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Are Microplastics Always Toxic? --Certain Concentrations of Polystyrene Are Promoters of Marine Algae Growth and Heavy Metal Bioremediation

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Abstract. The influence of microplastics on the bioremediation capabilities of algae in aquatic environments is an area of growing concern, particularly when these tiny plastic particles interact with heavy metals like cadmium (Cd). Such interactions could alter the algae's natural ability to detoxify and purify water, posing significant challenges to maintaining the health and stability of aquatic ecosystems. This study investigated the effects of different types (including Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyethylene Terephthalate (PET)) and concentrations (1-100 mg/L) of microplastics on the ability of algae to absorb Cd, focusing on whether the simultaneous presence of microplastics and heavy metals can affect both algal growth and heavy metal bio-uptake. The study results indicated that Cd or different types of microplastics individually inhibit algal growth. However, when algae are co-exposed to Cd and microplastics, a PS concentration of 1 mg/L combined with Cd significantly promotes algal growth, demonstrating an antagonistic effect, whereas the other two types of microplastics exhibit a synergistic effect with Cd. Additionally, regardless of exposure duration, when PS is present at 1 mg/L, it enhances the bio-uptake of Cd in algae compared to treatments with Cd alone. Among the various types and concentrations of microplastics studied, the combination of 1 mg/L PS and Cd was found to not only promote algal growth in aquatic environments but also enhance heavy metal bio-uptake, thereby mitigating heavy metal pollution. Consequently, the presence of microplastics at certain concentrations (such as 1 mg/L PS) may not necessarily be considered "pollutants" in aquatic environments. These findings provide a new perspective for re-evaluating the role of microplastics in aquatic ecosystems and suggest a strategy for leveraging microplastics to enhance algal bioremediation of heavy metals.

Keywords: Microplastic, heavy metal, algae, bio-uptake, bioremediation.

1. Source of the Subject

The sound of the waves, the feel of the sand between my toes, and snorkeling to observe marine life fostered my deep love for the sea. Every time I went on vacation, I would urge my parents to take me to the beach, but every time I went, I was disheartened to see trash all over the beach. This prompted me to start researching marine pollution, realizing that the plastics we invented a century ago are now threatening our ecosystem. We dump 8 million tons of plastic into the ocean every year, harming marine life.[1] Documentaries and news stories, such as the one about a pregnant whale that died from ingesting plastic, deeply affected me. Determined to make a difference, I joined as an environmental volunteer. I

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interviewed Hannah Hao, the leader of a nonprofit organization that led trash picking events. She confirmed that beaches have become hotspots for plastic pollution due to the high volume of foot traffic, and that microplastics are entering our food chain, endangering wildlife and even humans. The pollution not only affects wildlife and future generations, but also our current society. We ingest microplastics without even realizing it, with Europeans ingesting as many as 11,000 microplastics per year.[2] We can't live without plastics and we are producing more and more of them, so if it is irreversible that microplastics are entering the oceans, is it possible to utilize what is already in the oceans to deal with microplastics? Is it possible to reduce the damage of pollution to the oceans through human control or even find ways to produce some benefits? So, I decided to do this research regarding the combined toxicity of Cadmium and different types of microplastics.

2. Introduction

The marine environment is a crucial component of the global life support system, providing not only abundant resources but also playing a key role in regulating the climate and maintaining biodiversity. However, with the accelerated pace of industrialization, the issue of heavy metal pollution in the oceans has become increasingly severe. Heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg) enter the marine environment through industrial wastewater, agricultural runoff, and other pathways. Due to their high toxicity, persistence, and ability to bioaccumulate, these metals pose serious threats to marine ecosystems and human health.[3] These harmful metals can be biomagnified through the food chain, ultimately affecting top predators, including humans.[4] To address this environmental issue, bioremediation technologies have emerged and are increasingly being regarded as cost-effective and environmentally friendly solutions. Algae, due to their high efficiency in adsorbing and accumulating heavy metals and their rapid growth, have become a focus of attention as biological tools in bioremediation technology.[5] Studies have shown that certain algae, such as *Chlorella*, *Macrocystis pyrifera*, and *Gracilaria spp*., can effectively remove heavy metals from water by forming complex reactions between the polysaccharides, proteins, and lipids in their cell walls and heavy metal ions.[6] Furthermore, after algae adsorb heavy metals, their biomass can be recycled for metal resource recovery, providing dual benefits for environmental management and resource recycling.[7]

However, in recent years, the issue of marine microplastic pollution has become increasingly prominent, adding new complexities to heavy metal bioremediation. Microplastics are plastic particles less than 5 mm in diameter, primarily derived from the degradation of plastic waste, industrial raw materials, and the release of daily products.[8] These microplastics are widely distributed in the ocean and, due to their high surface area and chemical activity, can adsorb toxic substances such as heavy metals from seawater.[9] The interactions between microplastics and heavy metals may alter the chemical forms and bioavailability of heavy metals, thereby affecting the efficiency of algae in absorbing heavy metals.[10] Existing studies on the mechanisms by which microplastics and heavy metals jointly affect algal growth and heavy metal absorption remain controversial. For example, Bhattacharya et al. found that negatively charged plastic nanoparticles may reduce heavy metal absorption by binding with metal ions on the algal cell surface.[11] In contrast, Rochman et al. suggested that different types of microplastics, after adsorbing heavy metals, may be ingested by algae through biological pathways, thereby increasing the bioavailability of heavy metals.[12] Additionally, some studies have indicated that the combined effects of microplastics and heavy metals on algal growth may vary with concentration, potentially inhibiting algal growth at high concentrations while causing slight or even promotive effects at low concentrations. [13]

In light of this, the present study selects Cd as a representative heavy metal due to its high toxicity, and focuses on three types of microplastics: polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET), chosen for their widespread use and environmental occurrence.[2, 14] This research aimed to explore the combined effects of microplastics and heavy metals on marine algal growth and their ability to absorb heavy metals. Firstly, by analyzing the effects of different concentrations and types of microplastics and heavy metals on algal growth, the study revealed potential mechanisms underlying the synergistic effects of marine pollutants. Secondly, the research assessed

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changes in algal heavy metal bio-uptake in the presence of microplastics, aiming to clarify the impact of microplastics on the heavy metal remediation capabilities of algae. Finally, the study examined whether microplastics can enhance the bioremediation of heavy metals by marine algae based on the results of the study, which could provide a new solution to the problem of marine heavy metal pollution.

3. Materials and Methods

3.1. Algae Culture and Sample Preparation

The microalgae species used in this study was *Chlorella vulgaris* (FACHB-6), obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The study employed the f/2 culture medium to cultivate *Chlorella vulgaris*. The medium was prepared with an artificial seawater solution with a salinity of 30‰ and several nutrients (Nitrate, trace metals, and vitamins). Then, each sample was maintained under the light intensity of 4000 lux, a photoperiod of 12-hour light/12-hour dark alternating cycle, and a constant temperature at 25 ºC. To prevent algal sedimentation, the culture flasks were shaken three times daily, maintaining homogeneity and ensuring that algae receive adequate light and nutrients.

With a concentration of 100mg/L Cd, the stock cadmium (Cd) solution was prepared using a standard reagent from Sigma-Aldrich. The PS, PVC, and PET particles (with a diameter of three micrometers) used in the study were purchased from Macklin. A stock suspension of 1 g/L was prepared for PS, PVC, and PET. Prior to the experimentation, ultrasonic treatment was employed to ensure uniform dispersion of the particles.

3.2. Toxicity Experiments

Exposure experiment was conducted according to OECD201 guidelines.[15] This study included 11 experimental groups, with each group's initial algal cell density controlled at 2×10^6 cells/mL, corresponding to an absorbance value of 0.1 at 560 nm. Each treatment was set up in triplicate. As shown in Table 1, the first group served as the control group, meaning no toxic substances were added. The second group was exposed to 100 μg/L Cd alone. This experiment utilized a concentration of 100 μg/L Cd as it reflects levels that are higher than typical Cd levels. In marine contexts, cadmium concentrations are generally less than 3 μg/L,[16] though levels can rise to 50 μg/L in polluted areas. The concentration 100 μg/L was also pertinent because it aligns with other studies addressing heavy metal toxicity. Tunali et al. used 0.25 mg/L of metal which, while slightly higher, signifies a common trend in experimental toxicology involving heavy metals where concentrations are typical in toxicity assessments.[17] Such experiments contributed to a better understanding of the ecological risks posed by cadmium in marine ecosystems, suggesting that concentrations like 100 μg/L may be beneficial in simulating potential real-world scenarios where marine organisms are subjected to toxic metal influences. Groups 3-5 investigated the effects of exposure to different concentrations of PS alone. Groups 6-8 and 9-11 were treatments with exposure to PVC and PET alone, respectively. Groups 12-14 maintained the same Cd concentration (100 μg/L) while exploring the effects of different PS concentrations. Groups 15-17 and 18-20 were for PVC and PET treatments, respectively. This study used three distinct levels of MP concentrations: 1 mg/L, 10 mg/L, and 100 mg/L. Comparing high and low concentrations, the results showed differences in the different levels of MP concentrations. Previous studies also employed MP concentrations between 10 mg/L and 100 mg/L to investigate MP's effects on algae.[18, 19] Figure 1 shows pictures of algal growth of different treatment groups in a light incubator.

Table 1. Treatment groups of this study.

Group	$CdCl_2$ (μg/L)	PS (mg/L)	PVC (mg/L)	PET (mg/L)
1	0	0	0	0
2	100	0	0	0
3	0	1	0	0
4	0	10	0	0

Table 1. (continued).

5	0	100	0	0
6	0	0	1	0
7	0	0	10	0
8	0	0	100	0
9	0	0	0	1
10	0	0	0	10
11	0	0	0	100
12	100	1	0	0
13	100	10	0	0
14	100	100	0	0
15	100	0	1	0
16	100	0	10	0
17	100	0	100	0
18	100	0	0	1
19	100	0	0	10
20	100	0	0	100

Figure 1. Culture pictures of different algal treatment groups

3.3. Determination of Toxicity Indicators.

At the end of both the short-term (48 hours) and long-term (14 days) experimental periods, 200 µL samples from each treatment were placed in a 96-well plate for biomass measurement. The mircroplate reader (Varioskan LUX) was set to the wavelength of 560 nm.[20] The reader shook for 30s before measuring the absorbance values, preventing algal cell precipitation.

After short-term and long-term exposure experiments, algal cell suspensions (3mL) from each treatment group were collected and centrifuged at the rate of 10000 rpm for 5 minutes to remove the culture medium in the solutions. The algal precipitate at the bottom of the tube was washed three times with MQ water. The settled algal cells were then digested with 1 mL HNO_3 under 180 ºC for 1 hour, followed by acid removal. The analyte solution was diluted to 5 mL, and the heavy metal concentrations measured represent the amount of Cadmium absorbed by *Chlorella vulgaris*. The heavy metal content in the digestion solution was determined using ICP-MS (NexION 350). The formula for calculating the heavy metal content in algal cells is as follows:

$$q_a = (C_i \times V_d \times 1000)/(C_c \cdot V_a)$$

Where q_a represents the internalization amount of Cd by the algal cells (μg/cell); C_i is the concentration of Cd in the eluent and digestion solution (mg/L); V_a and V_d are the volumes of the algal suspension and digestion solution, respectively; C_c is the cell density of the algae (cells/mL, calculated based on absorbance).

3.4. Statistical Analysis

This experiment was repeated three times to arrive at three experimental data for each group, and the data is presented as mean ± standard deviation. Origin 2024 was used to conduct differential analysis of algal cell density among different treatment groups, employing the Tukey test method. Differences were considered significant when $p < 0.05$.

4. Results and Discussions

4.1. Single Toxicity of Cd and Microplastics

Regardless of exposure time and concentration, each treatment that only added microplastics PS or PET showed inhibitory effects on algal growth (Figure 2). This indicates that PS and PET, are harmful to algal growth. It is highly likely that when microplastics adhere to algal cells. Similarly, Tunali et al. found that PS up to 50 mg/L significantly inhibit algal growth and photosynthesis, whereas 1 and 5 mg/L had no effect.[17] The reason for the difference at the same exposure concentration (1 mg/L) may be due to the different sensitivities of the algal species.

For PVC, at low concentrations, it may trigger a stress response in algae, increasing their metabolic or physiological activities, such as increasing photosynthesis and cell division rates, to cope with environmental pressure. However, this stress response at low concentrations may exceed the algae's tolerance, resulting in toxic effects. At high concentrations of PVC 100 mg/L, nutrients or other organic substances adsorbed on the surface of microplastics may be released into the water, increasing the algae's nutrient sources. Additionally, high concentrations of PVC microplastics may alter the chemical environment of the water, such as adjusting pH and dissolved oxygen content, which may be beneficial for algal growth. Simultaneously, small molecular additives or chemicals released during the degradation of PVC microplastics may also have a promoting effect on algae at specific concentrations.

With only Cd added, both the short-term and long-term treatments inhibited the growth of *Chlorella vulgaris* (Figure 3). As typical toxic metal, Cd could easily enter algae cells. Once cadmium ions enter the algal cells, they induce oxidative stress in algae and decrease photosynthetic activity, thereby achieving the effect of inhibiting growth.[18] In the short term, the absorbance of biomass in the control group was 0.196 AU, which decreased to 0.182 AU under Cd treatment (Figure 3a), implying that the toxicity of Cd inhibited the growth of the algae. However, in the long term, there was no significant difference ($p > 0.05$) between the biomass of the Control group and that of the Cd exposure alone (Figure 3b), suggesting that in the long term, the algae became tolerant to the heavy metals.[21]

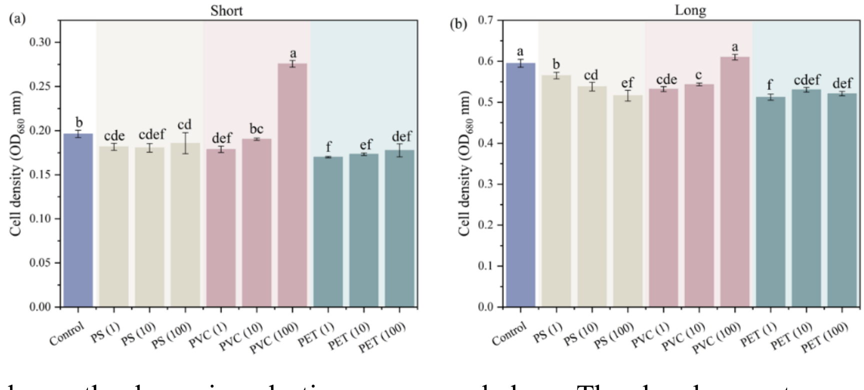

Figure 2. Algal growth when microplastics are exposed alone. The absorbance at a wavelength of 560 nm was used in this study to represent algal biomass, expressed using absorbance units (AU). It is worth noting that different letters on the bar graph represent significant differences ($p < 0.05$).

4.2. *Combined Toxicity to Algae (Cell Density)*

In the short term, for PS, both 1 and 10 mg/L were able to mitigate the toxicity of Cd and promote algal growth compared to Cd exposure alone (Figure 3a). As an example, the biomass of PS = 1 mg/L was 0.2 AU, which was 1.1 times higher than that of Cd exposure alone. The mitigation of Cd concentration by these two concentrations was mainly due to the mutual adsorption between them, which reduced the concentration of solution Cd ions.[22] However, the toxicity of Cd to algae was not mitigated when the PS concentration was 100 mg/L, which was attributed to the toxicity of the high concentration of PS itself. [23] It is noteworthy that PS and Cd were able to antagonize and promote algal growth only when the PS concentration was 1 mg/L compared to the control group. For PVC, both at 1 and 10 mg/L, the presence of PVC synergized with Cd to inhibit algal growth. Interestingly, at 100 mg/L, PVC significantly alleviated Cd toxicity and promoted algal growth. This is consistent with the findings when PVC (100 mg/L) was exposed alone, most likely because PVC may release certain nutrients and additives during degradation, providing essential nutrients to algae. For PET, either concentration was able to exacerbate the toxicity of Cd to algae, with growth inhibition and synergistic toxicity. Previous literature has also used PET and Pb at 40 mg/L for compound toxicity effects and found similar synergistic effects.

In the long term, for PS, consistent with the short term, 1 and 10 mg/L mitigated Cd toxicity compared to Cd exposure alone, while 100 mg/L was inhibitory (Figure 3b). It is noteworthy that in the long term, the combined toxicity with Cd was antagonistic at a PS concentration of 10 mg/L and synergistic in the short term compared to Control. This result may be due to the fact that short-term is the insufficient complexation of PS with Cd, while during the long-term experiments increased the surface adsorption of Cd by PS, thus reducing the free state Cd ions. [22] The toxicity of Cd was exacerbated at low concentration (1 mg/L), not affected at 10 mg/L, and alleviated at 100 mg/L for PVC. For PET, consistent with the short term, there was a synergistic effect with Cd regardless of concentration. In summary, PS at a concentration of 1 mg/L was able to alleviate the growth inhibition of algae by Cd in the aqueous environment and was able to antagonize with Cd to promote the growth of algae, regardless of the exposure term. Although PVC at 100 mg/L was able to promote algal growth in conjunction with Cd, this concentration was not environmentally relevant because such extremely high exposure concentrations are almost never present in real water environments.

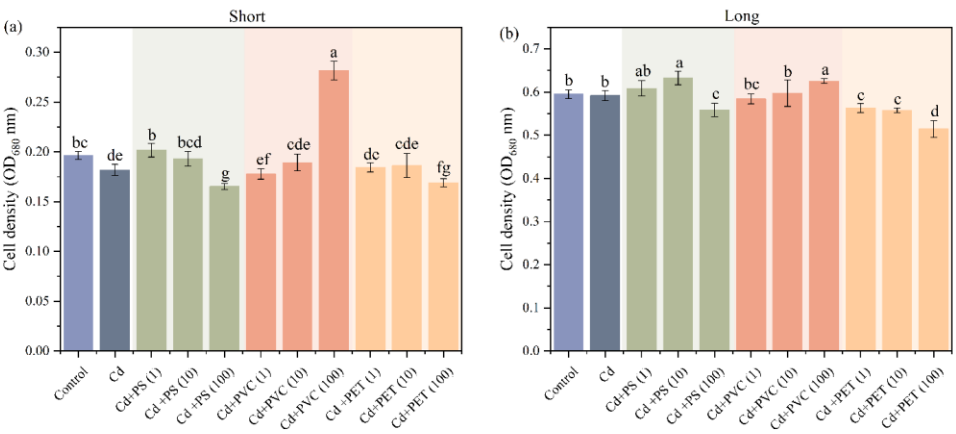

Figure 3. Algal growth during co-exposure of microplastics and Cd. Absorbance at 560 nm was used to quantify algal biomass in absorbance units (AU). Different letters on the bar graph represent significant differences ($p < 0.05$).

4.3. *Bio-uptake of Cd by Algae in the Presence of Microplastics*

In the short-term experiments, the effects of different types and concentrations of microplastics on Cd uptake by algae showed some differences (Figure 4a). For PS, only PS at 10 mg/L was able to significantly increase the uptake of heavy metals by algae compared to Cd alone treatment (2.17×10^{-8}

μg/cell). For 1 mg/L PS, the bioaccumulation of Cd by algae was slightly elevated close to 2.46×10^{-8} μg/cell, although this elevation was not significant, showing the potential promotion of Cd uptake by PS at low concentrations. In addition, 1 mg/L PS not only increased Cd accumulation by algae, but also significantly promoted algal growth, and the algal biomass of this treatment group was about 11% higher than that of the Cd-only treatment group in the short term. However, the effect of PS as a carrier on the toxicity of Pb to *C. reinhardtii* and Pb internalization was also evaluated by Sun et al.[24] The results showed that Pb-loaded microplastics had a greater negative effect on *C. reinhardtii* than Pb exposure alone, increasing the toxicity to the alga without an increase in its internalized Pb content. This difference may be due to differences in the types of heavy metals and algal species tested in this study, as well as differences in the interactions between microplastics and heavy metals. For PVC, the presence of 1 and 10 mg/L PVC did not increase Cd uptake by algae, while 100 mg/L significantly promoted Cd enrichment by algae. Regardless of the concentration, PET was able to increase the uptake of Cd by algae and showed a stoichiometric effect relationship. That is, Cd bioaccumulation by algae was highest at 1 mg/L of PET, reaching 3.50×10^{-8} μg/cell, while it was weakened at high concentrations (100 mg/L).

Under long-term exposure conditions (Figure 4b), Cd accumulation by algae increased to 5.08×10^{-8} μg/cell, which was 2.34 times higher than that of the short-term Cd alone treatment, indicating the enhancing effect of exposure time on Cd uptake by algae. For PS, 1 mg/L PS exhibited elevated Cd accumulation to 5.46×10^{-8} μg/cell, and the algal biomass remained higher than that of the Control group, which further verified the dual promotion of Cd uptake and algal growth by low concentration of PS microplastics. The middle and high concentrations of PS showed inhibitory effects on algal heavy metal uptake. For PVC, the uptake of Cd by algae was inhibited regardless of the concentration. Although Cd accumulation was also higher in the PET treatment group at 1 mg/L, the combined effect of PET and Cd significantly inhibited algal growth under long-term exposure, and the algal biomass was reduced by about 6.78%, which made PET unsuitable for use as a medium for heavy metal bioremediation.

Combining the results of short-term and long-term experiments, 1 mg/L PS showed optimal results in the bioremediation of Cd. The low concentration of PS not only slightly enhanced cadmium uptake in the short term (12.09% increase), but also significantly enhanced cadmium bioaccumulation under long-term exposure (7.48% increase), and promoted algal growth in both periods. Although PET also promoted Cd uptake at 1 mg/L, it significantly inhibited algal growth, reducing its potential for bioremediation applications. Therefore, 1 mg/L PS microplastic is the most promising microplastic species and concentration for promoting algal uptake of heavy metals without impairing algal growth, and the synergy between the two can be used as one of the bioremediation strategies for marine heavy metal pollution.

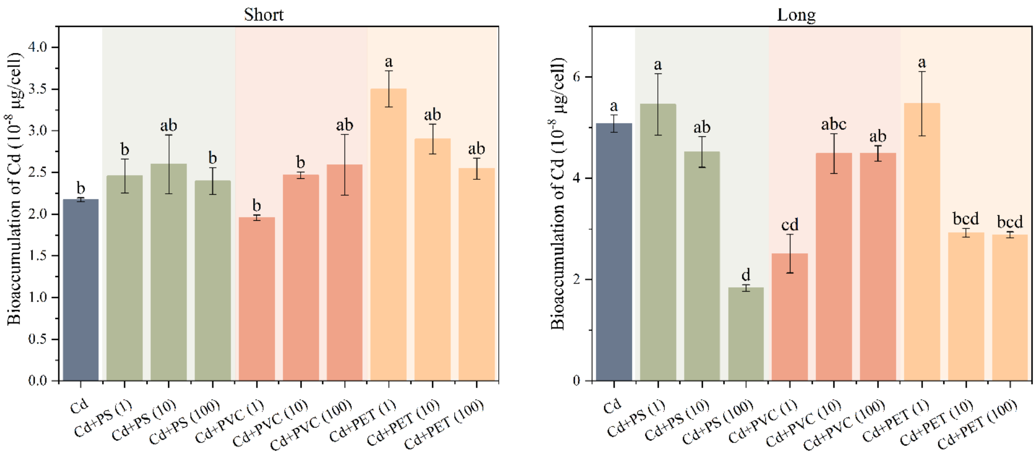

Figure 4. Effects of different types and concentrations of microplastics on the bio-uptake of heavy metals by algae. Significant differences ($p < 0.05$) are denoted by distinct letters on the bar graph.

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5. Conclusion

This study investigated the compound effects of different concentrations and species of microplastics and heavy metals on algae, with special attention to the bio-uptake of heavy metals by algae, with a view to proposing management strategies for the bioremediation of heavy metals in aquatic environments. The following conclusions were mainly obtained:

(1) Both Cd and different types of microplastics would inhibit the growth of algae when each was exposed alone. However, 100 mg/L PVC was beneficial to algal growth, mainly attributed to its nutrient release.

(2) Regardless of the exposure period, PS at 1 mg/L was able to significantly promote algal growth in conjunction with Cd, showing an antagonistic effect between the two. The remaining two types of microplastics synergized with Cd.

(3) In the short term, all microplastic treatments except PVC (1 mg/L) were able to promote the uptake of Cd by algae; in the long term, only PS (1 mg/L) and PET (1 mg/L) had a promoting effect.

(4) Combining the growth of algae and the uptake of Cd, when PS is 1 mg/L, its joint action with heavy metals can promote both the growth of algae and the uptake of heavy metals in the aquatic environment, and alleviate heavy metal pollution. Therefore, the presence of microplastics at a certain concentration (e.g., 1 mg/L PS) is not necessarily recognized as a “pollutant” in the aqueous environment.

Microplastics are widely recognized as a significant global pollutant, with well-documented negative impacts on the environment.[8, 19, 22] However, if their presence could be harnessed to enhance the ability of algae to absorb heavy metals, this would offer a novel approach to pollution management. Such a strategy not only has the potential to optimize bioremediation techniques but also to improve the efficiency of environmental purification efforts. Furthermore, the promotion of algal growth could increase primary productivity in ecosystems, contributing to both environmental restoration and resource utilization. This research direction not only mitigates the adverse effects of microplastics but also provides new opportunities for the advancement of environmental remediation technologies. Future studies should focus on optimizing the types and concentrations of microplastics/nano-plastics to maximize their potential in bioremediation applications.

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