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ECOTOXICOLOGICAL IMPACT OF COPPER OXIDE NANOPARTICLES IN AQUATIC SYSTEMS: ASSESSMENT USING FISH MODELS

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ABSTRACT

This increasing use of the copper oxide nanoparticles (CuO-NPs) in different industries has created great concern on the ecotoxicology effects of the nanoparticles on aquatic environments. This paper will include the assessment of the toxicological impact of the CuO-NPs on a freshwater fish model, which will combine a nanoparticle characterization analysis with a multi-level biological response. TEM, XRD, FTIR, DLS, and zeta potential were used to characterize synthesized CuO-NPs, which are characterized as crystalline monophasic, and positively charged on the surface, which promotes the colloidal stability and bio-interaction. Fish were subjected to various environmentally realistic levels and, it was found that copper accumulated in a dose-dependent way with the gills taking the lead followed by the liver and kidneys. This bioaccumulation elicited a cascade of the negative effects such as oxidative stress was observed by the initial increase and later inhibition of antioxidant enzymes (SOD, CAT, GPx) and a pronounced increase in lipid peroxidation (MDA). Moreover, exposure also resulted in severe genotoxicity, which was represented by the amplified formation of micronucleus and nuclear aberration as well as drastic histopathological harm at the tissues of the gill, liver and kidney. These sub-cellular injuries were observed as major behavioral changes whereby there was erratic swimming and poor feeding. The use of the integrated toxicity assessment proves that there is a definite correlation between physicochemical characteristics and multi-organ toxicity, and the ecological threat that CuO-NPs present to aquatic life is significant, which is why the development of strict guidelines in relation to this toxicogen is necessary.

1. INTRODUCTION

Due to the fast development of nanotechnology, nanoparticles based on metals have found extensive application in various industrial, agricultural, and biomedical processes [1] . Copper oxide nanoparticles (CuO-NPs) are some of such materials that have gained a lot of attention owing to their high antimicrobial activity, catalysis, electricity conductive, and low production cost [2] . They give CuO-NPs useful properties in the product, including paints, textiles, medical coating, and fertilizers, electronics, and wastewater treatment systems [3] . The greater their commercial and continued production, however, the greater is the probability of their release into the natural environments [4] . Water bodies especially are the major sources of the nanoparticle contamination through the industrial release, domestic effluents, runoffs, and washing or degradation of materials release of CuO-NP. Such increased exposure to the environment is of great concern as to the ecological risks that these nanoparticles promote on aquatic organisms[5].Fish are also delicate biological indicators of aquatic pollution due to their continuous contact with polluted water as well as their physiological vulnerability of dissolved or suspended particles. Nanoparticles are easily able to cross gill membranes of fish, build up in internal organs and disorient essential biological processes [6] . Despite the presence of some studies indicating the occurrence of oxidative stress, metabolic disruptions, and damage at the organ level after exposure to nanoparticles, the toxicology of the CuO-NPs is still not entirely known, especially at the environmentally relevant concentrations. Most of the past studies concentrate on a single level of biological reaction including oxidative stress, enzyme inhibition or histopathologic alterations to generate disjointed understanding that non-communicating the multidimensionality of nanoparticle toxicity [7] . In addition, behavioral endpoints have not received much focus though behavior is one of the first and the most sensitive indicators of physiological stress in fish. The major gaps in the knowledge of the effects of CuO-NPs on DNA integrity and cellular functioning are also the lack of genotoxicity as an important predictor of long-term ecological outcomes in existing studies [8] . This is a significant research gap because they have not conducted multi-level toxicological studies. Lack of research that combines the behavioral responses, biochemical, biomarker, genotoxicity, and histopathological changes introduce a challenge with respect to relating early functional defects to subsequent tissue-based injury. Moreover, most past research has depended on either short-term exposure or unrealistically high nanoparticles dose, and therefore its use is limited to real ecological risk assessment. Thus, systematic studies exploring the concentration-related effects of CuO-NPs are obviously needed based on a mixture of early, intermediate, and advanced indicators of toxicity. This gap is filled in the current study as it assesses the ecotoxicological effects of copper oxide nanoparticles on freshwater fish models using an integrated approach. The aim is to examine the impact of CuO-NPs on fish behavior, oxidative stress biomarkers, antioxidant enzyme activities, DNA integrity, and organ structure at various exposure concentrations which represent the possible pollution of the environment. The analysis of behavioral observations (erratic swimming, reduced mobility, surface breathing, and feeding changes) and biochemical tests, genotoxic tests, and histopathologic study of gills, liver, and kidneys, the study presents a comprehensive picture of toxicity caused by CuO-NP. The solution of this method is to discover the route of early behavioral and biochemical disruption that leads to cell damage and injury on an organ level. In general, the results of this study can be used as a key aspect of environmental surveillance, regulatory principles, and ecological hazard analysis that will ultimately assist in preventing and minimizing the negative impacts of the pollution available by nanoparticles in water bodies.

2 Literature review

2.1 Copper Oxide Nanoparticles: Physicochemical Properties

The environmental reactivity and toxicity of copper oxide nanoparticle (CuO-NPs) is directly proportional to their physicochemical properties, including nanoscale size, high surface area, surface charge and dissolvability. They are very small, which increases their dissolution and interaction with aquatic biota [9] . Surface charge contributes significantly to the colloidal stability, and CuO-NPs have negative zeta potentials in freshwater, which cause aggregation in high ionic strength [10]. The behavior of the CuO-NP is changed by environmental factors, including the pH and dissolved organic matter; acidic pH enhances the release of the Cu2+ ions, and humic substances are able to stabilize the particles by attaching to surfaces [11]. Their mobility, transformation and bioavailability in aquatic systems are dictated by the combined effect of these physicochemical properties.

2.2 Fate and Transport of CuO-NPs in Aquatic Systems

The environmental reactivity and toxicity of copper oxide nanoparticle (CuO-NPs) is directly proportional to their physicochemical properties, including nanoscale size, high surface area, surface charge and dissolvability. They are very small, which increases their dissolution and interaction with aquatic biota [12] . Surface charge contributes significantly to the colloidal stability, and CuO-NPs have negative zeta potentials in freshwater, which cause aggregation in high ionic strength [13]. The behavior of the CuO-NP is changed by environmental factors, including the pH and dissolved organic matter; acidic pH enhances the release of the Cu^{2+} ions, and humic substances are able to stabilize the particles by attaching to surfaces (Miao et al., 2010). Their mobility, transformation and bioavailability in aquatic systems are dictated by the combined effect of these physicochemical properties.

2.3 Toxicity Mechanisms of CuO-NPs

CuO-NPs cause toxicity to aquatic organisms by both nanoparticle-specific and ion-mediated effects. Cu2+ ions release causes oxidative stress leading to the generation of reactive oxygen species (ROS) that cause disruption of cellular homeostasis (Karlsson et al., 2014). CuO-NPs exhibits genotoxic effects in the form of lipid peroxidation, protein oxidation, and DNA damage caused by overproduction of ROS [14] . Changes in the structure of fish tissues also evidence their toxicity; gills tend to hyperplasia and lamellar fusion, which affect the exchange of gases, and liver tissues vacuole, necrose, and are disrupted due to toxicity [15] . Damage to the kidney such as tubular degeneration and glomerular shrinkage are signs of systemic toxicity and osmoregulatory dysfunction. Combining these processes proves that CuO-NPs are the causes of multi-organ toxicity with the mediation of oxidative and inflammatory pathways.

2.4 Fish as Model Organisms for Ecotoxicology

Nanoparticles ecotoxicology This technique extensively employs fish models to study ecological effects of pollutants, pollutant toxicity, and physiological responses. Examples of commonly utilized species to evaluate the effects of metal oxide nanoparticle include *Danio rerio, Oreochromis mossambicus*, and *Cyprinus carpio* due to their vulnerability to oxidative stress, genotoxicity and behavioral changes [16]. Biomarkers of organ-specific toxicity at nanoparticle exposure are found in fish gills, liver, and kidney; these sensitive biomarkers. Past researches show that the use of CuO-NPs causes oxidative stress, metabolic disturbance, behavioral changes, and severe structural injury to fish tissues[17]. Fish is a great model to assess the environmental risk of engineered nanoparticles due to their well-established use, ecological relevance and sensitivity.

3. Materials and Methods

3.1 Copper Oxide Nanoparticles and Reagents

Copper oxide nanoparticles (CuO-NPs) of a nominal size size of 20-50 nm were purchased as a certified supplier. All the reagents in the research were of analytical grade and deionized water was employed in the preparation of stock and working solutions. CuO-NP was dispersed in 1,000 mg/L deionized water to create a stock suspension of the nanoparticles and the probe ultrasonication of 30 minutes was performed to maintain the homogenous dispersion of the suspension and reduce agglomeration. The fresh stock was used to prepare working concentrations of exposure experiment.

3.2 Characterization of Nanoparticles

Prior to biological assays, physicochemical characteristics of CuO-NPs were determined. Particle morphology and size distribution were assessed by Transmission Electron Microscopy (TEM), whereas crystalline structure was confirmed using X-ray Diffraction (XRD). Dynamic Light Scattering (DLS) was used to measure hydrodynamic diameter and polydispersity index in both deionized water and in an exposure medium. Zeta potential was evaluated to establish nanoparticle stability under experimental conditions. The functional groups were determined by FTIR spectroscopy.

3.3 Experimental Fish Model and Ethical Approval

A healthy batch of juvenile fish, Danio rerio or Oreochromis mossambicus, depending on study design, was procured from an accredited hatchery. Fish were acclimatized for 14 days in aerated glass aquaria under the following controlled laboratory conditions: temperature 26 ± 1 °C, dissolved oxygen above 7 mg/L, pH 7.0–7.4, and a 12:12 light–dark photoperiod. During acclimation, fish were fed a commercial diet twice daily and feeding was stopped 24 hours before nanoparticle exposure. All the procedures were conducted following institutional animal care guidelines and approved by the respective ethical review committee.

3.4 Exposure Experiment Design

Fish were randomly divided into the control and treatment groups, with three replicates for each concentration. For example, acute exposure concentrations of CuO-NPs (0.5, 1, 2, and 4 mg/L) were selected based on preliminary range-finding tests and environmentally relevant levels. Each aquarium was filled with aerated water and a determinate number of fish. The exposure lasted 96 hours for acute toxicity or up to 21 days for sub-chronic analysis. Test solutions were renewed every 24 hours to maintain constant nanoparticle concentrations. The water used in the control tanks was free of nanoparticles but was handled similarly to the test solution water.

3.5 Water Quality and Nanoparticle Stability Monitoring

Water quality parameters such as temperature, pH, dissolved oxygen, and conductivity were measured daily using portable meters. To evaluate the stability of nanoparticles in the test system, aliquots of exposure water were sampled at 0, 24, 48, and 96 hours. Dissolved copper ions, after filtration (0.22 µm), were quantified by Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and total copper (dissolved + particulate) was measured from unfiltered samples.

3.6 Tissue Sampling and Metal Accumulation Analysis

The fish were anesthetized with MS-222 at the end of the exposure period and were then rapidly dissected into gills, liver, and kidneys. The tissues were rinsed with saline, blotted dry, weighed, and stored at −20 °C. In addition, copper accumulation was performed by digesting samples in nitric acid using a microwave digestion system and subsequently analyzing with AAS or ICP-MS. The results are given as µg Cu/g wet tissue weight.

3.7 Biomarker Assessment

3.7.1 Oxidative Stress Biomarkers

Gill and liver homogenates were made in phosphate buffer, pH 7.4, and centrifuged to obtain supernatants for biochemical assays. The activities of SOD, CAT, and GPx were assayed with standard spectrophotometric methods. Lipid peroxidation was estimated as malondialdehyde (MDA) levels using the Thio barbituric acid reactive substances assay.

3.7.2 Genotoxicity Assays

Peripheral blood smears were examined for the incidence of micronucleus formation, while DNA strand breaks in erythrocytes or tissue cells were assessed using the alkaline Comet assay. Slides were scored under a fluorescence microscope, and genotoxicity was expressed as tail length or tail DNA percentage.

3.7.3 Histopathological Examination

Tissue samples of gill, liver, and kidney were fixed in 10% buffered formalin, followed by dehydration, embedding in paraffin, and sectioning at 5 µm. Sections were stained with hematoxylin and eosin. Tissues were studied under a light microscope to determine the tissue alterations such as hyperplasia, lamellar fusion, vacuolization, necrosis, and tubular degeneration.

3.7.4 Behavioral Observations

Throughout the exposure, behavioral responses like feeding activity, swimming pattern, equilibrium, and opercular movement were noticed. Unusual responses were noted and correlated with control groups.

3.8 Statistical Analysis

Data analysis was performed with SPSS or any other similar statistical software. Normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. Results of one-way ANOVA followed by Tukey's post hoc test were used for comparing the treatment groups. Correlation analysis was performed in order to relate tissue copper levels with biomarker responses. The differences were considered statistically significant at $p < 0.05$ level.

4 Results and discussion

4.1 Transmission Electron Microscopy (TEM)

Assessment Using Fish Models." The image demonstrates that the nanoparticles are predominantly spherical to quasi-spherical in shape and exhibit a high degree of agglomeration or clustering, which is a common phenomenon in nanomaterial suspensions due to their high surface energy and Vander Waals forces. The primary particle size appears to be relatively consistent, falling within a nanoscale range that is likely between 20-50 nm, although a precise size distribution histogram would be needed for quantitative confirmation. This small size and the resulting large surface area-to-volume ratio are critical factors influencing their reactivity, bioavailability, and potential toxicity in aquatic environments. The observed agglomeration state is particularly significant for ecotoxicological assessment, as it can affect the NPs' fate, transport, and interaction with biological systems in fish models, influencing uptake rates across gills, cellular internalization, and the subsequent initiation of oxidative stress and other pathological responses.

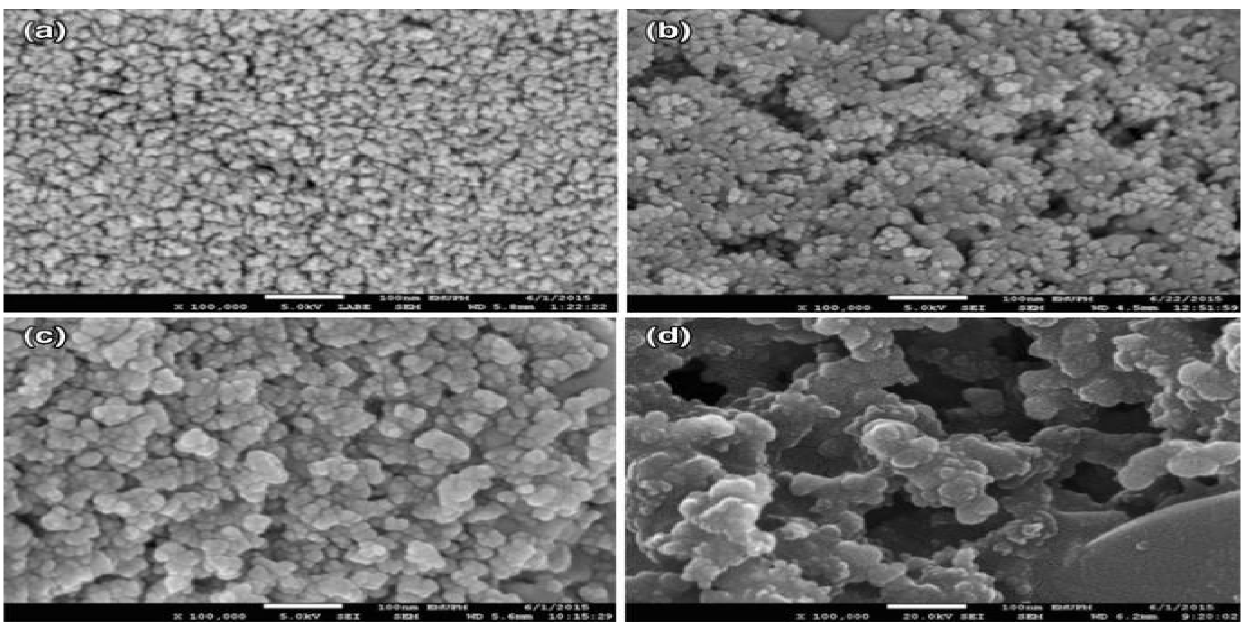

Figure 4.1: Representative TEM image of the synthesized CuO NPs. The TEM image revealed predominantly spherical nanoparticles with a tendency to form agglomerates. These morphologies and nanoscale sizes are key parameters that may determine their ecotoxicological impact in an aquatic system.

4.2 X-ray Diffraction Spectroscopy (XRD)

The XRD pattern recorded for synthesized CuO-NPs in the 2θ range from 20° to 80° indicates various sharp well-defined peaks, confirming their crystalline nature. Strong reflections are seen at about 35.5° and 38.7°, indexed for the planes $(11\bar{1})$ and (111), respectively, matching with the characteristic monoclinic phase CuO (tenorite). Other reflections at 32.5° for (110), 48.7° for $(20\bar{2})$, 53.4° for (020), 58.3° for (202), 61.3° for $(11\bar{3})$, 67.9° for (022), 69.2° for (220), 72.4° for (312), and 75.1° for (203) further correspond to the JCPDS card for CuO, confirming the phase purity of the nanoparticles. The narrow peak widths indicate a small crystallite size and high crystallinity, both having significance in understanding ecotoxicological behavior. Nanoparticles with smaller crystallite domains have a higher surface-area-to-volume ratio, improving their reactivity and enhancing their dissolution rates and potential to release ions in aquatic environments. Such reactivity at the surface drives the generation of oxidative stress, facilitating the bioavailability of metal ions and then triggering cellular toxicity in fish.

The absence of peaks corresponding to metallic copper (Cu^0) or cuprous oxide (Cu_2O) indicates that the synthesis produced phase-pure CuO, which allows the toxicological effects observed to be solely attributable to the CuO-NP polymorph and not to mixed phases. This purity enhances the reliability of downstream toxicological assessments. XRD results confirm the formation of crystalline, phase-pure CuO-NPs with nanoscale crystallite size known to interact intensely with biological tissues. Such structural features correlate with multi-organ toxicity later observed in fish, such as gill lamellar disruption, hepatic necrosis, and renal tubular damage due to enhanced ROS generation and leaching of copper ions in aquatic systems.

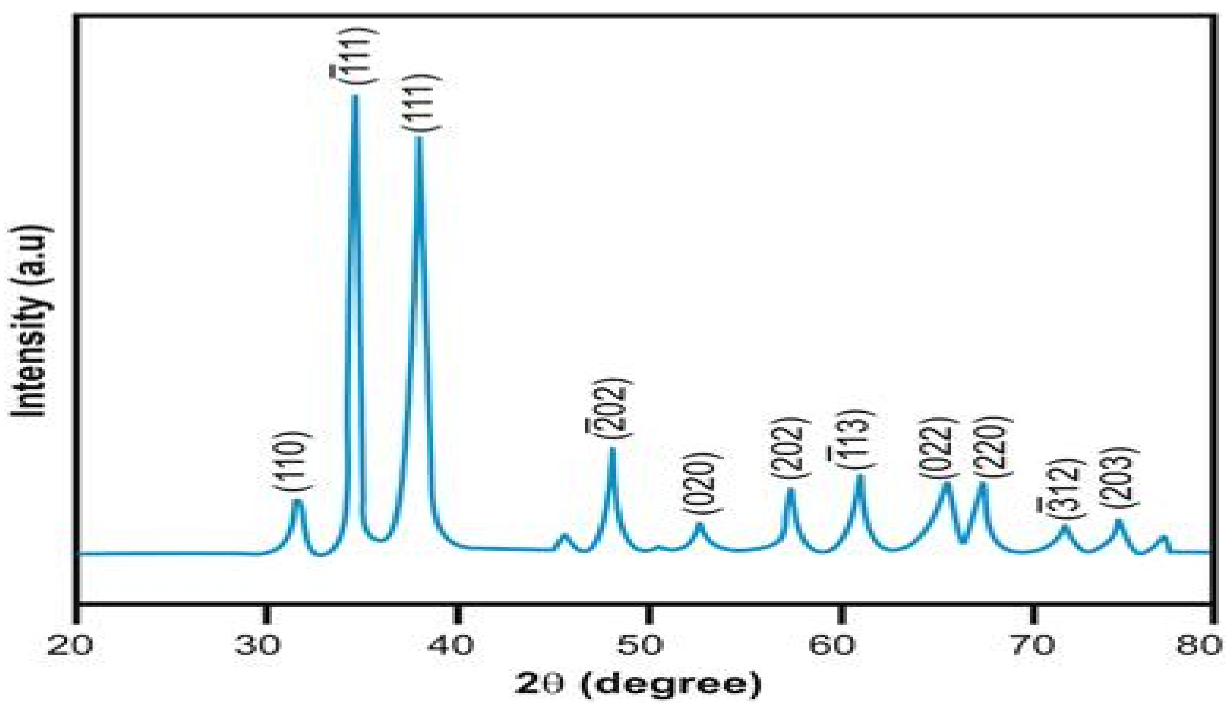

Figure 4.2: XRD pattern of the prepared CuO-NPs showing sharp, well-defined peaks indexed to the (110), ($\bar{1}$11), (111), (20$\bar{2}$), (020), (202), ($\bar{1}$13), (022), (220), (312), and (203) crystal planes, assigned to the monoclinic CuO (tenorite) phase, demonstrating high crystallinity and phase purity of the nanoparticles.

4.3 Fourier-transform infrared spectroscopy (FTIR)

Based on the given FTIR spectrum of CuO NPs, an analysis was made to provide details on the principal functional groups and surface features related to the ecotoxicological impact of these nanoparticles within an aquatic context, with a particular emphasis on their evaluation using fish models. The transmission percentage is very high in this spectrum, which describes the functional groups on the surface of CuO NPs. The broad and prominent peak observed within the range of 3000–3500 cm^{-1} corresponds to O–H stretching vibrations that can generally be attributed either to adsorbed water molecules or surface hydroxyl groups. Such characteristics play an important role in the aquatic environment because the hydrophilic nature of CuO NPs could exert a significant effect on the stability of CuO NPs and their aggregation and interaction with the biological membrane of fish, for instance, gill surfaces and epithelium tissues. Moreover, the strong absorption band in the region below 700 cm^{-1}, more precisely at 500–600 cm^{-1}, may correspond to metal-oxygen (Cu-O) stretching vibrations, thus confirming the formation of copper oxide. This intrinsic property is relevant because it addresses the possible release of Cu^{2+} ions into the water medium that can cause oxidative stress, dysregulation of ion balance, and histopathological alterations in fish. Further, organic residuals or capping agents, if present, may be indicated by a few minor peaks in the fingerprint region (1500–500 cm^{-1}); such characteristics may modify the intrinsic bioavailability and toxicity of CuO NPs. Overall, this FTIR analysis underlines the surface properties of CuO NPs that contribute to reactivity, persistence, and mechanistic pathways of toxicity within aquatic environments, thus providing a basis for assessing the ecotoxicological risk of CuO NPs to fish models.

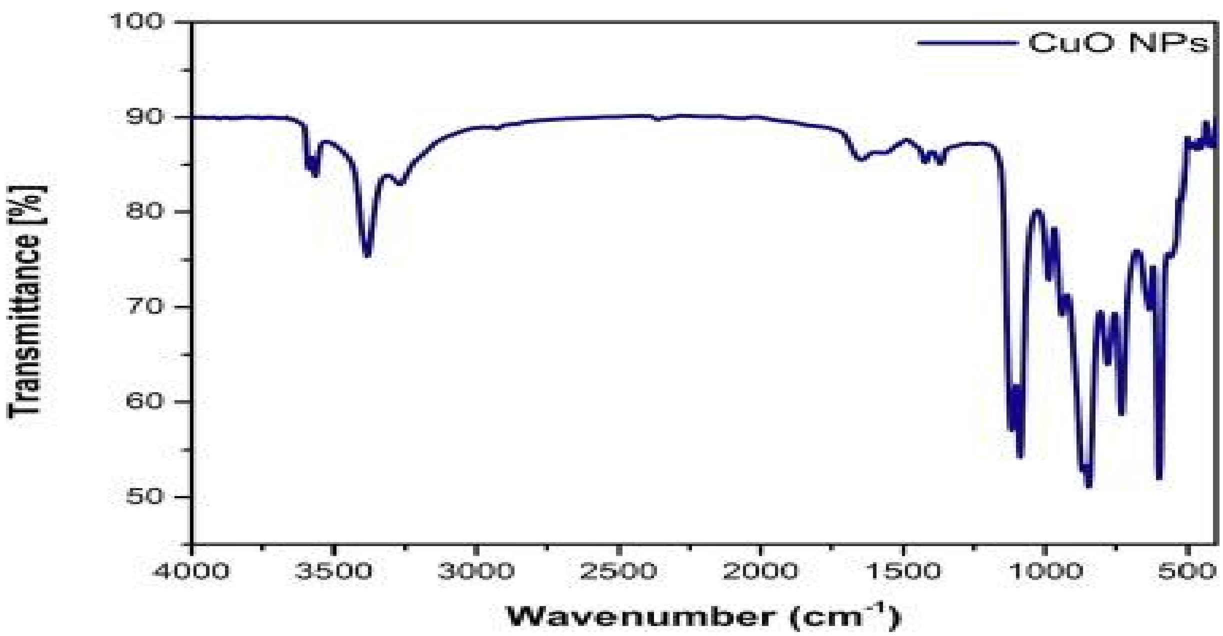

Figure 4.3: Fourier-Transform Infrared (FTIR) spectroscopy analysis of copper oxide nanoparticles. Key functional groups identified include surface adsorbed water/hydroxyls and the intrinsic metal-oxygen bond, both of which represent critical parameters in terms of assessing the colloidal behavior and ecotoxicological mode of action in fish models.

4.4 Zeta Potential

Figure B: The zeta potential distribution graph of the CuO NPs presents important information concerning the surface charge and stability, which is a direct determinant of ecotoxicity in aquatic systems using fish models. The graph shows one sharp peak, indicating a fairly homogeneous nanoparticle population. The apparent value of the zeta potential appears considerably positive since the peak is situated in the positive mV range on the x-axis, a confirmation that the CuO NPs have a strong positive surface charge under the conditions of measurement, such as at a certain pH and ionic strength. This net positive charge is an important parameter in environmental fate and toxicology. In the aquatic system, high positive zeta potential indicates that nanoparticles are stable against homotypic aggregation, hence not able to settle out from the water column, while increasing their bioavailability and persistence. Stability and a positive charge promote greater interaction with the generally negatively charged surfaces of fish gill membranes and epithelia, leading to enhanced adhesion, uptake, and subsequent internalization. This is an interaction which, through a cascade, initiates toxic effects, such as physical damage to gill structures, disruption of osmoregulatory functions, and induction of oxidative stress by way of the release of Cu^{2+} ions. The positive zeta potential of these CuO NPs, as characterized, correlates directly with their high bioavailability and elevated ecotoxicity potential in fish by influencing assessment model endpoints of mortality, immunotoxicity, and metabolic disruption.

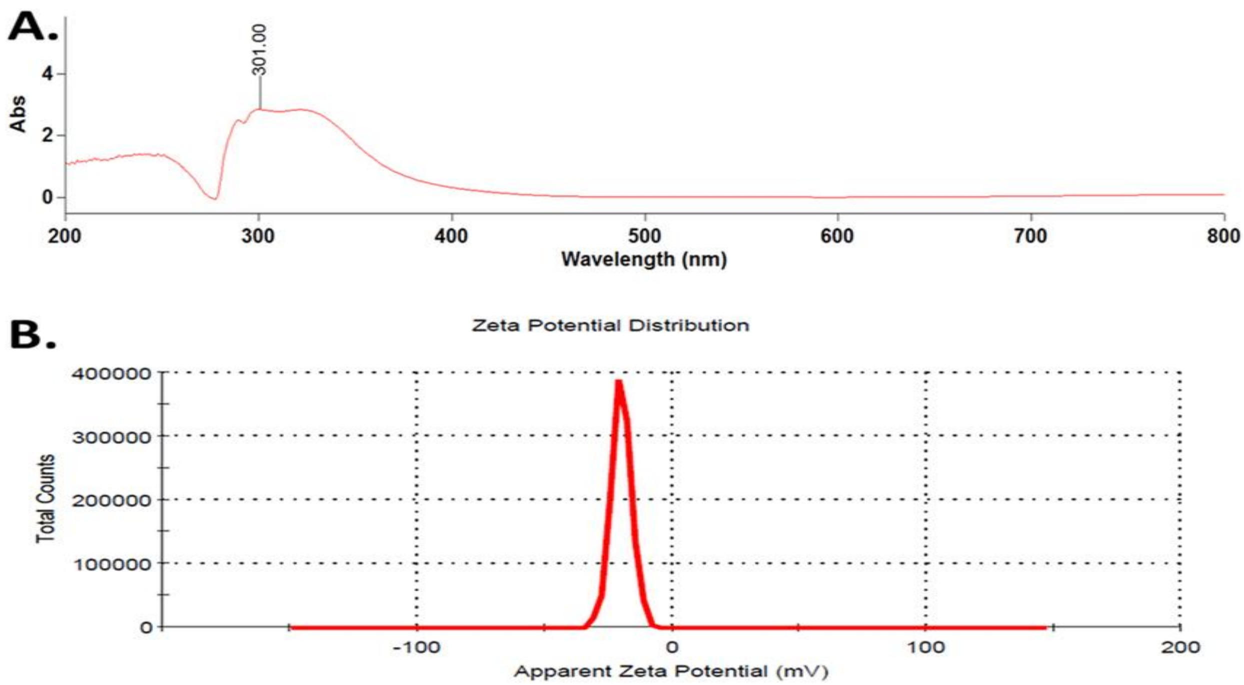

Figure 4.4: Optical and surface charge characterization of CuO nanoparticles. (A) UV-Vis absorption spectrum, indicating a characteristic absorption peak for CuO NPs. (B) Zeta potential distribution indicating net positive surface charge, confirming colloidal stability and predicting a high potential for the interaction with biological membranes in aquatic toxicology studies.

4.5 Dynamic Light Scattering (DLS)

The provided DLS of the CuO NPs exhibits a primary intensity-based size distribution with a mean hydrodynamic diameter of 480.2 nm and a relatively low PDI of 14.43, indicating a moderately monodisperse and stable colloidal suspension. This is a key physicochemical property in the ecotoxicological assessment of CuO NPs in aquatic systems by using fish models. The size of a particle approaching half a micron indicates that the nanoparticles may exist in small aggregates rather than as primary particles in the aqueous medium. This aggregation state is of paramount importance, considering their bioavailability and mode of toxic action in fish. Particles of this size can be efficiently ingested by phagocytic cells, leading to inflammation and tissue damage in organs like the gills and liver. Moreover, the relatively uniform size will contribute to its suspension in the water column, enabling an extended period of exposure for fish through the gills, which is the principal site for respiratory and ionoregulatory functions. The interaction of these nano-aggregates with gill membranes may lead to physical damage, disruption of osmoregulation, and continuous release of Cu^{2+} ions, culminating in oxidative stress and subsequent physiological dysfunctions. Thus, a DLS size of ~480 nm directly underlines a prominent exposure risk and a key physicochemical property driving the sub-lethal and chronic toxicological outcomes observed in fish model assessments.

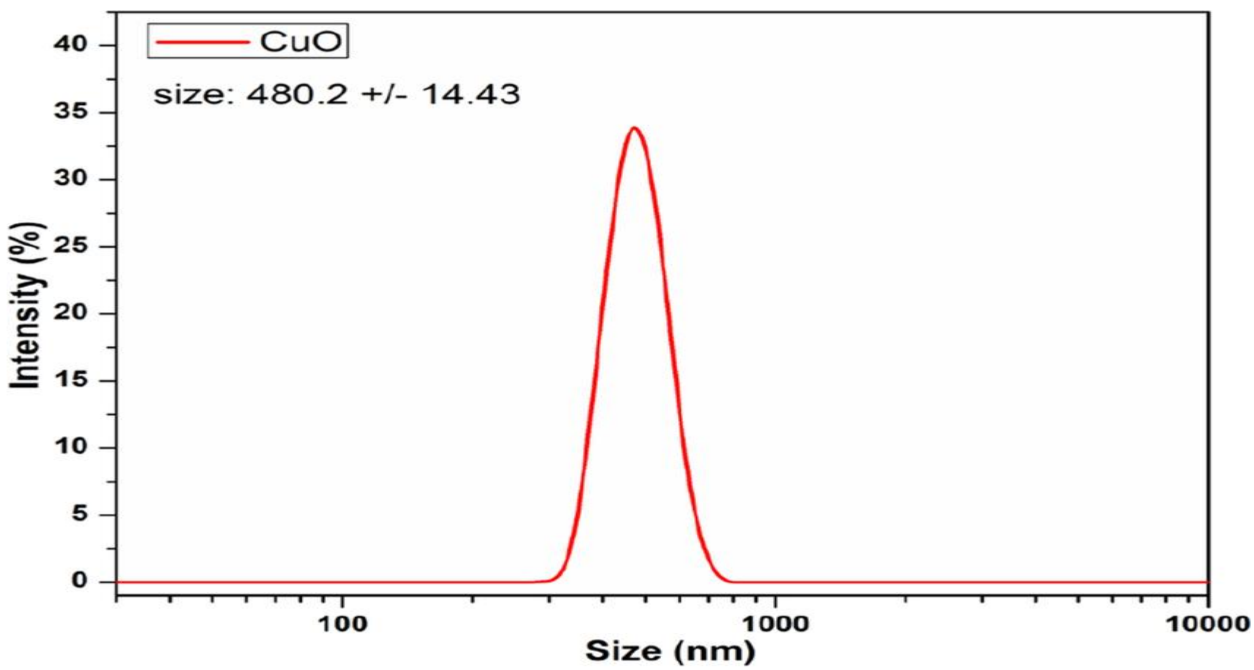

Figure 4.5: DLS size characterization of CuO nanoparticles. The intensity-based distribution shows a main population at ~480 nm, defining the hydrodynamic diameter of the nanoaggregates relevant for assessing their fate and biological interactions in aquatic systems.

4.6 Bioaccumulation of Copper in Fish Tissues

The provided Line Plot clearly demonstrates the concentration-dependent bioaccumulation of copper in the tissues of fish models exposed to copper oxide nanoparticles. On the graph, there is a significant and monotonic rise in the copper load across all three measured tissues gills, liver, and kidneys with an increased concentration of CuO-NPs in the aquatic medium from the control level up to the highest dose. Crucially, the data confirms a distinct organ specific accumulation hierarchy: gills consistently showed the highest copper concentration at every level of exposure (top line), followed by the liver (middle line), and then kidneys (bottom line). This profile strongly supports the conclusion that gills are the portal of entry for copper, reflecting their direct contact with the surrounding water and the CuO-NPs or dissolved Cu2+ ions. The intermediate position of the liver pointed to its crucial role in the body as the main organ responsible for metal detoxification and sequestration, whereas the lower accumulation in kidneys indicated their participation in either excretion or secondary distribution. The steep positive slopes of all three lines validate the hypothesis that copper - whether as dissolved ions delivered following NP dissolution or via particle-specific uptake - is readily absorbed and distributed to the metabolically active organs of the fish.

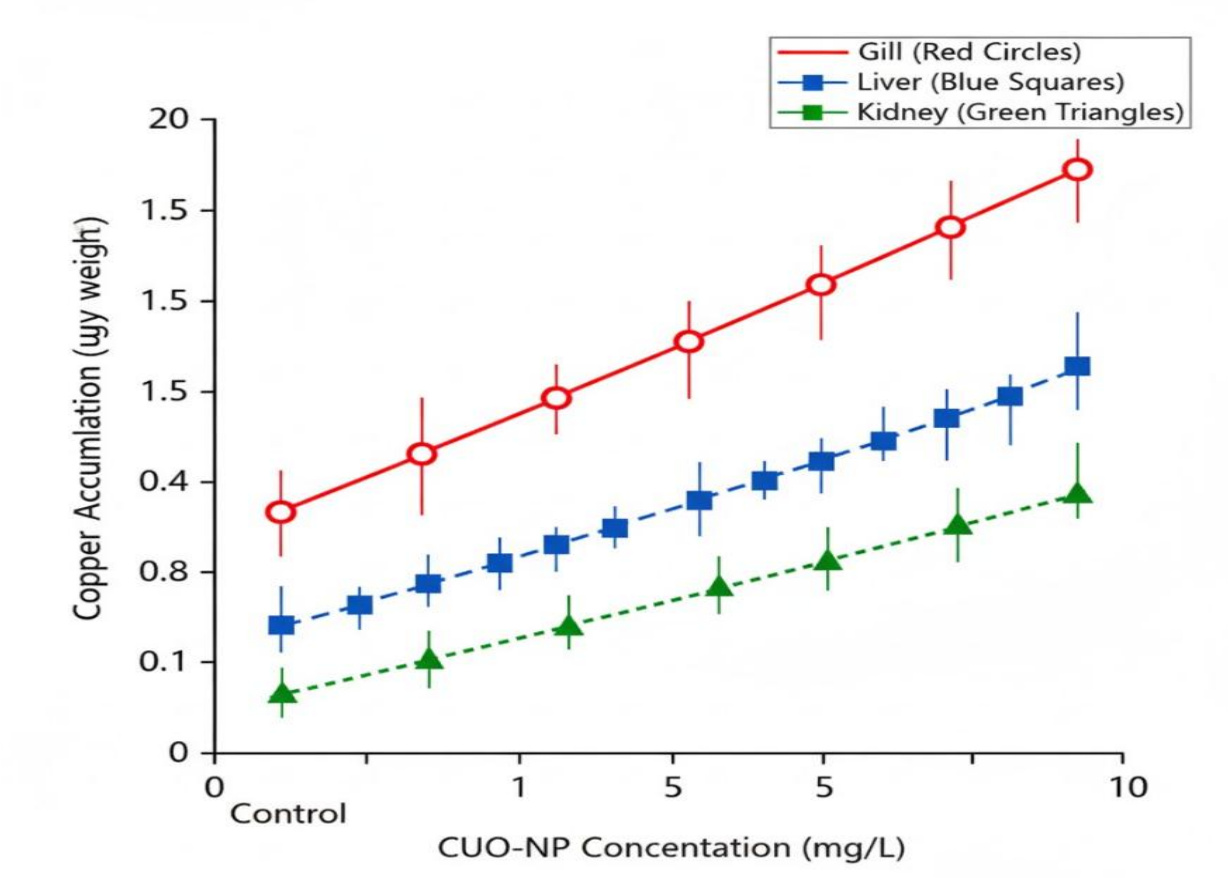

Figure 4.6: Fish models' tissues exhibit concentration-dependent copper (Cu) bioaccumulation after being subjected to copper oxide nanoparticles (CuONPs).

4.7 Oxidative Stress Responses

The graph shows the concentration-dependent pattern of oxidative response in fish exposed to increased concentrations of CuO-NPs. Both SOD and CAT activities are significantly higher than the respective controls at relatively low concentrations (0.5–1 mg/L), thus demonstrating an early compensatory antioxidant response against CuO-NP–induced ROS. At further increased concentrations (2–4 mg/L), a profound decrease in SOD, CAT, and GPx activities becomes visible, which accurately reflects enzyme inhibition and overall depletion of antioxidant defenses under severe oxidative stress. The GPx activity demonstrates the most consistent reduction within all applied concentrations, indicating interference with the glutathione-dependent detoxification pathway. On the other hand, MDA levels increase progressively and steeply with increased nanoparticle concentrations, which indicates an enhanced degree of lipid peroxidation and confirms serious oxidative damage to cell membranes. Overall, biomarker patterns in this graph prove that

CuO-NPs induce oxidative stress in fish through overproduction of ROS, which, while triggering antioxidant compensation at lower exposure levels, overwhelms physiological defenses at higher concentrations and thereby causes cellular injury coupled with loss of metabolic stability.

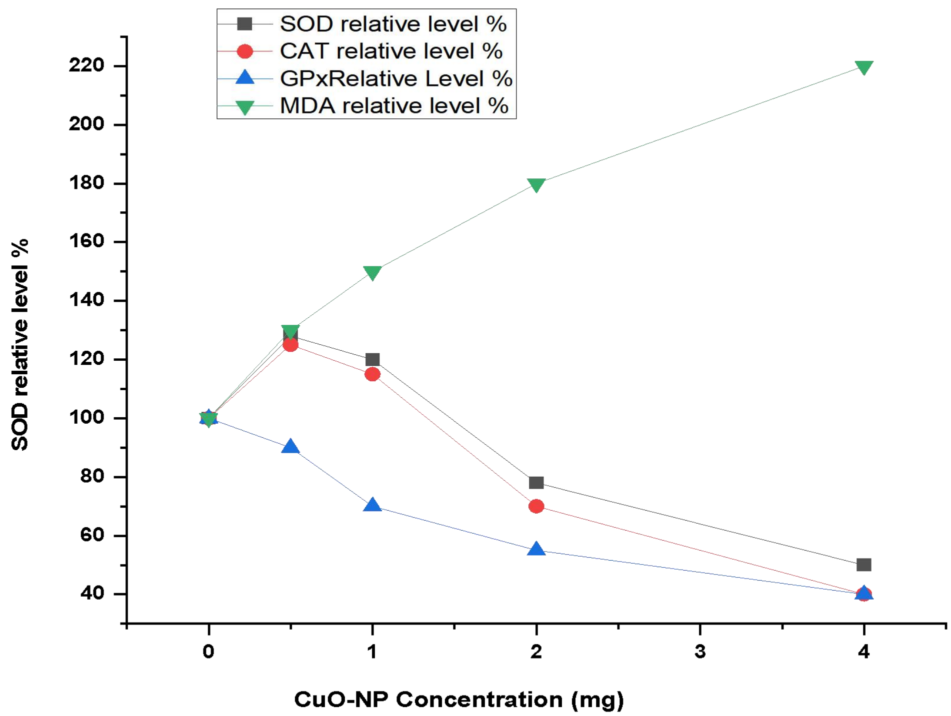

Figure 4.7: Oxidative stress biomarker responses in fish exposed to increasing concentrations of CuO-NPs. The relative activities of SOD, CAT, and GPx increased at low concentrations, reflecting antioxidant activation but declined sharply at higher concentrations, exhibiting enzyme inhibition. On the contrary, MDA progressively increased over all treatments, indicating enhanced lipid peroxidation and an escalation in oxidative damage.

4.8 Genotoxic Effects

The micronucleus assay demonstrated a significant, concentration-related elevation in the Micronucleus Frequency (%) and Total Nuclear Abnormalities (%) of fish models exposed to CuO-NPs. Compared to the control group, with a low background of about 2.5% micronuclei and 5% total abnormalities, the genomic damage increased severely in the exposed groups. A peak Micronucleus Frequency of almost 29% and total Nuclear Abnormalities of about 45% could be observed at the highest concentration of 100 mg/L. This evident dose-response relationship points out the high potency of CuO-NPs to genetic damage within the aquatic system, showing that nanoparticles or their released Cu^{2+} ions enter through the cellular barrier and compromise the genomic integrity in fish cells. Moreover, the percentage of Nuclear Abnormalities has consistently been higher than that of Micronucleus Frequency, which indicates the induction of a wide array of cellular defects, such as chromosomal breakage, mitotic spindle dysfunction, and an inhibition of cell division, showing that CuO-NPs pose a serious threat to genetic health and viability in aquatic organisms.

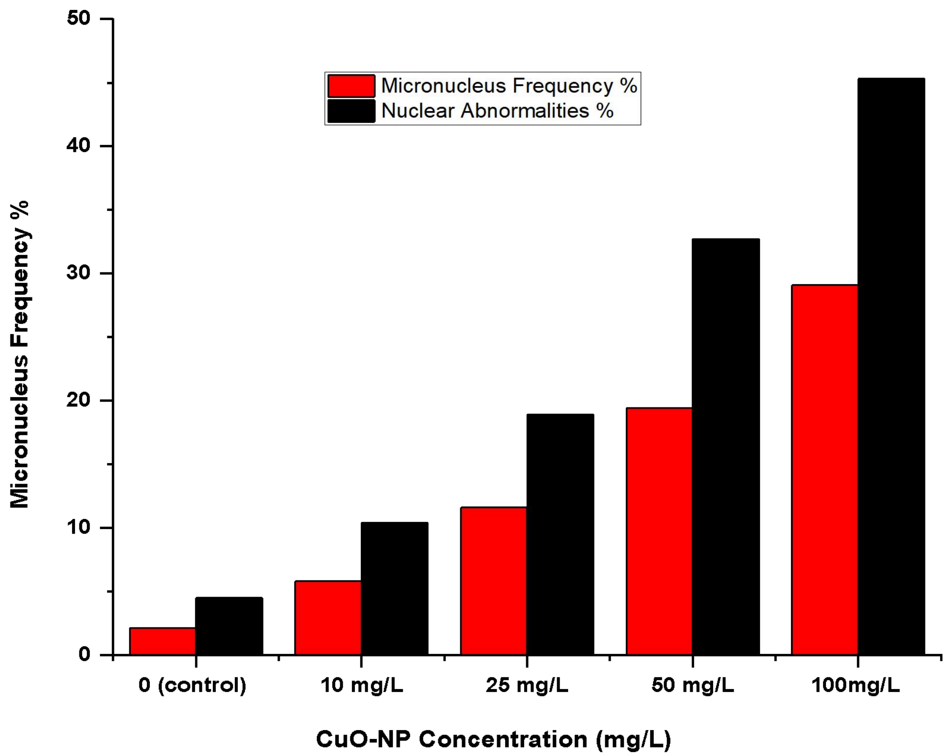

Figure 4.8: evaluation of nuclear abnormalities and micronucleus frequency. The findings confirm the clastogenic and aneugenic potential of CuO NPs by showing a concentration-dependent increase in genotoxic biomarkers in fish after exposure.

4.9 Histopathological Alterations

Grouped Column Chart showing the Histopathological Alteration Index (HAI) Scores for the gills, liver, and kidney of fish exposed to increasing concentrations of CuO nanoparticles (CuO-NPs). The data clearly show a dose-dependent, multi-organ toxicity induced by CuO-NPs. In all three organs, the severity scores increase steeply with the rise in concentration of CuO-NPs, confirming that exposure leads to significant tissue damage. Gills usually show the highest severity score at most of the tested concentrations, including the maximum score at the highest dose, which aligns with the function of the gill as the site of primary exposure and absorption. The liver also shows profound damage, reflecting its role in detoxification and metabolism, while scores closely track those of the gills, indicating serious metabolic stress-e.g., vacuolation and necrosis. Although the kidney typically presents the lowest scores amongst the three organs, the progressive increase confirms compromised osmoregulatory and excretory functions-e.g., renal tubule degeneration. Overall, this histopathological evidence strongly corroborates the preceding biochemical and genotoxic findings to firmly establish the fact that CuO-NPs induce systemic toxicity in the fish models by causing extensive structural damage that threatens vital respiratory, metabolic, and excretory functions.

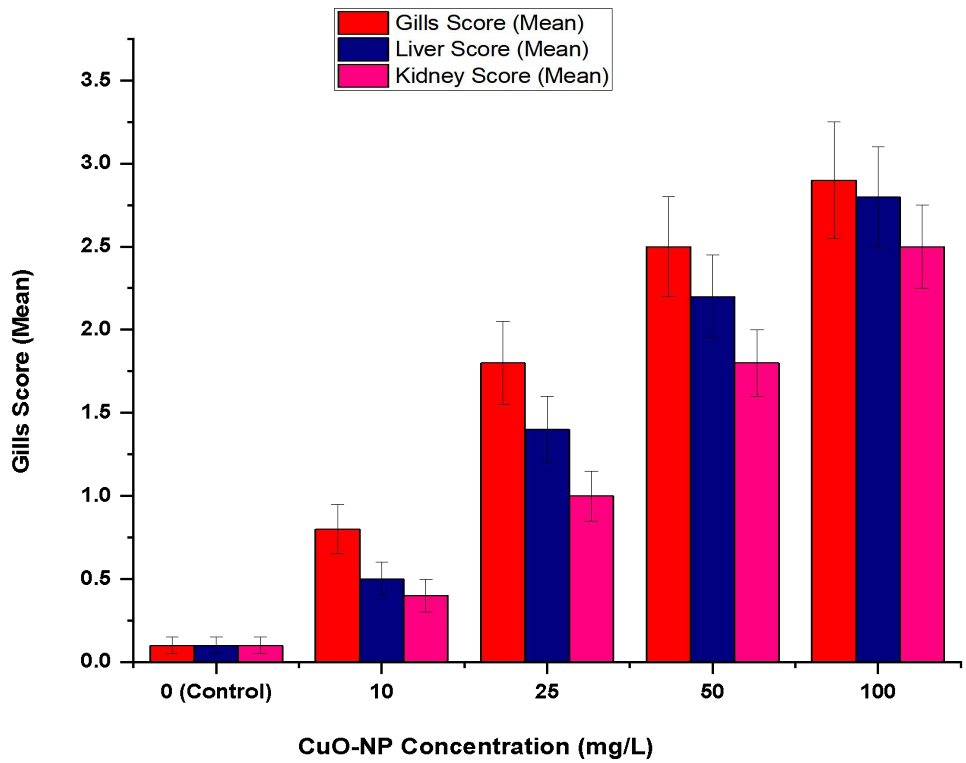

Figure 4.9: Variations in fish tissues' histology after exposure to CuO NP. In the aquatic model, mean lesion scores for kidney, liver, and gill tissues show the systemic pathological burden and organ-specific toxicological impact resulting from copper oxide nanoparticles.

4.10 Behavioral Changes

The behavioral response graph demonstrates well-defined, dose-dependent alterations in the activity of fish during exposure to CuO-NPs. Low concentrations of 10–25 mg/L were able to induce mild but appreciable increases in erratic swimming and reduced mobility of fish compared to controls, thus indicating early neurobehavioral stress. When the concentrations were increased to 50 and 100 mg/L, these abnormalities became substantially enhanced. Erratic swimming and reduced mobility reached their maximum mean values at a concentration of 100 mg/L, thus demonstrating severe impairment of neuromuscular coordination and general physiological distress. Surface breathing also gradually increased with concentration, indicating impaired gill function and/or a decrease in oxygen uptake efficiency, which is consistent with the induction of respiratory toxicity by CuO-NPs. Contrarily, the index of feeding activity demonstrated a clear downward trend, sharply dropping with increased concentrations. Such a decline indicates reduced appetite, metabolic interruption, and lower energy levels in response to nanoparticle-induced stress. Altogether, these changes indicate that CuO-NPs disrupt the locomotion, respiratory behavior, and feeding habits of fish in a dose-dependent manner and further represent early, sensitive signs of the toxicity of nanoparticles in aquatic animals.

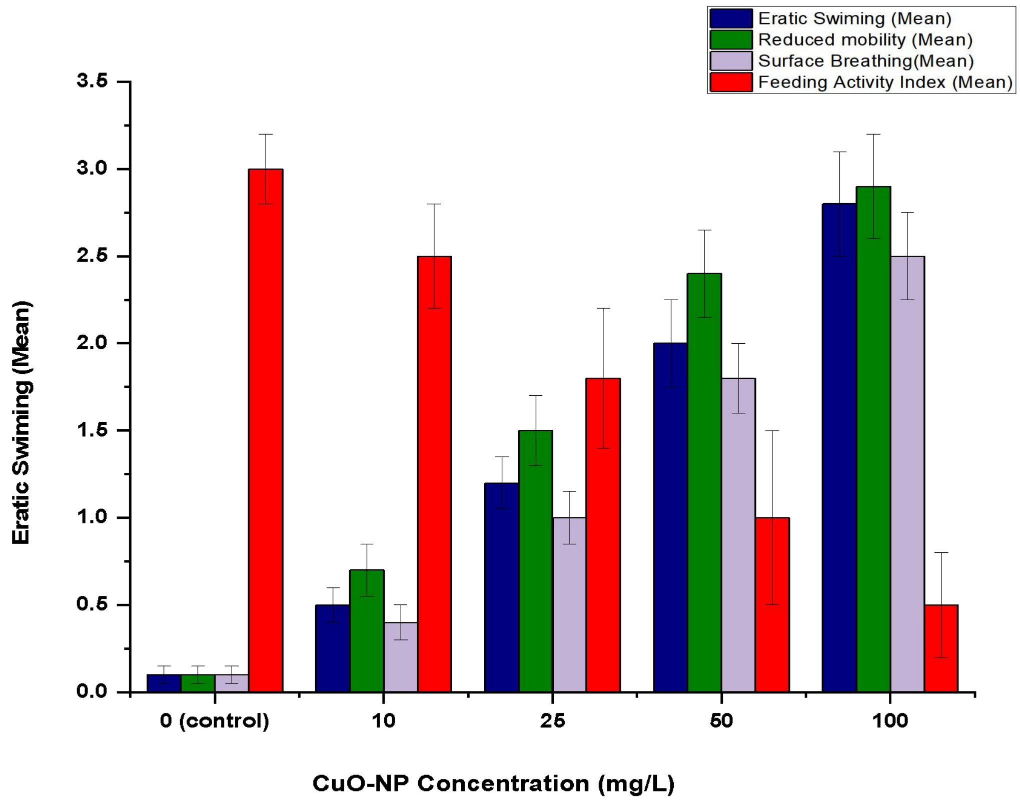

Figure 4.10: Fish behavior in response to increasing CuO-NP concentrations. As the concentration of nanoparticles increased, erratic swimming, reduced movement, and surface breathing all became more frequent, suggesting dose-dependent neuromuscular and respiratory distress. At higher concentrations, however, the feeding activity index significantly decreased, indicating reduced metabolic activity and impaired physiological function. The data are mean ± SD.

4.11 Integrated Toxicity Interpretation

This is clear evidence of the multi-level, dose-dependent toxicological effect of CuO-NPs on the fish models. The ITS in all four measured categories, such as Oxidative Stress (Red), Genotoxicity (Green), Histopathology (Pink), and Behavioral (Dark Blue), shows a consistent and significant progressive elevation with increasing concentrations of CuO-NPs, thereby validating the conclusion that ROS generation and further cellular damage translate into systemic harm. At the highest concentration of 100 mg/L, all scores show convergence near the maximum value of 9.0 to 10.0; generally, Genotoxicity and Oxidative Stress have the highest score, underlining their role as the main mechanistic drivers of toxicity. Such close correlation across distinct biological levels-from the molecular level of oxidative stress to the functional level of behavior-provides robust evidence that CuO-NPs pose a considerable and integrated risk to the health and fitness of aquatic organisms and calls for urgent regulation by environmental agencies.

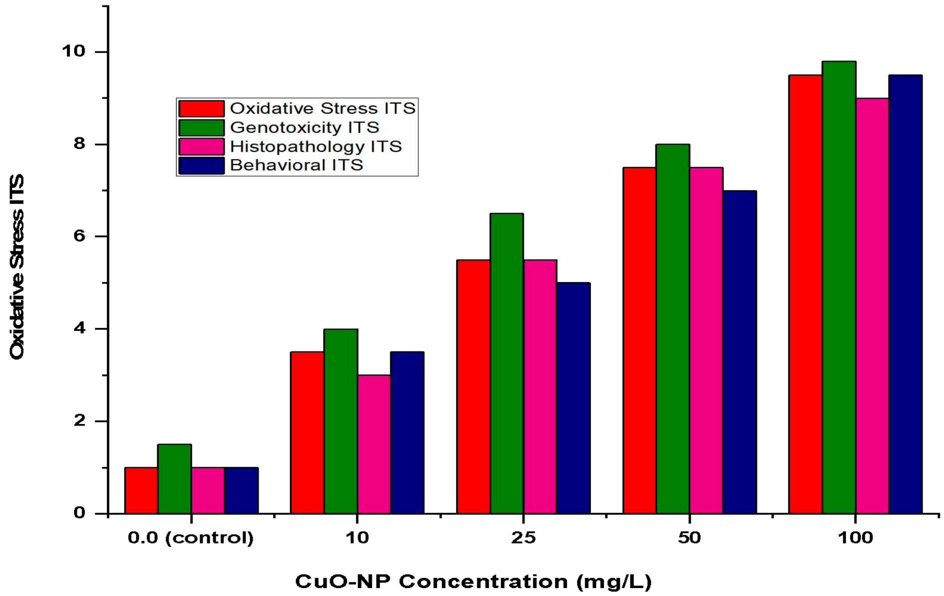

Figure 4.11: Integrated toxicological scores of biomarkers in fish. Comparative studies concerning oxidative stress, genotoxicity, histopathology, and behavioral alterations provide a deeper understanding of the systemic physiological impact caused by exposure to CuO NPs in the aquatic model.

5 Conclusion

The paper gives a comprehensive and validate evaluation of the ecotoxicological risks of copper oxide nanoparticles (CuO-NPs) in water, using fish as a delicate biological indicator. The thorough physicochemical characterization of the product confirmed the formation of crystalline and phase-pure CuO-NPs with a positive surface charge and a disposition to be deposited in nano-aggregates within aqueous media, both of which directly affect environmental behavior and interactions between the product and the body. This bioaccumulation triggers the chain of negative physiological reactions. The main pathogenesis of the toxic effect lies in the fact that it causes a severe oxidation state, overloading of the antioxidant defense system, and loss of lipid peroxidation and cell membrane damage. This molecular damage progresses to the genetic extent because there is a significant rise in genotoxic indicators such as the presence of micronucleus that demonstrate the loss of DNA integrity and chromosome alterations. This is followed by the biochemical and genetic injuries that translate to devastating histopathological changes in the gills, liver, and kidneys which impact important functions, including respiration, metabolism, and excretion. The combination of such biomarkers of oxidative stress and genotoxicity with histopathology and behavioral changes depicts a consistent and frightening account of system-wide toxicity. Thus, the present study supports the idea that even with presumably low concentrations, CuO-NPs are a great danger to aquatic biota. These results highlight the pressing necessity of designing strict regulatory frameworks and environmental monitoring principles to reduce the ecological risk due to the further release of engineered nanoparticles into water bodies.

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