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COPPER TOXICITY IN THE CARNIVOROUS FISH (*Wallago attu*) AND ITS RELATIONSHIP WITH THE WATER QUALITY VARIABLES

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ABSTRACT

Heavy metal-containing industrial effluents, primarily discharged illegally, are the primary cause of water contamination, posing a significant threat to aquatic life and water quality. To address this issue, the acute toxic effects of water-borne copper were evaluated in terms of the 96-hour median lethal concentration (LC50) and lethal concentrations for the carnivorous fish species *Wallago attu*. A static bioassay was performed separately with three replications, while the total hardness was maintained at 250mg/L. Fish mortality was observed in conjunction with water quality variables (pH, temperature, total hardness, total ammonia, carbon dioxide, dissolved oxygen, calcium, magnesium, sodium, and potassium) at 12-hour intervals. The results, computed using Probit analysis with a 95% confidence interval, revealed significant differences in the LC50 and lethal concentration values of copper for the fish. The value computed for the 96-hour LC50 was 27.08±0.63mg/L, while the lethal concentration was 47.48±2.77mg/L for *Wallago attu*. Hence, the study found a significant increase in total ammonia and carbon dioxide concentration with increased metal exposure, with total ammonia showing a direct relationship with carbon dioxide. At the same time, dissolved oxygen had an inverse relationship. This indicates a negative relationship between carbon dioxide and dissolved oxygen.

Keywords: *Wallago attu*; Acute toxicity; LC_{50} (median lethal concentration); Lethal; Copper.

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1. INTRODUCTION

Water contamination is a serious problem, predominantly because of the illegal release of industrial effluents containing heavy metals in several countries (Adnan et al., 2024). Due to enhanced and continual addition of heavy metals in freshwater bodies, it has detrimental effects on water quality and also poses a severe threat to fish and other aquatic organisms (Subhanullah et al., 2024; Sharma et al., 2025). Hence, it is essential to appraise the harmful effects of heavy metals on aquatic animals. It helps to evaluate the possible negative impacts on important fish species being used as food as well as on humans in all respects (Naz et al., 2023; Almafrachi et al., 2024; Ray & Vashishth, 2024).

Heavy metals possess a high density relatively and are considered pernicious, even in imperceptible amounts (Cheng et al., 2023; Nitansh et al., 2024). Zinc, selenium, and copper are a few heavy metals that are among trace elements and are indispensable for quotidian body activities. But these could become injurious to animals when the level exceeds the limits. Metals like copper, cadmium, chromium, lead, mercury, manganese, nickel, silver, and zinc are largely associated with environmental problems (Pandey & Madhuri, 2014) as they form enormous complexes when dissolved in water and have a pronounced effect on aquatic fauna (Thangam et al., 2014). They become carcinogenic for living organisms by forming reactive oxygen species in their bodies. Trace amount of copper (Cu) is essentially required for animals (Linder, 2001; Kek et al., 2025). It is significant for enzymatic activities and also play a vital role in cellular oxidation and reduction processes. Copper is commercially used in electrical appliances and is also a constituent of drugs and pesticides (Dehcheshmeh et al., 2024). Due to its fungicidal and algaecidal characteristics, it is extensively used to control the diseases of various crops, vegetables, and fruit orchards caused by algal, fungal, and bacterial attacks (Atamanalp et al., 2008; Das et al., 2025).

Increasing practices of reckless discharge of copper in natural ecosystems is polluting freshwater bodies (Campagna et al., 2008). Copper toxicity is immensely related to its ionic form i.e. Cu^{2+}. Due to increased concentration of copper in environment the formation of free radicals escalates in the body of aquatic organisms which leads toward certain chromosomal abnormalities and toxic conditions (Ghosh et al., 2024).

The level of pollution is examined in freshwater environments by using fish as it is ranked on the top of aquatic

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food chain, designating the impact of heavy metals on other living forms of water as well as on human. As a source of food fish is consumed by human. Fish cannot get rid of metal toxicants if water is contaminated, so may be harmful for consumer's health (Thangam et al., 2014). Therefore, the level of contaminants must be known in the natural waters for the heavy metal toxicity (Ahmed et al., 2022). *Wallago attu* is a carnivorous fish species of family "siluridae" and found in the natural waters of Pakistan, Nepal, Bangladesh, Afghanistan and India (Jayaram, 1999). Freshwater pollution is causing severe adverse effects on the food intake of krill species, which is leading to disturbance in the food pyramids of recent times. Having top position in the food chain of freshwater, *Wallago attu* prey upon others with accumulated heavy metals in high quantities (Olaifa et al., 2004). Considerable deterioration of water quality due to heavy metals has reduced the population size of carnivorous fish species (Javed et al., 2016).

In order to investigate the toxic effects of contaminants on aquatic ecosystems, toxicity testing is a necessary approach. The possible hazards to living organisms can be reduced by utilizing the knowledge about the acute toxic effects of heavy metals (Suchismita Das & Abhik Gupta, 2010). The vulnerability of fish to heavy metal contamination is assessed by computational features viz, lethality and survival (Ebrahimpour et al., 2010). Single metal exposures to fish are utilized to examine the adverse effects on the physiology (Bagdonas & Vosylienė, 2006). The LC_{50} value is used as a measure of the susceptibility of fish to several contaminants. The duration of fish exposure is also an essential aspect of toxicity. Various signs of fish physiological parameters are examined during acute toxicity testing, which ultimately cause lethality (Jezierska et al., 2009). The physicochemical parameters play a vital role in the dissemination and disposal of heavy metals to fish. Certain water quality variables such as water pH, dissolved oxygen, temperature, total ammonia, carbon dioxide, and total hardness, influence the copper toxicity (Jezierska & Witeska, 2001; Rai, Ullah, & Haider, 2015). The variations take place when water contains copper in dissolved form, with a low concentration of calcium carbonate and a low pH (Thangam et al., 2014; Tokhun et al., 2014). A considerable amount of research has been conducted to evaluate the acute toxicity of metals to herbivorous fish species in Pakistan. However, there is a need to determine the toxicity of metallic ions to carnivorous fish species. Therefore, the present research work was conducted under controlled laboratory conditions to assess the endurance of *Wallago attu* through 96-hour median lethal and lethal concentration toxicity trials. The physicochemical variables were also examined for their relationships in copper-exposed media.

2. MATERIALS AND METHODS

2.1. Copper Acute Toxicity Determination

Carnivorous fish species *Wallago attu* was tested for acute toxicity of copper in terms of 96-hr LC_{50} and lethal doses under controlled laboratory conditions, following all the institutional ethical regulations. For this purpose, fish from the experimental ponds of the Zoology and Fisheries Department at the University of Agriculture, Faisalabad, that had been hatched and raised for experimental purposes, were used. Fish fingerlings were fed during the time of adjustment with feed having 35% digestible protein and 2.90kcal g^{-1} digestible energy to their satiation every day. Fish were not provided with food during the research trial. The physico-chemical parameters, such as pH (8), temperature (28ºC), and total hardness (250mg/L) of the aquatic media, were kept constant during the whole experiment, with three replications for each concentration. The aquaria and all types of glassware were keenly washed to be used in trial with dechlorinated water. Ten fish per aquarium, having a water capacity of 35-L, were stocked for a separate dose of copper. Fish with average wet weight of 22.50±3.54g and total length of 199.50±6.40mm was used. An air pump with a capillary system was used for the continuous supply of air to the test media for the whole duration. Copper sulphate ($CuSO_4.5H_2O$) in pure chemical form was dissolved in deionized water for stock solution preparation. The dose of copper was started from zero and raised to 0.05 for the low and 5mg/L for the high concentration assessment of acute toxicity in terms of 96-hour trial. To evade the sudden stress on fish, 50% of test dose was gradually maintained in 3 hours and completed the application of dose in 6 hours in each aquarium. In order to keep the record, dead fish were removed from the aquatic media instantly.

2.2. Analysis of Water Quality Variables

The method of APHA (American Public Health Association) 1998 was followed for the determination of water quality variables viz. pH, temperature, total hardness, total ammonia, carbon dioxide, dissolved oxygen, calcium, magnesium, sodium and potassium at 12-hour interval.

2.3. Statistical Analyses

The probit analysis method was applied on the fish mortality data with a limit of 95% confidence interval for acute toxicity tests (Hamilton et al., 1977). In order to find a relationship between water quality variables, correlation and regression analyses, we followed (Steel & Torrie, 1984).

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3. RESULTS

3.1. Acute Toxicity of Copper

Values of median lethal and lethal concentration of copper attained by Probit analysis (for mortality of fish) are presented in Table 1. As the concentration of copper was raised, the rate of fish mortality also increased (Fig. 1). The value of LC50 computed for 96 hours of copper exposure for *Wallago attu* was 27.56±0.21mg/L for the first replication, whereas the 95% confidence interval was obtained as 23.59 - 30.66mg/L. Table 1 shows a 49.58±0.27mg/L value as the lethal concentration of metal for the fish with a confidence interval of 44.26-59.36mg/L (Table 1; Fig. 1A). The confidence interval range was found as 44.26 - 59.36mg/L.

Table 1: Calculated 96-hr LC_{50} and lethal concentrations of copper for the fish (*Wallago attu*)

Replication	Mean 96-hr LC_{50} (mg/L)	95% confidence interval (mg/L)	Mean lethal concentration (mg/L)	95% confidence interval (mg/L)
1	27.56±0.21a	23.59 - 30.66	49.58±0.27a	44.26 - 59.36
2	26.36±0.11a	21.82 - 29.54	48.52±0.25a	43.30 - 58.52
3	27.31±0.17a	24.08 - 29.90	44.34±0.19a	40.35 - 51.18
Average	27.08±0.63a		47.48±2.77a	

Mean±SD with similar letters in a column differ non-significantly (P<0.05).

The LC_{50} value of copper for the second replication was attained as 26.36±0.11mg/L with a range of 95% confidence interval 21.82 - 29.54mg/L (Fig. 1B). Whereas, Table 1 shows the results of lethal Cu concentration calculated as 48.52±0.25mg/L.

The value computed by Probit curve for median lethal concentration in terms of 96-hour toxicity trial was 27.31±0.17mg/L (95% confidence interval range of 24.08-29.90mg/L) for the third replication. The lethal concentration of Cu for *Wallago attu* was 44.34±0.19mg/L (Table 1; Fig. 1C). While 40.35 - 51.18mg/L was the range of confidence interval.

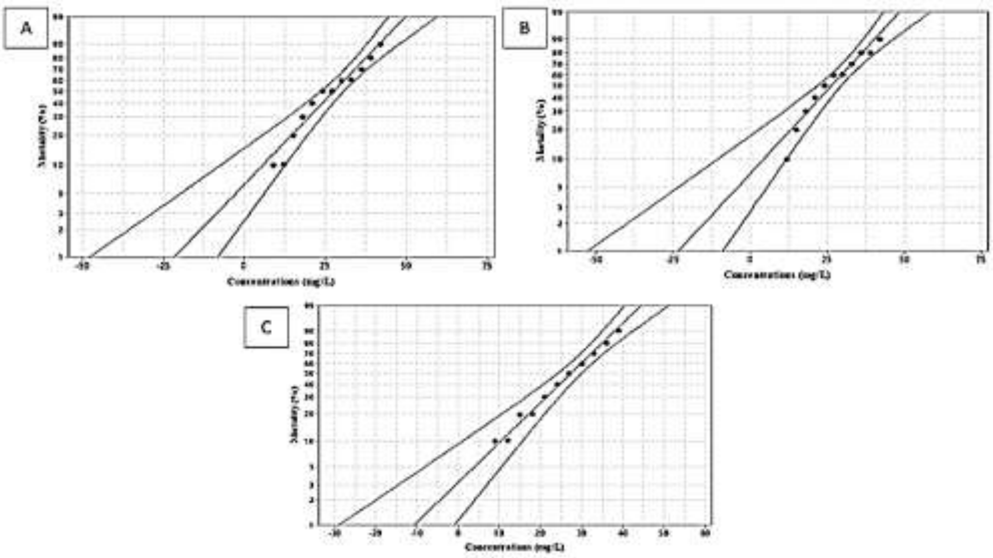

Fig. 1: Probability plot for mortality percent of *Wallago attu* under different exposure concentrations of copper.

Non-significant differences were found among all the three replicates of LC_{50} and lethal concentration experiments by analysis of variance. LC_{50} and lethal concentration values of Cu revealed statistically significant variation for the fish (Table 1).

3.2. Correlation Analyses

Herein, the data on mean values of all water quality variables of media with various copper concentrations for the fish species *Wallago attu*, during acute toxicity trials (Table 2). Correlation analysis was applied on the values obtained of all water quality variables in order to evaluate the relationship among variables and concentration of copper for the fish during toxicity tests (Table 3). Statistically positive and highly significant correlation was evaluated for ammonia and carbon dioxide with copper concentration. On the other hand, copper concentration exhibited negative and highly significant relation with dissolved oxygen. The contents like calcium, magnesium,

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sodium and potassium exhibited non-significant relation with concentration of copper. The relationship of total ammonia was found positive and highly significant with carbon dioxide while reverse was observed for dissolved oxygen. All other water quality variables of test media represented non-significant relationship with total ammonia. Carbon dioxide was observed to have a negative and highly significant relationship with dissolved oxygen content of test media whereas, non-significant correlation with other water quality variables. Dissolved oxygen revealed non-significant correlation with calcium, magnesium, sodium and potassium contents. Statistically significant correlation was observed between calcium and sodium whereas, calcium was found statistically negatively correlated and showed highly significant relation with magnesium. However, calcium and potassium showed non-significant relation. Negative and statistically significant correlation was assessed among magnesium and sodium contents. Sodium and potassium contents of test media were found non significantly correlated (Table 3). Fig. 1 shows a probability plot for the percent mortality of *Wallago attu* under different exposure concentrations of copper, a) Replication 1, b) Replication 2 and c) Replication 3.

Table 2: Mean±SD for physico-chemical variables of the copper-exposed test media recorded during 96-hr toxicity trials for *Wallago attu*.

Concentrations (mg/L)	pH	Temperature (°C)	Total hardness (mgL^{-1})	Total ammonia (mg/L)	Carbon dioxide (mg/L)	Dissolved oxygen (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)
3.00	8.00±0.00	28.00±0.00	250.00±0.00	0.16±0.03	0.40±0.11	5.78±0.08	22.00±0.56	48.75±0.35	238.00±7.22	11.24±0.45
6.00	8.00±0.00	28.00±0.00	250.00±0.00	0.19±0.04	0.43±0.04	5.72±0.05	24.00±2.37	47.50±1.48	255.30±6.19	10.99±0.89
9.00	8.01±0.01	28.00±0.01	250.01±0.01	0.14±0.03	0.47±0.06	5.69±0.33	25.12±1.36	46.80±0.85	293.00±14.12	13.16±1.47
12.00	8.00±0.01	28.01±0.00	250.00±0.00	0.25±0.08	0.51±0.05	5.63±0.07	22.44±1.95	48.47±1.22	212.00±9.37	12.34±0.10
15.00	8.00±0.00	28.00±0.01	250.01±0.01	0.27±0.05	0.39±0.04	5.61±0.09	26.00±1.43	46.25±0.89	287.34±8.10	15.25±1.18
18.00	8.00±0.00	28.00±0.00	250.00±0.00	0.18±0.05	0.56±0.05	5.57±0.12	23.11±2.15	48.06±1.34	309.02±9.32	11.00±0.22
21.00	8.02±0.01	28.00±0.00	250.02±0.01	0.31±0.07	0.59±0.07	5.54±0.11	23.03±2.23	48.11±1.39	249.23±15.80	14.32±1.68
24.00	8.01±0.01	28.02±0.01	250.00±0.00	0.35±0.07	0.41±0.06	5.52±0.27	26.13±0.80	46.17±0.50	218.49±6.44	12.54±1.31
27.00	8.00±0.00	28.00±0.00	250.00±0.00	0.39±0.07	0.62±0.14	5.61±0.18	23.00±2.38	48.13±1.49	257.00±12.16	11.45±1.22
30.00	8.00±0.00	28.00±0.00	250.00±0.01	0.51±0.06	0.65±0.17	5.46±0.08	27.00±0.01	45.63±0.08	312.44±7.50	16.87±0.21
33.00	8.00±0.00	28.00±0.01	250.01±0.01	0.69±0.07	0.73±0.13	5.43±0.06	22.32±2.17	48.55±1.36	211.68±9.73	13.81±1.74
36.00	8.01±0.01	28.00±0.00	250.00±0.00	0.73±0.05	0.77±0.14	5.40±0.08	21.00±1.54	49.38±0.96	244.78±19.28	15.33±1.39
39.00	8.00±0.00	28.00±0.00	250.01±0.01	0.88±0.08	0.83±0.09	5.37±0.07	27.21±0.50	45.49±0.31	321.12±6.46	14.00±1.71
42.00	8.00±0.01	28.01±0.01	250.00±0.00	1.02±0.10	0.89±0.08	5.33±0.10	25.00±2.56	46.88±1.60	222.45±7.42	11.43±0.91
45.00	8.01±0.01	28.00±0.01	250.00±0.00	1.17±0.12	0.90±0.05	5.27±0.05	21.66±1.52	48.96±0.95	209.19±7.63	13.56±2.23
Means±SD	8.00±0.01	28.00±0.01	250.00±0.01	0.48±0.34	0.61±0.18	5.53±0.15	23.93±2.01	47.54±1.26	256.07±39.38	13.15±1.80

Table 3: Correlation coefficients among exposure concentrations (mg/L) of copper and physico-chemical variables of the test media for *Wallago attu*

	Conc.	T. Amm.	CO2	DO	Ca	Mg	Na
T. Amm.	0.936 P<0.01						
CO2	0.920; P<0.01	0.932; P<0.01					
DO	-0.973; P<0.01	-0.932; P<0.01	-0.900; P<0.01				
Ca	0.040; NS	-0.038; NS	-0.128; NS	-0.053; NS			
Mg	-0.040; NS	0.038; NS	0.128; NS	0.053; NS	-1.000 P<0.01		
Na	-0.138; NS	-0.258; NS	-0.124; NS	0.149; NS	0.587; P<0.05	-0.587; P<0.05	
K	0.351; NS	0.253; NS	0.220; NS	-0.400; NS	0.277; NS	-0.276; NS	0.289; NS

Conc=Concentrations (mg/L); T. Amm=Total ammonia (mg/L); CO2=Carbon dioxide (mg/L); DO=Dissolved oxygen (mg/L); Ca=Calcium (mg/L); Mg=Magnesium (mg/L); Na=Sodium (mg/L); K=Potassium (mg/L); P<0.01=Highly significant; P<0.05=Significant; NS=Non-significant.

4. DISCUSSION

Being essential, Cu is a metal required for living forms. It is discharged into Pakistan's freshwater resources in various ways, consequently posing a threat to the survival of freshwater fauna, especially fish. The bio-amplification of substantial metals in natural ways of life make them progressively extraordinary at the higher trophic levels (Wyn et al., 2007; Naz et al., 2025). The 96-hr toxicity experiments are utilized to assess the toxic effects of specific toxicant that leads to fish toxicity during trial of short time period (Deshai et al., 2012). The tests were conducted to make a comparison of 96-hr LC_{50} and lethal concentrations of metal (Cu) by using static bioassay for *Wallago attu*.

The LC_{50} value was determined to be 27.08±0.63mg/L in the present experiment. Whereas, the mean lethal dose of Cu for the fish *Wallago attu* was 47.48±2.77mg/L. The research work found that with an increase in the duration of Cu exposure, its toxic effects also increased. Wani et al. (2013) evaluated the LC50 value of copper sulfate (CuSO4) for Clarias gariepinus, which was found to be 40.86mg/L, and toxicity was influenced by both the duration of exposure and concentration (Wani et al., 2013). The fish species *Rutilus frisii kutum* was examined for LC_{50} of Cu, and results were found as 2.310 (96-hr), 2.562 (72-hr), 2.756 (48-hr), and 2.944mg/L (24-hr) (Gharedaashi et al., 2013). For stripped dwarf catfish (*Mystus vittatus*), copper toxicity was assessed for the juvenile and adult fish. The median lethal concentration values were found as 18.62 (96-hr), 25.11 (72-hr), 28.84 (48-hr), and 38.90mg/L (24-hr) for juvenile *Mystus vittatus*. The LC_{50} values in terms of acute toxicity trials were found as 47.86 (96-hr), 56.23 (72-hr), 69.18 (48-hr) and 89.13mg/L (24-hr) for adult fish (Subathra et al., 2007). *Oncorhynchus mykiss* was tested for copper and zinc toxicity. 12.88mg/L was found as the value of LC_{50} for Zn, while for copper it was 0.094mg/L. Hence the results indicated that copper is more toxic to the fish (Gündoğdu, 2008). The LC_{50} value of copper for Brazilian fish species *Prochilodus vimboides* and *Leporinus microcephalus* were computed as 0.047 and 0.090mg/L, respectively during 96 hour toxicity trial (Gomes et al., 2009; Amouri et al., 2025). da Silva et al. (2014) worked to investigate the lethal and LC_{50} value of copper sulfate ($CuSO_4$) for *Hyphessobrycon eques* during 72-hr trial. The values of lethal and LC_{50} were found as 20 and 0.16mg/L for the fish species *Hyphessobrycon eques* (da Silva et al., 2014; Akash et al., 2025). Another scientist examined fish species Greater bony-lipped barb and Nile tilapia for acute toxic effects of copper (Tokhun et al., 2014). The LC_{50} values were calculated as 43.56 and 795.62µgL^{-1}, respectively. *Colossoma macropomum* was used to conduct experiments to assess copper toxicity. The median lethal concentration was computed as 17.5mg/L in terms of 96-hr trial (Tavares-Dias et al., 2011). The LC_{50} values for *Oreochromis niloticus* and *Clarias gariepinus* were observed as 58.837 and 70.135mg/L, respectively for 96-hr duration (Ezeonyejiaku et al., 2011). The LC_{50} value of Cu was evaluated as 502.95µg/L for *Poronotus triacanthus* and 73.83µg/L for *Danio rerio* (Campagna et al., 2008). Copper was found to be more toxic when *Mugil seheli* was tested against copper and cadmium concentrations, exhibiting an LC_{50} value of 1.64 and 5.36mg/L, respectively (El-Naga et al., 2005). Zarei et al. (2013) found mean median lethal concentration of Cu for black fish (*Capoeta fusca*) as 6.928mg/L (Zarei et al., 2013). Kumar et al. (2015) tested *Clarias batrachus* against 96-hr median lethal concentration of Cu and revealed the value as 33µg/L (Kumar et al., 2015). Another researcher found Cu more toxic metal when exposed *Rachycentron canadum* to concentrations of Cu and Zn, revealing LC_{50} values of 0.06 and 0.31mg/L, respectively (Cuong et al., 2004).

The fish *Prochilodus scrofa*, commonly known as a teleost, was examined for Cu toxicity. The median lethal concentrations at two different pH values 4.5 and 16 were observed as 98 and 88µg/L, respectively. The median lethal concentration was found as 14µg/L when Cu exposed test media was subjected to temperatures of 20 and 30°C at 8 pH (Carvalho & Fernandes, 2006). The water quality variables have an influence on the heavy metal toxicity to the fish (Subhanullah et al., 2024). The water hardness showed an inverse relation with the toxicity of copper-exposed media (Kiyani et al., 2013). With the rise in hardness of freshwater, Ca and mg. ions undergo antagonism, which lessens the metallic ion intake to the fish body (Kim et al., 2001; Pyle et al., 2002; Lipy et al., 2024). The present work exhibited that increased Cu concentration gives rise to increased concentrations of total ammonia and carbon dioxide in water. Abdullah et al. (2007) revealed that a rise in excretion of ammonia and CO_2 in water was resulted when metal's concentration was raised (Abdullah et al., 2007). Rai et al. (2015) examined reduced dissolved oxygen content with an increase in copper concentration of test media (Rai et al., 2015).

5. CONCLUSION

Herein, it was concluded that the fish mortality percentage increased concomitantly with a rise in Cu concentrations. The mean 96-hr median lethal (LC_{50}) and lethal concentration values of copper for the carnivorous fish species, *Wallago attu* were computed as 27.08±0.63 and 47.48±2.77mg/L, respectively. With a rise in Cu concentration, total ammonia and carbon dioxide contents were also increased significantly.

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Ethics Statement: This work did not involve any human data. The work was approved by the Institutional Biosafety and Bioethics Committee (IBC) by Permission No. 504/ORIC and by the Synopsis Scrutiny Committee by Permission No. DGS/535-539, University of Agriculture, Faisalabad, Pakistan. The animals in the present study were cared for and treated according to the Guidelines of the National Biosafety Committee 2005, Punjab Biosafety Act 2014, and Punjab Health Act 2019.

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