International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 3103-3108

Received: 19-05-2018
Accepted: 23-06-2018

Saravpreet Kaur
Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India

Kuldeep Singh Khera
Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India

Jasjit Kaur Kondal
Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India

Correspondence
Saravpreet Kaur
Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India

Effect of water contaminated with heavy metals on histopathology of freshwater catfish, *Clarias batrachus*

Saravpreet Kaur, Kuldeep Singh Khera and Jasjit Kaur Kondal

Abstract
In the present study, adult live specimens of edible freshwater catfish, *Clarias batrachus* were procured from local fish market at Hambran road, Ludhiana, Punjab from December 2016 to June 2017 and vital fish tissues (liver, muscle and kidney) were processed for heavy metal analysis and histopathological studies. The observations on histopathology revealed several hepatic lesions *viz*. Necrosis, infiltration of leukocytes, pyknosis and nuclear pleomorphism. Separation and degeneration of muscle fibres, atrophy of muscle bundles were well-noticed in muscle tissue. Renal alterations included, desquamation of renal epithelium, necrosis and disorganization of renal tubules. The results revealed that the content of toxic heavy metals (arsenic, chromium, cadmium, manganese and lead), which were beyond the permissible limits (WHO/FAO), might have caused the variations in histo-architecture of vital organs i.e. liver and kidney. However, the content of chromium and manganese exceeded the prescribed limits in muscle tissue which might have resulted in histological alterations.

Keywords: Heavy metals, *Clarias batrachus*, liver, muscle, kidney, histopathology

1. Introduction
The contamination of freshwaters with a wide range of pollutants has emerged as a serious environmental problem, worldwide [1, 2]. The rapid development of industry and agriculture has resulted in an increase in aquatic pollution with toxic chemicals, which has caused a significant environmental hazard in invertebrates, fish and humans [3]. Among aquatic pollutants, heavy metals being ubiquitous in nature, form a major stress related pollutant in aquatic bodies because of their non-biodegradable nature, toxicity, long persistence, ability to bio-accumulate and biomagnify in aquatic fauna [4, 5, 6]. These metal particulates enter the aquatic medium through effluents discharged from tanneries, textiles, metal finishing, mining, dyeing and printing industries, ceramic and pharmaceutical industries etc. [7]. Heavy metal contamination has devastating effect on the ecological balance of recipient environment and diversity of aquatic biota [8, 9].

Any metal or metalloid having relative atomic density more than 5 g/cm^3 is considered as heavy metal [10]. They are categorized as potentially toxic (arsenic, cadmium, lead, mercury, etc.), semi-essential (nickel, vanadium, cobalt) and essential (copper, zinc, iron, manganese). Harmful effects of toxic metals can be observed even at very low concentrations when ingestion occurs over a long period of time, however, essential metals can produce toxic effects when their intake is excessive [11] .

Among vertebrate aquatic fauna, fish are considered as one of the most indicative factors, in freshwater ecosystems, for the estimation of heavy metal pollution [12]. Fish are at a higher trophic level in the food web and can bioaccumulate large amounts of some metals particularly in liver, gills and kidney, upto concentrations several times higher than in the ambient water. Heavy metals are taken up through different organs of the fish because of the affinity between them [13]. Multiple factors including seasonal changes, exposure time, concentration of metals, physico-chemical parameters of water and metabolic activity of tissues might be the reason of significant augmentation of metals in different fish tissues [14, 15].

Histopathological changes have been widely used as a biomonitoring tool referring health status of fish exposed to environmental contaminants [16]. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining these specific target organs [17].

Fish liver is a good indicator of aquatic environmental pollution, where one of the important functions of the liver is to clean the pollutants from the blood coming from the intestine [18]. Also, it is the organ most affected by environmental contaminants in water, leading to histopathological changes in liver. However, kidney is a vital organ of body and its function is to maintain the homeostasis. It is not only involved in removal of wastes from blood but it is also responsible for selective reabsorption, which helps in maintaining volume and pH of blood and body fluid and erythropoieses [19]. Since a large volume of blood flows through the kidney, hence, it is a suitable organ for histological examination where lesions found in this organ can be useful as signs of environmental pollution [20]. Fish muscles are commonly analyzed for bioaccumulation of such toxic metals and pathological changes caused by it [21, 22]. These studies have been done for various reasons; many of them concerning food safety and public health interests where muscle tissues are generally the major edible portion of the fish [23].

Therefore, in the present study, heavy metal induced histopathological alterations were examined in different tissues (liver, muscle and kidney) of edible and commercially important catfish, *Clarias batrachus* procured from local fish vendors of Ludhiana, Punjab.

2. Material and Methods

2.1 Procurement of the fish

Live specimens of adult freshwater catfish, *Clarias batrachus*, irrespective of size, weight and sex were procured from local fish market at Hambran road in Ludhiana city (30°56´N; 75°52´S) during the period from December 2016 to June 2017. Source of captured fish, as told by fish vendors, was freshwater bodies from different districts of Punjab State (India) and local fish ponds of Ludhiana city and Sutlej River.

2.2 Analysis of heavy metals

Heavy metals in fish were estimated using Inductively Coupled Argon Plasma Atomic Emission Spectrophotometer (ICAP-AES, Thermo iCAP-6300) as recommended by Yousafzai *et al.* [13]. The samples (liver, muscle and kidney) were processed for the estimation of eight heavy metals i.e. arsenic, cadmium, chromium, copper, manganese, nickel, lead and zinc.

2.3 Histopathological studies

Adult fish specimens were dissected and different tissues (liver, muscle and kidney) were removed, excised of fat and adhering tissues. The representative samples were carefully preserved in labeled sample bottles containing aqueous Bouin's fixative for 24 hours.

2.3.1 Tissue processing, sectioning and staining

After complete fixation in aqueous Bouin's solution for 24 hours, the tissues were dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax (melting point between 58-60 °C). 4-6µm thick sections were cut with the help of rotary microtome.

After usual de-waxing with xylene followed by rehydration in descending series of ethanol, the sections were stained in haematoxylin, counterstained with eosin, dehydrated in ascending ethanol series, cleared in xylene and mounted in DPX [24]. The slides were observed under compound light microscope at 100X and 400X magnification and photomicrographs were taken using digital camera (Olympus CH20i).

3. Results and Discussions

3.1 Liver

In the present study, the liver of catfish displayed several histomorphological alterations such as mild necrosis, pyknosis, cytoplasmic degeneration and infiltration of leukocytes (Fig 1A, B). Branching of blood vessels, aggregation of erythrocytes was also well-marked (Fig 1C). Severe nuclear alterations such as nuclear pleomorphism, nuclear degeneration and hypertrophy of nuclei were also noticed (Fig 1D, E, F). The alterations in histo-architecture of liver might have occurred due to the accumulation of heavy metals i.e. arsenic, cadmium, chromium, lead and zinc beyond the certified limits as shown in Table 1.

Previous studies have demonstrated cause-to-effect links between exposure to pollutants and development of hepatic lesions and particularly, liver lesions in fish species are found to be effective.

Table 1: Level of heavy metals (ppm) in different tissues (liver, muscle and kidney) of market catfish

Tissues Elements	Liver	Muscle	Kidney	Permissible limits (WHO/FAO)
Arsenic	3.13±0.24*	0.68±0.14	3.09±0.29*	1.00
Cadmium	3.74±0.28*	0.24±0.06	4.45±0.42*	1.00
Chromium	3.69±0.47*	2.103±0.43*	2.14±0.13*	0.05
Copper	3.48±0.29	3.79±0.41	4.65±0.29	10.00
Manganese	2.71±0.38	1.47±0.202	3.29±0.29	5.00
Nickel	41.59±4.69	47.62±3.98	39.28±7.44	80.00
Lead	11.12±1.37*	4.48±0.16	8.04±0.49*	5.00
Zinc	67.78±2.78*	29.42±2.99	56.83±2.92*	50.00

Values are Mean ± SE Values with* (in a column) reveal the concentrations of heavy metals above the certified limits.

Biological markers of chemical exposures [25, 26]. The results of the present study are corroborated by the findings of Mahmoud and Abd El-Rahman. [27] Who noted similar hepatic variations in *Clarias gariepinus* and *Mugil capito*, inhabiting Manzalah Lake (Egypt) which included necrosis and degeneration of cells in *C.gariepinus* and hemolysis, haemorrhage and edema in *M. capito*. The alterations in liver of both the fish species might be the effect of pollutants which entered the lake with the drainage water as reported by Tayel *et al.* [28] the presence of necrosis is one of the most visible damages in tissues affected by pollutant [29]. The occurrence of necrosis is also the consequence of enzyme inhibition, damages in the cell membrane integrity and disturbances in the synthesis of proteins and carbohydrate metabolism [30].

Likewise, under laboratory conditions, Begum *et al.* [31] studied liver histology of *C. batrachus* after 28 days of exposure to sub-lethal concentrations of cadmium and chromium. Several degenerative changes such as atrophy of hepatocytes, shrunken and displaced nuclei, aggregation of nuclei and vacuolation of hepatocytes in cadmium-exposed fish, whereas, chromium-exposed fish showed alterations such as irregularly-shaped hepatocytes with pyknotic,

atrophied and necrosed nuclei. Morphological changes observed in the nucleus of hepatocytes are the evidence that this organelle can accumulate metals more intensely than other cellular organelles as proposed by Heath. [32] Similarly, Aly *et al.* [33] exposed *C. garipinus* (catfish) to acute lethal concentration of lead acetate for two weeks and found numerous histopathological alterations such as vacuolar degeneration, necrosis, hemolysis of erythrocytes and perivascular aggregations in liver tissue. Patnaik *et al*. [9] too, observed vacuolization, metal accumulation, many necrotic areas and pyknosis in the liver of *Cyprinus carpio communis*, following exposure to sub-lethal concentrations of lead and cadmium. Chavan and Muley. [34] Also, exposed *C. carpio* to sub-lethal concentration of lead acetate for 30 days and examined cytoplasmic vacuolation, intravascular haemolysis in blood vessels, focal necrosis, dilation and congestion in sinusoids and venules and cellular degeneration.

3.2 Muscle

Histological study of muscle tissue of catfish (*C. batrachus*) exhibited prominent alterations like degeneration of muscle fibres and many dystrophic changes (Fig. 2A, 2B). Aggregation of inflammatory cells, intercellular edema and separation of muscle bundles were also identified (Fig. 2C, 2D). Atrophy of muscle bundles was also observed (Fig. 2E, 2F).The results indicate that the alterations in muscle tissue of *C. batrachus* may probably be due to significantly high concentration of chromium and their accumulation beyond the prescribed limit in the muscle tissue (Table 1).

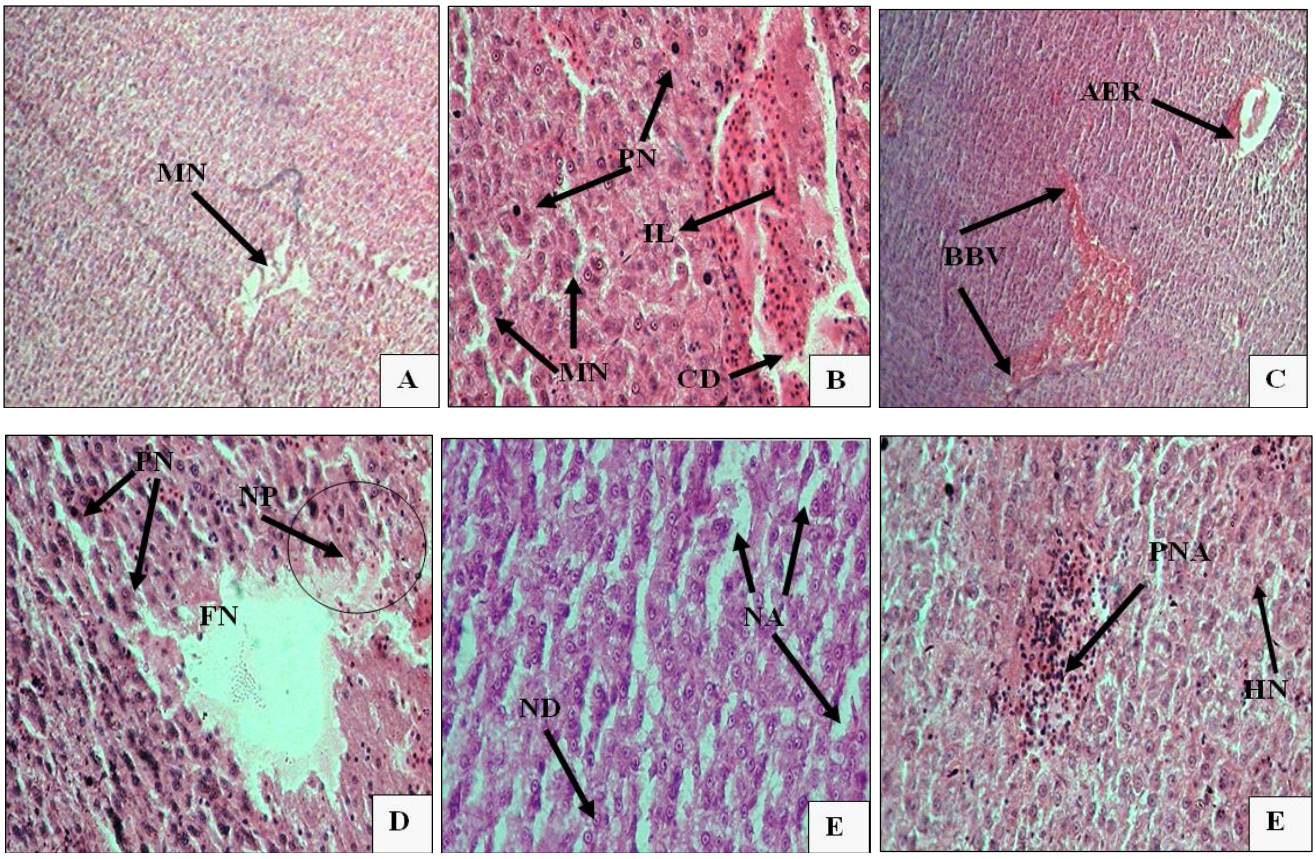
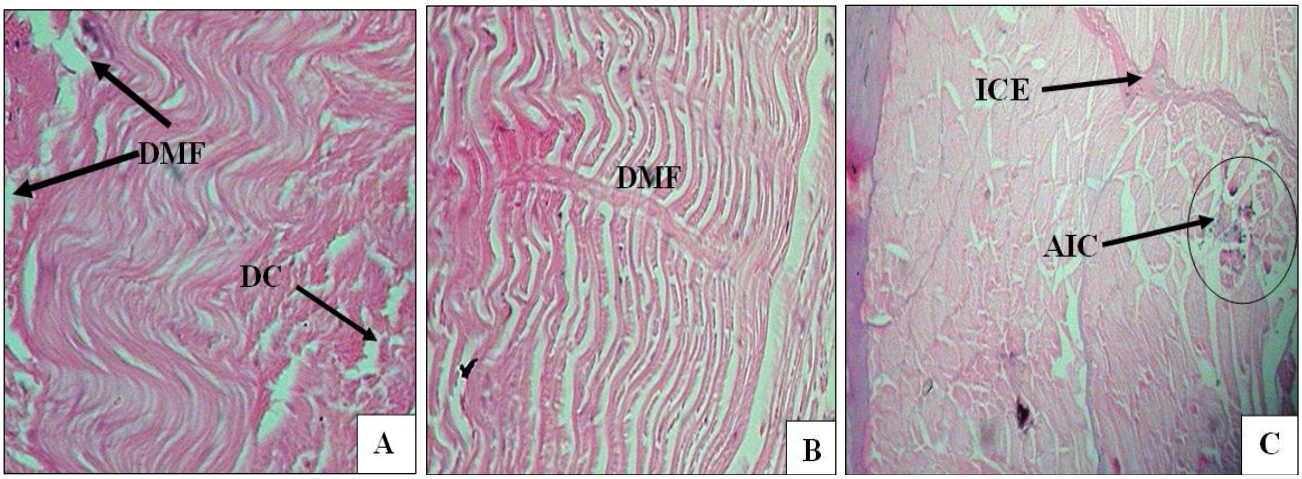


Fig 1: Transverse sections of liver (catfish) stained with H&E: (A, B) showing MN (mild necrosis), PN (pyknotic nuclei), CD (cytoplasmic degeneration) and IL (infilteration of leukocytes) at 100X and 400X. (C) Liver showing BBV (branching of blood vessels), AER (aggregation of erythrocytes) and HP (heterogenous parenchyma) at 100X. (D, E, F) liver showing NP (nuclear pleomorphism), FN (focal area of necrosis) and PN (pyknotic nuclei), NA (necrotic areas) and ND (nuclear degeneration), PNA (pyknotic nuclei aggregation) and HN (hypertrophy of nuclei) at 400X magnification.



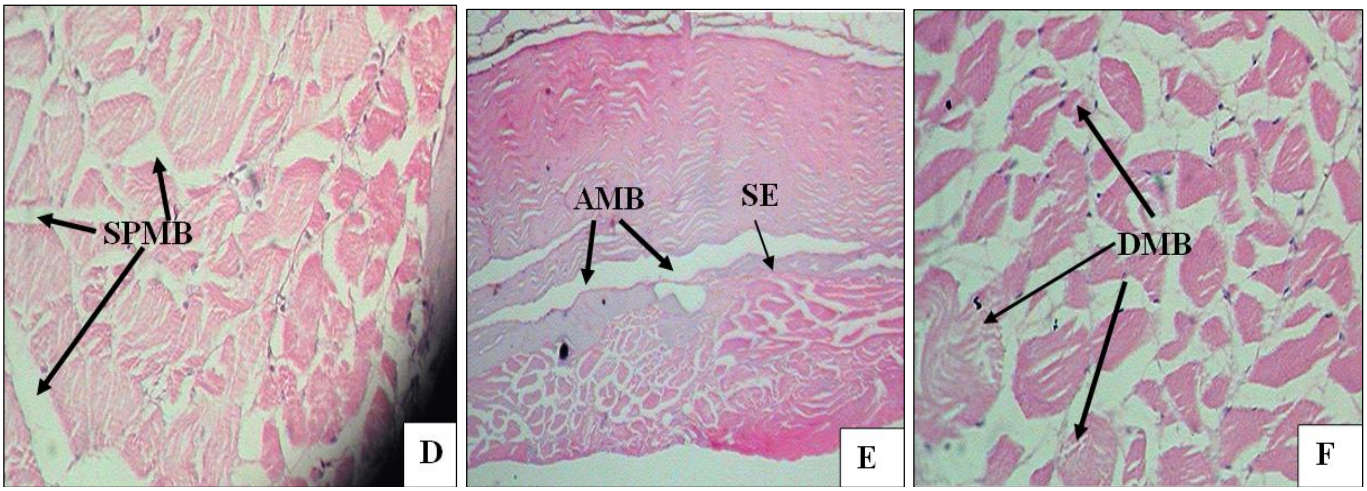


Fig 2: Vertical sections of muscle (catfish) stained with H&E [A, B] Section showing DMF (degeneration of muscle fibres) and DC (dystrophic changes) at 100X and 400X, respectively. [C, D] Section showing AIC (aggregation of inflammatory cells), IE (intercellular edema) and SPMB (separation of muscle bundles) at 100X and 400X, respectively. [E, F] Section showing AMB (atrophy of muscle bundles), SE (severe edema) and DMB (degeneration of muscle bundles) at 100X and 400X magnification, respectively.

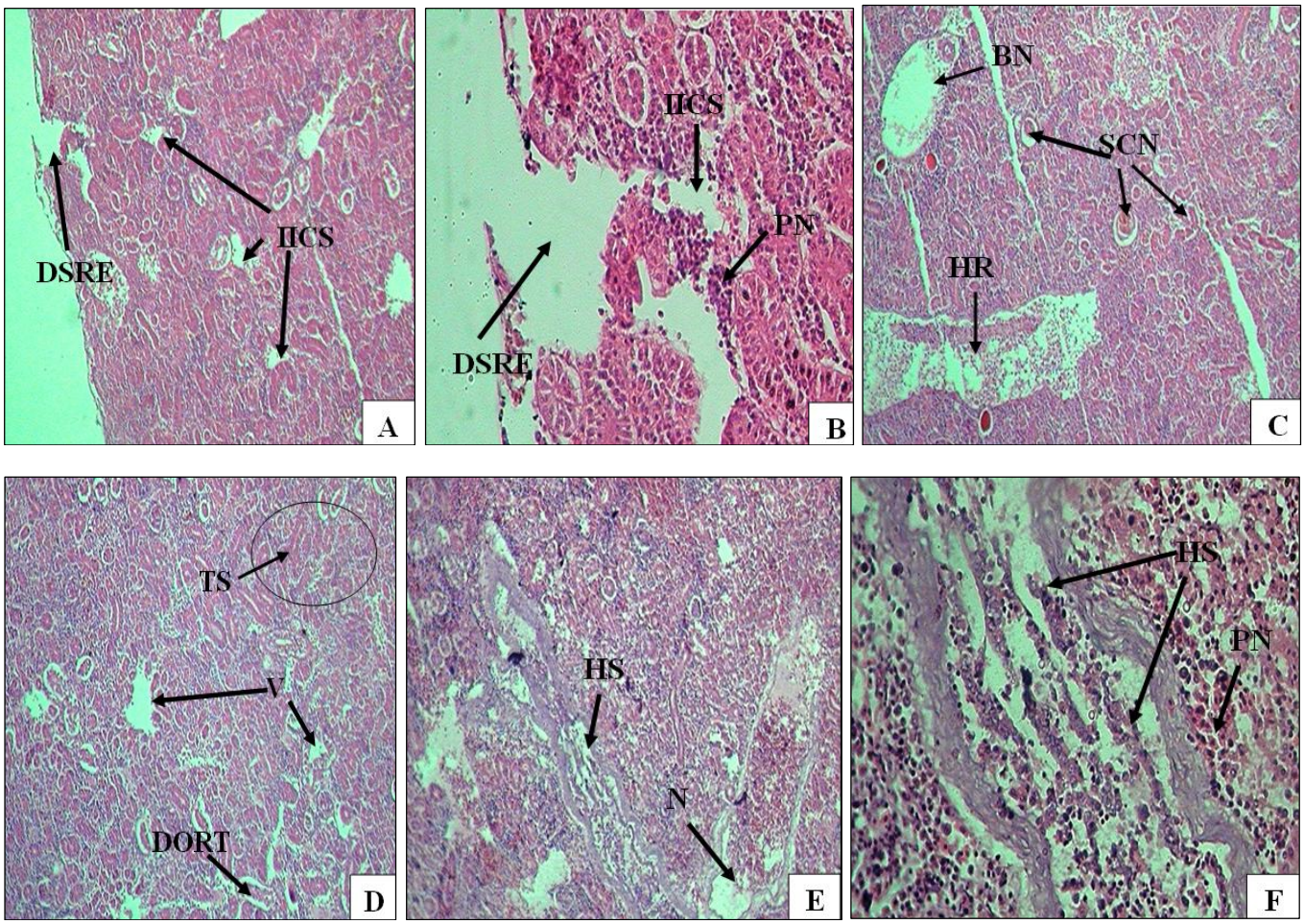


Fig 3: T.S. of kidney (catfish) stained with H&E - (A, B) showing DSRE (desquamation of renal epithelium), IICS (increased inter-cellular spaces) between renal tubules and PN (pyknotic nuclei) at 100X and 400X magnification, respectively. (C, D) showing BN (balloon necrosis), SCN (single cell necrosis) and HR (hemorrhage), TS (tubular shrinkage), DORT (disorganization of renal tubules) and V (vacuolization) at 100X magnification. (E, F) showing HS (hemosiderosis), N (necrosis) and PN (pyknotic nuclei) at 100X and 400X, respectively.

Since, fish fauna is an important component of the food chain and effect of pollutants on them, in the due course, would have adverse impact on the nutritive value of fish and on man through its consumption. Our results on muscle histology are in consonance with the findings of Dhevakrishnan and Zaman. [35] Who found numerous pathological alterations, induced by Cauvery river pollutants in muscle tissue of *Labeo rohita* which included disorganization of muscle bundles, severe intra muscular edema and necrosis of muscle bundles. All these changes indicated the fish under high environmental stress, due to more polluted region receiving effluents from industrial complex. However, severe changes such as edema and splitting of muscle fibres are found in muscles of *C. gariepinus* collected from El-Rahawy drain (Egypt) which might have occurred due to the high heavy metal concentrations in water and their accumulation in fish muscles

[36]. Similarly, degeneration of muscle bundles along with the aggregation of inflammatory cells between them, focal areas of necrosis, vacuolar degeneration in muscle bundles and atrophy of muscle bundles have been noted in *Tilapia zilli* exposed to different pollutants in Lake Qarun [37].

Patnaik *et al.* [9] studied the same in C.*carpio* under laboratory conditions, following exposure to sub-lethal concentrations of lead and cadmium. The authors reported many remarkable changes such as separation of muscle bundles, intramuscular edema and marked dystrophy. The results of present study are also corroborated by the findings of Abbas and Ali [38], who noticed various histological variations such as, destruction and vacuolation in the muscle cells of *Oreochromis* species, following exposure to chromium.

3.3 Kidney

Kidney is an important organ for the maintenance of homeostasis with respect to water and salts, metabolic waste excretion from blood and partially for the metabolism of xenobiotics [16, 39]. The effect of pollutants on fish kidney has been studied in some species and the severity of damage observed depends on the sensitivity of the species to the substances released into the environment [40].

In the present work, histology of kidney of market catfish exhibited desquamation of renal epithelium, increased inter-cellular spaces between renal tubules and pyknosis (Fig 3A, 3B). Some renal tubules are characterized by balloon necrosis, single cell necrosis, hemorrhage, tubular shrinkage. Glomeruli showed disorganized blood capillaries and vacuolization (Fig 3C, 3D). Hemosiderosis was also observed in renal tissue (Fig 3E, 3F). The histopathological alterations noticed in the kidney may be correlated with accumulation of heavy metals such as arsenic, cadmium, chromium, lead and zinc recorded in the tissue beyond the prescribed limit by WHO/FAO as depicted in Table 1.

The observations of present investigation are supported by the work of Mahmoud and Abd El-Rahman. [27] Who noted, degeneration, focal-necrosis, fibrosis in kidney tubules in the kidney of *C. gariepinus* and *M. capito* fish collected from El Manzalah Lake. These changes were attributed to the fact that kidney is one of the principal sites of detoxification in fish body as well as impaired blood supply due to toxic action of different pollutants in the lake, including heavy metals. Severe renal lesions were also reported by Tayel *et al.* [28] which included, disruption of tissue, hemorrhage and hemosiderosis in kidney of *Mugil cephalus* and *M. capito*, procured from Lake Manzala (Egypt). However, Dhevakrishnan and Zaman. [35] Recorded many pronounced histological variations such as, tubular shrinkage, degeneration of tubular epithelium, atrophy of renal tubules and formation of inter-cellular spaces in *L. rohita*, in response to Cauvery river pollutants.

Our results are also supported by the findings of Shah *et al.* [41] who studied cadmium-induced histological damage in kidney of freshwater catfish (*Heteropnuestes fossilis*). The authors noticed an increase in structural deformities such as, necrosis, loss of lumen, disorganization of renal tubules and obliterated lumen which might be due to bioaccumulation of cadmium in kidney. Similarly, histopathological changes i.e. enlargement of renal tubules, desquamation of epithelial lining, hypertrophied nuclei, edema, dilation of renal tubules, severe necrosis, pyknotic nuclei, vacuolization, disorganized blood capillaries in glomerulus were induced in kidney of *Channa punctatus* following exposure to sub-lethal Concentration of zinc [42]. The authors suggested that bioaccumulation of zinc would probably impair the detoxification mechanism of renal tissue and cause histopathological alterations in it. Likewise, Rana *et al.* [43] noted various changes such as aggregation of inflammatory cells, dilation in capillary tubes of renal tubules and hemolysis in kidney of *C. carpio*, following exposure to chromium at sub-lethal concentrations.

4. Conclusion

In conclusion, severe histological alterations were observed in major target organs (liver, muscle and kidney) of *C. batrachus*, which could be attributed to the significant bioaccumulation of toxic heavy metals in these tissues beyond the prescribed limits of WHO/FAO. Hence, there is a substantial need to understand the metal accumulation and its toxicity of different metals in various fish species as it forms the major source of protein all over the world. There should be stringent laws and policies to check the discharge of industrial effluents in freshwater bodies so as to avoid the contamination of water resources.

5. Acknowledgement

The authors thank Professor and Head, Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab (India) for providing financial assistance and infrastructure to carry out the research work.

6. References

1. Vutukuru SS. Acute effects of hexavalent chromium on survival, oxygen consumption, haematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. Int J Environ Res Public Health. 2005; 2:456-462.
2. Dirilgen N. Accumulation of heavy metals in freshwater organisms: Assessment of toxic interactions. Turk J Chem. 2001; 25(3):173-179.
3. Uluturhan E, Kucuksezgin F. Heavy metal contaminants in Red Pandora (*Pagellusery thrinus*) tissues from the Eastern Aegean Sea, Turkey. Water Res. 2007; 41:1185-1192.
4. Waqar A. Levels of selected heavy metals in Tuna fish. Arab J Sci Eng. 2006; 31:39-92.
5. Kamble GB, Muley DV. Effect of acute exposure of endosulfan and chlorpyriphos on the biochemical composition of the freshwater fish, *Sarothero mossambicus*. Indian J Environ Sci. 2000; 4(1):97-102.
6. [Dinodia GS, Gupta RK, Jain KL. Effect of cadmium toxicity on liver glycogen in some freshwater fishes. Proc XI Nat Symp Environ. 2002; 11:236-38.
7. Azmat R, Talat R. Metal contamination in edible carnivorous fishes of Arabian Sea. J Appl Sci. 2006; 6:1974-1977.
8. Farombi EO, Adelowo OA, Ajimoko YR. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Catfish (*Clarias gariepinus*) from Nigeria Ogun River. Int J Environ Res Public Health. 2007; 4:158-165.
9. Patnaik BB, Howrelia JH, Mathews T, Selvanayagam M. Histopathology of gill, liver, muscle and brain of *Cyprinus carpio communis* L. exposed to sublethal concentration of lead and cadmium. Afr J Biotechnol. 2011; 10(57):12218-12223.
10. Järup L. Hazards of heavy metal contamination. British Medical Bulletin. 2003; 68(1):167-182.

11. Szentmihalyi K, Then M. Examination of microelements in medicinal plants of carpathion basin. Acta Aliment. 2007; 36:231-236.
12. Rashed MN. Monitoring of environmental heavy metals in fish from Nasser Lake. Environ Int. 2001; 27:27-33.
13. Yousafzai AM, Chivers DP, Khan AR, Ahmad I, Siraj M. Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem. Pak J Zool. 2010; 42(5):537-544.
14. Romeo M, Siau Y, Sidoumou Z, Gnassia-Barelli M. Heavy metal distribution in different fish species from the Mauritania coast. Sci. Total Environ. 1999; 232:169-175.
15. Pandey S, Parvez S, Ansari RA, Ali M, Kaur M, Hayat F *et al*. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctatus* Bloch. Chem Biol. Interact. 2008; 174:183-192.
16. Thophon S, Kruatrachue M, Upatham E, Pokethitiyook P, Sahaphong S, Jaritkhuan S. Histopathological alterations of white sea bass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environ Pollut. 2003; 121:307-320.
17. Gernhofer M, Pawet M, Schramm M, Muller E, Triebskorn R. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. J Aquat Ecosyst Stress Recovery. 2001; 8:241-260.
18. El-Naggar AM, Mahmoud SA, Tayel SI. Bioaccumulation of some heavy metals and histopathological alternations in liver of *Oreochromis niloticus* in relation to water quality at different localities along the river Nile, Egypt. World J Fish Marine Sci. 2009; 1(2):105-114.
19. Iqbal F, Qureshi IZ, Ali M. Histopathological changes in the kidney of common carp, *Cyprinus carpio* following nitrate exposure. J Res Sci. 2004; 15:411-418.
20. Kadry SM, Yacoub AM, Siliem TA, Mabrouk DB. Injuries in kidney tissue of the mugil fish *Liza ramada* as a biomarker for environmental pollution in the Lake Manzalah. Egypt J Aquat Biol Fish. 2003; 7(3):1-24.
21. Andreji J, Stránai I, Massányi P, Valent M. Accumulation of some metals in muscles of five fish species from Lower Nitra River. J Environ Sci. Health, Part A: Toxic/Hazardous Substances and Environmental Engineering. 2006; 41(11):2607-2622.
22. Soegianto Hamami A. Trace metal concentrations in shrimp and fish collected from Gresik coastal waters, Indonesia. Sci Asia. 2007; 33:235-238.
23. Ashoka S, Peake BM, Bremner G, Hageman KJ. Distribution of trace metals in a ling (*Genypterus blacodes*) fish fillet. Food Chem. 2011; 125(2):402-409.
24. Humason GL. Animal tissues techniques 3rd edn. W.H Freeman and Company, San Francisco, CA, USA, 1972.
25. Stentiford GD, Longshan M, Lyons BP, Jones G, Green M, Feist SW. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar Environ Res. 2003; 55:137-159.
26. Stehr CM, Myers MS, Johnson LL, Spencer S, Stein JE. Toxicopathic liver lesions in English sole and chemical contaminant exposure in Vancouver Harbour, Canada. Mar Environ Res. 2004; 57:55-74.
27. Mahmoud SA, Abd El-Rahman AA. Eco-toxicological studies of water and their effect on fish in El Manzalah Lake. Res J Pharmaceutical, Biological and Chemical Sciences. 2017; 8(2):2497-2511.
28. Tayel SI, Ahmed NAM, EL-Hossiny MA. Impact of diffused pollution on histological and hematological properties of *Mugil cephalus* and *Mugil capito* collected from lake Manzalah, Egypt. Int J Environ Sci Eng. 2014; 5:51-67.
29. Rabitto IS, Costa JRMA, Randi MAF, Akaishi FM, Pelletier E, Oliveira Ribeiro CA. Dietary Pb(II) and TBT (tributyltin) exposures to neotropical fish *Hoplias malabaricus*: Histopathological and biochemical findings. Ecotoxicol Environ Safety. 2005; 60:147-156.
30. Manahan SE. Water Pollution Environment Chemistry, first ed. Lewis Publishers, London, 1991
31. Begum SA, Banu Q, Andhoque B. Effect of cadmium, chromium and mercury on the liver histology of *Clarias batrachus* L. The Chittagong Univ JB Sci. 2009; 4(1 &2):63-72.
32. Heath AG. Water pollution and fish physiology, first ed. Lewis Publishers, FL, 1995.
33. Aly SM, Zaki MS, El-Genaidy HM. Pathological, biochemical, haematological and hormonal changes in catfish (*Clarias gariepinus*) exposed to lead pollution. J Egypt Vet Med Assoc. 2003; 1:331-342.
34. Chavan VR, Muley DV. Effect of heavy metals on liver and gill of fish *Cirrhina mrigala*. Int J Curr Microbiol Appl Sci. 2014; 3(5):277-288.
35. Dhevakrishnan R, Zaman GMHU. Cauvery river pollutants induced histopathological changes in kidney and muscle tissues of freshwater fish, *Labeo rohita* (Hamilton 1822). Online Int Interdiscip Res J, 2012; 2(5):9-19.
36. Ibrahim SA, Authman MMN, Gaber HS, El-Kasheif MA. Bioaccumulation of heavy metals and their histopathological impact on muscles of *Clarias gariepinus* from El-Rahawy drain, Egypt. Int J Environ Sci Eng. 2013; 4:57-73.
37. Fatma ASM. Histopathological Studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World J Fish Marine Sci. 2009; 1(1):29-39.
38. Abbas H, Ali F. Study the effect of hexavalent chromium on some biochemical, cytological and histopathological aspects of *Oreochromis* spp. fish. Pak J Biol Sci. 2007; 10:3973-3982.
39. Mabrouk DR. Aspects of Manzalah Lake pollution on *Mugil* species. M. Sc. Thesis, Faculty of Girls, Ain Shams University, Egypt, 2004, 293.
40. Silva AG, Martinez CBR. Morphological changes in the kidney of a fish living in an urban stream. Environ Toxicol Pharmacol. 2007; 23(2):185-192.
41. Shah A, Kothari S, Parihar MS. Cadmium induced histological damage oxidative stress and antioxidative responses in kidney of freshwater catfish *H. fossilis* (Bl.). Int J Eng Technol Sci Res. 2017; 4(12):469-475.
42. Gupta P, Srivastava N. Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish *Channa punctatus* (Bloch). J Environ Biol. 2006; 27(2):211-215.
43. Rana MA, Jabeen F, Shabbir S, Naureen A, Sultana K, Ahmad I *et al*. Histopathological study of liver and kidney in common carp (*Cyprinus carpio*) exposed to different doses of potassium dichromate. Int J Biosci. 2015; 6(12):108-116.