single pollutants to the Ld biomass membrane and reduce the production of ROS to a certain extent. In addition, correlation analysis showed that H_2O_2 in Ld mycelia had a highly significant positive correlation with the oxalic acid and total hydrogen ion concentrations in the culture solution (P < 0.05). This indicates that strain Ld could respond to the oxidative damage caused by microplastics and Al by secreting oxalic acid and hydrogen ions into the culture medium.

Keywords: Microplastics, aluminum, ectomycorrhizal fungi, ecotoxicological effect

1. Introduction

Microplastics refer to plastic fragments and particles less than 5 mm in diameter (Wang *et al*., 2021). Because of their small size, they can migrate and diffuse under the action of wind, rivers, ocean currents and other external forces, which makes these small plastic particles easily enter water bodies and terrestrial ecosystems, causing serious harm to the organisms in the ecosystem (Gong *et al*., 2020). Microplastics can be absorbed by organisms through suspension in water or air and transmitted within the food chain, leading to changes in the living system, such as cellular biofilm stimulation, lipid metabolism, and oxidative stress (Chen *et al*., 2017; Lu *et al*., 2016; Della *et al*., 2014). Microplastics can also act as "carriers" to interact with pollutants such as antibiotics and metal ions to form more complex pollutants. Aluminum (Al) is the most abundant metal element in the Earth's crust, and Al^{3+} is dissolved in large amounts in acidified soils and has serious toxic effects on soil organisms (Magalhaes *et al*., 2018). Therefore, it is crucial to consider the synergistic pollution of microplastics

with Al when evaluating the harm of microplastics to soil plants, animals, and microorganisms.

In recent years, studies have found that microplastics themselves and the toxic substances they carry can have a serious impact on plants in the environment. Studies have shown that the adsorption of heavy metals by microplastics is closely related to the functional groups on the surface of microplastics, solution pH and ionic strength (Gong *et al*., 2019; Li *et al*., 2019). Microplastics may act as "carriers" for pollutants adsorbed on them, thereby transporting heavy metal pollutants into plants. Abbasi *et al*. (2020) demonstrated that polyethylene terephthalate particles (PET) can act as carriers to transport heavy metals to the rhizosphere region. The toxic effects of other pollutants in soil may also be enhanced when coexisting with microplastics (Ma *et al*., 2016). Wen *et al*. (2018) found that under the combined exposure of monodisperse polystyrene microplastics (PS-MPs) and cadmium (Cd), the activities of antioxidant enzymes in *Symphysodon aequifasciatus* in vivo were inhibited, resulting in severe oxidative damage. Wang *et al*. (2021) investigated the combined toxicity of pristine polyvinyl chloride (PVC) microplastics and Cd on *Vallisneria natans* and found that PVC microplastics could improve the growth inhibition of *Vallisneria natans* by Cd. In addition, studies have shown that under the combined pollution of heavy metals and PS-MPs, the community composition will be changed and bacterial diversity will be reduced (Feng *et al.,* 2022). Zhu *et al*. (2019) found that the competitive adsorption of triclosan (TCS) and microplastics would reduce the contact of harmful substances with microalgae, making the combined toxicity less than that of single microplastics and TCS on microalgae.

Ectomycorrhizal fungi are an important class of mycorrhizal organisms that can form a symbiotic relationship with plant roots. It is beneficial for plants to absorb water and insoluble mineral elements in the soil, such as phosphorus, potassium, calcium, and magnesium and enhance the resistance of plants to adverse environments (Kalsotra *et al*., 2018). At the same time, organic acids, enzymes, polysaccharide mucus and other components secreted by ectomycorrhizal fungi will affect the activity of heavy metals, reduce the mobility of heavy metals, and alleviate the damage of heavy metals to plants (Luo *et al*., 2014). However, there are few studies on the ecotoxic effects of ectomycorrhizal fungi due to the combined pollution of microplastics and Al. Therefore, this study selected *Lactarius delicious* (Ld) as the experimental subject under pure culture conditions, adding a single PS-MPs, Al^{3+} and their interaction, by analysing the impact of pollutants on the growth, antioxidant system, plasma membrane integrity, organic acid secretion and other indicators of ectomycorrhizal fungi. The purpose of this study was to analyse the growth and ecotoxicity mechanism of ectomycorrhizal fungi in the interaction of microplastics and Al, and to provide a theoretical basis for evaluating the ecotoxicity effect and mechanism of combined exposure of soil microorganisms to microplastics and metal ions.

2. Materials and methods

2.1. Material preparation

The laboratory-preserved ectomycorrhizal fungus Ld was selected as the test strain. The strain was collected from strongly acidic soil (pH 4.0-4.2) under the *Pinus massoniana* forest in Jinyun Mountain, Chongqing, which is located in the north subtropical zone. The strains were inoculated into Pachlewsk solid medium and cultured at 25 °C for 14 d for activation. The solid medium formula was as follows: 2 g of glucose, 2 g of agar, 0.1 g of potassium dihydrogen phosphate, 0.05 g of ammonium tartrate, 0.05 g of magnesium sulfate, 0.01 g of vitamin B_1, 0.1 mL of trace element liquid, and distilled water to make 100 mL. Adjust the pH to 5.5 and sterilize with high-pressure steam (121 °C, 30 min). The formula of trace element liquid was as follows: weigh 8.45 mg of boric acid, 6 mg of ferrous sulfate, 5 mg of manganese sulfate, 2.77 mg of zinc chloride, 0.625 mg of copper sulfate and 0.27 mg of ammonium molybdate to prepare a 1 L solution and store in sterilization.

Monodisperse polystyrene microsphere (PS-MPs) stock solution (concentration: 25 g L^{-1}) with a particle size of 80 nm was purchased from Jiangsu Zhichuan Technology Co., Ltd. The stock solution of PS-MPs was diluted into a 1000 mg L^{-1} stock solution with sterile water for use. Anhydrous aluminum trichloride was purchased from Tianjin Hengxing Reagent Co., Ltd., and an appropriate amount of anhydrous aluminum trichloride was taken to prepare a mother solution with an Al^{3+} concentration of 400 mg L^{-1}. After autoclaving (121 °C, 30 min), the samples were stored in a refrigerator at 4 ˚C for later use.

2.2. Experimental design

In the ultraclean workbench, a certain volume of PS-MPs and Al^{3+} mother solution were added to the sterilized Pachlewsk solid and liquid medium, respectively, and ultrasonic dispersion was carried out before and after dilution. Configured into a single PS-MPs concentration of 0 (control group), 20 (T1), 100 (T2) and 200 (T3) mg L^{-1}, and a single Al^{3+} concentration of 40 mg L^{-1} (T4), the interaction concentrations of PS-MPs and Al^{3+} were: 20+40 (T5), 100+40 (T6) and 200+40 (T7) mg L^{-1} in Pachlewsk solid and liquid medium. 20 mL culture medium was used to prepare solid plates and liquid medium. Then, a piece of activated solid bacteria block (8 mm in diameter) was inoculated in medium, and each treatment was repeated 5 times. After inoculation, the solid plate was cultured in an incubator at 25 °C for 14 days of static culture; the liquid medium was cultured on a shaking table (25 °C, 120 r min^{-1}) for 14 days, and the relevant indicators were measured.

2.3. Sampling and sample analysis

2.3.1. Determination of mycelial biomass

After culturing for 14 days, fungal growth on solid plates was observed. After liquid culture, the mycelia and the culture solution were separated by filtration, and then the mycelia were dried in an oven at 105 °C to a constant weight, which was the biomass of each flask of fungi (Huang *et al*., 2019).

2.3.2. Determination of antioxidant enzyme activity

An appropriate amount of mycelium was used to prepare tissue homogenate with 0.1 M phosphate buffer (pH 7-7.4) and centrifuged (4000× *g*, 8 min, 4 °C), and the supernatant was collected.

Superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium (NBT) method (Dhindsa *et al*., 1981). To determine SOD activity, a total of 3.0 mL of reaction mixture containing 50 mM phosphate buffer (pH 7.8), 130 mM methionine, 750 µM NBT, 100 µM EDTA-Na_2, 20 µM riboflavin, 50 µL enzyme extraction substance and 0.25 mL of deionized water was used. The absorbance of the reaction mixture was then measured at 560 nm with a spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT photoreduction by 50% (Hu *et al*., 2022), expressed as U mg protein^{-1}.

$$SOD\ activity = [(A_{CK} - A_E) \times V_{total}] / (0.5 \times A_{CK} \times FW \times V_t) \quad (1)$$

A_{CK}: absorbance value of the control group; A_E: absorbance value of the sample tube; V_{total}: total volume of extraction solution; V_t: amount of extraction solution used during measurement; FW: fresh weight of the sample; 0.5: 50% inhibition of NBT photoreduction.

The activity of peroxidase (POD) was determined by the guaiacol method (Chance and Maehly, 1955). To measure POD activity, a total of 4 mL of reaction mixture containing 100 mM phosphate buffer (pH 6.0), 5 mM guaiacol, 12.4 mM H_2O_2 and 1 mL of enzyme extract was used. Then, the absorbance change of the reaction mixture was measured at 470 nm for 40 seconds. One unit of POD activity was defined as an increase in absorbance (OD_{470} nm) of 0.01 per minute (Qiu *et al*., 2022), expressed as U min^{-1} mg protein^{-1}.

$$POD\ activity = (\Delta A_{470} \times V_{total}) / (FW \times V_t \times 0.01 \times t) \quad (2)$$

ΔA_{470}: change in absorbance value during reaction time; V_{total}: total volume of extraction solution; V_t: amount of extraction solution used during measurement; FW: fresh weight of the sample; t: reaction time; 0.01: 0.01 change in OD value per minute.

Catalase (CAT) activity was determined according to the method of Fathi *et al*. (2014). To assay CAT activity, a total of 3 mL of reaction mixture containing 150 mM phosphate buffer (pH 7.0), 50 mM H_2O_2, and 0.1 mL of enzyme extract was used. Then, the absorbance change of the reaction mixture was measured at 240 nm for 40 seconds. One unit of CAT activity was defined as a decrease in absorbance (OD_{240} nm) value of 0.1 per minute (Ali *et al*., 2021), expressed as U min^{-1} mg protein^{-1}.

$$CAT\ activity = (\Delta A_{240} \times V_{total}) / (FW \times V_t \times 0.1 \times t) \quad (3)$$

ΔA_{240}: change in absorbance value during reaction time; V_{total}: total volume of extraction solution; V_t: amount of extraction solution used during measurement; FW: fresh weight of the sample; t: reaction time; 0.1: 0.1 change in OD value per minute.

2.3.3. Determination of reactive oxygen species (ROS)

The H_2O_2 content was determined according to the method of Velikova *et al*. (2000). An appropriate amount of mycelia was used to prepare a tissue homogenate with 1 mL of 5% (w/v) trichloroacetic acid. Then, the homogenate was centrifuged (9000× *g*, 15 min, 4 °C). The supernatant was mixed with the reaction mixture containing 2.5 mM potassium phosphate buffer (pH 7.0) and 500 mM potassium iodide, and the absorbance was measured at 390 nm.

The superoxide anion radical ($O_2^{\cdot-}$) content was determined by the hydroxylamine hydrochloride oxidation reaction method (Lin *et al*., 2015). An appropriate amount of mycelia was used to prepare a tissue homogenate with 65 mM phosphate buffer (pH 7.8). The homogenate was filtered and centrifuged (9000× *g*, 15 min, 4 °C). A total of 2 mL of reaction mixture containing 1 mL supernatant, 0.9 mL 65 mM potassium phosphate buffer (pH 7.8) and 0.1 mL 10 mM hydroxylamine hydrochloride was reacted at 25 °C for 30 min. Finally, 1 mL 17 mM sulfanilic acid and 1 mL 7 mM α-naphthylamine were added, and the absorbance was measured at 530 nm.

2.3.4. Determination of malondialdehyde (MDA)

The MDA content was determined according to the method of Sharma *et al*. (2020). An appropriate amount of mycelia was added to 10% trichloroacetic acid and a small amount of quartz sand, ground to prepare 10 mL of tissue homogenate, and then centrifuged (4000× *g*, 10 min). An appropriate amount of supernatant was added to 0.6% thiobarbituric acid. Subsequently, the formed reaction mixture was subjected to 30 min of incubation in a 95 °C water bath, and 2 min of rapid cooling in an ice bath, followed by 10 min of centrifugation at 4000× g. At this stage, supernatant absorbance was determined at the following wavelengths (including 532, 600 and 450 nm) by a spectrophotometer. MDA content was computed according to the equation below:

$$C = 6.45\ (A_{532} - A_{600}) - 0.56\ A_{450} \quad (4)$$

C: MDA content in µmol g^{-1}; A: absorbance at different wavelengths.

2.3.5. Determination of oxalic acid content and total acidity in the culture solution

The collected culture medium was measured by the $KMnO_4$ titration method for the oxalic acid content in the filtrate (Massawe *et al*., 2018). The total acidity of the culture solution was determined by NaOH titration (total acidity refers to the amount of all hydrogen ions per culture solution) (Li *et al*., 2017).

2.3.6. Determination of the electrolyte leakage rate in mycelia

The electrolyte leakage rate was determined according to the method of Xu *et al*. (2022). An appropriate amount of mycelia was rinsed with deionized water. The mycelia were then soaked in deionized water, and the mycelial electrolyte leakage rate was measured. After boiling for 15 min, the electrolyte leakage rate of mycelia was measured

again, and the electrolyte leakage rate was computed according to the equation below:

$$Electrolyte\ leakage\ rate = R_1 / R_2 \times 100\% \quad (5)$$

R_1: electrolyte leakage rate before boiling in a water bath; R_2: electrolyte leakage rate after boiling in a water bath.

2.3.7. Determination of soluble protein in mycelia

The soluble protein was determined according to the method of Zhang *et al*. (2015). An appropriate amount of mycelia was added to 50 mM phosphate buffer solution (pH 7.8), ground to prepare 10 mL of tissue homogenate, and centrifuged (4000× *g*, 10 min). An appropriate amount of supernatant was added to 0.1 mg mL^{-1} Coomassie brilliant blue solution in a water bath at 30 °C for 10 min, and the absorbance was measured at 595 nm.

2.3.8. Determination of proline in mycelia

Proline was determined according to the method of Pingle *et al*. (2022). An appropriate amount of mycelia was added to 80% ethanol solution, ground to prepare 10 mL of tissue homogenate, extracted in the dark for 1 h, and shaken for 15 min. After centrifugation (3000× *g*, 5 min), glacial acetic acid and 25 mg mL^{-1} ninhydrin solution were added to the supernatant, and the solution was boiled for 20 min. After cooling, the absorbance was measured at 515 nm.

2.4. Data statistics and analysis

The experimental data were calculated and plotted using Excel 2010, and Duncan's method in SPSS 19.0 was used for multiple comparisons of the mean values ($P < 0.05$). In addition, Origin2021b software was used for two-factor correlation analysis.

3. Results

3.1. Growth of Ld

The effect of the interaction between PS-MPs and Al^{3+} on the growth of Ld is shown in Figure 1. In solid plate culture, when exposed to single PS-MPs or Al^{3+}, the growth of Ld was significantly inhibited compared with the control group. Under the interaction between PS-MPs and Al^{3+}, the growth of Ld was promoted with increasing PS-MPs concentration, which indicated that the interaction between two pollutants reduced the toxicity of single pollutants to a certain extent.

3.2. Biomass

Figure 2 shows the effect of the interaction between PS-MPs and Al^{3+} on the biomass of Ld. Under single PS-MPs exposure, compared with the control group, the biomass of strain Ld decreased gradually with increasing PS-MPs concentration. Under single Al^{3+} exposure, compared with the control group, the biomass of strain Ld decreased significantly. Under the combined exposure of PS-MPs and Al^{3+}, compared with the control group, the biomass of the T5 and T6 treatment groups decreased significantly, among which the T5 treatment group had the most significant decrease, which decreased by 49.9% (compared with the control group), while the biomass of the T7 treatment group significantly increased by 9.1% (compared with the control group). This shows that the interaction between high concentrations of PS-MPs and Al^{3+} basically shows an antagonistic effect on the biomass of Ld compared with the single pollution treatment. Namely, the combination of two pollutants to some extent reduces the toxicity of a single pollutant. The interaction between low and medium concentrations of PS-MPs and Al^{3+} basically showed a synergistic effect on the biomass of Ld.

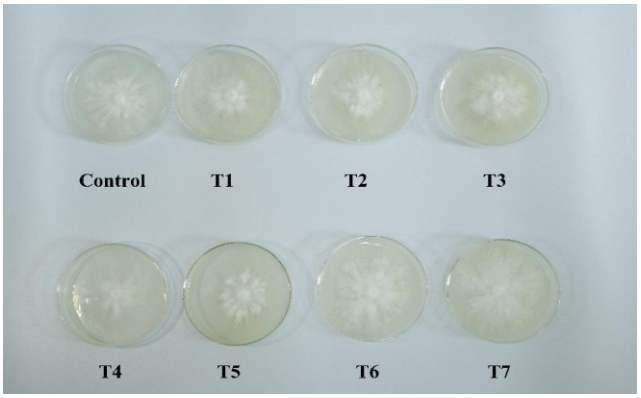


Figure 1. Effects of the interaction between PS-MPs and Al^{3+} on the growth of Ld

PS-MPs: monodisperse polystyrene microspheres; Al^{3+}: aluminum; Ld: *Lactarius delicious*; T1: 20 mg L^{-1} PS-MPs; T2: 100 mg L^{-1} PS-MPs; T3: 200 mg L^{-1} PS-MPs; T4: 40 mg L^{-1} Al^{3+}; T5: 20 mg L^{-1} PS-MPs+40 mg L^{-1} Al^{3+}; T6: 100 mg L^{-1} PS-MPs+40 mg L^{-1} Al^{3+}; T7: 200 mg L^{-1} PS-MPs+40 mg L^{-1} Al^{3+}, the same below.

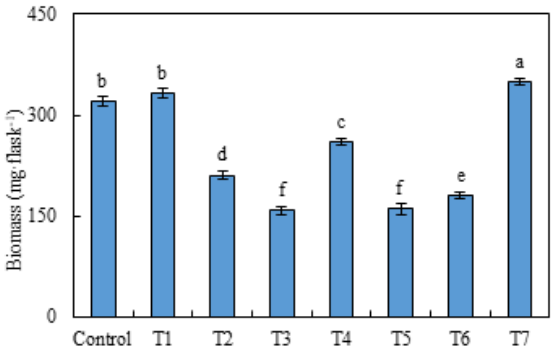


Figure 2. Effects of the interaction between PS-MPs and Al^{3+} on the biomass of Ld

Data are presented in the form of mean ± S.D. ($n = 5$ in each group); Different letters indicate significant differences among different treatments ($P < 0.05$), the same below.

3.3. Antioxidative enzymes and ROS

The effect of the interaction between PS-MPs and Al^{3+} on antioxidant enzymes and ROS in Ld mycelia is shown in Table 1. Under single PS-MPs exposure, compared with the control group, as the concentration of PS-MPs increased, SOD and POD activities in Ld mycelia showed a trend of "first increasing and then decreasing", while the CAT activity in Ld mycelia gradually decreased. Under single Al^{3+} exposure, compared with the control group, SOD and POD activities in Ld mycelia were significantly increased, while CAT activities were significantly decreased. Under the combined exposure of PS-MPs and Al^{3+}, the SOD activity in Ld mycelia showed a trend of "first increase and then decrease". Among them, under the T7 treatment, SOD activity decreased the most significantly, with a decrease of

45.4% compared to the control group. POD activity showed a trend of "first decreasing and then increasing", while CAT activity did not change significantly.

As shown in Table 1, compared with the control group, the contents of $O_2^{\cdot-}$ and H_2O_2 in Ld mycelia were significantly increased under single exposure to PS-MPs or Al^{3+}. Under the combined exposure to PS-MPs and Al^{3+}, the contents of $O_2^{\cdot-}$ and H_2O_2 in Ld mycelia also increased significantly compared with the control group. However, compared with the same concentration of single PS-MPs, the $O_2^{\cdot-}$ and H_2O_2 contents in Ld mycelia (except T5 compared with T1 treatment) were significantly reduced. This indicates that the interaction between PS-MPs and Al^{3+} basically shows an antagonistic effect on the production of ROS in Ld mycelia. Namely, the combination of two pollutants to a certain extent reduces the ecological toxicity of a single pollutant.

Table 1. Effects of the interaction between PS-MPs and Al^{3+} on antioxidant enzymes and ROS in Ld

Treatment	Concentration (mg L^{-1})	POD (U min^{-1} mg $protein^{-1}$)	CAT (U min^{-1} mg $protein^{-1}$)	SOD (U mg $protein^{-1}$)	$O_2^{\cdot-}$ (nmol g^{-1} FW^{-1})	H_2O_2 (nmol g^{-1} FW^{-1})
Control	0	41.6±1.2c	53.1±2.1a	74.5±2.4d	242.2±3.5h	11.6±0.5e
PS-MPs	20 (T1)	36.5±1.7c	49.3±1.8b	86.9±2.8c	821.7±.7.6b	13.2±0.7d
	100 (T2)	104.5±2.6a	50.1±2.4b	123.4±3.5a	741.9±10.1c	37.8±1.1b
	200 (T3)	25.3±0.9d	46.0±3.2c	107.6±1.6b	1140.7± 8.6a	68.8±2.1a
Al^{3+}	40 (T4)	66.5±1.4b	46.4±2.9c	89.0±2.1c	283.2±5.8g	13.9±0.4d
PS-MPs+Al^{3+}	20+40 (T5)	36.4±1.1c	46.1±1.5c	48.3±1.8e	462.7±4.7e	20.4±0.7c
	100+40 (T6)	15.8±0.8e	47.6±3.1c	82.8±2.6c	602.3±7.8d	19.8±0.5c
	200+40 (T7)	36.1±1.9c	47.4±2.5c	40.7±1.5f	323.1±5.3f	32.7±0.9b

Data are presented in the form of mean ± S.D. (n = 5 in each group); Different letters indicate significant differences among different treatments (P < 0.05), the same below.

Table 2. Effects of the interaction between PS-MPs and Al^{3+} on the electrolyte leakage rate, permeable substances and MDA contents of Ld

Treatment	Concentration (mg L^{-1})	Electrolyte leakage rate (%)	Soluble protein (mg g^{-1})	Proline (μg g^{-1})	MDA (μmol g^{-1})
Control	0	66.0±0.8d	24.2±0.5c	1.4±0.2e	5.2±0.3e
PS-MPs	20（T1）	74.1±1.2b	22.2±1.1d	2.7±0.3b	12.5±0.6a
	100（T2）	71.2±1.1c	24.3±0.8c	2.3±0.1bc	11.1±0.3b
	200（T3）	78.2±1.4a	29.0±1.4a	3.5±0.5a	11.8±0.7ab
Al^{3+}	40（T4）	78.4±2.1a	25.5±1.9b	1.7±0.2d	7.4±0.4d
PS-MPs+Al^{3+}	20+40（T5）	63.3±1.7e	24.6±0.6bc	2.1±0.4c	8.5±0.8c
	100+40（T6）	71.2±0.8c	30.7±1.2a	2.3±0.5bc	8.0±0.2c
	200+40（T7）	79.3±1.4a	29.0±1.8a	2.1±0.2c	5.8±0.4e

3.4. Electrolyte leakage rate, permeable substances and MDA content

The effects of the interaction between PS-MPs and Al^{3+} on the electrolyte leakage rate, permeable substances and MDA contents of Ld mycelia are shown in Table 2. Compared with the control group, single PS-MPs or Al^{3+} exposure treatment significantly increased the electrolyte leakage rate, proline and MDA contents in Ld mycelia. Compared with the control group, under single PS-MPs exposure, the content of soluble protein in Ld mycelia decreased significantly when the concentration of PS-MPs was 20 mg L^{-1}, while it increased significantly in other treatment groups. Compared with the control group, both the T6 and T7 treatment groups significantly increased the electrolyte leakage rate and soluble protein content in Ld mycelia. Compared with the control group, all PS-MPs and Al^{3+} combined exposure groups (T5, T6 and T7) had significantly increased proline content in Ld mycelia. Compared with the control group, both the T5 and T6 treatment groups had increased MDA content in Ld mycelia. In addition, compared with the same concentration of the single PS-MPs exposure group, the content of MDA was significantly decreased. This indicates that the interaction of PS-MPs and Al^{3+} reduces the production of membrane lipid peroxidation products in Ld mycelia compared with the single pollution treatment. Namely, the combined exposure of two pollutants to a certain extent reduces the destructive effect of a single pollutant on the Ld cellular biofilms.

3.5. Oxalic acid concentration and total acidity in culture medium

Effects of the interaction between PS-MPs and Al^{3+} on the secretion of oxalic acid and the concentration of total hydrogen ions in the culture medium are shown in Figure 3. Under single PS-MPs or Al^{3+} exposure, the concentrations of oxalic acid and total hydrogen ions in the liquid medium were significantly increased compared with the control group, with the T3 treatment group showing the most significant increase, which increased by 4.0-fold (oxalic acid) and 1.4-fold (total hydrogen ion concentration). Under the combined exposure of PS-MPs and Al^{3+}, the concentrations of oxalic acid and total hydrogen ions in the liquid medium were also significantly increased compared with the control group. However, in the low concentration PS-MPs and Al^{3+} exposure group (T5), the total hydrogen ion concentration in the liquid medium was significantly higher than that in the single PS-

MPs exposure group at the same concentration (T1). However, in the high concentration PS-MPs combined with Al^{3+} exposure group (T7), the total hydrogen ion concentration in the liquid culture medium was significantly lower than that in the single PS-MPs exposure group (T3) at the same concentration. This indicates that the interaction between high concentrations of PS-MPs and Al^{3+} basically has an antagonistic effect on the secretion of hydrogen ions by Ld, while the interaction between PS-MPs and Al^{3+} at low concentrations basically promotes the secretion of hydrogen ions by Ld.

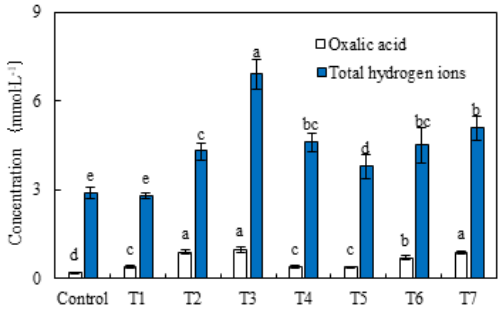


Figure 3. Effects of the interaction between PS-MPs and Al^{3+} on oxalic acid secretion and total acidity in culture medium

3.6. Correlation analysis

Figure 4 shows that MDA and proline in Ld mycelia were significantly positively correlated with $O_2^{\cdot-}$ ($P < 0.05$), and proline in Ld mycelia was significantly positively correlated with H_2O_2 ($P < 0.05$). H_2O_2 in Ld mycelia was significantly positively correlated with the oxalic acid and total hydrogen ion concentrations in the culture solution ($P < 0.05$).

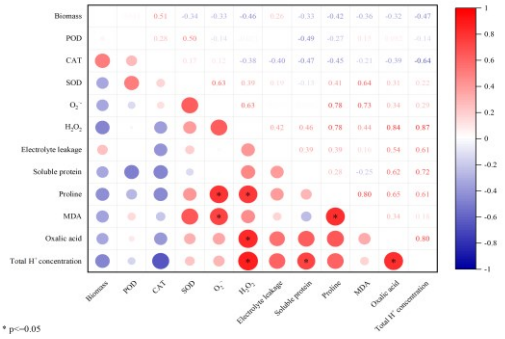


Figure 4. Correlation coefficients of various experimental factors
Note: * shows that correlations are significant at $P < 0.05$.

4. Discussion

4.1. Effect of the interaction between PS-MPs and Al^{3+} on the growth of Ld

Microplastics have become one of the emerging pollutants of global concern. At present, research on the ecotoxicity of microplastics is mostly concentrated on marine organisms, freshwater algae and soil plants. Due to the large specific surface area and strong hydrophobicity of microplastics, they can combine with other pollutants in the environment to produce compound toxicity. Many studies have shown that microplastics have an adsorption effect on heavy metals, such as Cd^{2+}, Zn^{2+}, Pb^{2+} and Al^{3+}, which can be adsorbed on the surface of PVC microplastics to form composite pollutants (Godoy *et al*., 2019). This means that the complex toxicity mechanism and environmental risk assessment of microplastics and other pollutants need more attention. Zhang *et al*. (2020) found that combined exposure to microplastics and Cd at different concentrations had inhibitory effects on zebrafish (*Danio rerio*) embryonic survival and heart rate. In addition, some studies have found that Cd treatment alone inhibits the growth of wheat seedlings more seriously than Cd-polystyrene nanomicroplastics combined treatment (Cd-PSNPs), indicating that the coexistence of PSNPs and Cd^{2+} could partially alleviate the ecological toxicity of Cd^{2+} on wheat seedlings (Lian *et al*., 2020). Studies have found that the amino groups of amino-modified polystyrene nanoplastics (200 nm) easily combine with the carboxyl groups of glyphosate to adsorb glyphosate. Therefore, when the two pollutants were jointly exposed, there was an antagonistic effect on the growth inhibition of *Microcystis aeruginosa* (Zhang *et al*., 2018). This study found that the growth of Ld was significantly inhibited and the biomass was significantly reduced when exposed to single PS-MPs or Al^{3+}. Under the combined exposure of PS-MPs and Al^{3+}, compared with the control group, the biomass of the T5 and T6 treatment groups were significantly decreased. At the same time, it was significantly lower than the single PS-MPs and Al^{3+} exposure group (T4 treatment group) at the same concentration, while the T7 treatment group had a significant increase in biomass, and it was significantly higher than the single PS-MPs and Al^{3+} exposure group (T4 treatment group) at the same concentration. This indicates that the interaction of high concentrations of PS-MPs and Al^{3+} on the biomass of Ld showed an antagonistic effect compared with the single pollution treatment. Namely, the combination of two pollutants to some extent reduces the toxicity of a single pollutant. However, the interaction between low and medium concentrations of PS-MPs and Al^{3+} showed a synergistic effect on the biomass of Ld. This may be due to the antagonistic effect of high concentrations of PS-MPs and Al^{3+}, and the binding of PS-MPs to Al^{3+} forms complexes or aggregates, causing Ld to be unable to absorb more PS-MPs or Al^{3+}, thereby reducing the toxicity of single pollutants. Zhu *et al*. (2020) studied the toxic effects of single copper nanoparticles (Cu-NPs) or PVC microplastics exposure and co-exposure on *Skeletonema costatum*. The results showed that the growth inhibition of *Skeletonema costatum* in the co-exposure group was lower than that in the single Cu-NPs exposure group, indicating that the addition of microplastics reduced the toxicity of Cu-NPs, which is consistent with the results of this study.

4.2. Effect of the interaction between PS-MPs and Al^{3+} on the activity of antioxidant enzymes and ROS in Ld mycelia

When biological cells are stressed by the external environment, more ROS are produced than normal, which causes membrane lipid peroxidation and damages the cell structure. SOD, POD and CAT are the main enzymes in the antioxidant system in the organism, and their activity level can reflect the degree of the organism being subjected to

external environmental pressure, and they are the main substances for scavenging ROS. Paul-Pont *et al*. (2016) assessed the combined toxicity of microplastics and fluoranthracene in mussels (*Mytilus edulis*) and found that mussel tissues suffered from pathological damage and increased levels of antioxidant markers, resulting in an oxidative stress effect. Fu *et al.* (2019) found that the interaction of perished PVC microplastics with Cu caused oxidative stress in *Chlorella* and decreased SOD activity in *Chlorella*. Abduro-Ogo *et al*. (2022) found that under combined MPs-Pb stress, the SOD and POD activities of two large submerged plants increased. Avio *et al*. (2015) showed that CAT activity in vivo was abnormal after mussels were exposed to microplastics and pyrene. In this study, under exposure to single PS-MPs, the activities of SOD and POD in Ld mycelia showed a trend of "first increasing and then decreasing", while CAT activity gradually decreased. Under single Al^{3+} treatment, compared with the control group, SOD and POD activities in Ld mycelia were significantly increased, while CAT enzyme activities were significantly decreased. Under the combined exposure of PS-MPs and Al^{3+}, the SOD activity in Ld mycelia showed a trend of "first increase and then decrease", the POD activity showed "first decrease and then increase", and the CAT activity did not change significantly. This indicates that the combined exposure of PS-MPs and Al^{3+} has different effects on the activity of antioxidant enzymes in Ld mycelia, resulting in the activation of the antioxidant system in the fungal cells and the production of more SOD to remove excess $O_2^{\cdot-}$. Due to the large specific surface area and surface energy of PS-MPs, as well as their charge carrying properties, which can fully contact Al^{3+} and adsorb Al^{3+} to form larger groups, thus being excluded from the extracellular surface. This means that the fungal cells cannot take up more PS-MPs or Al^{3+}, thereby reducing their toxicity to fungal cells. Thus, under the combined exposure of PS-MPs and Al^{3+}, the activity of antioxidant enzymes in Ld cells was lower than that of the single PS-MPs or Al^{3+} exposure group.

Under normal conditions, the production and scavenging of ROS in living organisms are maintained in a state of dynamic equilibrium. Under external environmental stress, the homeostasis of organisms is disrupted, and ROS are rapidly generated and accumulate, exceeding the self-regulation threshold and causing oxidative damage to organisms (Natarajan *et al*., 2022). Zong *et al*. (2021) found in the wheat hydroponic experiment that 100 mg L^{-1} PS-MPs (0.5 μm particle size) reduced their effectiveness in wheat roots by chemisorption of Cu and Cd. Compared with the single heavy metal treatments, the combined exposure of PS-MPs with heavy metals increased antioxidant enzyme activities and decreased the accumulation of ROS. Another study found that adding unmodified ordinary polystyrene microplastics and amino modified polystyrene microplastics to titanium dioxide nanoparticles (TiO_2-NPs) and the combined exposure of microplastics and TiO_2-NPs increased toxicity to *Chlorella* sp. and produced more ROS (Thiagarajan *et al*., 2019). In this study, under exposure to single PS-MPs, the $O_2^{\cdot-}$ content in Ld mycelia increased, and the H_2O_2 content in Ld mycelia showed a trend of "first decreasing and then increasing". The combined exposure of medium and low concentrations of PS-MPs and Al^{3+} resulted in a higher H_2O_2 content in Ld mycelia compared to a single treatment, while the combined exposure of high concentrations of PS-MPs and Al^{3+} resulted in a lower H_2O_2 content in Ld mycelia compared to a single treatment. However, the $O_2^{\cdot-}$ content in the mycelium was lower than that of a single PS-MPs treatment, which may be related to differences in the content of other nonenzymatic antioxidants in the mycelia. Therefore, the interaction between PS-MPs and Al^{3+} basically exhibits an antagonistic effect on the production of ROS in Ld mycelia. Namely, the combination of two pollutants to a certain extent reduces the ecological toxicity of a single pollutant and reduces the oxidative damage of reactive oxygen species to mycelial cells.

4.3. Effect of the interaction between PS-MPs and Al^{3+} on the electrolyte leakage rate, permeable substances and MDA content in Ld mycelia

MDA is the final product of lipid peroxidation, and its content level can be used as an indicator of the degree of peroxidation in the body (Tsikas. 2017), In addition, soluble protein and proline are important osmotic regulators and nutrients that play a protective role in cellular biofilms (Rahdari *et al*., 2012). Tunali *et al*. (2020) showed that microplastic and metal composite pollution led to a decrease in the content of soluble protein in *Chlorella*. In this study, it was found that under a single PS-MPs or Al^{3+} exposure, the content of soluble protein in Ld mycelia was significantly decreased when the concentration of PS-MPs was 20 mg L^{-1}, while the other treatment groups were significantly increased. At the same time, the combined exposure treatment group of PS-MPs and Al^{3+} (T6 and T7) also significantly increased the soluble protein content in Ld mycelia, which was significantly higher than that in the single PS-MPs or Al^{3+} exposure group at the same concentration. This indicated that the combined exposure of PS-MPs and Al^{3+} could increase the content of soluble protein in the mycelia to cope with the damage caused by the external stress environment. Mohsen *et al*. (2021) exposed *Apostichopus japonicus* to a diet containing microplastic fibers, and compared with the control group, the total antioxidant capacity (T-AOC) and MDA levels in juvenile and adult *Apostichopus japonicus* showed disturbances, significantly increasing the content of MDA. Khan *et al*. (2023) found that under the combined treatment of chromium and PVC microplastics, the content of proline in sweet potato leaves increased. In this study, it was found that the electrolyte leakage rate, proline and MDA contents in Ld mycelia were significantly increased under single exposure to PS-MPs or Al^{3+}, and the combined exposure group of all PS-MPs and Al^{3+} (T5, T6 and T7) significantly increased the proline content in Ld mycelia, compared with the control group. Both the T5 and T6 treatment groups increased the content of MDA in Ld mycelia. In addition, compared with the same concentration of the single PS-MPs exposure group, the content of MDA was significantly decreased. This indicates that the interaction of PS-MPs and Al^{3+} reduces the

production of membrane lipid peroxidation products in Ld mycelia compared with the single pollution treatment. Namely, the combined exposure of two pollutants to a certain extent reduces the destructive effect of a single pollutant on the Ld cellular biofilms. Zhang *et al*. (2019) studied the ecological effects of combined exposure to PS-MPs and roxithromycin (ROX) on freshwater red tilapia (*Oreochromis niloticus*). The MDA content was significantly decreased, antioxidant enzyme activity was increased, and the presence of microplastics alleviated oxidative damage to the tilapia liver by ROX. In addition, correlation analysis showed that the proline in Ld mycelia was significantly positively correlated with $O_2^{\cdot-}$ and H_2O_2 ($P < 0.05$), which indicated that oxidative damage occurs within Ld mycelia due to the accumulation of a large amount of ROS, and the mycelia could respond to the stress pressure generated by PS-MPs and Al^{3+} by increasing the content of osmoregulation substances.

4.4. Effect of the interaction between PS-MPs and Al^{3+} on the secretion of oxalic acid and the total acidity of the culture medium

Studies have shown that polystyrene nanoplastics (PS-NPs) can induce the roots of *Arabidopsis thaliana* to produce more organic acids. When the concentration of PS-NPs was 50 μg mL^{-1}, the content of oxalic acid secreted by the roots of *Arabidopsis thaliana* was approximately 2.6 times that of the control group (0 μg mL^{-1}) (Sun *et al.*, 2020). Low molecular weight organic acids are the most common and important plant root exudates. On the one hand, they can mobilize mineral elements such as Ca, K, P and Fe in the soil to provide nutrients for plant growth. On the other hand, they can form stable metal ligand complexes with Pb, Al^{3+}, Cd and other metal ions, which alter the mobility and bioavailability of heavy metals, prevent metal ions from entering plants or prevent their accumulation at sensitive points in roots. Studies have shown that organic acids such as oxalic acid, formic acid and citric acid secreted by ectomycorrhizal fungi are one of the important mechanisms for antagonizing Al. Among them, oxalate ions have strong chelating forces on divalent and trivalent cations (Kong *et al*., 2018). The stability constant of $[A1(C_2O_4)_3]^{3-}$ is 2.0×10^{16}, and its molecular weight is 9.8 times higher than that of Al, which not only reduces the activity of Al^{3+} around ectomycorrhizal fungal mycelia but also slows the rate of Al^{3+} passing through the hyphal cell membrane, inhibiting the absorption of Al. In the present study, it was found that the concentrations of oxalic acid and total hydrogen ions in the liquid medium were significantly increased compared with the control group under single exposure and combined exposure to both PS-MPs and Al^{3+}. However, in the low concentration PS-MPs and Al^{3+} exposure group (T5), the total hydrogen ion concentration in the liquid medium was significantly higher than that in the single PS-MPs exposure group at the same concentration (T1), while in the high concentration PS-MPs combined with Al^{3+} exposure group (T7), the total hydrogen ion concentration in liquid culture medium was significantly lower than that in the single PS-MPs exposure group (T3) at the same concentration. This indicates that the interaction between high concentrations of PS-MPs and Al^{3+} basically acts as an antagonistic effect on the secretion of hydrogen ions by Ld, while the interaction between low concentrations of PS-MPs and Al^{3+} basically exhibits a synergistic effect on the secretion of hydrogen ions from Ld. Studies have shown that high concentrations of CeO_2 nanoparticles can significantly increase the content of organic acid metabolites in spinach (*Spinacia oleracea*) leaves, and organic acids also play an important role in regulating the transport of metal ions and nanoparticles from roots to leaves and their accumulation in leaves (Zhang *et al*., 2019). In addition, correlation analysis revealed that there was a significant positive correlation between H_2O_2 in Ld mycelia and the concentration of oxalic acid and total hydrogen ions in the culture medium ($P < 0.05$). This indicates that strain Ld could regulate oxidative damage caused by PS-MPs and Al^{3+} by secreting acidic substances such as oxalic acid and hydrogen ions into the culture medium.

5. Conclusion

The results of this experiment showed that PS-MPs or Al^{3+} single and dual interactions had significant effects on the growth, biomass, antioxidant enzyme activity, ROS production, cell membrane permeability, oxalic acid secretion and total hydrogen ion concentration in the culture medium of ectomycorrhizal fungi.

1. The interaction of low concentrations of PS-MPs and Al^{3+} had a synergistic effect on the inhibition of Ld biomass, while the interaction of high concentrations of PS-MPs and Al^{3+} had an antagonistic effect, reducing the toxicity of a single pollutant.
2. Under a single exposure to PS-MPs/Al^{3+}, the antioxidant enzyme activity in Ld mycelia increased, but the ROS content increased. Under combined exposure, SOD activity showed a "first increase then decrease", POD activity showed a "first decreasing then increasing", while CAT activity showed no significant change. The ROS content in Ld mycelium was significantly lower than that of a single PS-MPs exposure group at the same concentration.
3. The electrolyte leakage rate, proline and MDA contents in Ld mycelia were significantly increased under the single PS-MPs/Al^{3+} and the combined exposure of PS-MPs and Al^{3+}, but the electrolyte leakage rate and MDA content in Ld mycelia under the combined exposure of PS-MPs and Al^{3+} were significantly lower than those in the single pollutant treatment group, which reduced the damage of a single pollutant to the Ld biomass membrane.
4. The Ld strain can secrete oxalic acid and hydrogen ions into the culture medium to respond to the stress produced by PS-MPs and Al^{3+}.

Author contributions

Conceptualization, J. Liu, Z.M. Cheng, Y.T. Xie, Y.L. Zhang, W.W. Jiang, Z.W. Jiang and L. Zhang; methodology, J. Liu and Z.M. Cheng; software, Y.T. Xie and Y.L. Zhang; validation, W.W. Jiang and Z.W. Jiang; formal analysis, Z.W. Jiang and L. Zhang; resources, J. Liu and Z.M. Cheng; data curation, Z.W. Jiang and L. Zhang; writing—original draft J. Liu and Z.M. Cheng; preparation, J. Liu and Z.M. Cheng; writing—review and editing, J. Liu, Z.M. Cheng and L. Zhang; supervision, L. Zhang; project administration, J. Liu, Z.M. Cheng and L. Zhang. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The current study was funded by the National Natural Science Foundation of China (32101118), the Natural Science Foundation of the Jiangsu Higher Education Institutions (20KJD180003), the Natural Science Foundation of Jiangsu Province (BK20200703), "the Horizontal Project of Taizhou College, Nanjing Normal University (KYC20230402)" after the Youth Science and Technology Talent Promotion Project of “Fengcheng Talents” in Taizhou of 2021 and the project of Innovation and Entrepreneurship Training Program for College Students in Jiangsu Province (202213843031Y).

Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

References

Abbasi S., Moore F., Keshavarzi B., Hopke P.K., Naidu R., Rahman M.M., Oleszczuk P. and Karimi J. (2020). PET-microplastics as a vector for heavy metals in a simulated plant rhizosphere zone. *The Science of the total environment*, **744**, 140984. Doi: 10.1016/j.scitotenv.2020.140984.

Abduro-Ogo H., Tang N., Li X.W., Gao X.Y. and Xing W. (2022). Combined toxicity of microplastic and lead on submerged macrophytes. *Chemosphere*, **295**, 133956. Doi: 10.1016/j.chemosphere.2022.133956.

Ali S., Akbar-Anjum M., Nawaz A., Naz S., Ejaz S., Shahzad-Saleem M., Tul-Ain Haider S. and Ul-Hasan M. (2021). Effect of gum arabic coating on antioxidative enzyme activities and quality of apricot (*Prunus armeniaca* L.) fruit during ambient storage. *Journal of Food Biochemistry.*, **45**, e13656. Doi: 10.1111/jfbc.13656.

Avio C G., Gorbi S., Milan M., Benedetti M., Fattorini D., d'Errico G., Pauletto M., Bargelloni L. and Regoli F. (2015). Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environmental pollution*, **198**, 211–222. Doi: 10.1016/j.envpol.2014.12.021.

Chance B. and Maehly C. (1955) Assay of catalase and peroxidases, *Methods Enzymol*, **11**, 764–775

Chen Q., Gundlach M., Yang S., Jiang J., Velki M., Yin D. and Hollert H. (2017). Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. *The Science of the total environment*, **584-585**, 1022–1031, Doi: 10.1016/j.scitotenv.2017.01.156.

Della-Torre C., Bergami E., Salvati A., Faleri C., Cirino P., Dawson K.A. and Corsi I. (2014). Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos Paracentrotus lividus. *Environmental science & technology*, **48**, 12302–12311. Doi: 10.1021/es502569w.

Dhindsa R.S., Dhindsa P.P. and Thorpe T.A. (1981). Leaf senescence correlated with increased levels of membrane permeability and lipid-peroxidation and decreased levels of superoxide dismutase and catalase. *Journal of Experimental botany*., **32**, 93–101. Doi:10.1093/jxb/32.1.93.

Fathi G.A., Gharineh H., Barzali M., Siadat S.A.A. and Tamadon-Rastegar M. (2014). Evaluation of water deficit stress on seedling growth, antioxidant enzyme activity and yield of four cultivars of cotton. *International Journal of Agriculture Innovations and Research*, **3**, 610–617.

Feng X.Y., Wang Q.L., Sun Y.H., Zhang S.W. and Wang F.Y. (2022). Microplastics change soil properties, heavy metal availability and bacterial community in a Pb-Zn-contaminated soil. *Journal of hazardous materials*, **424**, 127364. Doi: 10.1016/j.jhazmat.2021.127364.

Fu D.D., Zhang Q.J., Fan Z.Q., Qi H.Y., Wang Z.Z. and Peng L.C. (2019). Aged microplastics polyvinyl chloride interact with copper and cause oxidative stress towards microalgae Chlorella vulgaris. *Aquatic Toxicology*, **216**, 105319. Doi: 10.1016/j.aquatox.2019.105319.

Godoy V., Blázquez G., Calero M., Quesada L. and Martín-Lara M.A. (2019). The potential of microplastics as carriers of metals. *Environmental pollution*, **255**, 113363. Doi: 0.1016/j.envpol.2019.113363

Gong J. and Xie P. (2020). Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. *Chemosphere*, **254**, 126790. Doi: 10.1016/j.chemosphere.2020.126790.

Gong W., Jiang M.Y., Han P., Liang G., Zhang T.T. and Liu G.N. (2019). Comparative analysis on the sorption kinetics and isotherms of fipronil on nondegradable and biodegradable microplastics. *Environmental pollution*, **254**, 112927. Doi: 10.1016/j.envpol.2019.07.095.

Hu W.Z., Sarengaowa S. and Ke F. (2022). Effect of Edible Coating on the Quality and Antioxidant Enzymatic Activity of Postharvest Sweet Cherry (*Prunusavium* L.) during Storage, *Coatings*, **12,** Doi: 10.3390/coatings12050581

Huang W.W., Ratkowsky D.A., Cang H., Wang P., Su J.L. and Shi P.J. (2019). Leaf Fresh Weight Versus Dry Weight: Which is Better for Describing the Scaling Relationship between Leaf Biomass and Leaf Area for Broad-Leaved Plants? *Forests*, **10**. Doi: 10.3390/f10030256.

Kalsotra T., Khullar S., Agnihotri R. and Reddy M.S. (2018). Metal induction of two metallothionein genes in the ectomycorrhizal fungus Suillus himalayensis and their role in metal tolerance. *Microbiology*, **164**, 868–876. Doi: 10.1099/mic.0.000666.

Khan M.A., Kumar S., Wang Q.Q., Wang M.Z., Fahad S., Nizamani M.M., Chang K.L., Khan S., Huang Q. and Zhu G.P. (2023). Influence of polyvinyl chloride microplastic on chromium uptake and toxicity in sweet potato, *Ecotoxicology and Environmental Safety*, **251**. Doi: 10.1016/j.ecoenv.2023.114526.

Kong Q.P., Xie B.B., Preis S., Hu Y., Wu H.Z. and Wei C.H. (2018). Adsorption of Cd^{2+} by an ion-imprinted thiol-functionalized polymer in competition with heavy metal ions and organic acids. *RSC Advances*, **8**, 8950-8960. Doi: 10.1039/c7ra11811b.

Li R., Liu H.P., Hong C.L., Dai Z X., Liu J.W., Zhou J., Hu C.Q. and Weng H.X. (2017). Iodide and iodate effects on the growth and fruit quality of strawberry. *Journal of the Science of Food and Agriculture.*, **97**, 230-235. Doi: 10.1002/jsfa.7719.

Li Y.D., Li M., Li Z., Yang L. and Liu X. (2019). Effects of particle size and solution chemistry on Triclosan sorption on polystyrene microplastic. *Chemosphere*, **231**, 308–314. Doi: 10.1016/j.chemosphere.2019.05.116.

Lian J.P., Wu J.N., Zeb A., Zheng S.N., Ma T., Peng F.H., Tang J.C. and Liu W.T. (2020). Do polystyrene nanoplastics affect the toxicity of cadmium to wheat (*Triticum aestivum* L.)? *Environmental pollution, **263***, 114498. Doi: 10.1016/j.envpol.2020.114498.

Lin Y.F., Lin Y.X., Lin H.T., Zhang S., Chen Y.H. and Shi J. (2015). Inhibitory effects of propyl gallate on browning and its relationship to active oxygen metabolism in pericarp of harvested longan fruit. *LWT - Food Science and Technology*, **60**, 1122-1128. Doi: 10.1016/j.lwt.2014.10.008.

Lu Y.F., Zhang Y., Deng Y.F., Jiang W., Zhao Y.P., Geng J.J., Ding L.L. and Ren H.Q. (2016). Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (Danio rerio) and Toxic Effects in Liver. *Environmental Science and Technology*, **50**, 4054–4060. Doi: 10.1021/acs.est.6b00183.

Luo Z.B., Wu C.H., Zhang C., Li H., Lipka U. and Polle A. (2014). The role of ectomycorrhizas in heavy metal stress tolerance of host plants. *Environmental and Experimental Botany*, **108**, 47-62. Doi: 10.1016/j.envexpbot.2013.10.018.

Ma Y.N., Huang A.N., Cao S.Q., Sun F.F., Wang L.H., Guo H.Y. and Ji R. (2016). Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. *Environmental Pollution*, **219**, 166-173. Doi: 10.1016/j.envpol.2016.10.061.

Magalhaes J.V., Piñeros M.A., Maciel L.S. and Kochian L.V. (2018). Emerging Pleiotropic Mechanisms Underlying Aluminum Resistance and Phosphorus Acquisition on Acidic Soils. *Frontiers in plant science*, **9**, 1420. Doi: 10.3389/fpls.2018.01420.

Massawe V.C., Hanif A., Farzand A., Mburu D.K., Ochola S.O., Wu L.M., Tahir H.A.S., Gu Q., Wu H.J. and Gao X.W. (2018). Volatile Compounds of Endophytic Bacillus spp. have Biocontrol Activity Against Sclerotinia sclerotiorum. *Phytopathology*, **108**, 1373–1385. Doi: 10.1094/phyto-04-18-0118-r.

Mohsen M., Zhang L.B., Sun L.N., Lin C.G., Wang Q., Liu S.L., Sun J.C. and Yang H.S. (2021). Effect of chronic exposure to microplastic fibre ingestion in the sea cucumber Apostichopus japonicus. *Ecotoxicology and environmental safety*, **209**, 111794. Doi: 10.1016/j.ecoenv.2020.111794.

Natarajan L., Jenifer M.A., Chandrasekaran N., Suraishkumar G.K. and Mukherjee A. (2022). Polystyrene nanoplastics diminish the toxic effects of Nano-TiO_2 in marine algae Chlorella sp, *Environmental Research*, **204**, 112400. Doi: 10.1016/j.envres.2021.112400.

Paul-Pont I., Lacroix C., González-Fernández C., Hégaret H., Lambert C., Le-Goïc N., Frère L., Cassone A.L., Sussarellu R., Fabioux C., Guyomarch J., Albentosa M., Huvet A. and Soudant P. (2016). Exposure of marine mussels Mytilus spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. *Environmental pollution*, **216**, 724–737. Doi: 10.1016/j.envpol.2016.06.039.

Pingle S.N., Suryawanshi S.T., Pawar K.R. and Harke S.N. (2022). The Effect of Salt Stress on Proline Content in Maize (*Zea mays*) *Lafoba2*. Doi: 10.3390/environsciproc2022016064.

Qiu C.G., Wang W.D., Zhang Y.H., Zhou G.J. and Bi Y.H. (2022). Response of Antioxidant Enzyme Activities of the Green Microalga Chlorococcum sp. AZHB to Cu^{2+} and Cd^{2+} Stress. *Sustainability*, **14**. Doi: 10.3390/su141610320.

Rahdari P., Tavakoli S. and Hosseini S.M. (2012). Studying of Salinity Stress Effect on Germination, Proline, Sugar, Protein, Lipid and Chlorophyll Content in Purslane (*Portulaca oleracea* L.) Leaves. *Journal of Stress Physiology and Biochemistry*, **8**, 182-193.

Sharma A., Wang J.F., Xu D.B., Tao S.C., Chong S.L., Yan D.L., Li Z., Yuan H.W. and Zheng B.S. (2020). Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted Carya cathayensis plants. *Science of The Total Environment*, **713**. Doi: 10.1016/j.scitotenv.2020.136675.

Sun X.D., Yuan X.Z., Jia Y.B., Feng L.J., Zhu F.P., Dong S.S., Liu J.J., Kong X.P., Tian H.Y., Duan J.L., Ding Z.J., Wang S.G. and Xing B.S. (2020). Differentially charged nanoplastics demonstrate distinct accumulation in Arabidopsis thaliana. *Nat. Nanotechnol.*, **15**, 755–760. Doi: 10.1038/s41565-020-0707-4.

Thiagarajan V., Iswarya V., P.A.J., Seenivasan R., Chandrasekaran N. and Mukherjee A. (2019). Influence of differently functionalized polystyrene microplastics on the toxic effects of P_{25} TiO_2 NPs towards marine algae Chlorella sp. *Aquatic toxicology*, **207**, 208–216. Doi: 10.1016/j.aquatox.2018.12.014.

Tsikas D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges, *Analytical Biochemistry*., **524**, 13-30. Doi: 10.1016/j.ab.2016.10.021.

Tunali M., Uzoefuna E.N., Tunali M.M. and Yenigun O. (2020) Effect of microplastics and microplastic-metal combinations on growth and chlorophyll a concentration of Chlorella vulgaris. *Science of the Total Environment.*, **743**, 140479. Doi: 10.1016/j.scitotenv.2020.140479.

Velikova M., Bankova V., Sorkun K., Houcine S., Tsvetkova I. and Kujumgiev A. (2000) Propolis from the Mediterranean region: chemical composition and antimicrobial activity. *Z. Naturforsch. CJ Bioscience.*, **55**, 790–793. Doi: 10.1515/znc-2000-9-1019.

Wang J., Peng C., Li H.Y., Zhang P.P. and Liu X.H. (2021) The impact of microplastic-microbe interactions on animal health and biogeochemical cycles: A mini-review. *The Science of the total environment*, **773**, 145697. Doi: 10.1016/j.scitotenv.2021.145697.

Wang L., Gao Y.X., Jiang W., Chen J.X., Chen Y.S., Zhang X.H. and Wang G.X. (2021). Microplastics with cadmium inhibit the growth of *Vallisneria natans* (Lour.) Hara rather than reduce cadmium toxicity. *Chemosphere*, **266**, 128979. Doi: 10.1016/j.chemosphere.2020.128979.

Wen B., Jin S.R., Chen Z.Z., Gao J.Z., Liu Y.N., Liu J.H. and Feng X.S. (2018). Single and combined effects of microplastics and cadmium on the cadmium accumulation, antioxidant defence and innate immunity of the discus fish (*Symphysodon aequifasciatus*). *Environmental pollution*, **243**, 462–471. Doi: 10.1016/j.envpol.2018.09.029.

Xu L.X., Chen H., Zhang T.T., Deng Y.A., Yan J.X. and Wang L. (2022). Salicylic Acid Improves the Salt Tolerance Capacity of Saponaria officinalis by Modulating Its Photosynthetic Rate, Osmoprotectants, Antioxidant Levels, and Ion Homeostasis. *Agronomy*, **12**. Doi: 10.3390/agronomy12061443.

Zhang C., Liu F., Kong W.W. and He Y. (2015). Application of Visible and Near-Infrared Hyperspectral Imaging to Determine Soluble Protein Content in Oilseed Rape Leaves. *Sensors (Basel)*, **15**, 16576-16588. Doi: 10.3390/s150716576.

Zhang H.L., Lu L., Zhao X.P., Zhao S., Gu X.Y., Du W.C., Wei H., Ji R. and Zhao L.J. (2019) Metabolomics Reveals the Invisible Responses of Spinach Plants Exposed to CeO_2 Nanoparticles. *Environmental science and technology*, **53**, 6007–6017, Doi: 10.1021/acs.est.9b00593.

Zhang Q., Qu Q., Lu T., Ke M.J., Zhu Y.C., Zhang M., Zhang Z.Y., Du B.B., Pan X.L., Sun L.W. and Qian H.F. (2018) The combined toxicity effect of nanoplastics and glyphosate on Microcystis aeruginosa growth. *Environmental pollution*, **243**, 1106–1112. Doi: 10.1016/j.envpol.2018.09.073.

Zhang R., Wang M., Chen X.P., Yang C.M. and Wu L.L. (2020). Combined toxicity of microplastics and cadmium on the zebrafish embryos (*Danio rerio*), *Science of The Total Environment*, **743**, 140638, Doi: 10.1016/j.scitotenv.2020. 140638.

Zhang S.S., Ding J.N., Razanajatovo R.M., Jiang H., Zou H. and Zhu W.B. (2019). Interactive effects of polystyrene microplastics and roxithromycin on bioaccumulation and biochemical status in the freshwater fish red tilapia (*Oreochromis niloticus*). *The Science of the total environment*, **648**, 1431–143, Doi: 10.1016/j.scitotenv.2018.08.266.

Zhu X.L., Zhao W.H., Chen X.H., Zhao T., Tan L.J. and Wang J.T. (2020). Growth inhibition of the microalgae Skeletonema costatum under copper nanoparticles with microplastic exposure. *Marine environmental research*, **158**, 105005, Doi: 10.1016/j.marenvres.2020.105005.

Zhu Z.L., Wang S.C., Zhao F.F., Wang S.G., Liu F.F. and Liu G.Z. (2019) Joint toxicity of microplastics with triclosan to marine microalgae Skeletonema costatum. *Environmental pollution*, **246**, 509–517. Doi: 10.1016/j.envpol.2018.12.044.

Zong X.Y., Zhang J.J., Zhu J.W., Zhang L.Y., Jiang L.J., Yin Y. and Guo H.Y. (2021) Effects of polystyrene microplastic on uptake and toxicity of copper and cadmium in hydroponic wheat seedlings (*Triticum aestivum* L.). *Ecotoxicology and environmental safety*, **217**, 112217. Doi: 10.1016/j.ecoenv.2021.112217