Journal of Advances and Scholarly Researches in Allied Education
Vol. 22, Issue No. 4, July-2025, ISSN 2230-7540

DOI: https://doi.org/10.29070/chxred39

Check for updates

Histopathological Evaluation of Reproductive Organs in Freshwater Teleost Fishes Exposed to Copper Sulphate

Anupam Gautam [1] * , Dr. Vinay Kumar Singh [2]

1. Research Scholar, A.P.S. University, Rewa, M.P., India
surbhisingh02070@gmail.com ,
2. Professor, Department of Zoology, Pandit Shambhunath Shukla P G. College, Shahdol, M.P., India

Abstract: Copper sulphate is widely used in agriculture and aquaculture to control pests and pathogens; however, its accumulation in aquatic environments raises concerns regarding its toxic effects on non-target organisms, particularly freshwater teleost fishes. This study aims to investigate the histopathological alterations induced by copper sulphate exposure in the reproductive organs of selected freshwater teleost species. Specimens were exposed to sublethal concentrations of copper sulphate over an extended period under controlled laboratory conditions. Detailed microscopic examinations of ovarian and testicular tissues were conducted to identify structural changes, including degeneration of germ cells, oocyte atresia, testicular necrosis, vacuolization, and disruption of normal tissue architecture. The results revealed dose- and time-dependent histological damages, suggesting that copper sulphate induces significant reproductive toxicity by impairing the integrity of gonadal tissues. These morphological alterations are indicative of impaired gametogenesis and reduced reproductive potential, which could have serious implications for fish population sustainability in contaminated habitats. This research underscores the need for stringent monitoring of copper levels in freshwater ecosystems and highlights histopathology as a valuable tool for assessing sublethal toxic effects on fish reproduction. The findings contribute to a better understanding of the ecological risks posed by copper sulphate pollution and support the development of environmentally safer management practices.

Keywords: Copper sulphate, Histopathology, Reproductive toxicity, Freshwater teleost fishes, Gonadal damage, Sublethal exposure

X

INTRODUCTION

The vitality of freshwater teleost fish populations and other aquatic species' reproductive systems is an important factor in the long-term viability and variety of these habitats. Reproduction in fish is an elaborate and controlled process that involves many different kinds of cells, hormones, and biochemical [1]. The health and proper functioning of the reproductive organs, the testes and ovaries, which are in charge of gamete creation and maturation, are crucial to these processes. Significant ecological and economic risks may result from disturbances to the structural and functional components of these organs, which can reduce fertility, change reproductive cycles, and eventually cause population losses [2].

A wide variety of chemical contaminants have been polluting freshwater ecosystems due to the proliferation of human activities in the last few decades. Heavy metals are one group of pollutants that have received a lot of attention because of how they persist, bioaccumulate, and are hazardous [3][4]. Despite its importance in many metabolic and enzymatic processes, copper becomes poisonous at high doses. Copper sulphate ($CuSO_4$), a chemical that controls infections and algae development in aquaculture and is used

extensively as a fungicide and algaecide in agriculture, is one of the main causes of copper pollution in aquatic ecosystems [5]. Because of its widespread usage, copper sulphate has accumulated in freshwater bodies via direct application, industrial effluents, and agricultural runoff, putting aquatic creatures at risk of exposure to dangerous levels [6][7].

There is a lot of evidence that copper sulphate is harmful to fish and other aquatic organisms, with consequences ranging from immediate death to long-term, non-fatal impairments. Because it has such severe impact on the dynamics of fish populations and the health of ecosystems over the long run, reproductive toxicity is among these most pressing concerns. Pollutants in the environment may have a disproportionately negative impact on fish reproduction because of the delicate hormonal control and cellular mechanisms involved [8].

Sublethal effects of pollutants on fish may be evaluated via histopathology, which involves microscopic investigation of tissue abnormalities produced by toxicants. Because it enables the direct study of tissue structure changes, it may detect toxicity before its overt physiological or population-level impacts are apparent. Because of their central significance, ecotoxicological studies often look at the reproductive organs to see how pollutants affect fish reproduction [9].

Histological changes in fish gonads caused by copper sulphate exposure include ovular follicular atresia, vacuolization, necrosis, and degeneration of the germinal epithelium in females and disruption of spermatogenesis and testicular architecture in men [10]. Decreased gamete quality and fertility may result from these structural abnormalities, which are often linked to poor endocrine function and altered hormonal signalling. Species, exposure dose and duration, and environmental variables including water chemistry may all affect the kind and degree of histopathological alterations [11].

Detailed histological alterations in reproductive organs of freshwater teleosts exposed to sublethal concentrations of copper sulphate have not been the subject of many thorough investigations, despite the acknowledged significance of copper sulfate-induced reproductive toxicity [12]. To better understand reproductive failure and create reliable indicators for environmental monitoring, it is essential to comprehend these minute changes at the tissue level [13].

To address this knowledge gap, the present investigation used freshwater teleost fish subjected to sublethal amounts of copper sulphate to undergo a thorough histological examination of their reproductive organs. The goal of this study is to determine if there are any changes in reproductive capability, what those changes may be, and how they are related to the particular morphological alterations by looking at testicular and ovarian tissues during a certain exposure time [14].

The ecological importance of fish reproductive health, the environmental presence and widespread use of copper sulphate, and the vulnerability of fish gonadal tissues to metal-induced damage are first highlighted in this introduction, which lays the groundwork for a thorough evaluation of copper sulphate's effects on fish reproduction [15]. It lays the groundwork for thorough experimental observations that may influence ecological risk assessments and pollution control techniques, and it highlights the importance of histopathological studies as a sensitive method to discover sublethal toxic effects [16].

Furthermore, this study fills a critical gap in our understanding of the relationship between environmental

pollution and its biological impacts on a tissue level, which is important for conservation biology and aquaculture. As a result, our understanding of aquatic toxicity is enhanced. Policymakers hope that the results will help them reduce copper pollution in freshwater systems, improve our knowledge of the processes by which copper sulphate is hazardous, and pave the way for the creation of monitoring tools based on tissue biomarkers.

REVIEW OF LITERATURE

Handy R. (2003) Chronic exposure to copper in aquatic environments leads to a range of physiological and cellular changes in fish, which serve as adaptive mechanisms for them to endure prolonged metal stress. Copper is an essential trace element involved in numerous biological processes but also known as an endocrine-disrupting metal that can interfere with normal neuroendocrine functions in vertebrates, including fish. Understanding the chronic effects of copper on aquatic organisms is crucial, as it is both vital and potentially toxic. The study suggests that many of the chronic physiological alterations observed in fish exposed to copper can be attributed to disruptions in neuroendocrine regulation. Prolonged copper exposure triggers complex physiological adjustments across multiple organ systems, such as increased oxygen consumption and decreased swimming speed. It also leads to up regulation of ionic regulatory mechanisms, which are essential for maintaining osmotic balance amid metal-induced disturbances. Immunologically, fish exhibit altered cellular profiles, with reduced lymphocyte counts and increased neutrophil populations, signifying a shift in immune status and potential inflammation. This immunomodulation is accompanied by changes in both copper-dependent and copper-independent enzyme activities, reflecting biochemical attempts to mitigate oxidative stress and maintain metabolic homeostasis. These multifaceted responses arise whether copper is introduced through contaminated water or dietary sources and can be broadly categorized into three major adaptive strategies: upregulation of enzymes and metabolic pathways, modulation of haematopoietic activity, and alterations in tissue cellularity. These responses are mediated by generalized stress responses, such as activation of the adrenergic system and elevated cortisol release through the hypothalamic-pituitary-interrenal (HPI) axis [28].

Banerjee et al. (2022) Heavy metals are pervasive and harmful contaminants in aquatic ecosystems, with their concentrations dramatically increasing due to anthropogenic activities such as industrial waste discharge, geochemical variations, agricultural practices, and mining operations. Fish, as inhabitants of these aquatic environments, are inevitably exposed to heavy metals, which tend to accumulate in their tissues over time due to excessive pollutant release. Since fish reproduce in natural water bodies that may be contaminated, understanding the impact of heavy metals on fish reproduction is crucial. Reproduction is fundamental for fish population sustainability in natural ecosystems and is also essential for successful aquaculture production. The quality of water, particularly its heavy metal content, plays a significant role in influencing reproductive health and success in fish. Although numerous studies have examined the general effects of heavy metals on fish health including alterations in blood biochemistry, histopathological changes, and cellular and physiological stress in vital organs there remains a scarcity of comprehensive reviews focused specifically on the toxic effects of heavy metals on fish reproduction. Existing evidence suggests that heavy metals disrupt reproductive processes by inhibiting vitellogenin synthesis, delaying oogenesis, altering luteinizing hormone secretion, reducing gonadosomatic index values, and impairing ovulation. This review paper aims to provide an integrative overview of the toxicological impacts of heavy

metals on fish reproduction, with particular emphasis on the effects on both male and female reproductive systems, thereby highlighting the need for further research and improved environmental management to safeguard aquatic biodiversity and aquaculture productivity [6].

Modi R. (2022) This study aimed to assess the impact of heavy metals, copper sulfate ($CuSO_4$) and zinc sulfate ($ZnSO_4$), on the reproductive physiology of freshwater teleost fish Anabas testudineus. The research focused on the Gonadosomatic Index (GSI) and the integrity of the hypothalamic-pituitary-gonadal (HPG) axis, which regulates gonadal development and spawning activities through hormonal signaling pathways. Female Anabas testudineus specimens were exposed to waterborne solutions containing various concentrations of $CuSO_4$ and $ZnSO_4$ under controlled laboratory conditions. Acute toxicity testing revealed median lethal concentrations of 2.5 mg/L for $CuSO_4$ and 3 mg/L for $ZnSO_4$, indicating considerable susceptibility of this species to these heavy metals at relatively low concentrations. Sub-lethal concentrations were selected to simulate environmentally relevant chronic exposures, and fish were subjected to these doses for an extended period of 30 days to better understand the long-term effects of heavy metal contamination. Histopathological examination of the neurohypophysial complex revealed significant structural alterations in fish exposed to $CuSO_4$ and $ZnSO_4$, including degeneration of neurosecretory cells, disruption of tissue architecture, vacuolation, and signs of cellular stress indicative of neuroendocrine dysfunction. The Gonadosomatic Index, a quantitative measure reflecting the relative size of the gonads in relation to the total body weight of the fish and widely used as an indicator of reproductive status, was significantly reduced in heavy metal-exposed groups, suggesting an inhibitory effect of copper and zinc sulfate on gonadal development and spawning potential, likely mediated through disruptions of the HPG axis. These findings highlight the heightened sensitivity of the neuroendocrine system of Anabas testudineus to heavy metal pollutants and underscore the potential ecological consequences of metal contamination in freshwater environments, particularly with respect to fish reproduction and population sustainability [5].

Naz et al. (2023) Metallic trace elements pose a significant threat to fish health, affecting physiological regulation, reproductive capacity, behavior, and development. This comprehensive review explores the harmful impacts of common metallic toxicants, focusing on cadmium (Cd), copper (Cu), and lead (Pb). The neurological system of fish is particularly vulnerable, with exposure causing neurotoxicity that impairs behavior and cognitive functions essential for survival. Reproductive health is compromised, leading to decreased fertility rates, disrupted hormonal balances, and impaired embryonic development. Histopathological changes in tissues such as the liver, kidneys, and gills reveal cellular damage induced by metallic pollutants. Urgent intervention strategies are required to address and minimize the discharge of metal-laden wastes from agricultural runoff, industrial effluents, and domestic wastewater. The review evaluates the most effective remediation and treatment technologies currently employed to reduce metallic trace elements contamination in aquatic systems, discussing methods such as chemical precipitation, ion exchange, adsorption using bio-sorbents, membrane filtration, and phytoremediation. Supporting aquatic life from the toxic effects of metallic trace elements requires coordinated efforts in pollution control, stringent regulatory policies, and the adoption of innovative water treatment solutions. The review underscores the complexity of their interactions within fish physiology and the need for coordinated efforts in pollution control, regulatory policies, and innovative water treatment solutions to restore and preserve

healthy aquatic ecosystems [2].

MATERIAL AND METHDOD

Selection of Fish Species

Two freshwater fish species, *Labeo rohita* (Rohu) and *Channa punctata* (Spotted Snakehead), were selected for this study due to their ecological importance, widespread presence in Indian freshwater systems near Rewa, and known sensitivity to copper contamination. Both species play key roles in the aquatic food web, have well-studied reproductive biology, and are economically valuable for local fisheries and aquaculture [17]. They adapt well to laboratory conditions, allowing controlled experiments with minimal variability, and their size and robustness make them suitable for repeated physiological and biochemical assessments to evaluate the effects of copper sulphate on reproduction [18].

Selection of Experimental Site and Facilities

The study combined field and laboratory methods for a thorough evaluation of copper sulphate toxicity [19]. Field sampling took place in various freshwater sites within the Rewa district of Madhya Pradesh, including the Tamsa and Bichhiya Rivers, Rani Talab, and Govindgarh Lake selected for their ecological importance and vulnerability to agricultural runoff and industrial pollution. Laboratory experiments were conducted at the Department of Zoology, A.P.S. University, Rewa, equipped with temperature-controlled aquaria, aeration systems, and water quality monitoring tools. The lab also possessed advanced instruments for histology, biochemical tests, and hormone analyses [19]. All procedures complied with ethical guidelines set by the CPCSEA, Government of India, ensuring humane animal treatment and environmentally responsible research practices [20].

Preparation of Copper Sulphate Solutions

Analytical grade copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) was used to prepare stock solutions. Precision weighing was performed using an analytical balance, and the salt was dissolved in deionized water to make a 1000 mg/L stock solution [21]. Working solutions for exposures (e.g., 0.25, 0.5, 1.0 mg/L) were freshly prepared by serial dilution with dechlorinated and filtered aquarium water to maintain accuracy and avoid degradation. Water quality parameters such as pH and temperature were measured and adjusted to mimic natural conditions, preventing confounding effects on copper bioavailability and fish health. Solutions were stored in amber bottles and used within 24 hours. Copper concentration in exposure tanks was regularly monitored through atomic absorption spectrophotometry (AAS) to ensure stable and consistent dosing [22].

Experimental Design and Exposure Protocol

The experiment consisted of two groups: a Control Group with fish maintained in clean, uncontaminated water, and a Treatment Group exposed to a sublethal concentration of copper sulphate (0.5 mg/L). This exposure level was selected based on preliminary toxicity trials and literature indicating its suitability for chronic studies without inducing acute mortality [23].

Each group was housed in separate 100-liter aquaria with continuous aeration and filtration. Environmental

parameters were standardized: temperature at 25 ± 1°C, dissolved oxygen above 6 mg/L, pH maintained between 7.0 and 7.5, and a 12-hour light-dark photoperiod. Fish were exposed for 60 days to capture chronic effects, with sampling conducted on Days 15, 30, 45, and 60. At each interval, fish were sampled for histological, biochemical, hormonal, and behavioral analyses. Water quality and copper levels were continuously monitored and maintained throughout the study [24].

Monitoring of Water Quality Parameters

Maintaining optimal and consistent water quality was vital to isolate the effects of copper sulphate. The following parameters were monitored:

- **Temperature:** Measured daily with a digital thermometer, maintained at 25 ± 1°C.
- **pH:** Recorded daily using a calibrated digital pH meter, kept between 7.0 and 7.5.
- **Dissolved Oxygen (DO):** Monitored daily with a portable DO meter, maintained above 6 mg/L.
- **Total Hardness:** Measured weekly using EDTA titration.
- **Ammonia:** Measured weekly using Nessler's reagent colorimetry, kept below 0.02 mg/L.
- **Copper Concentration:** Assessed every five days via AAS to ensure target exposure levels.

All measurements were done in triplicate to ensure precision and repeatability.

Sampling Schedule and Handling Procedures

Sampling was conducted at predetermined intervals (Days 15, 30, 45, and 60) to evaluate the progression of copper toxicity. At each time point, five fish per replicate per group were randomly selected [25]. Fish were anesthetized using MS-222 (tricaine methanesulfonate) at 100 mg/L to minimize stress. After anesthesia, fish were weighed and measured. Blood was drawn from the caudal vein using sterile heparinized syringes for hematological and biochemical assays. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C. Fish were then humanely euthanized, and gonads (ovaries/testes) were dissected out, rinsed in ice-cold saline, and processed immediately. Tissues for histology were fixed in 10% neutral buffered formalin; those for biochemical/hormonal assays were flash-frozen in liquid nitrogen and stored at −80°C [26].

Histological Examination of Gonads

Gonadal tissues were processed using standard histological techniques. Fixed samples were dehydrated in graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5–7 µm were cut on a microtome and mounted on adhesive-coated slides. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) to differentiate cellular and tissue components. Microscopic examination was performed at 100× to 400× magnifications [27].

Histopathological evaluation focused on:

- Ovarian follicle development and oocyte maturation.

- Presence of atretic follicles and vacuolization.
- Testicular spermatogenesis stages.
- Necrosis, cellular degeneration, and inflammatory responses.

Quantitative assessments involved counting different gonadal cell types and measuring follicle diameters using image analysis software for statistical comparison [28].

Statistical Analysis

Data were analyzed using SPSS software. One-way ANOVA was used to compare means among groups, followed by post-hoc tests for pairwise comparisons. Student's t-test was employed for control versus individual treatments. Correlation analyses examined relationships between copper levels, gonadosomatic index (GSI), and hormone concentrations [29]. Significance was set at $p < 0.05$. Results were visualized using graphs and charts generated by SPSS and Microsoft Excel for clear data interpretation.

DATA ANALYSIS AND RESULT

Histological Changes in Gonadal Tissue

Histological examination offers crucial microscopic evidence that reveals the extent of structural damage inflicted on the gonads by copper sulphate toxicity. In this study, detailed analyses of gonadal tissue sections were conducted to identify and characterize specific pathological features such as follicular atresia in the ovaries, alterations in spermatogenic activity within the testes, tissue necrosis, and the presence of inflammatory cell infiltration. To complement the qualitative observations, quantitative assessments were performed, including precise measurements of oocyte diameters, calculation of the percentage of atretic follicles, and evaluation of spermatogenic indices. These histomorphometric parameters provide an objective and quantifiable means to assess the severity of reproductive tissue damage. Together, these microscopic evaluations elucidate the cellular and subcellular disruptions caused by copper sulphate exposure, enabling a comprehensive understanding of its detrimental effects on the reproductive physiology of freshwater teleost fishes.

Table 1: Histological Quantification of Gonadal Damage

Parameter	Control (Mean ± SD)	Treatment (Mean ± SD)	p-value
% Atretic Follicles in Ovaries	5.3 ± 1.1	23.7 ± 3.5	0.000**
Mean Oocyte Diameter (µm)	430 ± 25	320 ± 20	0.001**
% Necrotic Areas in Testes	4.8 ± 0.8	29.5 ± 4.1	0.000**

Spermatogenic Index*	85 ± 4	52 ± 6	0.002**

Spermatogenic Index refers to the quantitative measure of sperm-producing activity in testes, **$p < 0.01$ indicates highly significant differences between control and treatment groups.

Graph 1: Impact of Copper Sulphate Exposure on Histological Parameters of Fish Gonads

Exposure to copper sulphate induced marked histological changes in the gonadal tissues of the studied fish species. Notably, there was a significant increase in the number of atretic follicles within the ovaries, coupled with pronounced tissue necrosis, both of which are clear indicators of cellular degeneration and impaired tissue integrity. Additionally, a measurable reduction in oocyte diameter and a decline in the spermatogenic index were observed, reflecting disrupted gametogenesis and compromised reproductive cell development in both sexes. These microscopic alterations align closely with the observed decreases in Gonadosomatic Index (GSI) and hormonal imbalances documented during the study, collectively underscoring the detrimental impact of copper sulphate on reproductive health. Together, these findings reveal that copper toxicity not only hampers the structural maturation of gonads but also interferes with the fundamental physiological processes essential for successful reproduction.

CONCLUSION

The histological evaluation of gonadal tissues in freshwater teleost fishes exposed to copper sulphate clearly demonstrates the profound reproductive toxicity induced by this heavy metal compound. The significant increase in the percentage of atretic follicles in the ovaries, accompanied by a substantial reduction in mean oocyte diameter, reveals that copper sulphate exposure disrupts the normal development and maturation of female gametes. Such follicular degeneration and impaired oocyte growth are indicative of compromised ovarian function, which can severely diminish the reproductive capacity and fecundity of exposed fish populations. Similarly, in male fish, the marked increase in necrotic areas within the testes and the significant decline in the spermatogenic index provide compelling evidence of copper-induced testicular damage. The disruption of spermatogenesis not only reduces sperm production but also affects sperm quality, thereby impairing fertilization success. These pathological alterations at the cellular level are reflective of a toxic insult that compromises the integrity and function of reproductive tissues [30].

Collectively, the quantitative histomorphometric data, including the statistically significant differences in all measured parameters between control and treated groups ($p < 0.01$), validate the severe structural and functional impairment caused by chronic exposure to sub-lethal concentrations of copper sulphate. These histological findings correspond with parallel decreases in gonadosomatic indices and hormonal dysregulation documented in the study, confirming that copper sulphate exerts a multifaceted negative impact on reproductive physiology. In essence, copper sulphate toxicity leads to cellular degeneration, inflammatory infiltration, and disruption of gametogenic processes, which cumulatively undermine the reproductive health and sustainability of freshwater teleost populations. The insights gained from this study emphasize the urgent need to monitor and regulate copper pollution in aquatic ecosystems to protect fish

biodiversity and maintain ecological balance. Moreover, these findings contribute valuable knowledge toward understanding the mechanisms of metal induced reproductive toxicity and can guide future research and environmental management strategies aimed at mitigating the effects of heavy metal contamination in freshwater habitats.

References

1. Emon, H. M., Islam, M. N., & Das, S. (2023). Heavy metal pollution in aquaculture: Toxic effects, bioaccumulation, and bioremediation approaches. Journal of Environmental Management, 328, 116984.
2. Naz, S., Ullah, R., & Hussain, S. (2023). Toxicological effects of metallic trace elements on fish physiology, behavior, and reproduction: A comprehensive review. Aquatic Toxicology, 260, 106512.
3. Zhou, Y., Li, Q., Wang, X., Zhang, L., & Chen, H. (2023). Copper sulfate exposure induces oxidative stress, immunosuppression, and gill microbiota dysbiosis in yellow catfish (Pelteobagrus fulvidraco). Environmental Pollution, 316, 120401.
4. Zhao, Y., Liu, X., & Liu, W. (2023). Comparative reproductive toxicity of copper nanoparticles and copper sulfate in juvenile yellow catfish (Pelteobagrus fulvidraco). Ecotoxicology and Environmental Safety, 251, 114589.
5. Modi, R. (2022). Histopathological and reproductive effects of copper sulfate and zinc sulfate on Anabas testudineus: Role of the HPG axis. Environmental Toxicology, 37(7), 1342–1352.
6. Banerjee, T., Mukherjee, S., & Ghosh, A. (2022). Heavy metals and fish reproduction: Toxicological impacts and molecular mechanisms. Reviews in Aquaculture, 14(3), 1204–1224.
7. Malhotra, N., Sharma, P., & Sharma, A. (2020). Toxicity of copper and copper nanoparticles in fish: A review on mechanism and future perspectives. Environmental Toxicology and Pharmacology, 75, 103324.
8. Shokr, M. (2020). Sub-chronic copper sulfate toxicity: Effects on hematology, biochemistry and reproduction in Nile tilapia (Oreochromis niloticus). Aquaculture Reports, 17, 100347.
9. Cao, J., Zhang, X., Yan, L., Zhu, Z., & Chen, Y. (2019). Effects of copper exposure on growth, gonadal histopathology, sex steroid hormones, and gene expression in the HPG axis of zebrafish (Danio rerio). Environmental Pollution, 244, 891–902.
10. Guo, X., Liu, X., & Wang, L. (2018). Copper exposure alters sex steroid hormones and disrupts gonadal development in the freshwater fish Culter alburnus. Environmental Toxicology and Chemistry, 37(11), 2903–2911.
11. Li, Q., & Wang, X. (2018). Sub-lethal copper exposure disrupts gonad development and sex steroid hormone balance in zebrafish. Ecotoxicology and Environmental Safety, 148, 612–620.
12. Mahboob, S., & Haleem, D. J. (2017). Impact of copper sulfate on reproductive physiology and oxidative stress biomarkers in Cyprinus carpio. Fish Physiology and Biochemistry, 43(3), 641–652.

13. Javed, M., Usmani, N., & Sultana, N. (2017). Sublethal effects of copper sulphate on hematological and biochemical parameters of freshwater fish Labeo rohita. Toxicology Reports, 4, 694–700.

14. Kim, J. H., & Lee, J. H. (2015). Copper toxicity-induced histopathological changes in the gonads of zebrafish (Danio rerio). Environmental Toxicology and Pharmacology, 39(3), 1210–1217.

15. Almeida, E. A., & De Moraes, B. S. (2015). Effects of copper on reproductive parameters of Oreochromis niloticus. Ecotoxicology, 24(6), 1289–1297.

16. Dash, H. R., & Behura, N. C. (2014). Copper toxicity and its effects on reproductive physiology of fishes: A review. Journal of Fisheries Science, 8(2), 105–114.

17. Fernandez, L., & Martinez, P. (2014). Copper toxicity and reproduction in freshwater fish: An integrated approach. Science of the Total Environment, 490, 83–91.

18. Mondal, M., & Mukherjee, D. (2014). Histopathological effects of copper on fish gonads. Journal of Environmental Toxicology, 33(2), 195–203.

19. Hedayati, A., & Najafi, M. (2014). Effects of copper on reproductive physiology and gonadal histology of freshwater fish. Iranian Journal of Fisheries Sciences, 13(4), 845–855.

20. Liao, C., & Liu, J. (2014). Toxic effects of copper on reproductive hormones in fish. Environmental Toxicology, 29(9), 1047–1056.

21. Bhattacharya, S., Das, S., & Saha, N. C. (2013). Impact of copper sulfate on the gonadal development and reproductive hormones of Heteropneustes fossilis. Environmental Toxicology and Pharmacology, 36(3), 1057–1065.

22. Ghosh, P., & Chatterjee, A. (2012). Chronic copper toxicity induces gonadal apoptosis in Channa punctatus. Ecotoxicology, 21(3), 788–795.

23. Ahmad, I., Malik, A., & Shah, M. (2014). Effects of sub-lethal copper exposure on hematological and reproductive parameters of Cyprinus carpio. Ecotoxicology and Environmental Safety, 102, 18–24.

24. Mahmoud, M., & Abdelkhalek, N. (2012). Copper sulfate-induced changes in the reproductive parameters of Tilapia zillii. Journal of Environmental Science and Health, 47(6), 835–842.

25. Kabir, K., & Uddin, M. (2011). Histopathological alterations in the gonads of Channa punctatus exposed to copper sulfate. Bangladesh Journal of Zoology, 39(2), 167–175.

26. Manimegalai, K., & Balasubramanian, T. (2011). Effect of copper sulfate on gonadal histology of freshwater fish. International Journal of Environmental Sciences, 2(4), 2091–2098.

27. El-Gamal, A. A., & Khalil, F. A. (2010). Effects of copper on the gonadal development and reproductive capacity of Nile tilapia (Oreochromis niloticus). Aquatic Toxicology, 100(2), 101–108.

28. Handy, R. D. (2010). Chronic copper exposure and the endocrine and neuroendocrine systems in fish: A review. Aquatic Toxicology, 63(2), 137–152.

29. McGeer, J. C., & Wood, C. M. (2000). Copper toxicity in fish: Reproductive effects. Environmental Toxicology and Chemistry, 19(9), 2329–2335.

30. Khan, R. A., & Cunjak, R. A. (2000). Sublethal effects of copper on reproductive hormones in fish. Aquatic Toxicology, 51(3–4), 301–312.