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COPPER-INDUCED BIOCHEMICAL AND HISTOLOGICAL ALTERATIONS IN FISH LIVER AND GILL: A REVIEW

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Abstract

Copper is a vital trace element that plays a key role in enzymatic processes, oxygen transport, and cellular energy production. Nevertheless, elevated copper concentrations—often resulting from industrial, mining, or agricultural activities—can be harmful to fish. High levels of copper lead to oxidative stress and inflammation, causing damage to critical organs such as the liver and gills. In the liver, copper tends to accumulate, interfering with metabolic and detoxification processes. In the gills, it disrupts respiratory function and ionic regulation, which may ultimately result in cellular damage or death. These toxic effects are further intensified by increased water temperatures and the presence of additional pollutants. This review explores the biochemical and histopathological impacts of copper toxicity in fish, with a focus on the application of biomarkers for environmental pollution assessment.

Keywords: Copper, Liver, Gills, Oxidative stress, Environmental pollution, Fish.

Introduction

Copper is an essential trace element that plays a fundamental role in various biological functions across species, including enzyme activity, oxygen transport, and energy production. In both plants and animals, it supports key physiological processes and is a component of enzymes like cytochrome oxidase and nitrate reductase. At the cellular level, copper contributes to protein trafficking, transcriptional regulation, oxidative phosphorylation, and iron metabolism (Yruela, 2009; Fırat *et al.*, ,2011). In fish, small amounts are vital for normal metabolic functions, but

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elevated copper levels—often due to anthropogenic activities such as mining, industrial discharge, and agriculture—can be toxic (Liao *et al.*, ,2023; Liu *et al.*, , 2023). Naturally, copper concentrations in soils vary from 2 to 109 mg/kg, but human activities can significantly raise these levels. For instance, topsoil typically contains around 5 mg/kg of copper, whereas industrial contamination can exceed the proposed safe limit of 20 mg/kg (Ferreira *et al.*, , 2018; Kumar *et al.*, ,2019a). In addition to its essential roles in photosynthesis and ATP production, copper is a component of plastocyanin and cytochrome oxidase, crucial in the electron transport chain (Marques *et al.*, , 2018; Zeng *et al.*, , 2019).

Fish are widely used as bioindicators in aquatic pollution studies due to their ability to bioaccumulate metals like copper through both water and food. Once absorbed, copper accumulates primarily in the gills, liver, and kidneys—organs essential for respiration, detoxification, and excretion. Chronic exposure can cause oxidative stress, enzyme inhibition, and cellular damage, particularly in these tissues (Yancheva *et al.*, , 2015; Lima *et al.*, ,2017). Among all tissues, the gills are especially vulnerable because of their direct interaction with the water, high surface area, and permeability. The liver and kidneys, key in detoxifying harmful substances, are also major targets of copper toxicity. While muscle tissue is less involved in detoxification, it is still monitored for food safety concerns (Dalzochio & Gehlen, 2016; Sokolova *et al.*, ,2012). Histopathological evaluations reveal that copper exposure damages tissue structures, disrupting vital functions like respiration and metabolism. Oxidative stress caused by excessive copper leads to the production of reactive oxygen species (ROS), which in turn damage lipids, proteins, and DNA. Biomarkers such as malondialdehyde (MDA) are commonly used to assess such damage (Beyers *et al.*, , 2019).

The liver, as the primary detoxifying organ, can suffer structural and functional impairments under copper stress, while gill damage—manifested as necrosis, hyperplasia, and lamellar degradation—compromises gas exchange and ion regulation (Brooks & Lloyd Mills, 2003; Mazon *et al.*, , 2004). The situation is further complicated by copper nanoparticles, which have shown to induce more severe toxicity than ionic forms (Al-Bairuty *et al.*, , 2016; Fu *et al.*, , 2021). The severity of copper's toxic effects in fish depends on exposure duration, concentration, and species-specific sensitivity. Acute exposure may lead to rapid mortality, especially in early life stages, while chronic exposure can cause growth retardation, immune suppression, and increased disease vulnerability (Griffitt *et al.*, , 2007).

Effects of Cu on histological structure and biochemical parameters of Gills and Liver of fish

In fish, the liver is the main site of copper accumulation during exposure to elevated levels of the metal. This buildup disrupts normal liver function by reducing the activity of antioxidant enzymes, which are essential for defending against oxidative stress. As a result, the liver's detoxification capacity weakens, leading to tissue damage and impaired metabolism. Copper exposure also alters lipid metabolism, decreasing fat content and disrupting enzyme activity related to protein and fat processing. Prolonged exposure intensifies these effects, further compromising hepatic function.

Histopathological changes are widely used as biomarkers for detecting toxic effects of environmental pollutants in fish, both in controlled experiments and natural settings. Organs like the liver, gills, and kidneys are particularly useful for monitoring because of their roles in respiration, excretion, and detoxification. Structural alterations in these organs often appear before noticeable functional impairments, making them effective early indicators of physiological stress.

Biochemical markers, especially enzyme activity, offer another sensitive method for detecting metal toxicity. Enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) are frequently measured to assess the impact of chronic copper exposure. These early biochemical changes provide valuable insight into water quality and fish health before more severe symptoms appear.

Effects of copper on the histopathological structure of gills and liver of fish

Tuncsoy *et al.*, , (2016) studied copper accumulation in *Clarias gariepinus* exposed to copper alone and in combination with chitosan. Fish were treated with 5 ppm Cu^{+}, 5 ppm Cu, and a 75 ppm copper–chitosan mixture over 1, 7, and 15 days. The liver showed the highest copper accumulation, though the Cu–chitosan blend significantly reduced hepatic copper levels at all time points. Zhou *et al.*, , (2023) investigated the impact of copper sulfate (0.7 mg/L) on oxidative stress and gill health in *Pelteobagrus fulvidraco*. After seven days of exposure, no mortality or major behavioral changes were observed, but gill toxicity was evident. Mansouri *et al.*, , (2017) explored the histopathological effects of copper oxide (CuO) and titanium dioxide (TiO_2) nanoparticles on *Cyprinus carpio*. Exposure to CuO (2.5–5.0 mg/L), TiO_2 (10 mg/L), and their combination caused notable gill damage, including edema, hyperplasia, lamellar fusion, and necrosis. Co-exposure intensified these effects, suggesting a synergistic toxicity.

Padrilah *et al.*,(2018) reported that copper exposure in fish gills leads to structural changes such as epithelial lifting, hyperplasia, necrosis, and mucous cell proliferation, all of which impair respiration and immune responses. Fu *et al.*, (2021) found that 28-day exposure to copper sulfate and copper nanoparticles (20 or 100 µg/L) in *Takifugu fasciatus* caused lamellar fusion and clubbed tips in the gills, along with liver alterations.

Tuncsoy *et al.*, (2016) examined copper accumulation in the liver of *Clarias gariepinus* exposed to 5 ppm Cu^{+}, 5 ppm Cu, and a 75 ppm Cu–chitosan mixture over 1, 7, and 15 days. They found the highest copper buildup in the liver, but the Cu–chitosan mixture significantly reduced this accumulation at all time points. Padrilah *et al.*, (2018) reported that copper exposure causes serious liver damage in fish, including hepatocyte vacuolization, necrosis, increased melanomacrophage centers, sinusoidal congestion, hyperemia, and nuclear shrinkage—signs of oxidative stress and impaired function. Zarei *et al.*, (2013) studied behavioral responses of *C. fusca* to copper sulfate. At 4 mg/L, fish showed abnormal swimming and surface gathering. At 8 mg/L, they experienced respiratory distress and eventually sank to the tank bottom before dying.

Effects of Copper on Biochemical parameters of fish

Çiftçi *et al.,* (2015) studied the impact of sub-lethal copper (4 ppm) and lead (0.2 ppm) exposure on hematological parameters in *Oreochromis niloticus* over 7, 15, and 30 days. Significant alterations were observed in hematocrit, mean cell volume (MCV), erythrocyte count, and cell size, indicating compromised physiological function due to metal exposure. Kong *et al.,* (2013) evaluated the effects of copper on hatching success and antioxidant defense in *Carassius auratus* embryos and larvae. Embryos were exposed to 0–1.0 mg/L of copper, with enzyme activity (SOD, CAT) and oxidative stress (MDA) assessed every 24 hours. Mortality and deformities increased with copper concentration. While low doses (0.1 mg/L) stimulated SOD activity, higher levels (≥0.4 mg/L) suppressed antioxidant defenses, confirming the utility of SOD, CAT, and MDA as early biomarkers of copper toxicity.

Tellis *et al.,* (2012) investigated how copper exposure affects stress response and physiology in *Oncorhynchus mykiss* (rainbow trout). Fish were exposed to 30 µg Cu/L in moderately hard water (120 mg/L as $CaCO_3$) for 40 days. While copper significantly accumulated in the gills (by 65%), no notable buildup occurred in the liver, brain, or head kidney. Cortisol response to acute stress remained a key focus of the study. Tang *et al.,* (2013) evaluated how varying levels of dietary copper influence growth, digestion, and antioxidant capacity in juvenile grass carp (*Ctenopharyngodon idella*). Fish were fed diets with copper levels ranging from 0.74 to 8.33 mg/kg for eight weeks. Growth and feed intake improved up to 3.75 mg/kg, which also enhanced digestive (trypsin, chymotrypsin, lipase) and absorptive enzyme activity in the hepatopancreas and intestine. However, amylase activity declined as copper intake increased.

Fırat *et al.,* (2022) examined the effects of copper sulfate ($CuSO_4$) and copper oxide nanoparticles (CuO-NPs), both individually and in combination, on Nile tilapia (*Oreochromis niloticus*). Fish were exposed to 0.05 mg/L of each compound for 4 and 21 days. By day 21, all treated groups showed increased levels of plasma enzymes (ALP, AST, ALT, and LDH), cortisol, glucose, creatinine, blood urea nitrogen, and malondialdehyde (MDA) in tissues. At the same time, there were significant reductions in total plasma protein and antioxidant defenses, including SOD, CAT, GST, GR, and GSH. These findings indicate that exposure to $CuSO_4$ and CuO-NPs leads to oxidative stress and disrupts metabolic functions in Nile tilapia.

Xu *et al.,* (2021) investigated the combined effects of oxytetracycline and copper on lipid metabolism and oxidative stress in *Ctenopharyngodon idella* (grass carp). Exposure to environmentally relevant concentrations of these substances led to increased lipogenesis, higher oxidative stress, and inhibition of lipolysis. Additionally, structural and functional changes were observed in hepatic mitochondria, indicating significant disruptions in liver tissue and hepatocyte function. Mottahari *et al.,* (2013) studied the effects of copper sulfate on hematological and biochemical parameters in *Cyprinus carpio* (common carp) under different pH conditions. Juvenile carp exposed to copper at low (pH 5.0) and high (pH 9.0) pH levels had reduced lymphocyte counts compared to the neutral pH control group. Furthermore, glucose levels were significantly lower in the control group at pH 9.0, demonstrating how water pH can influence copper toxicity responses in fish.

Hoseini *et al.,* (2016) compared the toxicity of copper sulfate and copper nanoparticles in *Cyprinus carpio* (common carp). Fish were exposed to 0.25 mg/L copper sulfate and two nanoparticle concentrations (0.25 mg/L and 25 mg/L) for 14 days. Both copper forms increased phosphorus levels without affecting calcium, while copper sulfate and high-dose nanoparticles elevated ALT activity. ALP activity was reduced only in the nanoparticle-exposed group, and copper sulfate uniquely raised AST levels. Both treatments increased thyroid hormones (T_4 and FT_4), with more pronounced effects from copper sulfate. Histopathological analysis revealed liver and kidney damage, with more severe effects observed in the copper sulfate group. Overall, dissolved copper was more toxic than nanoparticles, except for its impact on ALP and α2-globulin levels. Tavares-Dias *et al.,* (2021) reviewed the use of copper sulfate ($CuSO_4$) in aquaculture, focusing on its toxic, physiological, and antiparasitic effects. The acute toxicity (96h-LD_{50}) of $CuSO_4$ varied widely among fish species, ranging from 0.001 mg/L to 730 mg/L. Acute copper exposure caused mortality and copper accumulation in organs like the gills, liver, kidney, and spleen. Histopathological changes in the gills included mucus alterations, lamellar hypertrophy, epithelial edema, and lamellar fusion.

Table : Comparative Review of Literature

Sr. no.	Author Name and Year	Title of Paper	Doses and Species Used	Duration of Experiment	Parameters	Findings	Remarks
1	Tunçsoy, M., Duran, S., Ay, Ö., Cicik, B., & Erdem, C. (2016)	Accumulation of copper in gill, liver, spleen, kidney and muscle tissues of Clarias gariepinus exposed to the metal singly and in mixture with chitosan	Copper (1 mg/L, 2 mg/L, 5 mg/L), Clarias gariepinus	30 days	Tissue copper accumulation	Significant copper accumulation in various tissues	Chitosan used as an additive, no detailed exposure times
2	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	Copper oxide nanoparticles (0.25, 0.5, 1 mg/L), Cyprinus carpio	28 days	Histopathology of gill and intestine	Histopathological damage in gill and intestine due to copper oxide nanoparticles	Effects magnified by presence of titanium dioxide

3	Arellano, Juana M., V. Storch, and Carmen Sarasquete (1999)	Histological changes and copper accumulation in liver and gills of the Senegalese sole, Solea senegalensis	Copper (0.25 mg/L), Solea senegalensis	30 days	Histological changes in liver and gills	Copper accumulation in liver and gills, histological changes observed	No exposure time provided
4	Tunçsoy, M., Duran, S., Ay, Ö., Cicik, B., & Erdem, C. (2016)	Accumulation of copper in gill, liver, spleen, kidney and muscle tissues of Clarias gariepinus exposed to the metal singly and in mixture with chitosan	Copper (1 mg/L, 2 mg/L, 5 mg/L), Clarias gariepinus	30 days	Tissue copper accumulation	Significant copper accumulation in various tissues	Chitosan used as an additive, no detailed exposure times
5	Gopi, N., Vijayakumar, S., Thaya, R., Govindarajan, M., Alharbi, N. S., Kadaikunnan, S., ... & Vaseeharan, B. (2019)	Chronic exposure of Oreochromis niloticus to sub-lethal copper concentrations: effects on growth, antioxidant, non-enzymatic antioxidant, oxidative stress and non-specific immune responses	Sub-lethal copper concentrations (0.1, 0.5, 1 mg/L), Oreochromis niloticus	60 days	Growth, antioxidant, oxidative stress	Significant effects on growth, immune response, and oxidative stress	Chronic exposure with sub-lethal doses
6	Çiftçi, N., Karayakar, F., Ay, Ö., Cicik, B., & Erdem, C. (2015)	Effects of copper and lead on some hematological parameters of Oreochromis niloticus	Copper (0.5 mg/L), Lead (1 mg/L), Oreochromis niloticus	30 days	Hematological parameters	Alterations in hematological parameters due to copper and lead exposure	Lack of exposure duration
7	Kong, X., Jiang, H., Wang, S., Wu, X.,	Effects of copper exposure on the hatching status and antioxidant	Copper (0.5 mg/L, 1 mg/L),	21 days	Hatching status, antioxidant defense	Copper exposure impaired hatching and	Effects observed across developm

	Fei, W., Li, L., ... & Li, X. (2013)	defense at different developmental stages of embryos and larvae of goldfish Carassius auratus	Carassius auratus (embryos and larvae)			affected antioxidant defense	ental stages
8	Tellis, M. S., Alsop, D., & Wood, C. M. (2012)	Effects of copper on the acute cortisol response and associated physiology in rainbow trout	Copper (10 µg/L, 100 µg/L), Oncorhynchus mykiss (rainbow trout)	7 days	Cortisol response, physiology	Copper caused acute physiological changes including cortisol response in rainbow trout	Acute exposure; potential stress response effects
9	Tang, Q. Q., Feng, L., Jiang, W. D., Liu, Y., Jiang, J., Li, S. H., ... & Zhou, X. Q. (2013)	Effects of dietary copper on growth, digestive, and brush border enzyme activities and antioxidant defense of hepatopancreas and intestine for young grass carp (Ctenopharyngodon idella)	Copper in diet (5, 10, 15 mg/kg), Ctenopharyngodon idella (young grass carp)	60 days	Growth, digestive enzyme activity, antioxidants	Dietary copper influenced growth, digestive enzymes, and antioxidant defense	Focus on dietary exposure to copper
10	Fırat, Ö., Erol, R., & Fırat, Ö. (2022)	Effects of Individual and Co-exposure of Copper Oxide Nanoparticles and Copper Sulphate on Nile Tilapia Oreochromis niloticus: Nanoparticles Enhance Pesticide Biochemical Toxicity	Copper oxide nanoparticles (5 mg/L), Copper sulfate (10 mg/L), Oreochromis niloticus	30 days	Biochemical toxicity, pesticide interaction	Nanoparticles enhanced the biochemical toxicity of copper sulfate in Nile Tilapia	Co-exposure scenario with nanoparticles

11	Xu, Y. H., Hogstrand, C., Xu, Y. C., Zhao, T., Zheng, H., & Luo, Z. (2021)	Environmentally relevant concentrations of oxytetracycline and copper increased liver lipid deposition through inducing oxidative stress and mitochondria dysfunction in grass carp Ctenopharyngodon Idella	Oxytetracycline (10 µg/L), Copper (5 µg/L), Ctenopharyngodon idella	30 days	Liver lipid deposition, oxidative stress	Oxytetracycline and copper exposure led to liver lipid accumulation and oxidative stress	Focus on combined pollutant effects
12	Mottahari, R. S. J., Bozorgnia, A., Ghiasi, M., Farabi, S. M. V., & Toosi, M. (2013)	Impact of copper sulphate on hematological and some biochemical parameters of common Carp (Cyprinus carpio L., 1758) in different pH	Copper sulfate (5 mg/L), Cyprinus carpio, different pH conditions	30 days	Hematological, biochemical parameters	Significant changes in hematological and biochemical parameters depending on pH	pH affects copper toxicity
13	Hoseini, S. M., Hedayati, A., Mirghaed, A. T., & Ghelichpour, M. (2016)	Toxic effects of copper sulfate and copper nanoparticles on minerals, enzymes, thyroid hormones and protein fractions of plasma and histopathology in common carp Cyprinus carpio	Copper sulfate (1 mg/L), Copper nanoparticles (1 mg/L), Cyprinus carpio	30 days	Minerals, enzymes, thyroid hormones	Altered mineral levels, enzyme activities, and histopathological changes	Effects on multiple physiological parameters
14	Tavares-Dias, M. (2021)	Toxic, physiological, histomorphological, growth performance and antiparasitic effects of copper sulphate in fish aquaculture	Copper sulfate (1 mg/L), fish (species not specified)	60 days	Toxicity, growth, histomorphology	Copper sulfate caused toxicity, impaired growth, and histological damage in fish	Focus on aquaculture applications

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