

Article

Synergism or Antagonism in Toxicity Induced by Co-Exposure to Polyamide Microplastics and Cadmium Is Dose-Dependent in the Submerged Macrophyte *Vallisneria natans*

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Abstract

The contamination of microplastics (MPs) and heavy metals (HMs) in water has caused widespread concern, while their effects on submerged macrophytes have rarely been reported. Experiments were carried out to investigate the toxic effects of polyamide microplastics (PAMPs; 0.1%, 0.3%, and 1.0% *w/w*) and cadmium (Cd; 0.3 and 1.0 mg/L), alone or in combination, on the submerged macrophyte *Vallisneria natans* (*V. natans*). The results showed that PAMPs significantly reduced Cd accumulation in leaves (decrease of 2.38% ~ 26.12%) but elevated Cd accumulation in roots. Both Cd exposure and high PAMP exposure alone inhibited plant growth. The combined stress showed concentration-dependent effects: the low Cd concentration (0.3 mg/L) and PAMPs synergistically exacerbated toxicity (synergism, MDR > 1.3), as PAMPs disrupted the sediment structure and enhanced the bioavailability of Cd, whereas when *V. natans* was co-exposed to the high Cd concentration (1.0 mg/L) and PAMPs, the PAMPs blunted the toxicity of Cd by efficiently adsorbing it (antagonism, MDR < 0.7). Both individual and combined exposures decreased chlorophyll *a* and chlorophyll *b* synthesis and increased superoxide dismutase (SOD) and peroxidase (POD) activities as well as malondialdehyde (MDA) content in plant tissues. However, exposure to low and medium concentrations of MPs (0.1% and 0.3% *w/w*) alone had positive effects on plant growth and photosynthesis systems, while combined exposures exacerbated the damaging effects of PAMPs on the antioxidant defense system in *V. natans*. These results allow for a better understanding of the synergistic effect of co-contamination of microplastics and heavy metals in freshwater ecosystems, and highlight the necessity of ecological risk assessment during phytoremediation using submerged macrophytes.

Keywords: polyamide microplastics; cadmium; submerged macrophyte; toxicity; *Vallisneria natans*

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1. Introduction

Plastics are synthetic organic polymers derived from the polymerization of monomers extracted from petroleum or natural gas [1–3], and are widely used as a new class of materials in a variety of fields due to their low density, low thermal conductivity, low electrical conductivity, and corrosion resistance. However, the mass production of plastics has led to serious environmental problems [4]; in particular, small-sized plastic particles from manufacturing production (primary source) and the degradation of large plastic fragments (secondary source) are prone to accumulation in the environment [5], causing unpredictable impacts on ecosystems. It is estimated that up to 53 million tons of plastics will enter the aquatic environment each year by 2030 [6], which will degrade through physical and chemical corrosion into small plastic particles, including microplastics (particle size less than 5 mm [7]) and nanoplastics (particle size less than 1 μm [8]). Freshwater environments are more susceptible to receiving micro/nanoplastics (MPs/NPs) than oceans [9], with domestic waste dumping, surface runoff, wastewater treatment plant discharge, as well as industrial, agricultural, and animal husbandry activities all contributing to the release and transport of MPs in freshwater systems [10].

Recently, many studies have been conducted on the biological effects of MPs in aquatic environments, including alterations to the growth and development of aquatic organisms, oxidative damage, and death [11–13]. MPs pose a threat to the growth of aquatic plants, especially submerged plants. Aquatic plants are the first interface connecting the abiotic and biotic components of ecosystems [14], and they play a crucial role in intercepting MPs in advance and preventing their further spread along the food chain. MPs have been shown to impede nutrient accumulation in *Myriophyllum spicatum* L. and *Elodea canadensis* Michx., leading to a decrease in the shoot-to-root ratio of both plants [15]. High concentrations of polystyrene microplastics (PSMPs) can significantly affect the morphological features and photosynthetic pigments of the aquatic carnivorous plant *Utricularia vulgaris* L. and alter the structure and diversity of its associated microbial communities [16,17]. Polyvinyl chloride microplastics (PVCMPs) were shown to inhibit the length, chlorophyll content, and chlorophyll fluorescence parameters of *Utricularia aurea* Lour., whereas no significant changes were observed with PSMPs at the same concentrations [18]. Therefore, exploring the mechanism of interaction between microplastics and aquatic plants is vital to understanding the toxicity of this ubiquitous emerging contaminant in aquatic ecosystems.

Meanwhile, micro(nano)plastics, which are characterized by a large surface area and low surface polarity [19], can accumulate, migrate, and transfer to organisms [20], directly or indirectly affecting the structure and function of ecosystems [21] and altering the growth and reproduction behavior of organisms [22]. It is worth noting that MPs do not exist in isolation in the aquatic environment and can easily adsorb pollutants in the surrounding environment and become vectors of pollutant dissemination; these pollutants include polychlorinated biphenyls [23], perfluorooctanoic acid [24], antibiotics [25], and heavy metals [26]. In recent years, researchers have been giving increasing attention to the potential role of MPs in altering the bioavailability of heavy metals [27,28]. PVCMPs have been found to have no effect on Cd accumulation in *Vallisneria natans*, a submerged macrophyte species that is a widely used tool in freshwater ecoremediation; not only did it not reduce the Cd concentration, but it also did not increase the antioxidant enzyme activities of the plants [26]. In one study, PSNPs attenuated the Cd-induced growth inhibition of *Ceratophyllum demersum* L. and decreased the Cd-enrichment capacity [29]. A large number of PSNPs also adhered to the surface of *Ceratophyllum demersum* L. under co-exposure conditions, but not under individual exposure conditions. PSNPs have also been demonstrated to significantly reduce the accumulation of As in *Myriophyllum verticillatum* L.; both As-alone and As–PSNP co-exposure treatments significantly inhibited plant

growth, but PSNPs attenuated arsenic toxicity at low As concentrations and reduced toxicity at high concentrations [30]. Research on the behavior and biological effects of MPs and heavy metals under co-exposure environmental conditions is still in the exploratory stage, and further studies on their co-toxicity and mechanisms of action on submerged plants are needed.

Submerged macrophytes, as important primary producers, often play an important role in maintaining the structure and function of aquatic ecosystems, especially in shallow water ecosystems [31–33]. Compared with floating-leaved and floating plants, submerged macrophytes can simultaneously establish close coupling relationships with overlying water and benthic sediments, endowing them with unique comprehensive ecological advantages in in situ phytoremediation. *Vallisneria natans* is one of the most common submerged macrophytes with a high purification capacity and is thus widely used for aquatic eco-remediation in China [34]. Different from single-pollutant-purification materials, the remediation process of *V. natans* provides multiple ecological services, which greatly enrich the ecological effects of phytoremediation technology. *V. natans* can remove, transform, or immobilize common organic pollutants through interactions between its root system and inter-root microorganisms [35]. Its well-developed root system can absorb water from underwater silt and reduce the water content of the substrate to improve the flow of surface substrates and inhibit the suspension of black-odor substances; this sediment regulation effect effectively alleviates the internal pollution release of water bodies and contributes to a stable benthic environment for long-term ecological restoration. *V. natans*' leaves have purification effects in eutrophic water bodies through the absorption of both nitrogen and phosphorus; *V. natans* also acts as bait for fish and other aquatic animals; thus, it is commonly found in a variety of freshwater bodies in China. This dual role of pollutant purification and food source means that *V. natans*-based phytoremediation can realize both pollution control and aquatic biodiversity conservation, highlighting its great potential for freshwater habitat restoration.

A variety of microplastics, such as polypropylene (PP), polystyrene (PS), and polyamide (PA), have been deposited into aquatic environments and have impacted aquatic organisms [36]. Among these microplastic types, most studies have focused on the effects of PP and PS rather than PA on aquatic vascular plants. Polyamide is the main material used in the production of various types of artifacts, such as ropes, nets, clothes, and other textiles, and PA is the most dominant microplastic category in several rivers [37,38]. Due to the high density of PA, it tends to settle on the surface of plant bodies and in sediments, which may affect submerged plants by altering their physiological characteristics and changing the sediment microbiome [39]. Heavy metal pollution of water bodies occurs occasionally, with Cd considered the most harmful metal due to its high toxicity and bio-availability [40]. It exhibits high mobility in sediment pore water and poses severe toxic risks to submerged vegetation, making it a model pollutant for investigating combined microplastic-heavy metal toxicity. Distinct from hydrophobic microplastics, the PA matrix contains an abundance of polar amide groups that can strongly bind coexisting contaminants, leading to unique composite toxicity characteristics. Herein, we explored the combined toxic mechanism of PAMPs and Cd on the submerged macrophyte *Vallisneria natans*; specifically, we examined (i) whether microplastics enhance or attenuate the toxicity of cadmium toward the growth and physiological responses of submerged macrophytes, and (ii) how the level of PAMPs presented in sediment modifies the toxicity and accumulation of cadmium in submerged macrophytes. The results from the current study could provide a theoretical basis for studying the behavior of MPs as carriers of heavy metals on submerged macrophytes.

2. Materials and Methods

2.1. PAMPs, Plants, and Sediment Collection and Preparation

PAMPs were purchased from Tesulang Chemical Materials Ltd. in Dongguan, China, and were immersed in 2% HNO₃ and rinsed with distilled water to remove potentially absorbed metal elements [41]. Scanning electron microscopy (Taisi Ken Trading Co., Ltd, Brno, Czech Republic) was used to observe the particle size and morphology of the microplastics and the adsorption of MPs on plant surfaces. The particle size distribution and functional group composition of the MPs were measured using a laser particle size meter (Malvern Instruments Limited, Malvern, UK) and Fourier infrared spectroscopy (Thermo Fisher Scientific, Waltham, MA, USA), respectively.

Vallisneria natans seedlings and sediments were collected in May from Xukou Bay (31°12'50", 120°28'49"), a macrophyte-dominated lake area without cadmium pollution in the east part of Lake Taihu, which is the third largest freshwater lake in the lower reaches of the Yangtze River. It has a surface area of 2338 km², a mean depth of 1.9 m, and a maximum depth of ≤3 m [26]. The collected *Vallisneria natans* seedlings were rinsed with ultrapure water to remove any attached sediment particles, benthic fauna, or other impurities. The water quality parameters dissolved oxygen (DO), electric conductivity (EC), total dissolved solids (TDS), salinity, and pH were measured in situ using a multifunctional water quality tester (Hengxin Industrial Co., Ltd, TW, CHN), while the total nitrogen (TN) and total phosphorus (TP) contents of the overlying water were determined in the laboratory using alkaline potassium persulphate UV spectrophotometry and ammonium molybdate spectrophotometry, respectively. The TN, total carbon (TC), and total organic carbon (TOC) concentrations in sediment were determined using an elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

To avoid interference from plastic containers, macrophyte cultivation was performed in glass bottles with a height of 23.0 cm and a diameter of 7.8 cm, which were carefully cleaned with 2% HNO₃ and then rinsed with distilled water before use. The sediments were dried naturally and passed through a 160-mesh stainless steel sieve to remove coarse particles and plant parts. Seedlings of similar sizes were pre-cultured in an incubator for 14 days at a temperature of 25 ± 1 °C, light intensity of 6000 Lx, and a 12/12 h light/dark cycle to simulate their natural growing environment. Cadmium chloride hemi (pentahydrate) was purchased from Shanghai Aladdin Biochemical Technology Co(SH, CHN). Both CdCl₂·2¹/₂H₂O and PAMPs were stored at 4 °C in the dark before being used in experiments to prevent physicochemical property changes in the microplastics and photodegradation of the cadmium salt.

2.2. Experimental Setup of Treatments

The Cd concentrations were set at 0, 0.3, and 1.0 mg/L following Wang et al. (2010) [42], which represent moderate and serious aquatic cadmium pollution and the actual Cd exposure levels in polluted freshwater sediment. The PAMP concentrations were set at 0, 0.1%, 0.3%, and 1.0% of the dried sediment mass [15] based on the reported environmental background levels. To have a fair chance of finding environmentally relevant effect thresholds, low, realistic doses as well as high doses were used. Twelve treatments (four PAMP levels combined with three Cd levels) were carried out with four replicates for each treatment, all of which were independent experimental units. A 500 g sample of dry sediment was mixed with the appropriate amount of the PAMP and CdCl₂ solutions and added to each glass bottle. The cadmium stock solution was only added to the overlying water in each microcosm, without directly spiking it into the sediment. Deionized water was slowly added before the seedlings were transplanted. After two weeks of exposure, the

plants from each treatment were collected, and the following were measured: fresh weight, root length, biomass, chlorophyll content, and antioxidant enzyme activities.

2.3. Plant Growth Characterization

The plants were gently rinsed with deionized water three times and dried with absorbent paper before measuring their morphological and physiological parameters. The relative growth rate (RGR) of the plants was calculated based on the difference in fresh weight before and after treatment. Chlorophyll content was determined using the ethanol method according to the method of Li [43] (2000). The chlorophyll a, chlorophyll b, and carotenoid contents (mg/g fresh weight) were calculated according to Lichtenthaler [44] and Wellburn (1983). After 1 d and 14 d of treatment, plant leaves were randomly selected to measure the fluorescent parameters F_v/F_m (Driving-pam, Germany) to determine the maximum photo-yield.

2.4. Oxidative Stress Parameters

Fresh leaves were washed with deionized water before being used to determine the malondialdehyde (MDA) content and antioxidant enzyme activities (superoxide dismutase (SOD) and the peroxidase (POD)) using the thiobarbituric acid (TBA), WST-8, and guaiacol methods, respectively, according to the protocols of Wang [26]. The soluble sugar content was measured using assay kits based on the anthrone colorimetric method (Suzhou Keming Biotechnology Co. Ltd., Comin, China).

2.5. Cadmium Accumulation

The macrophytes were washed with deionized water and freeze-dried for 2 weeks, and then digested with a 3:1 (v/v) mixture of HCl and concentrated HNO_3 to quantify the Cd concentration using an inductively coupled plasma mass spectrometer (ICP-MS) [26]. The biological concentration factor (BCF) was then calculated using the concentrations of Cd in the plant tissue versus the culture medium.

2.6. Combined Toxicity of PAMPs and Cd

The Independent Action (IA) model was employed to assess the combined toxicity of PAMPs and Cd towards *Vallisneria natans*. Plant height inhibition rate (%) was selected as the toxic response endpoint, and the theoretical mixture effect was calculated via the IA formula: $E(c_{mix}) = 1 - \prod_{i=1}^n (1 - E(c_i))$, where c_{mix} is the total mixture concentration, $E(c_i)$ is the toxic effect induced by the single i -th component at concentration c_i , and $E(c_{mix})$ is the mixture toxicity predicted by the IA model.

The model deviation ratio (MDR) was calculated to quantitatively estimate the difference between the predicted and actual toxicity according to the method of Phyu [45]. Within the IA framework, MDR represents the deviation between the actual observed value and the model-predicted value. Using a 30% model deviation tolerance threshold, the interaction type is classified based on toxicity criteria: if $\text{MDR} < 0.7$, it indicates that the mixture toxicity is antagonistic; if $\text{MDR} > 1.3$, it indicates that the mixture toxicity is synergistic; if $0.7 \leq \text{MDR} \leq 1.3$, it indicates that the combined toxicity is a combination of the independent effects of each component [45].

2.7. Statistical Analysis

The Shapiro–Wilk test and Levene’s test were used to test all data for normality and homogeneity of variance, respectively, before statistical analyses were performed. To evaluate the individual and combined impacts of the treatments, a two-way analysis of variance (ANOVA) was performed using SPSS 21.0 to test the main effects of Cd concentration, PAMP concentration, and their interaction on the physiological and biochemical

parameters of *V. natans*. When significant main or interaction effects were detected, Tukey's post hoc multiple comparisons were used to identify specific differences between treatment groups. Statistical significance was defined as a confidence limit of 95% ($p < 0.05$). Following one-way ANOVA screening of environmental factors, specific sediment (TP, TOC, TC, and TN) and overlying water (TP, TN, TDS, salinity, conductivity, and DO) parameters showing stable patterns were selected for correlation analysis. Pearson's correlation analysis was then conducted between these environmental indicators and ten plant physiological indicators: plant height, relative growth rate (RGR), light and fluorescence parameters, chlorophyll a, total chlorophyll, SOD, POD, soluble sugars, and MDA. All the graphs were drawn using Origin 2025 and were typeset and optimized in Adobe Illustrator 2023.

3. Results and Discussion

3.1. Characterization of PAMPs

SEM showed that the PAMPs exhibited an amorphous, fragmented structure with a rough surface (Figure 1). FTIR analysis of the PAMPs showed characteristic peaks at 1538.7, 1642.2, 2863.3, 2939.4, and 3302.8 cm^{-1} [46,47], which correspond to the amide II band (N-H bending vibration and C-N stretching vibration), amide I band (C=O stretching vibration), aliphatic C-H stretching vibrations, and amide N-H stretching vibration, respectively. Laser particle size analysis showed that the volume-averaged particle size was $78.16 \pm 0.10 \mu\text{m}$, with a deviation of less than 3% from the nominal value.

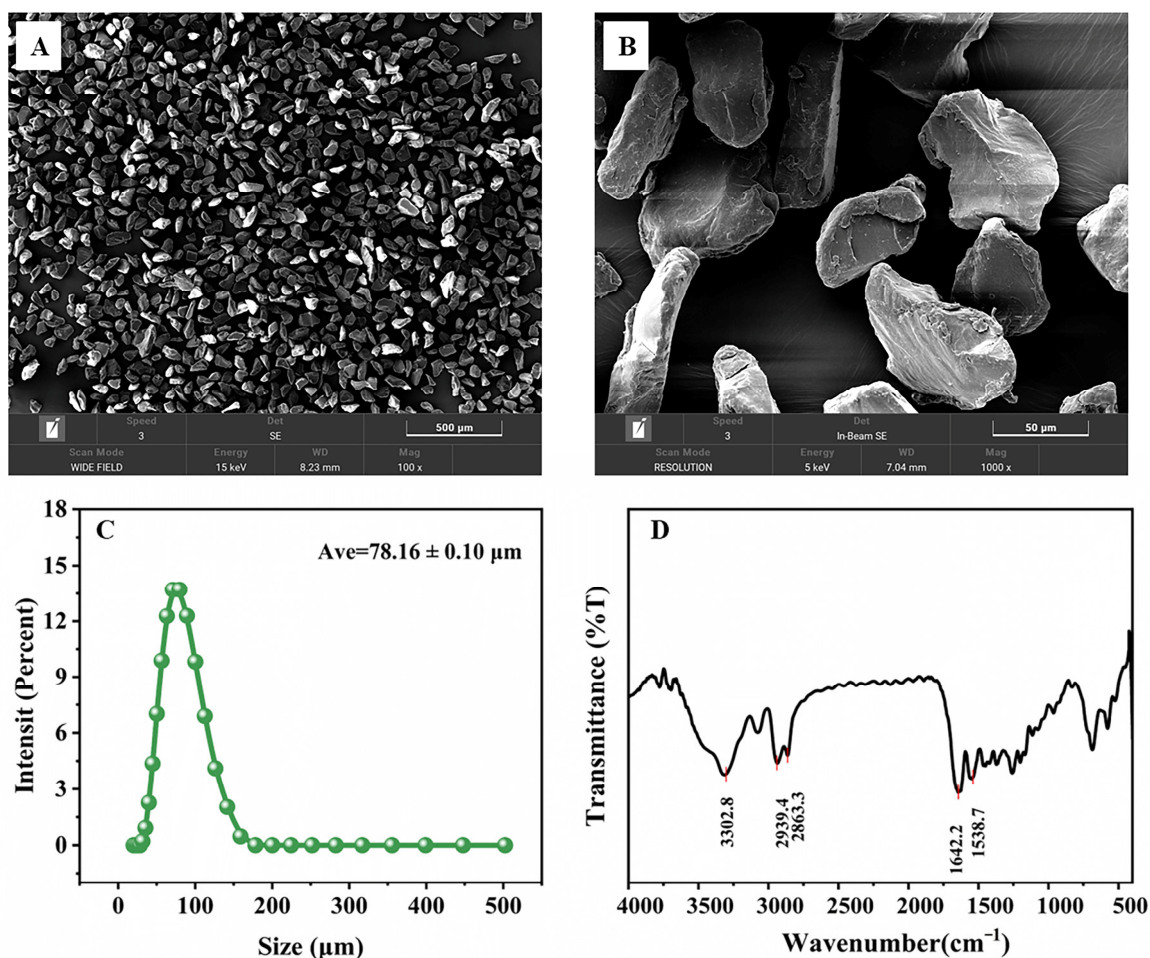


Figure 1. Characterization of polyamide microplastics (PAMPs) used in this study. SEM images of PAMPs at 500 and 50 μm scales (A,B), particle size distribution of PAMPs (C), and FTIR spectra (D).

3.2. PAMPs Attenuate Cd Toxicity Toward Plant Growth and Photosynthesis

The response of *Vallisneria natans* to Cd, PAMPs, and their mixture is shown in Figure 2. Plant height is highly sensitive to external environmental stress, which induces changes in the biochemical processes in vivo. Compared with the control, high cadmium concentrations significantly reduced plant height and RGR by 49.63% and 65.25% ($p < 0.01$) and reduced plant growth (Figure 2A,B). Under PAMP treatment, plant height and RGR showed a negative correlation with PAMP concentration. Specifically, compared with the CK group, plant height and RGR increased by 28.15% and 12.59% under the low (0.1%) concentration and 44.46% and 8.02% under the medium (0.3%) concentration treatment. By contrast, plant height and RGR under high concentration (1.0%) treatment decreased by 31.11% ($p < 0.01$) and 15.67%, respectively. Previous studies have shown that PAMPs significantly reduced the growth of *Lemna minor* L. and decreased the activity of root cells through mechanical blockade [48], indicating that low concentrations of MPs may exert a slight promoting effect on aquatic plant growth. Similarly, exposure to low concentrations of PSMPs alone increased RGR in *V. natans* by 25.61% ($p < 0.05$) compared to the control [15]. Compared with 1.0% MP treatment, the combined exposure to Cd and PAMPs increased plant height and RGR, indicating a slight promoting effect on submerged plant growth. This result indicates that cadmium toxicity dominates under high-concentration conditions, whereas the addition of microplastics, which serve as adsorptive carriers for cadmium, partially mitigates the toxic effects of cadmium on *V. natans* through adsorption.

The plant photosynthetic system is highly sensitive to stress, especially heavy metal stress [49], and the results showed that the chlorophyll content decreased significantly with increasing Cd concentration. Compared to the control, the chlorophyll a, chlorophyll b, and carotenoid contents under high Cd treatment decreased by 35.09%, 76.83%, and 17.65%, respectively (Figure 2C–E). Under PAMP treatment, the chlorophyll a, chlorophyll b, and carotenoid contents all exhibited a negative correlation with PAMP concentration. At the high PAMP concentration (1.0%), these three indices decreased by 51.26%, 55.19%, and 35.51% compared to the control ($p < 0.01$). Under cadmium treatment, F_v/F_m and the soluble sugar content showed a decreasing trend, with decreases of 2.63% and 5.33%, respectively, at high concentrations compared with the control. They decreased by 4.42% and 12.52% under the high MP concentration treatment, respectively, compared with the control. These decreases can be attributed to the shading effect of high concentrations of PSMPs adsorbed on the leaves of submerged plants, which blocks light, and to the damage caused by the accumulation of PSMPs in chloroplasts [50]. High concentrations of PSMPs can inhibit leaf growth and photosynthetic capacity. This inhibition is linked to stress-induced impairment of chloroplast metabolic functions [51]. The increase in intracellular ROS triggered by high concentrations of PAMPs may cause oxidative stress, disrupt chloroplast and cyst structures, and inhibit photosynthesis, thus hindering chlorophyll synthesis.

Combined exposure to PAMPs and Cd reduced the chlorophyll content and limited chloroplast and photosystem formation. This could be caused by alterations to pigments and proteins [16]. Reduced soluble sugar and protein contents will directly lead to a lack of intracellular energy sources, slowing or even preventing many important intracellular physiological activities, which ultimately manifests as slowed or stagnant cell growth [52,53]. Compared to the 1.0% PAMP treatment, combined exposure increased the F_v/F_m of the *V. natans* seedlings by 1.18% and 3.60%, respectively ($p > 0.05$). A slight increasing trend in chlorophyll content was also observed. These changes could be attributed to the efficient adsorption of cadmium by the high concentration of PAMPs, which reduced Cd bioavailability, thereby alleviating its damaging effects on the photosynthetic system.

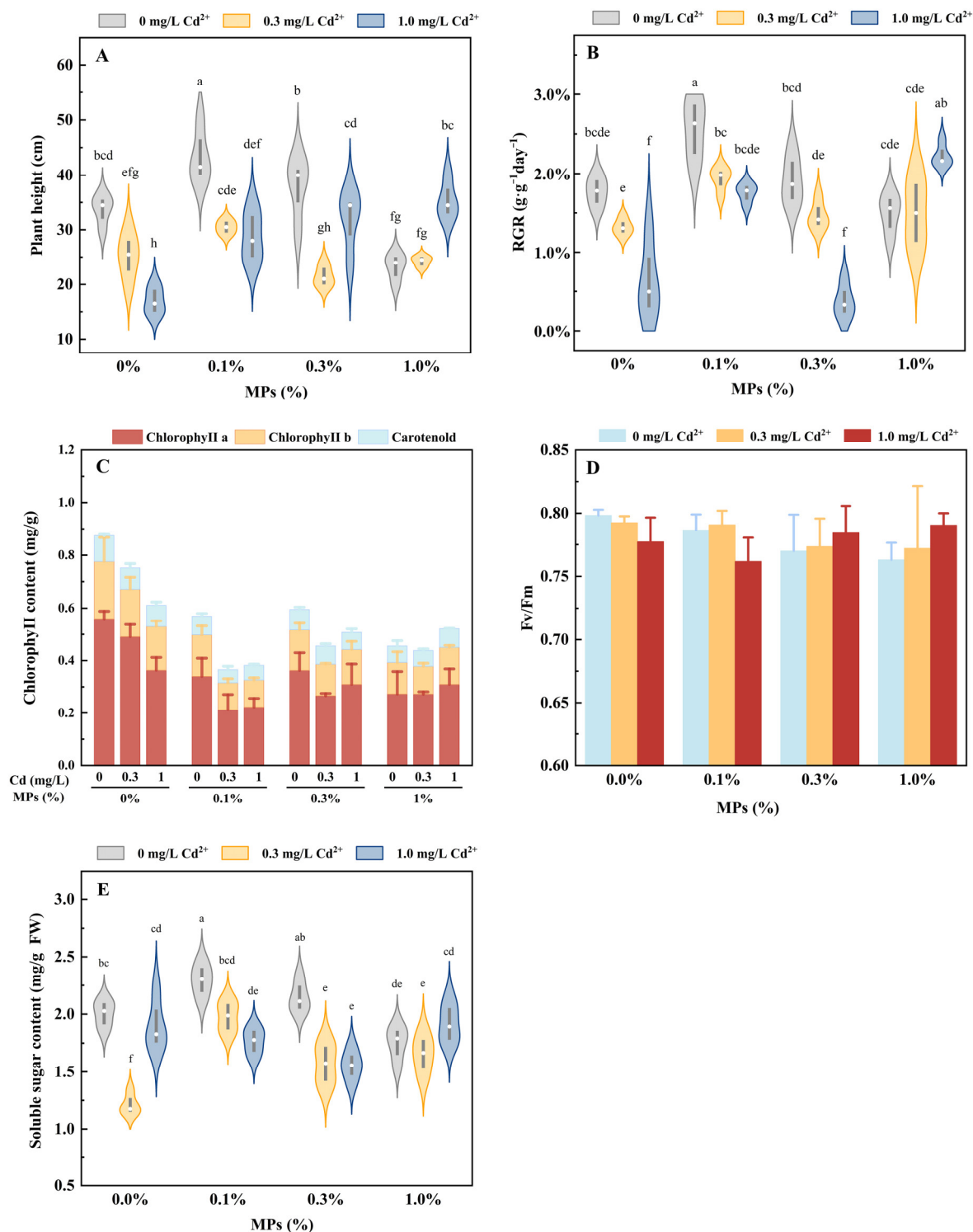


Figure 2. Plant height (A), relative growth rate (RGR) (B), chlorophyll content (C), Fv/Fm (D), and soluble sugar content (E) of *Vallisneria natans* seedlings exposed to cadmium, polyamide microplastics, or their combination. Values represent means $\pm$ standard deviations ($n = 4$). Different uppercase and lowercase letters indicate significant differences according to Tukey's test with a confidence limit of 95%.

3.3. PAMPs Intensify Cd-Induced Lipid Peroxidation and Oxidative Stress

The level of MDA, a natural product of lipid peroxidation, and changes in the permeability of the cytoplasmic membrane are important indicators of the degree of lipid peroxidation of cellular membranes and the degree of plasma membrane disruption [54].

Exposure to the high concentration (1 mg/L) of cadmium resulted in a significant increase in MDA levels, which increased by 22.52% ($p < 0.01$) compared to the control (Figure 3A). Under MP treatment, the MDA content showed an increasing trend with increasing PAMP concentration, which increased by 19.00%, 11.13%, and 25.13% ($p < 0.05$), respectively, compared with the control. Compared to the individual exposures, combined exposure significantly increased the MDA content; specifically, the addition of PAMPs increased the toxicity of Cd by 14.43% to 31.89%. This may be because the submerged plants received severe damage that could not be rapidly mitigated, resulting in an imbalance between lipid peroxidation-free and antioxidant systems [26]. A previous study found that exposure to PVCMPs and Cd showed a similar trend in *V. natans*. Exposure to higher concentrations of PEMP in the soil increases the accumulation of heavy metals, which have a toxic effect on oilseed rape plants, thereby exacerbating oxidative damage in plants [55].

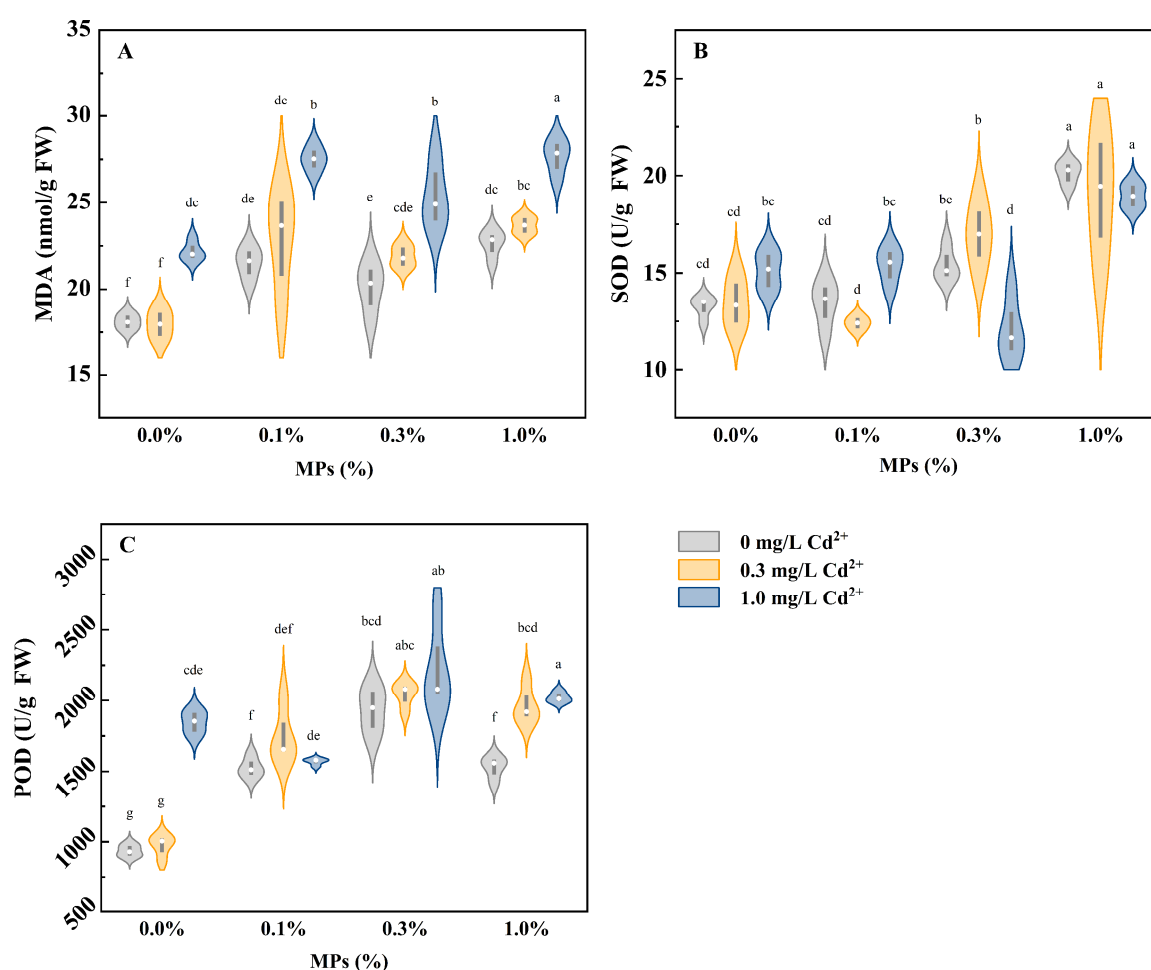


Figure 3. Malondialdehyde (MDA) content (A), superoxide dismutase (SOD) activity (B), and peroxidase (POD) activity (C) of *Vallisneria natans* seedlings exposed to cadmium, polyamide microplastics, or their combination. Values represent means $\pm$ standard deviations ($n = 4$). Different uppercase and lowercase letters indicate significant differences according to Tukey's test with a confidence limit of 95%.

SOD and POD are the main antioxidant defense enzymes in plant cells, and they are mainly associated with plant tolerance to abiotic stresses. SOD is part of the endogenous antioxidant defense system, which prevents and mitigates oxidative stress by catalyzing the conversion of ROS to H₂O₂ and O₂ [56]. When plants are stressed, an increase in SOD

activity leads to a decrease in ROS levels [26,57,58]. SOD activity under both individual and combined exposure showed an increasing trend, especially under a high concentration of MPs, where the SOD activity increased by 51.95% ($p < 0.001$) compared to the control (Figure 3B), suggesting that the MPs induced the generation of superoxide radicals [27]. POD can maintain intracellular redox homeostatic balance by catalyzing the reduction in H_2O_2 and other organic hydroperoxides [59]. Both individual and combined exposure induced a significant increase in POD activity, with increases ranging from 4.10% to 106.63% under the individual treatments compared to the control (Figure 3C). The cadmium-induced increases in SOD and POD enzyme activities increased further with the addition of PAMPs.

Both MPs and cadmium cause oxidative damage to plants [29]. Under PAMP or cadmium stress, the photosynthetic capacity of plants is weakened, and the efficiency of electron transfer is reduced, leading to the transfer of electrons to O_2 , forming reactive oxygen species (ROS), and resulting in oxidative stress. Excessive ROS production causes peroxidation of polyunsaturated fatty acids and disrupts cell membrane permeability and the structure of molecules, such as lipids, proteins, and DNA. Damage to biomolecules triggers a series of reactions, such as cell death, tissue damage, and changes in the metabolic system of plants.

It was found that co-exposure further enhanced oxidative stress in *V. natans*, with higher MDA and POD contents in the co-treated group (6.43–27.87% and 3.02–32.00%, respectively) compared to PAMP treatment alone. Active antioxidant enzymes provide a better defense mechanism against the oxidative damage induced by the combined action of PAMPs and Cd, and various antioxidant defense systems are activated in submerged plants, leading to the accumulation of defense-related metabolites [26]. The interaction of PAMPs with Cd further enhanced oxidative damage, possibly because the effect of MPs on enzyme activities may be closely related to plant habitat, plasticity, and plant species [60].

3.4. PAMPs Decrease Cd Uptake and Bioconcentration in Leaves

A dose-dependent effect on Cd uptake was observed in *V. natans* (Figure 4). Under Cd treatment alone, Cd uptake by both roots and leaves increased significantly ($p < 0.001$) with increasing exposure concentration, and the addition of PAMPs reduced Cd uptake by leaves by 2.38% ~ 26.12%. The bioconcentration factor (BCF) reflects the uptake efficiency of heavy metals and is a commonly used indicator for evaluating the toxicity and transfer of heavy metals from the environment to plants [61]. The BCF of roots increased significantly ($p < 0.001$) with increasing Cd concentration, and the addition of MPs did not significantly reduce the toxicity of Cd in the roots. Microplastics are known to reduce the adsorption capacity of soil for Cd and increase Cd bioavailability and plant uptake [62,63], and Lian et al. found that MPs partially reduced the Cd concentration in wheat, which in turn reduced the toxicity of Cd [64].

In contrast to roots, the BCF of *V. natans* leaves was significantly decreased ($p < 0.001$), indicating impaired Cd enrichment capacity in the leaves. This impairment may be attributed to Cd-induced plant damage, potentially manifesting as slow growth [65]. Notably, PAMPs exerted opposite regulatory effects on Cd bioaccumulation in roots compared to leaves. On the one hand, PAMPs possess strong adsorption affinity for dissolved Cd ions in overlying water; the abundant PAMP–Cd complexes attach to root epidermal cells, trapping more Cd on root surfaces and elevating root Cd accumulation and the BCF. On the other hand, co-exposure to PAMPs and Cd exacerbates oxidative damage to leaf tissues, suppresses the xylem-mediated root-to-shoot Cd translocation pathway, and further damages leaf metal uptake systems, ultimately weakening the leaf Cd enrichment capacity. Critically, under high Cd exposure concentrations, PAMPs reduced the Cd uptake and

the BCF of *V. natans*. This reduction is likely due to the oxidative damage and diminished Cd enrichment capacity induced by MPs [61]. A similar effect was observed in *Chlorella*, where PAN reduced Cu uptake [66]. MPs also inhibited the accumulation of Pb in *Chlorella vulgaris* due to the adsorption of pollutants by the MPs and the aggregation that occurs in adsorption, thus reducing the toxicity of Pb [67].

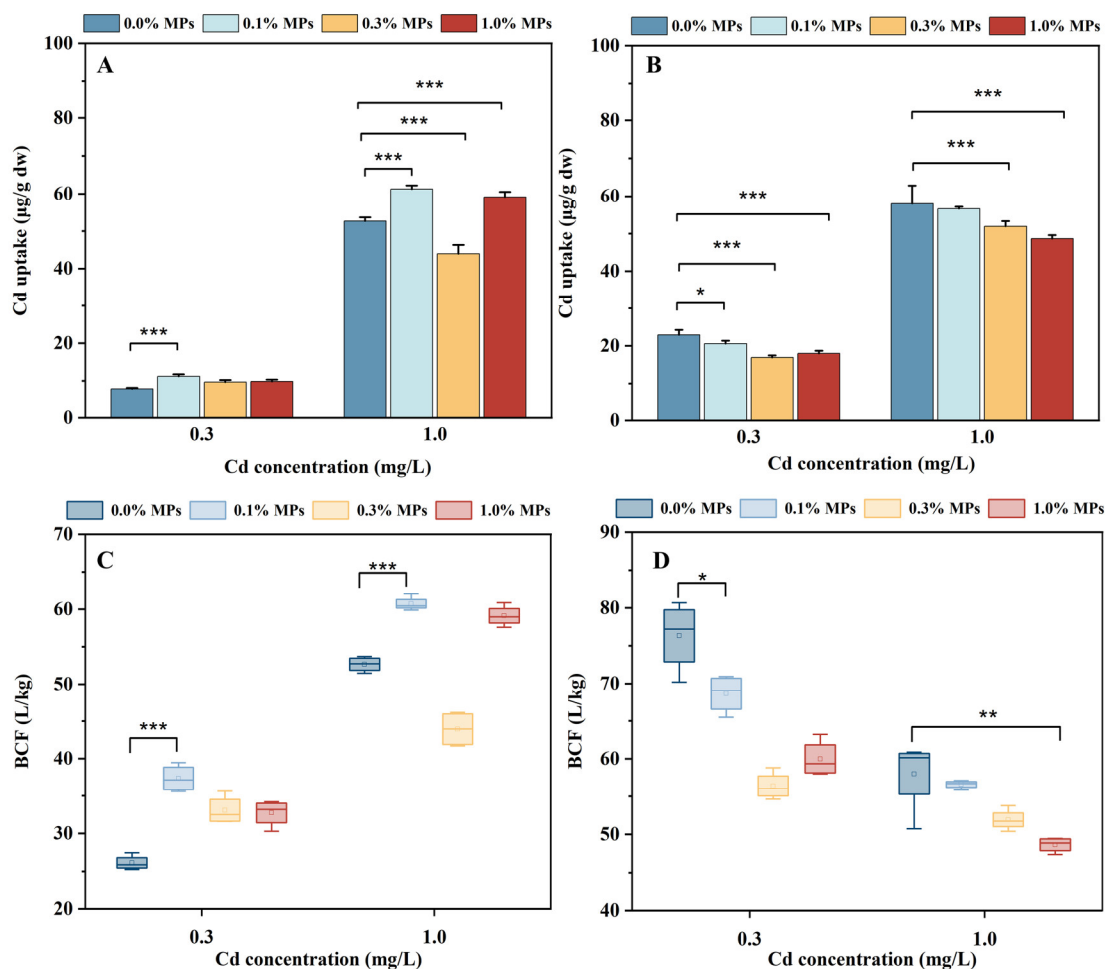


Figure 4. Cadmium uptake in roots (A) and leaves (B), and bioconcentration factor (BCF) in roots (C) and leaves (D) of *Vallisneria natans* seedlings exposed to cadmium, polyamide microplastics, or their combination. Asterisks indicate statistically significant differences compared to the control group (0.0% MPs) for the same Cd concentration: $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

In this study, the correlation between the addition of PAMPs and Cd uptake, as well as other physiological and biochemical responses, was analyzed to investigate the effect of MPs on *V. natans* exposed to the same Cd concentration. The addition of PAMPs was negatively correlated with Cd uptake and the chlorophyll content of leaves and significantly positively correlated with SOD activity (Figure 5). Additionally, Cd uptake in *V. natans* was found to be positively correlated with MDA content, soluble sugar content, and POD activity. These results suggest that PAMPs aggravated the damage to *V. natans*, probably due to the sharp edges of MPs, which resulted in physical damage. As shown in the SEM images (Figure 6), the surface structure of *V. natans* exhibited distinct morphological damage under the different treatments. Compared to the intact and smooth surface of the control group (Figure 6A), the plants exposed to Cd alone (Figure 6B) showed significant structural degradation, characterized by increased surface roughness. In the

PAMP exposure group (Figure 6C), microplastic particles extensively adhered to and embedded into the plant epidermal layer. Under combined exposure (Figure 6D), the surface damage was further exacerbated, presenting deeper tissue crevices and a massive accumulation of PAMPs. SEM images revealed visible physical adhesion between PAMPs and tissues of *V. natans*. While these observations indicate a direct physical interaction, this evidence cannot fully confirm whether this interaction has a dominant causal role in the altered Cd bioaccumulation. We posit that adhered PAMPs could obstruct light capture and root nutrient uptake channels, which could partially contribute to the modified Cd enrichment capacity under combined exposure. In both the MP exposure and co-exposure groups, the adhered PAMPs could have formed localized covering layers that directly obstructed incident light and reduced the light capture efficiency of leaves. This aligns with the observed decline in chlorophyll content, indicating that the addition of PAMPs inhibited chlorophyll synthesis, thereby impairing photosynthesis [68]. Thus, three independent modes of action can be clearly distinguished: surface adsorption, physical blockage, and physiological toxicity. Adsorption describes the attachment of PAMPs onto plant tissue surfaces; physical blockage refers to mechanical obstruction of light penetration, root pores, and internal transport pathways; and physiological toxicity arises from disrupted cell membranes, suppressed respiration, and altered rhizosphere microenvironments. Due to their relatively large particle size and high density, PAMPs are prone to settling and accumulating in sediments. This could lead to the roots becoming enveloped by PAMPs, which could not only reduce the effective root absorption area but also cause physical abrasions, damaging the plasma membranes of root epidermal cells. This membrane damage could compromise the structural integrity essential for active transport mechanisms [69]. Furthermore, PAMP deposition in the rhizosphere can alter the micro-environment, for instance, by reducing porosity and impeding oxygen diffusion. These changes can inhibit root aerobic respiration, triggering the accumulation of toxic metabolites such as lactate and ethanol under hypoxic conditions. Ultimately, these combined effects can exacerbate the functional damage to the root system.

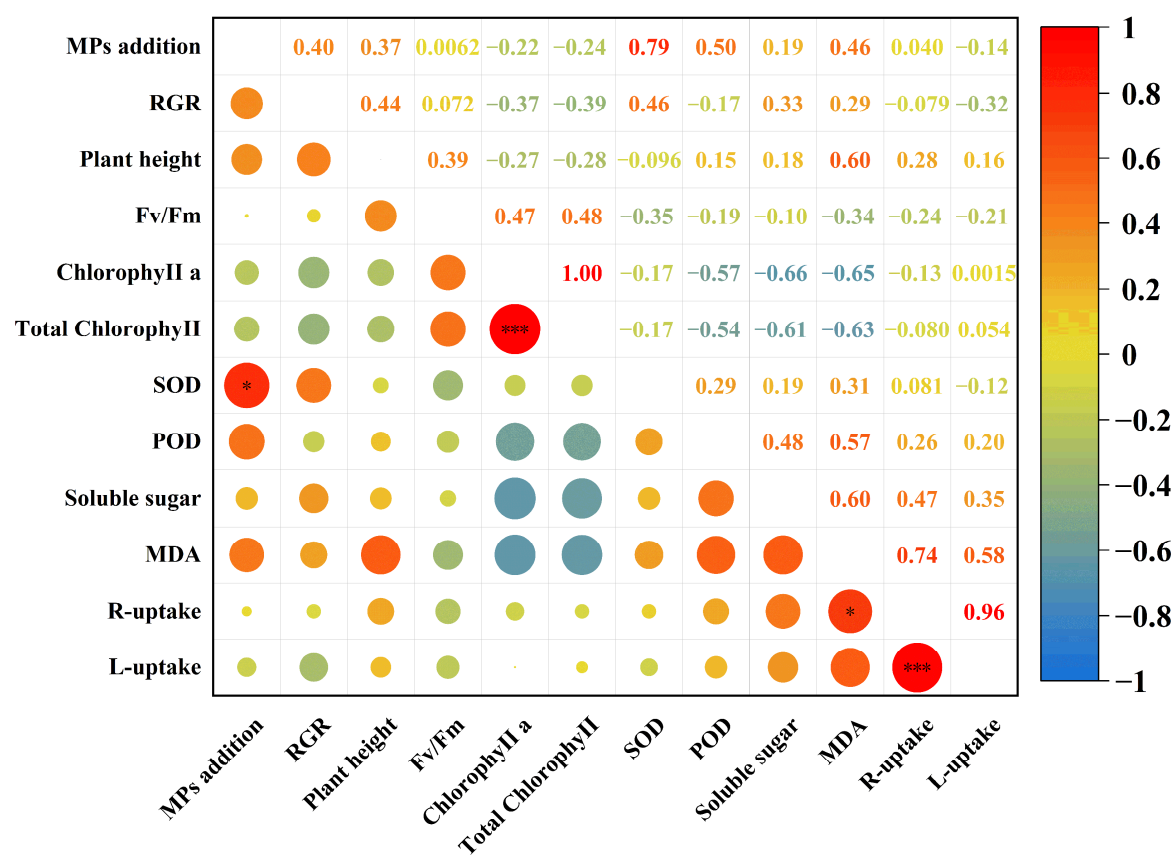


Figure 5. Correlation of physiological and biochemical indicators. Asterisks indicate statistically significant differences at $p \leq 0.05$ (*), $p \leq 0.001$ (**).

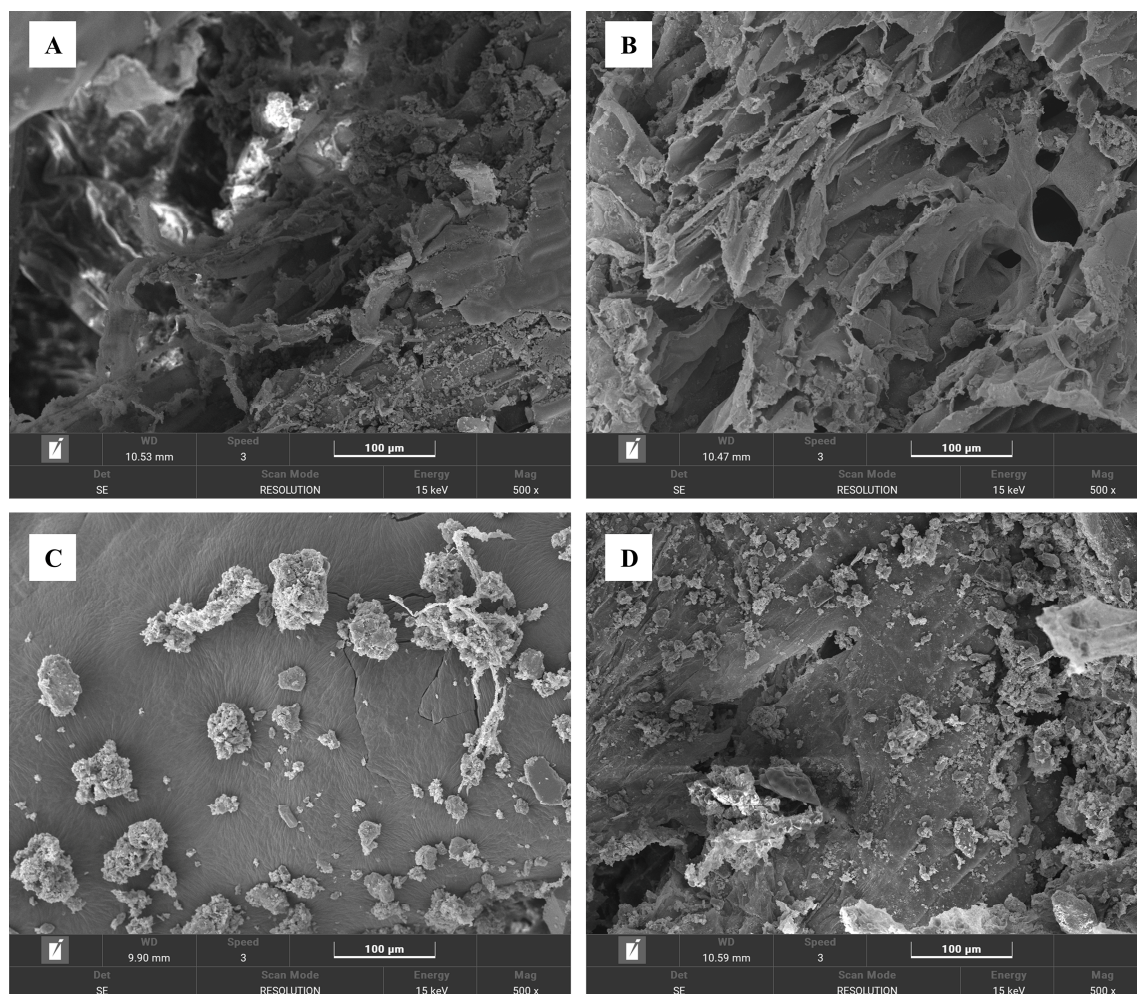


Figure 6. Scanning electron microscopy image of longitudinal sections of *Vallisneria natans* seedlings treated with control treatment (A), Cd (B), polyamide microplastics (PAMPs) (C), and their combination (D).

3.5. Synergism or Antagonism of Toxicity During Co-Exposure Is Dose-Dependent

The IA model is commonly used to assess the joint effects of pollutants with different modes of action. Previous studies have confirmed its feasibility for investigating interactions between nanoparticles and heavy metals [70]. The combined toxicity of microplastics (MPs) and heavy metals towards aquatic organisms was found to be dose-dependent, with toxic effects influenced by both the contaminant dosage and mixture ratio. The plant height inhibition rate was chosen as the sole toxicity endpoint because it integrates overall plant growth impairment, shows stable responses to PAMP–Cd co-stress, and is a standard endpoint in toxicology research on the submerged macrophyte *Vallisneria natans*. The IA model calculation results are presented in Table 1. Among the co-exposure groups, significant synergistic effects ($MDR > 1.3$) were observed for the combinations PA 0.1% + Cd 0.3 mg/L and PA 0.3% + Cd 0.3 mg/L. This result indicates an enhancement of toxicity by MPs when low concentrations of Cd are used. It is hypothesized that MPs may increase the bioavailability of low-concentration Cd through adsorption, which is consistent with the BCF results for *V. natans* leaves exposed to low Cd concentrations (Section 3.4). Conversely, all combinations involving high Cd concentrations exhibited antagonistic effects ($MDR < 0.7$). This suggests that the high concentration of Cd was mainly responsible for the toxicity, while MPs adsorbed some Cd, reducing the concentration of free Cd ions and

creating a “passivation effect”. Previous research on the combined toxicity of gold nanoparticles and MPs showed antagonistic effects on *Daphnia magna* at lower doses but synergistic effects at higher doses [71], which is consistent with the results of the present study.

Table 1. Assessment of the combined effects of polyamide microplastics (PAMPs) and Cd on the submerged macrophyte *Vallisneria natans* using the Independent Action (IA) model.

Treatment	Predicted Value	Observed Value	MDR	Consequence
PA 0.1% w/w + Cd 0.3 mg/L	4.1%	9.6%	2.334	Synergism
PA 0.1% w/w + Cd 1.0 mg/L	35.5%	14.8%	0.418	Antagonism
PA 0.3% w/w + Cd 0.3 mg/L	15.8%	36.3%	2.302	Synergism
PA 0.3% w/w + Cd 1.0 mg/L	43.3%	5.2%	0.120	Antagonism
PA 1.0% w/w + Cd 0.3 mg/L	48.5%	28.1%	0.581	Antagonism
PA 1.0% w/w + Cd 1.0 mg/L	65.3%	4.4%	0.068	Antagonism

3.6. Oxidative Stress Is Positively Correlated with Sediment Conditions and Not Overlying Water Conditions

As shown in Figure 7, SOD was significantly and positively correlated with the sediment indicators; both POD and MDA were positively correlated with the TP, TOC, and TC of the sediment; and all plant growth parameters were significantly and positively correlated with the TP and DO of the overlying water, and negatively correlated with the various physical and chemical indicators of the sediment.

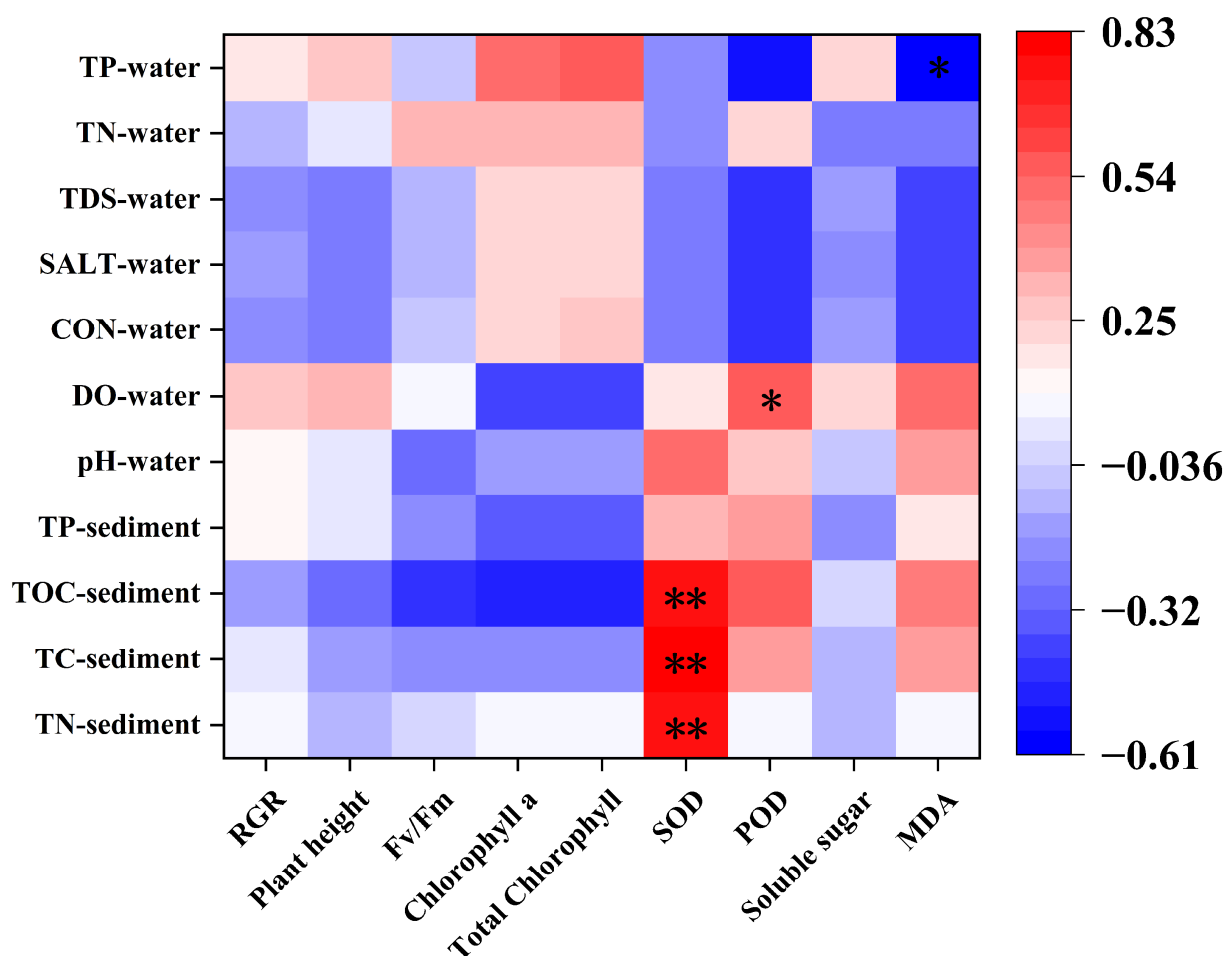


Figure 7. Person correlation analysis of changes in plant and environmental factors under combined treatment. Asterisks indicate statistically significant differences at $p < 0.05$ (*), $p < 0.01$ (**).

Submerged plants tend to live in a homogenized state since the whole plant lives in the water body. Nitrogen is an important nutrient for submerged plants, and water body TN content has been reported to be positively correlated with photosynthetic indicators [72]. In plants, photosynthesis is one of the most sensitive physiological processes to environmental changes, and chlorophyll content is an important metric for measuring the photosynthesis rate. Nitrogen is an important component of the chlorophyll molecule, and higher TN concentrations are often accompanied by elevated chlorophyll contents [73]; one plausible explanation is that plants may alleviate environmental stress by accumulating more chlorophyll. As documented in prior studies, an increased TN content in the overlying water and increased phytoplankton biomass can lead to nutrient and spatial competition, which negatively affects the growth of *V. natans* due to phytoplankton shading that reduces light availability [74,75]. Phosphorus in the sediment mainly affects the growth of organisms by providing them with nutrients, and elevated TP levels can promote plant growth by increasing the biomass of planktonic algae and submerged phytoplankton [76]. However, high TP levels may be detrimental, possibly through endogenous nutrient release, which maintains high water column concentrations [77]. In our study, sediment TP levels exhibited a negative correlation with plant RGR and a positive correlation with plant antioxidant enzyme activities (SOD and POD) and MDA content. Notably, these correlation trends only reflect synchronous variation in the tested indicators and cannot prove definite causal relationships. Based on the above correlative results and the published physiological evidence, we speculate that elevated sediment TP levels may coincide with oxidative stress and growth limitation in *V. natans* under the experimental conditions; subsequent independent verification experiments are needed to confirm this proposed causal association.

4. Conclusions

In summary, Cd concentration is the main determinant of the toxicity to *V. natans*, while MPs act as a modifier by adsorbing free Cd²⁺, producing a passivation effect that mitigates Cd bioavailability. This mitigation paradoxically reduces Cd in leaves but enhances root accumulation, and co-exposure exacerbates growth inhibition and oxidative stress (e.g., induced SOD/POD) compared to the individual treatments. However, the observed antagonistic and synergistic effects are concentration-dependent, warranting further large-scale studies to fully assess their ecological implications.

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