The Scientific Temper (2024) Vol. 15 (1): 1652-1660
E-ISSN: 2231-6396, ISSN: 0976-8653
Doi: 10.58414/SCIENTIFICTEMPER.2024.15.1.12
https://scientifictemper.com/

RESEARCH ARTICLE

Environmentally relevant concentration of copper elated hematological impairment, branchiotoxicity, myotoxicity, nephrotoxicity and antioxidants imbalance in fish *Channa punctatus*

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Abstract

Copper is a mineral that organisms need to stay healthy on one hand and on the other hand prolonged overexposure may result into various dangerous implications. The present study was outlined to assess various deleterious effects being caused to the edible fish *C. punctatus* exposed to an environmentally relevant concentration of copper (ERCC). For this purpose, well-acclimatized fish were classified into four groups. Group I was maintained as control while groups II, III, and IV were exposed to the ERCC (0.85 mg/L), 10% increase in ERCC (0.935 mg/L) and 20% increase in ERCC (1.02 mg/L), respectively, for 15, 30, 45 and 60d. A significant ($p < 0.05$) reduction in Hb% and RBC count while escalation in WBC counts was documented after all exposure periods and the maximum change was recorded after the longest exposure period of 60 days, respectively. Enzymatic antioxidants viz., superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and lipid peroxidation (LPO) levels were significantly ($p < 0.05$) raised and non-enzymatic antioxidant, i.e., glutathione (GSH) activity was reduced in all exposed groups in a dose-dependent manner. Severe histopathological modifications were observed in the treated fish's gills, muscles, and kidneys. The outcomes of the present investigation substantiate that ERCC induces toxicity in fish at various levels and warn about the possible repercussions of increased ERCC in the near future as the concentration of copper continuously rises with the ever-increasing pollution.

Keywords: *Channa punctatus*, ERCC, Antioxidants, hematology, histopathology, oxidative stress.

Introduction

Copper (Cu) is supposed to be a mixed blessing to the cell due to its built-in redox (reduction-oxidation) property that makes it beneficial in one way and toxic in other ways (Ge *et al.*, 2022). Though it is requisite for numerous physiological operations, cellular functions and biochemical activities (J. Wang *et al.*, 2020), its prolonged overexposure may result into various destructive consequences (Liu *et al.*, 2020). It is a malleable and ductile trace element that is copiously found in the earth's crust and has a wide range of applications. Over the last few years, Cu and Cu-nanoparticles (CuNPs) have been increasingly employed for various industrial purposes, drug delivery, agricultural and food maintenance, anti-microbial agents, sensors, electrical equipment, alloy formation, construction materials, water treatment, etc. (Malhotra *et al.*, 2020). In aquaculture practices, copper sulfate ($CuSO_4$) is frequently utilized for the elimination of phytoplankton and filamentous algae (Guo *et al.*, 2017). As a result of several natural (such as volcanic activity, geological deposits, weathering, soil erosion, etc.) and anthropogenic activities (such as industrial effluents, mining, fertilizers manufacturing, domestic sewage, etc.), it enters in aquatic ecosystems (de Paula *et al.*, 2021) and eventually incorporates in food chains (Y. Wang *et al.*, 2017). Water-inhabiting (M. Kumar *et al.*, 2022), terrestrial life-forms (Bui

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How to cite this article: Bakhasha, J., Yadav, K. K., Saxena, V., Arya, N., Trivedi, A. (2024). Environmentally relevant concentration of copper elated hematological impairment, branchiotoxicity, myotoxicity, nephrotoxicity and antioxidants imbalance in fish *Channa punctatus*. The Scientific Temper, **15**(1):1652-1660.

Doi: 10.58414/SCIENTIFICTEMPER.2024.15.1.12

Source of support: Nil

Conflict of interest: None.

Received: 09/01/2024 **Accepted:** 15/02/2024 **Published** : 15/03/2024

et al., 2016) and consequently, humans (Malhotra *et al.*, 2020) are unfavorably impacted as they are exposed to heavy metals via different routes. Basically, industrial workers are vulnerable to Cu-toxicity due to occupational exposure (Jomova & Valko, 2011).

As heavy metals have non-biodegradable, highly toxic and persistent traits, they cause mutagenicity, cytotoxicity and carcinogenicity in aquatic life forms (Kaur *et al.*, 2018). Fish occupy the top-notch position in the water trophic system and have the potential to accumulate metals in their vital tissues, which is why they are the critical bio-indicators of aquatic metal pollution (Saglam *et al.*, 2014; Trivedi *et al.*, 2021). Many investigations has validated that elevation in aquatic Cu levels may result in raised Cu accumulation in the gills, kidney, liver and muscles of fishes, which further leads to physiological impairments (Eyckmans *et al.*, 2011; Ma'rifah *et al.*, 2019; Tunçsoy & Erdem, 2014, 2018). Excess metals are gradually removed from the body of an organism via blood; therefore, hematology is adversely affected due to metal toxicity (Singh *et al.*, 2008). Hence, blood parameters viz. Hb%, RBCs, WBCs, etc., have been considered as susceptible bio-indicators of distress in fish (Naz *et al.*, 2021).

Earlier studies interpreted that the production of ROS is one of the most perilous impacts of Cu-intoxication (Guo *et al.*, 2017), which subsequently leads to increased oxidative stress (M. Kumar *et al.*, 2022). Various enzymatic (SOD, CAT, GR, etc.) and non-enzymatic antioxidants (like GSH) play a significant role in reducing oxidative stress (Kumar *et al.*, 2023). As oxidative stress is a reflection of the imbalance between ROS generation and antioxidant mechanisms (Kumar *et al.*, 2023; Liu *et al.*, 2020), it has been used as a noteworthy indicator for evaluating Cu-toxicity. When the ROS levels exceed the cells' capacity, it impairs membrane fluidity and functions (Liu *et al.*, 2020), which in due course provokes LPO via the formation of Malondialdehyde (MDA) that is responsible for membrane disruptions (Ma'rifah *et al.*, 2019). Accumulation of metals in the vital organs of life forms subsequently leads to histological destruction. Hence, analyzing alterations in the tissue structures is an insightful bio-marker for evaluating the toxic potential of metals (Trivedi *et al.*, 2022).

As Moradabad region is a hub of brass industries, their metal-containing effluents (especially Cu) are ultimately disposed into the river Ramganga and with the acceleration in industrialization and metal use, contamination of river water with copper is also shooting up continuously (Batar, 2016; Pathak & Alam, 2022). This rising metal pollution is perturbing the biological functions of fish and causing destructive effects (B. Kumar & Gupta, 2014; Sarah *et al.*, 2019). The test fish *C. punctatus* is a source of food for many populations inhabiting places near the river. The damages caused to fish will eventually affect the humans as they are holding the upper trophic level. Therefore, this work was planned to assess the jeopardous consequences on fish health at ERCC in the river Ramganga. The present investigation unfolded the threats being caused in terms of hematological anomalies, branchio-, myo- and nephrotoxicity mediated impaired antioxidant defense system in the fish *C. punctatus* exposed to ERCC.

Materials and Methods

Sites of investigation

We conducted our study in the district of Moradabad (Uttar Pradesh, India). Suitably, three localities, i.e., Nawabpura, Katghar and Pitalnagri, were considered as the sampling sites (S1, S2 and S3, respectively), since they exist at upstream, midstream and downstream sites of the river Ramganga, respectively. The sites are represented in Figure 1. The sampling stations were well-selected because the river Raganga is the ultimate receptor for the effluents of metal industries and other human activities occurring in the city.

Estimation of Cu in water samples and determination of ERCC

For the estimation of Cu acidification of water samples was done following the method of Kumar *e al.*, 2020. Acidified samples were carried to the laboratory and prepared to estimate Cu concentrations (APHA, 2017). The analysis was performed using an atomic absorption spectrophotometer (Model No, Varian AAS 240-FS, India) applying C2H2-air flame and the functioning variables were adjusted per the manufacturer's guidelines. The concentration of Cu in water samples is expressed as mg/L and the values recorded are given in Table 1. ERCC was calculated by calculating the mean of Cu concentration reported in the water samples of S1, S2 and S3 sites and it was 0.85 mg/L.

Test Chemicals

For the present study, test chemicals Cupric sulfate ($CuSO_4.5H_2O$) of Qualigens fine chemicals, Bombay; 2',7´-dichlorodihydrofluorescein (DCFH-DA) of Sigma Aldrich; Dithiothreitol (DTT), Phenylmethanesulfonyl fluoride (PMSF), 5,5'dithio 2-nitrobenzoic acid (DTNB), phenazinemethosulphate (PMS), nitroblue tetrazolium

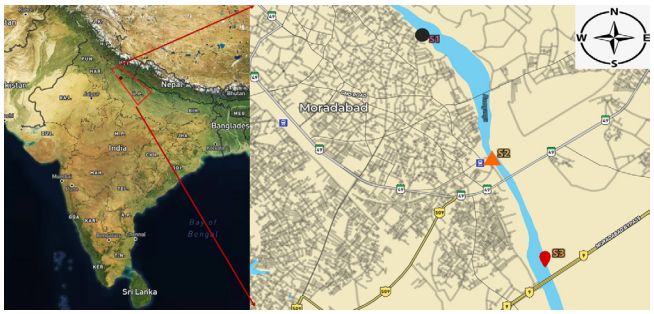

Figure 1: Map showing the location of sampling sites S1-Nawabpura (upstream), S2-Katghar (midstream) and S3-Pitalnagri (downstream).

Table 1: Cu concentration in water samples (mg/L)

Sample	*Concentration of Cu (mg/L)*			
	at S1	*at S2*	*at S3*	*Mean ± S.E.M.*
Water	0.72	0.86	0.97	0.85 ± 0.02

chloride (NBT), nicotinamide adenine dinucleotide (NADH), reduced NADP(H), thiobarbituric acid (TBA), trichloroacetic acid (TCA), May Grunnwald's, ethylene-diaminetetraacetic acid (EDTA) of Himedia; Giemsa stain of Merck and perchloric acid (PCA), nitric acid (NA), sulfuric acid (SA) of Rankem were procured through a local dealer of Bareilly.

Test Fish and experimental set-up

Test fish *Channa punctatus* (36 ± 3.0 g; 15.5 ± 1.0 cm) was caught with the help of local fishermen and imported to the laboratory. Fish were extensively rinsed with tap water then treated with 0.05% $KMnO_4$ to eliminate possible cutaneous infections (Ratn *et al*., 2018). The acclimatization and feeding processes were performed in line with Kumar *et al*., 2023. The aquaria water, to which acclimatized fish were transferred, was assessed for physicochemical parameters [total dissolved solids (TDS) 182.34 ± 3.2 mg/L, hardness 188.62 ± 4.0 as $CaCO_3$ mg/L, dissolved oxygen (DO) 6.9 ± 0.3 mg/L, temperature (T) 26.0 ± 1.5^{0}C, and pH 7.1 ± 0.3], adhering to the standard methods (APHA, 2017).

Finely habituated fish were divided into four triplicate groups containing 10 specimens each. Group I (G I) was not treated, so it served as control. Group II (G II), Group III (G III) and Group IV (G IV) were exposed to the ERCC (0.85 mg/L), 10% increment in ERCC (0.935 mg/L) and 20% increment in ERCC (1.02 mg/L), respectively for the duration of 15, 30, 45 and 60d. Fish from each experimental group were euthanized with 0.01% diethyl ether post-completion of the specified exposure duration. Fish blood and tissues (gill, muscle and kidney) were collected for further investigations.

Hematological analysis

Sahli's method was followed to estimate the Hb% (Godkar and Godkar, 2003), which is represented as g/dL. Neubauer's hemocytometer was used to count the total leucocytes and erythrocytes (Shah and Altindag, 2004). Blood was diluted with Hayem's fluid (1:200) and Turk's fluid (1:20) for counting of erythrocytes and leucocytes, respectively (Mishra *et al*., 1977). Leucocytes and erythrocytes were determined as 10^3/mm^3 and 10^6/mm^3, respectively (Masud and Singh, 2013).

Estimation of enzymatic and non-enzymatic antioxidants activities

Withdrawn tissues (gill, muscle and kidney) were weighed and rinsed with phosphate buffer saline (PBS) after the completion of the stipulated exposure period. Tissues were homogenized in a homogenization buffer and prepared as per the method of Ratn *et al*., 2018. Cell lysate was stored for further assessments like measurement of antioxidants activities. The levels of enzymatic antioxidants viz. SOD, CAT, GR were measured at the wavelength of 560, 240 and 340 nm, following the methods of Kakkar *et al*., 1984; Aebi, 1984 and Carlberg & Mannervik, 1985; respectively while the activity of non-enzymatic antioxidants, i.e., GSH was evaluated at 412 nm following the procedure described by Moron *et al*., 1979. The extents of the above-mentioned biomarkers were recorded with a UV-vis spectrophotometer (Shimadzu, UV-1900i) and expressed in Units/min/mg of protein. The method of Samanta *et al.,* 2014 was followed to estimate the activity of LPO. The method was based on the reaction between TBARS (Thiobarbituric acid reactive substance) and (MDA) malondialdehyde. The sample's absorbance was measured at 532 nm using an UV-vis spectrophotometer (Shimadzu, UV-1900i).

Histopathological analysis

Gill, muscle and kidney were properly drawn from each group's fish. Tissues were washed with distilled water and fixed in 10% neutral buffered formalin (NBF) for 48 hours. After the dehydration of tissues, paraffin blocks were prepared and kept at room temperature (RT) overnight. Sections were sliced using a microtome (YSI062 Yorco Precision Rotary Microtome, India). Slides were prepared after de-waxing, dehydration and staining of the sections. An oil immersion microscope (Nikon Corporation K-12,432) with 10/40X magnification was employed to capture the histological findings.

Statistical Analysis

Data from each replicate of all groups were depicted as mean ± standard error mean (SEM). The significance ($p<0.05$) of all results was examined with a one-way analysis of variance (ANOVA) applying Tukey's post hoc test. Data analytics was conducted with the help of SPSS software (version 20.0, SPSS Company, Chicago, USA). Regression and correlation examinations were also carried out to justify and substantiate interrelationships among different physiological considerations of copper-induced toxicity.

Results

Physicochemical parameters of sampling sites

Physicochemical parameters (viz., temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), and total hardness) of the water samples taken from the river Ramganga were ascertained and illustrated in Table 2. The obtained values were above the desirable limit and within the permissible limit for drinking water by the Bureau of Indian Standards (BIS, 2012).

Haematological analysis

In the present study, hematological parameters such as Hb%, RBCs and WBCs count of ERCC-exposed fish *C. punctatus* were estimated at various intervals of exposure viz. 15, 30, 45

Table 2: Physicochemical parameters of sampled water (mean values of all sites).

S. No.	*Water quality parameters*	*Values of parameters from the sampling site*	*Permissible limit BIS (BIS, 2012)*
1.	pH	7.8 ± 0.2	No relaxation from the acceptable limit (6.5–8.5)
2.	Temperature (°C)	24 ± 4	-
3.	Alkalinity (mg/L)	247 ± 6	600
4.	TDS (mg/L)	514 ± 8	2000
5.	DO (mg/L)	5.3 ± .25	4–6
6.	Total hardness (mg/L)	187 ± 4	600

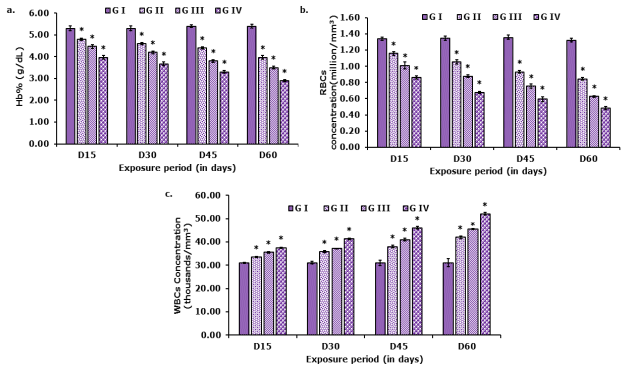

Figure 2: Showing changes in hematological parameters of *C. punctatus* in different Cu-exposed groups. Figure 2a shows Hb%, Figure 2b shows RBCs count and Figure 2c shows WBCs count after 15, 30, 45 and 60 days of exposure.

and 60d and represented in Figure 2 (a-c). Hemoglobin level under Cu-stress was significantly ($p < 0.05$) decreased from 5.4 ± 0.07 (GI) to 3.96 ± 0.8 g/dL (GII), 3.5 ± 0.5 g/dL (GIII) and 2.9 ± 0.5 g/dL (GIV) after 60d exposure as represented in Figure 2a.

Similar decreasing trend in RBCs count was also recorded in all the Cu exposed groups and it was maximum significantly ($p<0.05$) decreased from 1.31 ± 0.02 $\times 10^6$/mm^3 (GI) to 0.84 ± 0.01 $\times 10^6$/mm^3 (GII), 0.62 ± 0.01 $\times 10^6$/mm^3 (GIII) and 0.48 ± 0.01 $\times 10^6$/mm^3 (GIV) after 60d of exposure period (Figure 2b). While WBCs' count was increased significantly ($p < 0.05$) from 31 ± 1.7 $\times 10^3$/mm^3 (GI) to 42 ± 0.0.28 $\times 10^3$/mm^3, 45 ± 0.28 $\times 10^3$/mm^3 and 52 ± 0.57 $\times 10^3$/mm^3 in GII, GIII and GIV, respectively after 60d exposure as illustrated in Figure 2c.

Activities of antioxidants

The contents of enzymatic antioxidants viz. SOD, CAT and GR in gill, muscle and kidney were significantly ($p < 0.05$) escalated in a concentration- and duration-related manner and the utmost increase was reported in GIV after 60d exposure. The activity of non-enzymatic antioxidants was diminished significantly ($p < 0.05$) during exposure, and the lowest level was recorded in GIV after 60 days. Figures 3, 4 and 5, respectively.

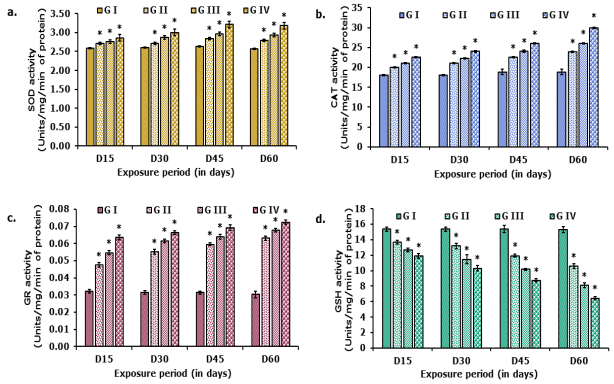

Figure 3: Activities of SOD (a), CAT (b), GR (c)and GSH (d) in the gill of test fish *C. punctatus* after 15, 30, 45 and 60 days of exposure.

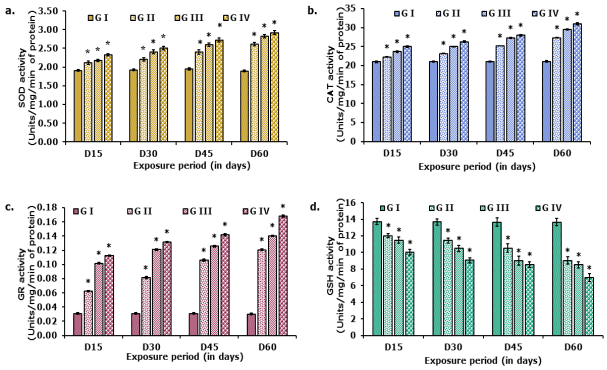

Figure 4: Activities of SOD (a), CAT (b), GR (c)and GSH (d) in the muscle of test fish *C. punctatus* after 15, 30, 45 and 60 days of exposure.

Quantification of LPO

The measured LPO activity in tissues of test fish is displayed in Figure 6. With increasing concentration of Cu, LPO activity was found to be increased significantly ($p < 0.05$) in a time-sensitive way and the highest elevation was documented in GIV at 60 days.

Modifications in histological architecture

Gill, muscle and kidney of Cu-intoxicated *C. punctatus* exhibited several alterations in their histology as compared to control. In Figure 7a, GI displayed the untreated gill structures viz. secondary lamellae (SL); mucous cell (MC); cartilaginous core (CC) and GII, GIII, GIV denoted the histopathological changes in the gill after 60d exposure. The deformities reported in gill were aneurysm (AN); complete loss of primary lamellae (CPL); primary lamella (PL); oedema (OE); necrosis (N); telangiectasis (T); degenerative primary lamellae (DPL); congestion (C); lamellar fusion (LF); hyperplasia (HP) and epithelium lifting (EL). Similarly, in Figure 7b, GI represented the unexposed muscle histology viz. septum (SPM); myotomes (MYT) and GII, GIII, GIV illustrated the alterations in the muscle after 60d treatment. The distortions observed in muscle were disintegrated

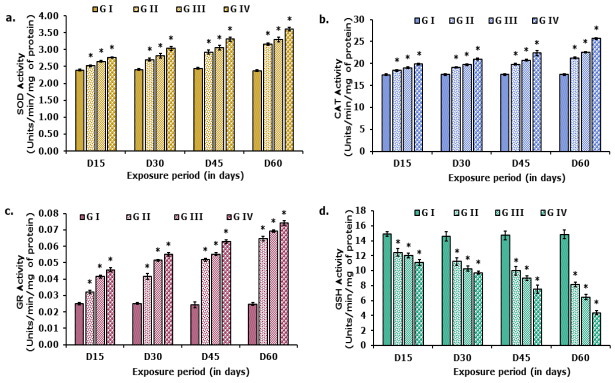

Figure 5: Activities of SOD (a), CAT (b), GR (c)and GSH (d) in the kidney of test fish *C. punctatus* after 15, 30, 45 and 60 days of exposure.

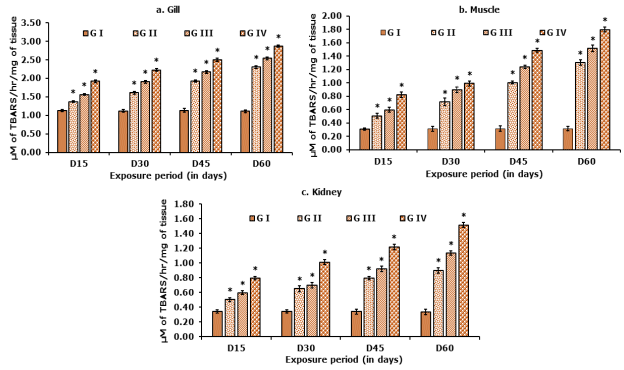

Figure 6: Activity of LPO in the gill (a), muscle (b) and kidney (c) of test fish C. punctatus after 15, 30, 45 and 60 days of exposure.

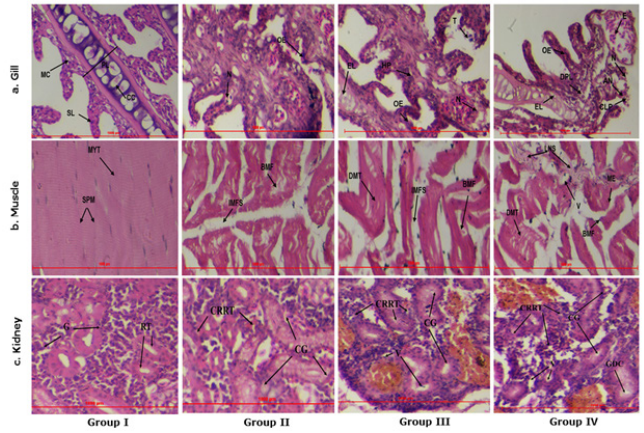

Figure 7: Histological alterations in gill (a), muscle (b) and kidney (c) of test fish C. punctatus after 60d exposure. Abbreviations used: E-erythrocytes; DPL-degenerative primary lamellae; CPL-complete loss of primary lamellae; PL-primary lamella; CC-cartilaginous core; SL-secondary lamellae; MC-mucous cell; AN-aneurysm; HP-hyperplasia; OE-edema; C-congestion; T-telangiectasis; EL-epithelium lifting; N-necrosis; LF-lamellar fusion; MYT-myotomes; BMF-broken myofibrils; DMT-disintegrated myotomes; LNS-lesions; SPM-septum; IMFS-inter myofibrillarspace; ME-muscle edema; V-vacuolization; CRRT-cavity reduction in the renal tubule, CG-compact glomerulus, GDC-glomerulus degenerative cells.

myotomes (DMT); lesions (LNS); vacuolization (V); broken myofibrils (BMF); muscle oedema (ME) and inter myofibrillar space (IMFS). Likewise, in Figure 7c, GI showed the regular histology of the kidney viz. renal tubules and glomerulus. Cavity reduction in renal tubule (CRRT), compact glomerulus (CG) and vacuolization (V) were observed in GII, GIII and GIV in a dose-dependent manner. Glomerulus degenerative cells (GDC) were reported in GIV only.

Regression and correlation analyses

Data from correlation analyses between selected chosen parameters displayed a compacted association of molecular and physiological turbulences with higher correlation coefficient (R) values in tissues of Cu^{+2} exposed groups. In particular, excessive ROS production causes a disturbed antioxidant system. Thus, a strong positive correlation was found between SOD and CAT, LPO and GR, while a strong negative correlation was observed between GSH and SOD, CAT in gill, muscle and kidney of test fish. The data are presented in Table 3.

Discussion

This investigation was planned to determine the ERCC in the river Ramganga water and its perilous consequences on the gill, muscle and kidney of edible fish *C. punctatus* in terms of hematological anomalies, oxidative distress and histopathological perturbations. The ERCC in water samples of S1, S2 and S3 was recorded as 0.72, 0.86 and 0.97 mg/L,

Table 3: Correlation coefficients by linear regression analysis

Gill					
	SOD	*CAT*	*GSH*	*GR*	*LPO*
SOD	1.000**				
CAT	0.99488	1.000**			
GSH	-0.9689	-0.9843	1.000**		
GR	0.88972	0.93018	-0.966	1.000**	
LPO	0.93376	0.96444	-0.9854	0.99413	1.000**
Muscle					
	SOD	CAT	GSH	GR	LPO
SOD	1.000**				
CAT	0.99699	1.000**			
GSH	-0.9878	-0.9903	1.000**		
GR	0.99393	0.99708	-0.998	1.000**	
LPO	0.99507	0.99715	-0.9979	0.9999	1.000**
Kidney					
	SOD	CAT	GSH	GR	LPO
SOD	1				
CAT	0.98837	1			
GSH	-0.95066	-0.98103	1		
GR	0.999562	0.990678	-0.95167	1	
LPO	0.966608	0.991886	-0.99761	0.968417	1

respectively and their mean concentration evaluated was 0.85 ± 0.02 mg/L and it was within the allowable limit [2, 1.5 and 1.3 mg/L as per WHO, BIS and USEPA], respectively (BIS, 2012; National Research Council (US) Committee on Copper in Drinking Water, 2000).

To ascertain the precarious possibilities upon exposure of toxicants, hematological anomalies in fish are apt biomarkers (Al-Akel *et al*., 2010). Present findings depicted the diminution in Hb% along with the reduction in RBCs count and the inflation in WBCs count in Cu-treated test fish *C. punctatus,* which is in accordance with the previous study conducted on the Cu- and Cd-exposed major carp *Catla catla* by (Naz *et al*., 2021). A reduction in the Hb% and RBCs amounts suggests the state of anemia in fish due to stress(Trivedi *et al*., 2022). As WBCs are involved in the mechanism of body defenses against several xenobiotics(Ates *et al*., 2008), elevated WBCs signify the immune response generated upon Cu-intoxication in test fish. Similar outcomes were documented in freshwater fish treated with Cd and Ni (Hedayati and Ghaffari, 2013), in Ni-exposed *Cyprinus carpio*(Alkahemal-Balawi *et al*., 2011), in *Oreochromis mykiss* stressed with Pb and Cu (Ates *et al*., 2008) and in *Oreochromis niloticus* treated with Cd (Mekkawy *et al*., 2011). Relatable trends were observed in rats upon acute exposure of Pb and Cd (Andjelkovic *et al*., 2019).

Raised concentrations of metals lead to the high production of ROS (such as hydroxyl radicals, hydrogen peroxide and superoxide radicals) by way of Fenton and Haber–Weiss reactions which subsequently results into elevated oxidative stress that causes oxidative damage to cell or even cell death (Atli & Canli, 2010). Cells have an inherent antioxidant defense system which, with the help of enzymatic and non-enzymatic antioxidants, neutralizes or removes the free radicals or ROS up to certain level (Awasthi *et al*., 2019). SOD is involved in the lowering of raised superoxide radicals via the formation of H_2O_2 which is further transformed into H_2O by the CAT(Trivedi *et al*., 2022). The observations of this exploration illustrated that escalated amounts of SOD and CAT enzymes in the gill, muscle and kidney of ERCC-exposed fish were observed in a duration-sensitive manner which further validated their role as the first line of defense. GR, another enzymatic stress biomarker, reduces the GSSG available in the body into GSH to combat with the expedited oxidative stress more efficiently (Trivedi *et al*., 2022). In present findings, GR activity was found elevated in all sample tissues of treated fish in a time-dependent manner, indicating that Cu induced oxidative distress at ERC. GSH, a non-enzymatic antioxidant, also helps lessen free radicals and often diminishes them during their neutralization (Kumar *et al*., 2023). Results of this experimentation demonstrated the reduction in the GSH amount in gill, muscle and kidney of exposed *C. punctatus*. Present outcomes concluded that disturbed extents of SOD, CAT, GR and GSH undoubtedly reflected the intensified oxidative stress. Likewise, higher levels of SOD and CAT were found in the gills of Cu-exposed *Oreochromisniloticus*(Ma'rifah *et al*., 2019). In the same way, increased levels of enzymatic antioxidants were documented in the gills, muscles, brains, and livers of Cd-treated *Labeo rohita* (Kumari *et al*., 2014). Similar trends were recorded in the liver of *C. punctatus* upon intoxication of chromium (Awasthi *et al*., 2019) and zinc (Ratn *et al*., 2018). Atli & Canli, 2010 also observed disturbances in the activities of antioxidants in *O. niloticus* when exposed to various heavy metals (Cd, Cu, Cr, Zn and Fe). Relatable findings were reported in the liver and kidney of fish exposed to $HgCl_2$ (Trivedi *et al*., 2022) and rat intoxicated with Cd and Pb (Andjelkovic *et al*., 2019).

When the ROS level surpasses the cells' tolerance limit, it disrupts the fluidity of the cellular membrane (Liu *et al*., 2020), eventually eliciting lipid peroxidation (Ma'rifah *et al*., 2019). As LPO causes hydrolysis of phospholipids, the increment in the quantities of LPO validates the perturbations in cell membrane structure (Mekkawy *et al*., 2011). Results of the present experimentation demonstrated the increased quantity of LPO in the tissues of experimental fish, which were in accordance with Mekkawy *et al*., 2011 who exposed *O.niloticus*to Cd. Simonato *et al*., 2016 also found elevations in the activity of LPO in fish *Prochilodus lineatus* upon Cu exposure. Similar results were observed in Cu-intoxicated rats (Mandil *et al*., 2020).

As copper enters and gets accumulates in the fish, it causes inhibition of various biological activities and severe disruptions in the histological structure of various tissues (Padrilah *et al*., 2018). In the present findings, gill, muscle and kidney of Cu-intoxicated *C. punctatus* exhibited several alterations in their histology as compared to control. The disfigurements reported in gill were aneurysm, complete loss of primary lamellae, primary lamella, edema, necrosis, telangiectasis, degenerative primary lamellae, congestion, lamellar fusion, hyperplasia and epithelium lifting. Relatable structural perturbations were evinced in the Cu-exposed gills of *O.niloticus* (Figueiredo-Fernandes *et al*., 2007), *Oncorhynchus mykiss* (Khabbazi *et al*., 2014), *Catla catla* (Bose *et al*., 2015), *Cyprinus carpio* (Al-Tamimi *et al*., 2015), *Ctenopharyngodan idella*(Atabati *et al*., 2015). Correspondingly, the deformities observed in muscle were disintegrated myotomes, lesions, vacuolization, broken myofibrils, muscle edema and inter myofibrillar space. Earlier studies have evinced similar trends in the Cu-exposed muscles of *C. carpio* (Al-Tamimi *et al*., 2015) and *Lates calcarifer*(Maharajan *et al*., 2016). Similarly, cavity reduction in renal tubule, compact glomerulus, vacuolization and glomerulus degenerative cells were the distortions observed in the kidneys of experimental fish. These consequences were in tune with the findings of Kumar *et al*., 2023 and

Naz *et al*., 2021. In another related research conducted on *Labeorohita*, stress under various concentrations of heavy metals, detrimental histological modifications were reported in various vital tissues (Kaur *et al*., 2018).

Considering the outcomes of this study, it is well-established that the food fish *C. punctatus* is vulnerable to high damage due to the exposure of Cu even at ERC in the river Ramganga. Although ERCC was found to be below the permissible limit in the present study, it will inevitably exceed the allowable extent in the near future due to the immensely increasing contamination of river Ramganga with the ever-rising industrialization and commercialization in Moradabad city. Our study has also revealed the consequences after exposure of 10 and 20% increased ERCC on the test fish. These explorations will unlatch the new perspectives of ERCC-induced toxicity. Further research is required to gain deep knowledge about toxicity caused by environmentally relevant copper and other heavy metal concentrations.

Ethical Approval

All techniques and operations were carried out for the study per the Institutional Animal Ethics Committee (IAEC) Regd. No. MJPRU/PY/IAEC/22/20 CPCSEA of M.J.P. Rohilkhand University, Bareilly, set under the provisions of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India.

Acknowledgment

The authors feel grateful to the Department of Higher Education, Research and Development, Government of Uttar Pradesh, India, for providing the Research and Development project 2021-2022 via grant no. 108/2021/2585/sattar-4-2021-4(28)/2021. We are also thankful to the Head, Department of Animal Science, M.J.P. Rohilkhand University, Bareilly (243006) for providing laboratory facilities and encouragement throughout the studies.

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