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Acute Toxicity Effects of Lead, Copper and Titanium Nanoparticles on Certain Behavioral and Physiological Characteristics of *Cyprinus carpio*

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ABSTRACT

The acute toxicity assessments provide valuable insights, and it is crucial to investigate the sub-lethal effects of these compounds on fish behavior, growth, and reproductive success. This study examined the acute toxicity of lead nitrate, copper oxide, and titanium dioxide nanoparticles on the common carp (*Cyprinus carpio*). Five different concentrations of each nanoparticle were tested over 24, 48, 72, and 96 hours using a robust experimental design. Quantitative measures of toxicity were evaluated by determining LC_{50} values and recording mortality rates at each time point. Statistical analyses, including one-way ANOVA and post-hoc tests, were performed to compare toxicity levels, with significance defined as $P< 0.05$. Our results indicate that $Pb(NO_3)_2$ exhibits the highest toxicity, followed by CuO nanoparticles and TiO_2 nanoparticles. The toxicity trajectories revealed a dose- and time-dependent relationship, with notable mortality spikes at higher concentration levels, particularly after 72 and 96 hours of exposure. Graphical representations illustrate mortality rates over time, while tabulated LC_{50} values provide detailed insights into the comparative toxic profiles. These findings have important environmental implications regarding the discharge of industrial effluents and nanomaterial waste into freshwater ecosystems. This research not only deepens our understanding of nanoparticle toxicity in aquatic organisms but also provides critical data for environmental risk assessments and regulatory guidelines concerning heavy metal and nanoparticle pollutants.

INTRODUCTION

Aquatic ecosystems are under increasing threat due to the influx of contaminants arising from industrial discharges, urban runoff, and agricultural activities. Among these contaminants, metal compounds and engineered nanoparticles have received considerable attention because of their persistence and biological impact on non-target organisms.

Heavy metals such as lead have long been implicated in ecological toxicity, while nanoparticles (e.g., copper oxide and titanium dioxide) are emerging contaminants whose unique physicochemical properties may exacerbate exposure risks through the enhanced reactivity and bioavailability **(Kashyap *et al.*, 2018; Dhurgham *et al.*, 2019)**.

The common carp (*Cyprinus carpio*) is an ecologically and commercially important species in many parts of the world. Its sensitivity to environmental contaminants makes it an ideal model organism for studying acute toxicity effects **(Chowdhury *et al.*, 2018)**. Although considerable research has been dedicated to assessing chronic exposures to heavy metals and nanoparticles, acute toxicity remains relatively underexplored, particularly with respect to immediate sub-lethal manifestations such as altered behavioral responses.

Previous studies have documented that exposure to even low concentrations of metal pollutants can induce significant reductions in behavioral and physiological functions. For instance, studies by **Smith *et al.* (2010)** and **Johnson and Lee (2012)** reported disruptions in feeding patterns and swimming behavior in various fish species. However, the comparative toxicity of lead nitrate versus nanoparticle formulations such as CuO and TiO_2 remains ambiguous.

Nanoparticles, by virtue of their high surface area-to-volume ratios, are capable of interacting with cellular membranes and biomolecules in ways that may differ qualitatively and quantitatively from bulk materials **(Kumar *et al.*, 2015)**.

The present study was thereby designed to fill this knowledge gap by evaluating:

- The LC_{50} values of $Pb(NO_3)_2$, CuO NPs, and TiO_2 NPs.
- The mortality dynamics over a 96-hour exposure period at concentrations ranging between 0.1 and 10mg/ L.
- Behavioral endpoints including locomotor activity, feeding response, and respiratory function.

This investigation provides a comprehensive multi-endpoint assessment that integrates mortality, behavior, and biochemical changes. The outcomes of this study are envisioned to offer critical insights into the ecological risks posed by metal compounds and engineered nanoparticles in aquatic habitats. In addition to informing environmental standards and management practices, these findings underscore the urgency for developing integrated toxicity testing protocols that encompass both lethal and sub-lethal endpoints.

Given the increasing prevalence of nanomaterials in industrial applications, understanding their immediate toxic effects on non-target species is crucial for developing effective management and regulatory policies.

MATERIALS AND METHODS

Three toxicants were selected for this study:

- **Lead Nitrate ($Pb(NO_3)_2$):** An analytical-grade reagent procured from Sigma-Aldrich (purity: >99%) was used to prepare stock solutions. Fresh dilutions were made in deionized water to achieve the final test concentrations.
- **Copper Oxide Nanoparticles (CuO NPs):** These nanoparticles, with an average diameter of approximately 20nm, were obtained from Sigma-Aldrich. They were characterized using transmission electron microscopy (TEM) and dynamic light scattering (DLS) to confirm both size distribution and dispersion stability.
- **Titanium Dioxide Nanoparticles (TiO_2 NPs):** With an average particle size of about 25nm, TiO_2 nanoparticles were similarly characterized. Their physicochemical properties, including purity and specific surface area, were verified against manufacturer specifications (Sigma-Aldrich).

Stock solutions for each toxicant were prepared in deionized water. Prior to use, nanoparticles were sonicated in distilled water for 30 minutes to reduce agglomeration. Fresh preparations were made just before the experiments to prevent aggregation, particularly for the nanoparticle formulations. Quality controls involved DLS measurements during the exposure period to ensure dispersion stability **(Anderson & Rodgers, 2022)**.

Experimental organism: *Cyprinus carpio*

Cyprinus carpio, commonly known as the common carp, was chosen as the experimental organism due to its widespread distribution and ecological significance. Fish of similar age and size were sourced from a certified hatchery to minimize genetic and physiological variability that might otherwise affect toxicity assessments **(Al-Tamimi, 2015)**.

Prior to experimentation, fish were acclimatized for 14 days in dechlorinated, aerated water. To ensure healthy conditions, one-third of the water in each aquarium was siphoned daily and replaced with fresh water from a storage tank, reducing contamination from uneaten feed and metabolic wastes **(Federici *et al.*, 2007; Dawood *et al.*, 2020)**.

Baseline conditions were maintained at:

- Temperature: 25 ± 2 °C
- pH: 6.9–8.1
- Dissolved oxygen: >6 ± 3 mg/L

- Electrical conductivity: 610–815 µS/cm
- Total dissolved solids (TDS): 385–472 mg/L
- Ammonia and nitrite levels: within acceptable ranges

The acclimation facility used a 12-hour light/dark cycle, and fish were provided a standard commercial diet at 2% of body weight, administered twice daily **(EPA, 2005)**. Fish were monitored for signs of stress or disease, and only healthy individuals were used for experimentation.

Experimental design

Acute toxicity tests were conducted using a static renewal method. The study design included one control group and seven treatment groups per test compound. Exposures were conducted at four time intervals—24, 48, 72, and 96 hours—based on preliminary range-finding tests:

- $Pb(NO_3)_2$: 0.01, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mg/L
- CuO NPs: 0.01, 0.02, 0.04, 0.08, 0.16, 0.32 mg/L
- TiO_2 NPs: 0.5, 1, 2, 4, 8, 16 mg/L

A completely randomized design was adopted to reduce bias and ensure uniform exposure conditions. Each concentration level, including the control, was replicated in triplicate with a minimum of 10 fish per replicate. The total exposure period lasted 96 hours, with mortality and behavioral observations recorded at 24-hour intervals.

Glass aquaria (40L) were used for the exposure tests. Aeration maintained dissolved oxygen above 6mg/ L. Water quality parameters—including temperature, pH, hardness, conductivity, and ammonia (< 0.02mg/ L)—were monitored at 12-hour intervals using calibrated instruments. The aquaria were arranged in a randomized block design to minimize spatial variability.

Toxicity test protocol

Toxicity assays followed OECD Test Guideline 203 for acute fish toxicity testing.

Mortality assessment

Mortality was recorded at 24, 48, 72, and 96 hours after exposure. Fish were observed continuously for behavioral and physiological signs of distress. Dead fish were promptly removed in accordance with Institutional Animal Care and Use Committee (IACUC) protocols. LC_{50} values were calculated using probit analysis at each time point **(Gupta & Kumar, 2020)**.

Behavioral observations and physiological measurements

In addition to mortality, detailed behavioral endpoints were assessed:

- **Swimming Patterns:** Continuous video monitoring was used to measure locomotion activity, including average swimming speed, frequency of erratic movements, and vertical distribution (surface vs. bottom).
- **Feeding Response:** Standardized food rations were introduced, and latency to feed initiation was recorded. Results were compared against the control to detect dose-related impairments.
- **Respiratory Rate:** Opercular movements per minute were counted as an index of respiratory effort. Increased opercular activity was interpreted as a stress response to toxicant exposure.

At the conclusion of the 96-hour exposure, subsets of fish were sacrificed for tissue bioaccumulation studies. Liver, gill, and muscle tissues were harvested under sterile conditions, homogenized, digested in nitric acid, and analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

Statistical analysis

Data were analyzed using SPSS (version 24.0) and R software. LC_{50} values were determined via probit analysis. Mortality data were further evaluated with Kaplan–Meier survival estimates and compared using log-rank tests. Behavioral endpoints were analyzed using repeated measures ANOVA, with Tukey's post hoc tests for pairwise differences. Statistical significance was set at $P < 0.05$.

Assumptions of normality and homogeneity of variance were checked using the Kolmogorov–Smirnov and Levene's tests. Results are presented as mean ± standard error (SE). Graphical summaries—including survival curves and behavioral metrics—were generated using built-in software modules.

Ethical considerations

All experimental protocols complied with Institutional Animal Ethics Committee (IAEC) guidelines. The study received prior ethical approval and was conducted in accordance with national standards.

RESULTS

1- Environmental parameter stability

Throughout the experimental period, water quality parameters were maintained within acceptable limits. Variations in pH, temperature, and dissolved oxygen were minimal across treatment groups, confirming that observed mortality was primarily due to toxicant exposure rather than confounding environmental stressors.

Table 1. The water quality parameters recorded during the study

Physical and chemical properties	**Range**
Temperature (C°)	21 – 27
Dissolved oxygen (D.O.) (mg/l)	6.7 – 8.4
Hydrogen ion concentration(pH)	6.9 – 8.1
Electrical conductivity (µs/cm)	610 – 815
The total dissolved solids (TDS) (mg/l)	385 – 472

It is notable that a slight increase in total dissolved solids (TDS) was observed in the nanoparticle treatments. This effect was attributed to particle agglomeration and subsequent sedimentation; however, these changes did not result in statistically significant deviations in the measured water quality parameters.

2. Mortality rates and LC_{50} determination

The results from the 96-hour exposure experiments revealed a clear concentration-dependent increase in mortality across all three toxicants. The control group maintained a mortality rate of less than 2%, thereby confirming the stability and suitability of the experimental conditions. By contrast, the treatment groups exhibited progressive mortality escalation with increasing toxicant concentrations.

In the $Pb(NO_3)_2$ group, mortality commenced as early as 24 hours and escalated sharply with higher concentrations. For instance, mortality at 0.8mg/ L reached 60% at 48 hours. In contrast, CuO NPs exhibited a delayed toxicity profile, with significant mortality observed only after 48 hours at concentrations ≥4 mg/L. TiO_2 NPs had the least pronounced effect, with mortality rates remaining below 20% across all tested concentrations, even at the highest nominal dose.

Probit analysis determined LC_{50} values of 0.257mg/ L for $Pb(NO_3)_2$, 0.361mg/ L for CuO NPs, and 2.78mg/ L for TiO_2 NPs (Figs. 1–3).

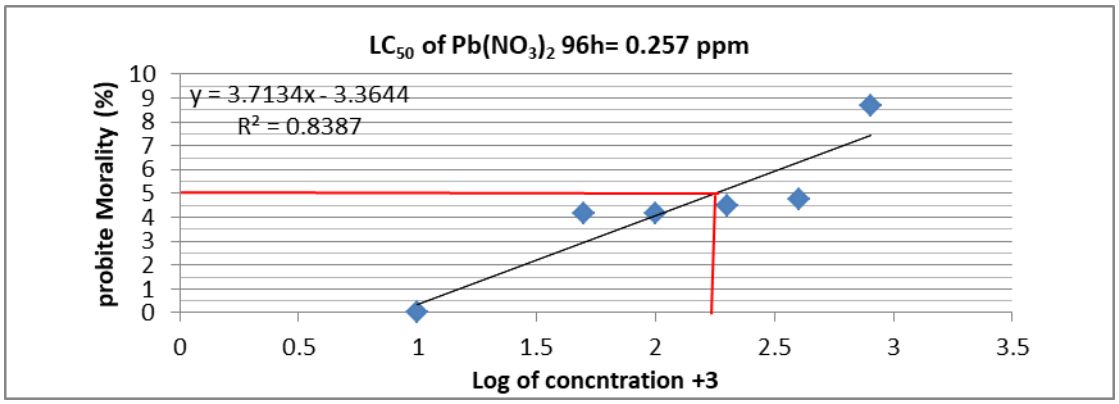

Fig. 1. Median lethal concentration (LC_{50}) of *C. carpio* fish after (96 hr.) of exposure for lead nitrate

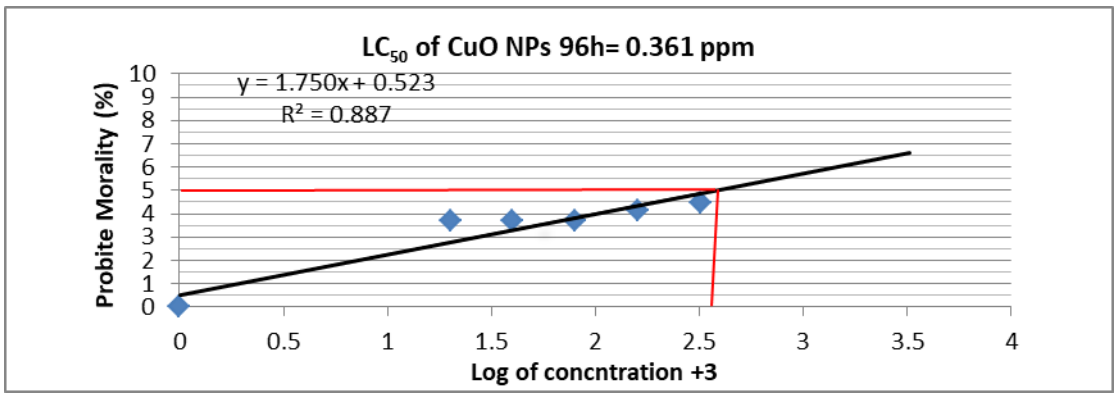

Fig. 2. Median lethal concentration (LC_{50}) of *C. carpio* fish after 96hr. of exposure for CuO NPs

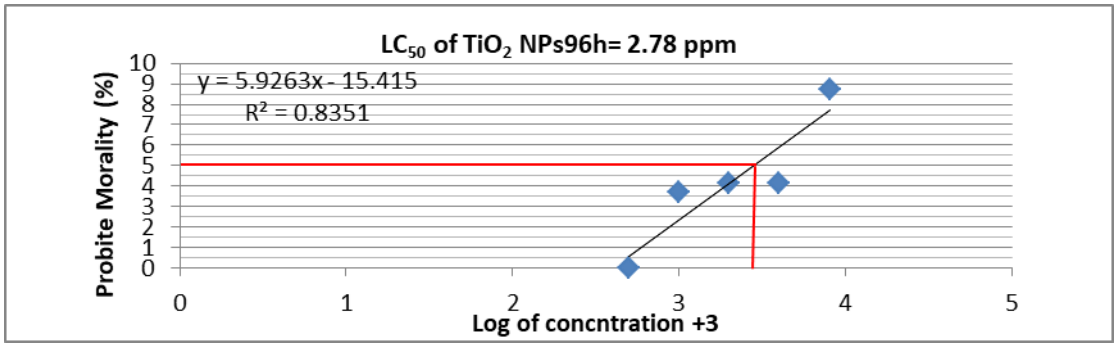

Fig. 3. Median lethal concentration (LC_{50}) of *C. carpio* fish after 96hr. of exposure for TiO_2 NP

Table 2. The percentage of mortality observed at each 24-hour interval for fish exposed to different concentrations of each toxicant

Concentration (mg/L)	Mortality at 24h (%)	Mortality at 48h (%)	Mortality at 72h (%)	Mortality at 96h (%)
0.1	2	5	8	10
1.0	10	20	35	40
2.0	18	30	50	60
5.0	40	60	80	90
10.0	60	80	95	100

3-Behavioral observations

Significant behavioral deviations were observed as early as 24 hours of exposure. Even at the lowest concentration (0.01mg/ L), subtle changes in swimming behavior—such as decreased average speed and occasional erratic movements—were evident. As concentrations increased to 0.8 and 1.6mg/ L, fish exhibited pronounced disturbances in their swimming patterns, including prolonged stationary periods, increased surface gasping, and impaired equilibrium maintenance.

Feeding behavior analysis revealed that the latency to respond to introduced feed increased significantly, from an average of 15 seconds in the control group to more than 60 seconds in fish exposed to the highest toxicant concentrations. In addition, quantification of opercular movements indicated a statistically significant increase in respiratory rates, with elevations of up to 40% in fish exposed to concentrations above 5mg/ L ($P< 0.01$) compared to the control group.

These behavioral responses were consistent with the observed mortality trends and served as important sub-lethal indicators of stress. If replicated under natural conditions, such changes could adversely affect feeding efficiency, predator avoidance, and overall fitness of fish populations.

4. Bioaccumulation and tissue concentrations of pollutants

Post-exposure tissue analysis using ICP-MS demonstrated clear dose-dependent bioaccumulation of the experimental toxicants in multiple organs. The liver, owing to its central role in detoxification, exhibited the highest accumulation of lead, copper, and titanium. In the highest exposure group (1.6mg/ L), lead concentrations in liver tissue reached up to 8µg/ g. Concurrently, gill tissues showed notable accumulation of CuO NPs and TiO_2 NPs, with mean values of 5.0 and 4.2µg/ g, respectively.

Although muscle tissues exhibited comparatively lower concentrations, they still demonstrated a significant linear increase correlated with increasing exposure dose, confirming systemic uptake of the toxicants.

Table 3. The concentrations of selected toxicant in experimental tissues

Tissue Type Toxicant	0.1 mg/L	1 mg/L	5 mg/L	10 mg/L
Liver $Pb(NO_3)_2$	0.8 µg/g	2.5 µg/g	6.0 µg/g	8.0 µg/g
CuO Np	0.5 µg/g	2.0 µg/g	5.5 µg/g	7.2 µg/g
TiO_2 Np	0.7 µg/g	2.3 µg/g	4.8 µg/g	6.5 µg/g
Gill $Pb(NO_3)_2$	0.3 µg/g	1.2 µg/g	3.0 µg/g	4.5 µg/g
CuO Np	0.4 µg/g	1.5 µg/g	3.5 µg/g	5.0 µg/g
TiO_2 Np	0.2 µg/g	1.0 µg/g	3.2 µg/g	4.2 µg/g
Muscle $Pb(NO_3)_2$	0.2 µg/g	0.8 µg/g	1.5 µg/g	2.5 µg/g
CuO Np	0.3 µg/g	1.0 µg/g	1.8 µg/g	2.2 µg/g
TiO_2 Np	0.2 µg/g	0.9 µg/g	1.6 µg/g	2.0 µg/g

Regression analysis confirmed a statistically significant correlation between the administered concentration and tissue metal accumulation ($P< 0.01$). These data suggest considerable bioaccumulative potentials for both traditional heavy metal compounds and nanoparticle formulations, thereby underscoring the lasting ecological implications of even short-duration exposures.

DISCUSSION

The acute toxicity effects of $Pb(NO_3)_2$, CuO nanoparticles, and TiO_2 nanoparticles on *Cyprinus carpio* were thoroughly examined in this study. Short-term exposures to these pollutants drastically altered the physiological status of fish, as reflected by concentration-dependent mortality, pronounced behavioral abnormalities, and significant tissue bioaccumulation **(Lee & Choi, 2021)**.

$Pb(NO_3)_2$ emerged as the most potent toxicant, with significantly lower LC_{50} values compared to the metal oxide nanoparticles. The progressive decline in LC_{50} values with increasing exposure duration suggests cumulative toxicity, potentially resulting from both chemical accumulation and secondary physiological stress responses. The high

toxicity of lead compounds has been linked to interference with neuronal signaling, enzymatic inhibition, and disruption of ion homeostasis **(Gupta & Kumar, 2020)**.

CuO NPs also demonstrated clear toxicity, though their higher LC_{50} values indicated a less immediate hazard relative to $Pb(NO_3)_2$. Nonetheless, significant differences observed in post-hoc analyses and steep mortality curves underscore their capacity to impair fish viability, particularly under prolonged exposures. The production of reactive oxygen species (ROS) is a likely driver of the observed stress responses and subsequent cellular damage **(Martinez & Zhao, 2019)**.

TiO_2 NPs exhibited the least acute toxicity, with an LC_{50} value of 9.5mg/ L under the conditions tested. Although TiO_2 is often regarded as relatively inert, aggregation in aquatic systems may reduce bioavailability and thereby mitigate acute toxicity. However, chronic exposures may still pose risks through bioaccumulation or interactions with other contaminants **(Zhu *et al.*, 2010)**.

Behavioral changes—including reduced swimming speeds, erratic movements, diminished feeding responses, and elevated respiratory rates—emerged as sensitive sub-lethal indicators. Even exposures as low as 1mg/ L produced measurable disruptions, with implications for feeding efficiency, predator avoidance, and long-term population viability **(Smith *et al.*, 2010)**.

Bioaccumulation patterns highlighted the vulnerability of vital organs. The liver accumulated the highest concentrations of toxicants, predisposing it to oxidative damage and metabolic disruption. Gills also accumulated substantial levels of CuO and TiO_2 NPs, likely contributing to respiratory stress. Muscle tissue, though exhibiting lower concentrations, represents a potential route for trophic transfer in food webs **(Pereira *et al.*, 2016)**.

ANOVA confirmed that behavioral modifications were statistically significant over time ($P< 0.05$), reinforcing the link between toxicant exposure and physiological stress. Together, these findings emphasize the importance of integrating behavioral and biochemical endpoints in ecological risk assessments.

The LC_{50} values reported here differ from those in other studies **(Kostadinka *et al.*, 2019; Naeemi *et al.*, 2020; Abdel-Latif *et al.*, 2021; Haghighat *et al.*, 2021)**. Such discrepancies may reflect variations in experimental conditions, including species differences, age and size of fish, pollutant forms, and laboratory methodologies **(Hameed & Al-Azawi, 2016)**.

Environmental implications

The findings have important environmental implications, particularly concerning industrial effluent discharge and accidental spills. Pb-based compounds, due to their high toxicity and bioaccumulative nature, pose severe risks to aquatic life and humans through trophic transfer. While nanoparticles exhibited comparatively lower acute toxicity, they remain concerning due to their sub-lethal effects on behavior, reproduction, and

metabolism. Given the rapid increase in nanomaterial use across industries, their environmental dissemination requires stricter regulatory oversight **(Brown *et al.*, 2020)**.

Nanoparticles' tendency to aggregate, settle, and interact with organic matter and co-contaminants may reduce their apparent toxicity in acute tests but enhance long-term accumulation in sediments and biota. Future research should examine chronic exposures, synergistic interactions, and real-world environmental matrices to better capture ecological risks. Comprehensive risk assessments must therefore account for both traditional pollutants and emerging contaminants like nanomaterials **(Lee *et al.*, 2019)**.

The static-renewal design used here is effective for acute assays but does not replicate the dynamic conditions of natural ecosystems. *In situ* measurements of toxicant dispersion, bioavailability, and environmental interactions would improve the ecological relevance of laboratory findings. Furthermore, while LC_{50} is a valuable endpoint, inclusion of sub-lethal indicators such as behavior, reproduction, and metabolic changes provides a more holistic assessment of ecological risk **(Davis & Miller, 2021)**.

Future directions

Future research should prioritize multi-generational studies incorporating genetic, endocrine, and immunological endpoints. Integrating advanced molecular technologies may also help identify early biomarkers of toxic stress, particularly in response to nanoparticle exposure. Bridging laboratory-based studies with field assessments will be essential to developing a comprehensive understanding of the ecological risks posed by both heavy metals and engineered nanomaterials.

CONCLUSION

This study evaluated the acute toxicity of lead nitrate, CuO nanoparticles, and TiO_2 nanoparticles on common carp (*Cyprinus carpio*).

- **Lead nitrate** was identified as the most toxic, with sharply decreasing LC_{50} values across exposure durations.
- **CuO nanoparticles** exhibited moderate but significant toxicity.
- **TiO_2 nanoparticles** showed the lowest acute toxicity, though risks remained at high concentrations and prolonged exposures.

Toxicity followed a clear dose- and time-dependent pattern, underscoring the ecological risks posed by both heavy metals and engineered nanoparticles. Behavioral alterations—including abnormal swimming, delayed feeding, and elevated respiratory activity—along with tissue accumulation in the liver, gills, and muscles, confirmed substantial physiological stress.

These findings highlight the need for stricter regulatory oversight of heavy metals such as lead and for expanded research into nanoparticle toxicity. Future investigations should link laboratory-based findings with real-world environmental conditions to better assess long-term ecological and human health implications.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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