Histopathological anomalies in Testis, Ovary, Muscle and Heart of fish, *Channa punctatus* exposed with Heavy Metal Copper Sulphate

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Abstract
Copper is an important trace element for living things to develop and function normally. However, if this element is employed past its safe range, it can transform into continuous metal compounds that can build up in water and disrupt the biological system. Activities related to aquaculture may also be impacted by its rise in environmental contamination. Because of its toxicity, which can be assessed at structural levels of the organism, copper is known to have the potential to harm the non-target animals like fish. The present study aims to assess the histopathological alterations in gonads (testis, ovary), muscle and heart of metal copper sulphate exposed fish *Channa punctatus*. The acclimatized fishes were exposed to 10% of 96 h-LC_{50} (0.40 mg/L) of copper sulphate along with a control. After the completion of time intervals, 7, 14 and 21 days, the fish organs, testis, ovary, muscle and heart were dissected out for further evaluation of histopathological manifestations. The results exemplified the conspicuous histopathological anomalies in testis, ovary, muscle and heart of copper sulphate exposed fish *Channa punctatus*. These organ-specific alterations can be used as structural indicators for evaluating the metal copper sulphate contamination in the aquatic milieu. Moreover, they will be helpful in the management of aquatic environment and its diversity.

Keywords: Copper Sulphate; *Channa punctatus*; Histopathological manifestations; Gonads; Muscle; Heart

1. Introduction

Heavy metal pollution introduces a wide spectrum of toxicities with negative effects on aquatic faunal populations. It is a major problem for aquatic habitats (Mahmuda et al., 2020). The majority of the heavy metals that build up in aquatic water bodies are caused by human activities including farming, landfill erosions, docking and embarking, sewage from home and industrial wastes and some natural processes (Sarkar et al.,2016; Ezemonye et al., 2019). The metals accumulate into the tissues of aquatic animals through aquatic food chains, where they can be concentrated. Fish reproduction is greatly hampered by metal pollution (Steinhagen et al., 2004). Reduced GSI, fecundity, hatching rate, fertilization success, aberrant form of reproductive organs, and finally overall reproductive success have all been recorded as reproductive compromises as a result of a range of environmental factors (Faroon et al., 2012). The development of reproductive cells and organs was distorted by heavy metals. Fish reproductive abilities were negatively impacted by copper sulphate contamination through inhibition of spermatogenesis and oogenesis, including decreased egg and sperm quality and quantity, hatching, and fertilization. Apart from gonads, the histopathological anomalies in muscle and heart of fish determined. Therefore, this structural evaluation is one of the finest methods for defining the presence of copper sulphate pollution and its effects on aquatic fauna including fishes. These histopathological changes are often known as important biomarkers for biological measurements in aquatic environmental monitoring. It is well recognized that the heavy metal Copper Sulphate causes severe toxicity in aquatic organisms especially fish. Water serves as a medium and is crucial for the healthy development and survival of aquatic life. During the investigation of fish perturbations in the aquatic ecosystem, the water quality essentially remained constant. The rate of organism survival and reproductive ability are significantly impacted by heavy metal poisoning. Depending on the species, dose, and exposure time, some of them have been documented to be highly carcinogenic, mutagenic, and teratogenic (Malik and Maurya, 2014). Heavy metals are persistent in the natural ecosystem, once enter into the living organism, it can accumulate inside. These metabolic changes eventually lead to the histological changes in fish tissues. The evaluation of an organism's health is often aided by the histopathological characteristics, a helpful biomarker for determining the toxicological impact of environmental pollutants (Hossain, 2012; Kanaujiya et al., 2023; Trivedi et al., 2021). Notably, the heart of *Channa punctatus* is an important organ for blood circulation. The fish heart is found ventrally beneath the gill structures but in advance of the pectoral fins and peritoneum. The pericardial cavity, a membrane-lined sac, houses the heart in its normal location. Only deoxygenated blood is present in the two chambered, venous heart of *Channa punctatus*. The auricle and ventricle are the differentiating chambers. Particularly, the endocardium, or lining of endothelial cells, is present in every heart chamber.

The epithelium's outermost layer of connective tissue and cells is known as the epicardium. Surprisingly, collagen and elastic fibers can be found in the sub-epicardial area of loose connective tissue, and there is also a smooth muscle cell layer with lymphatic capillaries. The ovaries of *Channa punctatus* were hollow sac-like, paired and more or less elongated structure lying dorsal to the alimentary canal and ventral to the swim bladder. A posterior extension of tunica albuginea united both the ovaries to form an oviduct which opened to the exterior via the oval shaped urogenital papilla. The left ovary was always slight larger than the right in all the examined fish. The color of the ovary varied from reddish brown in immature ovaries to light yellowish in mature ovaries. The testis of *Channa punctatus* were white, elongated, paired and ribbed structures. The left lobe of testis was found to be slightly larger than the right one. Two parts of the testis joined posteriorly before entering the cloaca and where they formed the third lobe. They were attached to the body wall by mesenteries. The color of the testis ranged from pinkish to whitish depending on the maturity. Freshwater fish, *Channa punctatus*, have been known to die after being exposed to copper sulphate. The heart acts as an intermediary organ to circulate the blood contaminated with heavy metals throughout the body, which may result in structural heart defects that lower cardiac output and the ability of exposed fish to swim. Time-dependent reports of the increase of fish heart lesions were made. The frequency and severity of tissue lesions in fish are influenced by heavy metal concentrations and the length of the exposure period. Fish reproductive performance is significantly impacted by heavy metal pollution, leading to low quality gametes that may affect not only the success rate of fertilization but also the rate of hatching and survival of the offspring. Numerous studies have shown that fish have problems conceiving, including aberrant oocyte shape, unfilled follicles and thinning follicular lines, cytoplasmic retraction and condensation, lower total GSI, and more (Yan et.al, 2020). This study aims to determine the histopathological changes in gonads (testis and ovary), muscle and heart of freshwater fish, *Channa punctatus* exposed to copper sulphate.

2. Material and Methods

Chemicals

Heavy metal copper sulphate was used as a test chemical and other chemicals were procured from the local market of Daliganj, Lucknow.

Acclimatization of fish

The fresh water teleost fish specimen, *Channa punctatus*, was procured from nearby water bodies of district Lucknow. The collected fish weighed 20-35 g and measured 11-13 cm in length. In a 70 L aquarium, the fishes were treated with 2% $KMnO_4$ for the removal of dermal infections. Fish were given commercial food to eat every day while they were acclimatized for ten days. An air pump was used to aerate the aquarium's water, which was replaced every day.

Experimental design

For the duration of 7, 14, and 21 days, the acclimatized fish in three aquariums were exposed to a sub-lethal concentration of copper sulphate (10% of 96 h-LC_{50}; 0.40 mg/L) while one aquarium was kept as a control. The different physicochemical variables, like dissolved oxygen, alkalinity, hardness and chloride, were roughly maintained at a constant level throughout the experiment. All of these factors were monitored regularly, and the pH and temperature were both stabilized (APHA, 2017).

Determination of 96 h-LC_{50} of metal copper sulphate for fish *Channa punctatus*

For the analysis of median sub-lethal concentration (96 h-LC_{50}) of copper sulphate for fish *Channa punctatus*, the acclimatized fishes were initially exposed to its five different concentrations (0.001, 0.01, 0.1, 1.0 and 10.0 mg/L) on logarithmic scale, for approximating the effective range. Secondly, the fishes were separately exposed to definitive concentrations like 1.50, 2.50, 3.50, 4.50, 5.50, 6.50, 7.50, 8.50, 9.50 and 10.50 mg/L of copper sulphate along with a control. Also, fish mortality was recorded after 24, 48, 72 and 96 h. So, the 50% mortality of fish was regarded as median sub-lethal concentration (LC_{50}) after 96 h (Hamilton et al., 1977).

Physicochemical parameters of test water

The physicochemical parameters (Dissolved oxygen, hardness, alkalinity, chloride, pH and temperature) were enumerated by using the standard methods (APHA, 2017) in both control and treated group after 7, 14 and 21 days.

Histopathological abnormalities in gonads (testis, ovary), muscle and heart of copper sulphate exposed fish:

Gonads (testis and ovary), heart and muscle were taken out from both the control and exposed fish and stored in saline water for the histopathological criteria. The tissues were extensively cleansed, dehydrated and washed in series of progressively stronger alcohol solutions. They were then cleared in xylene, embedded in paraffin wax and sectioned into 3 µm thick sections using a rotating microtome. Then, hematoxylin (H) and eosin (E) were used to stain the slides containing tissue sections for 1 min and 2 min, respectively. The prepared slides were mounted in DPX (distyrene, plasticizer and xylene) and were examined as well as photographed under a light microscope with 10/40X magnification of its objective lenses.

3. Results and Discussion

Median sub-lethal concentration (LC_{50}) of copper sulphate for fish, *C. punctatus*

The semi-static assays were conducted to evaluate the median sub-lethal concentration for 96 h (96 h-LC_{50}) of copper sulphate for fish *C. punctatus.* Its value was calculated 4 mg/L with 95% lower and upper confidence limits as 3.11 mg/L and 5.09 mg/L, respectively.

Parameters of water

Water is an essential component of all living things and plays a vital role in daily activities. Water has special relevance for human existence. Analyzing water involves estimating its physical and chemical characteristics. Due to fish's higher sensitivity in nature, the physicochemical characteristics of water are significant since they play key roles in all the metabolic activities of aquatic life. The physicochemical characteristics were estimated and their values expressed in **Table 1**.

Table 1 Physicochemical parameters of test water of control and exposed group aquaria constantly maintained after 7, 14, 21 days, as compared to control.

Parameters	Control	7 Days	14 Days	21 Days
Temperature (°C)	24.53 ± 0.48	25.12 ± 0.32	26.65 ± 0.49	27.73 ± 0.51
pH	6.96 ± 0.29	7.41 ± 0.15	7.13 ± 0.18	6.94 ± 0.23
Dissolved Oxygen (mg/L)	6.67 ± 0.14	6.53 ± 0.28	6.84 ± 0.21	6.94 ± 0.36
Hardness (mg/L)	167.63 ± 14.57	166.77 ± 12.34	170.67 ± 12.92	168.36 ± 10.14
Chloride (mg/L)	43.27 ± 2.34	37.15 ± 6.95	36.07 ± 4.29	38.91 ± 3.17
Alkalinity (mg/L)	110.18 ± 0.28	109.81 ± 0.74	108.64 ± 0.46	110.84 ± 0.64

(Values were given as mean ± S.E.M.)

Evaluation of histopathological alterations

Histopathology, which is utilized to show the illness patterns in a population of fish, is the microscopic evaluation of changed morphology expressing a disease process in an organism. According to Liebel et al., (2013), histopathological events are effective because they may promptly identify water contamination and communicate the state of exposed tissue's health. Because they provide an early response or detectable biological event as a result of exposure to contaminants, they are suited for use as biomarkers (Awasthi et al., 2019; Miranda et al., 2008; Ribeiro et al., 2005). Apart from that, the main goal of a histopathological investigation is to track cellular alterations in the fish target organs. Fish are comparatively susceptible to environmental alterations, such as an increase in pollutants like heavy metals. Prior to major alterations in the fish's physical characteristics and behaviors, the early harmful impacts of pollution are only detectable at the cellular or tissue level. The histopathological analysis, on the other hand, seems to be a very sensitive parameter important in identifying the cellular changes taking place in the target organs such as gills, liver, kidney, brain, spleen, gonads and muscles (Al-Balawi et al., 2013; Gaber et al., 2014; Hadi and Ahwan, 2012; Ratn et al., 2018; Sabullah et al., 2014).

Effect of copper sulphate on Testis and spermatogenesis

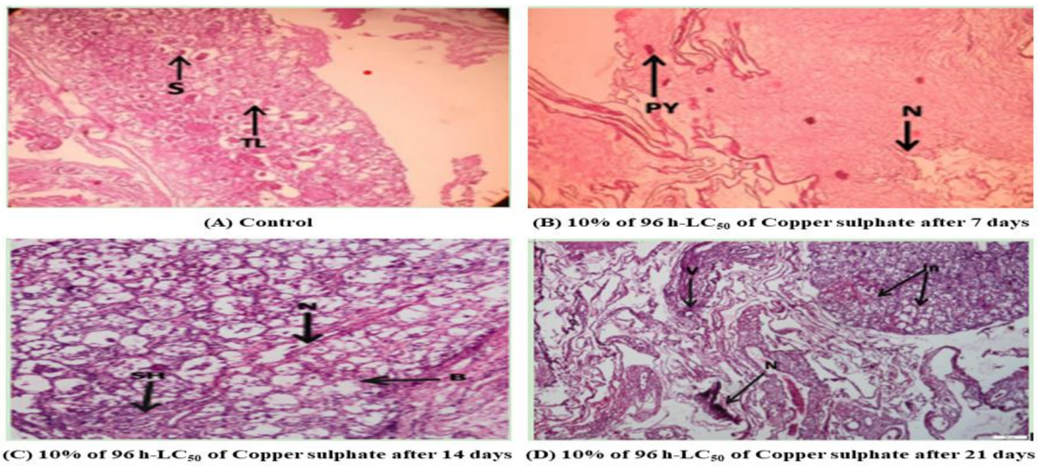

Fig. 1 Histological changes in testis of copper sulphate exposed fish *Channa punctatus* as compared to control (panel A) after 7 days (panel B), 14 days (panel C), and 21 days (panel D) of exposure intervals. Control (panel A) showing no abnormal changes with normal structure of spermatozoa (S) and testicular lobule (TL); Panel B illustrating the pycnotic cells (PY) and necrosis (N) after 7 days; Panel C expressing necrosis (N), bursting (B) and stromal hemorrhage (ST) after 14 days; Panel D showing necrosis (N), inflammation (In) and vacuolization (V) after 21 days. The testis sections stained with haematoxylin (H) and eosin (E) and examined under 10/40X magnification of objective lenses of light microscope.

Chronically, the exposure of copper sulphate posed prominent histopathological changes in testis of fish in a time-dependent manner (**Fig. 1**). The most complete sperm quality indicator is fertilization success, which is also one of the first markers of egg quality that can be observed (Bobe and Labbe, 2010). Spermatogenesis is the process through which a small number of diploid stem cells (spermatogonia) produce a large number of highly differentiated spermatozoa with a haploid, recombined genome and a structurally complete flagellum. According to several research (Marquez et al., 2019; Hayati et al., 2019; Zebral et al., 2019), heavy metals have an impact on the generation of sperm. Numerous fish species gonads have been discovered to be histologically damaged by heavy metals (Zulfahmi et al., 2018; Garriz *et al*., 2019). Histological investigations revealed pyknotic cells in the *Channa punctatus* testis in all heavy metal treatments. Pyknotic cells among spermatogonia have been shown to be a sign of sterility-causing germinal cell degeneration (Gárriz et al., 2017). Histological studies showed cell pyknosis, bursting, stromal hemorrhage, inflammation, vacuolization and necrosis after exposure to copper sulphate as comparison to control in which spermatozoa and testicular lobules are visible.

Effect of copper sulphate on Ovary and oogenesis

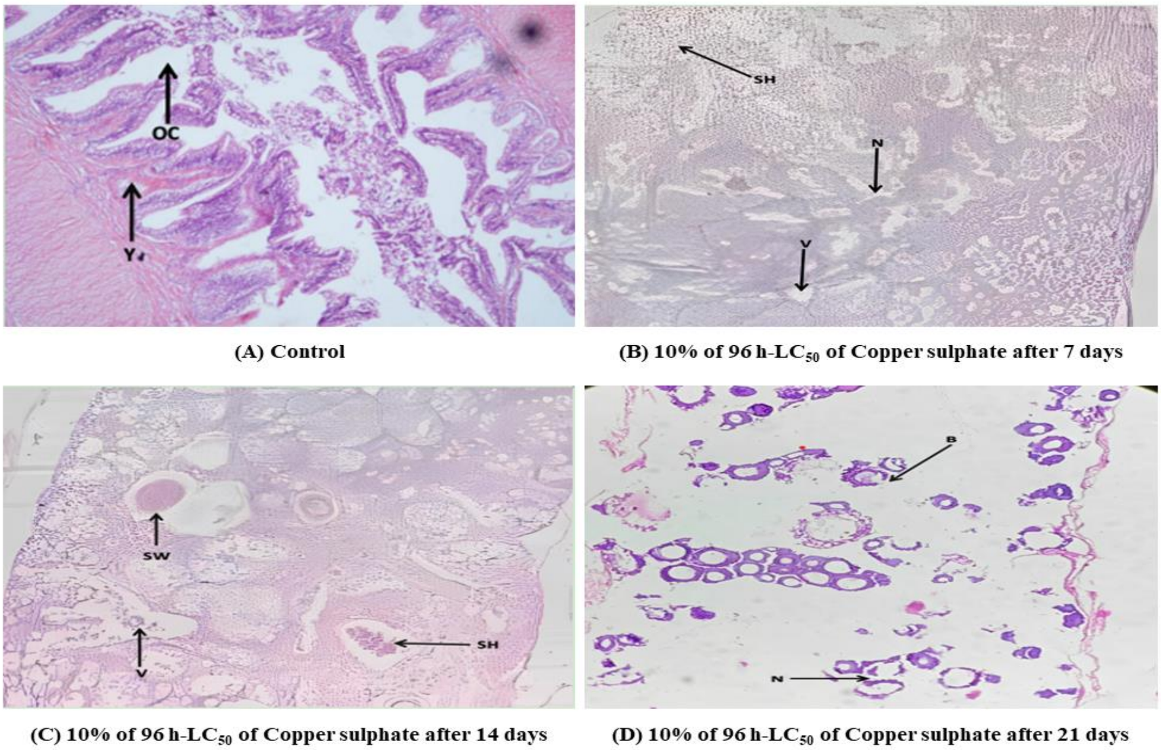

Fig. 2 Histological changes of ovary in copper sulphate exposed fish as compared to control (panel A) after 7 days (panel B), 14 days (panel C) and 21 days (panel D) of exposure intervals. Control (Panel A) exemplifying no abnormal changes with normal structure of ovarian cavity (OC) and yolky oocyte (Y); Panel B showing the vacuolization (V) and necrosis (N); Panel C illustrating the swelling (SW), vacuolization (V) and stromal hemorrhage (ST); Panel D displaying the necrosis (N) and bursting (b) of cell (H & E; 10/40X).

Persistently, the copper sulphate exposure engendered the histopathological anomalies in ovary of fish in a time-dependent manner (**Fig. 2**). Fish ovary and ova genesis are affected by heavy metals (Shobikhuliatul et al., 2013). Oogenesis is the process by which immature or previtellogenic oocytes evolve into mature oocytes in teleost. Oocyte maturation takes place prior to ovulation and is crucial for successful fertilization (Gautam and Chaube, 2018). According to Brraich and Jangu (2015), heavy metal toxicants produced from many sources have negative effects on the ovary and ultimately fish survival. Due to egg intoxication, metal accumulation in eggs, or direct effects of metal on the oogenesis process, heavy metal pollutants can alter fish oocytes (Brraich and Jangu, 2015). Oocyte maturation is primarily impacted by metal toxicity, which could lead to fewer and lower-quality oocytes being produced. Therefore, the quantity and quality of eggs are decreased as a result of different perturbations brought on by heavy metal pollution during oocyte development. In *Channa punctatus* ovary, an increase in the heavy metal copper sulphate results in greater atresia. Fish oocytes may change as a result of heavy metal pollution due to egg intoxication, metal buildup in eggs, or a direct impact of metal on the oogenesis process (Singh and Lal, 2015). In this investigation of histology, the exposure to copper sulphate, ovary cells exhibit stromal hemorrhage, vacuolization, necrosis, cell bursting and swelling as compare to control having normal structure of ovarian cavity and yolky oocyte are seen.

Effect of copper sulphate on fish Muscle

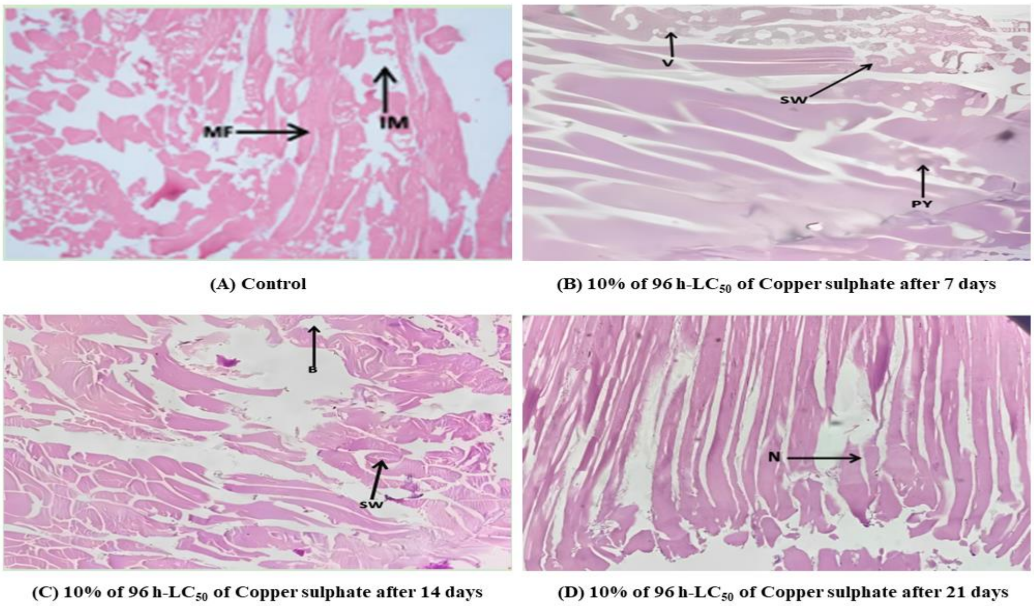

Fig. 3 Histological anomalies in muscle of copper sulphate exposed fish in comparison to control (panel A) after 7 days (panel B), 14 days (panel C) and 21 days (panel D) of exposure intervals. Control (Panel A) illustrating normal structure of myofibrils (MF) and interstitial material (IM); Panel B demonstrating vacuolization (V), swelling (SW) and pycnotic cells (PY); Panel C explaining the swelling (SW) and bursting (B) of cell; Panel D exemplifying necrosis (N) and bursting (B) of cell (H & E; 10/40X).

During prolonged time period, the copper sulphate toxication generated the histopathological abnormalities in muscle of fish in a time-dependent manner (**Fig. 3**). Muscle tissue comes into contact with heavy metal copper sulphate dissolved in water just like the gills and liver. In a study by Maharajan et al. (2016), the muscle displayed gradual structural deterioration, including thickening and muscular bundle separation along with significant intracellular edema. Similar abnormalities were also seen in the study by Das and Mukherjee (2000), where the separation of muscle bundles was thought to be an interesting observation. The degeneration of muscle bundles with aggregation of inflammatory cells between them and the focal area of necrosis, as well as vacuolar degeneration in muscle bundles and atrophy of muscle bundles, occurred as fish were exposed to toxicants, as found by Fatma (2009). When muscles are subjected to the heavy metal copper sulphate, this histological study reveals vacuolization, edema, and cell pyknosis. After some days, it shows cell rupture, swelling and necrotic cells on the exposure to copper sulphate. In contrast, normal myofibril and interstitial material structures are visible in the control group.

Effect of copper sulphate on fish heart

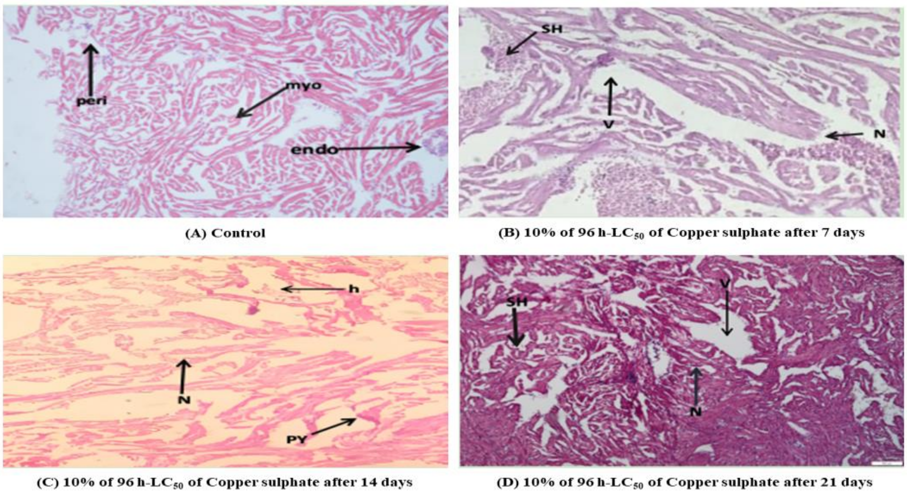

Fig. 4 Histological abnormalities of heart in copper sulphate exposed fish as compared to control (Panel A) after 7 days (Panel B), 14 days (Panel C) and 21 days (Panel D) of exposure intervals. Control (Panel A) showing normal structure of pericardium, myocardium and endocardium; Panel B illustrating vacuolization (V), stromal hemorrhage (ST) and necrosis (N); Panel C exemplifying necrosis (N), pyknosis (PY); Panel D explaining necrosis (N), vacuolization (V) and stromal hemorrhage (ST) (H & E; 10/40X).

Chronically, the exposure of copper sulphate elicited the prominent histopathological alterations in heart of fish in a time-dependent manner (**Fig. 4**). Gainey and Kenyon (1990) found that copper toxicity in *Mytilus edulis* resulted in a reduction in heart rate and cardiac function. The exposure to the heavy metal copper sulphate, the histological analysis of the heart reveals cell vacuolization, necrosis, and stromal hemorrhage. However, in the normal structure of the heart in control, the pericardium, myocardium, and endocardium are clearly visible. Pycnotic cells, stromal hemorrhage, necrosis and vacuolization are also apparent on the xenobiotic exposure in fish (Srivastava et al., 2022).

5. Conclusion

This investigation of the buildup of heavy metals provided insight into the level of environmental contamination. In addition, since fish is a significant source of copper for the general population, the degree of copper contamination in fish was very intriguing. The majority of the copper found in fish is present as copper sulphate, which is very absorbable. A number of factors, such as the concentration of copper absorbed and the duration of exposure, can affect the acute toxicity and physiological consequences on aquatic organisms following waterborne copper exposure. The histopathologic changes can be used as biomarkers for aquatic contamination with heavy metal copper. This study will be important for the conservation of aquatic fauna including fishes.

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Conflict of Interests

Authors have declared that no competing interests exist.

References

1. Al-Balawi, H.F.A., Al-Akel, A.S., Al-Misned, F., Suliman, E.A.M., Al-Ghanim, K.A., Mahboob, S. and Ahmad, Z. (2013), "Effects of sub-lethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of *Clarias gariepinus*", Brazilian Archives of Biology and Technology, Vol. 56 No. 2, pp. 293-302.
2. APHA/AWWA/WEF, "Standard Methods for the Examination of Water and Wastewater", 23rd ed., American Public Health Association, American Water Works Association, Water Environment Federation, Denver, 2017.
3. Awasthi, Y., Ratn, A., Prasad, R., Kumar, M., Trivedi, A., Shukla, J. P. and Trivedi, S.P. (2019), "A protective study of curcumin associated with Cr^{6+} induced oxidative stress, genetic damage, transcription of genes related to apoptosis and histopathology of fish, *Channa punctatus* (Bloch, 1793)", Environmental Toxicology and Pharmacology, Vol. 71, 103209.
4. Bobe, J. and Labbe, C. (2010), "Egg and sperm quality in fish", Gen. Comp. Endocrinol., Vol. 165, pp. 535-548.
5. Brraich, O.S. and Jangu, S. (2015), "Some aspects of reproductive biology on effect of heavy metal pollution on the histopathological structure of gonads in *Labeo rohita* (Hamilton-Buchanan) from Harike wetland, India", Inter. J. Fisher. Aqua., Vol. 7, pp. 9-14.
6. Das, B.K. and Mukherjee, S.C. (2000), "A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane", Veterinarski Archiv., Vol. 70 No. 4, pp. 169-180.
7. Ezemonye, L.I., Adebayo, P.O., Enuneku, A.A., Tongo, I. and Ogbomida, E. (2019), "Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin River, Nigeria", Toxicol. Reports, Vol. 6, pp. 1-9.
8. Faroon, O., Ashizawa, A., Wright, S., et al. (2012), "Toxicological Profile for Cadmium", Atlanta (GA): Agency for Toxic Substances and Disease Registry (US), pp. 11-15.
9. Fatma, A.S.M. (2009), "Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt", World Journal of Fish and Marine Sciences, Vol. 01 No. 01, pp. 29-39.
10. Gaber, H.S., Abbas, W.T., Authman, M.M.N. and Gaber, S.A. (2014), "Histological and biochemical studies on some organs of two fish species in Bardawil Lagoon, North Sinai, Egypt", Global Veterinarian, Vol. 12 No. 1, pp. 1-11.
11. Gainey, L.F. and Kenyon, J.R. (1990), "The effects of reserpine on copper induced cardiac inhibition in *Mytilus edulis*", Comp. Biochem. Physiol. Part C, Vol. 95 No. 2, pp. 177-179.
12. Gárriz, A., del Fresno, P. and Miranda, L. (2017), "Exposure to E2 and EE2 environmental concentrations affect different components of the Brain-Pituitary-Gonadal axis in pejerrey fish (*Odontesthes bonariensis*)", Ecotoxicol. Environ. Saf., Vol. 144, pp. 45-53.
13. Gárriz, Á.S., Pamela, P., Carriquiriborde and Miranda, L.A. (2019), "Effects of heavy metals identified in Chascomús shallow lake on the endocrine-reproductive axis of pejerrey fish (*Odontesthes bonariensis*)", Gene. Compa. Endocrin., Vol. 273, pp. 152-162.

14. Gautam, G.J. and Chaube, R. (2018), “Differential effects of heavy metals (cadmium, cobalt, lead and mercury) on oocyte maturation and ovulation of the catfish *Heteropneustes fossilis*: An *in vitro* study”, Turkish. J. Fish. Aquat. Sci., Vol. 18, pp. 1205-1214.

15. Hadi, A. and Ahwan, S. (2012), “Histopathological changes in gills, liver and kidney of freshwater fish, *Tilapia zillii*, exposed to aluminum”, International Journal of Pharmacy and Sciences, Vol. 3 No. 11, pp. 2071-2081.
16. Hamilton, M.A., Russo, R.C. and Thurston, R.V. (1977), “Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays”, Environmental science and technology, Vol. 11 No. 7, pp. 714-719.
17. Hayati, A., Wulansari, E., Armando, D.S., Sofiyanti, A., Amin, M.H.F.A. and Pramudya M. (2019), “Effects of *in vitro* exposure of mercury on sperm quality and fertility of tropical fish *Cyprinus carpio* L.”, Egypt. J. Aquat. Res., Vol. 45, pp. 189-195.
18. Hossain, M. (2012), “Effect of Arsenic (NaAsO2) on the Histological Change of Snakehead Fish, *Channa punctata*”, Journal of Life and Earth Science, Vol. 7, pp. 67-70.
19. Kanaujia, P., Ratn, A., Awasthi, Y., Trivedi, S.P., Kumar, M. and Sharma, Y.K. (2023), “Hexavalent chromium induced oxidative, histopathological and hematological changes in snakehead fish, *Channa punctatus*”, Journal of Environmental Biology, Vol. 44, pp. 569-577.
20. Liebel, S., Tomotake, M.E.M. and Ribeiro, C.A.O. (2013), “Fish histopathology as biomarker to evaluate water quality”, Ecotoxicology and Environmental Contamination, Vol. 8 No. 2, pp. 09-15.
21. Maharajan, A., Kitto, M.R., Paruruckumani, P.S. and Ganapiriya, V. (2016), “Histopathology biomarker responses in Asian sea bass, *Lates calcarifer* (Bloch) exposed to copper. The Journal of Basic and Applied Zoology, 77, 21–30.
22. Mahmuda, M., Rahman, M.H., Bashar A., Rohani M.F. and Hossain M.S. (2020), “Heavy metal contamination in tilapia, *Oreochromis niloticus* collected from different fish markets of Mymensingh District”, J. Agric. Food Environ., Vol. 01, pp. 01-05.
23. Malik, D.S. and Maurya, P.K. (2014), “Heavy metal concentration in water, sediment, and tissues of fish species (*Heteropneustis fossilis* and *Puntius ticto*) from Kali River, India”, Toxicol. Environ. Chem., Vol. 96 No. 8, pp. 1195-1206.
24. Marquez, L.S., Jose, E.A., Victor, A.G. and Jesus, O.V. (2019), “Effects of cadmium exposure on sperm and larvae of the neotropical fish *Prochilodus magdalenae*”, Comp. Biochem. Physiol. Part C., Vol. 225, 108577.
25. Miranda, A.L., Roche, H., Randi, M.A.F., Menezes, M.L., and Ribeiro, C.A. (2008), “Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish *Hoplias malabaricus*: histopathological, physiological, and immunological findings”, Vol. 34 No. 7, pp. 939-949.
26. Ratn, A., Prasad, R., Awasthi, Y., Kumar, M., Misra, A. and Trivedi, S.P. (2018), “Zn^{2+} induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver and kidney of the fish, *Channa punctatus* (Bloch, 1793)”, Ecotoxicology and Environmental Safety, Vol. 151, pp. 10-20.
27. Ribeiro, C.A.O., Vollaire, Y., Sanchez-Chardi, A. and Roche, H. (2005), “Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France”, Aquatic Toxicology, Vol. 74 No. 01, pp. 53-69.
28. Sabullah, M.K., Shukor, M.Y., Sulaiman, M.R., Shamaan, N.A., Syed, M.A., Khalid, A. and Ahmad, S.A. (2014), “The effect of copper on the ultrastructure of *Puntius javanicus* hepatocyte”, Australian Journal of Basic and Applied Science, Vol. 8 No. 15, pp. 245-251.
29. Sarkar, M., Islam, J.B. and Akter, S. (2016), “Pollution and ecological risk assessment for the environmentally impacted Turag River, Bangladesh”, J. Mater. Environ. Sci., Vol. 7, pp. 2295-2304.
30. Shobikhuliatul, J.J., Andayani S., Couteau J., Risjani Y. and Minier C. (2013), “Some aspect of reproductive biology on the effect of pollution on the histopathology of gonads in *Puntius javanicus* from Mas River, Surabaya, Indonesia”, J. Biol. Life. Sci., Vol. 4, pp. 191-205.
31. Singh, V.K. and Lal B. (2015), “Immunolocalization of nitric oxide synthase (NOS) isoforms in ovarian follicles of the catfish, *Clarias batrachus* and its relation with ovarian activity”, Gen. Comp. Endocrino., Vol. 220, pp. 98-102.
32. Srivastava, R.K., Singh, S., Mishra, S., Verma, P., Trivedi, S.P. and Ratn, A. (2022), “Analysis of Haematological and Histopathological manifestations in Gills and Muscle of Fish *Channa punctatus* (Bloch 1793) exposed to synthetic Pyrethroid Deltamethrin”, Uttar Pradesh Journal of Zoology, Vol. 43 No. 17, pp. 83-90.
33. Steinhagen, D., Helmus, T., Maurer, S., Michael, R.D., Leibold, W., Scharsack, J.P., et al. (2004), “Effect of hexavalent carcinogenic chromium on carp *Cyprinus carpio* immune cells”, Dis. Aquat. Organ., Vol. 62 No. 1-2, pp. 155-161.
34. Trivedi, S.P., Ratn, A., Awasthi, Y., Kumar, M. and Trivedi, A. (2021), “In vivo assessment of dichlorvos induced histological and biochemical impairments coupled with expression of p53 responsive apoptotic genes in the liver and kidney of fish, *Channa punctatus* (Bloch, 1793)”, Comparative Biochemistry and Physiology, Part C, Vol. 245, 109032.
35. Yan, W., Hamid, N., Deng, S., Jia, P.P. and Pei D.S. (2020), “Individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) perturb gut microbiota homeostasis and gonadal development in marine medaka (*Oryzias melastigma*)”, J. Hazard. Mater., Vol. 397, 122795.

36. Zebral, Y.D., Abou, I.S., Anni, A.S.V., Junior, C.C.D., Orcini, J.C., da. Silva, Caldas J.S. and Bianchini A. (2019), "Life-time exposure to waterborne copper IV: Sperm quality parameters are negatively affected in the killifish *Poecilia vivipara*", Chemosphere, Vol. 236, 124332.

37. Zulfahmi, I., Muliari, M., Akmal Y. and Batubara A.S. (2018), "Reproductive performance and gonad histopathology of female *Nile tilapia* (*Oreochromis niloticus* Linnaeus 1758) exposed to palm oil mill effluent", Egypt. J. Aquat. Res., Vol. 44, pp. 327-332.