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Heavy metal–microplastic co-exposure modulates toxicity in microalgae: A meta-analysis

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Article type: Meta-analysis

Running title: Heavy metal-microplastic toxicity in microalgae

Abstract

Background: Microplastics and heavy metals frequently co-occur in aquatic environments, but their joint effects on microalgae remain inconsistent across experimental studies. This uncertainty limits ecological risk assessment for combined pollution because microalgae form the base of many aquatic food webs and contribute substantially to primary production. Methods: We conducted a meta-analysis of combined exposure to heavy metals and microplastics in microalgae. Six databases (PubMed, Web of Science, ScienceDirect, CNKI, CQVIP, and Wanfang) were searched through December 2025. After screening 10,862 records, 28 studies and 1,151 observations were included. Seven physiological response categories were analysed: growth, oxidative response, photosynthesis, pigments, cellular metabolism, membrane damage, and toxin production. Interaction effect sizes were calculated as Hedges' d and pooled using multilevel mixed-effects models with study and response indicator as random effects. Moderator analyses examined heavy metal type, heavy metal concentration and speciation, microplastic polymer type, size and concentration, exposure time, algal phylum, and growth environment. Results: Combined exposure produced significant antagonistic effects on growth ($d = -1.73$, 95% CI: -3.23 to -0.22, $P = 0.024$) and oxidative response ($d = -1.29$, 95% CI: -2.47 to -0.11, $P = 0.032$). Effects on photosynthesis, pigments, cellular metabolism, membrane damage, and toxin production were not statistically significant. Moderator analyses showed that the direction of interaction depended strongly on exposure context. At low heavy metal concentrations, combined exposure shifted to synergism for growth ($d = 3.45$, 95% CI: 2.37 to 4.54, $P < 0.001$) and

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oxidative response (d = 1.67, 95% CI: 0.68 to 2.66, P = 0.001). Aged microplastics and several polymer types showed strong antagonistic effects for oxidative response, whereas short-term exposure (≤96 h) was associated with antagonism in growth. Random forest models identified exposure time and heavy metal concentration as the strongest predictors for growth, and heavy metal type and microplastic concentration as important predictors for oxidative response. Conclusions: The combined toxicity of heavy metals and microplastics to microalgae is concentration- and time-dependent. High-concentration acute studies tend to capture antagonism, whereas environmentally relevant low-concentration conditions may produce synergistic toxicity. Risk assessment should therefore incorporate concentration-dependent shifts in interaction direction and give greater attention to long-term exposure at environmentally realistic concentrations.

Keywords: heavy metals; microplastics; combined exposure; microalgae; meta-analysis

1. Introduction

Heavy metals are major pollutants in aquatic ecosystems. Industrial wastewater, agricultural runoff, and mining activities are among the main sources (Ali, Khan, & Ilahi, 2019; Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014; Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Metals such as cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), and chromium (Cr) persist in the environment, accumulate in organisms, and can be transferred through food webs (Edo et al., 2024; Parveen & Selvasembian, 2026). After entering water bodies, heavy metals can impair enzymatic activity, damage cell membranes, induce oxidative stress, and inhibit photosynthesis (Nowicka, 2022). Phytoplankton, especially microalgae, are sensitive to these stressors and are widely used as indicators of aquatic ecological risk (Chia, Lombardi, da Graça Gama Melão, & Parrish, 2015).

Microplastics are plastic particles smaller than 5 mm. Their small size, large specific surface area, and hydrophobicity allow them to occur widely in environmental media (Galloway, Cole, & Lewis, 2017; Prata, Da Costa, Lopes, Duarte, & Rocha-Santos, 2020; Thompson et al., 2024). They originate from the fragmentation of larger plastics, industrial pellets, and microbeads in consumer products (Auta, Emenike, & Fauziah, 2017). In aquatic systems, microplastics can affect organisms through physical contact, shading, ingestion, or carrier-mediated transport of contaminants (Bhattacharya, Lin, Turner, & Ke, 2010; Sjollema, Redondo-Hasselerharm, Leslie, Kraak, & Vethaak, 2016; Zhao, Deng, Wang, Ge, & Yang, 2024). They can adsorb heavy metals and organic pollutants, thereby altering contaminant bioavailability and toxicity (Brennecke, Duarte, Paiva, Caador, & Canning-Clode, 2016; Torres, Salinas, Pizarro, & De-La-Torre, 2020). The interaction between microplastics and metals depends on polymer type, particle aging, and water chemistry.

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Heavy metals and microplastics often co-occur in natural waters, where their joint effects may be synergistic, antagonistic, or additive (Crain, Kroeker, & Halpern, 2008; Khalid, Aqeel, Noman, Khan, & Akhter, 2021). Individual experimental studies have reported different interaction patterns (Devi, De Silva, Tyagi, & Aaryashree, 2025; Li et al., 2025). This variation likely arises from differences in species, endpoints, exposure time, contaminant type, and concentration (Warne & Hawker, 1995). Aged microplastics may be particularly relevant because weathering can increase surface roughness and introduce oxygen-containing functional groups that change metal sorption capacity (Zhang et al., 2024).

Ge et al. (2024) synthesized the combined toxicity of micro- and nanoplastics with conventional pollutants in microalgae and reported an overall antagonistic pattern, with additive effects accounting for a substantial fraction of interactions(Ge et al., 2024). However, that analysis combined heavy metals with pesticides, pharmaceuticals, and other organic contaminants. Because heavy metals act mainly through ion interference, protein binding, and oxidative damage, whereas many organic contaminants act through different molecular targets and metabolic pathways (Ebele, Abou-Elwafa Abdallah, & Harrad, 2017; Kalaivanan & Ganeshamurthy, 2015), a pollutant-specific synthesis is needed.

This study therefore focused on the combined exposure of heavy metals and microplastics in microalgae. We quantified interaction effect sizes across seven physiological categories using multilevel meta-analysis and examined moderators related to metal properties, microplastic characteristics, exposure duration, algal taxonomy, and growth environment. The aim was to provide a quantitative basis for ecological risk assessment of heavy metal-microplastic co-contamination.

2. Materials and methods

2.1. Literature search and study selection

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses in Ecology and Evolutionary Biology (PRISMA-EcoEvo) guidance (O'Dea et al., 2021). PubMed, Web of Science, ScienceDirect, CNKI, CQVIP, and Wanfang were searched through December 2025. The initial search identified 10,862 records: PubMed (n = 895), Web of Science (n = 5,300), ScienceDirect (n = 4,634), CNKI (n = 18), CQVIP (n = 0), and Wanfang (n = 15). After duplicate removal, 9,962 records were screened. We excluded 1,877 records because they were reviews, directories, guidelines, letters, commentaries, notifications, plans, editorials, or applied documents, and a further 8,028 records because they did not simultaneously involve microalgae, microplastics, and heavy metals. Fifty-seven reports were assessed for eligibility, and

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29 were excluded because they did not examine heavy metals, had unsuitable experimental designs, lacked extractable data, or did not report biological endpoints. The final dataset included 28 studies and 1,151 observations (Figure 1).

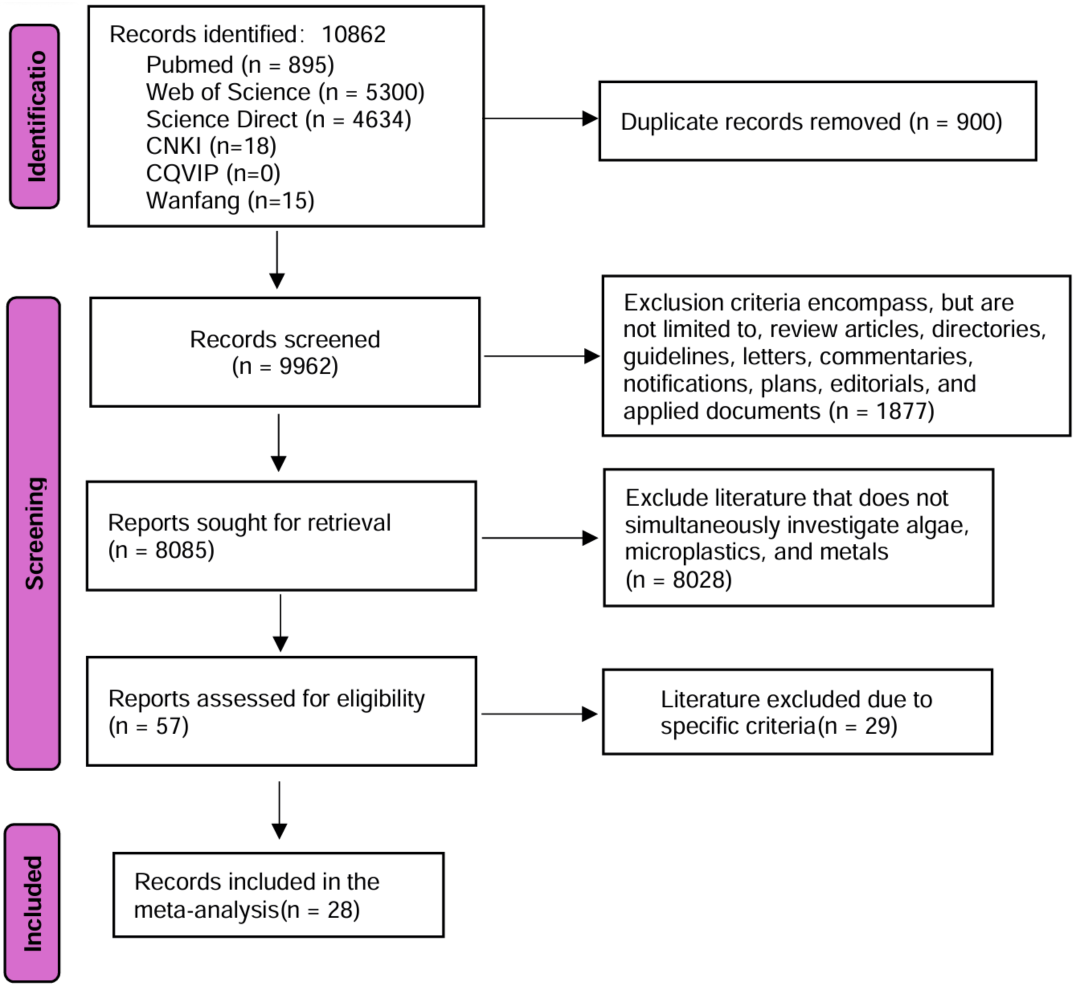

Figure 1. PRISMA-style flow diagram for study selection. The initial search included PubMed, Web of Science, ScienceDirect, CNKI, CQVIP, and Wanfang. Counts were harmonized with the screening steps used in the analysis: 10,862 records were identified, 900 duplicates were removed, and 28 studies were included in the meta-analysis.

2.2. Data extraction and endpoint classification

Two reviewers independently extracted data using a standardized form. Extracted information included study title, first author, publication year, mean response, standard deviation, and replicate number for the control, microplastic-only, heavy metal-only, and combined-exposure groups; algal phylum and species; exposure duration; growth environment; microplastic polymer type, average particle size, and concentration; and heavy metal type, speciation, and concentration. Disagreements were resolved by discussion or by a third reviewer. When data were presented only in graphs, numerical values were extracted using WebPlotDigitizer version 4.7.

Physiological endpoints were grouped into seven categories: (1) growth, including cell density, biomass productivity, growth ratio, average specific growth rate, and dry weight; (2)

photosynthesis, including maximum photosynthetic efficiency (Fv/Fm), oxygen-evolving complex activity, electron transport flux, performance index, and photochemical quenching coefficient; (3) oxidative response, including catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), reactive oxygen species (ROS), reduced glutathione (GSH), and total antioxidant capacity (T-AOC); (4) cellular metabolism, including total protein, soluble protein, extracellular polymeric substances, polysaccharides, soluble sugars, and lactate dehydrogenase; (5) membrane damage, including membrane permeability and abnormal-cell proportion; (6) pigments, including chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and phycobiliproteins; and (7) toxins, including microcystins.

2.3. Effect-size calculation

Hedges' d was used as the standardized interaction effect size (Hedges, 1981). For each observation, the interaction term compared the combined treatment with the expected additive response from single-pollutant treatments:

$$\mathrm{d}_{interaction} = \frac{(Y_{MH} - Y_M) - (Y_H - Y_C)}{2 \times \mathrm{s}_{pooled}} \times J \tag{2. 1}$$

$$V_{interaction} = \frac{1}{4}\left[\frac{1}{n_C} + \frac{1}{n_M} + \frac{1}{n_H} + \frac{1}{n_{MH}} + \frac{d^2_{interaction}}{2(n_C + n_M + n_H + n_{MH})}\right] \tag{2. 2}$$

where Y_{MH}, Y_M, Y_H, and Y_C are the means of the combined microplastic-heavy metal treatment, microplastic-only treatment, heavy metal-only treatment, and control, respectively. n_C, n_M, n_H, and n_{MH} denote the sample sizes of the control, microplastic-only, heavy metal-only, and combined microplastic-heavy metal treatment groups, respectively. spooled is the pooled standard deviation, and J is the small-sample correction factor. The calculation formulas for spooled and J are as follows:

$$\mathrm{s}_{pooled} = \sqrt{\frac{(n_C - 1)s_C^2 + (n_M - 1)s_M^2 + (n_H - 1)s_H^2 + (n_{MH} - 1)s_{MH}^2}{m}} \tag{2. 3}$$

$$J = 1 - \frac{3}{4\mathrm{m} - 1} \tag{2. 4}$$

$$m = n_C + n_M + n_H + n_{MH} - 4 \tag{2. 5}$$

where S_C, S_M, S_H, and S_{MH} are the standard deviations of the control, microplastic-only, heavy metal-only, and combined microplastic-heavy metal treatment groups, respectively; and m represents the degrees of freedom.

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For endpoints for which lower values indicate stronger toxicity, such as growth and photosynthesis, the sign of the effect size was reversed so that all endpoints shared the same interpretation. Negative values indicate antagonism, meaning that the combined toxicity was lower than the sum of the toxicities from the two pollutants exposed individually. Positive values indicate synergism, meaning that the combined toxicity was greater than the sum of individual toxicities (Gurevitch, Morrison, & Hedges, 2000).

2.4. Statistical analysis

Multilevel mixed-effects models were fitted in R version 4.5.2 using the rma.mv function in metafor version 4.8-0 (Viechtbauer, 2010). Study identity and response indicator were included as random effects to account for non-independence among effect sizes extracted from the same study or endpoint. Models were fitted by restricted maximum likelihood. The overall model estimated pooled effects for each physiological category, and category-specific moderator models examined factors that could explain variation in interaction effects.

Continuous moderators were grouped using thresholds specified in the analysis script. Exposure time was classified as low (≤96 h) or high (>96 h). Microplastic concentration was classified as low (≤10 mg/L) or high (>10 mg/L). Heavy metal concentrations were classified using metal-specific thresholds: Cu ≤1, Zn ≤2.0, Cd ≤0.01, Cr(VI) ≤0.1, Pb ≤0.1, Ag ≤0.5, and Au ≤10 (in the concentration units reported by the extracted dataset). Microplastic particle size was classified as nanoplastics (NPs, <1 μm) or microplastics (MPs, ≥1 μm). Heterogeneity was assessed using Cochran's Q test and multilevel I^2 statistics (Higgins, Thompson, Deeks, & Altman, 2003; Nakagawa, Yang, Macartney, Spake, & Lagisz, 2023). Funnel plots were used to evaluate small-study effects and publication bias (Rosenthal, 1979). Statistical significance was set at $\alpha = 0.05$.

Random forest analyses were conducted using randomForest version 4.7-1.2. Categorical variables, including phylum, polymer type, heavy metal type, heavy metal speciation, and growth environment, were numerically encoded; continuous variables, including exposure time, microplastic concentration, and heavy metal concentration, were retained in their original form. Separate models were fitted for physiological categories with at least 50 observations. Each model used 500 trees, Hedges' d as the response variable, and inverse variance as the weight. Predictor importance was evaluated using percentage increase in mean squared error (%IncMSE), and significance was assessed with 100 permutation tests.

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3. Results

3.1. Dataset overview

The final dataset contained 1,151 observations from 28 studies. Pigment endpoints were most frequent (314 observations, 27.3%), followed by growth (289, 25.1%), oxidative response (288, 25.0%), cellular metabolism (146), photosynthesis (66), membrane damage (40), and toxins (8, 0.7%). The total experimental sample size across treatment groups was 13,884, providing sufficient information for pooled estimates for the major endpoint categories.

3.2. Overall effects across physiological categories

Figure 2 shows pooled interaction effects for the seven physiological categories. Growth showed a significant antagonistic interaction (d = -1.73, 95% CI: -3.23 to -0.22, P = 0.024), indicating that the growth inhibition under combined exposure was lower than expected from the two single-pollutant treatments. Oxidative response also showed significant antagonism (d = -1.29, 95% CI: -2.47 to -0.11, P = 0.032). These two endpoints therefore provided the clearest evidence that microplastics can attenuate heavy metal toxicity under some experimental conditions, probably by reducing metal bioavailability through sorption.

Photosynthesis (d = -0.98, 95% CI: -2.25 to 0.30, P = 0.133), cellular metabolism (d = -0.42, 95% CI: -1.54 to 0.71, P = 0.469), membrane damage (d = -1.29, 95% CI: -3.20 to 0.63, P = 0.188), pigments (d = -0.89, 95% CI: -2.27 to 0.49, P = 0.208), and toxins (d = -0.65, 95% CI: -2.48 to 1.19, P = 0.490) showed non-significant trends toward antagonism. The wide confidence intervals for membrane damage and toxin production indicate limited precision, especially because these categories contained fewer observations.

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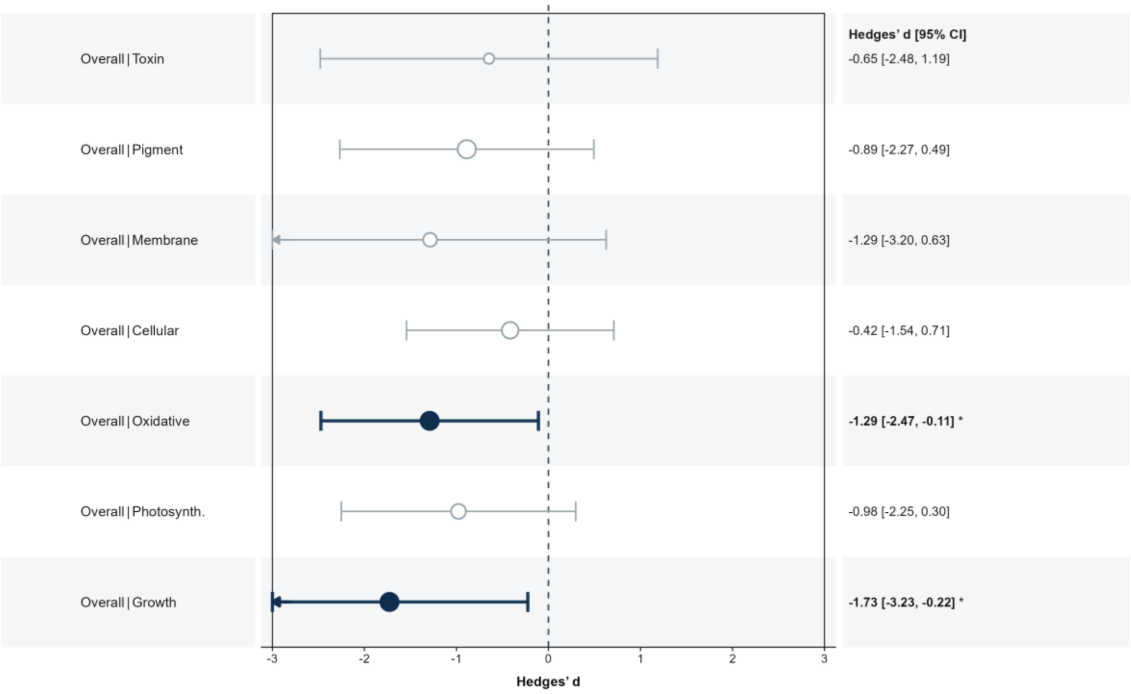

Figure 2. Overall interaction effect sizes for seven physiological categories. Points are pooled Hedges' d values and horizontal bars are 95% confidence intervals. The dashed vertical line marks d = 0, interpreted as additivity. Negative values indicate antagonistic interactions and positive values indicate synergistic interactions after endpoint-direction harmonization. Filled dark points and bold values denote statistically significant estimates. Open grey points denote non-significant estimates. Arrows indicate confidence intervals extending beyond the plotted axis range. Asterisks indicate significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.3. Heavy metal moderators

3.3.1. Metal-specific effects

Heavy metal type contributed to variation in combined toxicity (Figure 3). For growth, Cu (d = -0.05, 95% CI: -1.71 to 1.61, P = 0.951) and Pb (d = -1.62, 95% CI: -10.11 to 6.87, P = 0.709) tended toward antagonism, whereas Cd tended toward synergism (d = 1.17, 95% CI: -7.13 to 9.47, P = 0.783). None of these metal-specific growth estimates was statistically significant, and the wide confidence intervals indicate substantial uncertainty. For oxidative response, Cd (d = 2.63, 95% CI: -1.67 to 6.92, P = 0.230) and Cu (d = 1.56, 95% CI: -2.47 to 5.59, P = 0.449) tended toward synergism, whereas Pb tended toward antagonism (d = -2.39, 95% CI: -7.26 to 2.47, P = 0.335).

3.3.2. Concentration-dependent reversal at low heavy metal concentrations

Heavy metal concentration strongly altered interaction direction. Under low heavy metal concentrations, growth showed a significant synergistic interaction (d = 3.45, 95% CI: 2.37 to 4.54, $P < 0.001$). Oxidative response also showed significant synergism under low heavy metal concentrations (d = 1.67, 95% CI: 0.68 to 2.66, P = 0.001). This pattern indicates that environmentally relevant low-concentration conditions may differ from high-concentration laboratory exposures. At low metal concentrations, microplastics may act as carriers that increase

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cellular contact with metals or modify algal physiology in ways that increase metal sensitivity (Q. Liu et al., 2022).

3.3.3. Limited evidence for speciation effects

Heavy metal speciation was classified as particulate or ionic. Particulate metals showed no statistically significant effect in the analysed categories. Particulate metals may interact physically with microplastics through adsorption or aggregation, whereas ionic metals generally have higher bioavailability and interact with microplastics mainly through chemical sorption (Parveen & Selvasembian, 2026; X. Wang, Zhang, Li, & Yan, 2022). The current dataset did not provide enough precision to separate these pathways clearly.

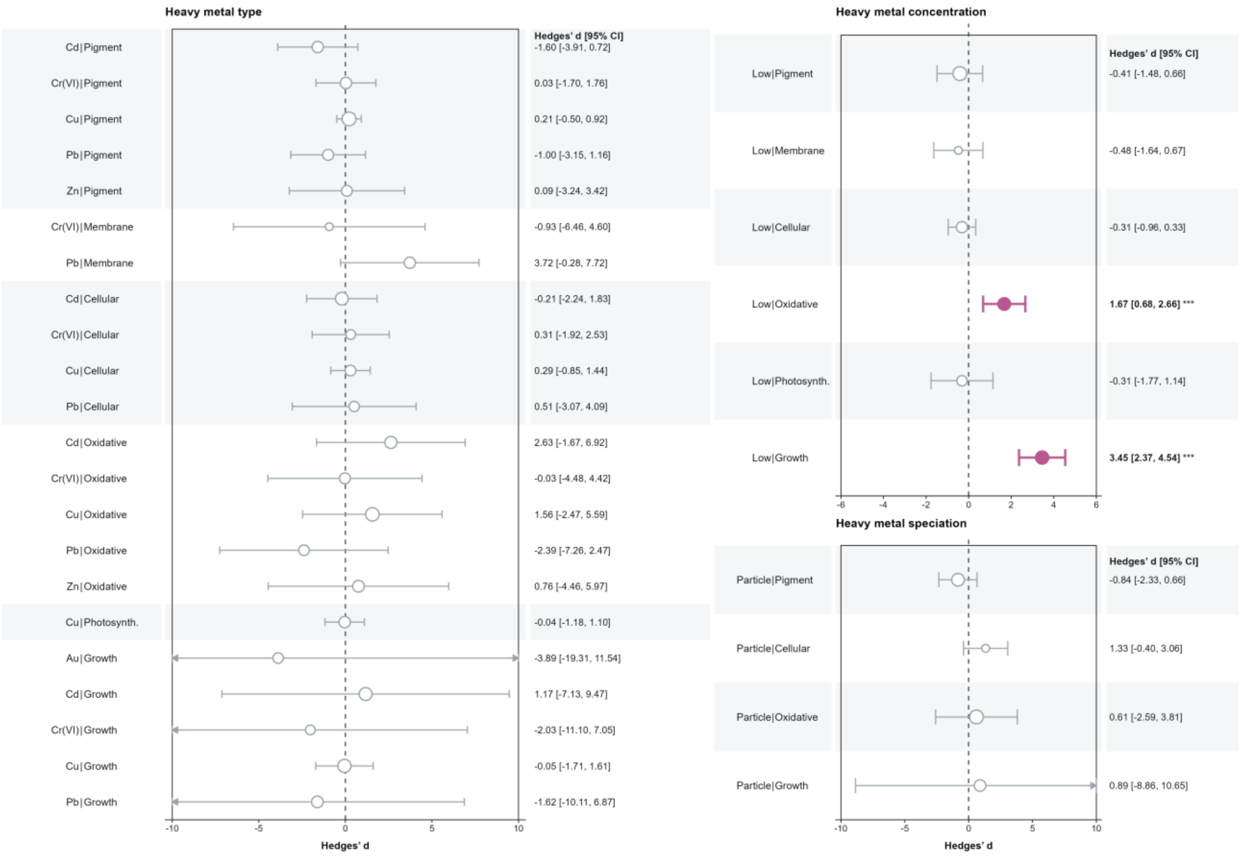

Figure 3. Heavy metal-related moderators of interaction effects. Panels show heavy metal type, heavy metal concentration, and heavy metal speciation. Points are Hedges' d estimates and horizontal bars are 95% confidence intervals. The dashed vertical line marks d = 0. Negative values indicate antagonism and positive values indicate synergism. Filled coloured points and bold values identify statistically significant estimates; open grey points identify non-significant estimates. Arrows indicate confidence intervals extending beyond the plotted axis range. Low heavy metal concentration follows the metal-specific thresholds used in the analysis script. Asterisks indicate significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.4. Microplastic moderators

3.4.1. Polymer type and aging

Microplastic polymer type was strongly associated with oxidative-response effects (Figure 4). Aged polystyrene (aged PS) showed significant antagonism (d = -8.93, 95% CI: -12.88 to -4.99, P

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< 0.001), and polyvinyl chloride (PVC) also showed significant antagonism ($d = -10.37$, 95% CI: -14.33 to -6.41, $P < 0.001$). Polyethylene (PE; $d = -8.93$, 95% CI: -13.01 to -4.86, $P < 0.001$), polypropylene (PP; $d = -8.31$, 95% CI: -12.59 to -4.03, $P < 0.001$), and polystyrene (PS; $d = -8.15$, 95% CI: -11.99 to -4.32, $P < 0.001$) also showed significant antagonistic effects for oxidative response. The strong effects for aged PS, aged PTFE, and aged PVC suggest that aging can increase the ability of microplastics to reduce metal bioavailability.

Environmental aging can increase surface roughness and introduce oxygen-containing functional groups such as carboxyl, hydroxyl, and carbonyl groups (P. Liu et al., 2020). These changes can increase heavy metal adsorption and reduce freely dissolved metal concentrations (Lang et al., 2020). UV-aged polystyrene has been reported to adsorb more Cu and Pb than pristine particles (L. Wang et al., 2023). Aging may also release additives that interact with metals and modify toxicity (Fred-Ahmadu et al., 2020).

3.4.2. Particle size

Microplastic size was classified as nanoplastics (NPs, <1 μm) or microplastics (MPs, ≥1 μm). For growth, NPs showed a weak and non-significant antagonistic trend ($d = -0.36$, 95% CI: -1.25 to 0.53, $P = 0.430$). For oxidative response, NPs also showed a non-significant antagonistic trend ($d = -0.29$, 95% CI: -0.87 to 0.30, $P = 0.335$). Although smaller particles have larger specific surface area and may have higher biological availability (Da Costa, Santos, Duarte, & Rocha-Santos, 2016), the meta-analysis did not indicate that particle size alone was a dominant moderator. Aggregation, sedimentation, surface modification, and cellular uptake may obscure simple size-based patterns (Xu et al., 2024).

3.4.3. Microplastic concentration

Microplastic concentration was classified as low (≤10 mg/L) or high (>10 mg/L). Under low microplastic concentrations, growth showed significant antagonism ($d = -3.28$, 95% CI: -4.85 to -1.71, $P < 0.001$), suggesting that even relatively low particle concentrations can reduce metal bioavailability when sorption sites are sufficient. For oxidative response, the effect of low microplastic concentration was close to zero ($d = -0.05$, 95% CI: -0.95 to 0.84, $P = 0.906$), indicating weaker concentration control at the oxidative-response level.

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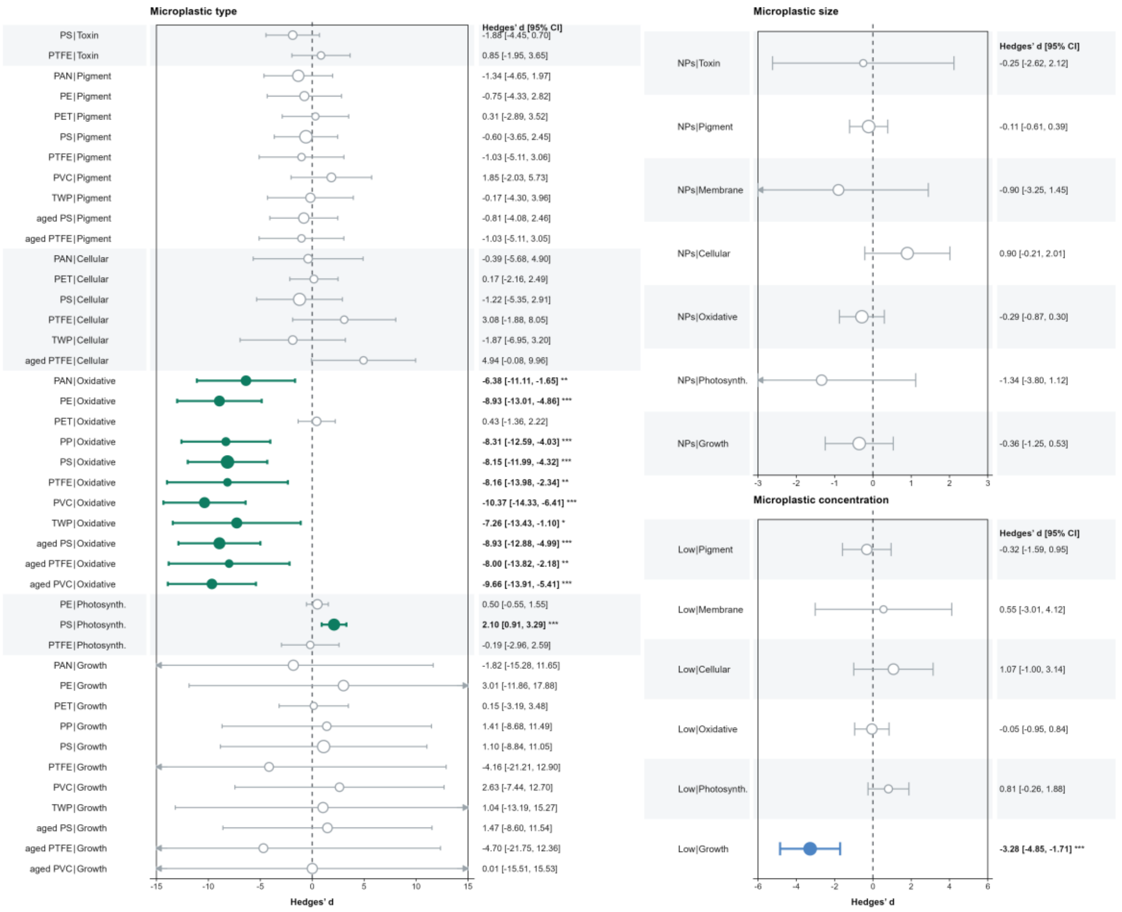

Figure 4. Microplastic-related moderators of interaction effects. Panels show polymer type, particle size, and microplastic concentration. Points are Hedges' d estimates and horizontal bars are 95% confidence intervals. The dashed vertical line marks d = 0. Negative values indicate antagonism and positive values indicate synergism. Filled coloured points and bold values identify statistically significant estimates; open grey points identify non-significant estimates. Arrows indicate confidence intervals extending beyond the plotted axis range. NPs denote nanoplastics (<1 μm). Low microplastic concentration denotes ≤10 mg/L. Asterisks indicate significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.5. Other moderators

3.5.1. Exposure time

Exposure duration was grouped into low (≤96 h) and high (>96 h) categories. For growth, short-term exposure showed significant antagonism (d = -1.82, 95% CI: -2.30 to -1.34, $P < 0.001$). This pattern is consistent with rapid sorption of metals to microplastic surfaces during acute exposure, which reduces direct contact between dissolved metals and algal cells. In longer exposures, slow desorption, particle aging, algal acclimation, and physiological exhaustion may produce more variable responses (W. Yang et al., 2021).

3.5.2. Algal phylum

Effect sizes varied across algal phyla (Figure 5). For growth, Chlorophyta (d = -1.23, 95% CI: -13.90 to 11.45, P = 0.850), Cyanobacteria (d = 1.31, 95% CI: -14.04 to 16.66, P = 0.867), Euglenophyta (d = -3.31, 95% CI: -16.03 to 9.41, P = 0.610), and Heterokontophyta (d = -4.44,

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95% CI: -18.06 to 9.17, P = 0.522) were all non-significant. The wide confidence intervals suggest high heterogeneity and limited precision. Differences among phyla may be related to cell wall composition, metabolic capacity, and stress defence systems (Ismaiel & Piercey-Normore, 2023; Y. Yang et al., 2026).

3.5.3. Growth environment

Growth environment was classified as seawater or freshwater. In seawater, growth showed a weak non-significant synergistic trend (d = 0.23, 95% CI: -1.02 to 1.48, P = 0.721), whereas oxidative response showed a non-significant antagonistic trend (d = -0.94, 95% CI: -2.50 to 0.61, P = 0.235). Seawater chemistry, including salinity, ionic strength, and pH, can affect metal speciation, microplastic aggregation, surface charge, and metal adsorption (Ashton, Holmes, & Turner, 2010; Rasool et al., 2026; X. Wang et al., 2022).

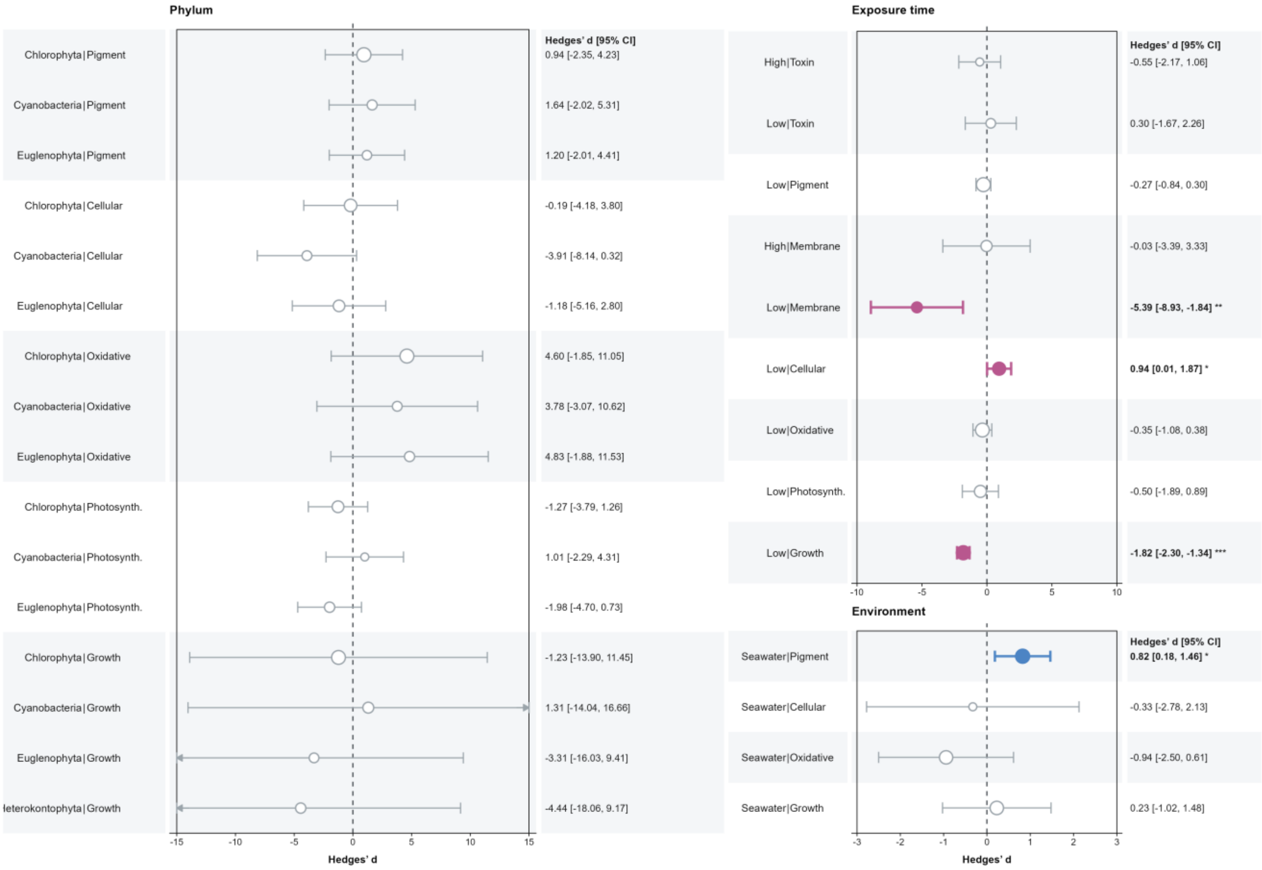

Figure 5. Other moderators of interaction effects, including algal phylum, exposure time, and growth environment. Points are Hedges' d estimates and horizontal bars are 95% confidence intervals. The dashed vertical line marks d = 0. Negative values indicate antagonism and positive values indicate synergism. Filled coloured points and bold values identify statistically significant estimates; open grey points identify non-significant estimates. Low exposure time denotes ≤96 h and high exposure time denotes >96 h. Arrows indicate confidence intervals extending beyond the plotted axis range. Asterisks indicate significance levels: * P < 0.05, ** P < 0.01, *** P < 0.001.

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3.6. Funnel plot assessment

Funnel plots were used to assess small-study effects and potential publication bias. The main-text funnel plots for the overall model, growth, oxidative response, and photosynthesis showed no obvious asymmetry (Figure 6). The effect-size distributions were broadly balanced around the central line, suggesting that the pooled estimates for the main categories were not strongly driven by small-study bias. Because funnel plot interpretation is qualitative and can be affected by heterogeneity, these plots were considered supportive rather than definitive evidence of low publication bias.

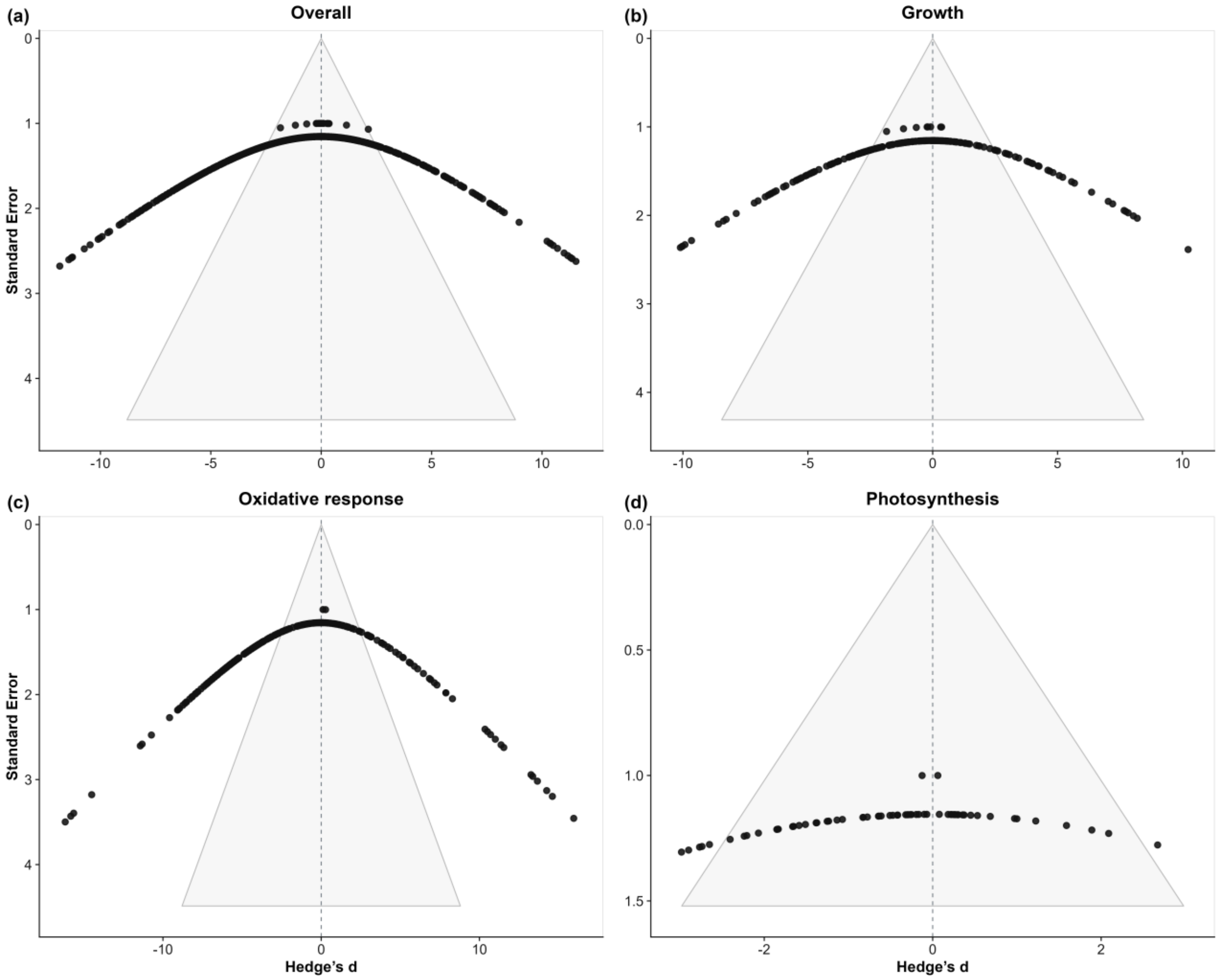

Figure 6. Funnel plots for the overall model and selected physiological categories. Panels show (a) overall, (b) growth, (c) oxidative response, and (d) photosynthesis. Each point represents an effect size. The x-axis is Hedges' d and the y-axis is standard error; smaller standard errors appear toward the top. The dashed vertical line marks d = 0, and the grey triangle shows the approximate 95% pseudo-confidence region expected in the absence of strong small-study effects.

3.7. Random forest variable-importance analysis

Random forest models were fitted for five categories with at least 50 observations: oxidative response, growth, photosynthesis, pigments, and cellular metabolism. The pseudo-R^2 values differed across categories. Growth had the best predictive performance (pseudo-R^2 = 0.42),

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followed by pigments (pseudo-$R^2 = 0.11$) and oxidative response (pseudo-$R^2 = 0.10$). Cellular metabolism (pseudo-$R^2 = -0.03$) and photosynthesis (pseudo-$R^2 = -0.13$) showed weak predictive performance, suggesting that the included moderators did not explain much of the effect-size variation for these endpoints.

For growth, exposure time (%IncMSE = 42.87) and heavy metal concentration (%IncMSE = 29.98) were the most important predictors, followed by heavy metal type (%IncMSE = 28.39) and phylum (%IncMSE = 25.45). Among microplastic-related variables, microplastic concentration (%IncMSE = 22.48) ranked above particle size (%IncMSE = 16.53) and polymer type (%IncMSE = 12.77). For oxidative response, heavy metal type (%IncMSE = 24.64) and microplastic concentration (%IncMSE = 18.62) ranked highest. None of the predictors in the growth or oxidative-response models passed the permutation significance test, indicating that their relative importance should be interpreted as exploratory.

Figure 7 presents the main-text random forest panels for oxidative response, growth, and photosynthesis. In the photosynthesis model, exposure time and heavy metal concentration were marked by the permutation test, but the model had negative pseudo-R^2, so these signals should not be overinterpreted. Negative %IncMSE values indicate that permuting the variable did not worsen model prediction and may reflect weak or unstable predictive contribution.

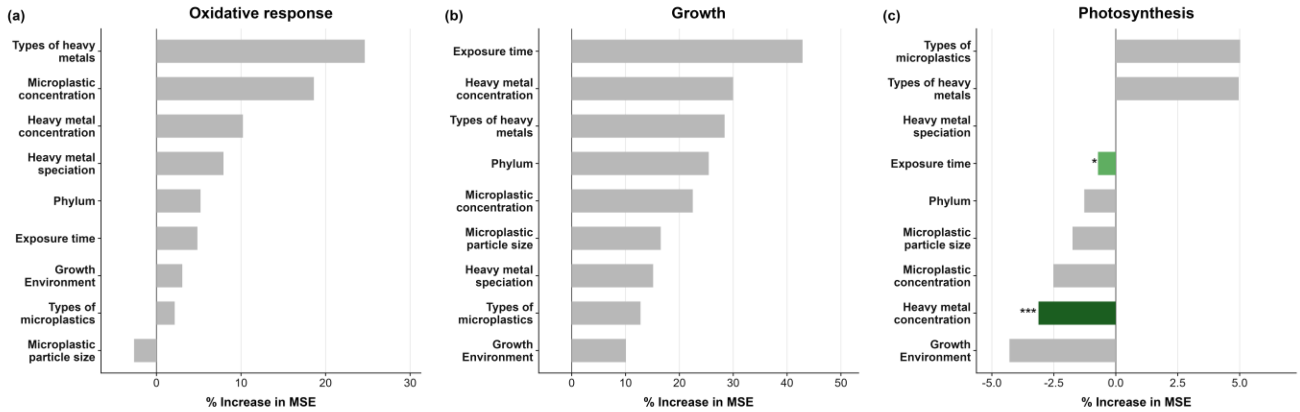

Figure 7. Random forest variable importance for selected physiological categories: (a) oxidative response, (b) growth, and (c) photosynthesis. Bars show percentage increase in mean squared error (%IncMSE) after permutation of each predictor. Larger positive values indicate stronger predictive contribution. Negative values indicate that permutation did not increase prediction error and therefore suggest little or unstable predictive contribution. Grey bars indicate non-significant predictors; green bars indicate predictors marked by the permutation test. Asterisks denote permutation-test significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4. Discussion

This meta-analysis synthesized 1,151 observations from 28 studies to evaluate how combined exposure to heavy metals and microplastics affects seven categories of microalgal physiological responses. The main pooled results showed significant antagonism for growth and oxidative response. However, moderator analyses revealed concentration-dependent shifts in interaction direction, especially under low heavy metal concentrations, where combined exposure became synergistic. These findings help explain why previous experimental studies have reported contrasting interaction patterns.

4.1. Strengths of the synthesis

The study has three main strengths. First, it restricted the analysis to heavy metals rather than combining several chemically distinct contaminant classes. Previous meta-analysis by Ge et al. (2024) included heavy metals, pesticides, pharmaceuticals, and other conventional pollutants(Ge et al., 2024). That broad approach increased the number of observations but may also have averaged across different mechanisms. By focusing on heavy metals, the present analysis detected clear antagonism for growth ($d = -1.73$) and oxidative response ($d = -1.29$), without dilution by organic contaminants that act through different toxicity pathways.

Second, the moderator structure was more specific to metal-microplastic interactions. Heavy metal concentration was classified using metal-specific thresholds, and the models included metal speciation and microplastic aging. This design made it possible to detect a reversal under low heavy metal concentrations, where growth showed strong synergism ($d = 3.45$, $P < 0.001$). This condition is particularly relevant to environmental risk assessment because many natural waters contain metals at low concentrations.

Third, the analysis treated physiological categories separately. Growth and oxidative response showed statistically significant antagonism, whereas photosynthesis, cellular metabolism, pigment endpoints, membrane damage, and toxin production did not. This pattern suggests that heavy metal-microplastic interactions do not affect all cellular pathways equally. Some endpoints may respond directly to changes in metal bioavailability, while others depend on downstream physiological regulation or require longer exposure to become detectable.

4.2. Limitations

The dataset was uneven across response categories. Toxin production included only eight observations, and membrane damage and photosynthesis also had relatively small sample sizes. The wide confidence intervals for these endpoints do not prove the absence of an interaction; rather, they indicate that the current evidence is insufficient to distinguish true effects from sampling variation.

Moderator confounding also limited causal interpretation. Heavy metal type and concentration were not fully independent, and microplastic polymer type was partly confounded with aging status. Random forest models provided exploratory information, but none of the variables in the

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growth or oxidative-response models passed permutation significance tests. The negative pseudo-R^2 values for photosynthesis and cellular metabolism indicate that unmeasured factors such as pH, temperature, light intensity, culture medium, and strain identity may be important.

Meta-analysis also depends on the quality and comparability of the included studies. Differences in light conditions, medium composition, exposure design, and algal source can introduce residual heterogeneity that multilevel models cannot fully remove. Controlled factorial experiments remain necessary for causal confirmation of the mechanisms suggested here.

The funnel plots did not show obvious asymmetry for the main models, supporting the robustness of the pooled estimates. Nevertheless, funnel plots are qualitative tools and can be difficult to interpret when true heterogeneity is high. Publication bias therefore cannot be ruled out completely.

4.3. Explaining differences among studies

The disagreement in the literature can be explained by pollutant scope, concentration, and exposure duration. Broad syntheses that combine heavy metals with organic pollutants may average over distinct mechanisms and obscure metal-specific patterns. In the present heavy metal-focused synthesis, the overall effect for growth was antagonistic, but low heavy metal concentrations produced synergism. Thus, studies reporting synergism may often represent environmentally relevant low-concentration conditions, whereas studies reporting antagonism may reflect higher laboratory concentrations where sorption and bioavailability reduction dominate.

Microplastic concentration adds another layer to this interpretation. The significant antagonism observed for growth at low microplastic concentrations ($d = -3.28$) suggests that adsorption can still dominate when enough sorption sites are available relative to dissolved metals. When metal concentrations are low but bioavailable and microplastics facilitate cellular contact or transport, carrier effects may become more important and produce synergistic toxicity. The balance between sorption-mediated detoxification and carrier-mediated delivery therefore likely controls the direction of interaction.

Exposure time further modifies the interaction. Short-term exposure (≤96 h) showed significant antagonism for growth, consistent with rapid binding of metals to microplastic surfaces. Over longer exposure periods, desorption, particle aging, biofilm formation, algal acclimation, and depletion of defence capacity may alter the response. The random forest model ranked exposure time as the strongest predictor for growth (%IncMSE = 42.87), supporting the

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view that acute toxicity data alone may underestimate risks under chronic environmental exposure.

Metal identity also matters. Heavy metal type ranked highest for oxidative response in the random forest model, indicating ion-specific toxicity pathways. This result supports the decision to analyse heavy metals separately from other pollutant classes and suggests that future risk models should not treat all co-contaminants as mechanistically equivalent.

4.4. Implications and future research

The environmental implication of this study is that risk assessment based mainly on high-concentration acute tests may miss synergistic effects that occur under low heavy metal concentrations. Water-quality criteria for combined pollution should consider non-linear changes in interaction direction across concentration ranges.

Future studies should prioritize three areas. First, mechanisms of low-concentration synergism should be tested using subcellular localization, isotope tracing, or metabolomics to determine whether microplastics increase metal uptake or alter intracellular distribution. Second, environmental aging should be studied dynamically rather than as a simple aged-versus-pristine contrast, because photodegradation, mechanical abrasion, and biofilm formation occur together in natural waters. Third, chronic exposure experiments at realistic concentrations are needed to improve extrapolation from laboratory tests to ecosystem risk.

5. Conclusions

Combined exposure to heavy metals and microplastics produced significant antagonistic effects on microalgal growth and oxidative response in the overall analysis, but moderator results showed that interaction direction is not fixed. Low heavy metal concentrations were associated with synergistic effects, whereas short-term exposure and several polymer types were associated with antagonism. These findings indicate that combined toxicity is concentration- and time-dependent. Ecological risk assessment should incorporate this context dependence and should not rely solely on high-concentration acute exposure data.

Acknowledgements

The author thanks Professor Chen Ke and Dr. Ren Peng for their guidance. Ren Peng provided instruction on meta-analysis methodology, model construction, code debugging, and figure preparation. Chen Ke offered expertise on experimental biology, including validation experiment design and physiological indicator classification. Both supervisors contributed to the conceptualization of this study and provided critical feedback on the manuscript.

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Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author contributions

Lin Liu: conceptualization, literature screening, data extraction, formal analysis, visualization, writing – original draft.

Juelin Chen: literature screening

Xianju Deng: data extraction

Peng Ren: conceptualization, methodology, writing – review and editing.

Ke Chen: conceptualization, writing – review and editing, supervision.

Competing interests

The authors declare no competing interests.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

Not applicable. This study is a meta-analysis of previously published data and did not involve human participants or vertebrate animals.

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