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Impact of biochemical parameters of freshwater fish *Cirrhinus mrigala* exposed to polycyclic aromatic hydrocarbon effluent

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Abstract

The purpose of this study was to estimate the acute toxicity of Polycyclic Aromatic Hydrocarbon (PAH) effluent on *Cirrhinus mrigala* and to evaluate the lethal levels. The 120 hrs median lethal concentration of Polycyclic Aromatic Hydrocarbon (PAH) Effluent were found to be 20ppt for *Cirrhinus mrigala*. Further experiments were proceeded with sub lethal concentration of ($1/10^{th}$ conc. of LC_{50}) Polycyclic Aromatic Hydrocarbon (PAH) Effluent which were evaluated from the LC_{50} value. After treatment the fishes were reared in ideal condition, then sacrificed dissected at different predetermined interval during the accumulation period, (i.e.) 1^{st} day to 20^{th} day, during the depuration period from 1^{st} day to 15^{th} day for *Cirrhinus mrigala* in Polycyclic Aromatic Hydrocarbon (PAH) Effluent treatment for Biochemical study. The biochemical studies carried out under sub lethal ($1/10^{th}$ concentration of LC_{50}) in gill, liver and muscle.

Keywords: Polycyclic Aromatic Hydrocarbon (PAH) Effluent, *Cirrhinus mrigala*, protein, carbohydrate, amino acid and lipid.

Introduction

Aquaculture contributes to the livelihoods of the poor through improved food supply, employment and income opportunities. The Fisheries and Aquaculture Department (FAO) has defined the role of fish aquaculture which contributes to national food self-sufficiency through direct consumption and through trade and exports.

In recent years, due to industrialization water pollution by discharging of effluents from various industries caused serious problems in many rivers and pounds. Most of the industries discharge their waste without proper treatment which cause change in physical, chemical and biological characteristic of water. The release of untreated industrial effluents into aquatic system seriously affects aquatic biota and their production. The effluents released by various industries are causing a lot of problems and disposal involves a complicated task.

Pollution of water is an important dimension of environmental degradation. The disposal of the industrial and agricultural wastes directly into the aquatic medium burdens the ecosystem and stresses

the need to analyse the concentration of these substances in the medium as well as in the organisms. The effluent water discharged from industries and agricultural fields containing toxic substances like heavy metals and pesticides. They, find their way to aquatic ecosystems, through surface runoff and discharge wastages into streams and rivers (Patil and Dhande, 2000).

Fish provide a good source of readily digested high quality animal protein, fat, mineral and vitamins specially vitamin A, D and E. Also fish plays important roles in the prevention and management of many human diseases such as heart disorders, neurological diseases, mood swings and when fish is substituted for beef, the nitrogen is utilized better resulting in a decreased excretion of uric acid in the urine (Thilsted and Roos, 1999 and Conquer and Holub, 2002). Fish protein produces a good influence on the assimilation of magnesium, phosphorous and iron. Fat in aquatic organisms are associated with a variety of function reflecting special biochemical and environmental conditions, fats are the major metabolic reserve in most fish (Lovell, 1989).

Carbohydrates, proteins and lipids play a major role as energy precursors for fishes under stress conditions (Idler and Clemens, 1959). Biochemical parameters were often used when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and toxic substances (Osman *et al*., 2001). In general biochemical profiles in fish and other aquatic organisms under stress serve as important bioindicators in the monitoring of aquatic environment.

Materials and Methods

Collection of Experimental Animal

The fish, *Cirrhinus mrigala* (Length 10±0.002cm, Weight 9±0.003 g) fresh water food fish were segregated and procured from Karanthai (Golden Fish Farm) farm, Thanjavur, Tamil Nadu, India and were transported in aerated polythene bags to the laboratory. The fishes were acclimatized to lab condition for 3 days before treatment with Polycyclic Aromatic Hydrocarbon (PAH) Effluent.

Evolution of Lc_{50} employing acute toxicity test

At first tentative experiment were conducted to fix the minimum concentration of Polycyclic aromatic hydrocarbon effluent to obtain maximum mortality for *Cirrhinus mrigala* over 120 hour's duration. After confirming the minimum concentration, identified size of *Cirrhinus mrigala* were placed in different tubs (each group consists of 6 animals in 10 litre capacity plastic tubs) and exposed to different concentration of Polycyclic aromatic hydrocarbon effluent which ranges from 10 ppt – 30 ppt at an interval of 5 ppt for *Cirrhinus mrigala* for a period of 120 hour. In addition to that a control was also maintained simultaneously.
The biochemical components level were carried out under sub lethal ($1/10^{th}$ conc. of LC_{50}) concentration of Polycyclic Aromatic Hydrocarbon (PAH) Effluent in various tissues (Gill, Liver and Muscle).

The total protein was estimated following the method of Lowery *et al.*, (1951). Carbohydrate was estimated by the method of Dubois *et al.,* (1950). Free Amino Acids was estimated by the Ninhydrin method Yemm and Cocking (1955). Lipids were estimated according to the method of Barnes and Blackstock (1973).

Results

Acute toxicity Lc_{50}

The 120 hrs median lethal concentration of Polycyclic aromatic hydrocarbon effluent on *Cirrhinus mrigala* was 20 ppt. During the experimental period the fishes were restless aggressive and have the tendency to leap out of the tubs (struggle for existence). This may be due to the suffocation out of oxygen deficiency. Secretion of mucus in the gill chamber should the lesions in gills of fishes. The 120 hrs median lethal concentration of Polycyclic aromatic hydrocarbon effluent were found to be 20 ppt for *Cirrhinus mrigala.*

Protein

Fig. 1 & Table 1 represent the level of protein in gill, liver and muscles of *Cirrhinus mrigala* in response to polycyclic aromatic hydrocarbon effluent. During the accumulation period ($1/10^{th}$ Conc. of LC_{50}) i.e. from 1^{st} day to 20^{th} day the mean protein was found to be decreased. During the depuration period, the mean of proximate values were found to be increased from 1^{st} day to 15^{th} day.

Fig 1: The Level of Protein content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

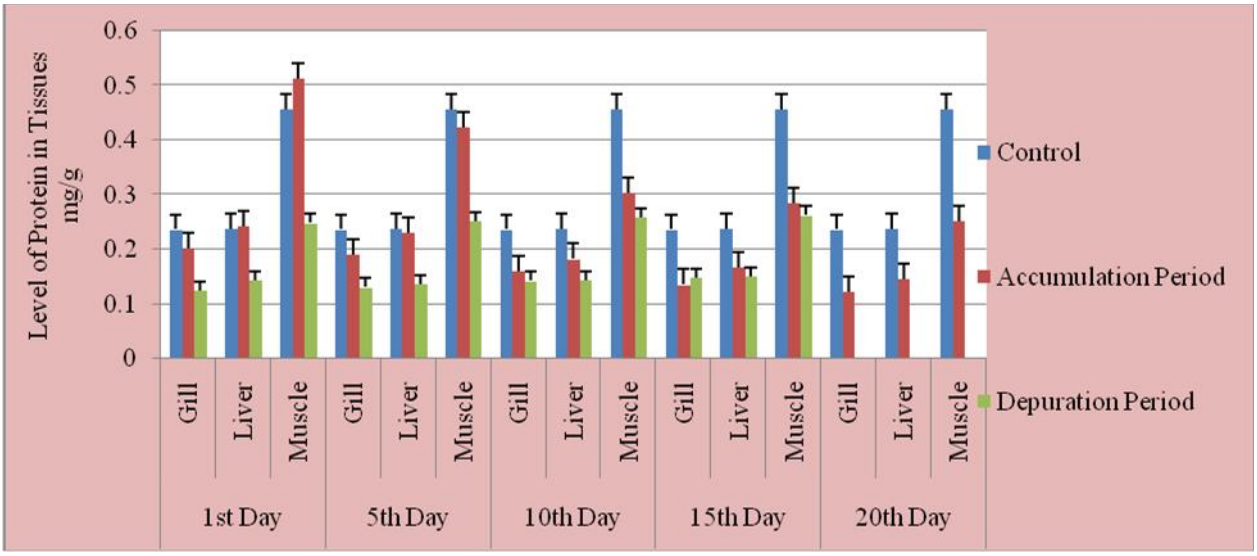

Table 1: The Level of Protein content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

S.No	Days	Tissues	Control (mg/g)	Experiments	
				Accumulation period (Mean ± SD) (mg/g)	Depuration period (Mean ± SD) (mg/g)
1	1st Day	Gill	0.236±0.002	0.202±0.002	0.124±0.002
		Liver	0.237±0.002	0.242±0.002	0.143±0.002
		Muscle	0.456±0.002	0.513±0.002	0.248±0.002
2	5th Day	Gill	0.236±0.002	0.190±0.002	0.130±0.000
		Liver	0.237±0.002	0.230±0.002	0.136±0.003
		Muscle	0.456±0.002	0.423±0.003	0.252±0.002
3	10th Day	Gill	0.236±0.002	0.160±0.002	0.142±0.002
		Liver	0.237±0.002	0.182±0.002	0.143±0.002
		Muscle	0.456±0.002	0.304±0.003	0.258±0.002
4	15th Day	Gill	0.236±0.002	0.135±0.002	0.148±0.002
		Liver	0.237±0.002	0.167±0.002	0.151±0.001
		Muscle	0.456±0.002	0.285±0.002	0.262±0.002
5	20th Day	Gill	0.236±0.002	0.122±0.002	
		Liver	0.237±0.002	0.145±0.002	
		Muscle	0.456±0.002	0.251±0.001	

Amino Acids

Fig. 2 & Table 2 represent the level of amino acids in gill, liver and muscles of *Cirrhinus mrigala* in response to polycyclic aromatic hydrocarbon effluent. During the accumulation period (1/10th Conc. of LC_{50}) i.e. from 1st day to 20th day the mean amino acids was found to be increased. During the depuration period, the mean of proximate values were found to be decreased from 1st day to 15th day.

Fig 2: The Level of Amino acids content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

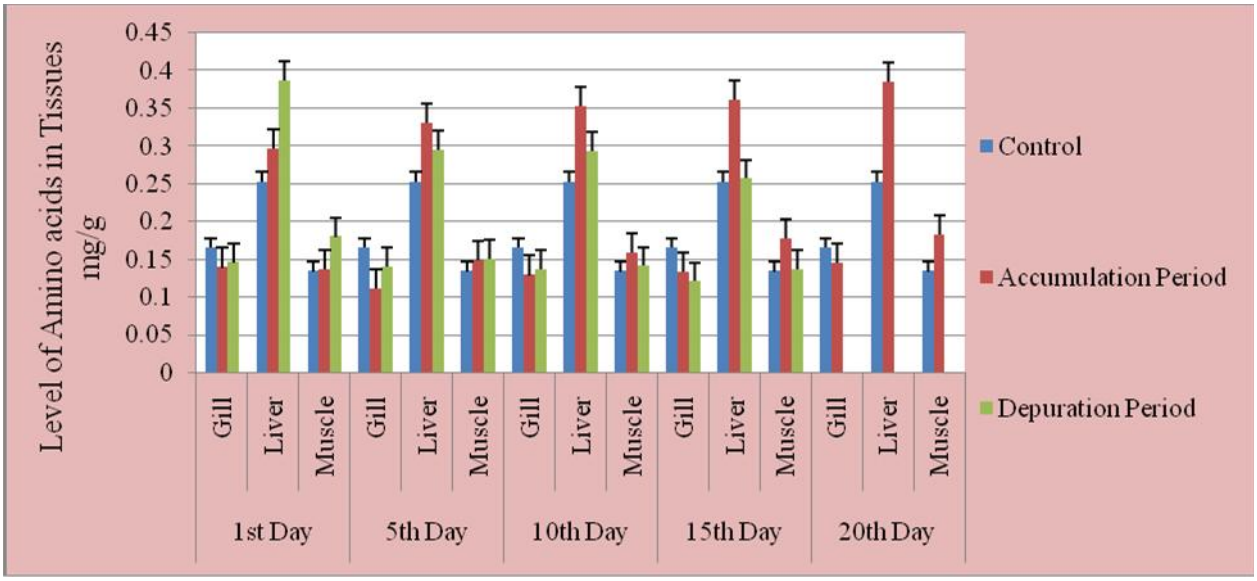

Table 2: The Level of Amino acids content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

S.No	Days	Tissues	Control (mg/g)	Experiments	
				Accumulation period (Mean ± SD) (mg/g)	Depuration period (Mean ± SD) (mg/g)
1	1st Day	Gill	0.165±0.002	0.139±0.000	0.146±0.002
		Liver	0.252±0.002	0.297±0.001	0.387±0.000
		Muscle	0.134±0.003	0.117±0.002	0.180±0.002
2	5th Day	Gill	0.165±0.002	0.111±0.001	0.121±0.001
		Liver	0.252±0.002	0.331±0.001	0.257±0.002
		Muscle	0.134±0.003	0.148±0.002	0.150±0.002
3	10th Day	Gill	0.165±0.002	0.129±0.001	0.137±0.003
		Liver	0.252±0.002	0.353±0.003	0.293±0.002
		Muscle	0.134±0.003	0.159±0.001	0.137±0.004
4	15th Day	Gill	0.165±0.002	0.133±0.003	0.140±0.002
		Liver	0.252±0.002	0.331±0.001	0.295±0.003
		Muscle	0.134±0.003	0.178±0.002	0.141±0.001
5	20th Day	Gill	0.165±0.002	0.145±0.001	
		Liver	0.252±0.002	0.385±0.003	
		Muscle	0.134±0.003	0.182±0.002	

Carbohydrate

Fig. 3 & Table 3 represent the level of carbohydrate in gill, liver and muscles of *Cirrhinus mrigala* in response to polycyclic aromatic hydrocarbon effluent.

During the accumulation period (1/10th Conc. of LC_{50}) i.e. from 1st day to 20th day the mean carbohydrate was found to be decreased. During the depuration period, the mean of proximate values were found to be increased from 1st day to 15th day.

Fig 3: The Level of Carbohydrate content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

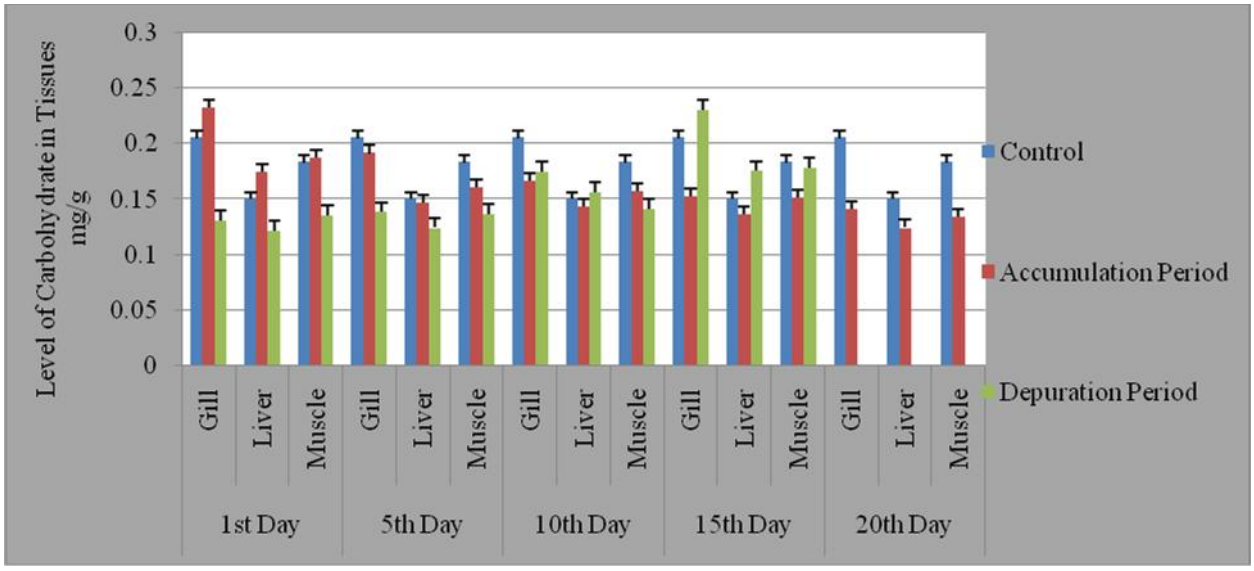

Table 3: The Level of Carbohydrate content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

S.No	Days	Tissues	Control (mg/g)	Experiments	
				Accumulation period (Mean ± SD) (mg/g)	Depuration period (Mean ± SD) (mg/g)
1	1st Day	Gill	0.205±0.002	0.191±0.001	0.230±0.002
		Liver	0.150±0.003	0.146±0.002	0.175±0.003
		Muscle	0.183±0.003	0.160±0.003	0.135±0.002
2	5th Day	Gill	0.205±0.002	0.152±0.002	0.174±0.002
		Liver	0.150±0.003	0.143±0.002	0.156±0.002
		Muscle	0.183±0.003	0.151±0.004	0.136±0.003
3	10th Day	Gill	0.205±0.002	0.141±0.003	0.138±0.000
		Liver	0.150±0.003	0.124±0.002	0.124±0.002
		Muscle	0.183±0.003	0.187±0.002	0.121±0.000
4	15th Day	Gill	0.205±0.002	0.166±0.002	0.130±0.002
		Liver	0.150±0.003	0.136±0.003	0.121±0.003
		Muscle	0.183±0.003	0.157±0.002	0.118±0.002
5	20th Day	Gill	0.205±0.002	0.232±0.002	
		Liver	0.150±0.003	0.174±0.003	
		Muscle	0.183±0.003	0.134±0.001	

Lipids

Fig. 4 & Table 4 represent the level of lipids in gill, liver and muscles of *Cirrhinus mrigala* in response to polycyclic aromatic hydrocarbon effluent. During the accumulation period (1/10th Conc. of LC_{50}) i.e. from 1st day to 20th day the mean lipid was found to be decreased. During the depuration period, the mean of proximate values were found to be increased from 1st day to 15th day.

Fig 4: The Level of Lipid content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

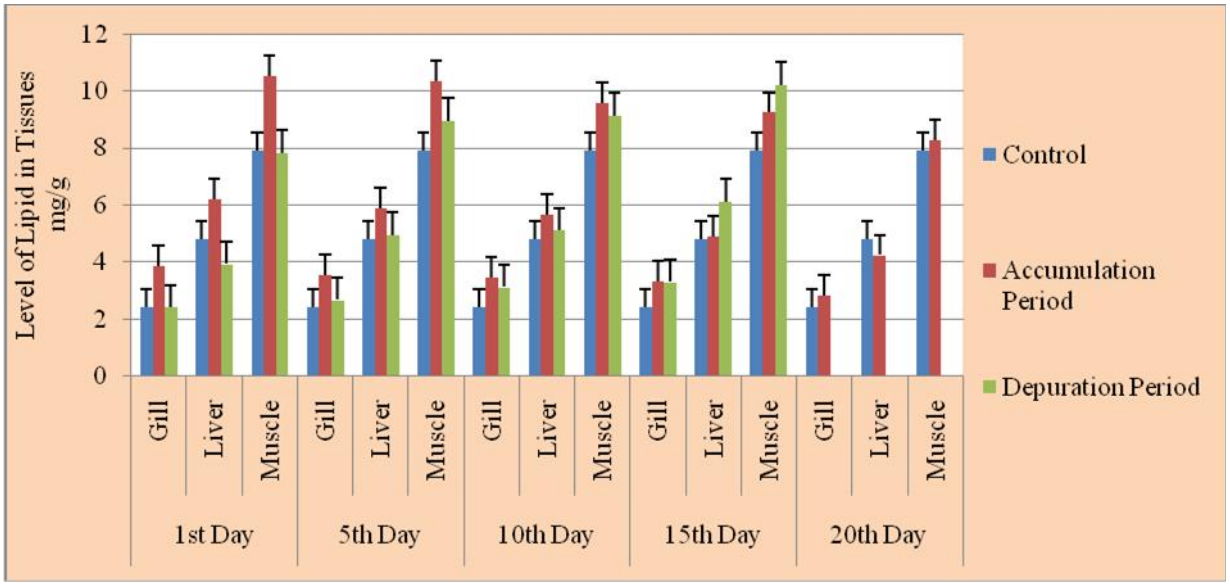

Table 4: The Level of Lipid content alterations in tissue of *Cirrhinus mrigala* during Accumulation and depuration period (1/10th Conc. of LC_{50}) of Polycyclic Aromatic Hydrocarbons.

S.No	Days	Tissues	Control (mg/g)	Experiments	
				Accumulation period (Mean ± SD) (mg/g)	Depuration period (Mean ± SD) (mg/g)
1	1st Day	Gill	2.437±0.002	2.826±0.002	3.303±0.003
		Liver	4.823±0.003	4.913±0.003	3.928±0.002
		Muscle	7.923±0.002	8.271±0.002	7.833±0.003
2	5th Day	Gill	2.437±0.002	3.866±0.002	3.121±0.002
		Liver	4.823±0.003	5.676±0.002	5.017±0.003
		Muscle	7.923±0.002	9.572±0.002	9.124±0.002
3	10th Day	Gill	2.437±0.002	3.335±0.005	2.671±0.003
		Liver	4.823±0.003	4.245±0.005	4.944±0.003
		Muscle	7.923±0.002	10.538±0.002	8.956±0.002
4	15th Day	Gill	2.437±0.002	3.562±0.002	2.405±0.002
		Liver	4.823±0.003	5.904±0.004	6.102±0.001
		Muscle	7.923±0.002	9.238±0.002	10.212±0.004
5	20th Day	Gill	2.437±0.002	3.444±0.004	
		Liver	4.823±0.003	6.211±0.002	
		Muscle	7.923±0.002	10.344±0.003	

Discussion

In the present study the sub lethal (1/10th conc. of LC_{50}) concentration of polycyclic aromatic Hydrocarbon effluent exposed to *Cirrhinus mrigala*, the value of Protein, Amino acids, Carbohydrate and Lipid level are Shown in (Fig. 1,2,3,4). Similar Observation were made by Singh and Bhati (1994) and Khare and Singh (2002).

The decreased protein content was observed throughout the exposure period. The two sublethal exposure results show the decrease in protein content and it depend upon the concentration. The toxicity of dimethoate also showed a direct correlation with the concentration and time exposure.

Amino acids are essential intermediate substances in the process of protein synthesis and its degradation products appear in the form of various nitrogenous compounds. The present study revealed that, free amino acids in the liver, kidney, gills, muscle showed a continuous reduction with both lethal and sub lethal concentrations.

The changes in biochemical constituents such as glycogen, carbohydrates, proteins, free amino acids and lipids are important to indicate the susceptibility of organ systems to toxicant by changing their function (Verma *et al.,* 1983).

The Carbohydrates are the main source of energy in the cells and play a vital role in the cellular metabolism by acting as fuel and providing energy to the body cells. In the present study, maximum carbohydrate depletion was observed in liver and muscle during lethal and sub lethal concentrations. The changes in carbohydrate metabolism that would meet the changing energy demands may be subjected to stress (Lacerda and Swaya 1986; Santos and Nay 1987).

Lipid form an essential component of protoplasm and during extreme starvation considerable amount can be extracted from tissues. Lipids are also the strong form of energy like glycogen. The lipid levels decreased in the tissues of the fish exposed to the sublethal and lethal concentration of toxicant.

In the present study, lipid had been found to decrease in all the tissues studied at short term exposures. The lipid content decreased in all the test tissues during the experimental period for toxicants studied justify the utilization of energy store house to meet the necessity of more energy for detoxification process and also to balance the hindrance of normal metabolism (Remia *et al*., 2008) similar observation was made in *Glarsogobiusgiuris* (Sreenivasa, 2002) has showed decreased lipid content in *Tilapia mossambica* on exposed to atazine.

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