

Oxidative Stress Response to Zinc Oxide, Copper Oxide and Iron (III) Oxide in *Gammarus pulex*

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Abstract: Nanoparticles (NPs) are increasingly used across various fields of science and technology, including food science from crop harvesting to final food consumption. With their widespread application in daily life, the environmental effects of nanoparticles have become an important area of academic investigation. In this study, *Gammarus pulex* species was selected as research material/bioindicator species to investigate the effects of zinc oxide (ZnO), copper oxide (CuO), and iron(III) oxide (Fe₂O₃) on aquatic life.

To conduct the experiments, separate setups were established for each of the nanoparticles (ZnO, CuO, and α -Fe₂O₃). Following ten organisms for each concentration group (10, 20, and 40 ppm) with corresponding control groups, exposures were performed for 24 and 96 hours. Samples were collected at both time points and biochemical analyses were conducted using homogenization procedures. The results were evaluated statistically.

According to the findings, SOD activity increased significantly after exposure to ZnO, CuO, and Fe₂O₃. CAT activity did not show any change under ZnO NP exposure, whereas a decrease was observed under CuO and Fe₂O₃ NP exposures. However, a significant increase in TBARS levels and a significant increase in GSH levels were observed following nanoparticle exposure. Based on the study data, exposure to ZnO, CuO, and Fe₂O₃ appears to induce oxidative stress in *G. pulex*.

Keywords: *Gammarus pulex*, biomarker, zinc oxide, copper oxide, iron (III) oxide.

Gammarus pulex'te Çinko Oksit, Bakır Oksit ve Demir (III) Oksit'e Karşı Oksidatif Stres Tepkisi

Öz: Nanopartiküller (NP'ler), ürün hasadından nihai nihai gıda tüketimine kadar gıda bilimi de dahil olmak üzere çeşitli bilim ve teknoloji alanlarında giderek daha fazla kullanılmaktadır. Günlük yaşamdaki yaygın uygulamalarıyla nanopartiküllerin çevresel etkileri önemli bir akademik araştırma alanı haline gelmiştir. Bu çalışmada, çinko oksit (ZnO), bakır oksit (CuO) ve demir(III) oksit (Fe₂O₃)'ün sucul yaşam üzerindeki etkilerini araştırmak için *Gammarus pulex* türü araştırma materyali/biyolojik göstergesi olarak seçilmiştir.

Deneyleri gerçekleştirmek için, her bir nanopartikül (ZnO, CuO ve Fe₂O₃) için ayrı kurulumlar oluşturuldu. Her konsantrasyon grubu (10, 20 ve 40 ppm) için on organizma ve bunlara karşılık gelen kontrol grupları takip edilerek maruziyetler 24 ve 96 saat boyunca gerçekleştirildi. Her iki zaman noktasında da örnekler toplandı ve homojenizasyon prosedürleri kullanılarak biyokimyasal analizler gerçekleştirildi. Sonuçlar istatistiksel olarak değerlendirildi.

Bulgulara göre, SOD aktivitesi ZnO, CuO ve Fe₂O₃'e maruziyetten sonra önemli ölçüde arttı. CAT aktivitesi ZnO NP uygulamasında değişim göstermezken, CuO ve Fe₂O₃ NP uygulamalarında azalma gözlemlenmiştir. Ancak, nanopartikül maruziyetinin ardından TBARS ve GSH seviyelerinde önemli bir artış gözlemlendi. Çalışma verilerine dayanarak, ZnO, CuO ve Fe₂O₃ maruziyetinin *G. pulex*'te oksidatif stresi indüklediği söylenebilir.

Anahtar kelimeler: *Gammarus pulex*, biyobelirteç, çinko oksit, bakır oksit, demir (III) oksit.

1. Introduction

An increasing variety of industrial, agricultural, and other chemical pollutants are entering aquatic environments and accumulating in the tissues of both invertebrates and vertebrates (Bopp et al., 2008; Solé et al., 2000). These contaminants include persistent and emerging chemicals of concern such as polynuclear aromatic hydrocarbons, organochlorine pesticides, industrial products like hexachlorocyclohexanes (Walker & Livingstone, 2013), and heavy metal waste. Uptake can occur via sediments, suspended particulate matter, the water column, and dietary sources. The primary exposure route depends on

the dietary habits and ecological characteristics of the organisms (Serdar et al., 2024).

Iron oxide nanoparticles (Fe₂O₃ NPs) are used in many industrial and commercial fields such as solar energy conversion, water separation, and cement production (Gui et al., 2015). These nanoparticles can be released into the environment during production, transportation, use, and disposal, reaching various locations. Furthermore, their use in agricultural applications and phytoremediation studies has been reported to be rapidly increasing (Martínez-Fernández et al., 2016; Sebastian et al., 2017). This increase raises

concerns about the possibility of Fe₂O₃ NPs accumulating over time, particularly in agricultural ecosystems (Bao et al., 2019).

Copper oxide nanoparticles (CuO-NPs) are widely preferred in various fields due to their strong catalytic properties, suitable thermophysical structure, and significant antibacterial effects. Furthermore, their more economical nature compared to expensive metals like gold, platinum, and silver further increases their use (Ighalo et al., 2021; Saad et al., 2015; Khan et al., 2021). However, high production and consumption volumes, combined with inadequate waste management, lead to the transport of CuO-NPs into the environment via industrial and domestic wastewater, posing a global environmental risk. Aquatic ecosystems are often the final destinations for these pollutants (Bystrzejewska-Piotrowska et al., 2009). In aquatic environments, CuO-NPs can interact with each other to form larger particle aggregates. These aggregates can settle over time, accumulating in bottom sediments or adhering to the surfaces of aquatic organisms (Thit et al., 2015; Keller et al., 2018). Thus, CuO-NPs found in both the water column and sediment become accessible to organisms and can potentially cause toxic effects (Scola et al., 2021; Ghosh et al., 2024).

Zinc oxide nanoparticles (ZnO NPs) exhibit antimicrobial properties and are widely used in products such as sunscreens, cosmetics, coatings, pastes, and adhesives (Ali et al., 2012). Although zinc is an essential trace element for fish nutrition, excessive levels in aquatic environments can lead to significant toxicological effects. These include morphological alterations in gill structure, osmoregulatory dysfunctions, and liver damage in fish species (Giardina et al., 2009).

Metal-induced toxicity is often linked to the generation of ROS that can cause extensive oxidative damage to proteins, nucleic acids, and lipids and potentially result in cell death (Bai et al., 2003). The role of oxygen-derived free radicals and other ROS in disrupting cellular homeostasis referred to as oxidative stress has become a key area of interest in environmental toxicology (Serdar et al., 2025). The interplay between pro-oxidant factors (both endogenous and exogenous) and antioxidant defenses serves as an indicator of environmental stress, especially in the context of chemical pollutant exposure (Valavanidis et al., 2006). Superoxide dismutase (SOD) is the primary antioxidant enzyme that neutralizes superoxide radicals, while catalase (CAT) converts the resulting hydrogen peroxide into water and oxygen (Atli

& Canli, 2007; Elia et al., 2006; Lui et al., 2012; Eyckmans et al., 2011). Lipid peroxidation, typically measured by thiobarbituric acid reactive substances (TBARS), results in the accumulation of malondialdehyde that is a major end product of oxidized polyunsaturated fatty acids (Romeo et al., 2000). Reduced glutathione (GSH) functions as an electron donor in glutathione peroxidase reactions, converting peroxides into water and generating oxidized glutathione (GSSG). GSSG is subsequently reduced back to GSH using NADPH via glutathione reductase. The GSH/GSSG ratio is widely used as a biomarker of oxidative stress and cellular redox balance (Arslan & Güngördü, 2024; Sevgiler et al., 2007).

Gammarus pulex was selected as the research material for this study because amphipods are widely recognized as reliable bioindicators of environmental metal bioavailability, primarily due to their capacity to accumulate trace metals (Khan et al., 2011). Freshwater gammarids are aerobic organisms frequently used in ecotoxicological assessments of metal exposure because of their broad distribution, high population density, distinct sexual dimorphism, ease of collection, and sensitivity to a variety of toxicants (Felten et al., 2008; Alonso & De Lange, 2010). Moreover, they play key ecological roles in leaf litter decomposition, nutrient cycling, and freshwater food web dynamics (Kelly et al., 2002).

This study presents a unique approach aimed at revealing the biochemical responses elucidated by different nanoparticles and filling a gap in this field by comparatively investigating the oxidative stress effects of ZnO, CuO, and Fe₂O₃ nanoparticles on *Gammarus pulex*.

2. Materials and Method

2.1. Nanoparticles

The nanoparticle (NP) materials Fe₂O₃, CuO, and ZnO used in this study were purchased from SkySpring Nanomaterials Inc. Analytical-grade chemicals were used without any further purification. The shape and size specifications provided by the manufacturer were taken as reference for use in the bioassay experiments. Representative transmission electron microscopy (TEM) images of CuO (40 nm), ZnO (10–30 nm), and α-Fe₂O₃ nanoparticles (α-20–40 nm) are given in Figure 1. The DLS and zeta potential results (Table 1 and Figs. 2–3) provide information on nanoparticle size distribution and colloidal stability, while SEM images (Fig. 4) support morphological characterization.

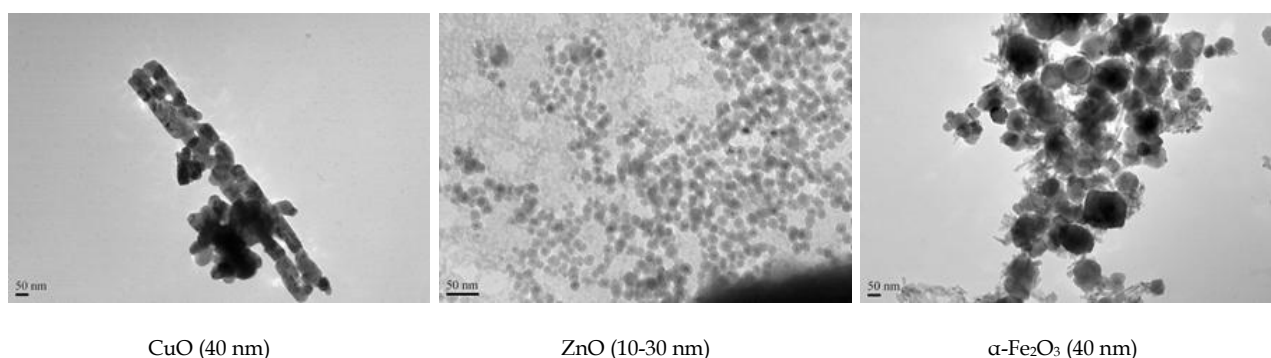


Figure 1. Representative transmission electron microscopy (TEM) images of CuO (40 nm), ZnO (10–30 nm), and α-Fe₂O₃ nanoparticles (α-20–40 nm).

Table 1. Physicochemical properties of ZnO, CuO, and α -Fe₂O₃ nanoparticles in aqueous medium, including hydrodynamic diameter (Z-average), polydispersity index (PDI), and zeta potential obtained from dynamic light scattering (DLS) measurements.

Nanopartiküller	DLS Boyut [Z-Average (d.nm)]	PdI*	Zeta Potansiyeli (mV)
CuO-NP'leri (40 nm)	404,1	0,418	+17,1
α -Fe ₂ O ₃ NP'leri (20-40 nm)	196,5	0,141	+32,1
ZnO-NP'leri (10-30 nm)	459,1	0,460	+20,2

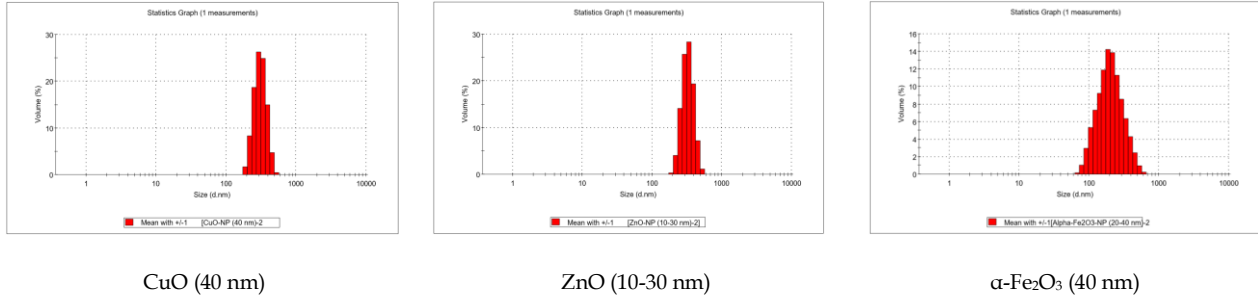


Figure 2. Particle size distribution profiles of ZnO, CuO, and α -Fe₂O₃ nanoparticles in aqueous medium obtained by dynamic light scattering (DLS) analysis.

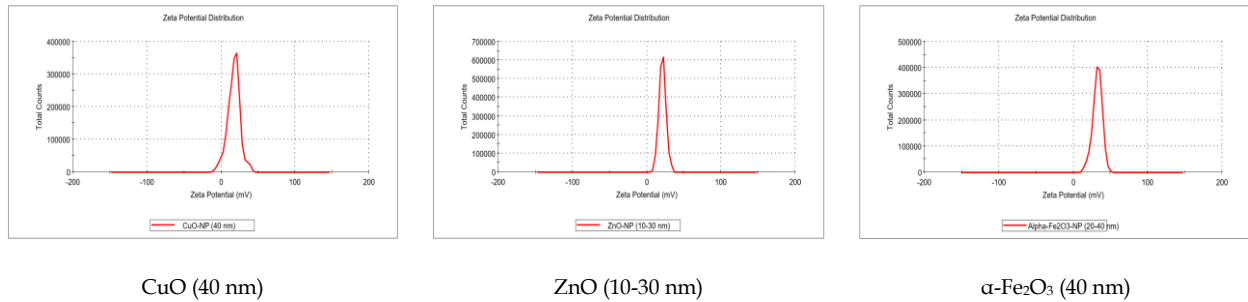


Figure 3. Zeta potential distribution of ZnO, CuO, and α -Fe₂O₃ nanoparticles in aqueous medium, indicating surface charge characteristics and colloidal stability.

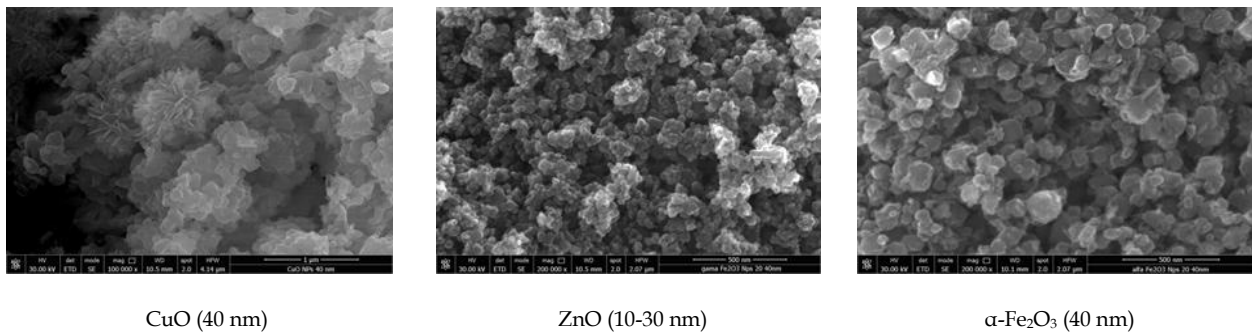


Figure 4. SEM micrographs of ZnO, CuO, and α -Fe₂O₃ nanoparticles illustrating particle morphology, surface structure, and aggregation behavior.

Comprehensive physicochemical characterization of ZnO, CuO, and Fe₂O₃ nanoparticles, including transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), and zeta potential analyses, has been previously conducted and reported by our research group. As the same nanoparticle materials obtained from the same supplier were used in the present study, their physicochemical properties are expected to remain consistent. These images were adapted from previous studies (Ates et al., 2019; Ates et al., 2020; Cimen et al., 2020) in which the same nanoparticles obtained from the

same supplier were comprehensively characterized using TEM, SEM, FTIR, and DLS analyses.

Thus, previously published data are referenced and partially presented here to support nanoparticle characterization. Representative images and analytical results (Figs. 1–4 and Table 1) are included to confirm particle size distribution, morphology, surface characteristics, and colloidal stability, thereby ensuring reproducibility and methodological consistency.

2.2. Organism Procurement and Adaptation

Gammarus pulex specimens used in this study were

collected using a bottom scoop from tributaries of the Munzur Stream in Tunceli Province, Türkiye and transported to the research laboratory at the Faculty of Fisheries, Munzur University. The organisms were placed in aquaria measuring 40 × 20 × 20 cm and acclimated to laboratory conditions for four weeks.

To ensure the proper acclimation of *G. pulex* to laboratory conditions, environments were created that mimicked their natural habitat. Sediments collected from their native habitat were rinsed with distilled water and placed in stock aquaria. Additionally, natural stream water was added to these tanks. Oxygenation was provided via an air pump and a 12:12 hour light-dark photoperiod was maintained. Water quality parameters were continuously monitored throughout the acclimation and exposure periods. The dissolved oxygen level was 9.33 ± 0.71 mg/L and the pH was 8.25 ± 0.37 , indicating well-oxygenated and moderately alkaline conditions. These environmental parameters were maintained within a stable range to ensure optimal living conditions for *Gammarus pulex* and to minimize potential confounding effects on the experimental outcomes. The temperature was regulated at 18°C using a thermostatic air conditioning system.

Once the acclimation setup was prepared, *G. pulex* individuals collected from Munzur Stream were introduced into the stock tanks and allowed to adapt. Approximately 70% of the aquarium water was replaced weekly. Willow tree leaves were collected and left to decompose, serving as a food source for the organisms. The animals fed solely on willow leaves.

2.3. Acute Toxicity Experiments and Trial Design

Lethal concentration 50 (LC₅₀) values were determined based on a literature review considering environmental pollutant exposure scenarios (Çiçek Çimen & Serdar, 2022).

Throughout the experiment, 0.5 L of non-chlorinated water obtained from the organisms' natural habitat was used in 1 L glass aquaria. No feeding was applied to any group, including the control group, during the exposure period. The study was conducted in triplicate, with each group designed to contain 10 individuals.

The experimental groups were as follows:

- Control 1: No ZnO exposure
- Group 2 (ZnO): 10 ppm ZnO
- Group 3 (ZnO): 20 ppm ZnO
- Group 4 (ZnO): 40 ppm ZnO
- Control 2: No CuO exposure
- Group 6 (CuO): 10 ppm CuO
- Group 7 (CuO): 20 ppm CuO
- Group 8 (CuO): 40 ppm CuO
- Control 3: No Fe₂O₃ exposure
- Group 10 (α-Fe₂O₃): 10 ppm Fe₂O₃
- Group 11 (α-Fe₂O₃): 20 ppm Fe₂O₃
- Group 12 (α-Fe₂O₃): 40 ppm Fe₂O₃

To evaluate nanoparticle elimination, organisms exposed for 96 hours were transferred to spring water collected from their natural habitat and held for an additional 24 hours. Elimination levels were subsequently assessed.

2.4. Biochemical analysis

Whole-body tissues of the organisms were collected after 24 and 96 hours of exposure. The samples were weighed and a 1:5 (w/v) ratio of phosphate-buffered saline (PBS) was added. Homogenization was performed on ice using a tissue homogenizer to prepare the samples for antioxidant parameter analysis (Serdar et al., 2024). The homogenates were centrifuged at 17,000 rpm for 15 minutes and the resulting supernatants were stored at -80°C until further analysis.

Superoxide dismutase (SOD), catalase (CAT) activities, thiobarbituric acid reactive substances (TBARS), and glutathione (GSH) levels were measured using ELISA kits (Cayman Chemical Company, USA) (Aydın & Serdar, 2025).

2.5. Statistical Analysis

Statistical analyses were conducted using SPSS version 24.0. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) was applied to evaluate differences among the biochemical parameters.

3. Results

3.1. SOD Activity

Changes in SOD activity in *G. pulex* exposed to ZnO, CuO, and Fe₂O₃ nanoparticles are presented in Figure 5. The increase in SOD activity in all treatment groups was found to be statistically significant ($p < 0.05$). After 24 hours, the highest increase in SOD activity was observed in the Fe₂O₃ group, whereas after 96 hours, the most pronounced effect was observed in the CuO group.

3.2. CAT Activity

Changes in CAT activity in *G. pulex* following exposure to ZnO, CuO, and Fe₂O₃ nanoparticles are shown in Figure 6. No statistically significant differences were observed in CAT activity in the ZnO treatment group. However, CAT activity significantly decreased in both CuO- and α-Fe₂O₃-exposed groups compared to their respective controls ($p < 0.05$).

3.3. TBARS Level

Figure 7 shows the changes in thiobarbituric acid reactive substances (TBARS) levels in *G. pulex* following exposure to ZnO, CuO, and α-Fe₂O₃ nanoparticles. TBARS levels increased significantly in all treatment groups compared to the controls ($p < 0.05$). The most pronounced increase was observed in the CuO group after 24 hours, whereas at 96 hours, the highest TBARS level was recorded in the α-Fe₂O₃ group.

3.4. GSH Level

Figure 8 shows the changes in GSH levels in *G. pulex* after exposure to ZnO, CuO, and α-Fe₂O₃ nanoparticles. GSH levels decreased significantly in all treatment groups compared to the control ($p < 0.05$) indicating oxidative stress in response to nanoparticle exposure.

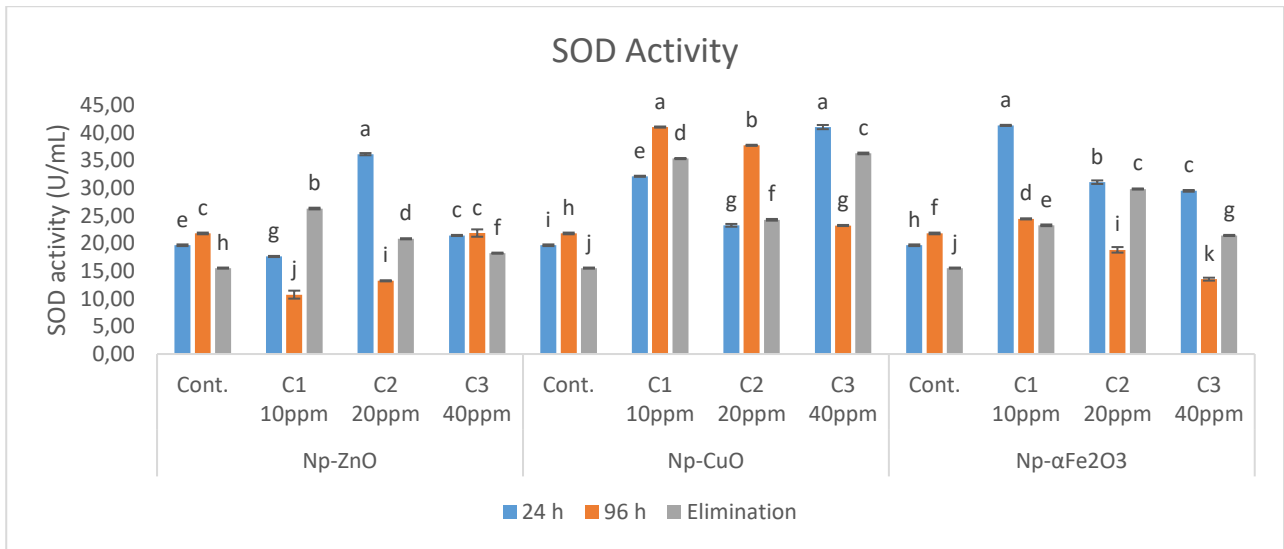


Figure 5: SOD activity (U/mL tissue) in *G. pulex* exposed to ZnO, CuO, and Fe₂O₃ nanoparticles. Different letters above the bars indicate statistically significant differences among treatments ($p < 0.05$), ($n = 9$ for each group).

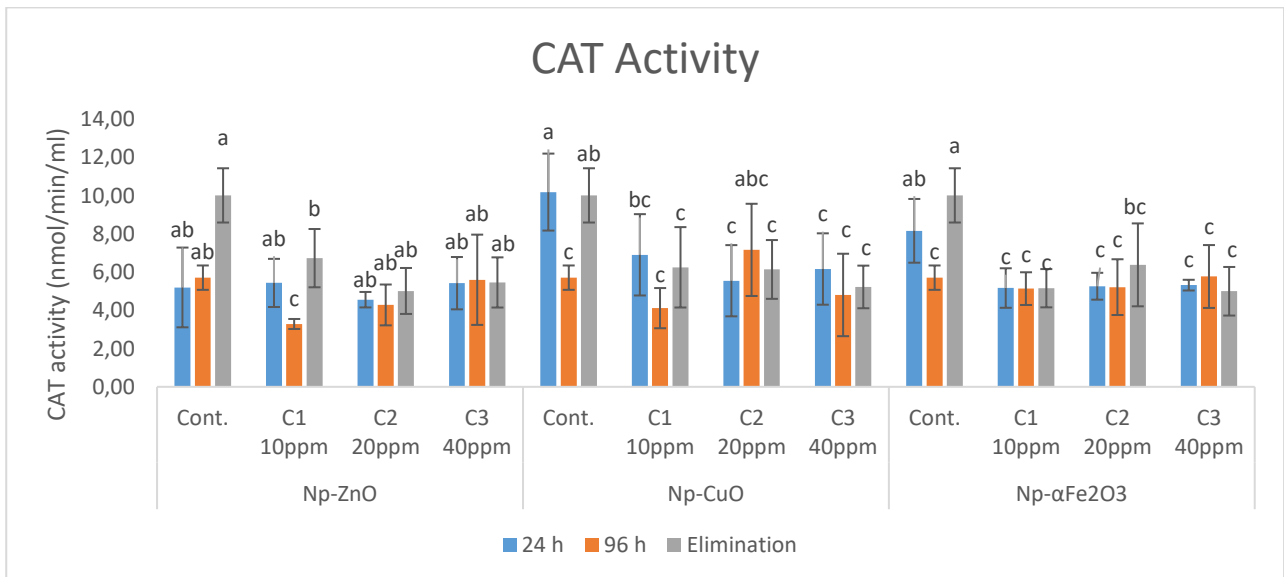


Figure 6: CAT activity (nmol/min/mL tissue) in *G. pulex* exposed to ZnO, CuO, and Fe₂O₃ nanoparticles. Different letters above the bars indicate statistically significant differences among groups ($p < 0.05$) ($n = 9$ for each group).

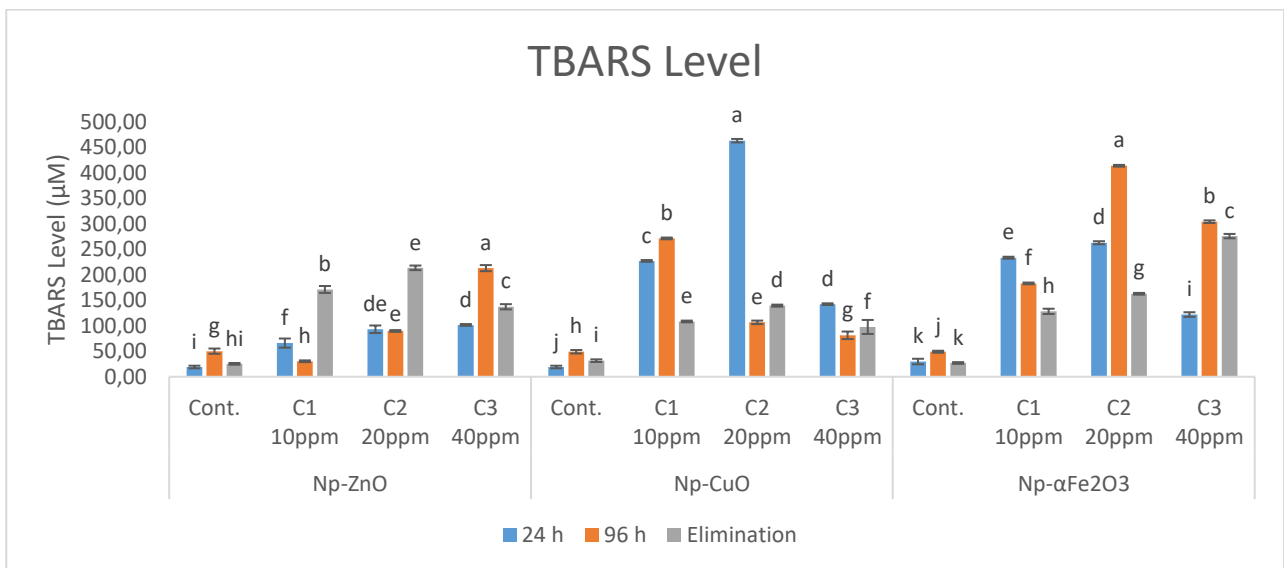


Figure 7: TBARS level (μM tissue) in *G. pulex* exposed to ZnO, CuO, and Fe₂O₃ nanoparticles. Different letters above the bars indicate statistically significant differences among groups ($p < 0.05$) ($n = 9$ for each group).

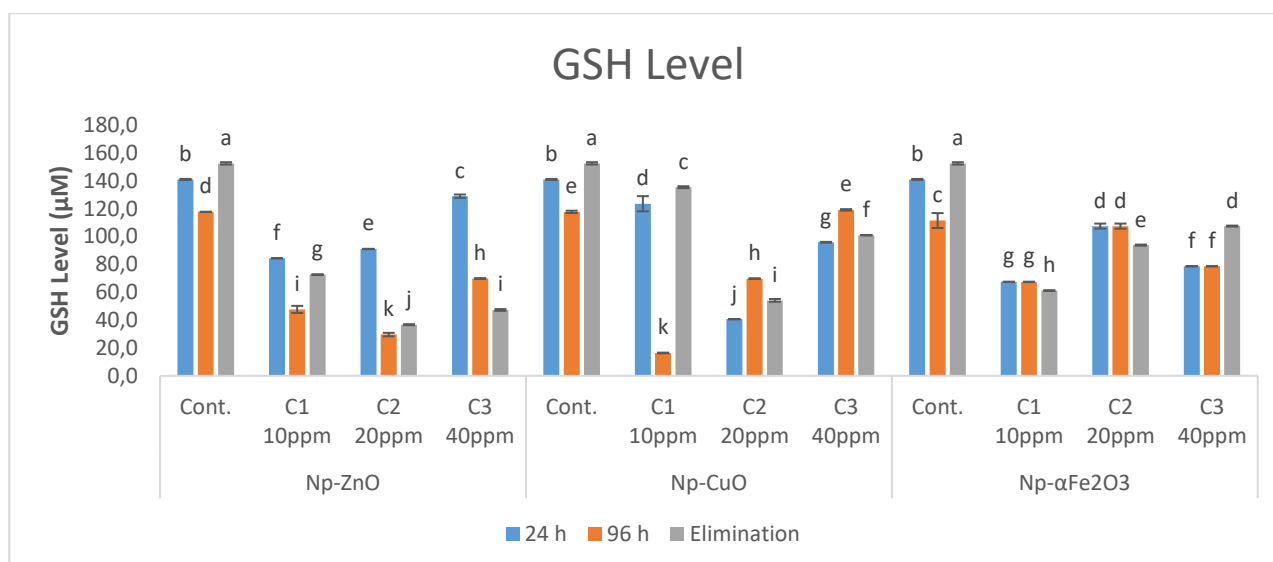


Figure 8: GSH level (μM tissue) in *G. pulex* exposed to ZnO, CuO, and Fe_2O_3 nanoparticles. Different letters above the bars indicate statistically significant differences among groups ($p < 0.05$) ($n=9$ for each group).

4. Discussion

Zn, Cu, and Fe are essential trace elements required for the normal functioning of living organisms. However, excessive accumulation of these metals in biological systems can have detrimental effects, potentially leading to cellular damage (Burke & Handy, 2005). To investigate such toxicological effects, various aquatic species are commonly used as model organisms in experimental studies.

SOD is a key antioxidant enzyme that catalyzes the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) (Ruas et al., 2008). CAT subsequently converts H_2O_2 into water and oxygen and its activity is often linked to SOD function (Cao et al., 2012). Together, these enzymes constitute the primary enzymatic defense system against oxidative stress (Asagba et al., 2008).

In the present study, a significant increase in SOD activity was observed in *G. pulex* following exposure to ZnO, CuO, and Fe_2O_3 nanoparticles. The observed changes in SOD activity are primarily due to the organism's defense response against reactive oxygen species (ROS). When nanoparticles such as ZnO, CuO, and Fe_2O_3 enter the cell or interact with the cell surface, they can disrupt electron transfer processes and increase the production of free radicals such as superoxide radical (O_2^-). These findings are consistent with previous studies: Ghazi et al. (2021) reported increased SOD activity in *Oreochromis niloticus* after ZnO exposure; Rajkumar et al. (2022) found similar results in *Cyprinus carpio*; and Koner et al. (2021) observed elevated SOD levels in *Clarias magur*. CuO exposure was also linked to increased SOD activity in *O. niloticus* (Abdel-Latif et al., 2021), *C. carpio* (Naeemi et al., 2020), and *Elodea nuttallii* (Regier et al., 2015). Regarding Fe_2O_3 , elevated SOD activity was noted in *Artemia salina* (Wang et al., 2017), *Chlorella sorokiniana* (Dehghanipour & Zamani, 2024), *Caridina fassarum* (Shahryari et al., 2025), and *A. salina* exposed to Fe_3O_4 (Zhu et al., 2017).

CAT is an antioxidant enzyme present in aerobic organisms that catalyzes the breakdown of hydrogen peroxide into water and oxygen, thereby preventing cellular oxidative damage (Chelikani et al., 2004). CAT

activity can vary depending on the type and intensity of pollutant exposure (Serdar et al., 2024).

In the current study, CAT activity in *G. pulex* showed no significant change following ZnO exposure. However, significant decreases were observed in CAT activity in the CuO and Fe_2O_3 treated groups. While CAT activity can generally increase defensively under low or moderate oxidative stress conditions, the decrease observed in this study, particularly with CuO and Fe_2O_3 exposure, can be explained by enzyme inhibition, structural damage, or suppression of the antioxidant system due to excessive ROS production. The lack of change with ZnO exposure can be attributed to the oxidative load not reaching a level that would affect the CAT system. Similar findings were reported in previous studies. Hao and Chen (2012) observed a reduction in CAT activity in *Cyprinus carpio* after ZnO exposure. Kaya et al. (2015) and Koner et al. (2019) noted fluctuations and decreases in CAT activity in *O. niloticus* and *Clarias magur*, respectively. Additional studies reported decreased CAT activity in *O. mossambicus* (Shahzad et al., 2019), *Tilapia zillii* (Saddick et al., 2017), and *Labeo rohita* (Aziz & Abdullah, 2023) under ZnO or CuO exposure.

CAT inhibition under CuO exposure was also demonstrated in *Biomphalaria alexandrina* (Fol et al., 2024), *C. carpio* (Noureen et al., 2018), *O. niloticus* (Tunçsoy et al., 2017), and *Epinephelus coioides* (Wang et al., 2015). Regarding Fe_2O_3 , decreased CAT activity was reported in *Nannochloropsis oculata* (Fazelian et al., 2023), *O. mykiss* (Ucar et al., 2022; Ucar et al., 2024), and *O. mykiss* again under Fe_3O_4 exposure (Özgür et al., 2018).

TBARS measurement is a commonly used indirect method to assess oxidative stress, reflecting the interaction between reactive oxygen species (ROS) and cellular lipid membranes (Serdar et al., 2021). In this study, exposure to ZnO, CuO, and Fe_2O_3 nanoparticles led to significant increases in TBARS levels in *G. pulex* which indicates enhanced lipid peroxidation. Changes in TBARS levels are primarily due to increased lipid peroxidation resulting from increased ROS. Nanoparticle exposure damages and breaks down polyunsaturated fatty acids in the cell

membrane, a process reflected in increased TBARS levels. Therefore, an increase in TBARS is an indicator of increased oxidative damage and impaired membrane integrity within the cell.

These results are supported by previous studies. For instance, Ali et al. (2012) observed increased TBARS levels in *Lymnaea luteola* after ZnO exposure. Similarly, Kaya et al. (2015) and Koner et al. (2019) reported elevated malondialdehyde (MDA, a TBARS indicator) levels in *O. niloticus* and *Clarias magur*, respectively. CuO and ZnO nanoparticles were also shown to raise TBARS levels in *Daphnia magna* (Mwaanga et al., 2014). However, some contrasting findings exist; for example, Aziz and Abdullah (2023) reported decreased TBARS levels in *Labeo rohita* under CuO exposure.

Additional studies demonstrated increased TBARS or MDA levels in *C. carpio* (Noureen et al., 2018), *Artemia salina* (Cimen et al., 2020), and *O. niloticus* (Badran & Hamed, 2024) under CuO or Cu exposure. Similar oxidative responses were reported in *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Daphnia magna* (Janani et al., 2020). With respect to iron-based nanoparticles, increased TBARS levels were observed in *Nannochloropsis oculata* (Fazelian et al., 2023), *A. salina* (Wang et al., 2017), and juvenile yellow catfish (Wei et al., 2025). In *O. mossambicus*, Fe₂O₃ exposure led to increased lipid peroxidation (Balakrishnan et al., 2024) and similar findings were reported for *Leuciscus cephalus* exposed to iron and multiple elements (Hermenean et al., 2015).

GSH is a key non-enzymatic antioxidant that plays a central role in cellular defense by neutralizing free radicals and peroxides, thereby protecting cells from oxidative damage (Serdar, 2019). In this study, GSH levels were significantly decreased in *G. pulex* following exposure to ZnO, CuO, and Fe₂O₃ nanoparticles that indicates oxidative stress.

These results are consistent with previous studies. For example, Ali et al. (2012) reported reduced GSH levels in *Lymnaea luteola* exposed to ZnO. Fluctuations in GSH levels were also noted in *O. niloticus* by Kaya et al. (2015) and similar reductions were observed in *C. vulgaris* (Suman et al., 2015) and the gill tissue of *Wallago attu* (Dubey et al., 2015) under ZnO and TiO₂ NP exposure.

CuO-induced decreases in GSH levels were documented in *O. niloticus* (Badran & Hamed, 2024), *Epinephelus coioides* (Wang et al., 2015), *Mugil cephalus* (Akbar et al., 2018), *Biomphalaria alexandrina* (Fol et al., 2024), *C. carpio* (Noureen et al., 2018), and *Danio rerio* (Batista-Gallep et al., 2018).

Fe-based nanoparticle effects on GSH depletion were exhibited in *Prochilodus lineatus* exposed to metal mixtures (de Oliveira et al., 2018), *Oryzias latipes* (Li et al., 2009), *Channa punctatus* (Javed et al., 2016; 2017), *Notopterus notopterus* (Mohanty & Samanta, 2018), and *Leuciscus cephalus* (Hermenean et al., 2015).

5. Conclusion

The increasing use of NPs raises significant concerns regarding environmental contamination, as these particles can be released into aquatic ecosystems and exert toxic effects on non-target organisms. The findings of this study

demonstrate that ZnO, CuO, and Fe₂O₃ nanoparticles induce oxidative stress in *Gammarus pulex* by altering both enzymatic (SOD, CAT) and non-enzymatic (GSH, TBARS) biomarkers.

Given that *G. pulex* is a recognized bioindicator species used in ecotoxicological assessments particularly in clean water environments, these results suggest that all three tested nanoparticles possess considerable ecotoxic potential and may contribute to aquatic pollution and ecosystem disruption.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The author declares that there is no conflict of interest.

Author Contributions: Conception – O.S., I.C.Ç.Ç.; Design – O.S., A.N.A.; Supervision – O.S., I.C.Ç.Ç., A.N.A.; Fund – O.S.; Materials – O.S., I.C.Ç.Ç., A.N.A.; Data Collection or Processing – O.S., I.C.Ç.Ç., A.N.A.; Analysis Interpretation – O.S., A.N.A.; Literature Review – A.N.A.; Writing – A.N.A.; Critical Review – O.S., A.N.A.

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