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Altered biotoxicity of cadmium to freshwater green algae by different concentrations of polystyrene

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Abstract. Microplastic pollution has become a global problem, threatening water bodies and aquatic organisms around the world. Heavy metal pollution in the aquatic environment is an environmental issue of long-term concern, and relatively few studies have been conducted on the compound toxic pollution of freshwater algae by microplastics and heavy metals. Therefore, in this study, Cd and polystyrene (PS) were selected as representative heavy metals and microplastics in the environment, and the toxic effects of the two pollutants on freshwater algae were investigated by determining the biomass of algal growth, chlorophyll a content, and the bioconcentration of heavy metals. It was found that the two pollutants alone could inhibit the growth and photosynthesis of the algae, and the toxicity increased with the increase of concentration. When the two pollutants were combined, the toxicity of Cd depended on the concentration of PS, and both low (1 mg/L) and high (100 mg/L) concentrations of PS increased the toxicity of Cd in a synergistic manner, whereas the medium concentration (10 mg/L) of PS showed an antagonistic effect, which was able to inhibit the toxicity of Cd.

Keywords: Heavy metal, Microplastic, Freshwater green algae, combined toxicity.

1. Introduction

Plastic pollution is a global problem, with between 4.8 and 12.7 million tons of plastic being released into water bodies each year, threatening terrestrial and marine ecosystems around the world [1]. Plastics are broken down in the natural environment into tiny plastic particles with diameters ranging from 100 nm to 5 mm, known as microplastics (MPs) [1,2]. Due to the inert nature of microplastics, they can accumulate and become widely distributed in the environment, and MPs are currently found in a variety of freshwater and marine ecosystems, including lakes, rivers, deep-sea and continental shelf oceans [3]. Common types of MPs found in water are polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC) [2]. In natural aquatic ecosystems, MPs can be widely distributed in different water layers, and due to their similarity in size and appearance to zooplankton, MPs are easily taken up by aquatic organisms [4,5]. Detection of microplastic content in fish, shellfish, crustaceans, echinoderms and coelenterates revealed the presence of MPs in all biological samples, covering almost all trophic levels [3,6].

In aquatic environments, heavy metals can likewise have deleterious effects on aquatic organisms [7,8]. Heavy metals could interfere with the metabolism of aquatic plants, inhibit their photosynthesis, cause membrane damage, alter the structure of DNA, disrupt energy transfer, affect protein synthesis, and increase mortality [8,9]. Under high concentrations of heavy metal stress, the reactive oxygen species content in aquatic plant cells exceeds the cellular tolerance, which will cause cellular homeostasis disorders [9]. Under low concentrations of heavy metal stress, aquatic plants, especially algae, will accumulate heavy metals and transfer them to other trophic levels through the food chain, which will have a wider impact on aquatic ecosystems [10].

Notably, MPs and heavy metals co-exist and interact in aquatic environments, and their combined pollution of aquatic ecosystems has attracted global attention [11]. Freshwater green algae, one of the most important primary producers in aquatic ecosystems, are widely found in water bodies around the world and play an important role in maintaining the structure and function of the entire aquatic ecosystem [6,11]. Currently, relatively few studies have been conducted on the combined toxicity of MPs and heavy metals to microalgae, and there is still some controversy about their mutual toxic effects [11]. For example, the results of Wan et al. showed that copper and copper-microplastic combinations did not exhibit significant differences on the growth of the freshwater green alga *aphidocelis subcapitata* [12]. On the contrary, other studies have shown that the coexistence of microplastics and contaminants increases the toxicity of the contaminants due to increased bioaccumulation or uptake in the organisms. For example, Wang et al. demonstrated that the addition of 5 μm diameter microplastics increased the accumulation of Cd in microalgae, while microplastics were able to induce more severe oxidative damage [6]. Therefore, it is of great significance to investigate the toxic effects of microplastics and heavy metals alone and in combination on freshwater algae, which can provide important information for the management of microplastics in the aquatic environment and ecological risk assessment. In this study, PS and Cd were selected as representative microplastics and heavy metals for the toxicity investigation, mainly due to their high detection rate and high toxicity in the natural environment.

2. Data and methods

2.1. Algal culture and toxicity experiments

The experimental algae were cultured using BG-11 medium. Cd stock solutions were prepared using $CdCl_2$ (analytical grade) samples purchased from Sigma-Aldrich Company (USA).PS samples were purchased from Aladdin Biochemical Technology Co. Ltd. in Shanghai, China, and the sizes were 250 μm, respectively. The microplastic powders were dissolved in ultrapure water and dispersed homogeneously in an ultrasonic cleaner. A total of eight experimental groups were set up in this study, and the initial algal cell density of each experimental group was controlled to be 1×10^6 cells/mL, and three parallels were set up for each treatment, and the 16 experimental treatment groups were as follows: group 1 was a blank control group (no Cd and PS were added); group 2 was exposed to Cd alone (0.1 mg/L, 0.5 mg/L, and 1 mg/L); and groups 3-5 were exposed to PS alone (1 mg/L, 10 mg/L, and 100 mg/L), and groups 6-8 were exposed to a mixture of Cd and PS (where the Cd concentration was fixed at 0.5 mg/L and the PS concentrations were 1 mg/L, 10 mg/L, and 100 mg/L). The solution pH was varied in the range of 7.0 to 7.6 throughout the experiment. Where the incubation temperature was 25°C, the light intensity was 4000 lux, the light-dark ratio was 12h: 12h, and shaking was performed three times a day to prevent precipitation. In this experiment, the exposure concentration of Cd was 0.1-1 mg/L and that of PS was 1-10 mg/L. These two concentration ranges were chosen because of the environmental relevance of the low concentration treatment, which was aimed at exploring whether ambient concentrations of pollutants could affect algae, while the high concentration was chosen to simulate the characteristics of the biological response under special extreme pollution emission scenarios, such as point-source emissions from factories [13].

2.2. Determination of toxicity indicators

2.2.1. Cell density. At the end of one week's toxicity exposure, deionised water was used as a control, and the shaken homogeneous algal cell solution was poured into a 1 cm quartz cuvette and the absorbance was measured at 680 nm on a UV spectrophotometer, which is representative of the density of algal growth [11].

2.2.2. Chlorophyll a concentration. Chlorophyll a concentration was determined according to the national standard hot ethanol method. The procedure was as follows: firstly, 10 mL of algal samples were measured and centrifuged at 4500 rpm/min for 15 min, and after centrifugation, the medium solution was removed; in the centrifuge tubes, 10 mL of ethanol solution preheated at 85 ℃ was added and put into the water bath for extraction, which took a total of 30 min. At the end of extraction, the algal extracts were shaken well and centrifuged at 4500 rpm/min for 20 min, and the supernatant was collected for the determination of chlorophyll a. The calculation formula was based on the previous study [10].

2.3. Statistical analyses

Three replications were performed in this study and the results are presented as mean ± standard deviation. All the glass instruments used were soaked in 10% HNO_3 and cleaned with MQ water before use to ensure that the experimental process was free from contamination.

3. Results and discussions

3.1. Single toxicity of the heavy metal Cd to algae

Cd, as one of the most toxic heavy metals, can cause severe toxic effects on aquatic organisms even at low concentrations. Compared with zooplankton and fish, freshwater green algae showed higher sensitivity to heavy metals. As shown in Figure 1a, the growth of algal cells was inhibited at any Cd concentration compared to the blank control group. At a Cd concentration of 1 mg/L, the cell density absorbance value was 0.96, which was 1.67 times higher than that of the blank control group, showing significant growth inhibition. In addition, there was no significant difference in algal cell density between 0.1 mg/L and 0.5 mg/L, suggesting that the algal cells were tolerant to low doses of Cd.

Heavy metals mainly affect the photosynthetic process of algae by disrupting photosynthetic pigment secretion and inhibiting non-photochemical quenching [14]. Figure 1b shows the changes of chlorophyll a content in algal cells under the effect of different concentrations of Cd, and the change trend is consistent with the density of algal cells. Specifically, compared with the blank control group, the chlorophyll a content decreased by 43.75% at a Cd concentration of 1 mg/L, implying that the secretion of photosynthetic pigments was significantly inhibited. This experimental result was consistent with the previous results of Cd photosynthesis inhibition in different types of algae, i.e., Cd inhibited photosynthesis in freshwater algae in a dose-dependent manner [15]. The intracellular chlorophyll a concentration in the algal cells were 6.1 mg/L and 6.0 mg/L at Cd concentrations of 0.1 mg/L and 0.5 mg/L, respectively, which did not show concentration differences.

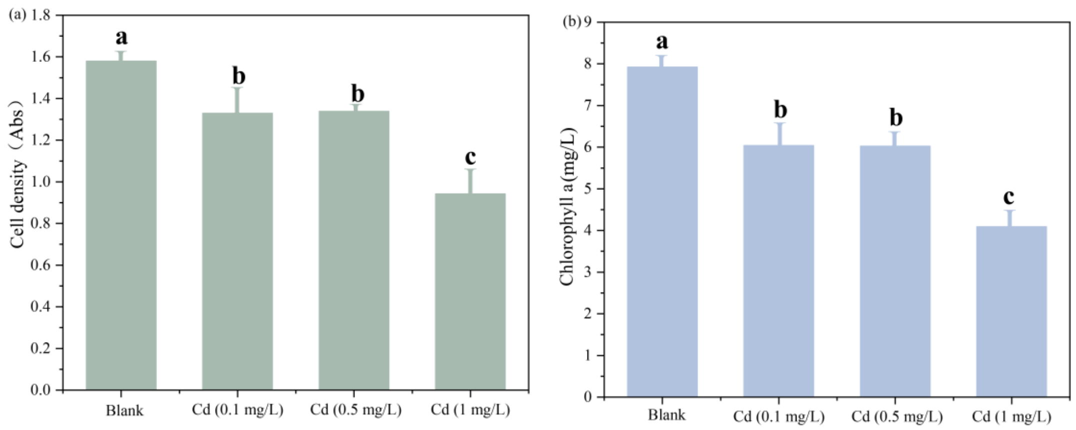

Figure 1. Cell density (a) and *chlorophyll a* (b) content under different concentrations of Cd

3.2. Single toxicity of microplastic to algae

Figure 2a shows the growth of green algae under the effect of different concentrations of PS. Compared with the blank control group, both concentrations of PS inhibited the growth of algae with 16.01%, 16.45% and 28.67%, respectively. In the present study, there was no significant difference between PS concentrations of 1 mg/L and 10 mg/L on algal growth, which is consistent with the results of previous studies. Wan et al. used PS at concentrations of 50, 100 and 1000 mg/L for the toxicity exposure of marine diatoms with the inhibition rates of 15.71%, 16.43% and 28.86% for each concentration, respectively, and the marine diatoms also showed a significantly high tolerance [12]. The above findings suggest that the toxicity of microplastics depends on a variety of parameters including exposure concentration, polymer type, size and presence of additives as well as the species, size and characteristics of the tested microalgae [16,17]. Currently, a considerable part of the literature has found that the presence of microplastics affects the production of photosynthetic pigments in algae thereby disrupting the algal photosynthesis mechanism [4]. As shown in Figure 2b, the chlorophyll content in algal cells under the effect of PS showed a trend consistent with the number of algal cells, i.e., PS inhibited chlorophyll a synthesis. Some previous studies have also reported the reduction of chlorophyll content in algae in the presence of microplastics.

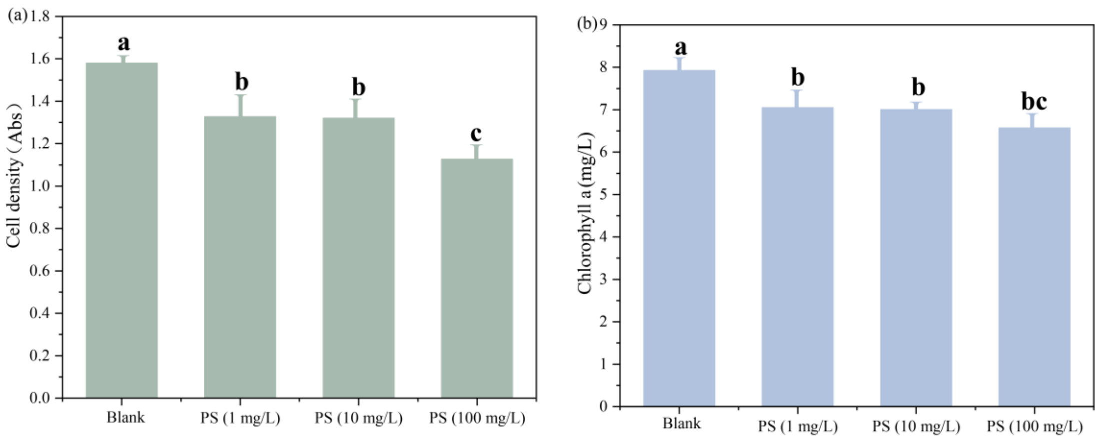

Figure 2. Cell density (a) and *chlorophyll a* (b) content under different concentrations of PS

3.3. Effects of microplastics and heavy metals in combination on algae

As shown in Figure 3a, the order of cell density among different treatment groups was blank control > Cd (0.5 mg/L)+PS (10 mg/L) > Cd (0.5 mg/L) > Cd (0.5 mg/L) > Cd (0.5 mg/L)+PS (1 mg/L) > Cd (0.5 mg/L)+PS (100 mg/L) (algal cell density: 1.58 > 1.40 > 1.34 > 1.27 > 1.16 Abs). 1.27 > 1.16 Abs), indicating that the presence of different concentrations of PS had different effects on the effects of both compound toxicity on drum algae. When PS was 10 mg/L, both showed antagonistic effects compared to the treatment group exposed to Cd only, i.e., PS was able to attenuate the biotoxicity of Cd on green algae. The reason for this result may be due to the ability of microplastics to adsorb heavy metals, thus reducing the bioconcentration of heavy metals by algae. Both were able to increase the cytotoxicity of Cd to the drum alga when the PS concentrations were 1 mg/L and 10 mg/L, showing obvious synergistic effects, but the mechanisms of action involved were different. At low concentrations of PS (1 mg/L), the combination of the two can change the permeability of the cell membrane, thereby increasing the uptake of the complex by the algae and thus increasing the toxicity of the complex pollution [18]. When high concentration of PS acted (100 mg/L), although PS could adsorb with Cd to reduce Cd uptake by algae, the increase of toxicity in this treatment group was mainly due to growth inhibition triggered by high concentration of microplastics. Chlorophyll a content showed that whatever concentration of PS was added exacerbated the disruption of algal photosynthesis by Cd (Figure 3b).

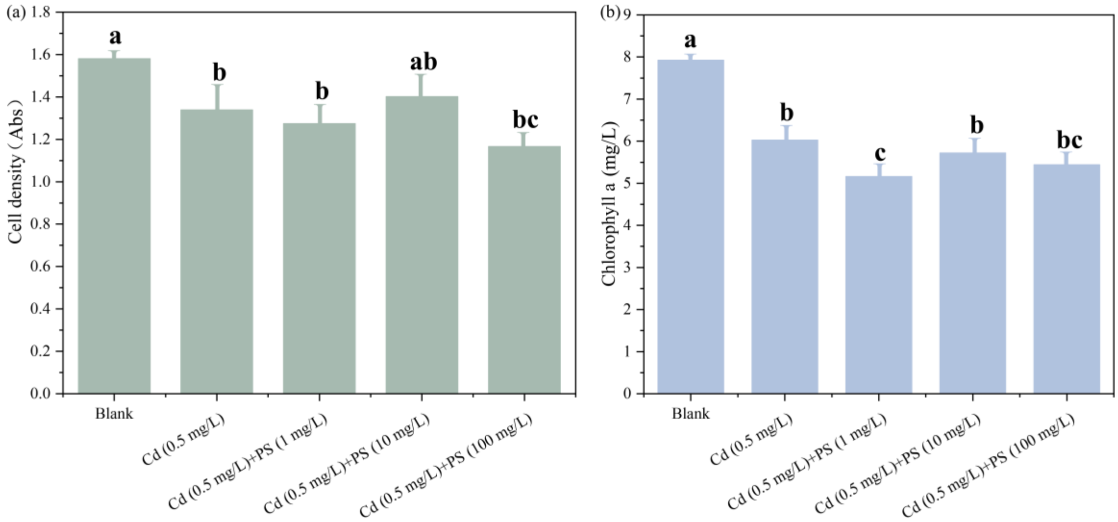

Figure 3. Cell density (a) and *chlorophyll a* (b) content under different combined Cd and PS

4. Conclusion

In this study, Cd and PS were selected as the representative heavy metals and microplastics in the environment, and the toxicity effects of the two pollutants on freshwater algae were investigated through the determination of algal growth biomass and chlorophyll a content. Both heavy metals and microplastics, when exposed to single toxicity, the biotoxicity of freshwater green algae increased with the increase of exposure concentration, in which the algae showed higher tolerance to microplastics, and did not show any significant difference in the low and medium concentrations. From the point of view of algal growth, different concentrations of microplastics had different effects on the toxicity of heavy metals. When the PS was 10 mg/L, the composite toxicity effect of the two showed antagonism; when the PS concentration was 1 mg/L and 100 mg/L, both of them were able to increase the cytotoxicity of Cd on algae, showing obvious synergistic effect.

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