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Biochemical Alteration in Themuscle, Liver, Kidney and Brain of a Fresh Water Fish, *Catlacatla* (Ham.) Exposure of a Heavy Metal Toxicant Ferrous Sulphate

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ABSTRACT

The present study evaluates toxicity of metal, ferrous sulphate and its impact on biochemical constituents like total sugars, total proteins, and total lipids in the fresh water edible carp *Catla catla*. Biochemical constituents weredetermined by standard biochemical procedures in the four tissues i.e., muscle, liver,brain and kidney of unexposed (Control) healthy fish and the fish exposed to 96-hr LC^{50} (Lethal) and sub-lethal concentration of the lethal dose for zero day, 4^{th} day and 7^{th} days of exposure of heavy metal, ferrous sulphate. The elevatedlevels of sugars are apparently indicative of the organism's response to the toxicant stress.

Key words: Toxicity, Heavy metal, Ferroussulphate, Biochemical, *Catlacatla.*

INTRODUCTION

Human population growth and industrial development have been the major causes of coastal contamination around the world during recent years (Caussy *et al.,* 2003).The subsequent accumulation of xenobiotic compounds in sediment, seawater or prey organisms. Another type of anthropogenic impact is manifested by pollution of the tributaries where there is an inflow of wastewater from household and industrial facilities, fecal pollution, detergents and other chemical waste material. The ecological effects of pollutants in aquatic ecosystems and their bioavailability and toxicity are closely related to species distribution, both in the solid and the liquid phase of the aquatic ecosystem. Pollutants are transferred to the plankton, aquatic plants, mollusks and fish. A wide range of microscopic and macroscopic animals and plants live in and on bottom sediments of the aquatic ecosystems, and a great number of these organisms ingest organic matter form these sediments (Mosisch and Arthington, 2001).The poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental long-term effect may result in the incidence of poisoning of fish and other aquatic life forms (Jyothi and Narayan, 1999).

Fishes being one of the most ancient from of aquatic life, as a food item have been reported to have a twofold nutritional advantage of being able to provide high proportions of their dry weight as proteins of relatively good quality due to the presence of essential amino acids and also being easily digestible unlike those of beef and other livestock because of low collagen. Pollution of aquatic environment from industrial, domestic and agricultural waste has exposed these important aquatic organisms to contaminants which not only endanger their lives but also eventually enter the food chain leading to serious public health hazards.

Catla catla is one of the major fresh water carps native to India, Bangladesh,Myanmar, Nepal, Pakistan and introducedin many other countries as exotic species. *C.catla* is a very rich source of proteins and is reported to attain a maximum size of 182 cm and weight of about 50 Kilograms (these figures vary). It is a surface and mid-water feeder,

mainly omnivorous with juveniles feeding on aquatic and terrestrial insects, detritus and phytoplankton. It has a characteristically large, upturned mouth with a prominent protrudingjaw. Because of its high nutritive value, it is a highly priced food fish and of great demandin the market.

Rapid industrialization lead to contamination of natural waters with metals due to dumping of untreated wastes in the aquatic habitats, causing deleterious effects to fish (Javed, 2004).The accumulation of metals in an aquatic environment has direct consequences to man and to the ecosystem also. Intensive activity in industrial and agricultural sectors has inevitably increased the levels of heavy metals in natural waters (Jordao *et al.,* 2002). Heavy metals play a major role among pollutants of environmental concern (Singer *et al.,* 2005). Heavy metals are serious pollutants of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms (Veena *et al.,*1997).Fish constitute a valuable commodity from the standpoint of human consumption; aquatic pollution undoubtedly affects fish health and survival. Heavy metals are common pollutants of the aquatic environment because of their persistence and tendency to concentrate in aquatic organisms (Hoo *et al.* 2004; Ayas *et al. 2007*; Kumar and Achyuthan, 2007, Shukla *et al.* 2007, Verma and Srivastava, 2008a,b and Srivastava and Verma, 2009).

Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (More *etal*., 2003). Aquatic organisms have the ability to accumulate heavy metals from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and discharges of wastewater (Labonne *et al*., 2001; Goodwin *et al*., 2003).Therefore, accumulation of heavy metals in aquatic organisms can pose a long lasting effect on biogeochemical cycling in the ecosphere. Heavy metalscan also adversely affect the growth rate in major carps (Hayat *et al*., 2007)Fish are often at the top of aquatic food chain andmay concentrate large amounts of some metals from the water (Mansour and Sidky, 2002).

Heavy metals are being passed on into aqueous environments through industrial processes, sewage disposal, soil leaching and rainfall.The concentrations of these heavy metals are sub-lethal or lethal to aquatic organisms when the duration of exposure to these metals are prolonged (Eisler and Gardener, 1973). It is well documented that effect of heavy metals are dependent upon the physical and chemical conditions of the environment especially water salinity, hardness, pH and dissolved oxygen and can act synergistically.

Heavy metals from several industrial mining and other sources enormously contribute to the pollution problem in rivers and streams resultingthe adverse impacts on biota including fish. Fish population is generally considered very sensitive to all kinds of environmental changes to which it is exposed as they are exclusively aquatic with external mode of fertilization. Certain stages in the life cycle of fresh water fish are more susceptible to environmental and pollution stresses (Von Westernhagan, 1988). Aquatic pollution causes significant harm to fisheries and aquaculture industries. The changes of physical, chemical and biological parameters of water alter the behaviour of fishes besides causing mortality (Singh and Singh, 2006).

Although, trace metals are essential for normal physiological process, abnormally high concentrations can be toxic to aquatic organisms (Javed, 2003). Heavy metals being non-biodegradable primarily necessitate knowledge on their uptake, distribution and persistence in tissues of organisms (Lewis *et al.,* 2004).

Iron is the fourth most abundant, by weight, of the elements that make up the earth's crust. Common in many rocks, it is an important component of many soils, especially clay soils where it is usually a major constituent. At certain concentrations, iron can also be toxic to aquatic life. The most common form of iron in solution in anoxic groundwater is the ferrous ion. Soluble ferrous iron typically enters surface waters from ground waters or mines (or waste deposits and tailings) when they are pumped or drained. Ferrous iron can also be found in the deep waters of stratified lakes with anaerobic hypolimnia.

For this study, a heavy metal , Ferrous sulphate were chosen as the toxicants and one of the commercially important Indian major carps, *Catla catla* selected as test organism to assess the toxic and pathological effects of the toxicants in an independent manner on the target animal.

MATERIALS AND METHODS

Catla fingerlings of the same size (8 - 10 cm in length and 6 - 8 g in weight) were procured from private culture ponds and brought to the laboratory in oxygen packs. The fish were acclimatized and maintained in ferro-cement tanks (3'L x 2'W x 2'H) filled with bore well water. The stock fish were fed with pelleted feed prepared with tapioca powder, groundnut oil cake, rice bran and mineral mixture (Omprakasam & Manohar, 1991) at 5% body weight in two split doses. Feeding was stopped 24 hr prior to experimentation.

Apparently healthy fish were selected for experiments and maintained in disinfected glass aquarium tanks (2'L x 1'W x 1'H) filled with water at the rate of 2 litres per fish. During the period of study the room temperature fluctuated from 29º to 32ºC. The dissolved oxygen content of water used for the study was 4.4 to 4.8 ml / litre and salinity of 0.82 - 0.85 ppm. The pH of water was in the range of 7.2 - 7.4.

Ferrous sulphate 'AR' Grade was used for LC_{50} determination. The fish without any structural, behavioral and clinical symptoms were chosen for experiments, after careful observation. Fish were divided into groups of ten each and exposed to a heavy metal compound (Ferrous sulphate) independently. To determine LC_{50} for the different groups based on the cumulative percentage mortality at the end of 96hr of experimentation the standard graphic method was followed.One fourth of LC_{50} values obtained from the above experiments was taken as the sub lethal concentration (SLC).

Observations were made for structural behavioral and internal pathological conditions. Ten fish from control as well as experimental groups were sacrificed for the study of selected parameters on Day zero, 4th day and 7th day of experimentation. Standard protocols were followed for the analyses.

For the analyses of biochemical parameters, muscle, liver, brain and kidney tissues were dissected out from the control and experimental fish. Colorimetric method was followed for the biochemical analyses using Spectronic-21 (Bausche & Lomb) spectrophotometer. Total sugars was estimated by anthrone method (Carrol *et al.,* 1956) and the total protein content in the tissues was done by folin phenol method (Lowry *et al.,* 1951), while for the estimation of total lipids, the method of Bligh and Dyer (Jayaraman, 1988) was followed.

Four different tissues *viz.,* muscle, liver, brain and kidney were dissected out carefully for biochemical studies. By using K-Roy Single pan electrical balance tissues were weighed and kept in an ice box till taken out for homogenization. Biochemical studies like total carbohydrates, total proteins, and total lipids were analyzed on zero day, 4^{th} and 7^{th} day in control groups; on 4^{th} and 7^{th} day after exposing the fish with the toxicants in the experimental groups of independent toxic exposure.

The data obtained was subjected to statistical analysis to arrive at Arithmetic Mean, Standard Deviation and Standard Error of Mean. To test the significance of differences observed between control and experimental groups, the data was subjected to Student 't' test. Statistical analyses were done applying statistics package software SPSS v 11.5.

RESULTSANDDISCUSSION

The use of biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed organisms. The health of any organism is influenced by the physiological activities taking place in the body. The physiological state of the organism is determined by the metabolic activities in the liver. If any toxic substance enters the liver, the physiological activities are immediately disturbed.

The fingerlings of *Catla catla* were exposed to ferrous sulphate in the concentrations of 2 to 20ppm and the pathological symptoms observed. White precipitate formation was seen in

experimental tanks. Loss of equilibrium, changes in opercular movement, change of orientation, erratic swimming were the other symptoms observed. No mortality was recorded in all the four days at the concentration of 2ppm. 10% mortality was noted at 4ppm on the first day of experiment. With regard to 6ppm concentration 20% mortality was observed on second day and later it reached 30%. The highest mortality of 100% was observed at 18ppm and 20ppm concentrations. 50% mortality was recorded in 8 and 9ppm concentration and 50% of mortality line intercepted the graph to show 8.4ppm as corresponding LC_{50} for the toxicant. SLC was calculated as 2.1ppm from the 96hr LC_{50} value. (Table.1)

Table 1: Determination of 96 hr LC50 for Ferrous sulphate from the freshwater fish *Catla catla*

S. No.	Conc. (ppm)	Percentage Mortality				Cumulative % Mortality
		Day 1	Day 2	Day 3	Day 4	
1	2	0	0	0	0	0
2	4	0	0	10	10	20
3	6	0	0	10	20	30
4	8	0	10	20	10	50
5	10	10	10	10	20	50
6	12	10	20	20	10	60
7	14	10	10	20	30	70
8	16	10	20	20	30	80
9	18	20	30	30	0	80
10	20	40	50	10	0	100

Condition LC16 LC50 LC84 Slope ƒLC50 Upper Confidence Limit Lower Confidence Limit
*Catlacatla*vs FeSO4 2.58.4 14.0 2.51 0.35 2.94 24.0
LC50 = 8.4 ppm

Total sugars

The tissues *viz.,* muscle, liver, brain and kidney were weighed and ground with 3ml of 5 % Trichloroacetic acid (TCA). The homogenates were carefully collected and centrifuged for 30 min at 3000 rpm. The clear supernatants were saved for the analysis of total carbohydrates by the method of Carrol *et al.* (1956). 10 ml of anthrone reagent was added to 1ml of supernatant and kept in a boiling water bath for 30 min and later cooled to room temperature. The intensity of the colour developed was read at 620nm in a photo colorimeter. The values are calibrated from the standard graph, and expressed as milligram per gram wet weight of the tissues.

Total proteins

The tissues were homogenized in 3ml of 5 % TCA in glass homogenizer and centrifuged for 30 min. at 3000 rpm. The precipitated pellet was taken for the analysis of protein by the method of Lowry et al. (1951), using Folin-Phenol reagent. The precipitate was dissolved thoroughly in 3ml of 1N sodium hydroxide, which was treated as sample for the analysis of protein. 0.2ml of this sample was made up to 1ml by the addition of 1N NaOH and 5ml of copper reagent mixture was added to the same. After 10min., at room temperature, 0.5ml of Folin-Phenol reagent was added and left undisturbed for 20 min at room temperature. The colour developed was read in a photo colorimeter at 500nm and the protein content of the tissues was calculated from the corresponding values in the standard graph and expressed as milligram per gram wet weight.

Total lipids

The tissues were weighed and homogenized in 3ml of chloroform-methanol reagent (chloroform : methanol at 2:1) with the

addition of 0.5ml of 0.9 % NaCl (Blight and Dyer method, as given in Jayaraman, 1988). The homogenates were mixed well and centrifuged at 2000 rpm for 20 minutes. Lower layer was carefully pipetted out into a clean test tube for all the tissues. From this extract, 0.5 ml was taken in another test tube and left over night to dry. Next day, 0.5 ml of concentrated sulfuric acid was added into each tube and mixed well. The test tubes were kept in water bath for 10 minutes and air cooled to room temperature. Finally 2.5ml of vanillin reagent was added to each sample, and allowed to stand for 20 min. The intensity of the colour developed was read at 620nm. The lipid content of the tissues was calculated from the standard graph and expressed as milligram per gram wet weight.

The total sugar content was estimated in muscle tissue of treated group at 4th day and 7th day respectively. In the fish exposed with a heavy metal ($FeSO_4$), the total sugar was increased on 4th day 7th day and these increased values were significant.

In the fish treated with heavy metal, ferrous sulphate, the carbohydrate content of the liver tissue was not significant on 4th day, where as the sugar level was significantly increased on 7th day. When the fish were treated with $FeSO_4$, the sugar content of the tissue increased on 4th day and 7th day with significant level of 2% on 7th day. Treatment of $FeSO_4$ increased the sugar value of kidney significantly on 4th day and decreased on 7th day.

In comparison with the control group of fishes, a significant ($p<0.001$) decline in the protein content was witnessed in the muscle tissues of metal exposed groups of fish on 4th day and 7th day. An elevation in the protein content was noted in $FeSO_4$ exposed fish on 4th and 7th days which was insignificant on 4th day and highly significant on 7th day. The total protein content of the brain tissue was 43.25mg/gm in the untreated fish and compared to this value on 4th day and 7th day experimental groups exposed to the $FeSO_4$ witnessed a significant decline in the total protein content of the liver on 4th day while the protein content was significantly increased at the end of 7th day. When the fish were treated with $FeSO_4$, the values decreased on 4th day and increased on 7th day. In the fishes exposed to sub-lethal concentration of $FeSO_4$, the kidney tissue showed a significant decline ($p<0.001$) in protein content of exposed fish when compared to the control groups.

Total lipid content in the muscle of the normal fish was estimated at 28.38 mg/gm. Exposure to the toxicants, $FeSO_4$, had resulted in a very significant increased on 4th and 7th days.On the 4th day of exposure there was a significant reduction in the lipid content of liver of the experimental group involving the toxicant. Similarly an increased trend was observed on 7th day and the variations were significant at 0.01% level.The total lipid was estimated at 170.03 mg/gm in the brain tissue of the normal fish. Comparatively, the lipid content was found to be reduced on the 4th and 7th days of exposure to the toxicant. These variations were significant at $p<0.001$.The total lipid of the kidney was 73.63 mg/gm in the fish untreated with toxicant. In the fish treated with $FeSO_4$, a decline in the lipid content was recorded on 4th and 7th days of exposure and significant at $p<0.001$.

The interaction between the contaminants and biomolecules is the first step in the generation of toxic effects. Understanding the alterations induced by the exposure to pollutants may contribute to the prediction of toxic effects that may occur at higher levels of biochemical organization (Rosety-Rodriguez *et al.,* 2005).Toxicant affect biologically active molecules such as carbohydrates, proteins and lipids (Ghosh and Chatterjee, 1985).

Normally carbohydrates, proteins and lipids which constitute the major components of the body play an important role in growth and energy metabolism. Carbohydrates serve as the immediate source of energy for animals.

Fishes use glycogen, the storage form of carbohydrates for their immediate energy requirement during stress (Vijayan and Moon, 1992). Liver and muscle are the two active sites where storage and metabolism of carbohydrate reserves take place. Generally, depletion in carbohydrate content is directly proportional to the exposure period of the toxicant.

In the present investigation, total sugar content in the muscle, liver, brain and kidney revealed a mixed trend in the different experimental groups at different days of exposure. Similar observations of mixed trend in the sugar content were reported in the muscle, liver, brain and kidney of *Catla catla* treated with heavy metal and fungicide (Sujatha, 2006). There was a decreased level of sugar content of the 4th day exposed fish in the muscle, liver and kidney showing a state of recovery in the 7th day.

(Catla) Consolidate Table : Total Sugars in Muscle (Mg / G Wet Wt.)

Experimental Condition		Zero Day Control	4th Day Control	4th Day Treated	7th Day Control	7th Day Treated
(Exposed To $Feso_4$)	Mean	5.37	3.41	4.20	1.48	2.62
	S.d.	0.522	0.300	0.189	0.412	0.683
	P <		N.S.		0.001	

(Catla) Consolidate Table : Total Sugars in Liver (Mg / G Wet Wt.)

Experimental Condition		Zero Day Control	4th Day Control	4th Day Treated	7th Day Control	7th Day Treated
(Exposed To $Feso_4$)	MEAN	9.97	13.63	12.46	2.04	4.42
	S.D.	0.339	1.875	1.286	0.294	0.485
	P <		0.001		0.002	

(Catla) Consolidate Table : Total Sugars in Brain (Mg / G Wet Wt.)

Experimental Condition		Zero Day Control	4th Day Control	4th Day Treated	7th Day Control	7th Day Treated
(Exposed To $Feso_4$)	MEAN	5.68	2.66	5.74	0.96	1.40
	S.D.	0.395	0.324	0.77	0.28	0.42
	P <		0.001		0.001	

(Catla) Consolidate Table : Total Sugars in Kidney (Mg / G Wet Wt.)

Experimental Condition		Zero Day Control	4th Day Control	4th Day Treated	7th Day Control	7th Day Treated
(Exposed To $Feso_4$)	MEAN	5.29	4.56	5.65	2.02	1.95
	S.D.	0.648	1.036	0.538	0.630	0.360
	P <		0.001		0.001	

(Catla) Consolidate Table : Total Protiens in Liver (Mg / G Wet Wt.)

Experimental Condition		Zero Day Control	4th Day Control	4th Day Treated	7th Day Control	7th Day Treated
(Exposed To $Feso_4$)	MEAN	77.65	99.18	101.46	142.87	190.68
	S.D.	5.70	4.36	8.17	9.58	3.68
	P <		0.001		0.001	

(Catla)		Consolidate Table : Total Protiens in Muscle (Mg / G Wet Wt.)				
Experimental Condition		**Zero Day Control**	**4th Day Control**	**4th Day Treated**	**7th Day Control**	**7th Day Treated**
(Exposed To $Feso_4$)	MEAN	119.57	146.85	92.70	414.32	133.32
	S.D.	1.48	6.65	2.06	9.99	6.42
	P <		0.001		0.001	

(Catla)		Consolidate Table : Total Protiens in Brain (Mg / G Wet Wt.)				
Experimental Condition		**Zero Day Control**	**4th Day Control**	**4th Day Treated**	**7th Day Control**	**7th Day Treated**
(Exposed To $Feso_4$)	MEAN	49.51	43.25	18.93	99.27	153.25
	S.D.	3.21	7.17	2.33	11.05	1.80
	P <		0.001		0.001	

(Catla)		Consolidate Table : Total Protiens in Kidney (Mg / G Wet Wt.)				
Experimental Condition		**Zero Day Control**	**4th Day Control**	**4th Day Treated**	**7th Day Control**	**7th Day Treated**
(Exposed To $Feso_4$)	MEAN	72.84	111.44	16.18	92.84	132.61
	S.D.	2.40	17.36	1.57	9.49	5.95
	P <		0.001		0.001	

(Catla)		Consolidate Table : Total Lipids in Muscle (Mg / G Wet Wt.)				
Experimental Condition		**Zero Day Control**	**4th Day Control**	**4th Day Treated**	**7th Day Control**	**7th Day Treated**
(Exposed To $Feso_4$)	MEAN	28.38	51.96	163.26	55.87	102.80
	S.D.	5.02	7.98	4.03	4.85	3.12
	P <		0.001		0.001	

(Catla)		Consolidate Table : Total Lipids in Liver (Mg / G Wet Wt.)				
Experimental Condition		**Zero Day Control**	**4th Day Control**	**4th Day Treated**	**7th Day Control**	**7th Day Treated**
(Exposed To $Feso_4$)	MEAN	108.39	137.61	98.18	164.76	222.27
	S.D.	5.34	23.42	3.91	5.67	2.95
	P <		0.001		0.001	

(Catla)		Consolidate Table : Total Lipids in Brain (Mg / G Wet Wt.)				
Experimental Condition		**Zero Day Control**	**4th Day Control**	**4th Day Treated**	**7th Day Control**	**7th Day Treated**
(Exposed To $Feso_4$)	MEAN	170.03	252.97	311.18	296.46	422.32
	S.D.	3.39	14.45	2.76	14.70	3.64
	P <		0.001		0.001	

(Catla)		Consolidate Table : Total Lipids in Kidney (Mg / G Wet Wt.)				
Experimental Condition		**Zero Day Control**	**4th Day Control**	**4th Day Treated**	**7th Day Control**	**7th Day Treated**
(Exposed To $Feso_4$)	MEAN	73.63	107.87	119.54	144.20	181.34
	S.D.	7.62	7.38	3.40	10.42	2.42
	P <		0.001		0.001	

The total sugar content of brain and kidney on the 4th day of experimental groups was more when compared to control groups which gradually reduced on 7thday in kidney. The increased and decreased levels were more pronounced in the fish exposed to $FeSO_4$. This clearly indicates the severe toxic nature of $FeSO_4$. The decline in the sugar content of muscle, liver, brain and kidney tissues could thus be attributed to the increased rate of glycolysis in tissues under stress. Kumari and Ram Kumar (1997) and Sastry and Siddiqui (1983) also reported a significant decrease in the sugar content of muscle, liver, brain and heart tissues of *Channa punctatus* inhabiting the polluted waters of HussainSagar lake of Hyderabad.

Carbohydrates are the chief sources of energy for any organism (Saravanan *et al.,* 2000). These are found in large amounts in the liver and muscle tissues and in small amounts in kidney and brain. Carbohydrate, protein, lipid metabolism of fishes is disturbed under the condition of toxic stress (Shaffi, 1978).The decrease in carbohydrate content in the muscle and brain may be due to glucose utilization to meet excess energy demand imposed by severe anaerobic stress of mercury intoxication. Similar results were observed in *Anabas testudineus* and *Anabas scandens,* when exposed to copper, lead nitrate and mercuric chloride respectively (Mary Chandravathy *et al.,* 1991).

Proteins are the most fundamental and abundant biochemical constituent present in fishes. Proteins are the most important energy source to spare during chronic period of stress (Yadav *et al.,* 2003). Animal exposed to toxicants even at the sub-lethal levels experience great stress at the metabolic level during the period of detoxification of the toxicant (Yadav *et al.,* 2003). Proteins are involved in major physiological events and therefore, the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Depletion of protein content has been observed in the muscle, intestine and brain of the fish, *Catla catla,* as a result of toxicity. When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted (Neff, 1985).The depletion of total protein content may be due to breakdown of protein into free amino acid.

The depletion of tissue protein in fishes exposed to toxicants is a physiological strategy played by the animal to adapt itself to the changed environment. Decreasing trend in total protein was reported in the liver, brain and gill tissues of catlaexposed to fenvalerate (Anita Susan *et al.,* 1999). It has been suggested that acute or chronic treatment of pesticide causes biochemical alteration in the organs involved in detoxification mechanisms (Shobha Rani *et al.,* 2001; Prabhakar *et al.,* 2002). Heavy metal-induced biochemical alteration in rohuwas reported by Neha *et al.* (2004) and impaired carbohydrate and protein metabolism (Sivakumar *et al.,* 1997; De Smet and Blust, 2000).

According to Moliwain and Bachelard (1971) any change in the environment may also alter the synthesis and utilization of protein in brain leading to behavioral change such as restlessness, tremor, ataxia, convusion and depression. Yadav *et al.* (2003) suggested that protein depletion leads to degradation processes such as proteolysis and utilization of its degradative products for increased metabolism in order to meet the energy requirements. Decrease in total protein content may be due to augmented proteolysis and the reduction

in total protein which might be related to the action of chemicals on nucleic acids.

A decrease in the glucose content of the liver, muscle and kidney tissue of *Clarias batrachus* was observed on exposure to sodium arsenite (Nimaichandra *et al.,* 2005). Neethirajan and Madhavan (2004) also reported changes in the carbohydrate of liver, muscle, and gill tissue of *Mystus vittatus* when exposed to sumidon.

Lipid profiles are important indicators of health and normal metabolic state in any animal. Lipid deposits come to the rescue of animals at times of crisis like stress and starvation. They are involved in the conversion to carbohydrates or proteins depending on the need to stabilize the metabolic processes and for physiological process to go on and on without compromise.

Elevated lipid contents are frequently associated with increased bioconcentration of lipophilic toxicants, which is usually correlated with enhanced toxicity of these compounds. High lipid deposits may exert protective effects by removing and inactivating organic chemicals from the metabolism or even actively sequestering them, thus improving toxicant tolerance and resistance. The abnormal accumulation of fats in experimental animals could be due to induced imbalance between fat production and utilization. It could be postulated that fatty liver changes are attributed largely to the reduced production of lipoproteins. A dietary imbalance is another especially common cause of fatty livers in cultured fishes. However, when the diet is normal, when the fishes are beyond the spawning seasons, and when a concurrent combination with other degeneration phenomena is observed, fatty livers must undoubtedly be considered pathological (Roberts, 1978).

Sujatha (2006) while studying on the effect of a fungicide and two metallic compounds in a synergistic and independent manner on catla found variations in the total lipid profiles, as observed in the present investigation by a bacterial pathogen, a pesticide and a metal in a similar study involving toxic synergism in catla.

Lipids are an important constituent of cellular structures. Lipids are also essential for maintenance of normal cell permeability and structural integrity of cell membranes. Synthetic pyrethroid, cypermethrin was also found to induce a decrease in lipid content of liver and muscle tissue of *Labeo rohita* (Das and Mukherjee, 2003). Govindan *et al.* (1994) reported a significant decline in the levels of total lipids in the muscle, liver and brain of *Gambusia affinis* exposed to a pesticide, phosphamidon. Freshwater fish, *Cyprinus carpio* exposed to sub lethal concentration of cypermethrin showed marked changes in protein fractions of liver, brain and muscle tissue (Asztalos *etal.,* 1990).

De Smet and Blust (2007) have reported that proteolysis is intended to increase the role of proteins in the energy production during cadmium stress. A reduction of carbohydrate, proteins and lipids were observed in freshwater fish *Labeo rohita* under the influence of heavy metal, lead (Neha *et al.,* 2004). The effluent from dye (Baskaran *et al.,* 1989), tannery (Ambrose *et al.,* 1994) and distillery (Maruthi and Rao, 2000) reported a severe reduction in the carbohydrate, protein and lipid contents among the fish population. Sujatha (2006) observed that the total sugars and proteins content in muscle, liver, brain and kidney revealed a mixed trend in *Catla catla* in the different experimental groups and different days of the exposure.

The alterations in the biochemical set up of fish during exposure to toxicants imposes stress on the fish which leads to rapid mobilization of these energy yielding biomolecules to yield excess energy required by the stressed animals (Saravanan *et al.,* 2003) to combat the heavy physical exercise in the form of erratic and rapid movements and high respiratory rates under conditions of toxicant stress.

Pollutant induced alteration in the cellular machinery could have altered the level of these biochemical constituents in the fishes. The potency of toxicity of the compounds differs in relation to the tissues, biomolecules and duration of exposure. The possible mode of action of the compounds is responsible for cellular disorganization of the internal tissue system affecting storage metabolism (Somaraj *et al.,* 2005).In the present study, in the

experiment involving successive synergism, the sugar, protein and lipid contents were more in all the experimental groups nearly in all the tissues. It clearly indicates very high metabolic rate that may be due to the initial stress exerted on the fish.

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