OPEN ACCESS

International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN 2091-2609

Available online at:

http://www.ijasbt.org

&

http://www.nepjol.info/index.php/IJASBT/index

Indexing and Abstracting

CrossRef, Google Scholar, Global Impact Factor, Genamics, Index Copernicus, Directory of Open Access Journals, WorldCat, Electronic Journals Library (EZB), Universitätsbibliothek Leipzig, Hamburg University, UTS (University of Technology, Sydney): Library, International Society of Universal Research in Sciences (EyeSource), Journal Seeker, WZB, Socolar, BioRes, Indian Science, Jadoun Science, Jour-Informatics, Journal Directory, JournalTOCs, Academic Journals Database, Journal Quality Evaluation Report, PDOAJ, Science Central, Journal Impact Factor, NewJour, Open Science Directory, Directory of Research Journals Indexing, Open Access Library, International Impact Factor Services, SciSeek, Cabell's Directories, Scientific Indexing Services, CiteFactor, UniSA Library, InfoBase Index, Infomine, Getinfo, Open Academic Journals Index, HINARI, etc.

CODEN (Chemical Abstract Services, USA): IJASKD

Vol-2(2) June, 2014

IC Value: **4.37**

SEM-Biotech Publishing

For any type of query and/or feedback don't hesitate to email us at: editor.ijasbt@gmail.com

G Bhuyan et al. (2014) Int J Appl Sci Biotechnol, Vol 2(2): 135-141
DOI: 10.3126/ijasbt.v2i2.10141

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

QUALITATIVE AND QUANTITATIVE ANALYSIS OF FISH TISSUE OF *OREOCHROMIS MOSSAMBICUS* COLLECTED FROM KEDILAM RIVER, CUDDALORE, TAMILNADU, INDIA

Gyaneswar Bhuyan, R. Anandhan* and V. Kavitha
Department of Zoology, Annamalai University, Annamalai Nagar

*Corresponding author email: raman_anandhan@yahoo.co.in

Abstract

Qualitative and quantitative histological alteration was taken to analyze in fish *Oreochromis mossambicus* collected from Kedilam River at three stations, which receive mostly industrial effluent and municipal runoff. Histoarchitecture of tissue alteration and percentage of prevalence was used as protocol for analysis. Results showed that number of histological alteration observed in gill like structural alteration of epithelium, epithelial lifting, fusion of secondary lamellae and hyperplasia. In liver blood congestion, regressive changes like degeneration of hepatocytes, vacuolation, and necrosis observed where as in kidney it shows glomerular congestion, tubular degeneration, progressive changes like hypertrophied epithelial cells, haemorrhage in bowman's space. Among the three stations severe histological alteration and percentage prevalence was observed in order of station 1> station 2 >station 3. Highest histological alteration and percentage of prevalence in three organs of fish *o. mossambicus* are kidney >gill >liver. The major reason for this histological alteration and contamination of fish is mostly due to industrial effluent. Repeated and continuous monitoring is must needed to protect the aquatic organism.

Key Words: Fish; Histopathology; Industrial Pollution; Kedilam River.

Introduction

The aquatic ecosystem has been contaminated since last two decade and the major reasons of this situation are industrial, agricultural and municipal effluents produced by human activities (Paul and Meyer, 2001). Due to this the aquatic organism are now affected severely, to determine the toxicity it is inevitable to know the aquatic status of organism where the pollutants been highly accumulated.

Fish is highly nutritious and major alternatives during this scarcity of food. Fish has been proved a successful tool for biomonitoring of aquatic pollution as it concentrates pollutants in their tissue directly from the water and food (Fisk *et al*., 2001; Boon *et al*., 2002). Histological studies in laboratory and field experiment are immensely practiced by eminent scientists, to know the degree of effect of various pollutants, on vital organ of fish tissue (Schwaiger *et al.*, 1997; Au, 2004; Yasser and Nasser, 2011).

In field studies fishes are exposed to various organic and inorganic pollutants where as in laboratory practice it is limited, but we cannot deny the significance of laboratory work, field studies confirm the actual status of aquatic organism which can explore the aquatic status of organism in relation to manmade and anthropogenic pollution. Histopathology is often the convenient method of accessory both short and long term effects (Hinton and Lawren, 1990; Teh *et al.*, 1997). Histocytological response in different organs of vertebrate and invertebrates has been observed a useful bioindicator (Geonhofer *et al.*, 2001; Vasanthi *et al.*, 2013). Furthermore, histopathological studies reflect the present health of fish by examine the specific target organ including gill, liver, kidney, intestine which are responsible for vital function like respiration, metabolism, excretion, and absorption of organism (Au, 1999; Camergo and Martinez, 2007; Vasanthi *et al.*, 2013). Histological changes like epithelial lifting of lamellae, mucous secretion, and hyperplasia are the early signal of contamination and the cellular level adaptation for short period of time due to wide variety of stress (Padmini and Usha rani, 2010). Randall and Tusi (2002) reported that exposure of fish to ammonia are associated with histopathological changes in gills and liver, whereas Muller et al (1991) observed that increase in ammonia and low pH induced gill damage in juveniles of *Salvelinus fontinalis*. Repeated and continuous exposure of pollutants in rivers and lakes can be enhancing the morphological and cellular changes which lead to mortality of fish.

Usha Damodhar (2012) observed the heavy metals on water whereas Bhuyan *et al.* (2013) showed that water had increase in turbidity, biological oxygen demand (BOD), chemical oxygen demand (COD). These feature indicated the pollution with organic and inorganic wastes. The main objective of the study is to characterize the histopathological effects of pollutants on gill, liver, and kidney of *Oreochromis mossambicus* collected at three different stations of Kedilam River. Due to availability, adaptability nature and commerciality purpose *O. mossambicus* was taken for this study (FAO). This is the first time comprehensive work on histopathology in Kedilam River. The results will provide a tool for biomonitoring of aquatic status and environmental health in natural aquatic ecosystem.

Materials and Methods

Collection of Sample

Live specimen of fish *Oreochromis mossambicus* and water was collected from the Kedilam River at three different stations (S1, S2, and S3). After collection of water by plastic bottles, samples were send to laboratory for analysis of physicochemical parameters, followed by Trivedy and Goel method (1986) and analyzed against standard WHO (1992) and IS (1993). Fishes were collected at three stations (S1, S2, and S3) irrespective of their sex from Kedilam River by the help of fisherman. For fish histological analysis purpose, immediately after being caught each fish was scarified and dissected out the vital organs (Gill, Liver and Kidney) and were fixed in 10% formalin on the spot.

After 24 hours the fixed tissues were taken for histological technique followed by Gurr (1958). For histological analysis section were cut at 5-6µm thickness and stained with Haematoxylin and Eosin (H&E). After stained the slides were examined under light microscope and photographed (Labomed).

Histological assessment

A qualitative histological assessment was done to identify histological alterations in the selected target organs (gill, liver and kidney) of fish *Oreochromis mossambicus* collected from Kedilam River. These results were assessed and analysed using a protocol developed by Takashima and Hibiya, 1995 and Bernet *et al.*, (1999). Each organ was assessed in terms of five reaction patterns including circulatory disturbances, regressive changes, progressive changes, inflammatory responses, and neoplasm.

Result and Discussion

Water quality analysis

Water quality parameters are determined at three stations are presented in Table 1. The result showed that parameters like conductivity and ammonia are above the permissible limit prescribed by WHO (1992) and BIS (1993) standard. Among the three stations S1 show more variation as compared to station S2 and S3.

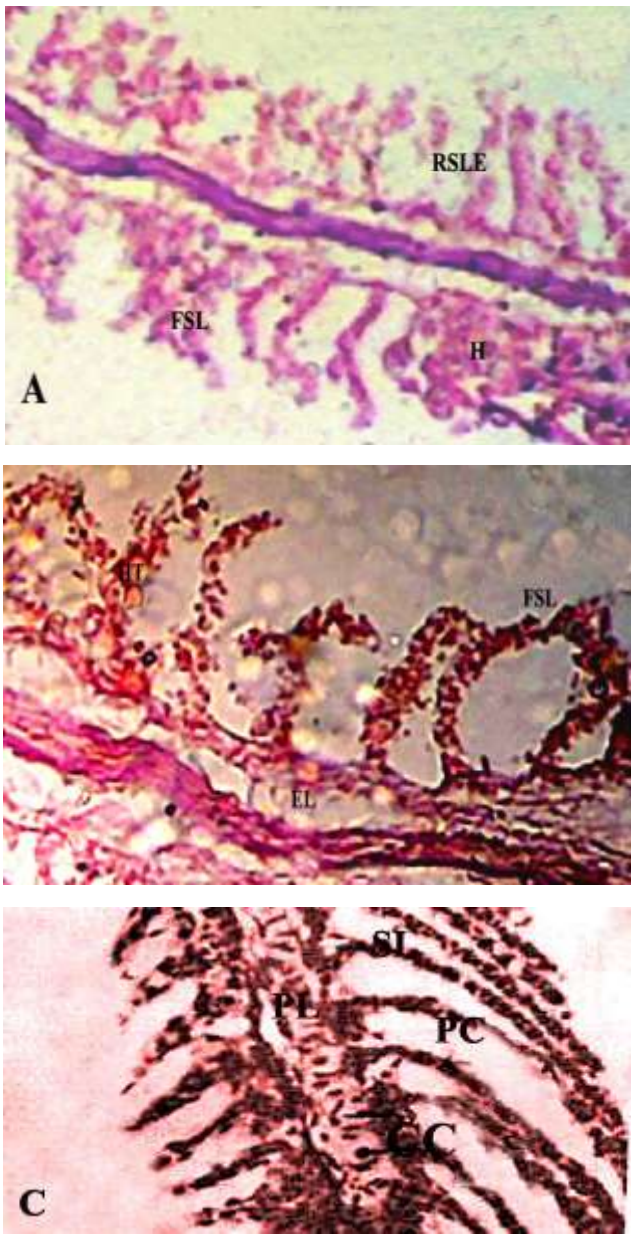

Fig. 1: (A-C) Photo microscope of gill O.mossambicus collected from station 1; 1.1(A) showing fusion of secondary lamellae (FSL), rupture of secondary lamellar epithelium (RSLE), hyperplasia (H). Gill histopathology at station 2: 1.1(B) severe epithelial lifting (EL), hypertrophy (HT), fusion of secondary lamellae (FSL). Gill histopathology at station 3; 1.1(C) primary lamellae (PL), secondary lamellae (SL), pillar cell (PC), chloride cell (CC) observed. Magnification 40X, H&E stain

Histological analysis

Details of fish tissue (gill, liver and kidney) histological alteration with percentage of prevalence are mentioned in Table 2. Among three stations highest to lowest alteration observed in order of station1 > station 2 > station 3.

Table 1: Water Quality at three stations (S1, S2, S3) of Kedilam River, Cuddalore, Tamilnadu

Parameters	S 1	S 2	S 3	WHO/BIS*
pH	6.6± 0.5	7.4 ± 0.8	7.5 ± 0.7	6.5-8.5
Temperature (°C)	27.5 ± 1.0	29.8± 0.5	28.4± 0.4	40*
Conductivity (µmhos/cm)	345.6± 2.6	310.45± 1.8	321.6±2.9	300
Dissolved oxygen (mg/l)	4.5±0.3	5.7±0.7	6.25±0.6	5.0/3.0*
Total hardness (ppm)	3520±5.8	3670±6.2	3450±4.7	-----
Salinity (ppt)	24±2.9	20±1.7	15±1.4	120/200*
Ammonia (ppm)	3.2±0.4	2.0±0.35	1.3±0.2	0.3/0.5*

#Mean±SD, All samples were analyzed in triplicates.

Table 2: Histological alteration observed in fish *O. mossambicus* at three stations (S1, S2 and S3) collected from Kedilam River, with frequency and percentage of changes

Organs	**Reaction pattern**	**Histological alteration observed**	**Station 1**		**Station 2**		**Station 3**	
			Frequency (n=12)	**Percentage**	**Frequency (n= 12)**	**Percentage**	**Frequency (n= 12)**	**Percentage**
Gill	Circulatory disturbance	Haemorrhage	7	58	6	50	5	42
		Epithelial lifting	8	67	5	42	5	42
	Regressive change	Fusion of secondary lamellae	9	75	7	58	6	50
		Structural alteration of epithelium	10	**83**	8	67	5	42
		Rupturing of secondary lamellae epithelium	9	75	8	67	7	58
	Progressive change	Hyperplasia	8	67	5	42	4	33
	Inflammation	Not observed	0	0	0	0	0	0
	Tumour	Not observed	0	0	0	0	0	0
Liver	Circulatory disturbance	Blood congestion	5	42	4	33	3	25
	Regressive change	Structural alteration	8	67	7	58	5	42
		Degeneration of Hepatocytes	7	58	6	50	5	42
		Irregular shaped nucleus	5	42	4	33	3	25
		Vacuolation	5	42	5	42	4	33
		Necrosis	5	42	4	33	2	17
	Progressive change	Not observed	0	0	0	0	0	0
	Inflammation	Not observed	0	0	0	0	0	0
	Tumour	Not observed	0	0	0	0	0	0

Table 2: Histological alteration observed in fish *O. mossambicus* at three stations (S1, S2 and S3) collected from Kedilam River, with frequency and percentage of changes (Cond.)

Organs	Reaction pattern	Histological alteration observed	Station 1		Station 2		Station 3	
			Frequency (n=12)	Percentage	Frequency (n= 12)	Percentage	Frequency (n= 12)	Percentage
Kidney	Circulatory disturbance	Glomerular congestion	9	75	6	50	4	33
	Regressive change	Narrowing of tubular lumen	8	67	8	67	7	58
		Tubular degeneration	9	75	7	58	7	58
	Progressive change	Hypertrophy of epithelial cells (Tubules)	8	67	7	58	5	42
		Hemorrhage in bowman's space	7	58	6	50	4	33
	Inflammation	Not observed	0		0		0	
	Tumour	Not observed	0		0		0	

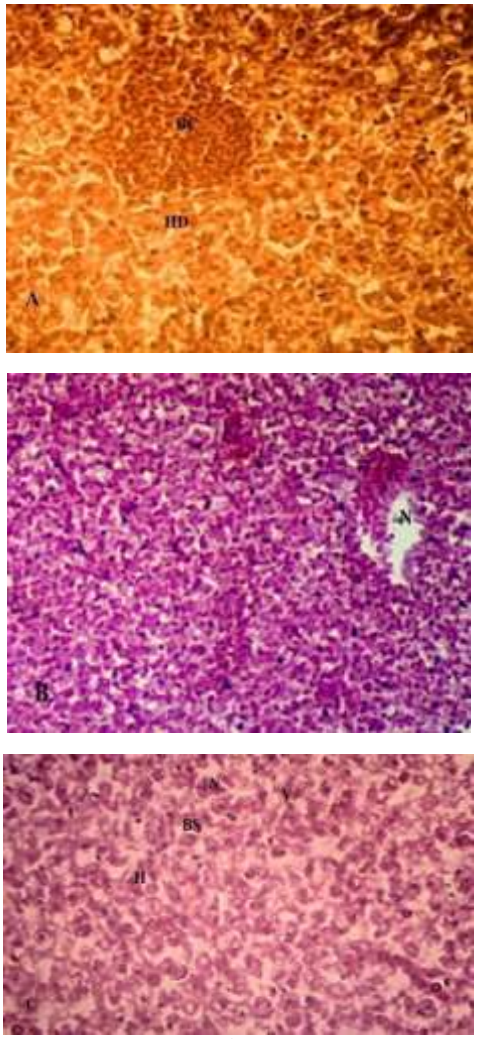

Fig. 1.2 (A-C) Photo microscope of liver *O. mossambicus* collected from station 1; (A) Blood congestion (BC), hepatocyte degeneration (HD) observed. Liver histopathology at station 2; (B) Necrosis (N). Liver histopathology at station 3; (C) showing clear structure of Nucleus (N), hepatocyte (H), vacuole (V) and blood sinusoid (BS) are observed. Magnification 40X, H&E stain

Gill is one of the good indicators of water quality (Rankin *et al.*, 1982). In present investigation gill shows severe histological lesions (Fig 1and Table 1), nearly 83% of fishes show the structural alteration of epithelium lesion at station 1, whereas 67% and 42% observed at station 2 and 3 respectively. Epithelial lifting (67% at station1) and fusion of secondary lamellae (75% at station 1) also observed, the reason for epithelial lifting alteration is due to potential difference of sodium and potassium ion activated ATPase or may be irregulation of sodium and chloride ions (Neiboer and Richardson, 1980; Evans, 1987) and fusion of secondary lamellae decrease in free gaseous exchange, which could be a barrier for normal growth of fish (Skidmore and Tovell, 1972). In addition due to Lamellar cell hypertrophy or fusion decrease the space between and increase the thickness of water-blood barrier which reduce oxygen intake capacity leads to haemorrhage (Novak, 1992; Jiraung *et al.*, 2002). At station1 progressive changes like hyperplasia (67%) observed which indicate the activity of cells or tissue in an organ due to severe stress on fishes. Hinton and Lauren (1990) reported that hyperplasia of epithelial cells are not specific alteration and it may be due to various pollutants such as heavy metals (Randi *et al.*, 1996; Abdel-Moenion *et al.*, 2012; Vasanthi *et al.*, 2013). Present results support the previously studied by researchers regarding water quality and presence of heavy metals in water and fish of Kedilam river (Damodaran and Readdy 2012; Muniyan and Ambedkar 2012; Bhuyan and Anandhan 2013).

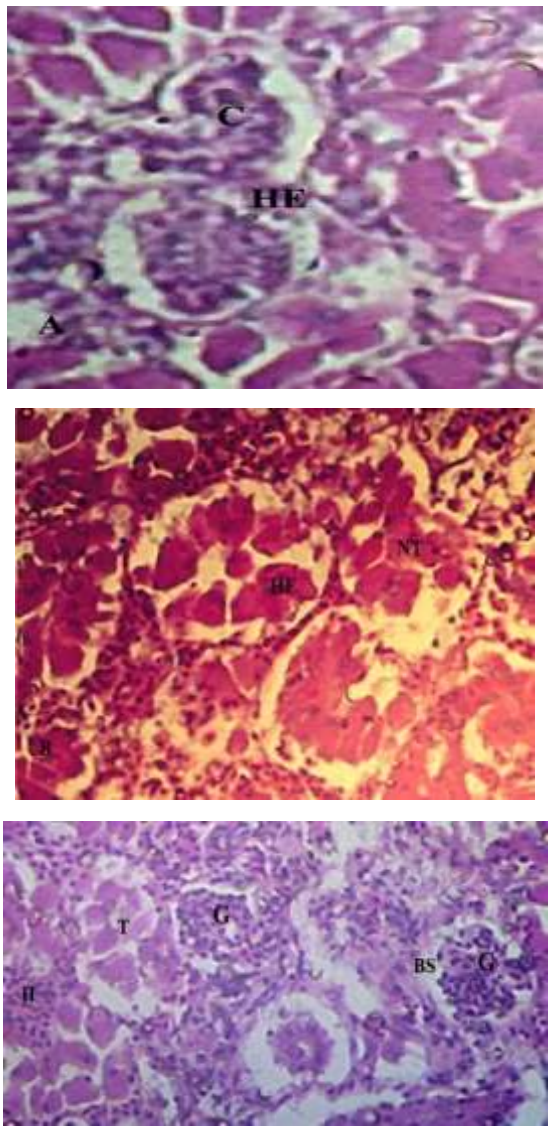

Fig. 3: (A-C) Photo microscope of Kidney *O.mossambicus* collected from station 1; (A) showing Glomerular congestion (C), Haemorrhage in Bowman's space (HE). Kidney histopathology at station 2; (B) Hypertrophied epithelial (HE) cell in tubules, Narrowing of tubular lumen (NT). Kidney histopathology at station 3; (C) Clear view of Glomerulus (G), Tubules (T), Haematopoietic cell (H), Bowman's space (BS). . Magnification 40X, H&E stain.

According to Hinton and Lauren (1990), the liver is a detoxification organ and is an essential for both the metabolism and the excretion of toxic substances in the body. Brusle and Gonza (1996) state that fish liver histology could serve as a model for studying the interactions between environmental factors and hepatic structures and function. During present study liver shows number and regressive changes like structural alteration of liver tissue (67%) is highest at station 1 in comparison to station 2 and 3 respectively but, Liver necrosis progressive changes, inflammation and neoplasm not observed in liver tissues of fish *O. mossambicus* (Fig 2). Among three station highest to lowest histological alteration observed in order of station1 (49%)>station 2(42%)>station 3(31%). Present results indicate, mode of contamination is due to polluted water other than food.

The kidney one of the vital organ of body and its primary function is osmotic regulation of water, salt, ions, which helps in maintaining volume and pH of blood and body fluids and erythropoieses (Iqbal *et al*, 2004). The kidney of the fish receives largest proportion of postbranchial blood, and its interregnal alteration is one of the good indicators of environmental pollution (Ortiz *et al*., 2003). During present investigation kidney of fish shows glomerular congestion, tubular degeneration, hypertrophy of epithelial cells (Tubules), haemorrhage in bowman's space (Fig. 3). Nearly 75% fish at station1 show glomerular congestion and tubular degeneration is might be due to presence of xenobiotics compound in tissue change the membrane potential and ion exchange, which increase the permeability of cells and also increase the cell shape, lastly rupture of membrane (Takshima and Hibiya, 1995; Silva and Martinez, 2007; Pal *et al.*, 2011). Hypertrophy of epithelial cells also observed 67% at station 1 which might be due to hyperactivity of interrenal cells by chronic stimulation of the HPI (hypothalamus-pituitary-interrenal cell) axis (Silva and Martinez 2007).

The level of alteration in fish tissue during present study are in order of kidney>gill>liver. After kidney and gill, liver is affected that means mode of contamination is water then food. Present result reported that Kedilam River is highly polluted, due to drainage of contaminated industrial effluent and municipal wastes directly to river. Furthermore a level of ammonia is above the permissible level by WHO at all the stations is one of reason for histological alteration (Devraj *et al.*, 2014). Among the three station the order of alteration are station 1>station 2>station 3. Station 1 is nearly industrial area which receives more contaminated water and cause for these histological lesions. There is decrease in trend of histological alteration reason might be the distance, which reduce the effect of pollutants by dilution and sedimentation process (Fig 1).

Conclusion

Present investigation observes that water of Kedilam River is polluted especially higher concentration of ammonia and conductivity which indicate that water is not suitable for fisheries and aquatic health. Histological alteration shows the adaptive and defensive character of fish against toxicants especially contaminated industrial effluent. It concludes that fishes are contaminated mostly by polluted water rather than food.

Acknowledgements

The authors are thankful to University Grants Commission (UGC), New Delhi, India for financial assistance through UGC Major Research Project (Project file no: 40-365/2011) and Dr. M. Sabesan, Professor Department of Zoology for the encouragement.

Reference

Abdel-Moneim AM, Al-Kahtani MA and Elmenshawy OM (2012) Histopathological biomarkers in gills and liver of Oreochromis niloticus from polluted wetland

environment, Saudi Arabia. *Chemosphere*. **88**: 1028-1035. DOI: 10.1016/j.chemosphere.2012.04.001

Ambedkar G and Muniyan M (2012) Analysis of Heavy metals in Water, Sediments and Selected Freshwater fish Collected from Gadilam River, Tamilnadu, India. *International Journal of Toxicology and Applied Pharmacology*. **2 (2)**: 25-30.

Au DWT (2004) The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. *Mar. Pollut. Bull*. **48**: 817-834. DOI: 10.1016/j.marpolbul.2004.02.032

Au DWT, Wu RSS, Zhou BS and Lam PKS (1999) Relationship between ultrastructural changes and EROD activities in liver of fish exposed to Benzol[a] pyrene. *Environ. Pollut.* **104(2)**: 235-247. DOI: 10.1016/S0269-7491(98)00176-6

Bernet D, Schmidt H, Meier W, Burkhardt-Holm P and Wahli T (1999) Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *J. Fish. Disease*. **22**:25-34. DOI: 10.1046/j.1365-2761.1999.00134.x

Bhuyan G and Anandhan R (2013) Bioaccumulation of Metals (Zn, Ni, Cr, Cd and Pb) in Tissue of Freshwater Teleost *Oreochromis mossambicus* (Tilapia) From Kedilam River, India. *Pollution Research*. **32 (4)**: 103-107.

Bhuyan G, Anandhan R and Kavitha V (2013) Seasonal variation of physico-chemical parameters in Kedilam River, Tamilnadu, India. International Journal of Scientific Research. **2:** 1-3.

Boon JP, Lewis WE, Choy MR, Allchin CR, Law RJ and De Boer J (2002) Levels of polybrominated diphenyl ether (PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. *Environ. Sci. Technol*. **36**: 4025-4032. DOI: 10.1021/es0158298

Camergo MMP and Martinez CBR (2007) Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology*. **5(3)**: 327-336. DOI: 10.1590/S1679-62252007000300013

Damodhar U and Reddy MV (2012) Assessment of trace metal pollution of water and sediment of River Gadilam (Cuddalore, South East Coast of India) receiving sugar industry effluents. *Continental J. Environmental Sciences*. **6 (3)**: 8 – 24.

Devaraj S, Arulprakasam C, Kandhan AP, Neelamegam K and Kalaiselvan R (2014) Toxicological effects of ammonia on gills of Cyprinus carpio var. communis (Linn.). *Journal of Coastal Life Medicine.* **2(2)**: 94-98.

Evans DH (1987) The fish Gill: Site of Action and Model for Toxic Effects of Environmental Pollutants. *Environmental Health Perspectives.* **71**: 47-58. DOI: 10.1289/ehp.877147

Fisk AT, Hobson KA and Norstrom RJ (2001) Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. *Environ. Sci. Technol*. **35**: 732- 738. DOI: 10.1021/es001459w

Goenhofer M, Pawet M, Schramm M, Muller and Triebskorn E (2001) Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *Journal of Aquatic Ecosystem, Stress and Recover*. **8**: 241-260. DOI: 10.1023/A:012958804442

Gurr E (1958) Method of Analytical Histology and Histochemistry. Leonard Hill Books Ltd, London.

Hinton DE and Lauren DJ (1990) Integrative histopathological approaches to detecting effects of environmental stressors of fishes. *Am. FishSoc. Sym.* **8**: 51-66.

Indian Standard Specification for Drinking Water; IS: 10500: 1992. (Reaffirmed 1993)

Iqbal F, Qureshi IZ and Ali M (2004) Histopathological changes in the kidney of common carp, *Cyprinus carpio* following nitrate exposure. *J. Res. Sci*.**15**: 411-418.

Jiraung KW, Upathama ES, Kruatrachuea M, Sahaphonge S, Vichasrigramsa S and Pokethitiyooka P (2002) Histopathological effects of round up, a glyphosate herbicide, on Nile tilapia (Oreochromis niloticus). *Sci. Asia*. **28**: 121-127. DOI: 10.2306/scienceasia1513-1874.2002.28.121

Mueller ME, Sanchez DA, Bergman HL, McDonald DG, Rhem RG and Wood CM (1991) Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. gill histology. *Can J Fish Aquat Sci*. **48**: 2016-2027. DOI: 10.1139/f91-240

Neiber E and Richardson DHS (1980) The replacement of nondescript term 'heavy metals' by a biologically and chemically significant classification of metals ion. *Environ. Poll*. **1**: 3-26. DOI: 10.1016/0143-148X(80)90017-8

Novak B (1992). HIstopathological changes in gills induced by residue of endosulfan. *Aqua. Toxicl*. **23**: 65-84. DOI: 10.1016/0166-445X(92)90012-C

Ortiz JB, Gonzalez de Canales ML and Sarasquete C (2003) Histopathological changes induced by lindane (γ-HCH) in various organs of fish. *Sci. Mar*. **67**: 53-61.

Padmini E and Usha Rani M (2010) Thioredoxin and HSP 90a modulate ASK1-JNK1/2 signalling in stressed hepatocytes of Mugil cephalus. *Comp. Biochem. Physiol Part C*. **151:** 187–193.

Pal S, Kokushi E, Cheikyula JO, Koyama J and Uno S (2011) Histopathological effects and EROD induction in common carp exposed to dietary heavy oil. *Ecotoxicology and Environmental Safety*. **74**: 307-314. DOI: 10.1016/j.ecoenv.2011.01.003

Paul MJ and Meyer JL (2001) Streams in the urban landscape. *Annual Review of Ecology and Systematic*. **32**: 333-365. DOI: 10.1146/annurev.ecolsys.32.081501.114040

Randall DJ and Tsui TK (2002) Ammonia toxicity in fish. *Mar Pollut Bull*. **45**: 17-23. DOI: 10.1016/S0025-326X(02)00227-8

Randi AS, Monserrat JM, Rodriquez EM and Romano LA (1996). Histopathological effects of cadmium on the gills of the freshwater fish Macropsobrycon uruguayanae Eigenmann

(Pisces, Atherinidae). *J. Fish. Dis*. **19**: 311-322. DOI: 10.1046/j.1365-2761.1996.d01-82.x

Rankin JC, Stagg RM and Bolis L (1982), Effects of pollutants on gills. In-Gills. Cambridge University Press. pp. 207-220.

Schwaiger J, Wanke R, Adam S, Pawert M, Honnen W and Triebskorn, R (1997) The use of histopathological indictors to evaluate contaminant- releted stress in fish. *J. Aquat. Ecosys. Recov*. **6**: 75-86. DOI: 10.1023/A:1008212000208

Silva AG and Martinez CBR (2007) Morphological changes in the kidney of fish living in an urban stream. *Environmental Toxicology and Pharmacology.* **23**: 185-192. DOI: 10.1016/j.etap.2006.08.009

Skidmore JF, Tovell PWA (1972) Toxic effect of zinc sulphate on the gill of rainbow trout. *Water Res*. **6**: 217-230. DOI: 10.1016/0043-1354(72)90001-2

Takashima F and Hibiya T (1995) An atlas of fish Histology. Normal and pathological features, SecondEdition Kodansha ltd., Tokyo, Japan.

Teh SJ and Adams SM Hinton DE (1997) Histopathological biomarkers in feral freshwater fish population exposed to different types of contaminant stress. *Aquatic Toxicology*. **37**: 51-70.DOI: 10.1016/S0166-445X(96)00808-9

Trivedy RK and Goel PK (1986) Chemical and Biological methods for water pollution studies (R. K. Trivedy and P. K. Goel Ed.) Environmental Publications, karad, India.

Vasanthi LA,Revathi P, Mini J and Munuswamy N (2013) Integrated use of histological and ultrastructural biomarkers in *Mugil cephalus* for assessing heavy metal pollution in Ennore Estuary. *Chemosphere.* **91**: 1156-1164. DOI: 10.1016/j.chemosphere.2013.01.021

World Health Organization (WHO). (1992). International standards for drinking water. Geneva.

Yasser A and Naser MD (2011) Impact of pollutants on fish collected from different parts of Shatt Al-Arab River: a histopathological study. *Environ Monit Assess.* **181**: 175-182. DOI: 10.1007/s10661-010-1822-8