



The Combined Effect of Copper Nanoparticles and Microplastics on Transcripts Involved in Oxidative Stress Pathway in Rainbow Trout (*Oncorhynchus Mykiss*) Hepatocytes

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Abstract

Copper nanoparticles (CuNPs) and microplastics (MPs) are two emerging contaminants of freshwater systems. Despite their co-occurrence in many water bodies, the combined effects of CuNPs and MPs on aquatic organisms are not well-investigated. In this study, primary cultures of rainbow trout hepatocytes were exposed to dissolved Cu, CuNPs, MPs, or a combination of MPs and CuNPs for 48 h, and the transcript abundances of oxidative stress-related genes were investigated. Exposure to CuNPs or dissolved Cu resulted in a significant increase in the transcript abundances of two antioxidant enzymes, catalase (*CAT*) and superoxide dismutase (*SOD*). Exposure to CuNPs also led to an upregulation in the expression of Na⁺/K⁺ ATPase alpha 1 subunit (*ATPIA1*). Microplastics alone or in combination with CuNPs did not have a significant effect on abundances of the target gene transcripts. Overall, our findings suggested acute exposure to CuNPs or dissolved ions may induce oxidative stress in hepatocytes, and the Cu-induced effect on target gene transcripts was not associated with MPs.

Keywords Copper nanoparticles · Microplastics · Oxidative stress · Hepatocytes · Rainbow trout

Introduction

In aquatic ecosystems, organisms are inevitably exposed to mixtures of environmental contaminants (Fleeger et al. 2003). To protect aquatic life, it is important to understand if contaminant mixtures can produce additive or non-additive effects. Investigating the additivity of mixtures of contaminants can assist regulatory authorities to evaluate the efficacy of environmental quality guidelines. Microplastics (MPs) and copper nanoparticles (CuNPs) are two contaminants of which the combined effect on aquatic organisms has yet to be fully understood.

The emergence of MPs as anthropogenic contaminants that enter aquatic systems from household and industrial effluent has raised concerns about not only the potential for MPs to be toxic on their own, which remains highly debated, but for microplastics to act as a vector for known toxicants (Ma et al. 2020). The physical risks associated with plastic pollution such as entanglement, ingestion, and blockage or disruption of respiratory or digestive function have been well substantiated; however, the toxicity of MPs and associated toxicants remains unclear (Foley et al. 2018). Microplastics exhibit a large surface area to volume ratio, allowing for a relatively large surface area for toxicants to sorb to while remaining small enough to be bioavailable to many species (Caruso 2019). Microplastic and metal mixture toxicity is an emerging area of research; however, this body of work is primarily focussed on ionic forms of metal with little work analyzing microplastic and metal nanoparticle interactions (Godoy et al. 2019).

Copper NPs with unique antimicrobial, catalytic, and conductivity activities are applied in many fields including textiles (Radetić and Marković 2019), biomedical (Akintelu et al. 2020), wastewater treatments (Dlamini et al. 2019),

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and aquaculture (Chari et al. 2017). Large-scale application of CuNPs in commercial and industrial processes increases the likelihood of their existence in aquatic environments. Copper NPs may undergo dissolution and release dissolved Cu — the most bioavailable form of Cu that exerts toxicity in living cells — to the surrounding solution. Nevertheless, many studies reported dissolved ions and nanocopper as both contributing to CuNP-induced toxicity in cells (Al-Bairuty et al. 2013; Griffitt et al. 2009; Razmara et al. 2021).

Many studies have demonstrated that fishes are highly susceptible to ingesting MPs. Microplastics can translocate from tissues such as intestine to hepatic tissue in a few freshwater fish species and accumulate (Collard et al. 2017; Lu et al. 2016). Some studies reported that accumulated MPs in fish liver can induce toxicity. For instance, long-term (35 days) exposure of zebrafish to 10 and 100 µg/L MPs resulted in a significant induction of oxidative stress and disruption of histological architecture in the liver (Umamaheswari et al. 2021). In rainbow trout hepatocytes, however, the toxicity of MPs was attributed to the adsorbed organic contaminants (Pannetier et al. 2019a). Our previous study suggested CuNPs can also translocate into fish liver (Lindh et al. 2019). Results indicated Cu was significantly accumulated in the liver of CuNP-injected and gavaged rainbow trout (Lindh et al. 2019). Exposure to CuNPs resulted in histopathological damage in fish liver (Al-Bairuty et al. 2013). A 30-day exposure of juvenile *Takifugu fasciatus* to 20 and 100 µg/L of CuNPs induced oxidative stress, immune responses, and apoptosis in liver (Wang et al. 2021). These findings underscored the liver's significance as one of the primary organs for the accumulation of Cu, leading to toxicity. Nonetheless, the combined effects of MPs and CuNPs on fish liver remains elusive. In the current study, our goal was to investigate the mRNA abundances of genes involved in the antioxidant response pathway in primary rainbow trout (*Oncorhynchus mykiss*) hepatocytes following acute exposure to MPs, CuNPs, and their mixture. In this study, we utilized a primary culture of rainbow trout to mitigate variability introduced by individual animals among treatments and to minimize the number of fish used in our experiments.

Materials and Methods

Animal Housing and Hepatocyte Culture Preparation

Rainbow trout ($n=4$, weight = 296 ± 7.4 (mean \pm SD)) were obtained from Sam Livingstone Fish Hatchery (Calgary, Alberta, Canada). Fish were transferred to the Aquatic Research Facility (ARF) of University of Lethbridge and were acclimated to the ARF water for two weeks (fish

holding tank capacity: 4 g/L; 16 h light: 8 h dark photoperiod; 12 °C). The ARF water quality was measured as follows (mean \pm SD; $n=3$): temperature, 12.3 ± 0.4 °C; dissolved oxygen, 8.9 ± 0.8 mg/L; conductivity, 331.1 ± 0.3 µS/cm; hardness, 155 ± 2.2 mg/L as CaCO₃; alkalinity, 130.1 ± 1.7 mg/L as CaCO₃; median pH, 8.2 (range 8.0–8.4); and dissolved organic carbon, 2.5 ± 0.6 mg/L. All experimental procedures were reviewed and approved by the University of Lethbridge Animal Welfare Committee (protocol # 1612). Fish were not fed 24 h prior to isolation of hepatocytes.

Hepatocytes were isolated according to a two-step collagenase perfusion protocol described elsewhere (Jamwal et al. 2016). In short, fish were euthanized by overdose of buffered tricaine methanesulfonate (AquaLife, Canada). Fish was cut open ventrally (from anus to pectoral girdle), the hepatic portal vein was cannulated with PE-50 (from the area of 8 cm away from the liver), and the liver was perfused with Hank's media (5.4 mM KCl, 137 mM NaCl, 0.81 mM MgSO₄·7H₂O, 0.44 mM KH₂PO₄, 0.33 mM Na₂HPO₄, 5.0 mM NaHCO₃, 5 mM Na-HEPES, 5 mM HEPES, pH was adjusted to 7.63). When the liver was entirely blanched, the perfusion solution was switched to Hank's medium containing 0.2 mg/L of collagenase (type IV, Roche LiveScience, USA). Once the liver was sufficiently degraded, it was removed from the body cavity and placed into a petri dish containing 2 ml of Hank's media and collagenase. To dissociate hepatocytes, the liver was chopped into small pieces with a razorblade and filtered through 260 and then 73 µm stainless steel mesh. To collect hepatocytes, the filtrate was centrifuged at 100 × g for 5 min at 4 °C. The hepatocyte pellet was then washed with a solution containing 2% bovine serum albumin and 1.5 mM CaCl₂ (adjusted pH of 7.63). The washed pellet was then resuspended in 25 ml of L-15 media (Fisher Scientific, Canada) containing antibiotic and antimycotic (ThermoFisher Scientific, Canada) and incubated for 30 min on an ice bath. After the incubation period, the supernatant was discarded, 25 ml of L-15 media (pH 7.63) was added to the cells, and cell viability was measured using a CytoSMART cell counter (Fisher Scientific, USA) using trypan blue exclusion assay (Jamwal et al. 2016). Hepatocytes with viability of > 85% were plated in a 6-well Nulcon delta plates (Fisher Scientific, Canada) at a density of $1.1\text{--}1.4 \times 10^6$ cells per well. To acquire a monolayer of hepatocytes, plates were incubated at 15.5 °C for 24 h in the dark.

Experimental Design and Hepatocyte Exposure

The median lethal concentration (48 h LC50) of CuNPs in rainbow trout was reported as 990 ± 150 µg/L (Song et al. 2015). In this study ~1/3 of the 48 h LC50 of CuNPs

(323 $\mu\text{g/L}$) was selected as the exposure concentration. Our previous study suggested 323 $\mu\text{g/L}$ of CuNPs were bioavailable for olfactory, gill, and intestinal epithelial cells and resulted in a significant Cu accumulation in these tissues (Lindh et al. 2019; Razmara et al. 2021). For the CuNPs exposure, a fresh stock suspension of CuNPs at the nominal concentration of 250 mg/L was prepared by suspending nanocopper powder (35 nm; 99.8% purity; Nanostructured & Amorphous Materials Inc, USA) in L-15 followed by a 15 min dispersion in water bath sonicator UD (150SH6LQ; 150 W; Eumax; USA). As the CuNP-induced toxicity is partly associated with released Cu^{2+} , the effect of CuNPs was compared to Cu^{2+} . A stock solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (> 98% purity; BHD, USA) was prepared at a concentration of 100 mg/L CuSO_4 in Milli-Q (EMD Millipore) and acidified to 0.4% HNO_3 (70%; TraceSelect grade, Sigma Aldrich, Canada). In the next step, a fresh solution of 1 mg/L CuSO_4 was prepared in L-15 and used as a source of Cu^{2+} . The nominal concentration of Cu^{2+} was 8 $\mu\text{g/L}$ which is equal to the average amount of dissolved Cu released from 320 $\mu\text{g/L}$ CuNPs over a 24 h period (Razmara et al. 2019). The hepatocytes were exposed to contaminants for 48 h (2 wells per biological replicates), and 90% of the exposure media was renewed at 24 h. The treatments were as follows: control (L-15 media only), CuNPs, dissolved Cu (Cu^{2+}), MP-620VF (MP1), MP-635XF (MP2), CuNPs + MP1, and CuNPs + MP2 ($n=4$).

The MP-620VF is reported by the manufacturer to be high density polyethylene with a mean particle size of 5–7 μm , a melting point of 114–116 $^\circ\text{C}$, density of 0.96 g/cc at 25 $^\circ\text{C}$, and an NPRI grind of 2.0–3.0 (MicroPowders Inc., USA). The MP-635XF characteristics are as follows: mean particle size: 4–6 μm , melting point: 123–125 $^\circ\text{C}$, density: 0.97 g/cc at 25 $^\circ\text{C}$, and NPRI Grind: 1.0–2.5 (MicroPowders Inc., USA). The MP-635XF has a greater fineness of grind, meaning that it has a greater surface area to volume ratio than MP-620VF. MPs were rinsed twice with 2 N HNO_3 (70%; TraceSelect grade, Sigma Aldrich, Canada), and subsequently rinsed three times with Milli-Q water before being dried in an oven at 60 $^\circ\text{C}$ to constant weight and resuspended in L-15 at a concentration of 2 mg/L.

Copper Analysis and CuNPs Characterizations

Total Cu concentrations in media were determined using graphite furnace atomic absorption spectrometry (GFAAS, Agilent Technologies) following the protocol described in Razmara et al. (2019). A certified reference material (CRM), SLRS-6 (National Research Council of Canada) was run every ten samples to evaluate accuracy, which was maintained above 90%. All samples and CRM were run in duplicate. The detection limit for Cu was previously estimated

utilizing this technique and found to be approximately 2 $\mu\text{g/L}$ Cu.

Nanocopper physicochemical properties were previously characterized (Razmara et al. 2019), and we used the same source of characterized CuNPs in this study. The average diameter of CuNPs was 32 ± 1 nm (mean \pm standard error of mean (SEM)). Polydispersity index (PDI), hydrodynamic diameter (HDD), and zeta potential, which are indicators of nanoparticles aggregation, showed the stability of CuNPs was reduced during the 24 h. The significant increase in the PDI and HDD along with the reduced zeta potential after 16 h revealed that CuNPs were less bioavailable over the last few hours of exposure (Razmara et al. 2019). The physicochemical parameters of CuNPs were initially assessed in fish tank water (Razmara et al. 2019). The L-15 media is a water-soluble solution with some properties akin to water. Nonetheless, it is possible that certain physicochemical attributes of CuNPs exhibit slight variations when suspended in L-15 rather than water.

Dissolution of CuNPs was measured through separating particulate Cu from the dissolved fraction using Amicon Ultra-4 centrifugal filter units (3 K regenerated cellulose membrane, Merck Millipore Ltd., USA). Total Cu concentration in the dissolved fraction was measured over a 24 h period using GFAAS (Razmara et al. 2019). The concentration of dissolved Cu was gradually increased over time and the averaged concentration of released Cu was 8 $\mu\text{g Cu/L}$ (Razmara et al. 2019).

Reverse Transcriptase Quantitative Polymerase Chain Reaction (qPCR) Analysis

Transcript abundance of oxidative stress regulating genes, including catalase (*CAT*), superoxide dismutase (*SOD*), glutathione peroxidase (*GPX*), glutathione-s-transferase (*GST*), and Na^+/K^+ ATPase alpha 1 subunit (*ATPIA1*) were determined following a protocol described in Razmara et al. (2021). Following exposures, hepatocytes (from 4 biological replicates) were collected from each well, flash-frozen in liquid nitrogen, and stored at -80 $^\circ\text{C}$ until further analysis. Total RNA was isolated using RNeasy Mini Kit (catalogue # 74,106; Qiagen). Purity and quantity of RNA samples were measured using a NanoDrop One spectrophotometer (Thermo Scientific), and all samples had high purity (> 1.8 and > 2 for 260/230 and 260/280 ratios). We used 1 μg of isolated RNA to synthesize cDNA using QuantiTect Reverse Transcription Kit, which contains a DNA elimination step (catalogue # 205,311, Qiagen). Characteristics of primers are described in Table S1 (Supplemental material 1). Reactions were performed in duplicate, and efficiency of reactions was determined using a serial dilution (5 \times) of a cDNA pool (made from pooling equal volumes from all

cDNA samples). The target gene transcripts were quantified using RT²SYBR Green qPCR master mix (catalogue # 330,503, Qiagen) and a Bio-Rad CFX96 Real-Time System (Bio-Rad). Denaturation of PCR mixtures were performed at 95 °C for 10 min, and it was followed by 40 amplification cycles (denaturation for 15 s at 95 °C and extension for 1 min at 62 °C). Using the Pfaffl method of relative quantification, the abundances of target gene transcripts were determined relative to the geometric means of reference gene transcripts (*ACTB* and *EF1a*) (Pfaffl 2001). The transcript abundance of reference genes did not significantly change among treatments.

Statistical Analysis

Statistical analysis was performed using R, version 3.6 (R Core Team 2019). Normality and homogeneity of variances of data were examined using a Shapiro-Wilks and a Bartlett test, respectively. Using an independent t-test, we compared the total concentration of Cu in the CuNPs and CuNPs + MPs exposure media to determine if CuNPs were adsorbed to MPs. To determine whether target genes were differentially expressed in response to different treatments, one-way analysis of variance (ANOVA) with Tukey's post hoc test were conducted on qPCR data.

Results

Copper Analysis

The total concentration of Cu in the CuNPs and CuNPs + MPs exposure suspensions was 232 ± 4 (mean \pm SEM; $n=3$) and 198 ± 11 ($n=4$) $\mu\text{g/L}$, respectively. The total Cu concentration in the Cu^{2+} solution was 8.0 ± 0.2 $\mu\text{g/L}$ ($n=3$). In the control and MPs treatments, the Cu concentration was below the instrument detection limit (2 $\mu\text{g/L}$ of Cu). The Cu concentration in the CuNPs + MPs suspension was significantly lower than CuNPs suspension ($p=0.03$). It is likely that some ionic and particulate Cu were adsorbed to the MPs, and consequently settled in the suspension containing MPs.

qPCR Analysis

In response to treatments, the transcript abundances of two oxidative stress indicators, *CAT* and *SOD*, were dysregulated in hepatocytes ($F_{CAT}(6, 21)=11.38$, $F_{SOD}(6, 21)=61.24$, $p<0.001$; Fig. 1A&B). The transcript abundances of *CAT* and *SOD* in the Cu containing treatments (i.e., Cu^{2+} , CuNPs, and CuNPs + MPs) was upregulated relative to the control or cells treated with MPs (Fig. 1A&B). The transcript

abundance of *ATP1A1*, which encodes a subunit of Na^+/K^+ ATPase pump, was also elevated in response to CuNP exposures, but not Cu^{2+} (Fig. 1F). Transcript abundances of *GPX* and *GST* did not significantly change among treatments ($p=0.775$; Fig. 1C&D). Our findings indicated that exposure to MPs alone did not have significant effects on the transcript abundances of target genes. Moreover, there were no significant differences in the gene transcript abundances in hepatocytes exposed to CuNPs or CuNPs + MPs.

Discussion

The potential interaction of nanoparticles and plastics in aquatic systems (Bhagat et al. 2021) prompted us to investigate the combined effect of CuNPs and MPs on the transcript abundance of genes involved in the oxidative response pathway. Previous studies showed that metal ions and nanoparticles can adhere to MPs (Ho and Leung 2021; Zhu et al. 2020). Depending on the properties of the surrounding medium and characteristics of microplastics, adsorption of contaminants to MPs may either reduce or increase toxicant bioavailability (Bhagat et al. 2021). In this study, although the Cu concentration in CuNPs + MPs suspension was relatively lower than CuNPs suspension, the co-exposure of MPs and CuNPs did not alter the effect of CuNPs on the target gene transcript abundances.

Recent studies have investigated the effect of combined exposure of Cu^{2+} and MPs on aquatic organisms; however, the influence of MPs on Cu^{2+} toxicity remains highly debated. Studies on zebrafish larvae have found the presence of MPs to not influence the toxicity of Cu^{2+} , as measured by levels of reactive oxygen species (Santos et al., 2020). Co-exposure of MPs and Cu^{2+} also did not change Cu^{2+} toxicity in the protozoan *Euglena gracilis* following a 96-h exposure period (Li et al. 2022). In snails, the presence of polystyrene MPs in a Cu^{2+} -containing solution did not exacerbate the Cu^{2+} -induced reproductive toxicity (Weber et al. 2021). To the contrary, many studies reported MPs having synergistic or antagonistic influence on the Cu^{2+} toxicity. In submerged microphytes, the presence of polyethylene MPs reduced the Cu^{2+} concentration in the CuSO_4 solution and decreased the Cu^{2+} -induced toxicity (Zhou et al. 2022). In another study, the combined exposure of MPs and Cu^{2+} resulted in a significant increase in Cu^{2+} accumulation and oxidative stress in goldfish (*Carassius auratus*) hepatopancreas relative to the Cu^{2+} -exposed treatment (Zhang et al. 2022). Following a 9-day exposure period, synergistic effects of MPs and Cu^{2+} were found in larvae exposed to high concentrations of Cu^{2+} (>270 $\mu\text{g/L}$), and antagonistic effects were observed in larvae exposed to low Cu^{2+} concentrations (Santos et al. 2022). These findings indicated the influence of MPs on

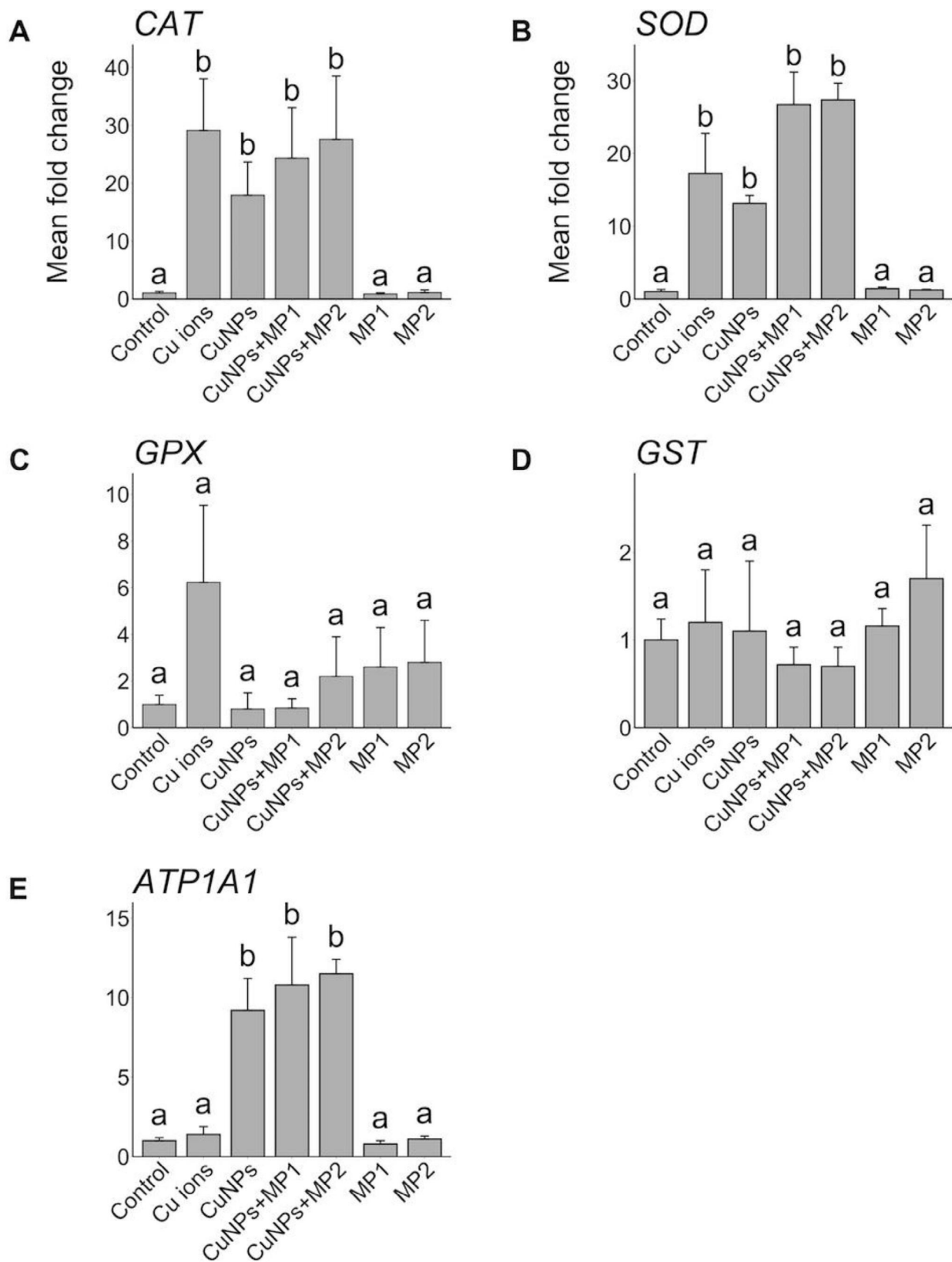


Fig. 1 Transcript abundances of target genes in rainbow trout hepatocytes following a 48 h exposure to Cu²⁺, CuNPs, CuNPs+MPs, or MPs. **(A)** Catalase (*CAT*), **(B)** superoxide dismutase (*SOD*), **(C)** glu-

tathione peroxidase (*GPX*), **(D)** glutathione-s-transferase (*GST*), **(E)** Na⁺/K⁺ ATPase alpha 1 subunit (*ATP1A1*). Lower-case letters indicate significant differences ($p \leq 0.05$, $n = 4$, error bars \pm SEM).

Cu²⁺-induced effects is associated with the type of MPs (e.g., polyethylene or polystyrene), size and concentration of MPs, concentration of Cu²⁺, exposure time and condition (e.g., water quality parameters), and organisms.

A limited number of studies have investigated the influence of MPs on toxicity of CuNPs to aquatic organisms. The combined effect of CuNPs and MPs was studied in microalgae *Skeletonema costatum* in which exposure to MPs or CuNPs alone resulted in growth inhibition, and co-exposure to MPs and CuNPs reduced the toxicity of CuNPs (Zhu et al. 2020). Authors argued the adsorption of dissolved Cu to the MPs and the aggregation between CuNPs and MPs were the primary reasons for the partial attenuation of toxicity of CuNPs in the presence of MPs (Zhu et al. 2020). A recent study evaluating the combined effect of CuNPs and polystyrene MPs on the transcriptome of zebrafish embryos reported that in the presence of CuNPs (980 µg/L), the toxicity-related pathway induced by 7 µm MPs was changed from cell cycle to hemostasis (Gao et al. 2021). Further, the cholesterol transport pathway was dysregulated in the fish exposed to MPs and CuNPs; however, oxidative stress pathway was not significantly affected in response to treatments (Gao et al. 2021).

Our findings also indicated that exposure to MPs alone did not cause changes in the abundances of transcripts of oxidative stress-related genes in rainbow trout hepatocytes. In agreement with our results, many previous studies reported that virgin MPs did not induce adverse effects in organisms (Beiras et al. 2018; Jovanovic et al. 2018; LeMoine et al. 2018; Pannetier et al. 2019a, b). In contrast, a 90-day dietary exposure of *Sparus aurata* to low-density polyethylene MPs (10% by weight; 200–500 µm) activated the antioxidant defense system and induced inflammatory responses in liver (Solomando et al. 2021). Locomotor activity of zebrafish was also inhibited by nanoplastics but not MPs (Chen et al. 2017). Thus, depending on the polymer composition, particle size and concentration, and exposure condition, plastic particles may induce toxic effects in aquatic animals.

Either form of Cu (Cu²⁺ and CuNPs) has been shown to induce oxidative stress in fish liver (Sanchez et al. 2005; Wang et al. 2021). Exposure to both Cu²⁺ and CuNPs resulted in a significant increase in the mRNA level of *CAT* and *SOD* (Fig. 1A&B). These results suggested the effect of CuNPs on the transcript abundance of *CAT* and *SOD* is probably associated with the dissolved ions released from CuNPs. In our previous study on rainbow trout olfactory mucosa, however, exposure to Cu²⁺ and CuNPs, did not induce lipid peroxidation or DNA fragmentation (Razmara et al. 2021). These findings suggested the Cu-induced effects are tissue-specific. It is also plausible that the oxidative stress may not have been sufficiently robust to cause oxidative

damage in the olfactory tissue. Expression of *CAT* and *SOD*, which are primary antioxidant enzymes, is increased in response to excessive concentrations of reactive oxygen species (ROS) (Lushchak 2016). Transcript abundance of *CAT* was increased in livers of zebrafish exposed to Cu²⁺ (15 µg/L) for 48 h (Craig et al. 2007). In contrast, activities of *CAT* and *SOD* and their mRNA abundances were reduced in hepatocyte primary culture of *Epinephelus coioides* after being exposed to 2400 µg/L of CuNPs or Cu²⁺ for 24 h (Wang et al. 2016). Exposure to high concentration of Cu and the consequent induction of oxidative stress (ROS concentration) in *E. coioides* hepatocytes resulted in impairment of the antioxidant defense system (Wang et al. 2016). Although greater abundances of *CAT* and *SOD* transcripts suggested that exposure to Cu²⁺ or CuNPs induced oxidative stress in rainbow trout hepatocytes, further investigation are required to measure the ROS and antioxidant enzyme activity. The Na⁺/K⁺ ATPase pump is an ion pump that maintains ion homeostasis in cell membrane and also plays a role in the generation of ROS (Liu et al. 2018). Oxidative stress has been suggested to inhibit the function of membrane enzymes such as Na⁺/K⁺ ATPase (Lopez-Lopez et al. 2011; Wang et al. 2003). Increased transcript abundance of *ATPIA1* in CuNP-treated hepatocytes is supported by a previous study indicating CuNPs can inhibit the activity of Na⁺/K⁺ ATPase in fish liver (Wang et al. 2019). As the transcript level of *ATPIA1* did not change in response to Cu²⁺ exposure, it is likely that *ATPIA1* expression regulation is sensitive to CuNPs.

Conclusion

This study provides insight into the combined impact of MPs and CuNPs on regulating oxidative stress in rainbow trout hepatocytes. Regardless of presence or absence of MPs, exposure to CuNPs increased transcript abundances of oxidative stress-related genes. Dissolved ions were probably the main driver of CuNP-induced effects on the oxidative stress-related genes. Exposure to virgin polyethylene MPs did not have a significant influence on the transcript level of target genes. Our findings suggested that while MPs adsorb Cu, the decrease in Cu concentration did not alter CuNP effect on gene transcripts. Further studies are needed to measure the oxidative stress and antioxidant responses in the CuNP- and CuNP+ MP-exposed hepatocytes.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00128-023-03811-8>.

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Declarations

Competing interests The authors declare no competing or financial interests.

References

- Akintelu SA, Folorunso AS, Folorunso FA, Oyebamiji AK (2020) Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. *Heliyon* 6(7):e04508. <https://doi.org/10.1016/j.heliyon.2020.e04508>
- Al-Bairuty GA, Shaw BJ, Handy RD, Henry TB (2013) Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 126:104–115. <https://doi.org/10.1016/j.aquatox.2012.10.005>
- Beiras R, Bellas J, Cachot J et al (2018) Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. *J Hazard Mater* 360:452–460
- Bhagat J, Nishimura N, Shimada Y (2021) Toxicological interactions of microplastics/nanoplastics and environmental contaminants: current knowledge and future perspectives. *J Hazard Mater* 405:123913. <https://doi.org/10.1016/j.jhazmat.2020.123913>
- Caruso G (2019) Microplastics as vectors of contaminants. *Mar Pollut Bull* 146:921–924. <https://doi.org/10.1016/j.marpolbul.2019.07.052>
- Chari N, Felix L, Davoodbasha M, Ali AS, Nooruddin T (2017) In vitro and in vivo antibiofilm effect of copper nanoparticles against aquaculture pathogens. *Biocatal Agric Biotechnol* 10:336–341
- Chen Q, Gundlach M, Yang S et al (2017) Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. *Sci Total Environ* 584–585:1022–1031. <https://doi.org/10.1016/j.scitotenv.2017.01.156>
- Collard F, Gilbert B, Compere P et al (2017) Microplastics in livers of european anchovies (*Engraulis encrasicolus*, L). *Environ Pollut* 229:1000–1005. <https://doi.org/10.1016/j.envpol.2017.07.089>
- Craig PM, Wood CM, McClelland GB (2007) Oxidative stress response and gene expression with acute copper exposure in zebrafish (*Danio rerio*). *Am J Physiology-Regulatory Integr Comp Physiol* 293(5):R1882–R1892
- Dlamini NG, Basson AK, Pullabhotla VSR (2019) Optimization and application of Biofloculant Passivated Copper Nanoparticles in the Wastewater Treatment. *Int J Environ Res Public Health* 16(12):2185. <https://doi.org/10.3390/ijerph16122185>
- Fleeger JW, Carman KR, Nisbet RM (2003) Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ* 317(1–3):207–233. [https://doi.org/10.1016/S0048-9697\(03\)00141-4](https://doi.org/10.1016/S0048-9697(03)00141-4)
- Foley CJ, Feiner ZS, Malinich TD, Hook TO (2018) A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Sci Total Environ* 631–632:550–559. <https://doi.org/10.1016/j.scitotenv.2018.03.046>
- Gao N, Huang ZH, Xing JN, Zhang SY, Hou J (2021) Impact and molecular mechanism of microplastics on zebrafish in the Presence and absence of copper nanoparticles. *Front Mar Sci* 8:1479 doi:ARTN 762530. <https://doi.org/10.3389/fmars.2021.762530>
- Godoy V, Blazquez G, Calero M, Quesada L, Martin-Lara MA (2019) The potential of microplastics as carriers of metals. *Environ Pollut* 255:113363. <https://doi.org/10.1016/j.envpol.2019.113363>
- Griffitt RJ, Hyndman K, Denslow ND, Barber DS (2009) Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. *Toxicol Sci* 107(2):404–415. <https://doi.org/10.1093/toxsci/kfn256>
- Ho WK, Leung KS (2021) The crucial role of heavy metals on the interaction of engineered nanoparticles with polystyrene microplastics. *Water Res* 201:117317. <https://doi.org/10.1016/j.watres.2021.117317>
- Jamwal A, Naderi M, Niyogi S (2016) An in vitro examination of selenium-cadmium antagonism using primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Metallomics* 8(2):218–227. <https://doi.org/10.1039/c5mt00232j>
- Jovanovic B, Gokdag K, Guven O, Emre Y, Whitley EM, Kideys AE (2018) Virgin microplastics are not causing imminent harm to fish after dietary exposure. *Mar Pollut Bull* 130:123–131. <https://doi.org/10.1016/j.marpolbul.2018.03.016>
- LeMoine CMR, Kelleher BM, Lagarde R, Northam C, Elebute OO, Cassone BJ (2018) Transcriptional effects of polyethylene microplastics ingestion in developing zebrafish (*Danio rerio*). *Environ Pollut* 243(Pt A):591–600. <https://doi.org/10.1016/j.envpol.2018.08.084>
- Lindh S, Razmara P, Bogart S, Pyle G (2019) Comparative tissue distribution and depuration characteristics of copper nanoparticles and soluble copper in rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 38(1):80–89. <https://doi.org/10.1002/etc.4282>
- Liu J, Lilly MN, Shapiro JI (2018) Targeting Na/K-ATPase signaling: a New Approach to control oxidative stress. *Curr Pharm Des* 24(3):359–364. <https://doi.org/10.2174/1381612824666180110101052>
- Li X, Wang Z, Bai M et al (2022) Effects of polystyrene microplastics on copper toxicity to the protozoan *Euglena gracilis*: emphasis on different evaluation methods, photosynthesis, and metal accumulation. *Environ Sci Pollut Res Int* 29(16):23461–23473. <https://doi.org/10.1007/s11356-021-17545-9>
- Lopez-Lopez E, Sedeno-Diaz JE, Soto C, Favari L (2011) Responses of antioxidant enzymes, lipid peroxidation, and Na⁺/K⁺-ATPase in liver of the fish *Goodea atripinnis* exposed to Lake Yuriria water. *Fish Physiol Biochem* 37(3):511–522. <https://doi.org/10.1007/s10695-010-9453-0>
- Lushchak VI (2016) Contaminant-induced oxidative stress in fish: a mechanistic approach. *Fish Physiol Biochem* 42(2):711–747. <https://doi.org/10.1007/s10695-015-0171-5>
- Lu Y, Zhang Y, Deng Y et al (2016) Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. *Environ Sci Technol* 50(7):4054–4060
- Ma H, Pu S, Liu S, Bai Y, Mandal S, Xing B (2020) Microplastics in aquatic environments: toxicity to trigger ecological consequences. *Environ Pollut* 261:114089. <https://doi.org/10.1016/j.envpol.2020.114089>
- Pannetier P, Cachot J, Clerandeanu C et al (2019a) Toxicity assessment of pollutants sorbed on environmental sample microplastics collected on beaches: part I-adverse effects on fish cell line. *Environ Pollut* 248:1088–1097. <https://doi.org/10.1016/j.envpol.2018.12.091>
- Pannetier P, Morin B, Clerandeanu C, Laurent J, Chapelle C, Cachot J (2019b) Toxicity assessment of pollutants sorbed on environmental microplastics collected on beaches: part II-adverse effects on japanese medaka early life stages. *Environ Pollut* 248:1098–1107. <https://doi.org/10.1016/j.envpol.2018.10.129>
- Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29(9):e45. <https://doi.org/10.1093/nar/29.9.e45>

- Radetić M, Marković D (2019) Nano-finishing of cellulose textile materials with copper and copper oxide nanoparticles. *Cellulose* 26(17):8971–8991
- Razmara P, Imbery JJ, Koide E et al (2021) Mechanism of copper nanoparticle toxicity in rainbow trout olfactory mucosa. *Environ Pollut* 284:117141. <https://doi.org/10.1016/j.envpol.2021.117141>
- Razmara P, Lari E, Mohaddes E, Zhang YY, Goss GG, Pyle GG (2019) The effect of copper nanoparticles on olfaction in rainbow trout (*Oncorhynchus mykiss*). *Environ Science-Nano* 6(7):2094–2104. <https://doi.org/10.1039/c9en00360f>
- R Core Team (2019) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria 2014. In: Team RC (ed)
- Sanchez W, Palluel O, Meunier L, Coquery M, Porcher JM, Ait-Aissa S (2005) Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. *Environ Toxicol Pharmacol* 19(1):177–183. <https://doi.org/10.1016/j.etap.2004.07.003>
- Santos D, Felix L, Luzzio A et al (2020) Toxicological effects induced on early life stages of zebrafish (*Danio rerio*) after an acute exposure to microplastics alone or co-exposed with copper. *Chemosphere* 261:127748. <https://doi.org/10.1016/j.chemosphere.2020.127748>
- Santos D, Perez M, Perez E et al (2022) Toxicity of microplastics and copper, alone or combined, in blackspot seabream (*Pagellus bogaraveo*) larvae. *Environ Toxicol Pharmacol* 91:103835. <https://doi.org/10.1016/j.etap.2022.103835>
- Solomando A, Capo X, Alomar C et al (2021) Assessment of the effect of long-term exposure to microplastics and depuration period in *Sparus aurata* Linnaeus, 1758: liver and blood biomarkers. *Sci Total Environ* 786:147479. <https://doi.org/10.1016/j.scitotenv.2021.147479>
- Song L, Vijver MG, Peijnenburg WJGM, Galloway TS, Tyler CR (2015) A comparative analysis on the in vivo toxicity of copper nanoparticles in three species of freshwater fish. *Chemosphere* 139:181–189. <https://doi.org/10.1016/j.chemosphere.2015.06.021>
- Umamaheswari S, Priyadarshinee S, Bhattacharjee M, Kadirvelu K, Ramesh M (2021) Exposure to polystyrene microplastics induced gene modulated biological responses in zebrafish (*Danio rerio*). *Chemosphere* 281:128592. <https://doi.org/10.1016/j.chemosphere.2020.128592>
- Wang T, Chen X, Long X, Liu Z, Yan S (2016) Copper nanoparticles and copper Sulphate Induced cytotoxicity in hepatocyte primary cultures of *Epinephelus coioides*. *PLoS ONE* 11(2):e0149484. <https://doi.org/10.1371/journal.pone.0149484>
- Wang T, Wei X, Sun Y et al (2021) Copper nanoparticles induce the formation of fatty liver in Takifugu fasciatus triggered by the PERK-EIF2 α -SREBP-1c pathway. *NanoImpact* 21:100280
- Wang T, Wen X, Hu Y, Zhang X, Wang D, Yin S (2019) Copper nanoparticles induced oxidation stress, cell apoptosis and immune response in the liver of juvenile *Takifugu fasciatus*. *Fish Shellfish Immunol* 84:648–655. <https://doi.org/10.1016/j.fsi.2018.10.053>
- Wang XQ, Xiao AY, Sheline C et al (2003) Apoptotic insults impair Na⁺, K⁺-ATPase activity as a mechanism of neuronal death mediated by concurrent ATP deficiency and oxidant stress. *J Cell Sci* 116(10):2099–2110. <https://doi.org/10.1242/jcs.00420>
- Weber A, von Randow M, Voigt AL et al (2021) Ingestion and toxicity of microplastics in the freshwater gastropod *Lymnaea stagnalis*: no microplastic-induced effects alone or in combination with copper. *Chemosphere* 263:128040. <https://doi.org/10.1016/j.chemosphere.2020.128040>
- Zhang C, Ye L, Wang C et al (2022) Toxic effect of combined exposure of Microplastics and Copper on Goldfish (*Carassius auratus*): insight from oxidative stress, inflammation, apoptosis and autophagy in Hepatopancreas and Intestine. *Bull Environ Contam Toxicol* 109(6):1029–1036. <https://doi.org/10.1007/s00128-022-03585-5>
- Zhou J, Liu X, Jiang H, Li X, Li W, Cao Y (2022) Antidote or trojan horse for submerged macrophytes: role of microplastics in copper toxicity in aquatic environments. *Water Res* 216:118354. <https://doi.org/10.1016/j.watres.2022.118354>
- Zhu X, Zhao W, Chen X, Zhao T, Tan L, Wang J (2020) Growth inhibition of the microalgae *Skeletonema costatum* under copper nanoparticles with microplastic exposure. *Mar Environ Res* 158:105005. <https://doi.org/10.1016/j.marenvres.2020.105005>

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