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Assessment of renosomatic index, histopathology and pathophysiology of the kidney of exotic freshwater fish, *Ctenopharyngodon idella* exposed to endosulfan

Renu Bala

Department of Biology, S.D. College, Barnala 148101, Punjab, India
E.mail: renubs23@gmail.com

ABSTRACT

Reckless use of pesticides and lack of a proper disposal system following the Green-Revolution in India, especially in the state of Punjab (India), resulted in the trickling down of pesticides to the surrounding ecosystems, including water bodies and caused lethal effects in the non-target species including fishes. The present study examined the effects of sublethal concentrations (0.00075 mg/L and 0.001 mg/L) of endosulfan on the histopathology of kidney, RBC count (a pathophysiological indicator), and renosomatic index (which shows gross anatomical changes) in the exotic freshwater herbivorous fish, *Ctenopharyngodon idella* after 15, 30, and 45 days exposure while also looking for the same in appropriately setup control. Severe renal damage; *significant* ($p<0.05$) alterations after 15 days of exposure to both selected sublethal concentrations and *highly significant* ($p<0.001$) alterations after 30 days of exposure in RBC count; significant ($p<0.05$) variations in the renosomatic index after 30 days have been observed showing that gross anatomical changes appear much after the functions of the kidney have been altered.

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1. Introduction

The organochlorine class of pesticides is widely used for the control of agricultural pests as well as the spread of vector-borne diseases. The use of pesticides, including organochlorine pesticides, became widely prevalent in the Punjab region of India after the 1960s following the Green Revolution endeavour and due to Government policy to promote the use of pesticides to ensure greater agricultural produce. The widespread indiscriminate use of pesticides coupled with the lack of proper disposal management has resulted in the excessive accumulation of the substances in the water bodies. This has caused ecological problems on gigantic proportions because of their hazardous effects on non-target organisms like amphibians, reptiles, snails, birds, bees, snails, earthworms, and humans (Elzen, 2001; Bostanian and Akalach, 2004; Benamu *et al.,* 2007; Cerrillo *et al.,* 2005). In the aquatic environment, these hazardous effects have been monitored using fish as biological indicators (Van der Oost *et al.,* 2003). Further, the visceral organs, such as the kidney (Bucher and Hofer, 1993) liver (Swarup *et al.,*1981), and blood (Rao and Murty, 1982) of fish are considered to be affected to the highest degree because of their role in circulation, metabolism, detoxification, and excretion. Histopathology (Kumar and Pant, 1984), haematology (for pathophysiological effects) (Sharma and Singh, 2004), and biometric assays (for gross anatomical effects) (Fernandes *et al.,*2019) have been employed to assess the toxicity to fishes in chronic exposure to organochlorine pesticides.

The present study is an attempt to correlate the timeline of loss of physiological function and appearance of the gross anatomical changes in the kidneys of the exotic freshwater fish, *Ctenopharyngodon idella,* on exposure to endosulfan. *Ctenopharyngodon idella* is a herbivorous fish and has been introduced in ponds of Punjab by farmers to keep the aquatic weeds in check as the cost of stocking phytophagous fish is lower than the cost of herbicidal or algicidal control; secondly, environmentalist groups would prefer to use biological rather than chemical control methods.

2. Materials and Methods

2.1 Fish sampling

Live, healthy fish having approximately the same length (12.56 ±1.52 cm) and weight (25.40±2.53 gms) were procured from local fish farms in the Barnala area in Punjab, India. These were dipped in 0.1% $KMnO_4$ to disinfect them, after which they were acclimatized to laboratory conditions for seven days in a glass aquaria provided with a single outlet aerator. After every 12 hours, the fish were fed with grass, barseem, and banana leaves. The aquarium was regularly cleaned every third day.

2.2 Chemical reagents

Endosulfan is an organochlorine insecticide of the cyclodine family (chemical name 6,7,8,9,10-hexachloro-1,5,5a.8,9,9a-hexahydro-6,9-methano-2,4,3-bezoldioxathiepin-3-oxide). Endosulfan 35 EC (commercial name) manufactured by Excel Industries Limited, India, was procured from the local market in Barnala, Punjab, India. Other reagents, stains, and chemicals for haematology and histology study were of analytical grades and were procured from local suppliers.

2.3 LC_{50} determination

Toxicity tests were done following the manual of APHA (2005). The fishes were exposed to seven different concentrations of endosulfan i.e 0.0032, 0.0036, 0.0040, 0.0044, 0.0048, 0.0052, 0.0056, 0.0060, 0.0064 mg/L. These concentrations were chosen based on the minimum and maximum mortality rates of the fish during 96 hours exposure period. Appropriate controls in endosulfan-free water were maintained simultaneously. LC_{50} was calculated using both graphical and arithmetic methods.

2.4 Experimental design

For the chronic toxicity tests, the fishes were exposed to endosulfan at levels much lower than those acutely

fatal but the duration of exposure was much longer. The concentrations for the same were selected from the LC_{50} as per the APHA (2005). The acclimatized fish were divided into 3 groups (6 fish each) and kept in separate syntax tanks of 10 liters capacity. Group I and II were exposed to 0.00075 mg/L and 0.001 mg/L for 15, 30, and 45 days and group III was maintained as the control in endosulfan free dechlorinated tap water. The tanks were cleaned and water was changed every alternate day to maintain the desired level of insecticidal concentration.

2.5 Biometric assay

Renosomatic index was used as a biometric assay and is expressed as:

$$RSI = \frac{\text{Weight of the kidney (g)}}{\text{Total body weight (g)} - \text{the weight of kidney (g)}}$$

2.6 Histopathological analysis

After the 15th, 30th, and 45th days, the kidneys of control and exposed fish were removed and passed through five key stages: fixation, processing, embedding, sectioning and staining for histological staining). Fixation was done in Bouin's fluid for 24 hours. Dehydrated using alcohol grades, cleared using benzene. The process of embedding is completed using paraffin wax. Subsequently, tissue blocks were made and 7µm sections were cut using a rotary microtome. These sections were fixed on glass slides thinly coated with Mayer's albumin. The sections were then treated with xylene, a descending series of ethanol and water. The staining was done by the hematoxylin-eosin staining method. Hematoxylin and eosine procedures could be used to study most histopathological processes (Titford and Bowman, 2012).).

2.7 Hematological studies

Free-flowing blood from the caudal vein was collected for hematological studies. Total erythrocyte count (TEC) was estimated using an improved Neubauer hemocytometer.

2.8 Statistical analysis

All the data were expressed as mean ± S.D. The mean value for each group of fish was tested for significance ($p < 0.05$ and $p < 0.01$) by student's t-test to establish the validity of the findings.

3. Results and Discussion

In present investigations, the LC_{50} of endosulfan for *C. idella* was 0.0050 mg/L.

3.1 Biometric Assay

The renosomatic index is considered as a parameter to determine the appearance of gross changes and lesions in the kidney of the fish. The alterations in RSI in control and exposed fishes are presented in table 1.

A declining trend has been observed in the RSI values throughout the study in the test organism; non-significant ($p > 0.05$) after 15 days of exposure, significant ($p < 0.05$) on exposure to 0.00075 mg/L after 30 days and highly significant ($p < 0.01$) on exposure to 0.00075 mg/L for 45 days; as well as to 0.001 mg/L for both 30 and 45 days. RSI has been found to increase with increased cadmium concentration and exposure duration in *Anabas testudineus* (Idrus *et al.,* 2022).

3.2 Renal histopathological changes

In the kidney of *Ctenopharyngodon idella* exposed to 0.00075 mg/L of endosulfan for 15 days (Fig. 3), there was loss of brush border of the proximal convoluted tubule, the appearance of a number of vacuoles in tubular cells, widening of tubular lumen, and atrophy of basal cytoplasm surrounding the tubule when compared to control (Fig. 1,2) When the dose was raised to 0.001 mg/L, the proximal tubules got displaced to one side and there was a separation of the renal tubular epithelium from the surrounding connective tissue; narrowing of the tubular lumen, and also increase in size of the peritubular spaces (Fig. 4).

Exposure to 0.00075 mg/L of endosulfan for 30 days (Fig. 5) caused narrowing of the tubular lumen, hypertrophy of tubular epithelial, and appearance of pyknotic nuclei in tubular epithelium of kidney of the test organism when compared to control in the present study. At 0.001 mg/L (Fig. 6), the tubular lumen disappeared altogether; cellular contours became indistinguishable; enlarged sinusoids within a decreased amount of hemopoietic tissue, eosinophilic exudates in the lumen as well as in peritubular space and focal necrosis have been observed in all concentrations on all exposure periods.

On 45 days of exposure to both concentrations of endosulfan, loss of tubular architecture of the kidney was observed (Fig. 7, 8). Similar results have been reported by earlier authors like (Thophon *et al.*,2003 in *Lates calcarifer* exposed to cadmium; Bhatia and Sandhu, 2005 in *Heteropneustes fossilis* exposed to endosulfan; Altinok and Capkin, 2007 in rainbow trout exposed to endosulfan; Capkin *et al.,*2010 in *Oncorhynchus mykiss* exposed to endosulfan) etc.

3.3 Haematological findings: Alterations in RBC count

The kidney is the chief hemopoietic organ in the fish (Bond, 1979; Smith, 1982; Heath, 1995; Johansson-Sjobeck and Larson, 1978.), hematological abnormalities (RBC count) can be considered as indications of pathophysiological changes in the kidney that lead to loss of its function (Seth and Saxena, 2003). In the present investigations, the erythrocyte count has been negatively correlated

Table 1. Effect of sublethal concentrations of endosulfan on renosomatic index (RSI) of *Ctenopharyngodon idella*

Exposure Period (days)	No. of Experimental fish	**RSI** in control fish	**RSI** in exposed fishes	
		Mean ± S.D.	Mean ± S.D.	
			0.00075 mg/L	0.001mg/L
15	6	0.576 ± 0.203	0.489 ± 0.102	0.405 ± 0.169
30	6	0.565 ± 0.414	0.326 ± 0.135*	0.295 ± 0.296**
45	6	0.570 ± 0.098	0.290 ± 0.106**	0.249 ± 0.522**

Level of significance *p < 0.05; **p < 0.01. Non significant (p > 0.05)

with the endosulfan concentration and the experiment length (Table 2).

All the results have shown a regular significant decrease ($p<0.05$) after 15 days and a highly significant decrease ($p<0.01$) in RBC count after exposure periods of 30 and 45 days. These changes in RBC count in the fish can be attributed to the loss of hematopoietic tissue in the kidney as has also been reported by (Bhatia *et al.,* 2004 (in *Heteropneustes fossilis* (Bloch) exposed to endosulfan); Jenkins *et al.,*2003 (*Cyprinus carpio* exposed to endosulfan)].

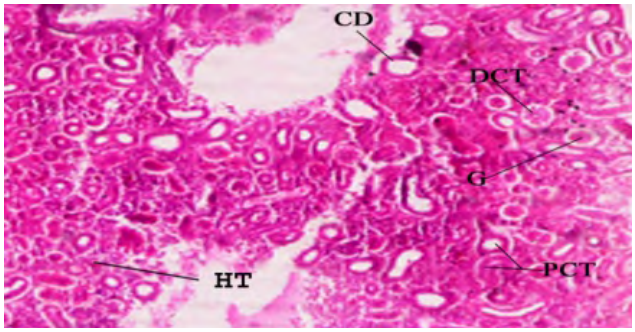

Fig. 1.

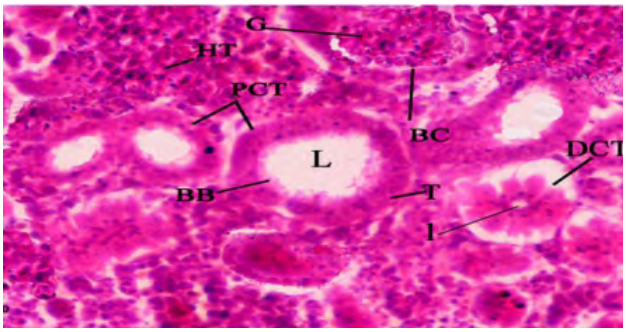

Fig. 2.

Renal tissue in the (1) control 40x and (2) control 100x**:** DCT: distal convoluted tubule ; CD: Collecting Duct; G: Glomerulus; HT: Hemopoietic tissue; PCT: Proximal Convoluted Tubule; T: Tubular cells; BB: Brush Border; L: Lumen in Proximal Convoluted Tubule; BC: Bowman's Capsule; l: Lumen In Distal Convoluted Tubule

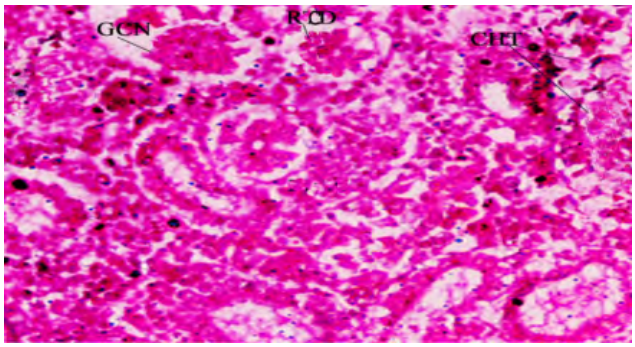

Fig. 3.

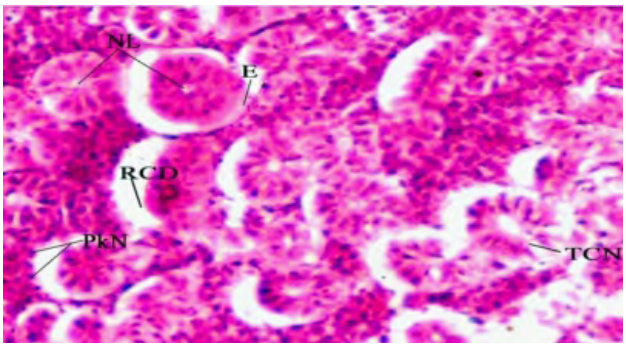

Fig. 4.

Renal tissue after 15 days of exposure to endosulfan (3) at 0.00075 mg/L and (4) at 0.001mg/L**:** CHT: Congestion of Hemopoeitc tissue; TCN: Tubular Cell Necrosis; GCN: Glomerular Cell Necrosis; NL: Narrowing of lumen; RCD :Renal Cell Displacement; PkN: Pyknotic nuclei; E: Exudate.

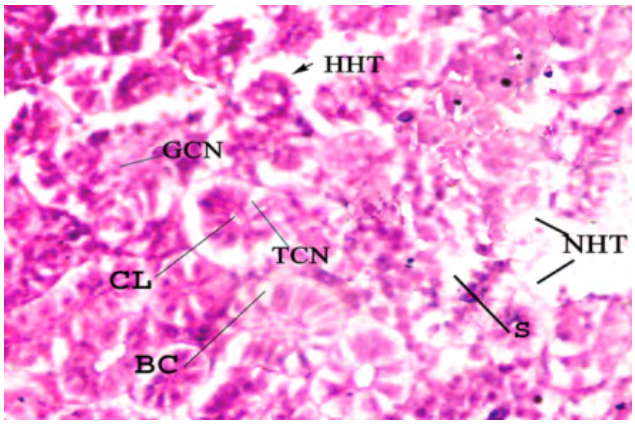

Fig. 5.

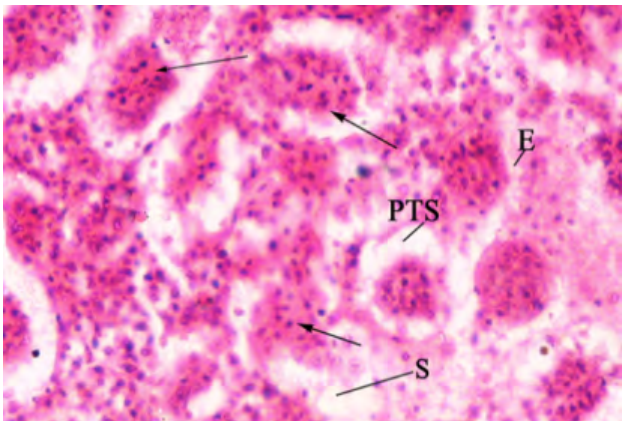

Fig. 6.

Renal tissue after 30 days of exposure to endosulfan (5) at 0.00075 mg/L and (6) at 0.001mg/L, CL: Constricted Lumen CHT: Congestion of Hemopoeitc tissue; TCN: Tubular Cell Necrosis; GCN: Glomerular Cell Necrosis; NL: Narrowing of lumen; RCD: Renal Cell Displacement; PkN: Pyknotic nuclei; E: Exudate; NHT and HHT: Necrosis of Hemopoeitic Tissue; V: Vacuole in Tubular Cells; S: Sinusoids; Arrows: Loss of Tubular Contours; PTS: Peritubular Space;

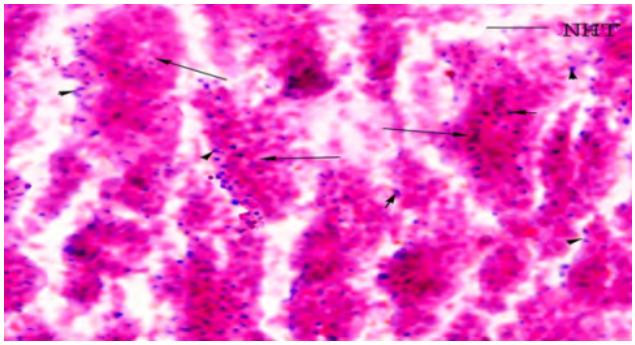

Fig. 7.

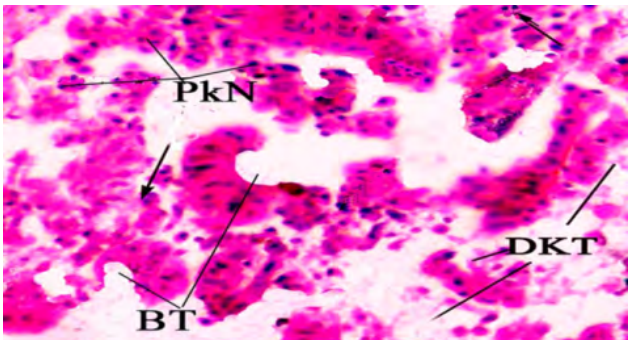

Fig. 8.

Renal tissue after 45 days of exposure to endosulfan (7) at 0.00075 mg/L and (8) at 0.001mg/L, PkN: Pyknotic nuclei; NHT: Necrosis of Hemopoeitic tissue; DKT: Disintegration of Tubules; Short Arrows: Pyknotic Nuclei; Long Arrows: Loss of Tubular Architecture; BT: Broken Tubule

Fig. 1-8. Showing histopathological alterations in the kidney of the control as well as the exposed fish

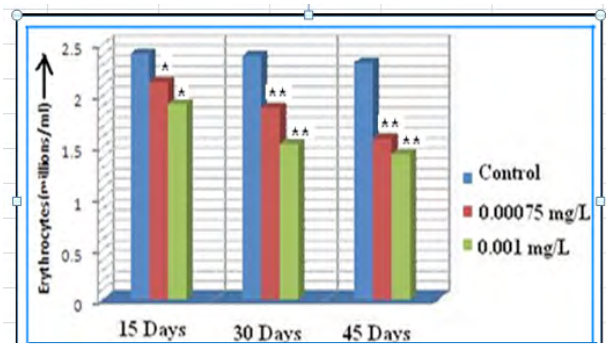

Fig. 9. Variations in total erythrocyte count in *Ctenopharyngodon idella* upon exposure to different sublethal doses of endosulfan for 15, 30 and 45 Days

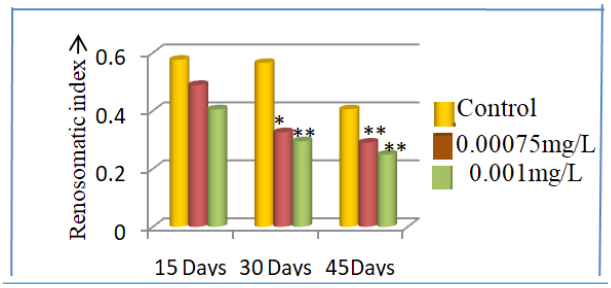

Fig. 10. Variations in renosomatic index in *Ctenopharyngodon idella* upon exposure to different sublethal doses of endosulfan for 15, 30 and 45 Days.

Table 2. Erythrocyte count in *Ctenopharyngodon idella* exposed to different sublethal concentrations of endosulfan for 15 days, 30 days and 45 days

Exposure Period (days)	No. of Experimental fish	RBC count in Control (millions/ml)	RBC count in Treated (millions/ml)	
		Mean ± S.D.	Mean ± S.D.	
			0.00075 mg/L	0.001mg/L
15	6	2.41 ±0.128	2.13 ± 0.482*	1.92 ± 0.488*
30	6	2.39 ±0.414	1.88 ± 0.845**	1.57 ± 0.964**
45	6	2.37 ±0.169	1.59 ± 0.576**	1.43 ± 0.522**

Level of significance *p<0.05; **p<0.01.Non significant (p > 0.05)

4. Conclusion

Based on the present studies, it can be concluded that in *Ctenopharyngodon idella*, exposure to endosulfan caused gross anatomical changes much after the functions of the kidney had been altered. This is because while the renosomatic index showed significant variations from the control organisms after 30 days, histopathological and haematological changes were significantly altered even after 15 days of exposure. The renosomatic index is considered an indicator of gross anatomical changes, while histopathological changes and changes in RBC count indicate loss of functions in the kidney. Damage to renal hemopoietic tissue, hence malfunctioning of the kidney, seems to be the leading cause of erythropenia in the test fish on exposure to different sublethal concentrations of endosulfan.

5. References

Altinok, I. and Capkin, E. 2007. Histopathology of rainbow trout exposed to sublethal concentrations of methiocarb or endosulfan. Journal of toxicologic pathology, 35: 405-410.DOI: 10.1080/01926230701230353

APHA, AWWA, WPCF. 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. (eds. S.C. Lenore, E.G. Arnold and D.E. Andrew). American Public Health Association, Washington, DC.

Benamu, M.A., Schneider, M.I., Pineda, S., Sanchez, N.E. and Gonzales, A. 2007. Sublethal effects of two neurotoxican insecticides on *Araneus pratensis* (Araneae, Araneidae). Communications in agricultural and applied biological sciences,72 (3): 557-559.

Bhatia, N.P. and Sandhu, G.S. 2005. Histopathological changes induced by endosulfan in the kidney of *Heteropneustes fossilis* (Bloch). *In: Proc. Nat. Se Feb. 16-18. 'New Trends in Fishery Development in India' (ed. M.S. Johal).* (pp. 185-192), Chandigahrh: Panjab University.

Bhatia, N.P., Sandhu, G.S. and Johal, M.S. 2004. Haematological alterations in *Heteropneustes fossilis* (Bloch) on exposure to endosulfan. Pollution Research, 23 (4): 633-636.

Bond, C. 1979. *Biology of Fishes.* Philadelphia, USA: Biology of Fishes. Saunders College Publishing.

Bostanian, N.J. and Akalach, M. 2004. The contact toxicity of indoxacarb and five other insecticides to *Orius insidiosus* (Hemiptera: Anthocoridae) and *Aphidius colemani* (Hymenoptera: Braconidae), beneficials used in the greenhouse industry. Pest Management Science,5:1231-1236.DOI: 10.1002/ps.938

Bucher, F. and Hofer, R. 1993. Histopathological effects of sublethal exposure to phenol on two variously pre- stressed populations of bullhead (*Cottus gobio L.*). Bulletin of Environmental Contamination and Toxicology, 51: 309-316.https://doi.org/10.1007/BF00198897

Capkin, E., Terzi, E., Boran, H., Yandi, I. and Altinok, I. 2010. Effects of some pesticides on the vital organs of juvenile rainbow trout (*Oncorhynchus mykiss*).Tissue and Cell, 42 (6): 376-382.DOI: 10.1016/j.tice.2010.10.001

Cerrillo, I., Granada, A., Lopez-Espinosa, M.J., Olmos, B., Jimenez, M., Cano, A., Olea, N. and Fatima Olea-Serrano, M. 2005. Endosulfan and its metabolites in fertile women, placenta, cord blood, and human milk. Environmental Research, 98 (2): 233-239.https://doi.org/10.1016/j.envres.2004.08.008

Elzen, G. 2001. .Lethal and sublethal effects of insecticide residues on *Orius insidiorus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). Journal of economic entomology, 94 (1): 55-59.DOI: 10.1603/0022-0493-94.1.55

Fernandes, C.E., Marcondes, S.F., Galindo,G.M.and Franco-Belussi, L. 2019. Kidney anatomy, histology and histometric traits associated to renosomatic index in *Gymnotus inaequilabiatus* (Gymnotiformes: Gymnotidae). Neotropical Ichthyology, 17(4): 10. DOI:1590/1982-0224-20190107.

Idrus,S. N.S., Abdullah,M.R., Mohd, F.H., Othman, M.S. 2022.Hepatosomatic and renosomatic indices of *Anabas testudineus* exposed to various levels of cadmium . Journal of environmental protection science, 13: 161-170. DOI: 10.4236/jep.2022.131010 .

Heath, A. 1995. Water Pollution and Fish Physiology. 2nd edition. Florida: CRC Press.

Jenkins, F., Smith, V., Rajanna, B., Shameem, U., Umadevi, K., Sandhya, V. and Madhavi, R. (2003). Effect of sublethal concentrations of endosulfan on haematological and serum biochemical parameters in the carp *Cyprinus carpio*. Bulletin of Environmental Contamination and Toxicology, 70 (5): 993-997.DOI: 10.1007/s00128-003-0080-7

Johansson-Sjobeck, M.L. and Larson, A. 1978. The effect of cadmium on the haematology and the activity of δ- aminolevulinic acid dehydrates (ALA-D) in blood and haemopoietic tissue of the flounder, *Pleuronectes flesus* (L). Environmental Research, 17(2): 191-204. doi: 10.1016/0013-9351(78)90021-x.

Kumar, S. and Pant, S.C. 1984. Organal damage caused by aldicarb to a freshwater teleost *Barbus conchonius* (Hamilton).Bulletin of Environmental Contamination and Toxicology,33: 50-55.https://doi.org/10.1007/BF01625510

Rao, D.M.R. and Murty, A.S. 1982. Toxicity and metabolism of endosulfan in three freshwater catfishes. Environmental Pollution Series A, Ecological and Biological, 27(3): 223-231.https://doi.org/10.1016/0143-1471(82)90027-7.

Seth, N. and Saxena, K.K. 2003. Hematological responses in a freshwater fish *Channa punctatus* due to fenvalerate. Bulletin of Environmental Contamination and Toxicology, 71, 1192–1199.https://doi.org/10.1007/s00128-003-8732-1.

Sharma, G. and Singh, S. 2004. Studies on the effect of intoxicant indofil on the blood morphology of *Channa punctatus* (Bloch). Bionotes, 6(1): 20.

Smith, L.1982. Introduction to Fish Physiology. Neptune, USA, TFH Publications, Inc.

Swarup, P.A., Rao, D.M. and Murty, A.S. 1981. Toxicity of endosulfan to the freshwater fish, *Cirrhina mrigala*. Bulletin of Environmental Contamination and Toxicology,27: 850-855.DOI: 10.1007/BF01611106

Thophon, S., Kruatrachuc, M., Upathau, E., Pokcthitiyook, P., Sahaphong, S. and Jarikhuan, S. 2003. Histopathological alterations of white seabass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environmental Pollution,121: 307-320.DOI: 10.1016/s0269-7491(02)00270-1

Titford, M. and Bowman, B. 2012. What may the future hold for histotechnologists? LabMedicine,43:5-10.

Van der Oost, R., Beyer, J. and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology,13:57-149.DOI: 10.1016/s1382-6689(02)00126-6.