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SPECIALTY SECTION

This article was submitted to
Marine Pollution,
a section of the journal
Frontiers in Marine Science

RECEIVED 04 November 2022

ACCEPTED 02 December 2022

PUBLISHED 16 December 2022

CITATION

Liu F-f, Gao Z-y, Chu W-c and
Wang S-c (2022) Polystyrene
nanoplastics alleviate the toxicity
of CuO nanoparticles to the
marine algae *Platymonas*
helgolandica var. *tsingtaoensis*.
Front. Mar. Sci. 9:1089282.
doi: 10.3389/fmars.2022.1089282

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Polystyrene nanoplastics alleviate the toxicity of CuO nanoparticles to the marine algae *Platymonas* *helgolandica* var. *tsingtaoensis*

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The increasing presence of pollutants such as engineered nanoparticles (ENPs) and nanoplastics (NPLs) poses potential risks to the marine environment. However, there is a scarcity of information on their joint toxic effects. In this study, we investigated the toxicity of copper oxide nanoparticles (nCuO) combined with polystyrene nanoplastics (PS-NPLs) on the marine microalgae *Platymonas helgolandica* var. *tsingtaoensis*. The inhibitory effect of nCuO increased with increasing concentrations, and nCuO \geq 100 mg/L significantly restrained the growth and chlorophyll content of microalgae. nCuO could be adsorbed by algal cells, which was responsible for membrane lipid oxidation and the disruption of membrane permeability. Simultaneous exposure to nCuO and PS-NPLs had an antagonistic effect on the growth inhibition of the microalgae, and nCuO played a leading role in the joint toxicity of nCuO and PS-NPLs. In comparison to nCuO exposure alone, combined exposure decreased the oxidative stress and alleviated the increase in the cell membrane permeability of microalgae. PS-NPLs could heteroaggregate with nCuO, which reduced the interaction between nCuO and microalgae, inducing decreased joint toxicity. Findings of this study will clarify our understanding of the joint toxicity of ENPs and NPLs.

KEYWORDS

engineered nanoparticles, nanoplastics, antagonistic, aggregation, microalgae

1 Introduction

Nanoplastics (NPLs, size of 1 nm–1 μ m) have been regarded as one of the least investigated types of marine debris, but also potentially one of the most dangerous (Koelmans, 2019). NPLs are predominantly formed through fragmentation and degradation of larger plastic debris as a result of mechanical wear, UV irradiation, and

biodegradation (Liu et al., 2019; Roager and Sonnenschein, 2019; Enfrin et al., 2020). Only a small fraction of NPLs occurring in the environment are intentionally manufactured as an added component in some products (e.g., nanobeads for personal care products and industrial cleaners) (Alimi et al., 2018; Ivleva, 2021). Although recent works have reported the chemical signatures of NPLs contamination in ocean waters, the abundance of NPLs in the environment still remains largely unknown due to methodological challenges in their identification and quantification (Ter Halle et al., 2017; Materić et al., 2020; Xu et al., 2022). However, given the increasing distribution and concentration of microplastics (< 5 mm) in environmental samples, it can also be assumed that NPLs have a high abundance in the environment.

Microalgae are primary producers at the lowest nutritional level in the marine food chain. Due to their unique properties, NPLs were reported to exhibit high toxicity to microalgae. NPLs can cause physical damage to microalgae, reduce the synthesis of chlorophyll, induce high production of reactive oxygen species (ROS), or form heteroaggregates with algae cells, which significantly inhibit the normal growth of microalgae (Gomes et al., 2020; Nava and Leoni, 2021; Yang et al., 2021). Moreover, NPLs may adsorb large amounts of external toxic compounds because of their high surface-to-volume ratio (Feng et al., 2022; Xi et al., 2022), which contributes to the flux of contaminants in organisms. The joint toxicity of NPLs with heavy metals or organic pollutants to microalgae have been investigated (Cao et al., 2022; Gao et al., 2022; Liu et al., 2022). However, the natural environment is a complicated system, and the fate and behaviors of NPLs in the presence of other contaminants still need further investigation.

Engineered nanoparticles (ENPs) have been widely used in various fields due to their specific physicochemical and biological properties. The large-scale use of nanomaterials makes ENPs inevitably enter the environment during production, transportation, and utilization processes, generating potential environmental risks (Keller and Lazareva, 2014; Lead et al., 2018). It has been reported that the concentrations of ENPs in real water environment reach up to several tens of microgram per liter (Bathi et al., 2022), which can cause the mechanical damage to cell membranes that promotes the internalization of ENPs (Ma et al., 2015; Zhao et al., 2016; Yan et al., 2022). In addition, metal-based ENPs can be partly dissolved to release soluble metal ions, which increase their bioavailability to microalgae (Chen et al., 2012; Yung et al., 2015). ENPs can also adsorb nutrients in the medium and lead to nutrient depletion by microalgae, thus causing indirect toxicity to algae cells (Zhao et al., 2017; Yan et al., 2022). Although both NPLs and ENPs share some similar properties and toxicity mechanisms toward microalgae, NPLs still should be considered a unique class of contaminants, distinct from ENPs (Gigault et al., 2021). Therefore, considering the cooccurrence of

NPLs and ENPs in the natural environment, their joint toxicity toward organisms needs to be further studied.

To date, there have been some studies on the joint toxicity of microplastics and ENPs; however, research on the combined effects of NPLs and ENPs is limited, and the relative mechanism of action is unclear. It was found that microplastics could be adsorbed on the algal cells and inhibit the interaction between algae and ENPs (eg. nano-ZnO, nano-Cu) (Gunasekaran et al., 2020; Zhu et al., 2020). In addition, microplastics may also reduce the surface reactivity of ENPs, leading to a reduction in the ROS content generated by microalgae, which results in a decrease in their joint toxicity (Gunasekaran et al., 2020). Natarajan et al. (2022) also found that nano-TiO₂ and nanopolystyrene (nano-PS) had antagonistic interactions with the marine algae *Chlorella* sp. because the heteroaggregation of nano-TiO₂ and nano-PS reduced the bioavailability of the particles to the algae. Thiagarajan et al. (2021) obtained similar results that the carboxylated PS microplastics decreased the toxicity of nano-TiO₂ in *Chlorella* sp., but the aminated and plain PS microplastics enhanced the toxicity of nano-TiO₂. Huang et al. (2019) investigated the joint toxicity of Ag and PS nanoparticles to two kinds of microalgae, and found that nano-PS showed opposite effects on *Chlamydomonas rheinisch* (increased) and *Dinoflagellates* (decreased) in terms of intracellular Ag accumulation. However, the joint toxicity of AgNPs and nano-PS had synergistic interactions with the algae, regardless of the differences in Ag accumulation. Therefore, the complicated joint toxicity mechanism of ENPs and NPLs pollution to microalgae needs to be further explored.

Copper oxide nanoparticles (nCuO), one of the most common ENPs, have been widely used in agriculture, textiles, food preservation, anti-fouling coatings, and water treatment (Roubeau Dumont et al., 2022). By 2025, the global annual production of nCuO is estimated to reach 1600 tons/year (Hou et al., 2017). nCuO can be indirectly released into the aquatic ecosystem through runoff, leaching from agricultural and industrial sites, or directly into the marine environment through the application of antifouling paints (Muller-Karanassos et al., 2021). Although data on nCuO concentrations in the marine environment are scarce (Zhao et al., 2021), once present in the environment, the chance of nCuO contact with organisms and transfer between organisms of different trophic levels increases with time (Wu et al., 2020).

In this study, nCuO was used as a typical representative of ENPs to study its joint toxicity with PS-NPLs (PS, and PS-COOH representing aged PS). *Platymonas helgolandica* var. *tsingtaoensis* (*P. helgolandica* var. *tsingtaoensis*) was used as the test species because it distributes widely as aquaculture high-quality feed. The cell growth, chlorophyll content, oxidative stress indicators, and cell membrane permeability were evaluated in the presence of nCuO alone or in combination with PS-NPLs. Interactions among nCuO, PS-

NPLs, and microalgae were further characterized, and the type of joint action of PS-NPLs and nCuO on microalgae were clarified by both of Abbott and the independent action models. The findings will facilitate more realistic evaluations of the environmental risks of ENPs and NPLs.

2 Materials and methods

2.1 Material characterization

PS-NPLs including PS and PS-COOH were obtained from Jingjie Technology Co., Ltd. (Changchun, China), which have been fully characterized in our previous study (Gao et al., 2022). nCuO (< 50 nm) was purchased from Sigma-Aldrich and could form homogeneous aggregates of several hundred nanometers in *f/2* culture medium (Table S1) under the observation of scanning electron microscopy with focused ion beam (FIB-SEM; Crossbeam 550, ZEISS, Germany) (Figure S1). The zeta potential and hydrodynamic diameter of nCuO in the *f/2* culture medium were determined by dynamic light scattering (Zetasizer Nano ZS90, Malvern, UK). The amount of Cu(II) released by nCuO in the *f/2* culture medium was detected to investigate the contribution of soluble metal ions to the toxicity of nCuO (Supporting Information).

2.2 Microalgae cultivation and growth inhibition experiments

The microalgae were obtained from Guangyu Biotechnology Co. Ltd. (Shanghai, China), and the detailed cultivation method was provided in the Supporting Information. The growth inhibition experiments were first conducted using algal cells in log phase (2×10^5 cells/mL). Single nCuO exposure experiments were carried out with the nCuO concentrations of 10, 50, 100, and 200 mg/L according to the preexperiment (Figure S2). To determine the joint effect of PS-NPLs and nCuO, the concentration of PS-NPLs was fixed at 100 mg/L, and the nCuO concentration was set at 50, 100, and 200 mg/L. The changes in cell concentration were measured every 24 hours, and the percent growth inhibition rate (IR) was determined by $IR (\%) = 100 \times (C_0 - C_i) / C_0$, where C_0 and C_i represent the cell concentration of the control and experimental groups at average, respectively.

2.3 Determination microalgae physiological and biochemical responses

Contents of chlorophyll including chlorophyll *a* and chlorophyll *b* were detected by ethanol extraction method.

Three oxidative stress indicators were determined in this study, including superoxide dismutase (SOD) activity, reduced glutathione (GSH), and malondialdehyde (MDA) contents (Gao et al., 2022). Besides, the cell membrane permeability of microalgae was also assessed in both single and combined exposure experiments at 96 h by the fluorescein diacetate (FDA) method (He et al., 2019; Yan et al., 2022). To investigate the morphological changes in algal cells, microscopy analysis was carried out by optical microscopy and scanning electron microscopy (SEM) at 96 h of the experiment. The detailed measurement procedures of the above physiological and biochemical responses were provided in the Supporting Information.

2.4 Data analysis

All experiments were set up with three replicates, and the results were expressed as the mean \pm standard deviation (SD). One-way ANOVA was used to test the significant differences between the control and treatment groups, and $P < 0.05$ indicated a statistically significant difference. The independent action (IA) model and Abbott model were used to evaluate the results to clarify the type of combined action of PS-NPLs and nCuO (Gao et al., 2022). Detail on the two models was supplied in the Supporting Information.

3 Results and discussion

3.1 Growth inhibition

The growth inhibitory effect of nCuO on the microalgae increased with increasing exposure concentration and time (Figure S3). At 96 h, no obvious difference was observed in cell concentration between the nCuO ≤ 50 mg/L groups and the control group, while the cell concentrations of the 100 and 200 mg/L nCuO groups were significantly reduced compared with the control group, decreasing by 21.43% and 29.56% (Figure 1), respectively. Previous studies revealed that the toxic effects of metal-based nanoparticles could be attributed to the release of soluble metal ions (Chen et al., 2012; Yung et al., 2015; Huang et al., 2019). Therefore, the concentration of Cu(II) released from nCuO in *f/2* medium was detected at 24 h and 96 h (Figure S4). The highest Cu(II) concentration was 0.21 ± 0.032 mg/L released from 200 mg/L nCuO at 96 h. Our previous study reported that the calculated growth inhibition rate was lower than 7% on *P. helgolandica* var. *tsingtaoensis* with Cu(II) below 0.21 mg/L (Gao et al., 2022), indicating that a small part of the toxicity of nCuO to the microalgae was due to the released Cu(II). Zhao et al. (2016) found that nCuO inhibited the growth of *Chlorella vulgaris*, and the median effect

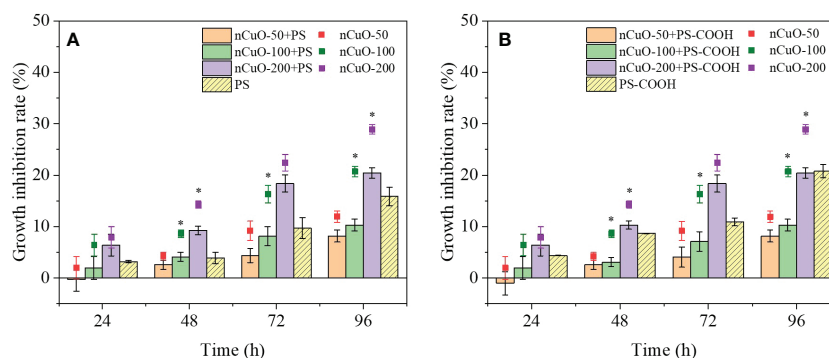


FIGURE 1

Combined effects of different concentrations of nCuO with 100mg/L PS (A) and PS-COOH (B) on the growth inhibition of *P. helgolandica* var. *tsingtaoensis*. Error bars represent standard deviations (n = 3). Asterisks indicate significant differences between single nCuO groups and the combined groups (P < 0.05).

concentration (EC₅₀) value at 72 h was 45.7 mg/L. However, in our study, the EC₅₀ value of nCuO on *P. helgolandica* var. *tsingtaoensis* was not obtained even when the concentrations reached 500 mg/L (Figure S2). High salinity could enhance the homogeneous aggregation of nanoparticles, which reduced the interaction between algal cells and nanoparticles (Callegaro et al., 2015; Conway et al., 2015). The mean hydrodynamic diameter for nCuO was 862.53 ± 59.90 nm in f/2 media, indicating the high aggregation of nCuO. In addition, nCuO underwent rapid aggregation and sedimentation after being added to the algal suspension (Figure S5); therefore, the strong aggregation of nCuO resulted in their relatively weakened toxicity to algal cells.

The results of the combined exposure to PS-NPLs and nCuO on the growth inhibition of microalgae are displayed in Figure 1. It was obvious that the growth inhibition rates of the combined exposure groups were positively correlated with the nCuO concentrations, indicating that the joint toxicity was mainly influenced by nCuO. The growth inhibition rates increased with increasing exposure time, and the highest values were 20.45% in the presence of 200 mg/L nCuO combined with PS-NPLs at 96 h. During the whole exposure period, the addition of PS-NPLs reduced the growth inhibition rates of single nCuO exposure. For example, at 96 h, compared with the single nCuO exposure results, the growth inhibition rates of 50, 100, and 200 mg/L nCuO combined with PS decreased by 3.70%, 10.32%, and 8.40%, respectively. Meanwhile, the antagonistic effects between nCuO and PS-NPLs were obtained by the IA and Abbott models (Table S2), which were in accord with the above results. These results may be attributed to the reduction in the nCuO effective concentration induced by the heterogeneous aggregation between PS-NPLs and nCuO (Conway et al., 2015), which will be further discussed in Section 3.5.

3.2 Effect on chlorophyll content

Figure S3B illustrates the chlorophyll content in *P. helgolandica* var. *tsingtaoensis* affected by nCuO. The chlorophyll content decreased at all tested concentrations during the experiment, and showed a decreasing trend with increasing nCuO concentration. At 24 h, compared with the control group, the chlorophyll *a* content in the four experimental groups (10, 50, 100 and 200 mg/L nCuO) significantly decreased by 24.53%, 27.89%, 30.87% and 29.75%, respectively. Chlorophyll *b* content showed a similar trend. At 48 h and 72 h, the chlorophyll content of the nCuO groups recovered with increasing exposure time and showed no significant difference from that of the control group. At 96 h, nCuO > 100 mg/L significantly inhibited chlorophyll synthesis, and the chlorophyll content in the 100 and 200 mg/L nCuO groups decreased by 19.11% and 31.81%, respectively. The surface coverage of nCuO on *P. helgolandica* var. *tsingtaoensis* could impede the access of algal cells to external light, oxygen and other substances (Zhao et al., 2016); alternatively, the turbidity of nanoparticles in the medium could produce a shading effect to inhibit the growth of microalgae (Chen et al., 2018).

The chlorophyll content in the 100 mg/L PS-NPLs treatment groups was higher than that in the control group at 96 h (Figure S6). However, compared with the control, the chlorophyll *a* content exposure to the combined nCuO (50, 100 and 200 mg/L) with PS and PS-COOH decreased by 9.86%, 23.46%, 20.62% and 5.87%, 21.39%, 20.66%, respectively (Figure 2). The chlorophyll *b* content showed a similar trend. No statistically significant difference was observed in chlorophyll content between the combined exposure groups and the single nCuO groups, which further indicated that the inhibition effect of combined

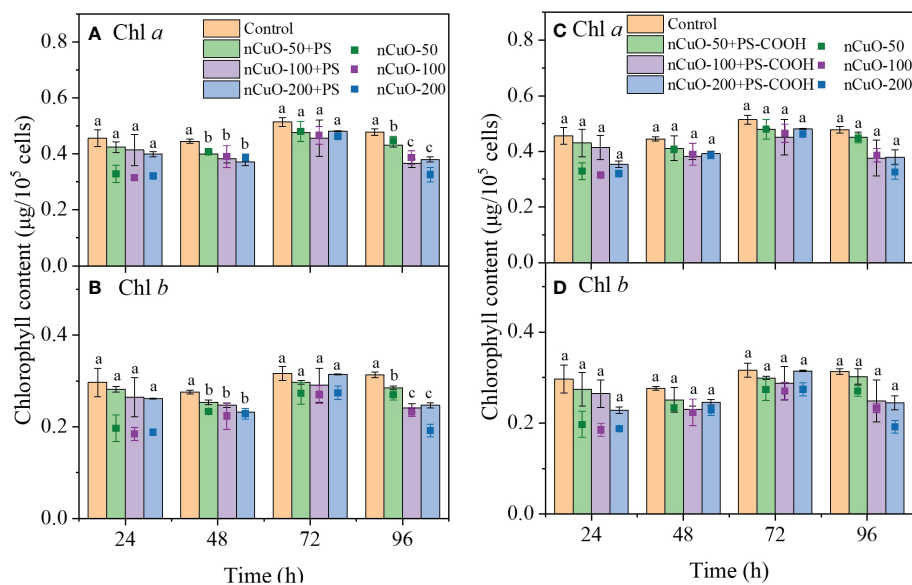


FIGURE 2

Combined effects of nCuO with 100mg/L PS (A, B) and PS-COOH (C, D) on the chlorophyll content of *P. helgolandica* var. *tsingtaoensis*. Error bars represent standard deviations (n = 3). Different letters indicate significant differences among the treatment groups (P < 0.05).

nCuO and PS-NPLs on the chlorophyll content was mostly affected by nCuO.

3.3 Effect on oxidative stress

Figure S7A shows that the SOD activity in all nCuO groups was higher than that in the control group, and the SOD activity increased with increasing nCuO concentration. This result indicated that nCuO induced oxidative stress in the microalgae, and higher exposure concentrations led to increased oxidative stress. Che et al. (2018) also found that nCuO could increase the ROS content and SOD activity of *Chlorella vulgaris*, and these two indicators were positively correlated. In our study, the difference in SOD activity between the nCuO groups and the control group gradually decreased as the exposure time increased, suggesting that the ROS inside the algal cells decreased with prolonged exposure time due to the self-adaptation and antioxidant defense of algae (Sabatini et al., 2009). Unlike SOD activity, nCuO at all concentrations resulted in a much lower GSH content compared with the control group at 24 h, and the higher the exposure concentration of nCuO was, the lower the GSH content of algal cells (Figure S7B). This might be attributed to the inhibition of GSH synthesis and the high consumption of GSH in algal cells. It has been reported that nCuO could cause dysfunction of intracellular biomolecules, alteration of antioxidant enzyme activity and depletion of GSH content (Naz et al., 2020). After 24 h, the GSH content in the 10 mg/L

nCuO groups had no significant difference from that in the control group. Meanwhile, GSH contents in the nCuO > 10 mg/L groups increased with the exposure time, and were not significantly different from those in the 10 mg/L nCuO groups at 96 h, indicating the recovery of GSH synthesis capacity and a decrease in GSH consumption due to the reduction in ROS in algal cells (Melegari et al., 2013).

During the exposure period, the MDA content was significantly higher in the nCuO groups than that in the control and showed a positive correlation with the nCuO exposure concentrations (Figure S7C). At 24 h, the MDA content of the four experimental nCuO groups (10, 50, 100, and 200 mg/L) was 1.17, 1.39, 1.48, and 1.79 times higher than that of the control, indicating that nCuO exposure caused lipid peroxidation to *P. helgolandica* var. *tsingtaoensis*. This was one of the reasons for the growth inhibition effect of nCuO. After 24 h, the MDA content of all nCuO groups did not change significantly with increasing exposure time. This suggested that the oxidative damage produced by nCuO decreased with time, which was related to the scavenging effect of SOD on ROS and the breaking of lipid peroxidation-related chains by GSH (Ribeiro et al., 2017; Estrela et al., 2021).

The combined effects of nCuO and PS-NPLs on the three oxidative stress indicators are presented in Figure 3. During the whole exposure time, the SOD activity of microalgae in the combined exposure groups significantly increased compared with that in the control group (Figs. 3A-B). SOD activity was positively correlated with the concentration of nCuO in the combined exposure groups, indicating that algae cells in the

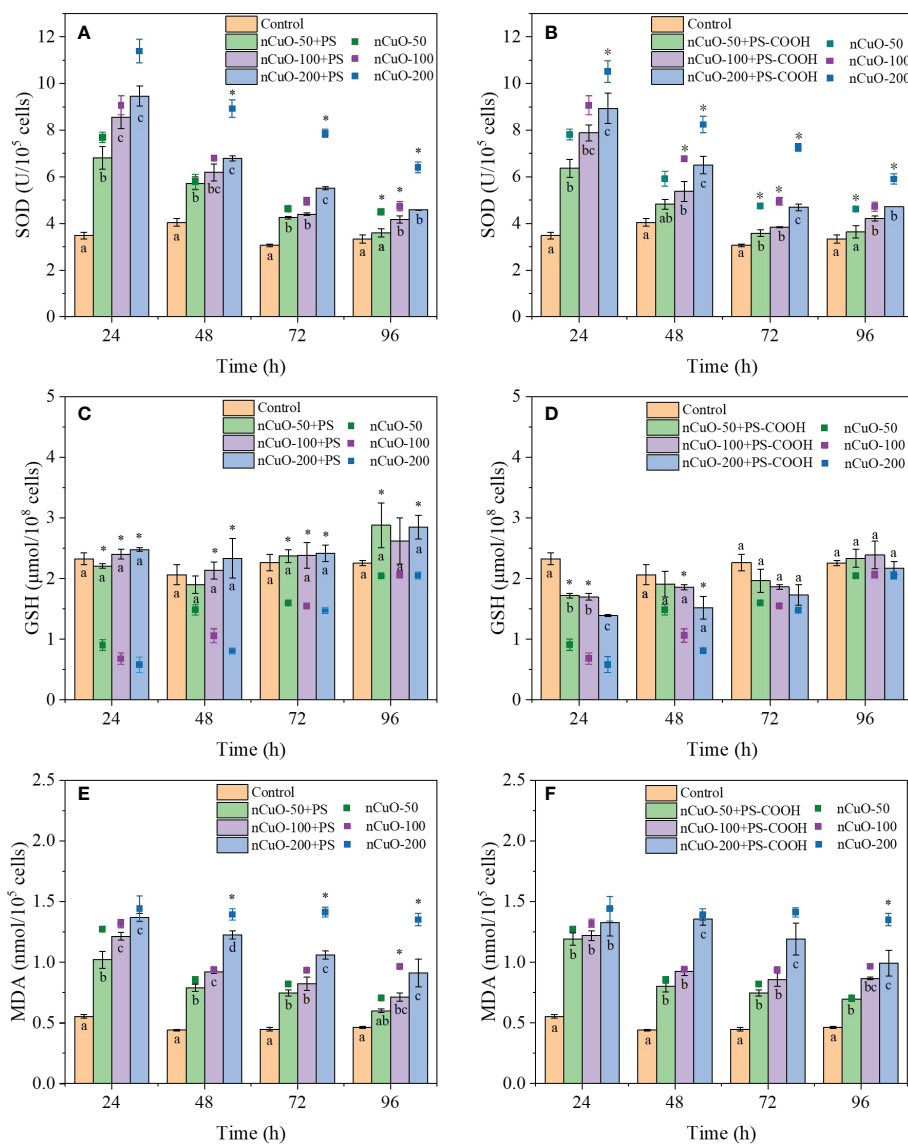


FIGURE 3

Combined effects of different concentrations of nCuO with 100mg/L PS and PS-COOH on SOD activity (A, B), GSH content (C, D) and MDA content (E, F) in *P. helgolandica* var. *tsingtaoensis*. Error bars represent standard deviations ($n = 3$). Different letters indicate significant differences among the treatment groups ($P < 0.05$). Asterisks indicate significant differences between single nCuO groups and the combined groups ($P < 0.05$).

combined exposure groups were also subjected to oxidative stress. Compared with nCuO exposure, the presence of PS-NPLs reduced the SOD activity of microalgae. Meanwhile, as shown in Figures 3C, D, the GSH content of algae cells in the combined exposure groups was always higher than that in the nCuO exposure groups. These results indicated that the oxidative stress in the combined exposure groups was weaker than that in the single nCuO exposure groups.

Figures 3E, F demonstrate the effect of the combined PS-NPLs and nCuO on the MDA content in the microalgae. The

MDA contents in the combined exposure groups were higher than those in the control group, and the MDA content was positively correlated with the nCuO concentration. However, MDA contents in the combined exposure groups were consistently lower than those in single nCuO exposure groups throughout the entire exposure period. MDA content in microalgae in the 200 mg/L nCuO combined with PS and PS-COOH groups was 34.91% and 28.12% lower than that in the nCuO group at 96 h. This indicated that PS-NPLs could reduce the membrane oxidative damage of algal cells caused by nCuO,

which was also the reason for the reduced combined toxicity. In addition, as shown in Table S3, the combined effect of nCuO and PS-NPLs was antagonistic, which was evaluated by the Abbott model based on the three oxidative stress indicators data (oxidative stress indicators of microalgae exposed to PS-NPLs are shown in Figure S8).

3.4 Effect on cell membrane permeability

To further investigate the degree of membrane damage when exposed to nCuO alone or in combination with PS-NPLs, the cell membrane permeability of the microalgae was evaluated. There was a negative correlation between the fluorescence intensity and the nCuO concentrations (Figure S9A), indicating that the cell membrane permeability in all nCuO groups significantly increased compared with that in the control group. The abrasiveness of particles can cause physical damage when they contact with algal cells (Pakrashi et al., 2013). Besides, the attachment of ENPs on the cell can disturb the electron/ion transport chains of cell membrane (Lei et al., 2016), resulting in an increase in membrane permeability. In addition, ENPs also induced lipid peroxidation of the cell membrane, leading to an increase in permeability. The dose dependent increase in MDA content in the nCuO groups (Figure S7C) demonstrated that nCuO at higher concentrations induced stronger cell membrane lipid peroxidation, thus leading to an increase in cell permeability. He et al. (2019) found similar results that the cell membrane permeability of *Chlorella pyrenoidosa* increased with increasing nThO₂ concentrations, which was attributed to increasing oxidative stress.

Figure S9B presents the combined effect of nCuO and PS-NPLs on the cell membrane permeability of *P. helgolandica* var. *tsingtaoensis*. PS-NPLs significantly alleviated the increase in cell membrane permeability caused by nCuO, which was in accord with the reduction in cell membrane oxidative damage. This result might be attributed to the reduced interaction between nCuO and algal cells in the combined exposure groups. Similarly, Zhao et al. (2018) observed that Al₂O₃ nanoparticles could heterogeneously aggregate with graphene oxide (GO), leading to reduced physical contact between GO and algal cells, thus significantly alleviating the cell membrane damage of GO on *Chlorella pyrenoidosa*.

3.5 Interactions among nCuO, PS-NPLs, and microalgae

As the cell growth and chlorophyll content in *P. helgolandica* var. *tsingtaoensis* was considerably reduced in the nCuO ≥ 100 mg/L groups at 96 h, optical microscopy observation was employed to examine the interaction between nCuO and algal cells in the 100 and 200 mg/L nCuO groups. As

shown in Figure S10, large heterogeneous aggregates composed of algal cells and nCuO were observed. SEM images also showed that nCuO was mainly in an agglomeration state and could be adsorbed on the surface of algal cells (Figures 4A, B). This could be explained by the electrostatic attraction between the negatively surface charged *P. helgolandica* var. *tsingtaoensis* (zeta potential = -7.75 ± 1.66 mV) and the positively surface charged nCuO (zeta potential = 3.66 ± 0.54 mV). Various ENPs have been found to form heteroaggregates with algal cells (Ma et al., 2015; Zhao et al., 2016; He et al., 2019); as a result of cell membrane damage, thus inhibiting algae growth. Meanwhile, there were a large number of deciduous flagella surrounding the algal cells (Figure S10), indicating that nCuO could reduce the cell activity by disrupting the integrity of *P. helgolandica* var. *tsingtaoensis*.

As the combined effects of PS-NPLs and nCuO on the growth and oxidative stress indicators were antagonistic according to Sections 3.1 and 3.3, we hypothesized that PS-NPLs might aggregate with nCuO and thus reduce the bioavailability of nCuO on the microalgae. Indeed, both types of PS-NPLs could interact with nCuO to form heteroaggregates (Figure S11). Natarajan et al. (2022) observed a similar heteroaggregation of nano-TiO₂ and PS-NPLs, and the toxic effects of nano-TiO₂ to marine algae *Chlorella* sp. was significantly reduced in the presence of PS-NPLs. In our study, both PS and PS-COOH were negatively charged with zeta potentials of -8.24 ± 1.5 mV and -15.15 ± 1.06 mV, respectively. Therefore, PS-NPLs and *P. helgolandica* var. *tsingtaoensis* might compete for the interaction with nCuO, and heteroaggregation between PS-NPLs and nCuO would reduce the interaction between nCuO and microalgae. As shown in Figures 4C-F, PS-NPLs in the combined groups formed heterogeneous aggregates with nCuO, resulting in reduced surface interactions between nCuO and algal cells. Therefore, the simultaneous exposure of nCuO and PS-NPLs showed antagonistic effects on the toxicity of *P. helgolandica* var. *tsingtaoensis*.

4 Conclusions

This study evaluated the toxic effects of nCuO with and without PS-NPLs to *P. helgolandica* var. *tsingtaoensis*, specifically focusing on the growth, chlorophyll, oxidative stress, and membrane permeability of the microalgae. The heteroaggregation of nCuO and *P. helgolandica* var. *tsingtaoensis* induced severe membrane lipid peroxidation, decreased the chlorophyll content, and increased the membrane permeability of microalgae, which were the toxic mechanisms of nCuO toward microalgae. In combined exposure experiments, nCuO and PS-NPLs had an antagonistic effect on *P. helgolandica* var. *tsingtaoensis*. The addition of PS-NPLs alleviated the growth inhibition, oxidative stress, and cell

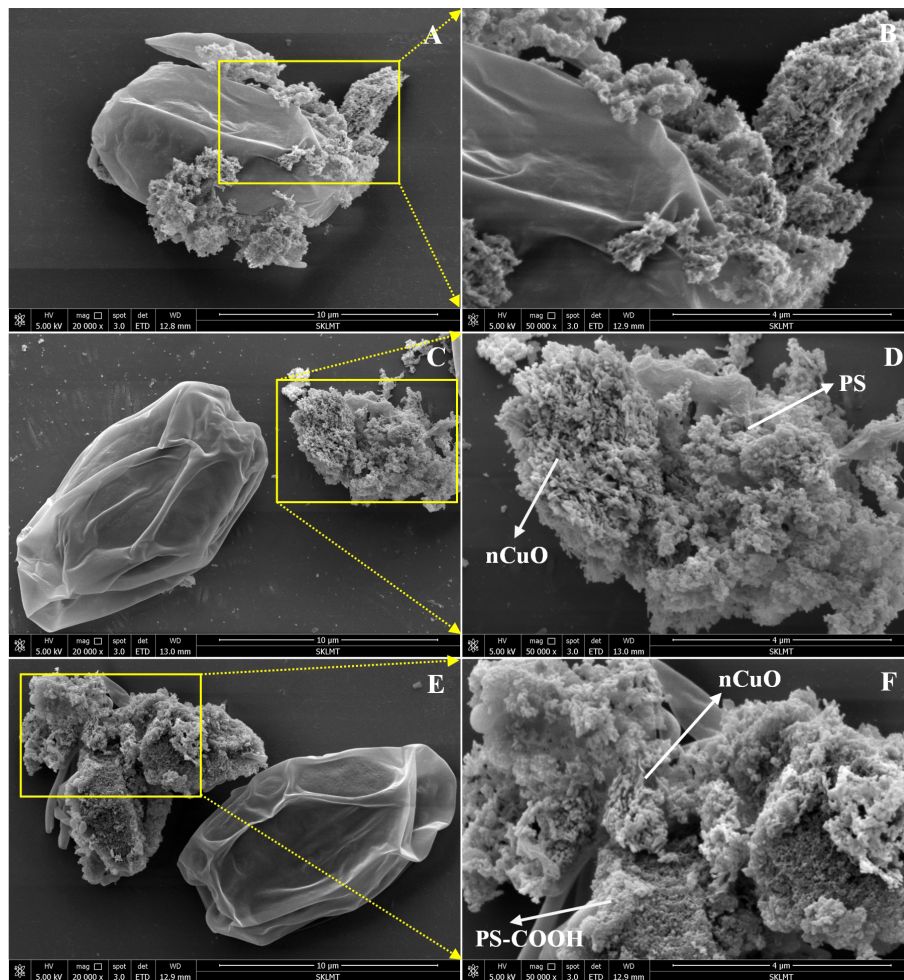


FIGURE 4
SEM images of *P. helgolandica* var. *tsingtaoensis* exposed to nCuO (A, B), nCuO + PS (C, D), and nCuO + PS-COOH (E, F).

membrane permeability caused by nCuO to microalgae. This could be attributed to the heterogeneous aggregation of PS-NPLs with nCuO, which inhibited the interactions between nCuO and microalgae. This study provides a better understanding of the joint toxicity of nanoplastics and nanoparticles to microalgae.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

F-FL: Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - original draft, Writing - review

and editing, Supervision, Project administration, Funding acquisition. Z-YG: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing - original draft. W-CC: Investigation, Writing - original draft. S-CW: Methodology, Writing - review and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was financially supported by the National Natural Science Foundation of China (U1906224), the Natural Science Foundation of Shandong Province (ZR2020MD114), Shandong Provincial Key Research and Development Program (2020CXGC011406), Young Scholars Program of Shandong University, and Shandong University Interdisciplinary Research and Innovation Team of Young Scholars (2020QNQT20).

Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.1089282/full#supplementary-material>

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