Research Article

Histopathological Effects of Cadmium on Various Tissues of Rohu (*Labeo rohita*) Fingerlings

Samia Azad[1], Iram Liaqat[1*], Riffat Iqbal[1] and Uzma Rafi[2]

[1]Microbiology Lab, Department of Zoology, Government College University, Lahore-54000 Pakistan; [2]Department of Zoology, Lahore Garrison University, Lahore, Pakistan.

Abstract | The present study documented the histopathological changes in liver, kidney and intestinal tissues of Rohu (*Labeo rohita)* exposed to sub lethal concentrations of heavy metal cadmium (Cd). $CdCl_2$ was used as cadmium (heavy metal) source. Five concentrations of $CdCl_2$ (0.2, 0.4, 0.6, 0.8 and 1.0 ppm of Cd) were tested in glass aquaria having 35 Rohu fingerlings in each. After exposure for six week, sections of intestine, kidney and liver were excised and studied histologically. Results revealed variation in histological changes from mild to severe depending on concentration of exposure. Shrinkage of sub mucosal tissue, enlarged flattened villi, increased apoptosis and degenerated nuclei alongwith missing cytoplasmic boundaries were observed at high concentration of exposure (0.4-1.0 ppm). Likewise, $CdCl_2$ at high concentrations (0.6-1.0 ppm) had toxic effects kidneys and severe pyknosis, degenerated renal tubule, loss of cell integrity in complete tissue were observed. Pronounced degeneration of liver tissues, severe vacuolization, remarkable cirrhosis, necrosis and karyolysis was observed in live tissue of fish exposed to high Cd concentrations (0.6-1.0ppm). In conclusion, long term exposure of Cd at high concentrations showed toxic effects on intestine, kidney and liver of Rohu (*Labeo rohita)* and confirmed it harmful heavy metal for aquatic environments.

Received | June 08, 2021; **Accepted** | August 02, 2021; **Published** | June 20, 2022
***Correspondence** | Iram Liaqat, Associate Professor, Department of Zoology, Government College University, Lahore-54000, Pakistan; **Email:** iramliaq@hotmal.com
Citation | Azad, S., I. Liaqat, R. Iqbal and U. Rafi. 2022. Histopathological effects of cadmium on various tissues of Rohu (*Labeo rohita*) fingerlings. *Journal of Innovative Sciences,* 8(1): 01-07.
DOI | https://dx.doi.org/10.17582/journal.jis/2022/8.1.01.07
Keywords | Rohu, Cadmium, Histopathology, Growth performance, Fingerlings

1. Introduction

Heavy metals are metallic elements and are essential for living organisms under control conditions. However, these become toxic due to exceeded concentration and associated health risks. Heavy metal environmental pollution becomes an alarming signal depending upon level of absorbance, route and duration of exposure. Furthermore, their bioaccumulations in aquatic systems greatly affect organisms morphologically and physiologically (Hoseini *et al.*, 2015).

Fresh water contamination with diversity of pollutants has become a focus of attention since the last many years. Release of heavy metals from

domestic, industrial and other man-made activities has extensively contaminated the natural aquatic bodies. Several devastating effects of heavy metal contamination have disturbed the ecological balance of both the environment and aquatic organisms of diverse types. Among these aquatic animals, fishes are the most deeply affected inhabitants that have no escape from harmful effects of the pollutants. Fishes are widely used as indicator organisms since pollutants build up in the food chain and adversely affects them causing deaths in severe cases (Siddiqui and Chang, 2014)

Cadmium, an important xenobiotic, has been considered as a problem causing metal that is present as persistent and non-biodegradable chemical pollutant in the aquatic environment. Among numerous causative agents and the sources of environmental pollution, some heavy metals like arsenic, zinc, lead, copper, nickel and cadmium are abundantly found in aquatic environment are numerous due to increased industrialization, urbanization, municipal wastes production and improper use of automobiles (Olaniran *et al*., 2013). Effluent from electroplating industries manufacturing vinyl plastics, metallurgical operations and mining are the major sources of cadmium into aquatic environments. Fishes occupy the higher trophic level in aquatic ecosystem and have greater chances to transfer cadmium to higher organisms including human. Cadmium is a recognized carcinogen in mammals. There is an increasing research trend on cadmium toxic effects to terrestrial and aquatic organisms (Shah, 2017).

The environmental pollution and toxicity induced from heavy metals particularly from cadmium requires a global initiative. The bioaccumulation of metals in particular cadmium is of great concern since it has deleterious effects on human health. Additionally, fish and seafood are the main possible link between environmental contamination and human exposure (Fraser *et al*., 2012). Hence, this study was planned to evaluate the toxic effects of various doses of $CdCl_2$ in soft tissues (liver, kidney and intestine) of Rohu (*Labeo rohita*) Fingerlings.

2. Materials and Methods

2.1 Fish stocking and experimental design

Fingerlings measuring 10-12 cm in length and 340 ± 10 g of a freshwater fish *Labeo rohita* locally called as Rohu were purchased from a commercial fish farm. The fish were brought to the laboratory in air filled polythene bags. The fish were given a bath in 0.1% methylene blue before transferring them in glass aquaria to avoid any infection and multiplication of bacteria that could adversely affect their health. The fish were then maintained in flow through system in glass aquaria having the water temperature at 27°C. All fishes were kept in glass aquaria at 27°C for one week prior to experiment. After a week the fish were transferred in 80 liters experimental aquaria. All fish were fed with the commercial pelleted aquarium feed twice daily at a rate of 2% of their body weight. Cadmium in the form of cadmium chloride ($CdCl_2$) in a solution was used as cadmium (heavy metal) source. The water used for acclimation and conduction of experiments was clear ground water. The hydrographical condition of the water was as follows: temperature 28 ± 2 °C, pH 7.8, total hardness 232 mgL^{-1}, chloride 30 mgL^{-1}, Ammonia 0.15 mg/1N NH_3, nitrate 0.06 mgL^{-1}, Nitrite 0.09 mg/1NNO_2/L, iron 0.05 mgL^{-1}, phosphate 0.112 mg L^{-1}, dissolved oxygen 9-10 mgL^{-1}, phenols nil. Standard protocols of APHA (2005) were followed for the collection of materials, storage, handling, feeding, and biomass loading and for the analysis of water. Mortality in each group was monitored on daily basis.

Cadmium as cadmium chloride was dissolved in distilled water and added in the water of aquaria. Five concentrations of Cd (0.2, 0.4, 0.6, 0.8 and 1.0 ppm) were maintained in experimental aquaria 2-6 respectively and control group was without cadmium dose. In each treatment and control aquaria 35 healthy fingerlings of Rohu were added.

2.2 Sampling and post-mortem of fish

Five fish of equal size were sampled from all the six treatment groups (five experimental and one control) after 6 weeks and sacrificed. A straight cut was made from operculum to the vent. The body wall was stretched with the help of forceps to expose the internal organs. The anterior half of intestine, lobes of liver and the kidneys were removed. They were fixed in aqueous Bouin's solution for 48 hours, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Sections of 5µm thickness were cut and stained with Ehrlich hematoxylin/ Eosin (dissolved in 70 % alcohol). After mounting in canada balsam Sections were observed using digital microscope (Intel Play QX3) at 200x magnification.

3. Results and Discussion

3.1 Cadmium effect on intestine

The intestinal sections of fish in control group exhibited normal structure of intestine having long tapering villi and tightly packed sub mucosal tissue (Figure 1A). However, slight changes were observed in villi and sub-mucosal tissues of fish group exposed to 0.2 ppm Cd (Figure 1B). Intestine of fish exposed to 0.4 ppm of Cd showed shrinkage of sub mucosal tissue and the villi are enlarged towards the tip (Figure 1C). Exposure to 0.6 ppm of Cd resulted in broad flattened villi alongwith some signs of deterioration (Figure 1D). Cell death in each villus facing lumen, lack of distinct nuclei and cytoplasmic boundaries was observed on exposure to 0.8 ppm of Cd (Figure 1E). In the last group, exposed to 1 ppm of Cd, the intestine showed severe structural damage indicating severe toxic effect (Figure 1F).

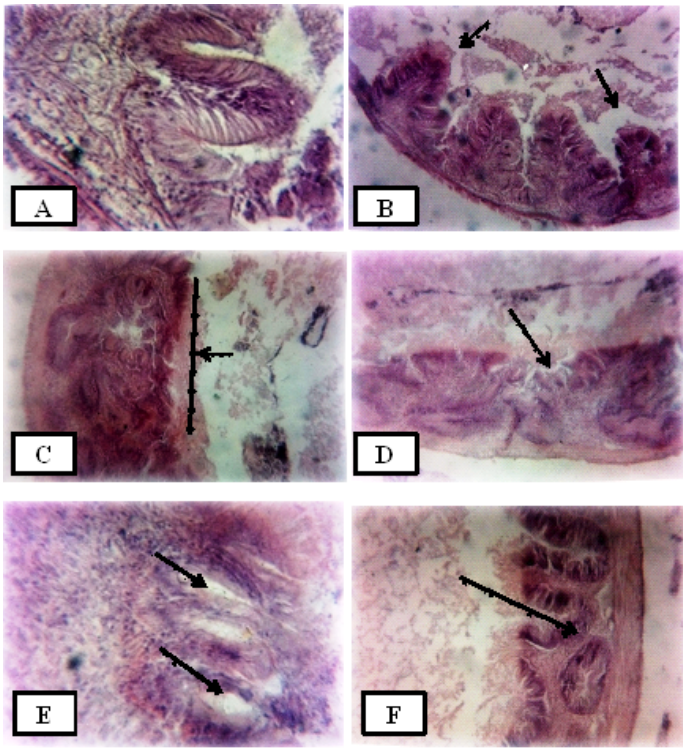

Figure 1: (A) Cross section of intestine without exposure to cadmium showing normal structure of intestinr; (B) Slight changes occurred in intestine villi; (C) Shrinkage of sub-mucosal tissue is quite visible; (D) Mucosal epithelium is collapsed; (E) Intestine inner lining shows damage and distortion at the basement membrane; (F) Intestine showing complete degeneration.

3.2 Cadmium effect on kidney

Regarding kidney histology, control group showed normal arrangement, normal organization of renal corpuscles, renal tubules and haemopoietic tissues (Figure 2A) while there observed a slight disorganization of epithelial cells of renal tubule and nuclear pyknosis in renal epithelial cells and haemopoietic tissues of fish group exposed to 0.2 ppm Cd (Figure 2B). The fish exposed to 0.4 ppm of Cd showed dilution of glomeruli, increased nuclear pyknosis and necrosis as observed in both epithelial cells of renal tubules and haemopoietic tissues (Figure 2C). Increased exposure to 0.6 ppm of Cd led to increase in nuclear pyknosis and necrosis with degraded tissue patches and degenerate glomeruli (Figure 2D). Renal tubules degenerated and lost their normal integrity and were not distinguished from haemopoietic tissues in fish group exposed to 0.8 ppm Cd (Figure 2E). Fish group exposed to 1 ppm Cd showed dilation of renal tubules and highly pyknotic nuclei indicating degenerated and disorganized of the whole tissue (Figure 2F).

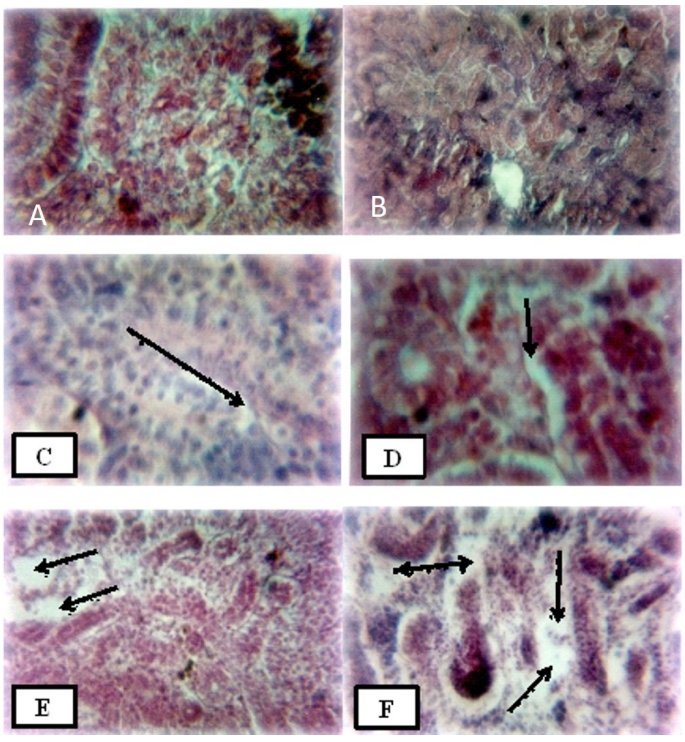

Figure 2: (A) Cross section of kidney without exposure to cadmium; (B) Slight disorganization of epithelial cells of renal tubule; (C) Significant nuclear pyknosis and necrosis is visible; (D) Glomeruli showing degeneration; (E) Renal tubules are degenerated and lost their normal integrity; (F) Tissues are degenerated and disorganized heavily.

3.3 Cadmium effect on liver

The fish exposed to 0.2 ppm of Cd, showed almost normal pattern in liver cells with very slight degenerative changes in cell arrangements (Figure

3B), compared to liver tissues of fish in control group (Figure 3A). The group exposed to 0.4 ppm Cd showed initial stage of cirrhosis and vacuolization of cytoplasm (Figure 3C). Degeneration in the liver parenchymal cells with severe damage, drastic karyolysis and necrosis was pronounced in group exposed to 0.6 ppm of Cd (Figure 3D). Significant degenerative changes were observed in the liver parenchyma and pronounced necrosis in fish exposed to 0.8 ppm of Cd (Figure 3E). Fish exposed to 1 ppm Cd showed complete degeneration of liver tissues, severe vacuolization, remarkable cirrhosis, necrosis and karyolysis (Figure 3F).

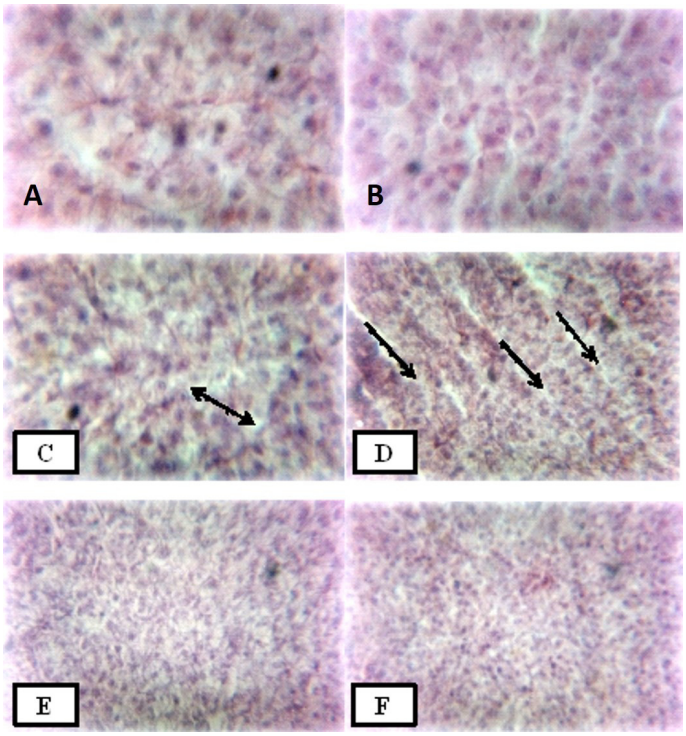

Figure 3: (A) Cross section of liver without exposure to cadmium; (B) Slight degeneration is visible in cell parenchyma; (C) Slight necrosis and karyolysis is visible; (D) Severe degenerative changes in tissue parenchyma can be seen; (F) Severe karyolysis and necrosis are shown; (F) Tissue are completely damaged and destroyed.

Environmental pollution in an aquatic ecosystem is the most serious chronic problem. Pollution caused by heavy metals is one of the major problems for human beings, not addressed properly in Pakistan. Heavy metal contamination of aquatic ecosystem has been reported to cause serious health hazardous effects and require direct attention. Natural phenomena such as volcanic eruptions, erosion and man-made activities including industrialization and mining are the major factors contributing to heavy metal contamination of aquatic bodies. Significant exposure to heavy metals at high concentrations has induced a diverse range of toxic effects in both animals and humans. Fish is an important source of human food and directly threaten human nutrition due to polluted aquatic ecosystems (Nikalje *et al.*, 2012).

Several studies have indicated that various metals including arsenic, copper, cadmium, chromium and mercury have caused metal induced fish intoxication (Younis *et al.*, 2012). Histopathological biomarkers have been a primary source in fish to identify and assess the toxic effects of exposure to pollutants (Bais *et al.*, 2012). The current study illustrated the toxic effects of cadmium intoxication in Rohu (*Labeo rohita)*. The fingerlings were kept in the similar environment as a natural water body receiving toxic heavy metals released by industries entering into the water system and the toxic effects of $CdCl_2$ on tissues like intestine, kidney and liver were studied. Rohu (*Labeo rohita)* fingerings were exposed to five different concentrations of cadmium (0.2, 0.4, 0.6, 0.8 and 1.0 ppm) and serious cytological changes were observed in intestine, kidney and liver.

Exposure to cadmium caused broadening and flattening of the villi observed in the anterior half of intestine indicating hyperactivity. Cell death, lack of distinct nuclei and cytoplasmic boundaries was noted in each villus facing the lumen. Shrinkage of sub mucosal tissue created clear spaces tips of the villi. This indicated that intestine was suffering from necrosis. Mohamed (2009) reported edema between submucosa and mucosa due to absorption of toxic metals. The histopathological alterations noted in the intestine of Rohu (*Labeo rohita*) fingerlings indicated severe degeneration and destruction of intestinal mucosa. Necrosis caused cell death in the columnar epithelial cells and shortening of villi was directly related to increased dose and exposure time of cadmium chloride. Hence pointing to the fact that though uptake of metals occurs mainly through gills but may also occur via intestinal epithelium (Mohamed, 2008). Previously, Kaoud *et al.* (2011) reported similar findings and observed that the intestine of *Oreochromis niloticus* treated with cadmium resulted in atrophy of muscularis, degeneration and necrosis of intestinal mucosa and submucosa. Authors further observed necrotized cells clustered in the lumen of intestine causing edema and atrophy of submucosa.

The fish kidneys act as filters and are exposed to cadmium through blood filtration. In present study the Cd intoxication produce histological and cytological changes to the kidneys. It affected both the renal tubules and haemopoietic tissues in fish, *Labeo rohita*. The major changes were nuclear pyknosis, necrosis and disorganization of renal epithelial cells. Nordberg *et al.* (2012) stated that environmental exposure to $CdCl_2$ affects various tissues notably kidney by causing cell death leading to problems in renal function. The observed features of damage to kidneys induced by Cd intoxication involved glomerular degeneration, occluded tubular lumen, loss of cellular parenchyma and necrosis. Similar findings were observed by Kondera and Witeska (2013) who reported that exposure to Cd caused kidney damage of the common carp *Cyprinus carpio* by affecting hematopoiesis. The presence of tubular degeneration combined with necrosis indicated detrimental damage to kidneys induced by the Cd exposure. Cadmium accumulation at high concentrations in kidney of fishes has been associated with nephrotoxic effects in human and animal populations. In fact, kidney is a highly dynamic organ and chronic cadmium exposure resulted in varying degrees of renal damage (Kondera *et al.*, 2013). Following exposure to Cd, cellular damage and decrease in number of precursor cells in the kidney has already been reported by Kondera and Witeska (2013). Cd exposure in the freshwater fish *C. macropomum* produced an abnormal kidney structure, and induced inflammation, affecting hematopoietic cell differentiation, affecting the overall function of organ.

Liver is among the most important sensitive organs to the biochemical disruption produced by diverse environmental pollutants (Younis *et al.*, 2012). It is involved in processes of detoxification and biotransformation and is greatly affected by water pollution/contamination due to its location and route to blood supply (Mohamed, 2009). In this work, sinusoidal dilatation in fish exposed to cadmium chloride was observed. Likewise, Nobuyoshi *et al.* (2005) reported that the liver being the center of detoxification in body receives cadmium very often resulting in increased arterial flow and causing sinusoidal dilatation.

This study reported various cytological changes in the liver following Cd intoxication leading to severe necrosis, cirrhosis vacuolization of cytoplasm, leukocyte infiltration and karyolysis. In the necrotic hepatic cells, the nucleus decreased in size and the chromatin was adherent to the nuclear membrane. The nucleus became pycnotic leading to karyolysis followed by cytolysis and phagocytosed by lymphocytes. The hepatocytes abnormalities were reported previously by a number of researchers following exposure to cadmium due to affinity of these with legends in proteins thus retarding their biochemical and physiological activities in living organisms (Patel and Bahadur, 2011).

Cadmium accumulation at high concentrations in liver of fishes induced various pathological changes in liver tissues such as engorgement of blood vessels, congestion, vacuolar degeneration of hepatocytes and necrosis of pancreatic cells (Dangre *et al.*, 2010). The most common damage due to Cd intoxication was vacuolar degeneration of hepatocyte having inflammation and necrosis thus causing dissociation of hepatocytes. Also, both central and periportal veins were congested and expended. This might be due to distended sinusoid between hepatocyte due to blood congestion and fibrosis (Salim and Majeed, 2014).

The bioaccumulation of metals by the fish and vital organs is variable depending upon the concentration of heavy metals in the water reservoir, sensitivity of fish, the age of the fish, the size of the fish, the physiological status of fish, their habitat preference, feeding behavior, and rate of growth. The results of the study showed that kidney and liver have maximum and almost equal concentration of Cd, affected equally. Though, histology of liver seems to be slightly more affected, possibly due to accumulation of Cd. It might be due to its capacity to react with other metabolites (Cuevas *et al.*, 2016). Recently, Mahboob *et al.* (2020), reported highest levels of heavy metals in liver and kidney, being the major organs involved in the detoxification and removal of toxic substances circulating in the blood stream.

Babu (2013), reported vascular degeneration of hepatocytes in his study due to cadmium intoxication. Likewise, observed necrosis in parenchyma cells of liver coupled with extensive cytoplasmic vacuolization and hepatocytes shrinkage around the bile ducts was noted. Selvanathan *et al.* (2013) showed histological alterations like vascular degeneration of hepatocytes, congestion of hepatic tissues, sub capsular vacuolization, necrosis, indistinct cell boundaries and

pyknotic nuclei in cat fish (*Clarias batrachus*) exposed to cadmium.

To summarize, present study produced hazardous effects of Cd exposure to Rohu (*Labeorohita*) Fingerlings and led to severe variations in histology. At high concentrations, cadmium badly effected kidney, liver and intestine leading to the death of fish. Histological changes observed in Rohu can be applied to monitor aquatic pollution using it as a model animal. Further, observation of degenerative changes in kidney, liver and intestine confirmed the adverse effects of Cd exposure on digestion, detoxification and excretion of fish in long run.

In conclusion, the presence of excess amounts of heavy metals in aquaculture is a major concern and fish cannot escape from the detrimental effects of toxic heavy metals. Consumption of fish poisoned with such heavy metals cause damage or decreased mental ability, reduced energy levels and damage to blood, liver, kidney, bones and other organs. Long term exposure may additionally result in slow progression of muscular dystrophy, multiple sclerosis, Alzheimer's disease and Parkinson's disease. Allergies are also common and even cancer may be the result. Heavy metal toxicity and fish poisoning if not properly treated, it will lead to significant illness and poor quality of life with ultimate result in death. Appropriate strategies such as technological measures, industrial and household waste management, holistic planning, environmental legislations and monitoring programs are recommended to overcome this emerging and increasing alarming threat (Amqam *et al*., 2020; Sonone *et al*., 2020).

Acknowledgments

The authors would like to thank Dr. Shafiq Ahmad, Professor, UVAS, for their technical support and guidance.

Novelty Statement

The bioaccumulation of metals in particular cadmium is of great concern since it has deleterious effects on human health. The present study provided evidence that long term exposure of Cd at high concentrations showed toxic effects on intestine, kidney and liver of Rohu (Labeo rohita) and confirmed it harmful heavy metal for aquatic environments.

Author's Contribution

Iram Liaqat: Conceptualization, methodology, formal analysis, writing-original draft.
Samia Azad: performed experimental work and analysed data.
Riffat Iqbal and Uzma Rafi: Data analysis, review of manuscript.
All authors read the final manuscript and approved the final version.

Conflicts of Interest

The authors have declared no conflict of interest.

References

American Public Health Association (APHA) 2005. *Standard methods for the examination of water and wastewater*. 21th Edn. Washington DC, USA.

Amqam, H., Thalib, D., Anwar, D., Sirajuddin, S. and Mallongi, A., 2020. Human health risk assessment of heavy metals via consumption of fish from Kao Bay. *Environ. Health Rev.,* 35(3): 257-263. https://doi.org/10.1515/reveh-2020-0023

Babu, R.A., 2013. Effects of Cadmium on Digestive Organs of Teleost Fish *Ophiocephalus* (Channa*). J. Environ. Res. Technol.,* 3(2): 173-180.

Bais, U.E. and Lokhande, M.V., 2012. Effect of cadmium chloride on histopathological changes in the freshwater fish, *Ophiocephalus striatus* (Channa). *Int. J. Zool. Res.,* 8(1): 23-32. https://doi.org/10.3923/ijzr.2012.23.32

Cuevas, N., Zorita, I., Franco, J., Costa, P.M. and Larreta, J., 2016. Multi-organ histopathology in gobies for estuarine environmental risk assessment: a case study in the Ibaizabal estuary (SE Bay of Biscay). *Estuar. Coast. Shelf Sci.,* 179: 145-154. https://doi.org/10.1016/j.ecss.2015.11.023

Dangre, A.J., Manning, S. and Brouwer, M., 2010. Effects of cadmium on hypoxia-induced expression of hemoglobin and erythropoietin in larval sheepshead minnow, *Cyprinodon variegates. Aquat. Toxicol.*, 99(2): 168-175. https://doi.org/10.1016/j.aquatox.2010.04.015

Fraser, M., Surette, C. and Vaillancourt, C., 2012. Fish and seafood availability in markets in the Baie des Chaleurs region, New Brunswick, Canada: A heavy metal contamination baseline

study. *Environ. Sci. Pollut. Res.*, 20(2): 761-770. https://doi.org/10.1007/s11356-012-1134-3

Hoseini, H., Tahami, M.S. and Askar Hesini, M., 2015. Bioaccumulation of Lead and Cadmium in liver and muscle tissues of Kutum fish (Teleostei: Cyprinidae) in the southern Caspian Sea. *Int. J. Instruct.*, 2(3): 165-171.

Kaoud, H.A., Zaki, M.M., El-Dahshan, A.R., Saeid, S. and El Zorba, H.Y., 2011. Amelioration the toxic effects of cadmium-exposure in nile tilapia (*Oreochromisniloticus*) by using Lemnagibba L. *Life Sci. J.*, 8(1): 185-195.

Kondera, E., and Witeska, M., 2013. Cadmium and copper reduce hematopoietic potential in common carp (*Cyprinus carpio* L.) head kidney. *Fish Physiol. Biochem.*, 39(4): 755-764. https://doi.org/10.1007/s10695-012-9738-6

Kondera, E., Lugowska, K. and Sarnowski, P., 2013. High affinity of cadmium and copper to head kidney of common carp (*Cyprinuscarpio* L.). *Fish Physiol. Biochem.*, 40(1): 9-22. https://doi.org/10.1007/s10695-013-9819-1

Mahboob, S., Al-Ghanim, K.A., Al-Balawi, H.F., Al-Misned, F. and Ahmed, Z., 2020. Toxicological effects of heavy metals on histological alterations in various organs in Nile tilapia (*Oreochromis niloticus*) from freshwater reservoir. *J. King Saud Univ. Sci.*, 32(1): 970-973. https://doi.org/10.1016/j.jksus.2019.07.004

Mohamed, F.A.S., 2008. Bioaccumulation of selected metals and histopathological alterations in tissues of Oreochromisniloticus and Latesniloticus from Lake Nasser, Egypt. *Global Vet.*, 2(4): 205-218.

Mohamed, F.A.S., 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. *World J. Fish Mar. Sci.*, 1(1): 29-39.

Nikalje, S.B., Muley, D.V. and Angadi, S.M., 2012. Histopathological changes in gills of a freshwater major carp *Labeo rohita* after acute and chronic exposure to textile mill effluent (tme). *Int. J. Environ. Sci.*, 3(1): 108-118.

Nobuyoshi, O., Hisaya, N., Kazuo, I., Saori, N., Yoshinoro, I., Makoto, O. and Isao, O., 2005. Telangiectatic focal nodular hyperplasia of the liver in the perinatal period: Case report. *Pediatr. Dev. Pathol.*, 8(5): 581-586. https://doi.org/10.1007/s10024-005-0037-4

Nordberg, G., Jin, T., Wu, X., Lu, J., Chen, L., Liang, Y., Lei, L., Hong, F., Bergdahl, I.A. and Nordberg, M., 2012. Kidney dysfunction and cadmium exposure–factors influencing dose–response relationships. *J. Trace Elem. Med. Biol.*, 26(2-3): 197-200.

Olaniran, A.O., Balgobind, A. and Pillay, B., 2013. Bioavailability of heavy metals in soil: Impact on microbial biodegradation of organic compounds and possible improvement strategies. *Int. J. Mol. Sci.*, 14(5): 10197-228. https://doi.org/10.3390/ijms140510197

Patel, J.M. and Bahadur, A., 2011. Histopathological Manifestations of Sub Lethal toxicity of Copper Ions in *Catla catla*. *Am. Eur. J. Toxicol. Sci.*, 3(1): 01-05.

Salim, F. and Majeed, S.K., 2014. Survey on histopathological changes in different organs of local fresh water fishes in Basra province. *J. Int. Acad. Res. Multidisc.*, 2(10): 236-256.

Selvanathan, J., Vincent, S. and Nirmala, A., 2013. Histopathology changes in freshwater fish *Clariasbatrachus* (Linn.) exposed to mercury and cadmium. *Int. J. Life Sci. Pharma* Res., 3(2): 422–428.

Shah, I.A., 2017. Assesment of cadmium toxicity on the red blood cells of aquatic life. *Int. J. Adv. Res.*, 5(1): 685-688. https://doi.org/10.21474/IJAR01/2799

Siddiqui, A.A. and Chang, S., 2014. Cadmium Chloride intoxication and evaluation of Protein changes in *Clarias batrachus* (Linn). *Int. J. Curr. Microbiol. App. Sci.*, 3(2): 787-794.

Sonone, S.S., Jadhav, S., Sankhla, M.S. and Kumar, R., 2020. Water contamination by heavy metals and their toxic effect on aquaculture and human health through food Chain. *Lett. Appl. NanoBioSci.*, 10(2): 2148-2166. https://doi.org/10.33263/LIANBS102.21482166

Younis, E.M., Abdel-Warith, A.A. and Al-Asgah, N.A., 2012. Hematological and enzymatic responses of Nile tilapia *Oreochromis niloticus* during short and long term sublethal expo https://doi.org/10.5897/AJB11.3987 sure to zinc. *Afr. J. Biotech.*, 11(19): 4442-4446.