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Effects of polystyrene microplastic on uptake and toxicity of copper and cadmium in hydroponic wheat seedlings (*Triticum aestivum* L.)

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A R T I C L E I N F O

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A B S T R A C T

Microplastics are widespread in freshwater environments, their biological effects and combined effects of other pollutants have attracted extensive attention. In this study, we investigated the adsorption properties of heavy metals onto polystyrene (PS) microplastics as well as the bioavailability and toxicity of microplastics and heavy metals by hydroponic wheat seedlings experiment. Results showed that PS microplastics (0.5 μm, 100 mg/L) had no significant effect on wheat seedlings growth, photosynthesis, and reactive oxygen species (ROS) content. However, PS microplastics could adsorb copper and cadmium, with a predominantly chemisorption. The accumulation of copper and cadmium in wheat seedlings reduced in the presence of PS microplastics, which meant the toxic effect by heavy metals might be mitigated. Compared with single heavy metals treatments, the combination of PS microplastics and heavy metals increased chlorophyll content, enhanced photosynthesis and reduced the accumulation of ROS. These findings suggest that PS microplastics (0.5 μm, 100 mg/L) have a mitigating effect on the bioavailability and toxicity of copper and cadmium.

1. Introduction

With the large-scale production and widespread applications of plastic products, increasing amounts of plastic are entering the natural environment (Belzagui et al., 2020; Veerasingam et al., 2020). Plastic debris becomes brittle and fragmented under the exposure of ultraviolet (UV) light and oxygen, as well as physical action from wind and waves (Andrady, 2015). These plastics gradually degrade into micrometer- and nanometer-scale particles. Many studies confirm the worldwide occurrence of microplastics in marine environments (Ory et al., 2020), sediments (Zhang et al., 2020), freshwater (Forrest et al., 2019), groundwater (Panno et al., 2019), and soil (Zhang and Liu, 2018). In freshwater, the main microplastics found are polypropylene (PP), polyethylene (PE), polystyrene (PS), and polyethylene terephthalate (PET) (Li et al., 2019a).

Microplastics affect plants in the environment (Prokić et al., 2019). Qi et al. (2018) found that both low-density PE and biodegradable plastic mulch films of macro- and micro-size were harmful to wheat (*Triticum aestivum*) growth. One of the toxicity mechanisms of microplastics to plants is the blockage of the root surface pores caused by adhesion to plant roots, which affects adsorption of nutrients (Gao et al., 2019b; Jiang et al., 2019). For nanoplastics, the uptake and accumulation of nanoplastics may affect the transport of nutrients. For instance, 100-nm PS nanoplastics block cell connections or cell wall pores, causing oxidative damage and genotoxicity in fava beans (*Vicia faba*) (Jiang et al., 2019). The adsorption and aggregation between microplastics and microalgae are also considered as the reasonable explanations for toxic effects of microplastic on marine microalgae (Zhang et al., 2017). Meanwhile, PE and biodegradable plastic mulches improve raspberry plant growth and yield (Zhang et al., 2019a). These findings suggest that the toxicity of microplastics may be related to their type, particle size and concentration.

Many experiments have demonstrated the adsorption of metals on microplastics. For example, Cu^{2+} and Zn^{2+} can adsorb on the surface of PET microplastics (Wang et al., 2020b). The adsorption isotherm of Cd^{2+} onto PE microplastics follow Langmuir model (Zhang et al., 2019b), while Freundlich model is more suited for that of Cd^{2+} onto PET microplastics (Abbasi et al., 2020). It has been found that the adsorption of cadmium onto microplastics is closely related to the functional groups on the surface of microplastics, the solution pH and the ionic strength (Guo et al., 2020; Li et al., 2019c). Microplastics may serve as "transfer carriers" for the pollutants adsorbed on them and improve the

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bioavailability of the pollutants once they enter the organism. Hodson et al. (2017) proved that microplastics could be used as a Zn carrier to increase metal exposure in earthworms. High Density Polyethylene (HDPE) as vectors facilitate the entrance of Hg in mussel but also promote Hg elimination (Fernández et al., 2020). Nevertheless, Wen et al. (2018) found that the adsorption of Cd onto PS microplastics resulted in a decrease of Cd accumulation in the discus fish (*Symphysodon aequifasciatus*), while the co-exposure induce severe oxidative stress and stimulated innate immunity. The toxic effects of other pollutants may be enhanced in plants as well, when microplastics coexist (Dong et al., 2020). The PS-metal combination (Cu, Zn, Mn) show greater inhibition than single PS microplastics on the growth and chlorophyll a content of *Chlorella vulgaris* (Tunali et al., 2020). However, some studies have found that microplastics can reduce or not affect the toxic effects of other pollutants. Davarpanah and Guilhermino (2015) confirmed that microplastics could actually decrease the Cu-induced inhibition of the average growth rate of the microalga (*Tetraselmis chuii*). The results of Li et al. (2020a) showed that PVC microplastics didn't significantly affect the absorption and biological effects of cadmium in mussel.

Heavy metals in water mainly come from industrial discharge, metal mining, improper use of chemical fertilizers and pesticides and atmospheric deposition of heavy metals (Khan et al., 2019; Patinha et al., 2018; Shahid et al., 2017). The accumulation of heavy metals may destroy enzyme activity, leading to impaired biological growth and metabolism, as well as oxidative damage (Esmaeilzadeh et al., 2017; Kovacevic et al., 2020). Moreover, heavy metals can biomagnify along the food chain, ultimately threatening human health (Tsybekmitova et al., 2017). Many studies have demonstrated that Cu and Cd affect chlorophyll content and increase reactive oxygen species (ROS) content, which indicated that they are toxic for photosynthesis and antioxidant systems (Rombel-Bryzek et al., 2017; Zhang et al., 2016). At present, research on the combined effects of microplastics and heavy metals attracts broad attention. In this study, we investigate the adsorption of Cu and Cd on PS microplastics. Meanwhile, we explore the biological effects of PS microplastics and effects of PS microplastics on the bioavailability of heavy metals by hydroponic wheat seedlings experiment.

2. Materials and methods

2.1. Synthesis of PS microplastics

PS microplastics were synthesized from styrene (99.5%; Aladdin Reagent Co., Ltd., Shanghai, China) according to a procedure described by Tian et al. (2019). Distilled water (140 mL) was placed in a three-necked flask and heated to 70 °C. After blowing nitrogen gas to remove oxygen, 10 g of styrene was added to the flask. The mixtures were stirred for 10 min and then charged with 10 mL of a solution containing 230 mg of $K_2S_2O_8$ (99.7%; Shanghai Sihewei Chemical Co., Ltd., Shanghai, China). Nitrogen was blown constantly, the temperature was charged at 70 °C, and the stirring speed was 140–150 rpm. After 24 h of reaction, the emulsion product was PS. The PS emulsion was poured into a 50-mL centrifuge tube, cooled to 25 °C, and frozen at − 40 °C for 2 h. The solid was then melted and sonicated for 10 min to allow for even dispersion of the mixture. The emulsion was centrifuged at 10,000 rpm and 4 °C for 20 min. It was then washed with ethanol (99.7%; Aladdin Reagent Co., Ltd., Shanghai, China) (20 mL × 4) and water by re-suspension and centrifugation to remove unreacted $K_2S_2O_8$ and styrene. After 16 h of drying in an oven, PS microplastic products were obtained.

The shapes of the products were characterized by scanning electron microscopy (SEM) (S-3400N II; Hitachi, Japan), operated at a 20 kV accelerating voltage. The composition of products were identified using Fourier-transform infrared spectroscopy (FTIR) (Nexus 870 spectrometer; Nicolet, USA) with attenuated total reflection (ATR) detection mode, and the acquisition spectral range is from 4000 to 500 cm^{-1}. For X-ray diffraction (XRD) (X'TRA; ARL, Switzerland) measurement, its operating voltage is 40 kV, current is 100 mA, and the scanning range is from 3.00° to 50.00°. The hydrodynamic size and zeta potential were analyzed using a Zetasizer Nano ZS-90 (Malvern Instruments Ltd., Malvern, UK).

2.2. Microplastics adsorption kinetics

2 mg/L Cd^{2+} adsorption solution was prepared. 1 mg of PS was weighed in a 20-mL glass bottle, and approximately 20 mL of the adsorption solution was added. The mixture was sonicated for 10 min for full dispersal, and then oscillated at 25 ℃ and 200 rpm in the dark. Samples were taken at 0.5, 1, 2, 3, 5, 7, 12, and 24 h. Each treatment had three replicates. Samples then passed through a 0.22 µm filter membrane. These steps were repeated using 2 mg/L Cu^{2+} adsorption solution.

The adsorption amount, Qt (mg/g), of PS for heavy metal ions was calculated as:

$$Q_t = \frac{C_0 \times V_0 - C_t \times V_t}{M} \tag{1}$$

where C_0 (mg/L) is the initial concentration of heavy metal ions; C_t (mg/L) is the concentration of heavy metal ions after t(h); V_0 (L) is the volume of the adsorption system before adsorption; V_t (L) is the volume after adsorption; and M (g) is the mass of PS.

The kinetics of Cu^{2+} and Cd^{2+} adsorption were modeled by applying the pseudo-first-order kinetic model (PFOM) and pseudo-second-order kinetic model (PSOM), expressed as follows:

$$\log(Q_e - Q_t) = \log Q_e - k_1 \times t \tag{2}$$

$$\frac{t}{Q_t} = \frac{1}{k_2 \times Q_e^2} + \frac{1}{Q_e} \times t \tag{3}$$

where Q_t (mg/g) is the adsorption amount of heavy metal ions to PS after t (h); Q_e (mg/g) is the adsorption amount of PS to heavy metal ions at adsorption equilibrium; k_1 is the primary adsorption rate constant; and k_2 is the secondary adsorption rate constant.

2.3. Hydroponic wheat seedlings experiment

To examine microplastic toxicity and the effect of microplastic adsorption on heavy metal toxicity to wheat seedlings (Huaimai 20) (compared with the effects of single heavy metals), five treatment groups were set up with 10% Hoagland culture medium (purchased from Qingdao Hi-Tech Industrial Park Haibo Biotechnology Co., Ltd, Qingdao, China) as follows: 100 mg/L PS, 2 mg/L Cu^{2+}, 1 mg/L Cd^{2+}, 100 mg/L PS and 2 mg/L Cu^{2+}, 100 mg/L PS and 1 mg/L Cd^{2+}. The control groups were only subjected to 10% Hoagland culture medium. Each group was sonicated for 30 min to evenly disperse the PS microplastics and then oscillated at 200 rpm at 25 ℃ for 2 days, the pre-adsorption of the culture system was complete. The plump wheat seeds were sterilized and rinsed, soaked for 10 h, and then put in a 20 ℃ incubator without light and accelerated germination. When the seedlings grew to about 5 cm, those with satisfactory, similar growth were selected and transferred to the culture medium with complete pre-adsorption. Six strains of wheat seedlings were cultured in each system, and the medium was supplemented regularly. The culture temperature was 25 ℃, and the daily light period was 12 h. All groups were prepared in triplicate and placed in random positions throughout the incubator.

2.4. Metal content of wheat seedlings

Metal element content was determined by wet digestion. After 8 days of culture, the wheat seedlings were dried to a constant mass. The 0.2 g dry sample was weighed and placed in a tall form beaker. After adding concentrated nitric acid and perchloric acid (3:1), the mixture was evenly mixed and put on an electric heating plate for digestion at 160 ℃. The fully digested solution was transferred to a 15 mL volumetric flask, which was mixed at a 15 mL and then passed through a 0.22 μm filter membrane. The determination was made with an atomic absorption spectrometer (M Series; Thermo, USA). The settings were as follows: air flow rate of 5.0 L/min, acetylene of 2.0 L/min, wavelength of hollow cathode lamp of 327.4 nm (Cu) and 228.8 nm (Cd), lamp current of 9 mA, and spectral passband of 0.7 nm.

The metal translocation factor from roots to leaves was calculated as follows:

$$\text{translocation factor} = \frac{[metal]_{leaves}}{[metal]_{roots}}.$$

2.5. Chlorophyll content and photosynthetic parameters

Firstly, 0.2 g of fresh wheat seedling leaves were ground in liquid nitrogen to homogenate, 1.5 mL of anhydrous ethanol and 80% acetone (1:1) extract was added, and the sample was placed in darkness for 12 h. Following these steps, chlorophyll measurements were made with a multimode reader (M200 PRO; TECAN, Shanghai, China) under 649 nm and 665 nm. Ethanol was used for blank correction. The formulas for calculating chlorophyll a (C_a) and chlorophyll b (C_b) are as follows:

$$C_a = 13.95A_{665}\text{-}6.88A_{649} \quad (8)$$

$$C_b = 24.96A_{649}\text{-}7.32A_{665} \quad (9)$$

Photosynthetic parameters were measured with a Li-6800 photosynthetic analyzer (LI-COR, USA) from 8:30 a.m. to 11:30 a.m. on sunny days. The wheat seedlings were moved to an area with sunlight for more than 30 min. When the leaves of the seedlings were fully expanded, three functional leaves with satisfactory growth were selected in each treatment, and the indexes—net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr)—were measured. The photosynthetic effective radiation was set at 400 mol/(m^2 s), and the CO_2 concentration was 1145 μmol/mol.

2.6. ROS content

For each treatment, 0.2 g samples of fresh wheat seedling leaves were ground in liquid nitrogen after 8 days of culture. The leaf powder was quickly transferred to a centrifuge tube at low temperature, and 15 mL of 50 mM PBS buffer was added. The sample was centrifuged at 4 ℃ for 15 min at 12,000*g*, and the supernatant was used for ROS determination. ROS content was determined using a ROS assay kit (chemical fluorescence method), which was purchased from Nanjing Jiancheng Bioengineering Institute.

2.7. Statistical analysis

The data were expressed as the means ± standard deviation of three replicates. Statistical differences among treatments were determined using independent sample *t*-tests by IBM SPSS Statistics 19.

3. Results

3.1. Characterization of PS

According to SEM images, the PS was spherical and the surface was relatively smooth (Fig. 1A). The FTIR (Fig. 1B) showed that the four peaks between 2800 and 3100 cm^{-1} corresponded to PS stretching bands. The peaks at 2920 and 2850 cm^{-1} corresponded to CH_2 stretching. Three peaks at 1600, 1490, and 1450 cm^{-1} indicated benzene ring stretching vibration. The two sharp peaks at 756 and 704 cm^{-1} indicated aromatic C-H out-of-plane bending vibration. These observations confirmed that the sample was PS (Brijmohan et al., 2005; Syakti et al., 2017). In the XRD image (Fig. 1C), amorphous halos appeared at 19.32°, which was consistent with PS (Sudha and Sivakala, 2009). The hydrodynamic particle size of PS was 584.2 ± 2.0 nm, and the zeta potential was − 37.2 ± 0.4 mV. Because the zeta potentials of all particles were lower than − 30 mV in the aqueous phase (Angel et al., 2015), the dispersion system was stable according to DLVO theory.

3.2. Heavy metal adsorption on PS

The adsorption of Cu^{2+} and Cd^{2+} on PS at different times was shown in Fig. 2. Adsorption occurred rapidly—within 3 h. The adsorption capacity of Cd^{2+} on PS reached about half of the total adsorption capacity. Within 3–7 h, adsorption rate decreased gradually. After 7 h, adsorption basically reached equilibrium. The adsorption curves of PS to Cu^{2+} were similar. The PFOM and PSOM were applied to describe the adsorption kinetics data. According to the kinetic parameters (Table 1), the value of R^2 was above 0.99 in the case of PSOM. Besides, the calculated Q_e value of Cd^{2+} was approximately 25.41 mg/g, which agreed with the experimental data of 25.40 ± 0.10 mg/g, while Cu^{2+} was approximately 26.20 mg/g to 26.17 ± 0.51 mg/g. These results suggested that PSOM were suitable to the adsorption data, and supported the assumption that the rate-limiting step of Cu^{2+} and Cd^{2+} onto PS may be chemisorption process.

3.3. Heavy metal accumulation in wheat seedlings

The contents of heavy metals were measured in wheat roots and leaves across all the treatments (Fig. 3A and B). In wheat roots, the Cu and Cd contents were 299.0 and 155.4 μg/g DW, respectively, and the Cu and Cd contents were 270.5 and 138.9 μg/g DW in the presence of PS, respectively, which represented significant decreases of 9.514% ($p < 0.05$) and 10.63% ($p < 0.05$). Similarly, in wheat leaves, the Cu and Cd contents were 29.52 and 15.68 μg/g DW, respectively, while the Cu and Cd contents were 20.72 and 11.86 μg/g DW when PS was added, respectively, which reflected significant decreases of 29.80% ($p < 0.05$) and 24.33% ($p < 0.05$). The translocation factor from roots to leaves was calculated (Fig. 3C and D). The transports of Cu and Cd were both decreased in the presence of PS microplastics. This may be the self-protective mechanism of wheat seedlings. When the metal content absorbed by roots decreased, wheat seedlings preferentially reduced the transport to the above-ground parts, and weakened the toxicity of metal on shoots. The specific mechanism needs further research.

3.4. Root and shoot length

The root and shoot lengths of wheat seedlings in each group were shown in Fig. 4. Single PS addition showed no clear effects on root and shoot lengths relative to the control. There was significant inhibition in root length in the Cu and Cd treatments compared with the control, however. No difference was found in the presence of PS. For shoot length, the Cu treatment showed significant inhibition compared with the control ($p < 0.05$), while the Cd treatment showed slight, but not statistically significant, inhibition. Both of these inhibitions were significantly relieved when PS was present ($p < 0.05$). In connection with the element content, the metal content in the root was probably high and thus had a significant inhibitory effect on root length. During growth, metal was transported from root to shoot and accumulated in the stem, which also inhibited shoot length. However, the Cd content in the stem may be within the tolerance content range of wheat seedlings, such that the inhibitory effect was not significant. With the addition of microplastics, the metal accumulation in roots and shoots decreased, and the inhibitory effects on root and shoot lengths were alleviated.

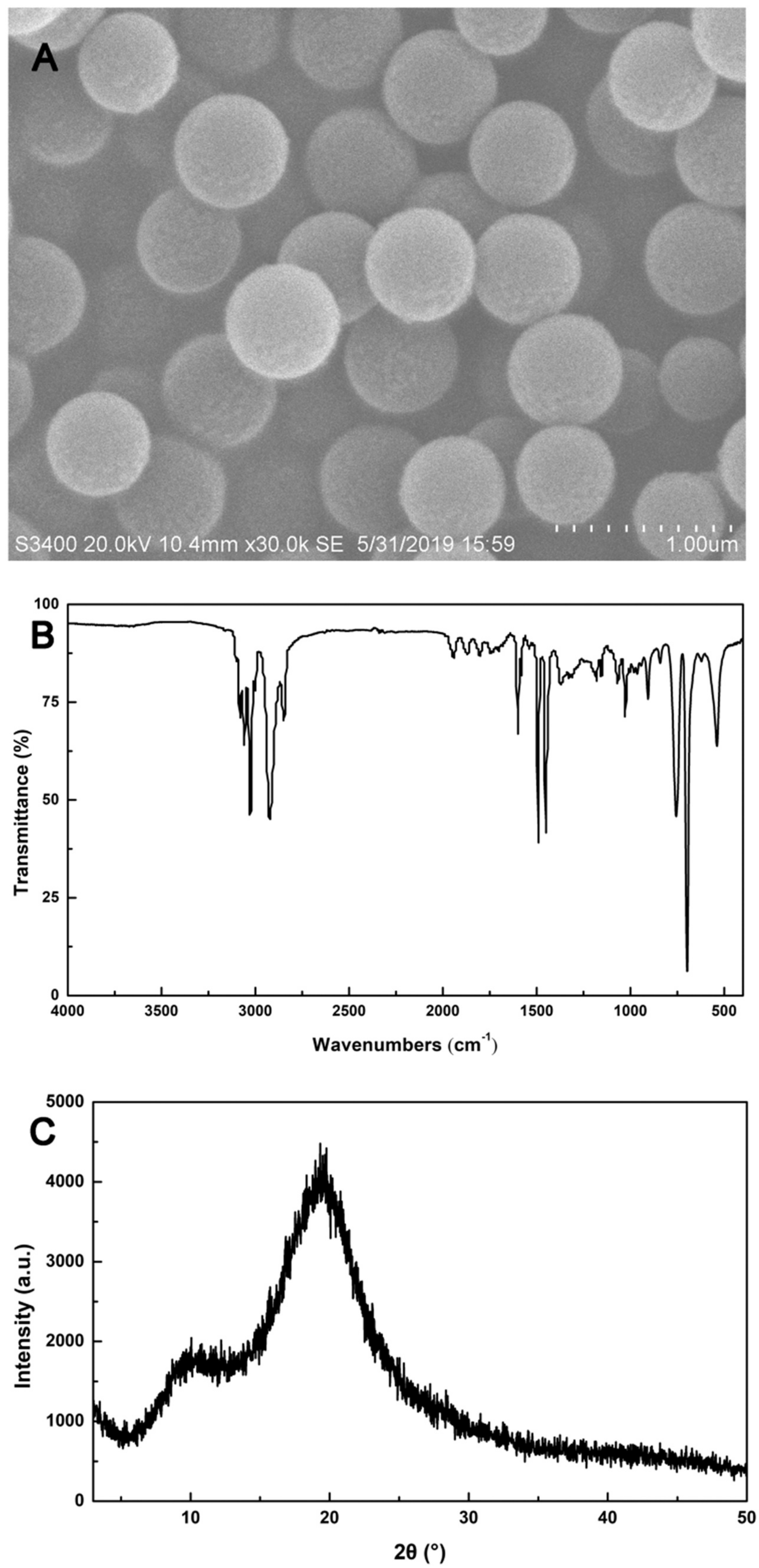

Fig. 1. SEM images (A), FTIR absorption spectra (B), and XRD pattern (C) of PS.

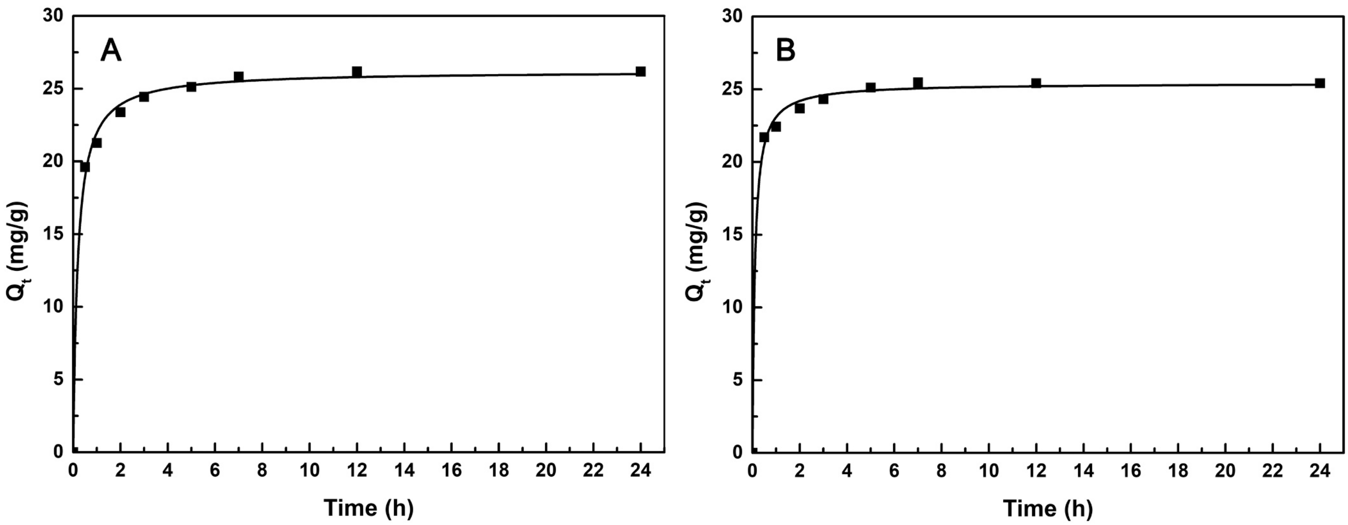

Fig. 2. Adsorption kinetics of Cu^{2+} (A) and Cd^{2+} (B) on PS (with PSOM curves).

Table 1
Pseudo-first-order kinetic model (PFOM) and pseudo-second-order kinetic model (PSOM) parameters for adsorption of Cu^{2+} and Cd^{2+} onto PS microplastics.

		Cu^{2+}	Cd^{2+}
PFOM	Q_e (mg/g)	25.04	24.68
	k_1	2.663	3.908
	R^2	0.9764	0.9889
PSOM	Q_e (mg/g)	26.20	25.41
	k_2	0.1986	0.3909
	R^2	0.9967	0.9971

3.5. Photosynthesis

Chlorophyll content (the content of chlorophyll a and b), chlorophyll a/b ratio, Tr, Pn, Gs and Ci were measured as indicators of photosynthesis performance in wheat seedlings. Chlorophyll data and other photosynthetic parameters from experimental plants were shown in Fig. 5.

The chlorophyll content and chlorophyll ratio were used as stress indicators. Chlorophyll content and chlorophyll a/b ratio both had no significant differences between the control and single PS group. Chlorophyll content and chlorophyll a/b ratio were significantly decreased when treated with either Cu or Cd, which meant chlorophyll content decreased under the action of heavy metals, and chlorophyll a decreased faster than chlorophyll b. When PS and metal co-existed, chlorophyll content significantly increased compared with the treatments with a single metal. This result was consistent with the lower heavy metal accumulation in leaves when PS was present. The difference was that the chlorophyll a/b ratio did not change in the PS-Cu group, whereas it significantly decreased in the PS-Cd group.

Similarly, the Tr, Pn, Gs, and Ci of wheat seedlings remained unchanged in the single PS treatment group, while they were significantly decreased in the treatments with only Cu or Cd. When treated with PS and metal, all four photosynthetic parameters increased compared with those of the single metal treatment groups. The Cd and PS treatment increased the Pn, Gs, Ci, and Tr of wheat seedlings by about 2.036%, 5.551%, 4.430%, and 11.28%, respectively, compared with those of the Cd treatment group. The Cu and PS treatment slightly increased the Pn, Gs, Ci, and Tr concentration by 1.172%, 13.77%, 15.90%, and 22.42%, respectively, compared with the Cu treatment group.

3.6. ROS content

ROS content was determined to reflect the degree of oxidative damage in wheat seedlings (Fig. 6). In the PS group, ROS content was not significantly different compared with the control. The ROS content under either the Cu or Cd treatment increased by 2.093 times and 1.821 times ($p < 0.05$) compared with the control group. Comparing the PS and metal treatment with the Cu and Cd metal treatments, the ROS content decreased by about 13.09% and 2.542% ($p < 0.05$), respectively.

4. Discussion

In our study, it was observed that PS microplastics (0.5 μm, 100 mg/L) did not affect growth, photosynthesis and ROS content on wheat seedlings. It was consistent with the research that PS nanoplastics (100 nm, 0.01–10 mg/L) had no effect on seed germination and even enhanced seeding growth (Lian et al., 2019). This phenomenon was also found in a study that PE microplastics (1–5 μm, 0.046–1.472 mg/L) had no significant effects on the growth rate of microalgae *Tetraselmis chuii* (Davarpanah and Guilhermino, 2015). However, some studies reported that the eco-toxicity of microplastics on plants (Jiang et al., 2019; Qi et al., 2018). This difference may be related to the size and concentration of microplastics studied. For example, 200 nm PS microplastics could enter lettuce from root and transport to stems and leaves, while 1 μm could not (Li et al., 2019b). HDPE microplastics (3 μm) can neither reach the vascular system nor translocate to the shoots (Urbina et al., 2020). The confocal images of Arabidopsis (*Arabidopsis thaliana*) and wheat (*Triticum aestivum*) showed no active absorption of 40 nm and 1 μm PS spheres by plants (Taylor et al., 2020). In this study, the microplastics had a particle size of about 500 nm and could not be absorbed by wheat, which might explain their unsignificant ecological effect on wheat seedlings. In addition, although microplastics were too large to be absorbed by plants, it could affect the soil density, water holding capacity, microbial activity in soil, and further the availability of nutrients (Machado et al., 2018, 2019), which may consequently cause damage to plants.

Our study showed that PS microplastics (0.5 μm, 100 mg/L) can adsorb Cu^{2+} and Cd^{2+} in solution. Some studies revealed that the adsorption and release of heavy metals by microplastics was related to the concentration, adsorption time and particle size of the metals (Town et al., 2018). Biofilms can also enhance the adsorption capacity of microplastics for heavy metals in solution, mainly through the complexation of the functional groups contained in the biofilm with

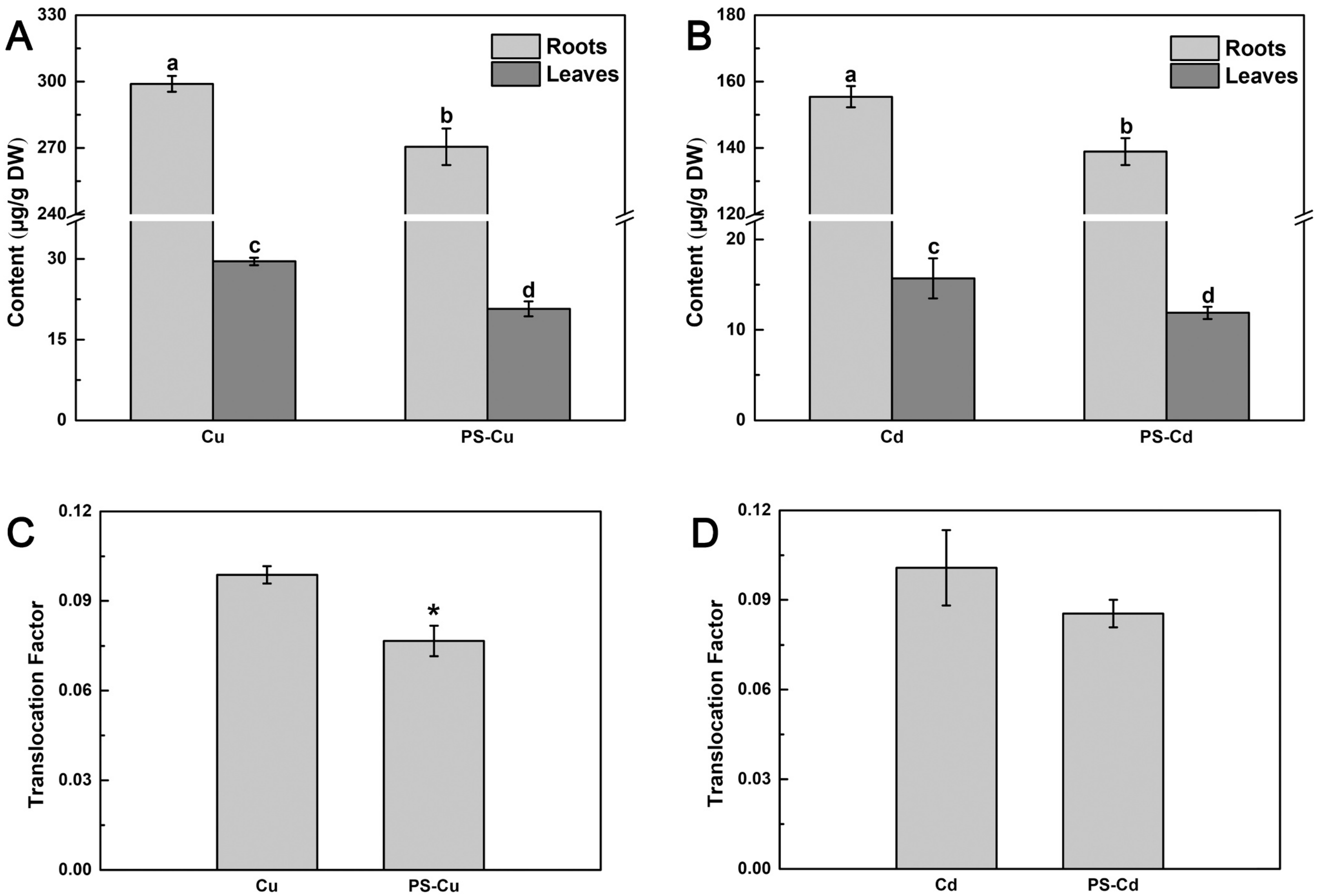

Fig. 3. Contents of Cu (A) and Cd (B) in roots and leaves. Translocation factors of Cu (C) and Cd (D) in wheat seedlings. Different letters indicate statistically significant differences ($p < 0.05$).

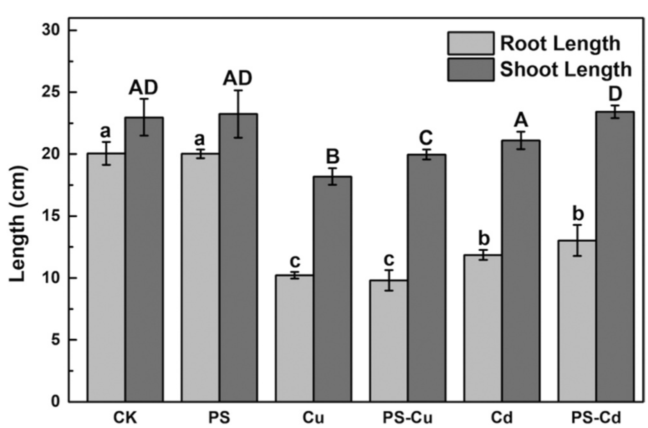

Fig. 4. Root and shoot length for all treatments. Different letters indicate statistically significant differences ($p < 0.05$).

heavy metals (Guan et al., 2020; Qi et al., 2021). Gao et al. (2019a) showed that microplastics had adsorb ability toward different heavy metals (Pb, Cu, and Cd), and the smaller size of the microplastics, the stronger adsorption capacity. At the same time, in an aqueous environment, as the particle size decreases, the desorption of adsorbed substances from the microplastics also increased (Town et al., 2018). When the size of the microplastics was small enough to enter the plant, the metal ions adsorbed by microplastics would be released, which may increase the accumulation of heavy metals in plant. The experiment in this study was in solution, but in fact, the cultivation of wheat is mostly in the soil environment. The environmental behaviors of microplastics and heavy metals in the soil were complicated. The adsorption or desorption of heavy metals by microorganisms in the soil was also related to soil aggregates, the metals and their concentration (Li et al., 2020b; Yu et al., 2021). PE microplastics (100 μm, 28%) promoted the conversion of heavy metals from bioavailable fractions to organic-bound fractions, which may reduce their bioavailability (Yu et al., 2021). Li et al. (2021) found that the concentration also affected the adsorption capability of soil to metals, like that high concentration (10%) of PE microplastics would decrease the adsorb ability and increase the mobility of metals, but low concentration (0.1%) had no significant influence on metal bioavailability.

Under heavy metal stress, heavy metals accumulated in wheat roots and leaves, while the content of copper and cadmium significantly reduced in the presence of PS. This phenomenon that microplastics decreased the accumulation of heavy metals was also found in an experiment involving polylactic acid (PLA) dose of 10% and cadmium on maize (*Zea mays* L.) in soil (Wang et al., 2020a). This phenomenon may be due to PS microplastics could adsorb Cu^{2+} and Cd^{2+} in solution which was demonstrated in this study, resulting in a decrease in the concentration of Cu^{2+} and Cd^{2+} in solution, and the content of Cu^{2+} and Cd^{2+} that wheat seedlings roots absorbed was also reduced. Furthermore, PS microplastics could accumulate at the root surface, and they might compete with heavy metals for their adsorption sites (Dong et al., 2020; Taylor et al., 2020).

The toxic effect of heavy metals on plants was closely related to the accumulation of metals in plants (Adejumo et al., 2018; Wang et al., 2019). In the present study, not only root and shoot length but also photosynthetic functions were invariably affected by heavy metal stress.

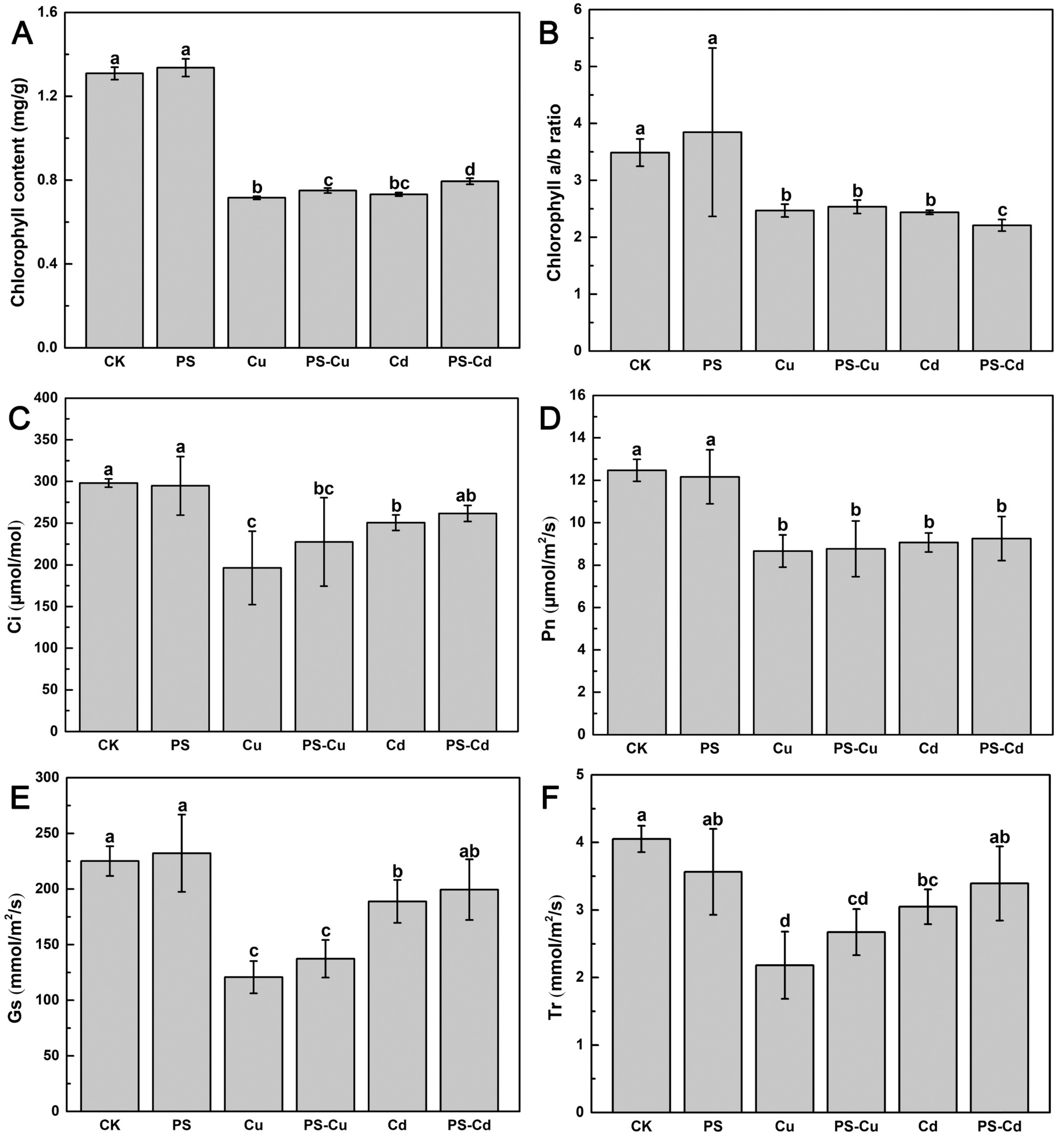

Fig. 5. Chlorophyll content (A), chlorophyll a/b ratio (B), inter-cell CO_2 concentration (Ci; C), photosynthesis rate (Pn; D), stomatal conductance (Gs; E), and transpiration rate (Tr; F) for all treatments. Different letters indicate statistically significant differences ($p < 0.05$).

Like that Pn was significantly decreased in Cu or Cd treatment, which indicated a reduction in the rate of photosynthesis, which was consistent with a significant reduction in chlorophyll content. According to the results of the root length, shoot length, Ci, Gs, Tr, and ROS, the effect of Cu^{2+} was greater than that of Cd^{2+}. On the one hand, the initial concentrations in the culture medium of Cu^{2+} and Cd^{2+} were 2 mg/L and 1 mg/L, respectively. On the other hand, the mechanisms of Cu^{2+} and Cd^{2+} affecting photosynthesis and antioxidative response were different. Cu could inhibit the activity of photosystem I (PSI) and photosystem II (PSII) to affect photosynthesis (Wu et al., 2020). Cd was shown to replace magnesium in chlorophyll molecule, which was one of the reasons for the inhibition of photosynthesis (Grajek et al., 2020). Thus, the degree of damage to photosynthesis may be different. Besides, it was reported that Cu induced the activation of the antioxidative response more than Cd (Chamseddine et al., 2009), which may be one of the reasons that ROS content was greater under Cu stress.

The accumulation of metals in wheat seedlings decreased by the presence of PS microplastics. Thus, these inhibitions on wheat seedlings were mitigated. For instance, the decreased metal content was consistent with the increased shoot length in the presence of PS microplastics.

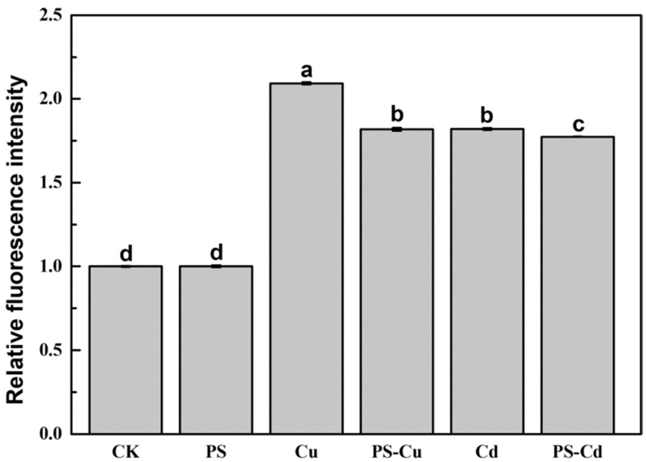

Fig. 6. ROS content (represented by the relative fluorescence intensity compared with the control group) of all treatments. Different letters indicate statistically significant differences ($p < 0.05$).

PE microplastics also presented the decrease of copper-induced inhibition on microalgae growth (Davarpanah and Guilhermino, 2015). In addition, the chlorophyll a/b ratio did not change in the PS-Cu group, whereas it significantly decreased in the PS-Cd group. It is reported that wheat was more sensitive to cadmium than copper (Metwali et al., 2013). In other words, the accumulation of cadmium in wheat seedlings decreased when PS was present, leading to the increased chlorophyll content. Given oxidative stress from exposure to Cu and Cd on wheat (Pena et al., 2015), ROS content was determined to investigate the antioxidant status. Results showed that the ROS content increased under Cu and Cd exposure, respectively, indicating the occurrence of oxidative stress. Similarly, comparing the PS and metal treatment with the Cu and Cd metal treatments, the ROS content decreased, which was mainly due to the reduced accumulation of metals in wheat seedlings. This result was similar to a study that Si nanoparticles could alleviate oxidative damage caused by Cd because of the lower contents of Cd in grains (Hussain et al., 2019). It was reported that the presence of PS microplastics may promote the capture of free radicals on organic molecules and enhance cell metabolism of sugars and amino acids that were important for plants to remove elevated ROS (Lian et al., 2020). The latter may be a metabolic mechanism by which microplastics reduced heavy metal-induced oxidative stress in wheat seedlings.

In conclusion, the adsorption of Cu^{2+} and Cd^{2+} onto PS microplastics in aqueous solution conformed to the pseudo-second-order kinetic model, which indicated that chemisorption was dominant. PS microplastics (0.5 µm, 100 mg/L) had no significant effect on hydroponic wheat seedlings. However, the combination of PS and heavy metals significantly reduced the accumulation of heavy metals in wheat seedlings, increased chlorophyll content and reduced the accumulation of ROS. These results suggested that the toxic effects of heavy metals on hydroponic wheat seedlings were reduced due to the adsorption of heavy metals on PS microplastics. The microscopic mechanism of the combined action of microplastics and metals needs further study.

CRediT authorship contribution statement

Xueying Zong: Investigation, Writing - original draft and Editing. **Juanjuan Zhang, Jinwei Zhu, and Linyu Zhang**: Investigation. **Lijuan Jiang**: Writing - reviewing & editing. **Ying Yin**: Conceptualization, Methodology, Writing - reviewing & editing. **Hongyan Guo**: Writing - reviewing & editing. All authors contributed to the subsequent development and approved the final manuscript.

Declaration of Competing Interest

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

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