Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

September 2010, 31(5) 755-758 (2010)
For personal use only
Commercial distribution of this copy is illegal

Studies on effects of chronic exposure of endosulfan to *Labeo rohita*

T.S. Saravanan*, P. Rajesh and M. Sundaramoorthy

Post Graduate & Research Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli - 20, India

(Received: May 11, 2009; Revised received: November 29, 2009; Accepted: January 01, 2010)

Abstract: *The major carp, Labeo rohita (Hamilton) upon chronic exposure to endosulfan showed hyperglycemia (60.11 to 117.71 mg 100 ml^{-1}) and hypercholestemia (51.78 to 76.87 mg 100 ml^{-1}) of blood when compared to the control. As a result of the insecticidal stress, the tissue metabolites and enzymes like AST and ALT revealed significant alterations. The hormones T3 (0.53 to 1.35 µml l^{-1}) and TSH (0.48 to 0.32 µml l^{-1}) were also affected by the endosulfan even in sub lethal concentration. Signficant histopathological alterations were observed in liver and gill of treated fishes. The reason for the significant alterations in tissue sugars, aminoacids, cholesterol, protein, enzymes and homones and the histopathological changes is discussed.*

Key words: *Labeo rohita, Endosulfan, Blood, Liver, Gill, Enzymes and Hormones*
PDF of full length paper is available online

Introduction

Any alteration in the chemical composition of natural aquatic environment usually induces changes in the biochemical aspects of the inhabitants particularly fishes (Edward, 1973). These alterations in body metabolism due to organochlorine intoxication have been extensively studied (Leonard *et al.,* 2000; Ramaneswari and Rao, 2008). These studies indicate that all organochlorine pesticides are highly toxic to fish when compared to organophosphate, carbamate and pyrethroid under same environmental conditions. The degree of toxicity produced by the poisonous substance is does independent upon environmental conditions such as temperature, pH, oxygen content and presence of residue molecules (Capkin *et al.*, 2006; Singh and Mishra, 2009; Gulfer *et al.,* 2009). It is well known that protein, carbohydrates and lipid play a major role as energy precursors in fish under stress conditions. Enzymes play significant role in food utilization and metabolism. The proteolytic enzymes participate in the break down of protein molecules into aminoacids and these aminoacids are inturn oxidized to give energy for body function (Saravanan *et al.*, 2000). Pollutants can produce metabolic changes at cellular levels by a way of influencing enzyme systems. Many authors have reported the changes in acid and alkaline proteases in the fish exposed to sublethal levels of pesticides. Baskaran *et al.* (1991) have studied the acid and alkaline protease activity in the fish *Oreocromis mossambicus* under organophosphate stress. Malla Reddy and Bashamohideen (1988) have observed the changes in protease activity when *Cyprinus carpio* exposed to fenvelerate, a pyrethroid insecticide.

Ramaswamy (1987) has studied the effects of sevin on the lipid content levels of liver and muscle of *Sarotherodon mossambicus* and noted lipids are accumulated. There are reports that the lipid levels are much altered when fish was exposed to toxicants (Saravanan *et al.,* 2000,2003). In the fish blood there are several Thyroxin (TH) binding proteins, including transthyretin (TTR) which has been recently identified in fish (Power *et al.*, 2000) It is known that some protein bind to human TTR, inhibiting TH binding and transport (Lans *et al.,* 1994). Coimbra *et al.* (2002) have reported decrease in plasma cortisol and low level of T_3 in plasma of treated fish, *Oreochromis niloticus.* A careful perusal of the literature reveal not much of work has been done on the chronic effects of endosulfan as an endocrine disrupting chemical on the major carp *Labeo rohita.* Further, endosulfan has been widely used in the fields of Trichy district because of its powerful insecticidal effect (Saravanan *et al.*, 2000). So an attempt has been made to study the influence of sublethal concentration of endosulfan on certain tissue metabolites, enzymes (AST and ALT) and hormones T_3 and TSH and histopathological changes of gill and liver of the fish, *Labeo rohita (*Hamilton).

* **Corresponding author:** thiruver@gmail.com

Materials and Methods

The major Carp, *Labeo rohita* were obtained from a local market at Thiruchirappalli, Tamil Nadu, India. The standard length and weight of fishes were in the range of 10.0 to 15.2 cm and 60 to 76.5 gm, respectively. They were acclimatized in the laboratory condition for more than two weeks. During this period they were fed with pelleted food (ricebran and groundnut oil cake) on alternate days and the water was renewed daily. The temperature, pH, salinity and dissolved oxygen of the water were found to be 27 $\pm$ 1°C, 7.55 $\pm$ 0.1, 0.76 $\pm$ 0.09‰ and 7.20 $\pm$ 0.12 ml l^{-1} respectively. After the acclimatization, two groups of five fishes each were introduced into control and experiment tanks with 40 litters of water in each tank. Endosulfan, technical grade (99%) obtained from the Scientific Insecticide Company, Guntur, Thiruchirappalli district. To obtain the sublethal concentration of 0.01 ppm of endosulfan, a stock solution was prepared by dissolving 50 mg of endoslufan in 50 ml of acetone. When 0.4 ml of this stock solution was mixed with 40 liters of water to yield 0.01 mg of pesticide (0.01ppm).

During the chronic study, the fishes were fed with pellets on alternate days and the water was also renewed an hour after providing the food. Soon after the change of water, 0.4 ml of acetone and 0.4 ml of endosulfan stock solution were added to the control and experimental tanks, respectively. At the end of 50th day, the fishes were caught individually and used for the analysis. The blood was collected individually using micropipette by cutting the fish at the caudal peduncle. Then the fishes were sacrificed for collecting tissues like gill and liver. The biochemical analysis of total free sugars, free aminoacids, cholesterol and protein were estimated in the tissues following the procedure Roe (1955), Yem and Cocking (1955), Zarrow (1964) and Gornall *et al*. (1949) respectively. ALT and AST enzymes of blood and liver were analysed following the procedure of Wooten (1964). The histopathological studies on liver and gill were done after fixing the tissues in bouins fluid by standard procedure. Then the tissue was embedded in paraffin, sections were cut at 8µ and stained in hematoxilin and counter stained with esosin. The permanent slides were photomicrographed using canon T-70 Model camera. The serum level of T_3 and TSH of serum were determined using chemiluminescence immuno Assay Kit. The statistical analysis include standard deviation and mean difference t' test using Statsoft (1997).

Results and Discussion

The mean blood total free sugar level in control group was 60.17±8.84 mg 100 ml^{-1} whereas in experimental group the mean level increased to 117.71±10.14 mg 100 ml^{-1} (Table 1). The increasing trend is also observed in blood cholesterol level in that mean level of blood cholesterol increased from 51.78±5.61 mg 100 ml^{-1} to 76.17 ± 7.70 mg 100 ml^{-1} in experimental group. The mean blood for amino acids level increased from 18.54 ± 3.72 mg 100 ml^{-1} to 24.55 ± 1.08 mg 100 ml^{-1} in exposed fish. It is of interest to note that during this period the mean protein level decreased from 47.50 ± 9.49 to 35.04±7.75 mg 100 ml^{-1}. The mean levels of Aspartate transaminase (AST) and Alanine transaminase (ALT) were also increased significantly from 278.23± 31.02 to 715.71 ± 26.08 IU L^{-1} and from 33.92 ± 5.23 to 55.16 ± 10.03 IU L^{-1}. respectively. The mean level of serum T_3 increased from 0.53 ± 0.07 in control to 1.35 ± 0.1IU L^{-1} to treated group whereas the TSH level decreased from 0.48 ± 0.32 to 0.08 ± 0.13 µmol l^{-1} in treated group. (Taple 1) The mean liver total free sugar level increased from 15.34±1.13 to 15.53 ± 2.96 mg g^{-1} wet. wt. The mean level of cholesterol in liver was 49.08±7.42 mg g^{-1} wet.wt in control group and 28.92±4.08 mg g^{-1} wet.wt in treated group. The average liver aminoacids level increased from 94.14± 4.71 to 154.44 ±5.53 where as the mean liver protein level decreased from 167.74 ± 10.32 to 70.78 ± 5.89 mg g^{-1} wet.wt. respectively. In liver both AST and ALT levels increased in that the mean ALT level significantly increased from 86.04 ± 7.48 to 115.86 ± 7.31IU L^{-1} g^{-1} wet.wt in treated fishes. In the present study the following histopathological changes were observed in the gills of treated fishes, the tip of the primary lamellae were shapeless and seemed to be eroded; the secondary lamellae varied in shape widely. Debris of inter lamellar epithelium, damaged secondary lamellae and red blood cells were often observed between secondary lamellae and primary lamellae. Haematomas and aneuryama were also observed in some seconday lamellae (Fig. 1,2). The liver of control fish appeared to be normal in that the liver showed a continuous mass of hepatic cells, which are hexagonal in shape with more or less centrally placed nucleus. Enlargement of liver cells with vacuolation, histolysis and necrosis were observed after 50 days of exposure (Fig. 3,4).

The major carp *Labeo rohita* on exposure to sublethal concentration of endosulfan for a chronic period of 50 days showed the following changes like hyperglycemia of blood in the treated fish, which is in agreement with earlier reports of Grant and Mehrle (1973) in *Salmo gairdneri;* Mukhopodhya and Dehadrai (1980) in *Clarias batrachus*; Sastri and Siddique (1982) in C*hanna punctatus*; Simon *et al.* (1983) in *Cyprinus carpio* and Saravanan *et al.* (2000) in *Oreochromis mossambicus*. The liver total free sugar decreased significantly which is in agreement with the report of Tilak *et al.* (2005) in major carps. The reason for the significant decrease in tissue sugar may be due to its utilization to meet the insecticidal stress (Umminger, 1970). Due to the endosulfan induced stress, the blood cholesterol level increased significantly which is in agreement with the earlier reports of Kaur and Kaur (2006) in *Channa Panctatus* and Kumar *et al.* (2005) in C*irrhinus mrigala*. On the other hand the liver cholesterol level decreased as a result of the insecticidal stress. Wasserman *et al.* (1970), Gill and Pant (1983) and Nirmala and Eliza (2005) have reported enhanced catabolism of cholesterol with resultant hypercholestemia as the result of toxicity to insecticides and heavy metals. In normal animals cholesterol synthesis in liver is to made to operate at a level below maximum due to continuous absorption of cholesterol from the digestive track and to negative feedback effect (Sabine, 1977). But, when any xenobiotics entering the liver will be interfering with this feedback mechanism.

The endosulfan even at sublethal concentration affected the levels of free amino acids of blood and liver,in that in both the tissue the level increased significantly. Toxicant induced alteration in the quantity of free amino acids has been reported by Shaffer (1976) in *Leucipus cephalus* and Mehrle *et al.* (1971) in *Salmo giardneri.* In fact during this period the protein level decreased in both the tissues strongly suggesting the insecticide induced proteolysis to meet the increased energy demand as opined by the earlier authors like (Ramalingam and Ramalingam (1982), Jayantha Rao *et al.* (1984), Saravanan *et al.* (2000) and Kumar *et al.* (2005).

The present study also reveals the significant increase in aspartate amino transferase (AST) and Alanine amino transferase (ALT) in blood and liver. Such an insecticide induced increase in AST and ALT has been reported by earlier authors. Tilak *et al.* (2005) have reported an increase in enzyme levels in the three major carps to the organophosphate insecticide, chlorpyrifos. They have also suggested that the increase in tissue AST and ALT was the indication of incorporation of amino acids by way of amino transferase activities of these enzymes into Kreb's cycle to over come the acute stress posed by the endosulfan. This may be reason for the increased in the enzymes levels in tissues of exposed fishes as observed in the present study. As a result of chronic exposure

Table - 1: Changes in the levels of organic constituents of blood and liver of *Labeo rohita* (Hamilton) on exposure to Endosulfan for 50 days

Name of tissue	TFS		CHO		FAA		Protein		AST		ALT		T_3		TSH	
	(mg 100 ml^{-1})								($IU\ l^{-1}$)				(m mol l^{-1})			
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Blood	60.17	117.71*	51.78	76.17*	18.54	24.55*	47.5	35.04*	278.23	715.71*	33.92	55.16*	0.53	1.35*	0.48	0.32*
	±8.84	±10.14	±5.61	±7.70	±3.72	±1.08	±9.49	±7.75	±31.02	±26.08	±5.23	±10.03	±0.07	±0.1	±0.08	±0.13
	mg g^{-1} wet wt.								($IU\ L^{-1}\ g^{-1}$)							
Liver	11.34	15.53*	49.08	28.92*	94.14	154.44*	167.74	70.78*	49.23	50.02*	86.04	115.86*	-	-	-	-
	±1.13	±2.96	±7.42	±4.08	±4.71	±5.53	±10.32	±5.89	±6.45	±9.93	±7.48	±7.31	-	-	-	-

Mean ± Standard deviation, C = Control, E = Experiment , TFS = Total free sugar, FAA = Free amino acid, AST = Aspartate transaminase, ALT = Alanine amino transferase, TSH = Thyroid stimulating hormone, T_3 = Triodothyronine, S = Significant at * 0.05 and ** 0.01, NS = Non significant, CHO = Cholesterol

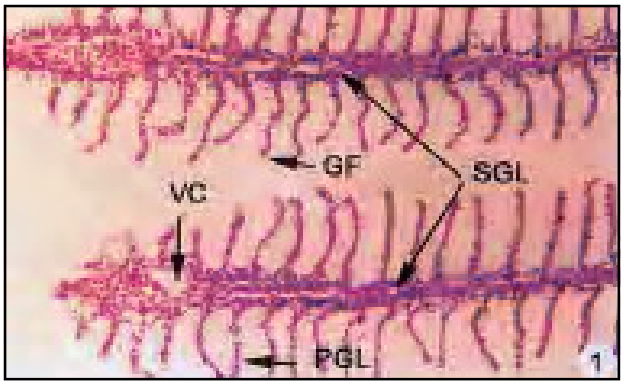

Fig. 1: T.S. of control gill of *Labeo rohita*, showing normal gill filament (GF) with primary and secondary gill lamellae (SGL and PGL). X 100

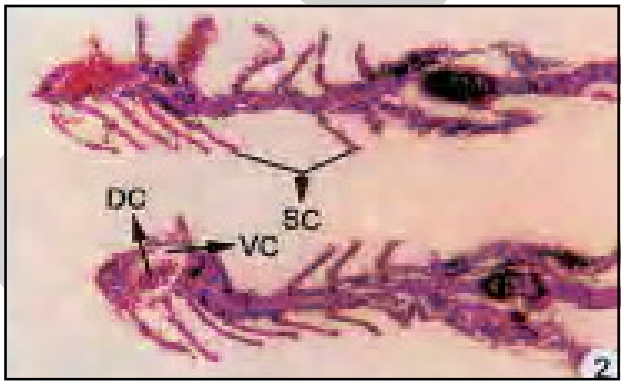

Fig. 2: T.S. of treated gill of *Labeo rohita* showing shrinkage(SC) disintegrated cells(DC) vacuolation (VC). X 100

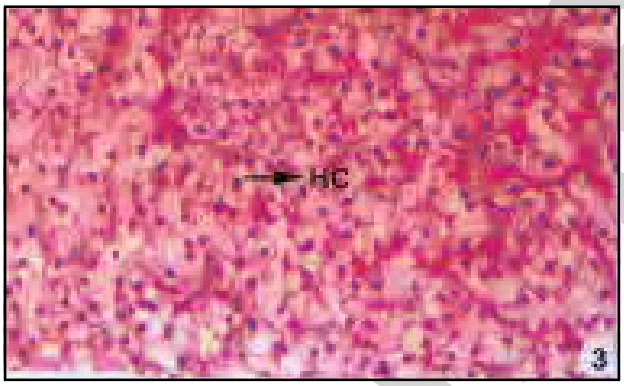

Fig. 3: T.S. of control liver of *Labeo rohita*, showing compact hexagonal cells(HC) X 100

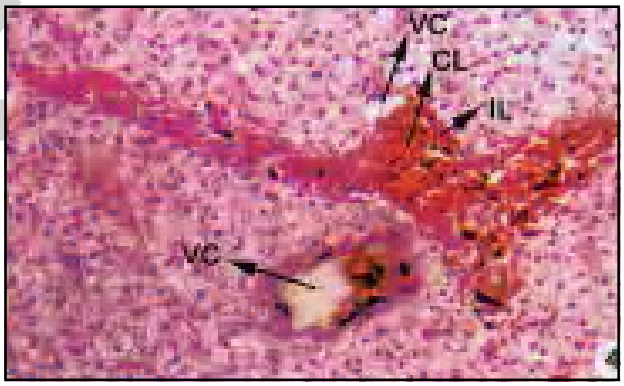

Fig. 4: T.S. of treated liver of *Labeo rohita* showing clowdy swelling and vacuolation (VC) of hepatic cells, destruction and separation of Islets of Langerhans (IL) X 100

(50 days), the serum T_3 (Triodothyronine) level increased but TSH level decreased in treated fish. The TSH is synthesized and secreted by the anterior pituitary in response to a negative feedback mechanism involving concentration of free plasma T_3. Such pesticide induced changes in circulatory thyroid hormones has been observed by earlier author, Sinha *et al.* (2005) in C*larias batrachus*. Further the authors have recognized the endosulfan being an endocrine disrupting chemical (EDC). Chakravarthy *et al.* (2005) have reported the effect of endosulfan on vitellogenesis and its modulation by different hormones in the vitellogenic cat fishes, C*larias batrachus.*

The gills, being delicate structure, get affected easily if the surrounding media is contaminated (Roy and Munshi, 1991). This is well reflected in the present study in that the gill of treated fish showed detachment of epithelial layer with reduction of interlamellar space and fusion of secondary lamellae. Such changes have also been reported by Dhanapakiam *et al.* (1998) in Channa punctatus exposed to industrial effluents. Further, the treated fish liver showed shrinkage (SC), disintegration (DC) and vacuolation (VC) ultimately resulting in necrosis when compared with normal hexagonal cells found in the control liver. This is in agreement with earlier reports of Saravanan *et al.* (2003) in

Oreochromis mossambicus and Nagarajan and Arunadevi (2006) in *Labeo rohita.*

Acknowledgments

We thank Dr. M. Sheik Mohamed, Principal and Dr. B.M. Gulam Mohideen, HOD of Zoology, Jamal Mohamed College, Tiruchy for the encouragement and facilities provided.

References

Baskaran, P., R. Rajeswari, S. Palanichamy and S. Muniandi: Effects of metacid (organo phosphate pesticide) on the physiology and biochemistry of *Oreochromis mossambicus* (*Ed*.: Gopal, B.V. Asthana). Aquatic science in India. pp. 299-218 (1991).

Capkin, E., I. Altinok and S. Karahan: Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. *Chemosphere*, **64**, 1793-1800 (2006).

Chakravarty, Geetanjali, R.P. Goyal, Shipra Sharma and Anjali Sharma: Haematological changes induced by a common non-permitted food colour Malachite Green (MG) in swiss albino mice. *Ind. J. Environ. Sci.*, **92**, 113-117 (2005).

Coimbra, A.M., M.R. Almeida, L. Silva and M.A. Reis-Henriques: Some effects of Endosulfan on Nile Tilapia (*Oreocromis niloticus*) Proc. 21st C.E.C.E. (Germany). pp. 75-79 (2002).

Dhanapakiam, P., V.K. Ramasamy and R. Sampoorani: A study on the histological changes in gills of Channa punctatus in Cauvery river water. *J. Envrion. Biol.*, **19**, 265-269 (1998).

Edwards, C.A.: Environmental pollution by pesticides Pleunum press, London. p. 250 (1973).

Gill, T.S. and P.C. Pant: Cadmium toxicity; Inducement of changes in blood and tissue metabolities in fish. *Toxicol. Letters*, **18**, 195-200 (1983).

Gornall, A.G., C.J. Brandnwill and M.M. David: Determination of total serum protein by means of biuret reaction. *Biol. Chem*., **177**, 751-766 (1949).

Grant, B.F. and P.M. Mehrle: Endrin toxicosis in rainbow trout (*Salmo gairdneri). J. Fish. Res. Bd. Can.*, **30**, 31-40 (1973).

Gulfer, B., M. Coskun, T. Kayis and I. Emre: Impact of organophosphorus insecticide, malathion on the progeny sex ratio of *Pimpla turionellae* L. *J. Environ. Biol.,* **30**, 727-730 (2009).

Jayantha Rao, K. Azhar Baig and K. Ramamurthy: Effects of a systemic pesticide phosphonidon some aspects of freshwater fish *Tilapia mossambica. Ind. J. Environ. Hlth.*, **26**, 60-64 (1984).

Kaur, A. and K. Kaur: Impact of nickel-chrome electroplating effluent on the protein and cholesterol contents of blood plasma of *Channa punctatus* (Bl.) during different phases of the reproductive cycle (2006).

Kumar Pradip, Y.G. Gupta, Vishal Tiwari, Shakti Tiwari and Ajay Singh: Haematological and biochemical abnormalities in *Cirrhinus mrigala* (Ham.) induced by lead. *J. Ecophysiol. Occupl. Hlth.*, **5**, 213-216 (2005).

Leonard, A.W., R.V. Hyne, R. Lim, F. Pablo and P. Van den Brink: Riverine endosulfan concentrations in the Namoi river, Australia: Link to cotton field runoff and macroinvertebrate population densities. *Environ. Toxicol. Chem.*, **19**, 1540-1551 (2000).

Lans, M.C., C. Spiertz, A. Brouwer and J.H. Koemam: Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBS, PCDDs and PCDFs. *Environ. J. Pharmacol.*, **270**, 109-136 (1994).

Malla Reddy, P. and M.D. Bashamohideen: Toxic impact of fenvelerate on the protein metabolism in the branchial tissue of a fish *Cyprinus carpio. Cur. Sci.*, **57**, 211-212 (1988).

Mehrle, P., D.L. Stalling and R.A. Bloomfield: Serum amino acids in rainbow trout Salmo Giardneri as affected by DDT and Dieldrin. *Comp. Biochem. Physiol.*, **38**, 273-377 (1971).

Mukhopaday, P.K. and P.V. Dehadrai: Biochemical changes in the air breathing cat fish *Clarius batrachus* (Linn) exposed to malathion. *Environ. Pollut., (Ser. A)*, **22**, 149-158 (1980).

Nagarajan, K. and P.S. Aruna Devi: Histological changes in Indian freshwater carp *Labeo rohita* due to distillery effluent. *Nat. Environ. Pollut. Technol.*, **5**, 23-27 (2006).

Nirmala Arc and J. Eliza: Effect of phosphamidon on the carbohydrate, protein and lipid contents in *Cirrhina mrigala. Asian J. Micrbiol., Biotechnol. Environ. Sci.*, **7**, 813-814 (2005).

Power, O.M., N.P. Elias, S.J. Richardson, J. Mendes, C.M. Soares and C.R.A. Somtos: Evolution of the thyroid hormone-binding protein, tronthyretin. *Gen. Comp. Endocrinol.*, **119**, 241-255 (2000).

Ramalingam, K. and K. Ramalingam: Effects of sub-lethal levels of DDT, Malathion and mercury on tissue protein of *Sarotherodon mossambius* (peters). *Proc. Ind.. Acad. Sci. (Animal Sci.)*, **91**, 501-505 (1982).

Ramasamy, M.: A shift in metabolic pathway of *Sarotherodon mossambicus* (peters) exposed to Thiodon (Endosulfan). *Proc. Ind. Acad. Sci. (Anim., Sci.)*, **95**, 55-61 (1987).

Ramaneswari, K. and L.M. Rao: Influence of endosulfan and monocrotophos exposure on the activity of NADPH cytochrome C reductase (NCCR) of *Labeo rohita* (Ham). *J. Environ. Biol.,* **29**, 183-185 (2008).

Roe, J.H.: The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem*., **212**, 335-343 (1955).

Roy, P.K. and J.S. Datta Munshi: Malathion induced structural and morphometric changes of gills of a fresh water major carp, Cirrhinus mrigala (Ham). *J. Envrion. Biol.*, **12**, 79-87 (1991).

Sabine, J.R.: Cholesterol – Mec Dekkar Inc: New York and Basel. p. 55-75 (1977).

Saravanan, T.S., M. Aneez Mohamed and R.Harikrishnan: Studies on the chronic effects of endosulfan on blood and liver of *Orechromis mossambicus. J. Ecol. Res. Biocon.*,**1**, 24-27 (2000).

Saravanan, T.S., R. Chanadrasekar and M. Aneez Mohamed: Endosulfan induced changes in the liver and ovary of freshwater fish, Oreochromis mossambicus (Trewaves) Malaysian. *J. Sci.*, **22**, 29-34 (2003).

Sastry, K.V. and A.A. Siddique: Effect of endosulfan and quinolphos on intestinal absorption of glucose in freshwater mussel, *Channa punctatus. Toxicol. Left (ADMST),* **12**, 289-294 (1982).

Shaffer, R.: The effects of pollutants on the face aminoacids contents in the fish, *Leuciscus cephalus.*(L). *Rev. Biol.*, **59**, 385-404 (1967).

Simon, L.M., J. Memcshok and L. Boross: Studies on the effect of paraquat on glycogen metabolism in liver of common carp. *Cyprinus carpio* (L). *Camp. Biochem. Physiol.*, **75C**, 167-169 (1983).

Sinha, N., T.P. Lal and B. Singh: Pesticides induced changes in circulating Thyroid hormones in the freshwater catfish *Clarias batrachus*. Demonstrates the importance of environmental awareness In Endocrinology. *The Int. J. Endocrinol.*, **18**, 26-34 (2005).

Singh, S. and R.N. Mishra: Occurrence of organochlorine pesticides residues in Kuano river of eastern Uttar Pradesh. *J. Environ. Biol.,* **30**, 467-468 (2009).

Statsoft, P.: Statistica for Windows. Version 5.1. Computer program manual. Statsoft, Tulsa, OK (1997).

Tilak, K.S., K. Veeraiah and D. Koleswara Rao: Biochemical changes induced by chlorpyrifos, an organophosphate compound in sublethal concentrations to the freshwater fish *catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. *J. Environ. Biol.*, **26**, 341-347 (2005).

Tilak, K.S. and R. Swarna Kumari: Acute toxicity of Nuvan®, an organophosphate to freshwater fish *Ctenopharyngodon idella* and its effect on oxygen consumption. *J. Environ. Biol.,* **30**, 1031-1033 (2009).

Umminger, B.L.: Studies on super cooled killi fish *Fundulus heteroditus* III carbohydrate metabolism and survival at sub zero temperature. *J. Exp. Zool.*, **173**, 159-174 (1970).

Wasserman, D., H. Wassermann and I. Aronovski: The effect of *orgnochlorine* insecticides on serum cholesterol level in people occupationally exposed. *Bull. Environ. Contam. Toxical.*, **5**, 368-372 (1970).

Wooten, I.D.P.: Microanalysis in medical biochemistry A Churchill Ltd., London W.I. (1964).

Yem, E.M. and E.C. Cocking: The determination of amino acid with ninhydrin. *Analyst.*, **80**, 209-213 (1995).

Zarrow, M.X. Yochim and J.H. Mccarthy: Experimental endocrinology: A source book of basic techniques Acd. Press. New York. p. 206 (1964).