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Effect of profenophos on the histology of freshwater climbing perch *Anabas testudineus*

Kanagavalli V[1], K Muthukumaravel[2*], O Sathick[2], N Vasanthi[2]
[1] Department of Zoology, A.V.V.M Sri Pushpam College, Poondi, Tamil Nadu, India
[2] Department of Zoology, Khadir Mohideen College, Adirampattinam, Tamil Nadu, India (Affiliated to Bharathidasan University, Tiruchirappalli), Tamil Nadu, India
*Correspondence Author: K Muthukumaravel

Abstract
Acute toxicity (96 h LC_{50} - 0.5 ppm) of profenophos was evaluated in the freshwater fish *Anabas testudineus* fingerlings in static bioassay over a 96 –h exposure period using probit method. Histopathological investigations revealed various degrees of pathological lesions in intestine, muscle, liver and kidney. An apparent aberration in the histological architectures of the tissues has been observed in the vital organs of *Anabas testudineus* after 28 days of treatment with profenophos. Sloughing of mucosal lining, hypertrophy of intestinal epithelium were documented in the intestinal tissue. In the muscle tissues splitting of muscular fibres, vacuolar degeneration and atrophy of muscle bundles were conspicuous. Disintegration of hepatocyte cells boundaries, dilation of blood sinusoids, vacuolation and necrosis of hepatocytes were registered in the liver tissue of profenophos treated fish. Dilation of glomerular capillaries, shrinkage of glomerulus and hypertrophy of renal tubules were perceived in the kidney tissue of profenophos treated *Anabas testudineus.* The histological alterations in the several tissues of fish could be used as an important tool for assessment of pesticide pollution.

Keywords: *Anabas testudineus*, acute toxicity, profenophos, histology

Introduction
Agrochemicals namely insecticide, fungicide, herbicide, etc., have offered tremendous benefits to mankind by increasing the production of food and also by controlling the vector of many diseases of man and his animals. But on the other side the use of these agrochemicals affected the health of aquatic organisms as it has directly posed a potential health hazards to the life of fishes and indirectly to human though consumption of fishes (Osman *et al*., 2011) [21] Agrochemicals are the major cause of concern for the aquatic environment due to their toxicity, persistence and the tendency towards their accumulating ability in the organisms (Joseph *et al.*, 2010) [12]. The impacts of these toxicant on aquatic organisms are due to their mobility from various pint or sources and are posing a great threat to aquatic fauna, particularly to fishes which constitute one of the major sources of protein-rich food for human (Ray *et al*., 2015) [26]. Hence the present study was carried out to evaluate the profenophos toxicity in the Indian major carp *Anabas testudineus*.

Materials and Methods
Healthy fresh water air breathing fish, *Anabas testudineus* fingerlings (6 - 7 cm length 4.5 - 5 g weight) were collected from MNR Aqua Farm, Orathanadu, Thanjavur district. The fishes, brought to the laboratory, were introduced in the glass tank containing aerated well water. The fishes were acclimated to the laboratory conditions (Temperature 28 ± 1^0C; total hardness 375 ± 12 mgs/litre as $CaCo_3$; salinity 0.8 ± 0.15 ppt and pH 7.4 ± 0.05).
Toxicity tests were conducted in accordance to the standard methods (APHA, 1992) [5]. The fish were starved for 24 hours prior to their utilization in the experiments as recommended by storage to avoid any interference in the toxicity of profenophos. After the addition of the toxicant into the test tank with 10 liters of water having twenty fish, mortality of the fish was recorded after 24, 48, 72 and 96 hours. Simultaneously five replicates were maintained. Per cent mortality was calculated and the values were fitted into probit scale. (Finney. 1971) [9]. Based on the acute toxicity test (96h LC_{50} - 0.5 ppm) sub lethal concentrations (10% and 30%) of profenophos were prepared and were used as the experimental concentration of the profenophos in the subsequent experiments.
After 28 days of subacute toxicity profenophos exposed fish *A. testudineus* was dissected. The target organs of the present study *viz*., intestine, muscle, liver and kidney were isolated and fixed in formal alcohol. After 24 hours of fixation, the tissues were passed through an ascending series of alcohol for dehydration and then cleared in xylene. The paraffin embedded wax block was sectioned at a thickness of 8 µm with the help of a rotary microtome. The sections were stained with Harri's hematoxylin and then counterstained with 70 % alcoholic eosin (Humason, 1967) [10]. After dehydration the tissue sections on the glass slide was mounted with cover slide using DPX mountant.

Result and Discussion
Histology of intestine in the control fish *Anabas testudineus*
The histological structure of the intestinal wall of *A.testudineus* comprised of four layers namey mucosa, submucosa, muscularis and serosa (Plate – 1 Fig. a) the mucosa was evaginated into a prominent finger like projections called intestinal villi. The mucosa composed of columnar epithelium having absorptive mucosa secreting cells or goblet cells. The submucosa was made up of loose connective tissue. The submucosa was vascularized and

extended into the villi as lamina propria thus forming the core of the villi. The muscularis was formed of the inner layer of circular and outer layer of longitudinal muscle fibres. Serosa, the outermost thin layer was formed of flattened epithelial cells (Plate – 1 Fig. a).

Histopathology of the intestine of 10% sub lethal profenophos treated *A.testudineus*

In the low sub lethal concentrated Profenophos treated fish, the intestinal histopathology showed marked variations. Degenerative changes were noticed in the mucosal lining and intestinal villi. Flattening of intestinal villi and sloughing off of the mucosal lining were observed. Hypertrophy of the epithelial cells and oedema of lamina propria were recognized. Hypertrophy of the intestinal columnar epithelium resulted in fusion of the villi and then ultimately led to the rupture of villi at their tips (Plate – 1 Fig. b).

Histopathology of the intestine of 30% sub lethal profenophos treated *A.testudineus*

The histopathology of 30% sublethal concentration of Profenophos treated fish showed well pronounced degenerative and necrotic changes in the intestinal mucosa and submucosa. The necrotized cells were accumulated in the intestinal lumen. Hemorrhage in the submucosa and amassing of inflammatory cells into the mucosa and submucosa resulted in edema among them. Dilation of blood vessels caused rupture of serosa layer. Vacuolation of circular and longitudinal muscle layers were apparent (Plate – 1 Fig. c).

Plate 1: Histopathological alterations in the intestinal tissue of profenophos treated *A. testudineus*
(Scale bar: 500µ)

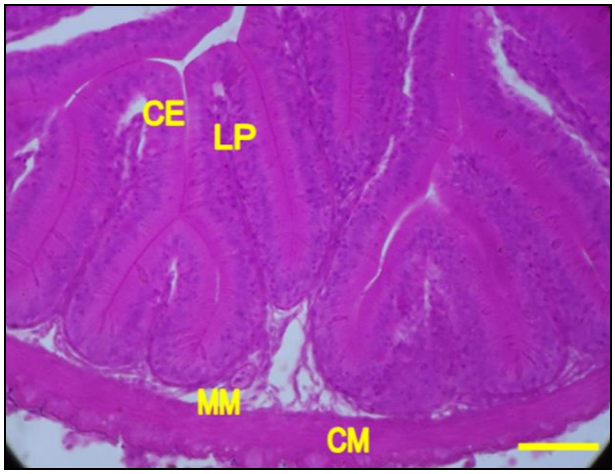

Fig a: Intestine of control fish

CE– Columnar Epithelium
LP– Lamina Propria
MM– Musculouis Mucosae
CM– Circular Muscle

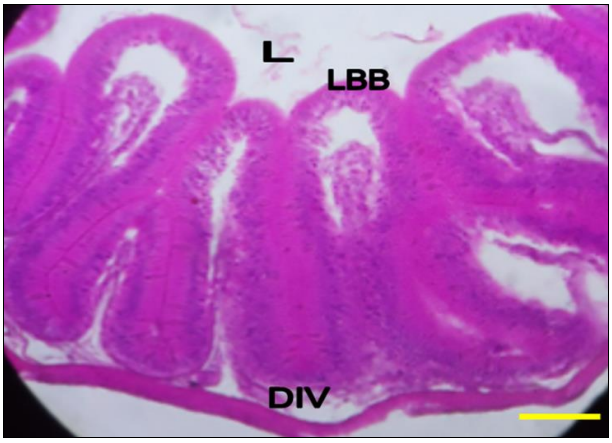

Fig b: Intestine of 10% profenophos treated fish
L– Lumen
LBB– Loss of Bruoh Border
DIV– Detachment of Intestinal Villi

7.5

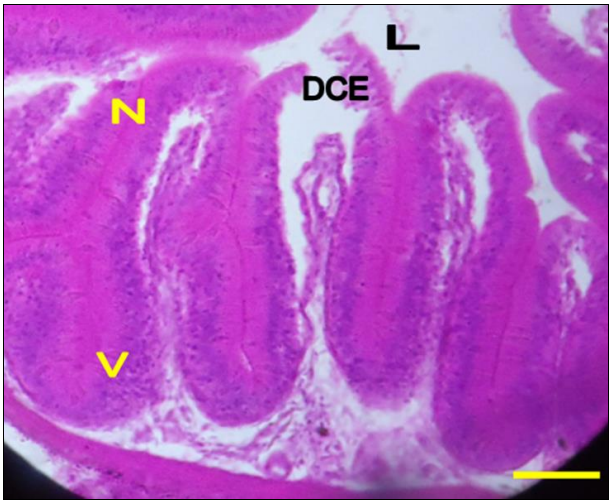

Fig c: Intestine of 30% profenophos treated fish

L– Lumen
N– Necrosis
DCE– Disintegration of Columnar Epithelium

Histology of muscle in the control fish *A.testudineus*

In the muscle tissue of the control *A.testudineus*, comprised of many muscle fibres which were arranged together to form muscle bundles that were separated from each other by connective tissue. The muscle tissue was segmented into myotomes. Each myotome was found to be bent into a single W with an anteriorly directed angle. Each myotome was formed of bundle of muscle fibres. Each muscle fibre was enclosed with in a thin membrane, the sarcolemma. The protoplasm of the muscle fibre called as sarcoplasm, which contained the longitudinal myofibrils that extend throughout the muscle (Plate – 2 and fig. a).

Histopathology of the muscle of 10% sub lethal profenophos treated *A.testudineus*

Depreciatory changes had been noticed in the muscle tissue of 10% sublethal profenophos treated fish. Splitting of muscle fibres was apparent in the low sublethal Profenophos treated fish. Inflammatory cells were found to be aggregated between the muscle bundles (Plate – 2, fig. b).

Histopathology of the muscle of 30% sub lethal profenophos treated *A.testudineus*

In the high 30% sublethal profenophos treated fish there was vacuolar degeneration of muscle bundles. Atrophy of the muscle bundles was also conspicuous (Plate – 2 and fig. c).

Plate 2: Histopathological alterations in the muscle tissue of profenophos treated *A. testudineus*
(Scale bar: 500µ)

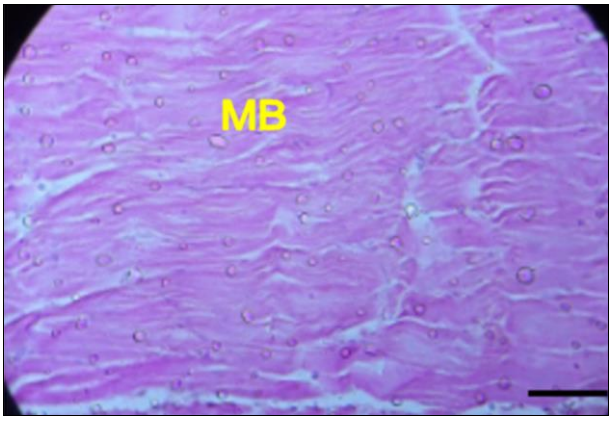

Fig a: Muscle Tissue of the Control Fish

MB– Muscle Bundle

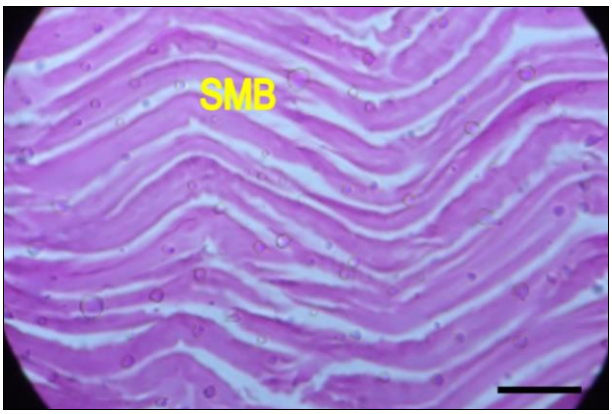

Fig b: Muscle tissue of the 10% profenophos treated fish

SMB– Splitting of Muscle Bundles

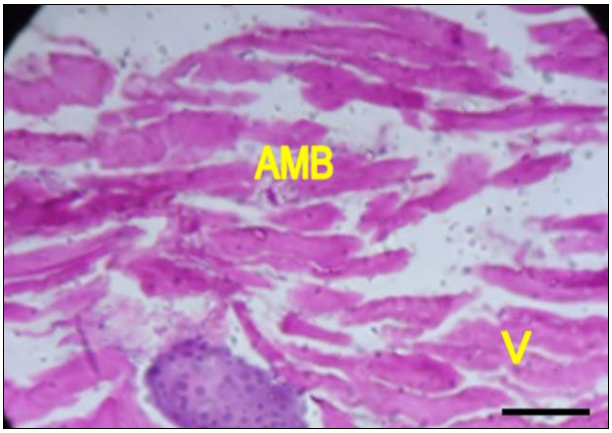

Fig c: Muscle tissue of the 30% profenophos treated fish

AMB– Atrophy of Muscle Bundles
V– Vacuolation

Histology of liver in the control fish *A. testudineus*

The liver tissue of the control fish *A.testudineus* exhibited a normal architecture of typical parenchymatous tissue. The parenchyma tissue itself was composed of epithelial cells (Hepatocytes) which had a large central nucleus homogenous cytoplasm. The sinusoids were areas in which the hepatocytes were located among blood capillaries. They formed a cord-like structure called hepatic cord. Erythrocytes were observed in the lumen of the sinusoids (Plate – 3 Fig. a).

Histopathology of the liver of 10% sub lethal profenophos treated *A.testudineus*

Subacute 10% sub lethal profenophos toxicity exposed fish witnessed disintegration of hepatocyte cell boundaries. Mild dilation of blood sinusoids were detected (Plate – 3 Fig. b).

Histopathology of the liver of 30% sub lethal profenophos treated *A.testudineus*

Significant histopathological aberrations were observed in the 30% sublethal profenophos treated fish. They included the hypertrophy as well as the vacuolation of hepatocytes. Multifocal areas of hepatocyte necrosis were also apparent (Plate – 3 Fig. c).

Plate 3: Histopathological alterations in the liver tissue of profenophos treated *A. testudineus*
(Scale Bar: 600µ)

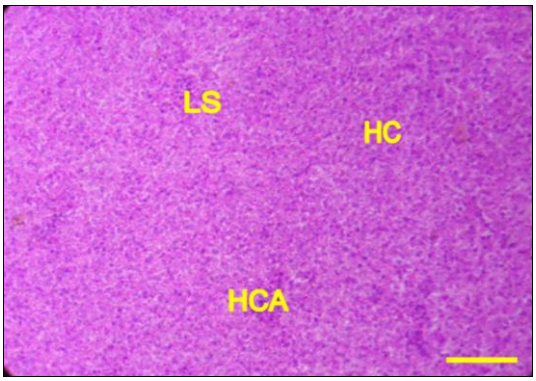

Fig a: Liver tissue of Control fish

LS– Liver Sinusoid
HC– Hepatocytes
HCA– Hepatic Capillary Artery

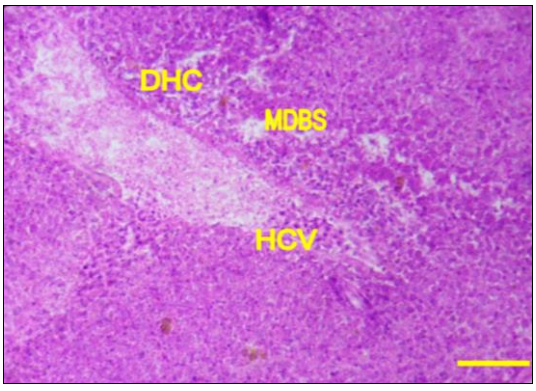

Fig b: Liver tissue of 10% profenophos treated fish

HCV– Hepatic central view
DHC– Disintegration of Hepatocytes
MDBS– Mild Dilation of Blood Sinusoid

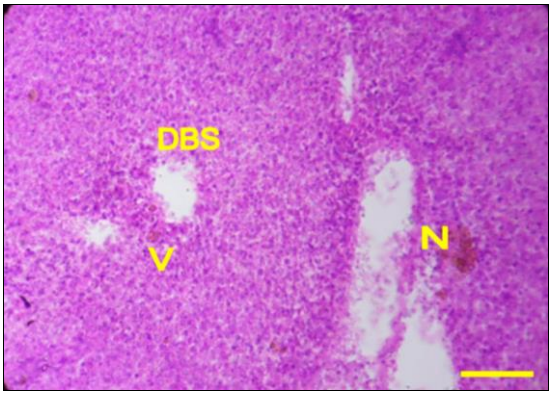

Fig c: Liver Tissue of 30% profenophos treated fish

V– Vacuolation
N– Necrosis
DBS– Dilation of Blood Sinusoids

Histology of kidney in the control fish *A. testudineus*

The histology of the kidney of control *A.testudineus* comprised of anterior kidney and posterior kidney. The anterior kidney contained the endocrine element. The posterior kidney comprised the nephrons with variable quantities of hemopoietic and lymphoid tissue in the interstation. Which is composed of renal corpuscles that were made up of the Bowman's capsules, glomeruli, renal tubules and collecting ducts (Plate- 4 Fig. a).

Histopathology of the kidney of 10% sub lethal profenophos treated *A. testudineus*

The 10% sublethal profenophos treated fish possessed eloquent histopathological anomalies which included the dilation of glomerular capillaries, increased space within the Bowman's capsule. Hypertrophy of the renal tubules was also recognized (Plate – 4 Fig. b).

Histopathology of the kidney of 30% sub lethal profenophos treated *A. testudineus*

Histopathological abnormalities observed in the 30% sub lethal Profenophos exposed fish were vacuolation of renal tubules. Necrosis of the renal tubules were also detected (Plate – 4 Fig. c).

Plate 4: Histopathological alterations in the kidney tissue of profenophos treated *A. testudineus*
(Scale Bar: 500µ)

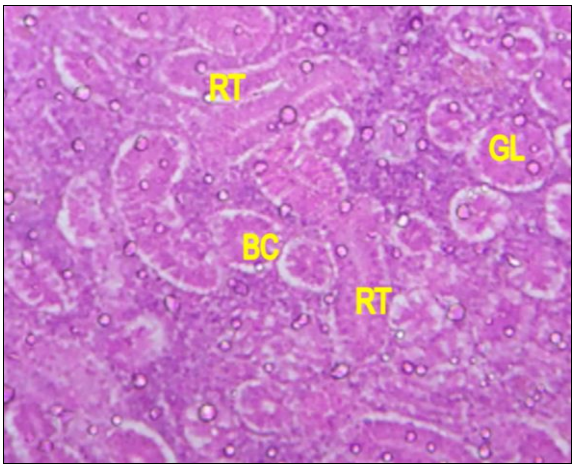

Fig a: Kidney tissue of Control fish

RT– Renal Tubles
GL– Glomerulus
BC– Bowman's capsule

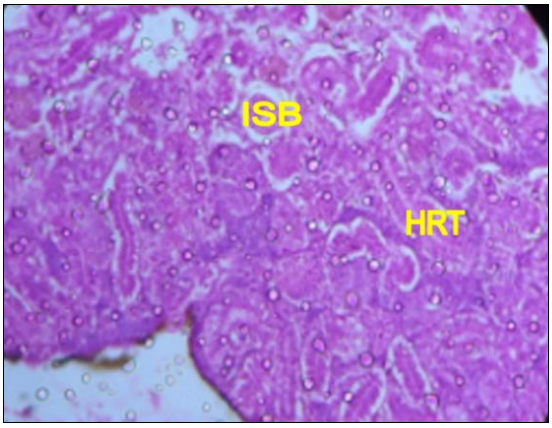

Fig b: Kidney tissue of 10% profenophos treated fish

ISB– Increased Space within Bowman's capsule
HRT– Hypertrophy of Renal Tubular

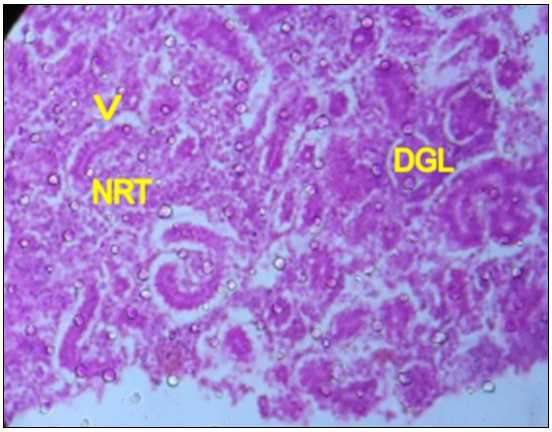

Fig c: Kidney tissue of 30% profenophos treated fish

V– Vacuolation
NRT– Necrosis of Renal Tubules
DGL– Degenerating Glomerulus

Discussion

The intestinal histology of pesticide profenophos toxicity imposed fish *Anabas testudineus* expounded expressive histological alterations with regards to control fish. In the low sub lethal pesticide exposed fish flattening of intestinal villi and sloughing off of mucosal lining can be noticed. Al-Mansoori, (2006) [4] propounded similar findings in the intestine of dimethoate treated fish. Hypertrophy of the epithelial cells and oedema of lamina propria were documented in the present study. Similar observations have been made by Virk *et al*, (1987) [31] in the intestine of endrin and carbaryl induced fish *Mystus tengara*. Fusion and rupture of villi of columnar epithelium was witnessed in the present investigation. The above observations are in harmony with that of Shawkat *et al.* (2010) [28] in the intestine of heavy metal inducted *Clarias batrachus*. In the 30% sublethal pesticide toxicity imposed fish necrosis of intestinal mucosa and accumulation of necrotized cells were visible. These findings were favoured by the contemplations of Shete and Palwari, (2012) [29] in the intestine of copper

imposed *Macrones cavasius*. Haemorrhage of intestinal mucosa, amassing of inflammatory cells between the edemated mucosa and submucosa were documented in the present study. The aforementioned histopathological anomalies are consonance with that of Fatma, (2009) [7] in the intestine of *Tilapia zilli* and *Solea vulgaris.*

In the present study, the muscle tissue of low pesticide profenophos concentration treated fish showed splitting of muscle fibres from muscle bundles. It is suggested that the pesticide could have damaged the connective tissues of the bundles namely endomysium and perimysium. The observation was favoured by Das and Mukherjee, 2000; Mahamed and Gad, 2008 and Mohamed, 2009) [6, 16, 7, 17]. Vacuolar degeneration and atrophy of the muscle bundles were conspicuous in 30% pesticide treated fish. In the chromium treated muscle of *Oreochromis sp.*, Abbas and Ali (2007) [1] have observed degeneration and vacuolation of muscle cell. Vacuolar degeneration and atrophy of the muscle bundles were contemplated by Fatma, (2009) [7].

Evaluation of the liver tissue histopathology of fish is an effective monitoring tool to assess the impacts of environmental toxicants on fish (Fernandes *et al*., 2008) [8]. Thence the liver plays a significant role in many vital function (Lang *et al.,* 2006) [14]. Liver controls metabolism, transforms, excretes xenobiotics and aids in detoxification. It forms a major storage organ for lipids and as site for metabolic process in fish. Hepatocytes of liver carry xenobiotics to bile for their elimination (Patel and Bahadur, 2011) [23]. The liver tissue of the 10% sub lethal pesticide profenophos exposed fish has epitomized a number of histopathological deviations when compared to control fish liver tissue. It was found that the degree of aberrations followed the concentrations of the pesticide profenophos toxicant. It has been suggested that the deviations of hepatocyte structure could be due to amassing of pesticide. Monteiro *et al.* (2005) [18] and Rajeshkumar and Munuswamy (2011) [25] suggested that the degenerative alterations of liver tissue might have been due to interruption in biochemical pathway which encompassed activation of enzymes and impaired ion regulations.

The liver tissue of high sublethal pesticide profenophos treated fish elucidated hypertrophy as well as the vacuolation of hepatocytes, pyknotic nuclei in the hepatocytes and necrosis of hepatocytes. Mishra *et al.* (2009) [15] revealed that the vacuolation of hepatocytes has been a common response correlated to the exposure of toxicants. They also suggested that the lesions represented as a result of histopathological alterations are epitomized due to inhibition of protein synthesis or depletion of energy. Moore *et al.* (1997) [19] attributed that the visible foci of vacuolation is associated with neoplasias. Ahamed *et al.* (2002) [3] suggested that disorders in the osmotic regulation of cellular membranes resulted in bulging of nuclei and nucleoli which ultimately led to necrosis of hepatocytes. Robert, (1978) [27] ascribed that necrosis can be characterized due to nuclear and cytoplasmic alterations caused by toxicants which is followed by the loss of cellular contours.

Kidney is a vital organ and it maintains the homeostasis. Kidney not only involved in the removal of wastes from blood but also responsible for selective re-absorption which aids in maintaining volume and pH of blood as well as body fluids (Iqbal *et al.,* 2004) [11]. In the 10% sub lethal pesticide profenophos treated fish kidney, the remarkable histopathological variations perceived were dilation of glomerular capillaries, shrinkage of glomerulus, increased space within the Bowman's capsule and hypertrophy of the renal tubules. Takashima and Hibiya (1995) [30] had reported similar pathological aberrations. Fish kidney is considered to be a significant target organ since it receives large proportions of post branchial blood. So the histopathological lesions in the kidney should be regarded as a good indicator of environmental pollution (Ortiz *et al.,* 2003 and Kurtovic *et al.,* 2008) [20, 13]. Pacheco and Santos (2002) [22] stated that the severity of the aberrations was corresponding to the sensitivity of the fish species to the pollutants released into the environment. Shrinkage of glomeruli, degeneration of renal tubules have also been reported by Afsar and Magar (2013) [2] by exposing *Channa punctatus* to the pesticide Malathion. In the 30% sub lethal pesticide profenophos exposed fish cytoplasmic degenerations, karyolysis and necrosis of renal tubules were apparent. The above observations have been falling in line with that of Ptashynki *et al.* (2002) [24] in the nickel treated silver carp.

Conclusion

From these results it is concluded that the histological changes in the structural integrity of the cells of several organs of fish was directly proportional to the exposed pesticide profenophos concentrations and the histological damages were also high.

Acknowledgments

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References

1. 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.
2. Afsar S, Magar RS. Effect of Malathion toxicity on detoxifying organ of fresh water fish *Channa punctatus*. IJPCBS.,2013:3(3):723-728.
3. Ahmad A, Pillai KK, Najmi AK, Ahmad SJ, Pal SN, Balani DK. Evaluation of hepatoprotective potential of jigrine post-treatment against thioacetamide induced hepatic damage. - Journal of Ethnopharmacology, 2002:79(1):35-41.
4. Al-Mansoori AF. Histological changes induced by cadmium ion in the Gills, Liver and Intestine of juvenile *Carassius carassius* (L.). Basrah J. Sci. (B),2006:24(1):32-46.
5. APHA. American Public Health Association, American Waterwork Association, Water Pollution Control Federation, standard methods for the examination of water and waste water, 1992, 1193.
6. Das B, Mukherjee S. A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. Vet. Arhiv,2000:70(4):169-180.
7. Fatma AS Mohamed. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarum, Egypt. World J. Fish. Mari. Sci.,2009:1(1):29-39.
8. Fernandes C, Fontaínhas-Fernandes A, Rocha E, Salgado MA. Monitoring pollution in Esmoriz–

Paramos lagoon, Portugal: liver histological and biochemical effects in *Liza saliens*. - Environmental Monitoring and Assessment,2008:145:315-322.
9. Finney DJ. Probit Analysis. Cambridge Univ. Press, London, 1971..
10. Humason. Animal tissue techniques.Fourth Edition, W.H.Freeman and Company, San Franscisco. USA, 1967.
11. 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.
12. Joseph B, Raj SJ, Edwin BT, Sankarganesh P, Jeevitha MV, Ajisha SU, *et al*. Toxic effect of heavy metals on aquatic environment. International Journal of Biological and Chemical Sciences,2010:4:939-952.
13. Kurtovic B, Teskeredzic E, Teskeredzic Z. Histological comparision of spleen and kidney tissue from farmed and wild Europen sea bass (Dicentrarchus labrax). Acta Adriatic,2008:49(2):147-154.
14. Lang T, Wosniok W, Baršienėc J, Broeg K, Kopecka J, Parkkonen J. Liver histopathology in Baltic flounder (*Platichthys flesus*) as indicator of biological effects of contaminants. - Marine Pollution Bulletin,2006:53:488-496.
15. Mishra AK, Mohanty B. Chronic exposure to sublethal hexavalent chromium affects organ histopathology and serum cortisol profile of a teleost, Channa punctatus (Bloch). Science of the Total Environment,2009:407:5031-5038.
16. Mohamed FA, Gad NS. Environmental pollution-induced biochemical changes in tissues of Tilapia zillii, Solea vulgaris and Mugil capito from Lake Qarun, Egypt. Global Veterinaria,2008:2:327-336.
17. Mohamed FAS. Histopathological studies on *Tilapia zilli* and *solea vulgaris* from lake Qarum, Egypt. World J. Fish Marine Sci.,2009:1:29-39.
18. Monteiro SM, Mancera JM, Fontaínhas-Fernandes A, Sousa M. Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*. - Comparative Biochemistry and Physiology,2005:141:375-383.
19. Moore MJ, Smolowitz RM, Stegeman JJ. Stages of hydropic vacuolation in the liver of winter flounder Pleuronectes americanus from a chemically contaminated site. - Diseases of Aquatic Organisms,1997:31:19-28.
20. Ortiz JB, González De Canales ML, Sarasquete C. Histopathological changes induced by lindane (γ-HCH) in various organs of fishes. - Scientia Marina,2003:67(1):53-61.
21. Osman AG, Abd El Reheema AM, Moustafa MA, Mahmoud UM, Abuel-Fadld KY, Kloas W. In situ evaluation of the genotoxic potential of the river nile: I. Micronucleus and nuclear lesion tests of erythrocytes of Oreochromis niloticus (Linnaeus, 1758) and Clarias gariepinus (Burchell, 1822). Toxicological and Environmental Chemistry,2011:93:1002-1017.
22. Pacheco M, Santos MA. Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel, *Anguilla anguilla* L. Ecotoxicology and Environmental Safety,2002:53:331-347.
23. Patel JM, Bahadur A. Histopathological manifestations of sub lethal toxicity of copper ions in Catla catla. American-Eurasian Journal of Toxicological Sciences,2011:4(1):01-05.
24. Ptashynski M, Pedlar R, Evan C, Baron M, Klaver Kamp J. Toxicology of dietary nickel in lake white fish (Coregonus clupeaformis). Aquatic Toxicology,2002:58:229-247.
25. Rajeshkumar S, Munuswamy N. Impact of metals on histopathology and expression of HSP 70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, South East Coast, India. – Chemosphere,2011:83(4):415-421.
26. Ray SNC, Mohan C, Sinha RC. Effect of methyl parathion on TSH, T4 and T3 in fish, Labeo rohita. International Journal of Applied Research,2015:5(3):554-557.
27. Roberts JR. The pathophysiology and systematic pathology of teleosts. In fish pathology. Bailliere Tindall, London,1978:(1):67-70.
28. Shawkat Ara Begum, Qamar Banu, Baharul Hoque. Histopathology of the Intestine of *Clarias batrachus* L. under heavy metal stress. Chittagong Univ. J. B. Sci,2010:5(1&2):45-53.
29. Shete PS, Patwari JM. Acute toxicity of CuSO4 in the freshwater fish: Histopathology of the Intestine. Trends Fisheries Res.,2012:1(1):22-23.
30. Takashima F, Hibiya T. An atlas of fish histology. Normal and pathogical features. Tokyo, Kodansha Ltd, 1995, 2.
31. Virk S, Kaur K, Kaur S. Histopathological and biochemical changes induced by endrin and carbaryl in the stomach, intestine and liver of Mystus tengara. Indian J. Ecol.,1987:14(1):14-20.