

Research Article

Induction of micronuclei in blood and histopathological alterations in gill, kidney and liver of *Channa punctatus* (Bloch, 1793) exposed to copper sulphate

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Abstract

Copper is one of the most toxic metals to fish and causes cytotoxic, mutagenic and carcinogenic effects. The accumulation of copper in an aquatic environment directly impacts man and the aquatic ecosystem. The present study aimed to determine the effect of copper sulphate (CuSO₄) accumulation on the induction of micronuclei in blood and histological changes in kidney and liver of the fish *Channa punctatus*. The test chemical LC₅₀ was determined after 96 hours during the first experiment. Later in the second experiment, the test animal was exposed to three sub-lethal concentrations of CuSO₄ [0.1 mg/l (96 h-LC₅₀/40), 0.2 mg/l (96 h-LC₅₀/20) and 0.4 mg/l (96 h-LC₅₀/10)]. Physico chemical parameters of the test medium, such as pH, temperature, hardness, alkalinity, and dissolved oxygen, were observed throughout the experiment, and all values were within the ranges necessary for the fish's survival. At intervals of 7, 14, 21, and 28 days, the control (without any test chemical) and copper sulphate exposed Groups' blood, liver, kidney, and gill tissues were taken to evaluate changes in genotoxic and histological parameters. Micronuclei (MN) induction and nuclear abnormalities (NAs) were observed at regular intervals. After 28 days, the MN frequency in Groups 1, 2, 3, and 4 was 0.78±0.006, 8.40±0.052, 10.37±0.098 and 10.90±0.024 respectively. A significant (P< 0.05) rise in MN frequencies and NAs indicated fish erythrocytes' DNA damage. Histological analysis of the liver, kidney, and gills revealed serious tissue injury such as necrosis, vacuolization, and degeneration after 28 days in the exposed Groups. The present study observed changes due to genotoxicity and histology, giving the most comprehensive understanding of CuSO₄ stress on the fish *C. punctatus* and its risks to human health.

Keywords: *Channa punctatus*, Copper, Micronuclei, Necrosis, Nuclear abnormalities

INTRODUCTION

Copper, an essential micronutrient vital for the well-being of all living organisms, plays a crucial role in a wide range of cellular functions. It acts as a necessary cofactor for antioxidant enzymes, aids in neurotransmitter production, and supports cellular respiration. These functions collectively underpin critical biological

processes (Jomova *et al.*, 2022); when obtained from the environment, either through diet or water intake, copper binds to α-globulin and is subsequently distributed throughout various body tissues (Mhaske and Shukla, 2023). Copper is required in very low (5-20 µg/g) concentrations (Kumar *et al.*, 2020), but it becomes toxic when it exceeds more than 20 µg/g, can harm fish, shellfish, and other aquatic organisms (Maurya *et*

al., 2019). Copper enters the aquatic world through many ways, such as mining and metallurgical activities, Urban runoff which includes stormwater and wastewater discharges from urban areas, copper-containing pesticides and fungicides used in agriculture, industrial processes such as electroplating and wastewater treatment (De Zwart et al., 2018; Rehman et al., 2019; Chowdhary et al., 2020; Elalfy et al., 2021). The persistent presence of copper, its resistance to biodegradation, and its potential to trigger the formation of harmful free radicals, teratogenic effects, and irregularities in chromosomal structure, all contribute to significant environmental and health risks (Mitra et al., 2022; Lawrence and Hemingway, 2008). Ingesting excessive amounts of copper has been associated with a range of negative health outcomes, including intravascular hemolysis, liver cirrhosis, rapid heart rate, and acute kidney failure (Karim, 2018).

Release of excessive copper sulphate into water sources can seriously harm the quality of the water. This makes it difficult for aquatic animals to live. Fishes are the most common part of aquatic ecosystems, and their health indicates the overall health of those who consume them. They are highly sensitive to changes in their surrounding environment. Monitoring their health and survival can provide early warning signs of potential health problems (Lieke et al., 2020). *Channa punctatus* called "poor man's" fish because of its excellent nutritional value and affordability. It exhibits a broad range of toxin affectability and is often recommended as a crucial model for eco-toxicogenomic testing (Trivedi et al., 2022). Gradually, when copper builds up in this organism over time, it starts causing a serious threat. This includes harmful changes in their haematological parameters, micronucleus formation and nuclear abnormalities. Moreover, different body organs, like the liver, kidneys, and gills, go through histological changes (Javed and Usmani, 2019). That is why it is important to bio-monitor aquatic systems at regular intervals. Thus, the present study aimed to determine the effect of copper sulphate (CuSO_4) on micronuclei induction and histological changes in the kidney and liver of *C. punctatus*.

MATERIALS AND METHODS

Test chemical

Analytical grade copper sulphate (CuSO_4), manufactured by Central Drug House (CDH) Pvt Ltd, New Delhi - 110002, was used for the experimental work.

Experimental animals and its acclimatization

Freshwater fish *C. punctatus* was obtained from Gomti River, Lucknow (latitude 26.84° N and longitude 80.94° E) in well-aerated, wide-mouth plastic tanks with water from the collecting site to reduce stress. The fish were

treated with a 0.05% KMnO_4 solution for 4-5 minutes to prevent cutaneous infection. They were kept in 10 days of pre-filled tap water in tanks (APHA, 2017) whose carrying capacity was 120 litres to maintain 4g/l body weight of fish. Before the experiment began, the fish were acclimated for 15 days and kept in aerated, dechlorinated water under natural photoperiod (12 hours light:12 hours dark) and laboratory settings (temperature 26°C). Water was changed regularly to remove waste metabolites. During acclimatization, fish were fed twice a day with artificial fish food called "Optimum" (produced by Perfect Companion Group Co., Ltd., Thailand). One day before the experiment began, the randomly selected fishes, which were approximately 15±2 cm long and weighed 35±5 g, were kept without feeding until the experiment ended (OECD, 2019).

Ethical approval

According to the guidelines set forth by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), the University of Lucknow, in Lucknow, established an Institutional Animal Ethics Committee (IAEC) with registration number 1861/GO/Re/S/16/CPCSEA. The authors experimented with the work following the CPCSEA's specified protocols.

Estimation of LC₅₀ Value of Copper sulphate

The fish were exposed in ten aquariums (consisting 10 fish in each Aquarium) with 10 different concentrations of CuSO_4 (3.00, 3.20, 3.40, 3.60, 3.80, 4.00, 4.20, 4.40, 4.60, 4.80 mg/l) on a logarithmic scale up to 96 h. to estimate the test chemical's approximate hazardous range. Fish deaths were noted after 24, 48, 72, and 96 hours, and the corpses were removed from the Aquarium right away. Based on mortality, the toxic range of CuSO_4 was determined between 3.88 and 4.88 mg/l. Again 10 nominal concentrations (4.00, 4.01, 4.02, 4.03, 4.04, 4.05, 4.06, 4.07, 4.08, and 4.09 mg/l) ranging in logarithmic series were used to establish the precise LC₅₀ value of CuSO_4 after determining the approximate hazardous range. The proportion of fish deaths was recorded up to 96 hours after exposure. To ensure that experiments could be reproduced, they were run three times. CuSO_4 96-hour LC₅₀ value and their 95% upper and lower confidence limits were calculated using the Trimmed Spearman-Kärber Method (Hamilton et al., 1977).

Experimental setup

After getting the LC₅₀ value of CuSO_4 , one hundred and forty-four (144) healthy fish *C. punctatus* were divided into 4 Groups with three replicas of each separately with 12 fish in each Aquarium. Group 1 served as control without any exposure, whereas Group 2 was exposed with 0.1 mg/l of CuSO_4 (96 h-LC₅₀/40), Group 3

with 0.2 mg/l of CuSO₄ (96 h-LC₅₀/20) and Group 4 with 0.4 mg/l of CuSO₄ (96 h-LC₅₀/10). No deaths were noticed during the investigational period of 28 days. One fish from each replicate was anaesthetized with 0.01% (v/w) diethyl ether and dissected lengthwise from the ventral side, where the necessary organs, such as the liver, kidney, and gills as well as blood, were collected for the genotoxic and histological study. This was done after each desired period of exposure (7, 14, 21 and 28 days).

Physico-chemical parameters

The physicochemical analysis of water throughout the experiment followed the standard methods (APHA, 2017). During the experimental period, pH, temperature (°C), hardness (mg/l), alkalinity (mg/l) and dissolved oxygen (mg/l) were estimated with their respective standard methods.

Micronuclei (MN) Induction and Nuclear Abnormalities (NAs)

Genomic instability was measured by micronuclei induction (Alimba and Laide, 2019). Slides that had already been washed were smeared with a drop of blood from the caudal region. All night, the smeared slides were left at room temperature. Following a 5-minute fix in absolute methanol, slides were stained for 3 and 5 minutes, respectively, with May-Grunwald's stain solutions 1 and 2 (0.125%; Himedia). The slides were now counterstained for 30 minutes with 5% Giemsa (produced in phosphate buffer, pH 6.8; Sigma Aldrich, USA) and then mounted in PX (a compound made of distyrene, plasticizer, and xylene). Two thousand erythrocytes were examined from each slide under light microscope (Nikon Corporation in Tokyo, Japan) and MN frequencies were calculated (MN% = Number of erythrocytes containing micronucleus × 100 / Total erythrocytes counted). Apart from the micronuclei induction, other abnormalities of nuclei (NAs) were also observed in the peripheral blood cells, which were identified previously by several authors (Anbumani and Mohankumar, 2011; Shahjahan *et al.*, 2020; Sarangi, 2021).

Histology

The required organs, such as the kidney, liver, and gills, were removed and rinsed in saline (0.9% NaCl) solution to eliminate pollutants. Tissues were first fixed for 48 hours in Bouin's fluid, and then for 4-5 days, they were washed twice daily with 70% ethanol to eliminate any remaining Bouin's fluid. Various ethanol concentrations (50%, 70%, 90% and 100%) were used to dehydrate the tissues. The tissues were washed in Xylool for 30 minutes, dipped in liquid paraffin wax heated to 60° C, and embedded in blocks. The sample blocks were cut into slices of 5 µm thickness using a rotatory microtome (Biocraft and Scientific Industries). Sections were

mounted on slides that had already been cleaned, flattened on a heated plate, stained with haematoxylin for a minute, and then counterstained with eosin for two minutes. After proper preparation, sections were mounted with DPX (Trivedi *et al.*, 2022). The images were captured using an oil immersion microscope from the Nikon Corporation in Tokyo, Japan, with a 40X objective lens magnification. Image J software was used to execute image analysis techniques.

Statistical analysis

Each exposure was carried out in triplicate using separate fish (N = 3) to guarantee the accuracy of the data. SPSS software (version 26.0, SPSS Company, Chicago, IL, USA) was used to analyze the data. One-way analysis of variance (ANOVA) and post hoc analysis were used to assess the results from each experiment. The Tukey-Kramer post hoc test was used to determine the Groups' significance. At P < 0.05, the findings were declared significant. The means and standard errors (SE) of the means were calculated for the Data.

RESULTS AND DISCUSSION

LC₅₀ of copper sulphate (CuSO₄)

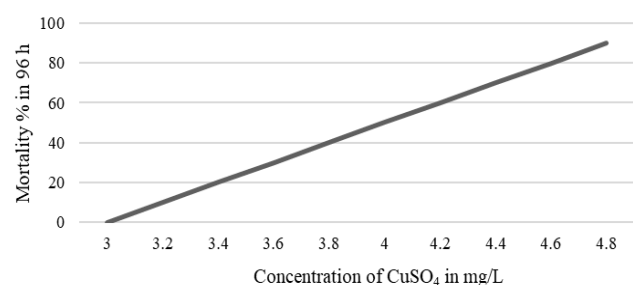
The estimated 96-hour LC₅₀ value of CuSO₄ for *C. punctatus* was 4.07 mg/l, with 95% lower and upper confidence limits of 3.88 mg/l and 4.28 mg/l, respectively, shown in Graph 1.

Analysis of physicochemical parameters

During the experimental period (i.e., on 7, 14, 21 and 28 days), analysis of physicochemical characteristics comprising temperature, DO, alkalinity, pH, and hardness made in all Groups, including the control and exposed Groups, is given in Table 1. All values were found to be within the parameters required for the fish's survival, as mentioned in APHA (2017).

Assessment of micronuclei induction and nuclear abnormalities (NAs)

After being exposed to three sublethal test concentrations of copper sulphate, MN frequencies in the eryth-



Graph 1. Showing percentage of test animal (*C. punctatus*) that died after being exposed to a range of CuSO₄

Table 1. Detailed analysis of physicochemical parameters of water or test medium

Groups	Conc. (mg ⁻¹)	Experimental time (in days)	Alkalinity (mg/l)	DO (mg/l)	Hardness (mg/l)	pH	Temp. (°C)
Group 1	Control	07	77.50±0.05	6.76±0.03	74.50±0.17	7.13±0.03	27.40±0.15
		14	76.30±0.10	6.66±0.03	74.56±0.08	7.16±0.03	26.40±0.05
		21	75.30±0.05	6.63±0.03	73.43±0.12	7.13±0.06	26.03±0.12
		28	73.80±0.05	6.66±0.03	73.36±0.03	7.16±0.08	25.46±0.08
Group 2	mg ⁻¹ (LC ₅₀ /40)	07	71.13±0.03	6.46±0.03	72.73±0.08	6.76±0.03	27.63±0.12
		14	69.40±0.05	6.13±0.03	71.56±0.12	6.66±0.03	26.30±0.05
		21	68.70±0.05	6.60±0.05	69.70±0.05	6.63±0.03	25.90±0.11
		28	68.16±0.03	6.20±0.05	68.50±0.05	6.46±0.03	25.46±0.06
Group 3	mg ⁻¹ (LC ₅₀ /20)	07	73.16±0.03	6.43±0.03	73.33±0.06	6.83±0.03	27.53±0.23
		14	72.36±0.03	6.50±0.05	73.70±0.05	6.73±0.06	26.60±0.15
		21	71.73±0.03	6.40±0.05	72.46±0.06	6.70±0.05	25.80±0.05
		28	71.46±0.03	6.20±0.05	71.66±0.03	6.60±0.05	25.33±0.18
Group 4	0.4 mg ⁻¹ (LC ₅₀ /10)	07	75.40±0.05	6.46±0.03	74.30±0.11	6.86±0.03	27.46±0.08
		14	74.80±0.05	6.36±0.03	73.36±0.03	6.80±0.05	26.70±0.05
		21	74.16±0.03	6.56±0.03	73.26±0.03	6.73±0.03	25.93±0.08
		28	73.53±0.03	6.13±0.03	72.30±0.05	6.76±0.03	25.33±0.12

rocytes of *C. punctatus* exhibited a significant difference ($P < 0.05$). After each exposure period of 7, 14, 21 and 28 days, the frequencies of MN in Group 1 or the control were noted as 0.78 ± 0.003 , 0.78 ± 0.003 , 0.79 ± 0.000 and 0.78 ± 0.006 . Group 2 exposed with 0.1 mg/l of CuSO_4 were estimated as 5.19 ± 0.048 , 6.17 ± 0.100 , 7.04 ± 0.054 and 8.40 ± 0.052 after each exposure period. Group 3 treated with 0.2 mg/l of CuSO_4 , showed the frequencies as 7.27 ± 0.083 , 7.83 ± 0.064 , 9.03 ± 0.045 and 10.37 ± 0.098 while Group 4, with highest 0.4 mg/l of concentration recorded the MN frequencies as 8.17 ± 0.043 , 9.53 ± 0.050 , 10.00 ± 0.079 and 10.90 ± 0.024 after the given exposure period. Following 28 days, Group 4 had the highest frequency (10.90 ± 0.024) of MN. Additionally, among all exposed Groups, there were significant ($P < 0.05$) differences in MN frequencies (Fig. 1; Graph II). To enhance the comprehensiveness and standardization of the micronucleus (MN) assay, a concurrent assessment of nuclear anomalies was conducted on the blood cells of the experimental species *C. punctatus*, as depicted in Figure 2. Various nuclear irregularities were identified, including (Fig. 2b) nuclear buds (NB) characterized by the presence of

micronucleus-like structures attached to the nucleus. (Fig. 2c) Nuclei exhibiting vacuoles and significant invagination, lacking nuclear material, designated as notched nuclei (NN). (Fig. 2d) Blebbed nuclei (BN) display minor invaginations in the nuclear membrane. (Fig. 2e) Karyorrhectic nuclei (KN) are marked by fragmented and disrupted nuclear structures. (Fig. 2f) Lobed nuclei (LN) presenting two or more differentiated lobes. (Fig. 2g) Shrunken nuclei (SN) indicative of reduced nucleus size due to hypoxic conditions. (Fig. 2h) Lysed nuclei (LN) display incomplete or ruptured nuclear integrity. These observations contribute collectively to a more comprehensive understanding of nuclear anomalies and their implications in *C. punctatus*.

Shah et al. (2021) have examined the effects of copper on the production of micronuclei and nuclear abnormalities in common grass carp (*Ctenopharynx godonidella Valenciennes*). After the fish were exposed to copper, the authors noticed a substantial rise ($P < 0.05$) in the frequency of micronuclei and nuclear abnormalities. In one more study by Canalejo et al. (2016), the authors examined the genotoxic effects of copper in *Dicentrarchus labrax*. The *Astyanax altiparanae* (Francisco et al.,

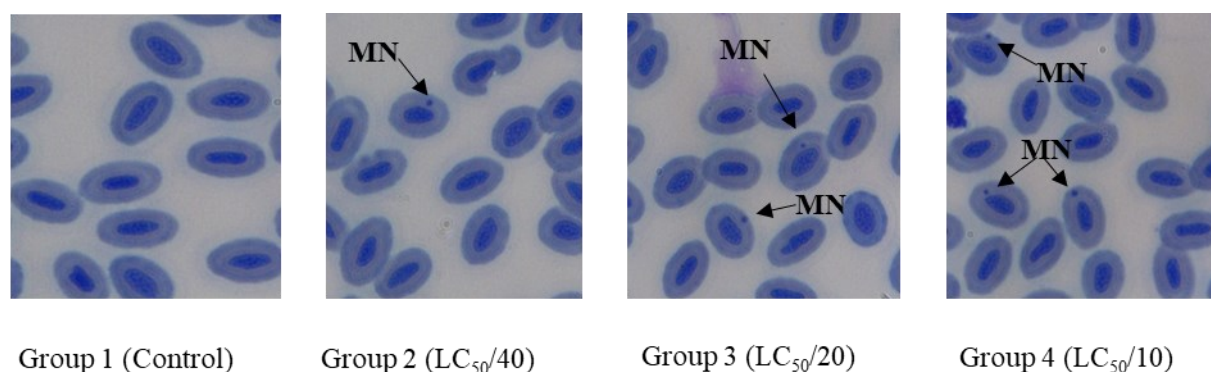
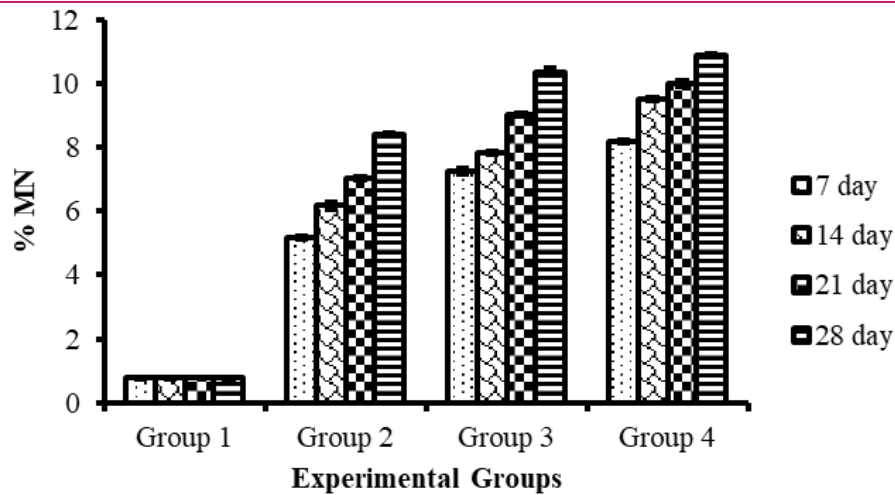


Fig. 1. Microphotograph showing Group 1 without exposure with no micronuclei (MN) formation while formation of MN in peripheral blood cells of *C. punctatus* in Group 2, Group 3 and Group 4 with exposure of CuSO_4 at 100X magnification



Graph 2. MN frequency induced by CuSO₄ in Group 2, 3 and 4 in comparison with Group 1 (control Group) for 7, 14, 21 and 28 days of exposure periods

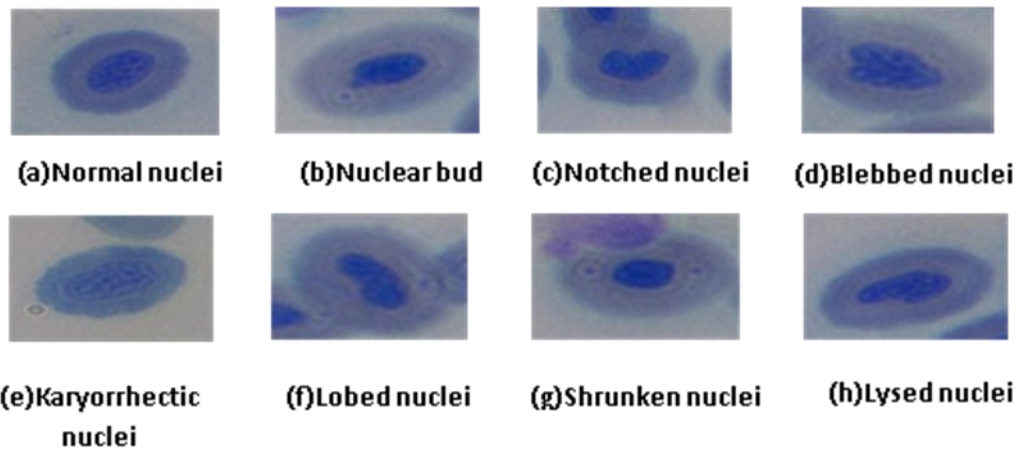


Fig. 2. Microphotograph showing nuclear abnormalities (NAs) in blood cells of *C. punctatus* in all the Groups at 100X magnification

2019), *C. punctatus* (Nagpure et al., 2015), and *On-corhynchus mykiss* (Stankevičiūtė et al., 2016) are just a few of the fish species that have been the subject of studies looking into the genotoxic effects of various heavy metals like copper, Chromium and zinc. These studies showed a large variation, indicating that copper buildup causes genotoxic consequences on fish species. The present study found a significant difference ($P < 0.05$) like observed above by authors, in the mean frequency of micronuclei following copper exposure to different concentrations of copper sulphate.

Histological analysis of kidney

The component of kidney tissue is altered by repeated exposure to CuSO₄, and the alterations in the test animal's kidney are expressed as a percentage of damage. Comparatively to the control group (Fig. 3a), the kidneys exposed to sublethal concentrations of CuSO₄ after 28 days showed multiple changes. Cavity reduction in renal tubule (CRRT) (Fig. 3b) was the most significant alteration seen in the renal tubule, while necrosis (Fig. 3c), hypertrophy (Fig. 3d) and vacuolization

(Fig. 3e) were the most frequent modifications in the tubules. Kidney tissue of Group 1 was healthy and did not show any damage. The CRRT damage percentages in Group 2 and Group 3 were 32% and 38%, respectively, while in Group 4, it was 51% after 28 days. However, the damage percentage of necrosis, hypertrophy and vacuolization (treated with the highest concentration, i.e., 0.4 mg/l of CuSO₄) was 46%, 43% and 37%, respectively, after 28 days. Among all four kinds of damage, the percentage of CRRT was the highest in the kidney tissue.

In fish, the kidney is essential for filtration, excretion, and maintaining osmotic balance. The present study aimed to look at the kidney histology in *C. punctatus* exposed to copper buildup. Numerous studies have been done on the effect of copper on the kidney histology of several fish species. Several studies have found varied degrees of damage depending on the amount and duration of copper exposure. The study by Wu et al. (2019) on the kidney histology of *Gobiocypris rarus* treated with copper showed moderate damage, including tubular necrosis, interstitial oedema, and cellular

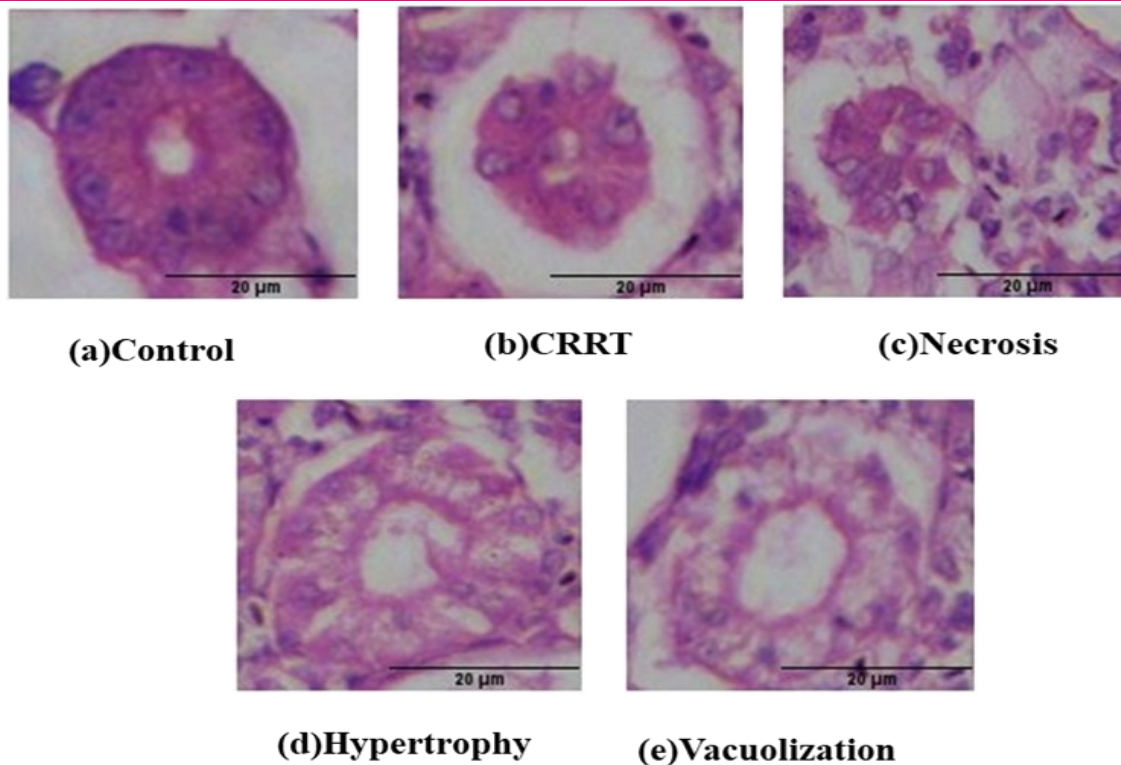


Fig. 3. Microphotographs of the kidney section taken from the exposed and control Groups of *C. punctatus*. Section of the kidney displaying the following conditions: (a) control; (b) cavity reduction in the renal tubule (CRRT); (c) necrosis; (d) hypertrophy; and (e) vacuolization brought on by CuSO_4 exposure shown at 40X

infiltration. The authors suggested that copper accumulation in the kidney might decrease renal function and ultimately lead to fish mortality. Abdel *et al.* (2021) reported kidney damage in *Oreochromis niloticus* exposed to copper. The kidney showed severe tubular necrosis and interstitial fibrosis, leading to decreased renal function. The study by Tavares-Dias (2021) on the kidney histology of fish *Labeo rohita* exposed to copper showed congestion of blood vessels, tubular necrosis of glomerulus, interstitial oedema, and cellular infiltration. The authors (Kumar *et al.*, 2023; Yu *et al.*, 2020; Malhotra *et al.*, 2020) suggested that copper accumulation might lead to oxidative stress and kidney damage. The present study is consistent with previous research on the effect of copper on different fish species. The results show multiple damages as earlier observed, such as reduction of cavity in renal tubules, necrosis where cells get injured, and hypertrophy causing tissue enlargement and vacuolization.

Histological analysis of liver

The liver's histology displays hepatocytes, or polygonal cells with rounded nuclei, which are Grouped in the liver parenchyma, blood arteries, and bile ducts. Figure 4 displays the histological findings of fish liver tissue from the *exposed and control groups of C. punctatus* after 28 days. The major modifications that originated in the liver were vacuolization (Fig. 4b), cytoplasm degeneration (Fig. 4c), pyknotic nuclei (fig 4d) and necrosis

(Fig. 4e). Liver tissue of the control Group was healthy and did not show any damage while Cytoplasmic degeneration shows the highest damage percentage among all kinds of damage. After a 28-day exposure, there was 31% necrosis in Group 2, 39% in Group 3, and 45% in Group 4. Similarly, the damage percentage of vacuolization and pyknotic nuclei in Group 2 was 15% and 10%, in Group 3 was 18% and 13%, while in Group 4, it was 23% and 16%, respectively, after the completion of the experimental period. Vacuolization, pyknosis, necrosis, and cytoplasmic degeneration were noted in all the treated Groups with an escalating pattern and dose-dependently; however, the greatest modifications were seen in Group 4, which was given the highest dosage.

Noureen *et al.* (2018) investigated the outcome of copper on the histology of the liver in *Cyprinus carpio*. They found that when fish were exposed to higher copper concentrations, liver damage, such as vacuolation, congestion, and necrosis, increased in a dose-dependent manner. Similarly, the study by Kaur *et al.* (2018) on the liver histology of *Labeo rohita* exposed to copper showed severe damage, including degeneration, necrosis, and fibrosis. It was discovered that there was a positive correlation between the concentration of copper exposure and the severity of liver damage. In contrast, the research done by Sangeetha and Aruljothi (2019) on the liver histology of common carp *C. carpio* exposed to copper showed mild damage, including vac-

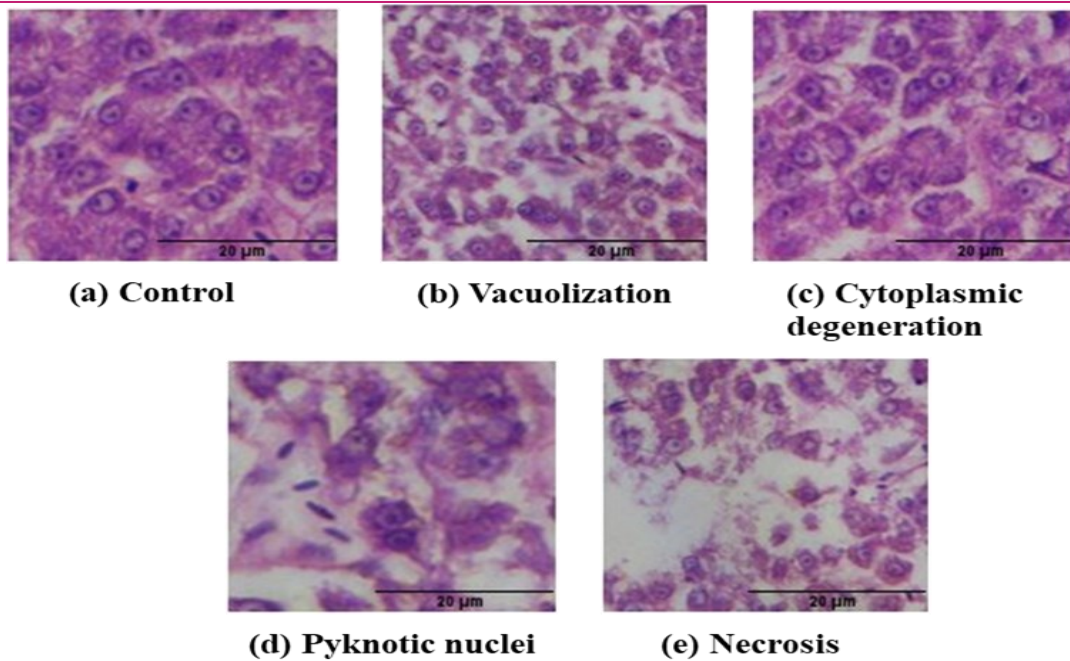


Fig. 4. Microphotographs taken from exposed Groups of *C. punctatus*'s liver segment. Liver section demonstrating (a) vacuolization, (b) Cytoplasmic degeneration, (c) Pyknotic nuclei, and (d) Necrosis induced by CuSO_4 observed at 40X

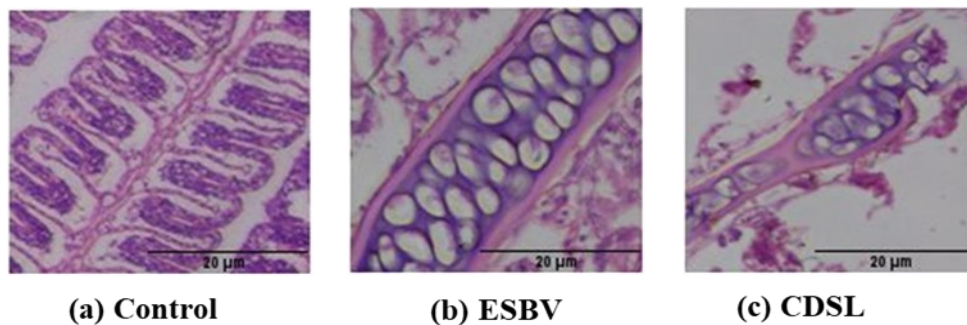


Fig. 5. Microphotographs of *C. punctatus*'s gill region taken from control and exposed Groups. Section of gills showing (a) control, (b) extreme swollen blood vessels (ESBV) and (c) complete degeneration of secondary lamellae (CDSL) observed at 40X

uolation and lipid accumulation. The authors (Zeng et al., 2020; Zebra et al., 2019) suggested that fish might have developed adaptive mechanisms to cope with copper exposure, preventing severe liver damage. During the present investigation, it was found that continuous exposure to copper sulphate leads to vacuolization, necrosis, and cytoplasmic degeneration, which causes collapsing of functioning liver cells and pyknotic nuclei where the nucleus becomes dense, compact and begins to fragment (karyorrhexis), similarly like previously observed.

Histological analysis of gill

Fish gills have a huge surface area separating the blood from water, which is essential for breathing, excretion, acid-base regulation, and osmoregulation (Murali et al., 2018). The gill tissue of control Group (Fig 5a) does not show any histological changes, while the exposed Groups show hyperplasia, oedema, Com-

plete disintegration of secondary lamellae (CDSL), and Extreme swollen blood vessels (ESBV), out of these CDSL (Fig 5c) and ESBV (Fig 5b) were the major histological alterations. Except Group 1, the other Groups (Group 2, Group 3 and Group 4 were exposed Groups with 0.1 mg/l, 0.2 mg/l and 0.4 mg/l of CuSO_4) were the exposed Groups, which showed multiple alterations in the gill tissue. CDSL was recorded at 28%, 39% and 45%, while ESBV was 31%, 38% and 42% in Group 2, Group 3 and Group 4 respectively. The damage percentage due to CDSL was higher than ESBV.

The gills of fish play a vital role in gas exchange, osmoregulation, and ion balance. Copper accumulation in fish gills can lead to damage and affect the normal functioning of the gills. A study by Aghamirkarimi et al. (2017) researched the adverse consequences of copper on the histology of gills in *Rutilus rutilus caspicus*. They found that when fish were exposed to higher concentrations of copper, there was a dose-dependent

increase in gill damage, including filament fusion, epithelial hyperplasia, and edema. Similarly, the study by Mansouri *et al.* (2017) on the gill histology of *Cyprinus carpio* exposed to copper showed severe damage, including hyperplasia, necrosis, and gill lamellae fusion. It was discovered that there was a positive correlation between the concentration of copper exposure and the degree of gill damage. Abdel *et al.* (2016) reported severe gill damage in *Oreochromis niloticus* exposed to copper. The gills showed congestion, epithelial lifting, and necrosis. The authors (Malhotra *et al.*, 2020; Ale *et al.*, 2018; Kumar *et al.*, 2015) suggested that copper accumulation in the gill might decrease respiratory function and ultimately lead to fish mortality. In the current study two types of damages were observed: first, extremely swollen blood vessels, where the blood vessels of primary lamellae got swollen and second, where complete disintegration of secondary gill lamellae were observed in the test animal *C. punctatus* due to continuous exposure of copper sulphate upto 28 days.

Conclusion

The present study concluded that copper accumulation in *C. punctatus* led to micronuclei induction, nuclear abnormalities, and histological changes in organs such as the fish's liver, kidneys, and gills. Though fish consumption has many health benefits, consuming toxic contaminated fish can harm organisms, including humans. This study highlights the potential negative effects of copper accumulation on fish health and thus emphasizes the need for effective measures to prevent such accumulation in aquatic environments. The findings can help develop strategies for the sustainable management of aquatic resources and ensure the long-term health of the fish population.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abdel-Khalek, A. A., Badran, S. R. & Marie, M. A. S. (2016). Toxicity evaluation of copper oxide bulk and nanoparticles in Nile tilapia, *Oreochromis niloticus*, using hematological, bioaccumulation and histological biomarkers. *Fish Physiology and Biochemistry*, 42, 1225-1236.
2. Abdel-Latif, H. M., Dawood, M. A., Mahmoud, S. F., Shukry, M., Noreldin, A. E., Ghetas, H. A. & Khallaf, M. A. (2021). Copper oxide nanoparticles alter serum biochemical indices, induce histopathological alterations, and modulate transcription of cytokines, HSP70, and oxidative stress genes in *Oreochromis niloticus*. *Animals*, 11(3), 652. <https://doi.org/10.3390/ani11030652>
3. Aghamirkarimi, S., Mashinchian Moradi, A., Sharifpour, I., Jamili, S. & GhavamMostafavi, P. (2017). Sublethal effects of copper nanoparticles on the histology of gill, liver and kidney of the Caspian roach, *Rutilus rutilus caspius*. *Global Journal of Environmental Science and Management*. DOI 10.22034/gjesm.2017.03.03.009Ale, A., Bacchetta, C., Rossi, A. S., Galdopórpóra, J., Desimone, M. F., de la Torre, F. R., ... & Cazenave, J. (2018). Nanosilver toxicity in gills of a neotropical fish: Metal accumulation, oxidative stress, histopathology and other physiological effects. *Ecotoxicology and Environmental Safety*, 148, 976-984. <https://doi.org/10.1016/j.ecoenv.2017.11.072>Alimba, C. G. & Laide, A. W. (2019). Genotoxic and cytotoxic assessment of individual and composite mixture of cadmium, lead and manganese in *Clarias gariepinus* (Burchell 1822) using micronucleus assay. *The Nucleus*, 62(3), 191-202. <https://doi.org/10.1007/s13237-019-00289-w>
4. Anbumani, S. & Mohankumar, M. N. (2011). Nuclear and cytoplasmic abnormalities in the fish *Catla catla* (Hamilton) exposed to chemicals and ionizing radiation. *Research Journal of Environmental Sciences*, 5(12), 867-877.
5. APHA, AWWA & WEF (2017). In: Rice EW, Baird RB, Eaton AD, Clesceri LS, editors. Standard methods for the examination of water and wastewater. 23rd ed. Washington, DC: APHA; 2017.
6. Canalejo, A., Diaz-de-Alba, M., Granado-Castro, M. D., Cordoba, F., Espada-Bellido, E., Galindo-Riaño, M. D. & Torronteras, R. (2016). Early genotoxic response and accumulation induced by waterborne copper, lead, and arsenic in European seabass, *Dicentrarchus labrax*. *Environmental Science and Pollution Research*, 23, 3256-3266. <https://doi.org/10.1007/s11356-015-5435-1>
7. Chowdhary, P., Bharagava, R. N., Mishra, S. & Khan, N. (2020). Role of industries in water scarcity and its adverse effects on environment and human health. *Environmental Concerns and Sustainable Development: Volume 1: Air, Water and Energy Resources*, 235-256. https://doi.org/10.1007/978-981-13-5889-0_12
8. De Zwart, D., Adams, W., Galay Burgos, M., Hollender, J., Junghans, M., Merrington, G., ... & Williams, R. (2018). Aquatic exposures of chemical mixtures in urban environments: Approaches to impact assessment. *Environmental Toxicology and Chemistry*, 37(3), 703-714. <https://doi.org/10.1002/etc.3975>
9. Elalfy, M. M., Abomosallam, M. S., Sleem, F. & Elhadidy, M. (2021). Copper and copper containing pesticide as copper oxychloride toxicity and its adverse effects on animal and human health. *MedicoResearch Chronicles*, 8 (2),89-98. <https://doi.org/10.26838 MEDRECH. 2021.8.2.486>
10. Francisco, C. D. M., Bertolino, S. M., De Oliveira Junior, R. J., Morelli, S. & Pereira, B. B. (2019). Genotoxicity assessment of polluted urban streams using a native fish *Astyanax altiparanae*. *Journal of Toxicology and Environ-*

- mental Health, Part A, 82(8), 514-523. <https://doi.org/10.1080/15287394.2019.1624235>
11. Javed, M. & Usmani, N. (2019). An overview of the adverse effects of heavy metal contamination on fish health. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 89, 389-403. <https://doi.org/10.1007/s40011-017-0875-7>
 12. Jomova, K., Makova, M., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2022). Essential metals in health and disease. *Chemico-biological interactions*, 110173. <https://doi.org/10.1016/j.cbi.2022.110173>
 13. Karim, N. (2018). Copper and human health-a review. *Journal of Bahria University Medical and Dental College*, 8(2), 117-122.
 14. Kaur, S., Khera, K. S. & Kondal, J. K. (2018). Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, *Labeorohita* (Hamilton). *Journal of Entomology and Zoology Studies*, 6 (2), 2137-2144.
 15. Kumar, M., Gupta, N., Ratn, A., Awasthi, Y., Prasad, R., Trivedi, A. & Trivedi, S. P. (2020). Biomonitoring of heavy metals in river Ganga water, sediments, plant, and fishes of different trophic levels. *Biological trace element research*, 193, 536-547. <https://doi.org/10.1007/s12011-019-01736-0>
 16. Kumar, M., Kumar, P. & Devi, S. (2015). To study the histopathological changes in the gills of *Clarias batrachus*, an air-breathing teleost after short term exposure of copper sulphate. *J Aquac Res Develop*, 6(10). DOI: 10.4172/2155-9546.1000369
 17. Kumar, M., Singh, S., Dwivedi, S., Trivedi, A., Dubey, I. & Trivedi, S. P. (2023). Copper-induced genotoxicity, oxidative stress, and alteration in transcriptional level of autophagy-associated genes in snakehead fish *Channa punctatus*. *Biological Trace Element Research*, 201(4), 2022-2035. <https://doi.org/10.1007/s12011-022-03301-8>
 18. Lawrence, A. J. & Hemingway, K. L. (Eds.). (2008). Effects of pollution on fish: molecular effects and population responses. *John Wiley & Sons.*
 19. Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L. & Steinberg, C. E. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Reviews in Aquaculture*, 12(2), 943-965. <https://doi.org/10.1111/raq.12365>
 20. Malhotra, N., Ger, T. R., Uapipatanakul, B., Huang, J. C., Chen, K. H. C. & Hsiao, C. D. (2020). Review of copper and copper nanoparticle toxicity in fish. *Nanomaterials*, 10 (6), 1126. <https://doi.org/10.3390/nano10061126>
 21. Mansouri, B., Maleki, A., Johari, S. A., Shahmoradi, B., Mohammadi, E. & Davari, B. (2017). Histopathological effects of copper oxide nanoparticles on the gill and intestine of common carp (*Cyprinus carpio*) in the presence of titanium dioxide nanoparticles. *Chemistry and Ecology*, 33 (4), 295-308. <https://doi.org/10.1080/02757540.2017.1301436>
 22. Maurya, P. K., Malik, D. S., Yadav, K. K., Kumar, A., Kumar, S. & Kamyab, H. (2019). Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. *Toxicology Reports*, 6, 472-481. <https://doi.org/10.1016/j.toxrep.2019.05.012>
 23. Mhaske, A., Sharma, S. & Shukla, R. (2023). Nanotheranostic: The futuristic therapy for copper mediated neurological sequelae. *Journal of Drug Delivery Science and Technology*, 104193. <https://doi.org/10.1016/j.jddst.2023.104193>
 24. Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., ... & Simal-Gandara, J. (2022). Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter toxicity. *Journal of King Saud University-Science*, 34(3), 101865. <https://doi.org/10.1016/j.jksus.2022.101865>
 25. Murali, M., Athif, P., Suganthi, P., Bukhari, A. S., Mohamed, H. S., Basu, H. & Singhal, R. K. (2018). Toxicological effect of Al₂O₃ nanoparticles on histoarchitecture of the freshwater fish *Oreochromis mossambicus*. *Environmental Toxicology and Pharmacology*, 59, 74-81. <https://doi.org/10.1016/j.etap.2018.03.004>
 26. Nagpure, N. S., Srivastava, R., Kumar, R., Dabas, A., Kushwaha, B. & Kumar, P. (2015). Assessment of pollution of river Ganges by tannery effluents using genotoxicity biomarkers in murrel fish, *Channa punctatus* (Bloch).
 27. Noureen, A., Jabeen, F., Tabish, T. A., Yaqub, S., Ali, M. & Chaudhry, A. S. (2018). Assessment of copper nanoparticles (Cu-NPs) and copper (II) oxide (CuO) induced hemato-and hepatotoxicity in *Cyprinus carpio*. *Nanotechnology*, 29(14), 144003. [10.1088/1361-6528/aaaaa7](https://doi.org/10.1088/1361-6528/aaaaa7)
 28. OECD. Test no. 203: fish, acute toxicity testing, section 2: Effects on biotic systems. Guidelines for the testing of chemicals. 2019;10.
 29. Rehman, M., Liu, L., Wang, Q., Saleem, M. H., Bashir, S., Ullah, S. & Peng, D. (2019). Copper environmental toxicology, recent advances, and future outlook: a review. *Environmental science and pollution research*, 26, 18003-18016. <https://doi.org/10.1007/s11356-019-05073-6>
 30. Sangeetha, P. & Aruljothi, B. (2019). The toxic effect of heavy metals (nickel, copper, and mixture) on histological parameters of the common carp (fingerlings) *Cyprinus carpio*. *Plant Arch*, 19(2), 4534-4542.
 31. Sarangi, P. K. (2021). Cytogenotoxic Effect of Pesticides Induces Variability in Micronucleus and Nucleo-Cytoplasmic Abnormalities in *Channa punctatus* in vivo. *Journal of Zoological Research* | Volume, 3(04).
 32. Shah, N., Khan, A., Habib Khan, N. & Khisroon, M. (2021). Genotoxic consequences in common grass carp (*Ctenopharyngodon idella Valenciennes, 1844*) exposed to selected toxic metals. *Biological Trace Element Research*, 199, 305-314. <https://doi.org/10.1007/s12011-020-02122-x>
 33. Shahjahan, M., Khatun, M. S., Mun, M. M., Islam, S. M., Uddin, M. H., Badruzzaman, M. & Khan, S. (2020). Nuclear and cellular abnormalities of erythrocytes in response to thermal stress in common carp *Cyprinus carpio*. *Frontiers in Physiology*, 11, 543. <https://doi.org/10.3389/fphys.2020.00543>
 34. Stankevičiūtė, M., Butrimavičienė, L., Valskienė, R., Greičiūnaitė, J., Baršienė, J., Vosyliienė, M. Z. & Svecevičius, G. (2016). Analysis of nuclear abnormalities in erythrocytes of rainbow trout (*Oncorhynchus mykiss*) treated with Cu and Zn and after 4-, 8-, and 12-day depuration (post-treatment recovery). *Mutation Research/Genetic Toxicology*

- gy and Environmental Mutagenesis, 797, 26-35. <https://doi.org/10.1016/j.mrgentox.2016.01.003>
35. Tavares-Dias, M. (2021). Toxic, physiological, histomorphological, growth performance and antiparasitic effects of copper sulphate in fish aquaculture. *Aquaculture*, 535, 736350. <https://doi.org/10.1016/j.aquaculture.2021.736350>
36. Trivedi, S. P., Singh, S., Trivedi, A. & Kumar, M. (2022). Mercuric chloride-induced oxidative stress, genotoxicity, haematological changes, and histopathological alterations in fish *Channa punctatus* (Bloch, 1793). *Journal of Fish Biology*, 100(4), 868-883. <https://doi.org/10.1111/jfb.15019>
37. Wu, L., Yu, Q., Zhang, G., Wu, F., Zhang, Y., Yuan, C., ... & Wang, Z. (2019). Single and combined exposures of waterborne Cu and Cd induced oxidative stress responses and tissue injury in female rare minnow (*Gobiocypris rarus*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 222, 90-99. <https://doi.org/10.1016/j.cbpc.2019.04.013>
38. Yu, Z., Zheng, Y. G., Du, H. L., Li, H. J. & Wu, L. F. (2020). Bioflocs protects copper-induced inflammatory response and oxidative stress in *Rhynchocypris lagowski Dybowski* through inhibiting NF- κ B and Nrf2 signaling pathways. *Fish & shellfish immunology*, 98, 466-476. <https://doi.org/10.1016/j.fsi.2020.01.048>
39. Zebal, Y. D., Roza, M., da Silva Fonseca, J., Costa, P. G., de Oliveira, C. S., Zocke, T. G., ... & Bianchini, A. (2019). Waterborne copper is more toxic to the killifish *Poecilia vivipara* in elevated temperatures: Linking oxidative stress in the liver with reduced organismal thermal performance. *Aquatic Toxicology*, 209, 142-149. <https://doi.org/10.1016/j.aquatox.2019.02.005>
40. Zeng, L., Ai, C. X., Zhang, J. S. & Li, W. C. (2020). Prehypoxia exposure inhibited copper toxicity by improving energy metabolism, antioxidant defence and mitophagy in the liver of the large yellow croaker *Larimichthys crocea*. *Science of the Total Environment*, 708, 134961. <https://doi.org/10.1016/j.scitotenv.2019.134961>