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ORIGINAL RESEARCH ARTICLE

Toxicity effects of microplastics and lead(II) ion co-exposure on *Chlorella*: Protein- and enzyme-level responses

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Abstract: Microplastics (MPs) synergistically interact with heavy metal contaminants, posing substantial ecological threats to biota. Hydrodynamic regimes and water-level fluctuations within the Three Gorges Reservoir (TGR) critically influence contaminant dynamics. Nevertheless, biomolecular responses to MP and heavy metal co-exposure at protein and enzymatic levels remain inadequately characterized. This study selected polyethylene (PE), polypropylene (PP), and lead(II) ions (Pb^{2+}) to examine the freshwater microalgae *Chlorella vulgaris* within the TGR. After 96 h of exposure, the half-maximal effective concentrations for PE and PP against *C. vulgaris* were determined to be 334 mg/L and 295.29 mg/L, respectively. PE, PP, and Pb^{2+} significantly reduced the accumulation of *C. vulgaris* biomass, deteriorated algal cell growth, and caused the algal cells to undergo membrane lipid peroxidation. Nitrate and phosphate assimilation were considerably inhibited across all treatments, with heightened suppression under co-exposure. MPs' addition significantly increased malondialdehyde levels. MP adsorption of Pb^{2+} appeared to attenuate Pb^{2+}-associated changes in nitrate reductase (NR) and alkaline phosphatase activities. This study provides a theoretical foundation for evaluating MP and heavy metal ecological risks in reservoir ecosystems.

Keywords: Three Gorges Reservoir; Combined toxicity, Polyethylene; Polypropylene; Lead ion; Enzyme response

1. Introduction

Rapid industrialization has precipitated microplastics (MPs) and heavy metal pollution as paramount environmental challenges, exerting multiscale ecosystem impacts. MPs (<5 mm) originate primarily from plastic degradation, industrial emissions, and microbeads in personal care products.[1,2] Their small size and diverse transmission pathways facilitate widespread migration across atmospheric, aquatic, and terrestrial environments. Large plastic items can fragment into MPs and even nanoplastics through physical (abrasion and wave action), chemical (ultraviolet radiation), and biological processes (degradation), thereby increasing adsorption capacity.[3,4]

Since its commissioning, the Three Gorges Reservoir (TGR) has profoundly altered local hydrological regimes and pollutant transport, resulting in the formation of a water-level fluctuation zone approximately 30 m deep.[5] This area, subject to continuous water-level variations and surface vegetation interception, has become a potential accumulation zone for MPs. Extensive investigations across different sections of the TGR have reported MPs concentrations averaging 4.895 ± 3.670 items/m^3 in water and 286 ± 229 items/kg in sediments.[6] The predominant types of polymer identified include polyvinyl chloride, polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), and polystyrene (PS).[7,8] In the Chongqing section of the TGR, MP concentrations in water are typically below 1000 particles/L, and lead(II) ion (Pb^{2+}) concentration is below 1 mg/L, with low-density MPs, such as PP and PE, dominating the water column. In contrast, higher-density PS is more prevalent in sediments.[9] The densities of PE and PP allow them to remain in suspension in slow-moving waters, whereas PS settles out. During the reservoir storage period (from October to April of the following year), the emergence of quasi-stagnant water bodies under elevated water levels likely enhances Stokes settling of higher-density MPs and reduces their resuspension from sediment layers.[10] Moreover, MPs in reservoir environments can adsorb co-occurring contaminants, such as heavy metals and persistent organic pollutants, leading to the formation of toxic aggregates with increased bioaccumulation potential. This process considerably elevates ecological exposure risks within the reservoir ecosystem.[11]

Rapid socioeconomic development and urbanization in the TGR region have led to large-scale pollutant discharge, including industrial and domestic wastewater. Flow regulation by the Three Gorges Project may promote the remobilization of heavy metals from sedimentary deposits,[12] leading to significantly increased heavy metal pollution in both aqueous and sediment environments.[13,14] The total concentrations of zinc and Pb in the TGR sediments far exceeded regional background levels. It is estimated that 72–95% of upstream-derived heavy metals are retained within the reservoir,[15] increasing ecological risks. In the Chongqing section of the TGR, the concentrations of PE and PP in surface water range from 120 to 850 particles/L, with higher levels observed during the dry season (October–April) due to reduced water flow. The concentration of Pb^{2+} in surface water is usually below 1 mg/L in the normal season, but can exceed 1.2 mg/L in the wet season (May–September) after heavy rains, mainly due to the remobilization of sediment-bound Pb, with a corresponding increase in Pb levels in fish observed post-rainy season.[16,17] Of particular concern is the formation of a composite toxic carrier, in which MPs and heavy metals interact. MPs can indirectly mobilize heavy metals by altering sediment environments, thereby increasing the potential for secondary release at the sediment-water interface.

Co-exposure to MPs and chemical contaminants often induces more severe biological impacts than exposure to individual stressors, highlighting the importance of assessing their combined toxicological effects.[18] Heavy metals are known to induce toxic responses in algae; for instance, Pb^{2+} exhibits a concentration-dependent effect on *Microcystis aeruginosa*, stimulating growth at low levels while inhibiting it at higher concentrations.[19] A decline in phytoplankton adaptability has also been linked to MP exposure.[20] In general, algal growth and photosynthetic performance decline under MP stress,[21] with adverse effects reported for green algae, diatoms, and cyanobacteria.[22-24]

Research on the co-exposure effects of MPs and metals on *Chlorella vulgaris* has revealed synergistic toxicity. For example, one investigation reported inhibition rates of 70.43% in growth and 64.09% in chlorophyll content under combined PS and heavy metal stress.[18] Another study indicated that the ternary combination of copper, polyacrylonitrile, and MPs suppressed algal development.[25] A study on PET–Pb^{2+} combined stress found that while the adsorption of Pb^{2+} onto PET MPs reduced the chemical stress from free Pb^{2+}, the physical presence of MP–Pb^{2+} complexes still posed a serious threat to algal survival.[26]

Despite these findings, significant research gaps remain. Most studies have focused on single pollutants, whereas research on combined MP–heavy metal pollution is relatively scarce. In real-world environments, such as the TGR, these pollutants coexist and can interact, producing complex synergistic or antagonistic effects.[27] There is a particular lack of comparative studies on different MP polymer types and of mechanistic investigations of changes in enzymatic activity. Therefore, in-depth research on the effects of combined pollution is of great practical significance.

The prevalent algal species, *C. vulgaris*, in the TGR was selected to investigate the combined effects and mechanisms of Pb^{2+} and MPs through acute and chronic exposure tests.[28] This study employed PE and PP because they are the dominant MP polymers in the TGR, accounting for ~45% of total MPs in surface waters.[9] Their low density (~0.9 g/cm^3) allows them to remain suspended in slow-moving reservoir waters, thereby increasing their exposure to pelagic algae, such as *C. vulgaris*. MP concentrations in TGR were reported to reach 1200 mg/L, which exceeded actual environmental levels.[9] The key measured endpoints included growth inhibition rate, chlorophyll a content, soluble protein, malondialdehyde (MDA) content, and the uptake capacity of nitrate (NO_3^-) and phosphate (PO_4^{3-}) ions. Furthermore, we investigated changes in the activities of key enzymes, such as alkaline phosphatase (AKP) and NO_3^- reductase (NR). The results can also help us to evaluate MP–heavy metal ecological risks in reservoir ecosystems.

2. Materials and methods

2.1. Materials and reagents

C. vulgaris and BG-11 medium were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Before use, the BG-11 medium was autoclaved at 121°C for 30 min. Algal cultivation was conducted at 25 ± 1°C, illuminated at 5000 lux under a 12-h light/12-h dark photoperiod. The cultures were manually shaken daily to ensure optimal growth.

Lead(II) nitrate was purchased from National Nonferrous Metals and Electronic Materials Testing Center (China). PE MPs (white powdery solids, 120–180 μm) and PP MPs (white powdery solids, 120–180 μm) were purchased from Total Energies (China). The MPs were modified by coating with Tween 80.[28] Specifically, PE and PP MPs were modified by adding Tween 80 at a final concentration of 0.1% (v/v) to the MP suspension. The mixture was stirred at 200 rpm for 2 h at 25°C and then sonicated for 30 min to ensure uniform coating. After modification, the surface charge of PE MPs changed from −2.3 mV (unmodified) to −5.1 mV, and that of PP MPs changed from −5.7 mV (unmodified) to −8.2 mV, as measured using a zeta potential analyzer. The hydrophobicity of modified MPs, determined by the contact angle method, increased by approximately 15% compared to unmodified MPs. Commercially available kits from Nanjing Jiancheng Bioengineering Institute (Soluble protein [A045-2-2], MDA [A003-1], NR [A096-1-2], AKP [A059-1-1], China) were used to determine the levels of soluble protein, MDA, NR, and AKP.

2.2. Design of exposure experiments

2.2.1. Acute toxicity study of MPs on C. vulgaris

C. vulgaris was subjected to a 96-h toxicity test in accordance with the acute aquatic hazard classification criteria. PE and PP particles (0, 0.5, 5, 10, 20, 40, and 60 mg) were weighed and added to a conical flask containing 25 mL of dispersant. Weighing of small PP/PE masses (0.5–60 mg) was performed using a microbalance with a sensitivity of 0.001 mg (XP2U, Mettler Toledo International Inc., Switzerland), calibrated with standard weights. To avoid static adhesion and weighing errors: (i) Weighing boats were pre-rinsed with 75% ethanol and dried to eliminate static electricity; (ii) For 0.5 mg samples (the lowest concentration), a cumulative weighing method was adopted: 5 mg of MPs were first weighed into a sterile 5 mL centrifuge tube, mixed with 5 mL of sterile ultrapure water, and vortexed for 1 min to form a homogeneous suspension. A 0.5 mL aliquot of this suspension (containing 0.5 mg MPs) was then pipetted into the experimental conical flask; (iii) All weighing operations were completed within 30 s in a low-humidity (40–60%) environment to minimize particle loss. Each concentration was weighed in triplicate, with a relative standard deviation (SD) < 5%. The conical flasks were placed on a vortex shaker for 1 min and sonicated for 30 min. An algae-containing solution (25 mL) was added to obtain samples with MP contents of 0, 10, 100, 200, 400, 800, and 1200 mg/L. Cells were seeded at 2.46×10^6 cells/mL in flasks sealed with gas-permeable membranes and subjected to daily agitation. Absorbance was measured after 96 h. Each concentration was tested in triplicate.

2.2.2. MP combined with lead(II) ion exposure experiment

The 96-h half-maximal effective concentrations (EC_{50}) were used as MP exposure concentrations in the co-exposure experiment based on the results presented in Section 3.1. The diluted lead(II) nitrate stock solution was added to the Pb^{2+} only, Pb^{2+} + PE, and Pb^{2+} + PP groups. The Pb^{2+} concentrations in each experimental group were 0, 0.05, 2, and 5 mg/L, respectively. Each concentration was tested in triplicate. The initial cell concentration was approximately 1×10^7 cells/mL, and the culture cycle was 28 days. Cell biomass, chlorophyll a content, and nutrient salt concentration were measured every 3 days, and soluble protein content and related nutrient salt enzyme activities were measured every 7 days.

2.3. Sample test

Algal cell concentration was determined using a fitting curve through absorbance at 680 nm.[26] The equation was expressed as $Y = 2.38 \times 10^7 X = 1.13 \times 10^5$, where X is the absorbance at a wavelength of 680 nm (L/[g·cm]) and is the number of algal cells (ind).

Chlorophyll a was extracted using hot ethanol and quantified spectrophotometrically at 680 nm.[29] The content of soluble protein, MDA, NR, and AKP was measured using commercial assay kits (A059-1-1, Jiancheng Bioengineering Institute, China). Before analysis, algal cells were disrupted via ultrasonication (KQ-500DE, Kunshan Ultrasonic Instrument, China) with the following parameters: power = 100 W, frequency = 40 kHz, duration = 30 min (continuous mode), temperature ≤25°C (maintained with an ice-water bath). Samples were vortexed for 1 min every 10 min during sonication to ensure uniform disruption, and the resulting homogenates were centrifuged at 3000 rpm for 10 min at 4°C to collect the supernatant.

Concentration-inhibition effect curves were plotted using the logarithm of the PE and PP concentrations as the horizontal coordinate and the growth inhibition rate as the vertical coordinate to obtain the 96-h EC_{50}. The growth inhibition rate was calculated using Equations (1) and (2):

$$\mu = \left(\frac{lnX_L - lnX_O}{t_L - t_O}\right) \quad (1)$$

Where μ is the average specific growth rate, N_t is the cell concentration at (ind/mL), N_0 is the initial cell concentration (ind/mL), t_t is the time of analysis, and t_0 is the time of commencement of the study.

$$I_{\mu i} = \left(\frac{\mu_c - \mu_i}{\mu_c}\right) \times 100 \quad (2)$$

where I_μ is the inhibition rate, μ_c is the average growth rate of the blank group, and μ_t is the average growth rate of the experimental group.

Nitrate concentrations were measured using gas-phase molecular absorption spectrometry.[30] PO_4^{3-} concentrations were measured using continuous flow analysis with ammonium molybdate spectrophotometry.[31]

2.4. Statistical analysis

All experiments were conducted with three replicates, and data were presented as means ± SD. Data processing and plotting were performed using Excel (2020, Microsoft Corporation, USA) and Origin (2022, OriginLab Corporation, USA). Statistical analysis (Duncan's multiple range test) was conducted using the Statistical Package for Social Sciences (22.0, IBM, USA), with p<0.05 considered significant and p<0.01 highly significant.

3. Results and discussion

3.1. Acute toxicity of PE and PP at 96 hours

The EC_{50} value after 96 h on *C. vulgaris* was 295 mg/L and 334 mg/L for PE MPs and PP MPs, respectively, as shown in Figure 1. These findings indicate that both PE MPs and PP MPs exhibit growth-inhibitory effects on *C. vulgaris*, with PP MPs showing stronger inhibitory activity. According to the aquatic toxicity classification criteria, substances with 96-h EC_{50} values between 100 mg/L and 1000 mg/L for algae are classified as "moderately toxic."[32] Physical factors, such as the small specific area and light density of MPs, tend to float on the surface, weakening underwater light and leading to insufficient photosynthesis in *C. vulgaris* and a decrease in oxygen production, thus inhibiting the growth of algal cells.

3.2. Growth of *C. vulgaris* under co-exposure to lead(II) ion and MPs

3.2.1. C. vulgaris cell density

As shown in Figures 1 and 2, growth of *C. vulgaris* was significantly inhibited by Pb^{2+}, PE, and PP exposures, as well as under combined exposures, with the strongest suppression observed under Pb^{2+} + PP exposure. The proliferation of algal cells decreased with increasing Pb^{2+} concentration, with the most pronounced inhibition observed at 5 mg/L. This inhibitory effect may be linked to metal toxicity that disrupted enzymatic processes during biosynthesis.[33] The growth inhibition rates of *C. vulgaris* under combined Pb^{2+} + PE and Pb^{2+} + PP exposure were 52.44% and 72.66%, respectively, compared with those of the blank at a Pb^{2+} concentration of 5 mg/L. These results align with the toxicity assays detailed in Section 3.1.

3.2.2. Chlorophyll a

As shown in Figure 3, the concentration of chlorophyll a in samples from each environment followed a similar pattern: Increased initially, decreased after the 7th day, and then gradually increased. However, the total amount of chlorophyll a was limited by the biomass of *C. vulgaris*. The chlorophyll a content at 28 days under each culture condition decreased with increasing Pb^{2+} concentration and with the addition of MPs, and the lowest chlorophyll a content was found in the algal mixture under the combined exposure to 5 mg/L Pb^{2+} + PP. This finding is consistent with previous studies on algal biomass under MP–heavy metal-combined

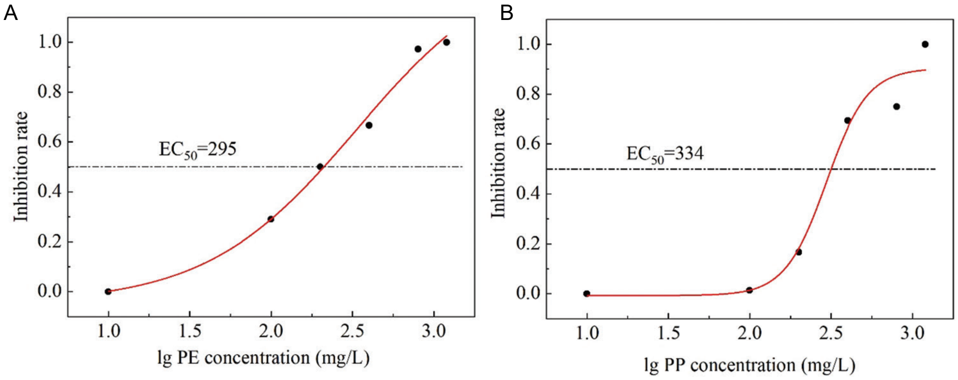

Figure 1. Fitted curves of 96 h toxic effects of (A) PE and (B) PP

Abbreviations: EC_{50}: Half-maximal effective concentration; PE: Polyethylene; PP: Polypropylene.

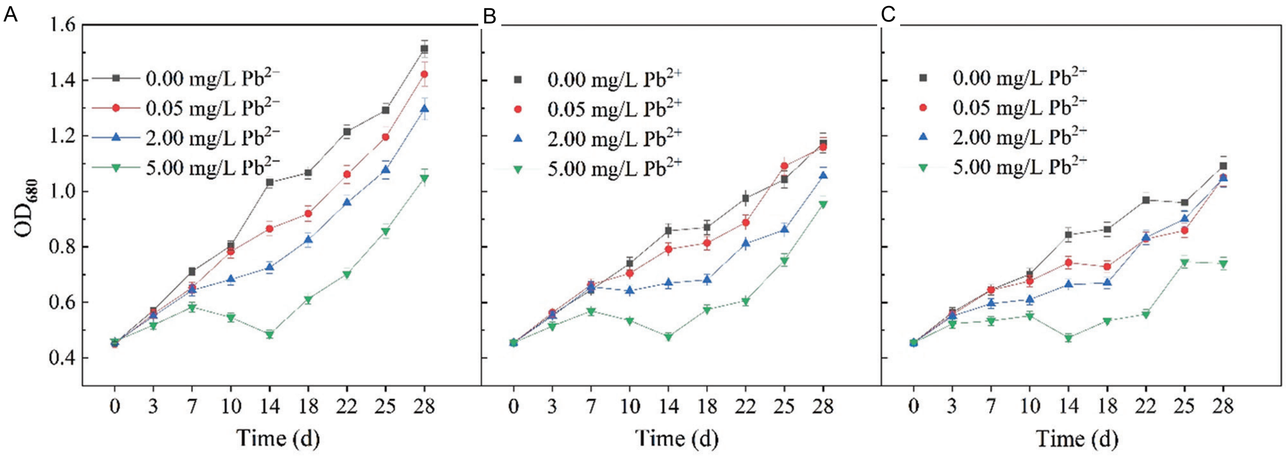

Figure 2. Growth curves of *Chlorella vulgaris* under (A) Pb^{2+}, (B) Pb^{2+} + PE, and (C) Pb^{2+} + PP conditions

Abbreviations: OD: Optical density; Pb^{2+}: Lead(II) ion.

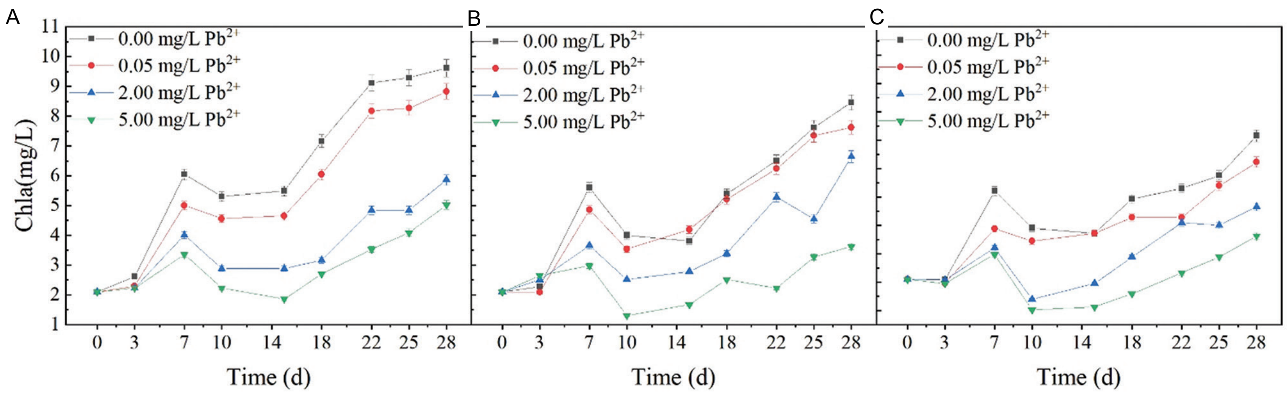

Figure 3. (A-C) Effect of incubation days on chlorophyll a concentration

Abbreviations: CK: Control; Pb^{2+}: Lead(II) ion; PE: Polyethylene; PP: Polypropylene.

stress.[34] For example, Tunali *et al.*[18] reported that combined exposure to PS MPs and heavy metals significantly reduced the biomass of *C. vulgaris*, and Yu *et al.*[26] observed similar inhibitory effects on *C. vulgaris* under PET MPs + Pb^{2+} stress.

3.2.3. Soluble protein content of C. vulgaris and MDA content

Figure 4A shows the variations in the content of soluble proteins in *C. vulgaris* under different conditions. Under Pb^{2+} exposure alone, soluble protein content

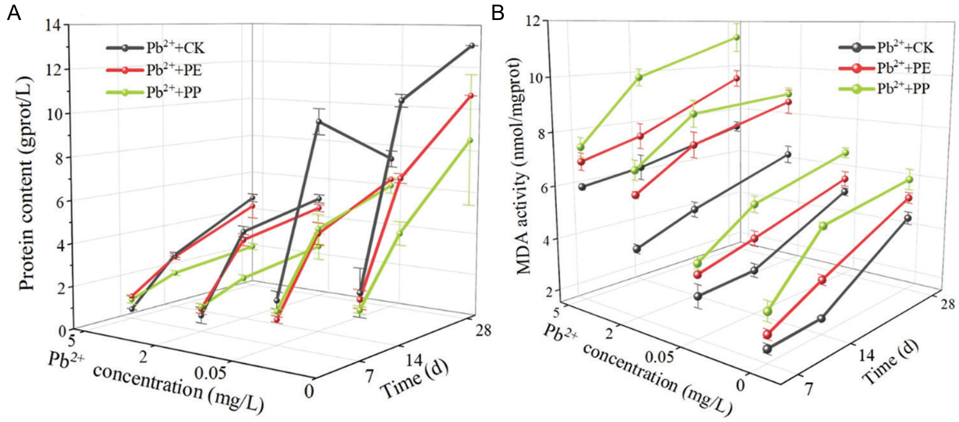

Figure 4. Dynamic effects of different microplastics-lead composite treatments on protein content and MDA activity. (A) Variations in the activity of soluble protein content of *Chlorella vulgaris* under different conditions. (B) Variation of MDA content under different conditions.

Abbreviations: CK: Control; MDA: Malondialdehyde; Pb^{2+}: Lead(II) ion; PE: Polyethylene; PP: Polypropylene.

in *C. vulgaris* declined progressively with increasing Pb^{2+} concentrations, with a significant reduction under 5 mg/L Pb^{2+}. A similar decreasing trend in soluble protein content was observed under co-exposure to MPs and Pb^{2+}; the soluble protein content of *C. vulgaris* algal cells also decreased with increasing Pb^{2+} concentration. Changes in soluble protein content were similar to the growth trend of *C. vulgaris*, suggesting a correlation between soluble protein content in algal cells and *C. vulgaris* growth. MDA, a product of membrane lipid peroxidation, serves as an indicator of oxidative damage; elevated MDA levels reflect increased peroxidation and altered membrane stability.[35] As illustrated in Figure 4B, exposure to high concentrations of heavy metals resulted in a more pronounced increase in MDA content. During the incubation period, MDA levels in algal cells increased with Pb^{2+} concentration, indicating Pb^{2}-induced membrane lipid peroxidation in *C. vulgaris*.[36] In addition, both MPs alone and combined exposure to each concentration of Pb^{2+} resulted in increased MDA production, and the strongest effect on the peroxidation of *C. vulgaris* membrane lipids was observed in the environment of Pb^{2+} + PP. Simultaneously, microscopy showed cellular damage, consistent with oxidative stress, indicated by elevated MDA.

3.3. Variation in the ability of *C. vulgaris* to absorb nutrient salts

3.3.1. Ability of C. vulgaris to absorb nitrate

As shown in Figure 5A, PE impeded the uptake of NO_3^- by *C. vulgaris*, whereas both PP and combined Pb^{2+} facilitated the uptake of NO_3^- by *C. vulgaris*. The best NO_3^- uptake capacity of *C. vulgaris* was achieved under PP and 2 mg/L Pb^{2+}, which was 2.67 times greater than that under PP only. In contrast, PE promoted NO_3^- uptake by *C. vulgaris* only at a Pb^{2+} concentration of 5 mg/L. The ability of *C. vulgaris* to increase NO_3^- uptake under the dual stress of MPs and the heavy metal Pb^{2+} was lower than that of the Pb^{2+} alone group.

The surface chemical properties of MPs determine their adsorption capacity for Pb^{2+}. MPs undergo environmental aging, which can alter their surface properties. This may also be one of the reasons why Pb^{2+} + PP has a stronger inhibitory effect on *C. vulgaris* growth than Pb^{2+} + PE. NR plays an important role in nitrogen metabolism.[37] As shown in Figure 5B, the nutrient salts absorbed by *C. vulgaris* during growth and reproduction cannot satisfy the needs of *C. vulgaris*, and the ability to transform elements can only be strengthened by increasing internal enzyme activity. Pb^{2+} and MPs did not directly increase enzyme activity but rather enhanced it by disrupting other aspects of *C. vulgaris*'s ability to activate internal regulatory mechanisms.

3.3.2. Ability of C. vulgaris to absorb phosphate

As shown in Figure 6A, the dual stress of MPs and the heavy metal Pb^{2+} may have an antagonistic effect on PO_4^{3-} uptake by *C. vulgaris*. The overall PO_4^{3-} concentrations across various exposure environments first increased, then decreased, indicating severe damage to primary algal cells. In the late stage of cultivation, the inhibitory effects of MPs and heavy metals on the absorption of PO_4^{3-} by *C. vulgaris* were significantly enhanced, indicating that cell damage had- reached a certain level and normal nutrient absorption function

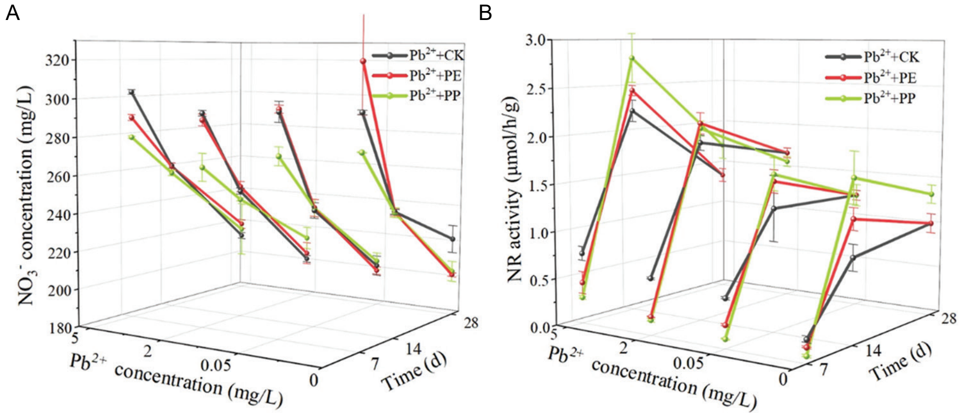

Figure 5. Effects of Pb^{2+} and microplastic co-exposure on nitrate concentration and NR activity. (A) Variation in NO_3^- concentration in the culture solution under different conditions. (B) Variation in NR activity under different conditions.

Abbreviations: CK: Control; MDA: Malondialdehyde; NO^{3-}: Nitrate; NR: Nitrate reductase; Pb^{2+}: Lead(II) ion; PE: Polyethylene; PP: Polypropylene.

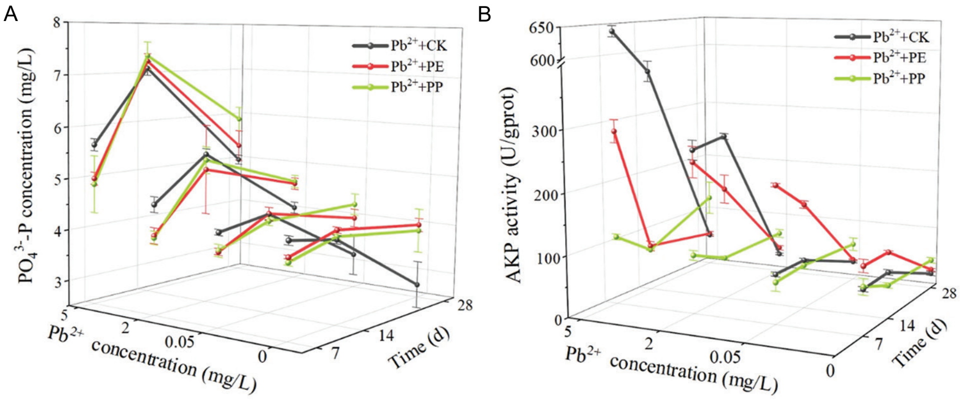

Figure 6. Effects of Pb^{2+} and microplastic co-exposure on phosphate concentration and AKP activity. (A) Variation in PO_4^{3-} concentration in the culture solution under different conditions. (B) Variation in AKP activity under different conditions.

Abbreviations: AKP: Alkaline phosphatase; CK: Control; MDA: Malondialdehyde; Pb^{2+}: Lead(II) ion; PE: Polyethylene; PO_4^{3-}: Phosphate; PP: Polypropylene.

could no longer be maintained. As shown in Figure 6B, high concentrations of Pb^{2+} significantly increased AKP activity in algal cells when *C. vulgaris* was exposed to a Pb^{2+} environment. However, the uptake of PO_4^{3-} by *C. vulgaris* cells exposed to Pb^{2+} was disrupted, leading to phosphorus deficiency in algal cells. This stimulated the organism to produce large amounts of AKP. This can also be corroborated by the results in Section 3.3.1. These findings indicate that MPs and high Pb^{2+} concentrations have antagonistic effects on AKP. This may be because PE and PP have a certain adsorption effect on Pb^{2+}.[26]

Our study found that the Pb^{2+} + PP had a significant inhibitory effect on *C. vulgaris* (lower EC_{50} value and higher degree of membrane lipid peroxidation), which may be related to the stronger adsorption ability of hydrophobic functional groups on the PP surface for Pb^{2+} ions. The Pb^{2+} + PP system exhibited sustained membrane damage after 28 d of cultivation (MDA content was 2.56 times that of the control group), which is closely related to the mechanism by which the microporous structure generated on the PP surface during environmental aging promotes the transport of Pb^{2+} into cells.

At the enzyme level, our study revealed dynamic changes in NR and AKP activity. Notably, enzyme activities increased despite impaired nutrient uptake, which is consistent with MP-induced disruption of membrane integrity and associated transport dysfunction. Pb^{2+} may further suppress nitrate assimilation by interfering with the biosynthesis or availability of NO_3^- reductase co-factors. Compared to the linear response of AKP activity in the Pb^{2+} + PET system,[26] we found that the Pb^{2+} + PP system showed an increase in AKP activity in the later stage of culture (up to 8.82 times that of the control group), which is closely related to the adaptive mechanism of PP-induced programmed cell death, releasing intracellular phosphorus and stimulating cells to recapture phosphorus by upregulating AKP activity. The non-linear characteristics of the enzyme level response provide a new perspective on the mechanism of toxicity from MP–heavy metal composite pollution.

4. Conclusion

We investigated the combined toxic effects and mechanisms of PE, PP, and lead ions Pb^{2+} on *C. vulgaris* from the TGR. PE and PP MPs (120–180 μm) exhibited acute toxicity to the algae, with 96-h EC_{50} values of 334 mg/L (PE) and 295.29 mg/L (PP), indicating that PP is more toxic. Single and combined exposures to PE, PP, and Pb^{2+} significantly suppressed algal biomass accumulation and cell proliferation, with the strongest inhibition observed under Pb^{2+} + PP co-exposure; Pb^{2+} toxicity was concentration-dependent, with the most pronounced growth inhibition at 5 mg/L. Chlorophyll a and soluble protein contents decreased with increasing Pb^{2+} concentration and the addition of MPs. MDA levels increased, reflecting membrane lipid peroxidation and oxidative damage to cells, which was most severe under Pb^{2+} and PP exposure. Nutrient assimilation of NO_3^- and PO_4^{3-} showed a trend of initial stimulation followed by significant suppression in the later stage of exposure, especially under combined treatment; high Pb^{2+} concentrations (5 mg/L) exacerbated this suppression, and MPs exhibited species-specific effects (e.g., PP promoted NO_3^- absorption under low Pb^{2+} levels, whereas PE only showed a promoting effect at 5 mg/L Pb^{2+}). Antagonistic interactions between PE/PP and Pb^{2+} were observed for NR and AKP activities; despite elevated NR and AKP levels under stress, nutrient assimilation remained compromised, attributed to membrane integrity damage by MPs and disrupted metabolic pathways by Pb^{2+}. Notably, the Pb^{2+} + PP system showed a secondary increase in AKP activity at a later stage of culture (up to 8.82 times that of the control group), closely related to the adaptive mechanism of PP-induced programmed cell death. This study provides critical insights into the composite toxicity mechanisms of MPs and heavy metals in reservoir ecosystems, supporting ecological risk assessment for similar aquatic environments. Limitations include the use of a single algal species (*C. vulgaris*), which may limit the generalizability of conclusions due to differences in physiological characteristics among algae, and the absence of natural organic matter (NOM) in the experimental system, which could alter the surface properties of MPs and the bioavailability of Pb^{2+} in real water environments. Future studies should construct complex systems that incorporate NOM and involve multiple algal species to improve the real-world relevance of results.

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Conflict of interest

Chongzhe Dong is an employee of Qingyuan Water Group Co., LTD. The authors declare that this affiliation did not influence the study design, data collection, analysis, interpretation, or the decision to publish. The remaining authors declare no competing interests.

Author contributions

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Investigation: Ran Tu, Yang Yang
Methodology: Yan Wu, Guoxin Lan
Writing–original draft: Ming Chen, Lianqi Huang
Writing–review & editing: Bin Yan, Ming Chen

Availability of data

Data are available from the corresponding author upon reasonable request.

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