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Effect of waterborne copper oxide nanoparticles and copper ions on guppy (Poecilia reticulata): Bioaccumulation and histopathology

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Original Article

Abstract

The aim of the present study was to investigate the effect of copper oxide nanoparticles (CuO-NPs) and copper ions (Cu^{++}) on guppy (Poecilia reticulata), in order to assess Cu uptake in the gill, and histopathology of gill and intestinal organs in semi-static regimes for 10 days. Guppy fish were assigned into three groups; one control group, and two experimental groups receiving 20 µg/l of either Cu^{++} or CuO-NPs in a semi-static aqueous culture for 10 days. Gill and intestinal tissue samples were obtained under a standard protocol for further histopathological examinations. The notable alterations observed in gill tissues in the experimental groups were aneurism, fusion, gill epithelial hyperplasia, increased mucous secretion, and necrosis. Noticeable anomalies in intestinal tissue were increase in the number of goblet cells, swelling of goblet cells, degeneration, vacuolation, necrosis, and erosion. Moreover, copper accumulation in gill tissue in the Cu^{++} treated group was higher than that in the CuO-NPs treated group. In contrast, the severity of histopathological damages in gill and intestinal tissues was greater in the CuO-NPs experimental group.

KEYWORDS: Gills, Goblet cells, Hyperplasia, Nanoparticles, Copper

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Introduction

Copper (Cu) in low amounts is considered as an essential micronutrient to all living organisms because it acts as a cofactor for various enzymes responsible for performing essential metabolic activity.[1] However, excessive concentrations of copper in aquatic ecosystems can exert adverse toxicological effects on freshwater organisms such as fish.[2] In the last decade, several studies reported that waterborne exposure to soluble Cu can induce endocrine disruption and affect metabolic rates,[3,4] oxidation stress, cell apoptosis, immune responses,[5] swimming behavior,[6] histopathology,[4,7] growth parameters, digestive enzymes, and body composition.[7] In recent decades, copper oxide nanoparticles (CuO-NPs) have found a wide spectrum of applications such as gas sensors,[8] catalytic processes,[9] solar cells and lithium batteries,[10] face masks, wound dressings, and socks.[11,12] There is a growing concern that these products and their byproducts may

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discharge hazardous biochemical particles into aquatic habitats which in turn can affect their biota.

Several studies have been conducted on the toxicity of copper and copper oxide nanoparticles to aquatic organisms, and these studies have shown that these substances can be toxic to aquatic organisms such as fish.[1,3,5,7] Wang et al. reported the toxic effect of either Cu-NPs or $CuSO_4$ exposure on juvenile Epinephelus coioides.[5] They found that either form of Cu exposure inhibited in digestive enzyme activities contribute to the diminished growth performance of Epinephelus coioides. In another study, Abdel-Khalek et al. studied the toxic effects of CuO [bulk particles (BPs) and nanoparticles (NPs)] at various concentrations and concluded that it can induce biochemical alterations and oxidative stress in Nile tilapia (Oreochromis niloticus).[13] Moreover, Shaw et al. showed significant Cu accumulation in gill over time after treating fish with Cu and $CuSO_4$ treatments.[14] They reported similar toxic effects for Cu-NPs and $CuSO_4$.

Fish are frequently used in toxicological studies as a biological indicator. Fish are sensitive to many variables in their environment; hence, they play a significant role in assessment of water quality.[15] In this regard, due to its unique characteristics, gill tissue is used routinely in aquatic toxicology studies. This tissue becomes the first target of waterborne NPs due to its direct contact with the external environment and the large surface area of gill exposed to the pollutants, as well as the main place for copper uptake.[16-19] Therefore, our study purposed to further investigate the effects of copper oxide nanoparticles (CuO-NPs) and copper ions (Cu^{++}) on guppy (Poecilia reticulata) to compare CuO-NPs and Cu^{++} bioaccumulation in the gill, and determine the histopathology of intestine and gill of guppy.

Materials and Methods

The CuO-NPs (CuO purity: 99+٪, 40 nm) used in this study were produced by US Research Nanomaterials, Inc. (3302 Twig Leaflane, Houston, TX77084) and purchased from Nanosany Co. (Mashhad, Iran). The purity, and morphology and mean unaggregated particle diameters of CuO-NPs were determined through transmission electron microscopy (TEM) and scanning electron microscopy (SEM), respectively (Figure 1). The other characteristics of CuO-NPs were 20 m^2/g specific surface area (SSA), 6.4 g/cm true density, and 0.79 g/cm bulk density. Moreover, the copper ions used in the form of cupric sulphate ($CuSO_4$ $5H_2O$) were produced by BDH Chemical Ltd Poole, England.

Guppy (Poecilia reticulata) with a mean total length of 3 ± 0.4 cm and mean weight of 2 ± 0.4 g were obtained from a local aquaculture shop in Sanandaj, Iran. Prior to the beginning of experiments, guppy fish were acclimatized in 50 l tanks supplied with continuously aerated tap water (22-27 ℃) under 12-hour light/12-hour dark cycles for a photoperiod of one month. Fish were fed with commercially available fish food (Tetra) at a rate of 2% body weight per day. The characteristics of the water used for the guppy were 7.3 ± 0.3 pH, 600 ± 10 µS/cm conductivity, 5° degrees of general hardness (dGH), 25.0 ± 1 °C temperature, and 6.2 ± 0.6 mg/l dissolved oxygen content (DO).

For our evaluation, 20 µg/l of either CuO-NPs or $CuSO_4.5H_2O$ was used to reflect the actual environmental concentration.[14] Fish were divided into three groups. One group served as control, and the other two groups were treated with CuO-NPs and Cu^{++}, respectively. Within each group, three fish were randomly selected for further studies. Using a 12 l aquarium, the effect of the interventions was assessed in two periods of exposure (5 and 10 days). To minimize degradation of CuO-NPs and Cu^{++} concentrations, half of the water in the aquariums was renewed every day. Moreover, during the exposure, the tanks were aerated to prevent the propensity of aggregation. At the end of the experiment,

bioaccumulation and histopathology studies were carried out for selected specimens.

For the evaluation of bioaccumulation, on day 5 and 10, dissections were performed to isolate gill organs (2 fishes were pooled). Gill samples were digested in a solution of nitric acid (HNO_3) and perchloric acid ($HClO_4$). Samples were accurately weighed and separated into 50-ml Erlenmeyer flasks and 5 ml nitric acid (65%) was added to each sample. Before adding 2.5 ml perchloric acid (70%) to each simple, they were left out overnight to be slowly digested. Digestion was performed on the bain-marie (water bath) at 100 ^{o}C until the solutions were cleared. Then, the digested samples were diluted with 25 ml deionized water.[20,21] Finally, the concentration of Cu was measured using a Phoenix 886 flame furnace atomic absorption spectrophotometer.

For the histopathology evaluation of each experimental group and control group, 4 fish were sacrificed to remove their gills. Gills were fixed in Bouin solution and their tissues were dehydrated using a series of graded ethanol solutions and were cleared in xylene. Samples were embedded in paraffin wax and portions of 5 µm were prepared from paraffin blocks using a rotary microtome. These portions were then stained with haematoxylin-eosin and examined microscopically.[22] The diameter and length of secondary gill lamellas as well as diameter of gill filaments were measured using the Axio Vision software (Release 4.8.2, Zeiss, Germany).

The SPSS software (version 16, SPSzA S Inc., Chicago, IL, USA) was used for data analysis. To compare the mean of copper concentration accumulated in the gill tissues between treatment groups one-way analysis of variance (ANOVA) was applied. Data were log transformed to meet the homogeneity of variance required by ANOVA. Ethical principles and animal rights were applied in this research and the study was approved by the Ethics Committee of the Kurdistan University of Medical Sciences, Iran (MUK.REC.1393.98).

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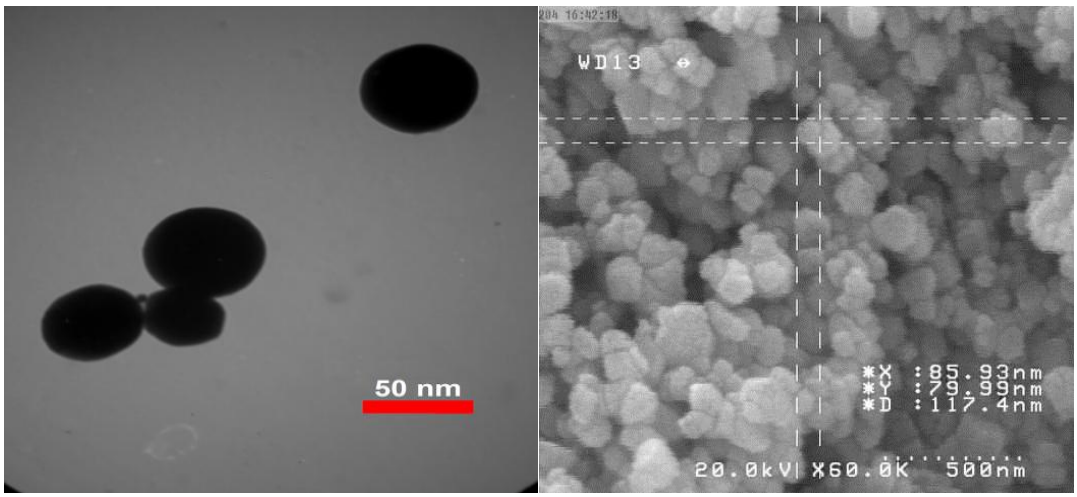

Figure 1. Transmission electron microscopy (TEM) (left) and scanning electron microscopy (SEM) (right) images of tested CuO nanoparticles

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Results and Discussion

For treatment groups, the accumulated copper in the gills of fish samples are given in table 1. Results are shown in the form of means ± standard deviation (SD). It appears that the Cu uptake was higher in the group treated with Cu^{++} than the CuO-NPs group, but the difference was not statistically significant at nominal 5% level (one-way ANOVA, $P > 0.05$). However, the accumulated copper in the gills of fish treated with either Cu^{++} or CuO-NPs were significantly higher than that observed in the control group (one-way ANOVA, $P < 0.05$).

Table 1. Copper accumulation in the gill of guppy following 5 or 10 days exposure to 20 µg/l of CuO-NPs and Cu^{++}

Tissue	Gill	
Groups	**5 days**	**10 days**
CuO-NPs	0.93 ± 0.01*	1.45 ± 0.11*
Cu^{++}	1.11 ± 0.13*	1.53 ± 0.12*
Control	0.24 ± 0.07**	0.20 ± 0.04**
P**	0.05	0.05

* In each column, the numbers with different letters differ significantly ($P > 0.05$); ** One-way ANOVA

The predominant gill responses to CuO-NPs and Cu^{++} exposure were an aneurism, fusion, gill epithelial hyperplasia, increased mucous secretion, and necrosis (Figure 2). Anomalies were also observed in the intestinal organs including increase in the number of goblet cells, swelling of goblet cells, degeneration, vacuolation, necrosis, and erosion (Figure 3). The results presented in tables 2 and 3 illustrate that the severity of damages to the gill (manifesting as fusion and necrosis) and intestine (manifesting as degeneration and necrosis) in the CuO-NPs treatment group was greater than those in the Cu^{++} group.

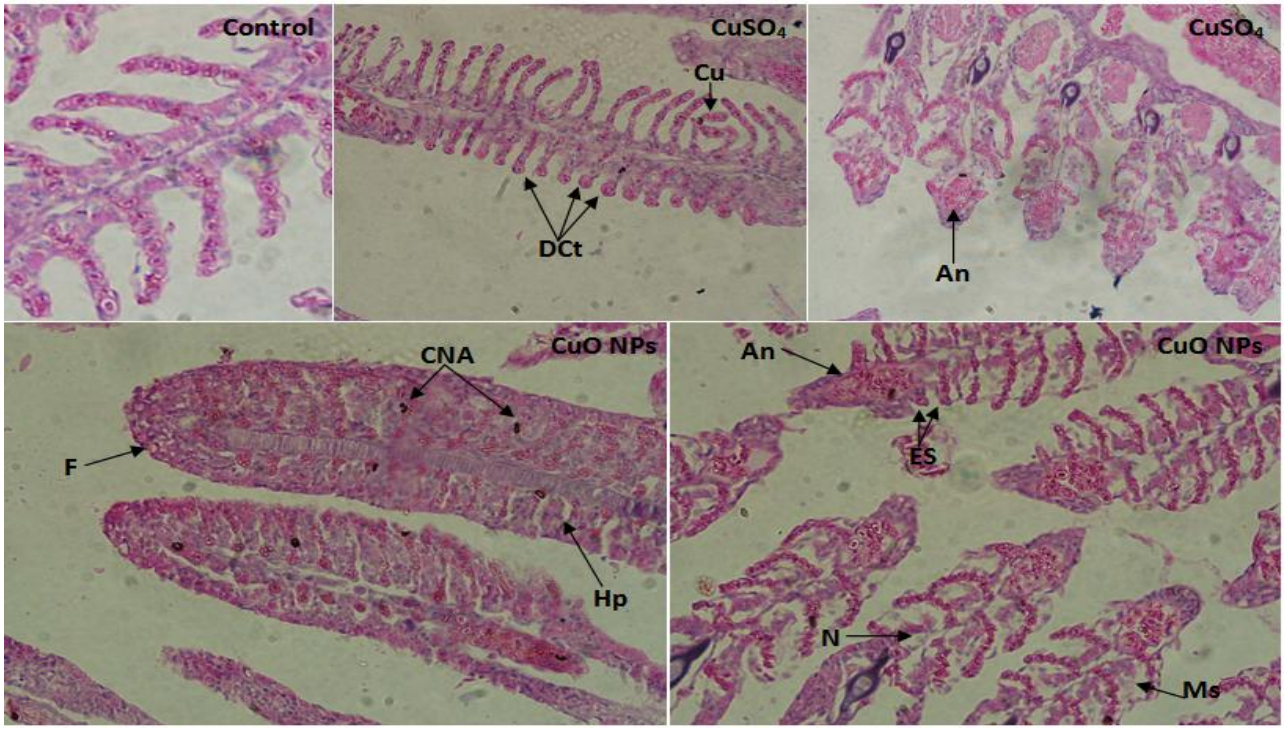

Figure 2. Histological alterations in the gills of guppy after 10 days of exposure to CuO-NPs and Cu^{++} (x40)
Gills of fish in the control group showed only some minor histopathological alterations, whereas treatment groups showed injuries including aneurism (An), dilated and clubbed tips (DCt), hyperplasia (Hp), epithelium shortening (ES), curvature (Cu), fusion of lamellae (F), increased mucous secretion (Ms), and necrosis (N).

Table 2. Summarized histopathological effects of 20 µg/l CuO-NPs and Cu^{++} on the gill of guppy

Damages / Groups	An	DCt	Hp	N	ES	Cu	F	Ms
Exposure								
Control	+	+	+	-	-	+	-	-
CuO-NPs	+++	+	+++	++	++	-	++	++
Cu^{++}	++	++	+	+	+	++	+	+

None (−), mild (+), moderate (++) and severe (+++)
An: Aneurism; DCt: Dilated and clubbed tips; Hp: Hyperplasia; ES: Epithelium shortening; Cu: Curvature; F: Fusion of lamellae; Ms: Mucous secretion; N: Necrosis

The uptake potential of nanoparticles by aquatic organisms is one of the most important factors in assessing the toxicity of nanoparticles. The uptake of nanomaterials by the body of aquatic organisms depends on several parameters such as NPs size and shape, species, organs, route of exposure, environmental conditions, exposure duration, and exposure concentration.[19,23] We found a consistent trend towards Cu accumulation in gill tissue over time as well as higher Cu^{++} uptake compared to CuO-NPs. Bioaccumulation of nanoparticles such as CuO-NPs, ZnO-NPs, and TiO_2-NPs by fish and other aquatic species has been reported previously.[19,23,24] Accumulation of pollutants in gill tissue occurs as the result of competing rates between chemical accumulate and depurate. Hence, bioaccumulation of CuO-NPs and Cu^{++} can occur when the rate of accumulate is higher than the rate of depurate. Bioaccumulation of metal oxide NPs and other pollutants in gill tissue suggests that fish can be used as an appropriate indicator to assess pollutants in aquatic environment.[24-26] Gills have a large surface area, and thus, can greatly accumulate CuO-NPs and Cu^{++}.

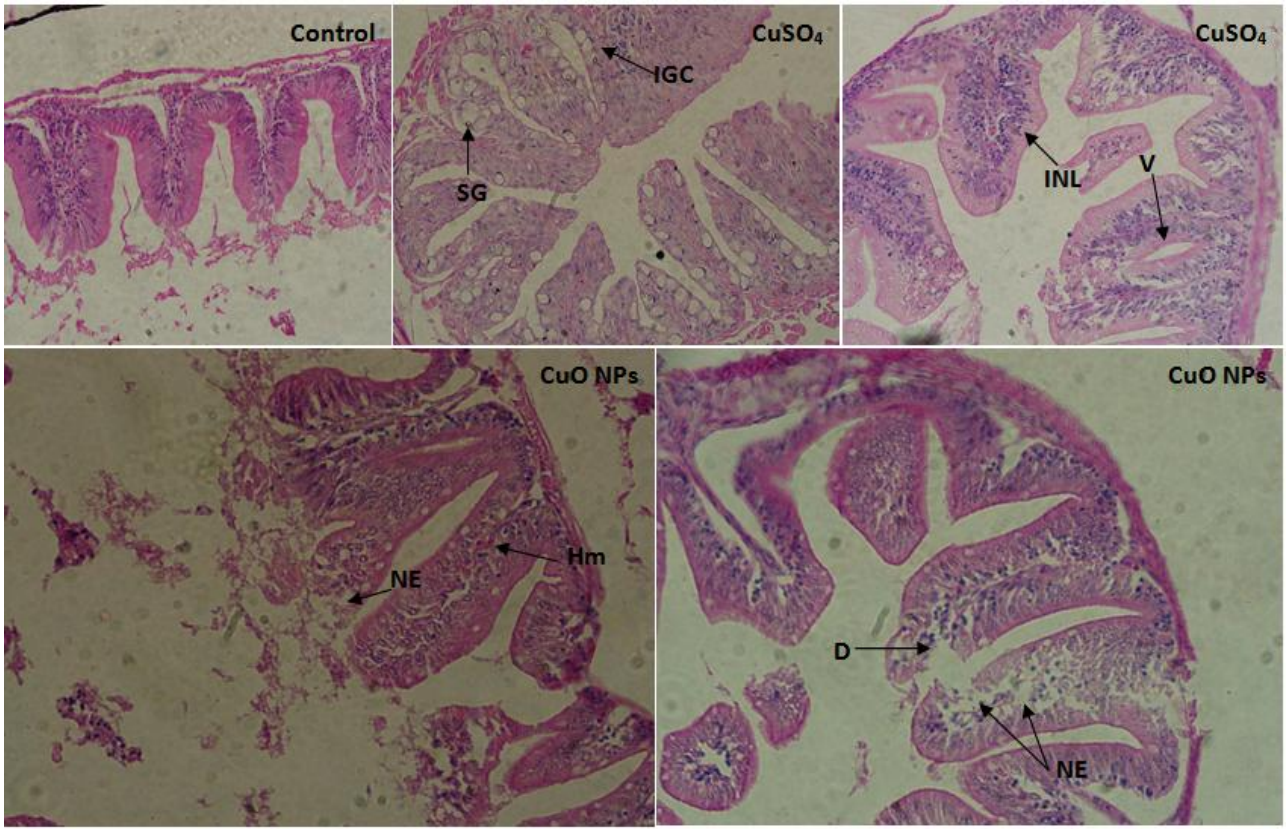

Figure 3. Histological alterations in the intestine of guppy after 10 days of exposure to CuO-NPs and Cu^{++} (x40)

The intestine of fish in the control group indicated only some minor histopathological alterations, whereas treatment groups showed injuries including degeneration (D), vacuolation (V), necrosis and erosion (NE), increase in the number of goblet cells (IGC), swelling of goblet cells (SG), hemorrhage (Hm), and increase in the number of lymphocyte (INL).

Table 3. Summarized histopathological effects of 20 µg/l CuO-NPs and Cu^{++} on the intestine of guppy

Damages / Groups	D	V	NE	IGC	SG	Hm	INL
Exposure							
Control	-	+	-	-	-	+	-
CuO-NPs	++	+	+++	+	+	+	+
Cu^{++}	+	++	+	++	+++	+	++

None (−); mild (+); moderate (++); severe (+++)

D: Degeneration; V: Vacuolation; NE: Necrosis and erosion; IGC: Increase in the number of goblet cells; SG: Swelling of goblet cells; Hm: Hemorrhage; INL: Increase in the number of lymphocyte

Histopathological changes are used to detect the effects of toxic substances on the organs of organisms.[27] Histopathological alteration in the gill may lead to the impairment of several functions, including respiration, osmoregulation, acid-base balance, and excretion of metabolite. Thus, gill histopathology appears to be a good biomarker for evaluating the effects of environmental stress on fish.[28,29] In the present study, aneurism, mucus, hyperplasia, and fusion of the filament lamellae were observed in the gills of guppy in both $CuSO_4$ and CuO-NPs experimental groups. Similar histopathological alterations have been reported as a result of exposure to copper sulphate[30,31] and copper nanoparticles[18] in other organisms. Al-Bairuty reported that exposure to copper nanoparticles resulted in edema, lamellar fusion, clubbed tips, and hyperplasia, aneurisms, and necrosis in the secondary lamellae of the gill filaments of

rainbow trout.[18] In the present study, significant differences were observed in gill histology of guppy between nanoparticulate and soluble forms of copper. The severity of histopathological lesions in the gill of guppy, such as aneurism, hyperplasia, fusion of lamellae, mucous secretion, and necrosis, was higher in the CuO-NPs treatment group than Cu^{++} treatment group. Moreover, exposure to CuO-NPs resulted in significantly greater thickening of the gill filaments. Griffitt et al. suggested that the effects of Cu-NPs and gill morphology are due to a combination of dissolution and particulate effect.[32] Cu-NPs exposure causes similar gill copper burdens as soluble copper exposure, this suggests that Cu-NPs is acting outer to the gill.

This study showed that CuO-NPs can cause lamellar fusion in gills. Lamellar fusion is a defence mechanism of fish gill that reduces total respiratory area when it is in contact with an external environment. This alteration could cause a decrease in oxygen-uptake for total metabolic activities, hence affecting the general health of fish.[27,33] Blanchard and Grosell[34] showed that exposure to copper can cause iono-regulatory impairment in fish due to its inhibitory effect on Na^+/K^+-ATPase activity causing proliferation of interlamellar cells in fish.[17] Moreover, studies suggest that the increased mucous secretion and hyperplasia of lamellae can make a barrier for NPs accumulated by gills and increase the diffusion distance for gas exchange.[5,35] In this study, we found aneurism to be a common alteration in gill resulted from exposure to both Cu^{++} and CuO-NPs. This is because of collapsing pillar cells in the secondary lamellae and swelling blood vessels and disturbances in blood flow in the gills.[33] It can therefore be concluded that necrosis of gill in guppy is the direct deleterious effect induced by CuO-NPs. This indicates that, for these forms of alteration, CuO-NPs are considerably more toxic than their sulphate equivalents.

The intestinal tissue is another main absorption site for chemical toxicants, such as nanoparticles, in fish. The intestine has the ability to uptake toxicants from ambient water[36] and may contribute to distribution of NPs in fish. This study showed histopathology lesions in intestinal organs including vacuolation, increase in the number of goblet cells, and swelling of goblet cells in the Cu^{++} treatment group, and degeneration, necrosis, and erosion in the CuO-NPs group. Perera and Pathiratne reported that Nile tilapia in the presence of TiO_2-NPs developed intestinal pathologies such as erosion of the villi epithelium, decline in mucous cells, and degeneration of the intestinal mucosa.[27] Federici et al. demonstrated that 14 days of exposure of rainbow trout to TiO_2-NPs caused several histopathological lesions in the intestine such as erosion of the villi, and fusion and vacuolation of the intestinal mucosa.[37] Both gill–blood route and intestine–blood route might contribute to the distribution of NPs in fish.[38] Nevertheless, to obtain a good knowledge of the distribution of NPs in organs of aquatic organisms and its mechanism in animals requires further studies.

Conclusion

In the present study, the effects of CuO-NPs and Cu^{++} were assessed on copper bioaccumulation in gill as well as histopathology of gill and intestine of guppy. The findings indicated that the Cu accumulation in the gill tissue was higher in the Cu^{++} exposure group than the CuO-NPs treatment group. However, the difference in the amount of copper uptake by the gill tissue between guppy fish in the Cu^{++} and CuO-NPs groups was not statistically significant. However, the severity of gill and intestine damages in the CuO-NPs exposed fish was higher than the Cu^{++} exposed fish. Therefore, it is recommended to be considered in toxicological study assessments in an aquatic environment.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

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