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A Study on the effect of electroplating effluent on biochemical changes in the fresh water fish, *Oreochromis mossambicus*.

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Abstract: *Biological monitoring techniques like biochemical variable have become alternative and useful for monitoring environmental quality, water pollution and the health condition of aquatic organisms. Biochemical parameters are frequently used as an indicator of the general state of health and early warming of stress in fish under stressful conditions. Biochemical changes induced by effluent stress lead to metabolic disturbances, inhibition of important enzymes retardation of growth and reduction in the fecundity and longevity of the organism. The fish was exposed to varying levels of the toxicant concentrations using static bioassay to determine the median lethal concentration. The LC_{50} value is 7.9 per cent the fish was exposed to short and long term periods in sub lethal concentration is 0.79 per cent. Biochemical characteristics like Protein, Carbohydrate and Lipid were estimated in gill, liver, kidney and muscle. The decrease of biochemical constituents from the control was noted. The results are statistically analysed and most of the values found to be significant.*
Keywords: *Protein, Carbohydrate, Lipid, effluent and Oreochromis mossambicus.*

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I. Introduction

Water pollution is one of the main problems presently facing in India and several efforts are being vigorously pursued to control various industries, agricultural, spanning length and breadth of the country. Pollution in the environment is mostly due to rapid progress in colonization, urbanization, industrialization, agricultural, mining, transportation and chemical technology. The freshwater habitats have become the depositors of pollutants released from all the anthropogenic activities [1].

Toxicity evaluation is an important and cost effective tool in water quality monitoring as it provides the complete response of test organisms to all the compounds in accumulative way. Toxicity of a substance refers to its capacity to cause adverse effects on living organisms and the term is more commonly used to compare the impact of two or more substances. Toxicity is nothing but a chemical's potency to cause an adverse impact on living organism and is dose and duration dependent. To evaluate the toxic impact of various pollutants, a number of bioassay procedures have been put to use in the study of concentration levels of poisonous substances on organisms.

Electroplating effluent from various processing industries have reported to contain high amounts of heavy metal ions such as nickel, iron, lead, zinc, chromium, cadmium, and copper [2]. The presence of these heavy metals in industrial waste water is of serious concern because they are highly toxic, non-biodegradable, and carcinogenic and their continuous deposition into receiving lakes, streams and other water sources within the vicinity causes bioaccumulation in the living organisms. These perhaps, could lead to several health problems like cancer, kidney failure, metabolic acidosis, oral ulcer, renal failure and many more. Biochemical parameters are very sensitive to sublethal concentration of many stress agents. Biochemical studies are good parameters, which help to see the effect of pollutants on biochemical composition of vital tissue of fish.
Proteins, carbohydrates and lipids which constitute the major component of the body play an important role in body construction and energy metabolism. They are involved in major physiological events and therefore the assessment of the protein, carbohydrate and lipid can be considered as diagnostic tool to determine the physiological phases of organisms [3].

II. Materials and Methods

Oreochromis mossambicus is a fish of the carp family Cyprinidae, found commonly in rivers and fresh water lakes in around South Asia and South East-Asia. Bulk of sample of fishes *Oreochromis mossambicus* ranging in weight from 14-17 gms and measuring 7-10 cm in length were procured from Aliyar fish farm. Fishes were acclimatized to the laboratory conditions for one month in large plastic tank (200 L).The fishes were fed with adlibitium, rice bran, wheat bran and oil cakes. Appropriate narrow range of concentration 0.79 ml was used to find the median lethal concentration and the mortality was recorded for every 24 hrs upto 96 hrs. It was found as 7.9 ml for 96 hrs using probit analysis method [5]. Three groups of fishes were exposed to 0.79 ml (1/10th of 96 hrs LC_{50} value) concentration of the electroplating effluent for short and long term periods respectively. Another group was maintained as control.

At the end of the each exposure period, fishes were sacrificed and tissues such as liver, gill, muscle and kidney were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, Centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters. Total protein concentration was estimated by the method of [5]. Carbohydrates were estimated by the method of [6] Cholesterol was estimated based on enzymatic method using cholesterol esterase, cholesterol oxidase and peroxidase [7].

III. Results

In the present investigation the effect of electroplating effluent and biochemical nature of protein, carbohydrate, and lipid in the different tissues such as gill, liver, kidney, and muscle of the fish *Oreochromis mossambicus*, have been studied and tabulated (1-3).

Total protein

The amount of protein estimated in different tissues of fish *Oreochromis mossambicus* subjected to different exposure periods are present in Tables 1.

Gill tissues showed 1.19,1.01,0.24, 0.20 mg/gm of protein in 7.9 ml of electroplating effluent and 2.24 mg/g of protein in control after 24, 48, 72 and 96 hours of exposure and also for long term exposure gills showed 0.20, 0.19, 0.12, 0.09 mg/g of protein after 7, 14, 21 days respectively.

Liver tissues shows the decreased value of protein content in liver 1.08, 0.97, 0.90, 0.84 mg/g in 7.9 ml of electroplating effluent and 1.71 mg/g in control after 24, 42, 72 and 96 hours of exposure and also for long term exposures kidney showed 0.53, 0.47 and 0.43 mg/g of protein after 7, 14 and 21 days respectively.

In the kidney tissues 0.81, 0.71, 0.69 and 0.60 mg/g of protein in 7.9 ml of electroplating effluent exposure and 1.14 mg/g in control after 24, 48, 72 and 96 hours of exposure and also for long term exposure kidney showed 0.53, 0.47 and 0.43 mg/g of protein after 7, 14 and 21 days respectively.

The protein content in the muscle is also reduced. In control the protein level is 2.18 mg/g it is decreased to 2.0, 1.69, 1.49 and 1.32 mg/g in 7.9 ml of electroplating effluent for 24, 48, 72 and 96 hours of exposure and also for long term exposure muscles showed 1.07, 0.40 and 0.18 mg/g of proteins respectively.

Protein is the most important constituent in living tissues, which is of considerable metabolic and structural value. Therefore any changes in the constituent indicate the stress inflicted on the metabolic functions required for maintaining a healthy physiological state. In this work the protein content of the *O.mossambicus* at different sublethal concentrations decreased in all exposure periods.

Proteins are mainly involved in the architecture of the cell. During chronic period of stress, they are also a source of energy. Since the fishes have the very little amount of carbohydrates, the next alternative source of energy is the protein to meet the increased energy demand. The depletion of the protein content in the gill, liver, kidney and muscle tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes. The depletion in tissue protein of *O.mossambicus* indicated rapid utilization of energy stores to meet the energy demands warrented by the environmental. The observed depletion in tissue protein on treatment with sub lethal doses of electroplating effluent was suggestive of proteolitic activity, possibly to meet the excess energy demands under toxic conditions. [8] Observed significantly decreased in protein content in all the tissues of the fish, *Cirrhinus mrigala* on exposed to pesticide Avanut. Similar results were observed by [9] in fish *Cirrhinus mrigala* on exposed to cypermethrin.

Carbohydrates

The amount of carbohydrate estimated in the different tissues of fish, *Oreochromis mossambicus* subjected to different exposure are presented in Tables 2.

The carbohydrate level in gill for 24, 48, 72 and 96 hours is reduced to 8.1, 7.9, 6.7 and 6.3mg/g from 10.7 mg/g in control of 7.9 ml of electroplating effluent and for 7, 14 and 21 days is reduced to 5.0, 4.2 and 3.4 mg/g of carbohydrate respectively.

In liver tissues decreased level of 12.1, 7.52, 6.4 and 5.2 mg/g of carbohydrate in 7.9 ml of electroplating effluent exposure and 17.7 mg/g in control after 24, 48, 72 and 96 hours respectively and for long term exposure it reduced to 4.3, 3.6 and 2.0 mg/g respectively.

The carbohydrate level in muscle during control is 27.1mg/g. It is reduced to 23.7, 20.7, 20.7 and 19.0 mg/g in 7.9 ml of electroplating effluent for 24, 48, 72 and 96 hours and for long term exposure 7, 14, and 21 days it reduced to 18.4, 10.1 and 9.3 mg/g respectively.

Carbohydrates which supply the major portion of the metabolites for the energy requirements in a normal individual is oxidised for the energy requisite. Carbohydrates may be converted into glycogen or shunted in the metabolic pathway to supply the carbon chain for the amino acids or converted into fat. At sublethal concentration, when the liver carbohydrate content decreased the blood sugar level increased which suggests the breakdown of liver glycogen (glycogenolysis). The mobilization of glucose from the liver to the blood and its availability for utilization by the needed tissues for ensuring normal metabolic processes in the body appears inevitable when the fish is exposed to toxic medium.

The carbohydrates of fishes comprised mainly glycogen and total free sugars and the fluctuations in the carbohydrate content may be due to accumulation and utilization of the glycogen and free sugars at different phases of life like growth, gametogenesis and spawning. In the fishes, generally the carbohydrates reserves may be rapidly utilized under unfavourable conditions and the great variations found in the tissues indicate that the level of mobilizable carbohydrate reserves may fluctuate widely and rapidly in response to fluctuations in the nutritional state of the animal. [10] Have observed that the fenvalerate exposed *Ctenopharyngodon idellus* showed a decrease in the carbohydrate content in the various tissues.

[11] Reported that the synchronized fall of carbohydrate level in the fish *Oreochromis mossambicus* may be due to the expenditure of energy for the constant movements aided by muscular action. Fall in carbohydrate level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to dairy effluent toxicants. Similar results of the present investigation further confirm that the alteration in the carbohydrate levels may be due to physiological activity. Decrease in carbohydrate content may be due to enhanced breakdown of glycogen to glucose through glycogenesis in the silver cat fish *Rhmbia quelen* on exposed to glycophosphate to the existing stress condition mediated by catecolamine and adenocortical hormone.

[12] Assumed that decrease in carbohydrate content may be due to the inhibition of hormones, which contribute to glycogen synthesis. Carbohydrate decline might be partially due to utilization in the formation of glycoprotein and glycolipids exposed to Dimethoate in the fish, *Pantius ticto*. [13] Studied the effect of Ekalux on the fish *Labeo rohita* and reported that carbohydrates showed the maximum decrease as (-64.96%) in liver during 72 hours exposure and minimum as (-7.69%) in the kidney during 24 hours exposure.

Lipid

The amount lipid estimated in different tissues of *Oreochromis mossambicus* subjected to exposure are present in Tables 3.

The lipid content in the gill tissues exposed to 7.9 ml of electroplating effluent were 16.4, 10.6, 10.1 and 7.8 mg/g and 21.5 mg/g in control experiment after 24, 48, 72 and 96hours exposures and for long term 7, 14 and 21days it reduced to 7.4, 6.4 and 5.3 mg/g respectively.

Liver tissue recorded lipid level of 14.6, 12.5, 11.1 and 8.10 mg/g when exposed to 7.9 ml of electroplating effluent. It is recorded 16.4 mg/g in control for 24, 48, 72 and 96 hours exposure periods and for long term 7, 14 and 21 days it reduced to 3.2, 2.0, and 1.7 mg/g respectively.

Kidney tissue was found to contain 10.8 mg/g of lipids in control sample and recorded 7.61, 7.21, 5.4 and 4.3 mg/g in 7.9 ml electroplating effluent exposure after 24, 48, 72 and 96hours and in long term 7, 14, and 21days it reduced to 3.2, 2.0 and 1.17 mg/g respectively.

The amount of lipid in muscle tissue were 17.8, 17.0, 9.7 and 8.6 mg/g in 7.9ml of electroplating effluent exposure periods of 24, 48, 72 and 96 hours and for long term exposure it reduced to 7.2, 6.4 and 5.3 mg/g. In the control the amount of lipid was found to be 20.5 mg/g.

Lipid is an important constituent of animal tissue, which plays a prime role in energy metabolism. Lipids are also important in cellular and sub-cellular membranes. A gradual decrease in lipid content in various tissues of *L.rohita* after chronic treatments of monocrotophos of various periods of exposure. Earlier [14] also suggest that the decrease in lipid content in *Channa punctatus* may be due to the uptake of lipid by tissue for utilization at cellular levels or due to increased lipolysis or mitochondrial injury, which affect the fatty acid oxidation metabolism suggested by [15].

[16] Reported that the decrease in the lipid content was noticed in the fish Mystus vitlatus on exposed to mercuric chloride, might have resulted from the enhanced breakdown through glycogenolysis to meet the high energy demand due to stress. [17] Reported that the percentage decreased was found to be more in gill. Decreased in lipid contents in the tissues indicates that lipid hydrolysis might be accelerated to drive energy to overcome pesticide toxicity. [18] Reported that there is drastic decrease in the lipid content in all tissues especially in liver of the cement factory effluent treated fish *C.mrigala*.

V Conclusion

Acute and chronic exposure to electroplating effluent proved to be highly toxic to *O.mossambicus* and induced cumulative deleterious effects at vital functional site like haematological indices. Through significant changes observed both at short and long term exposure periods these changes are more pronounced at the end of long term exposure periods suggestive of time dependent toxicity. Te chemicals in electroplating effluent induced decreases of protein, carbohydrate and lipid content to control could possibly affect the enzyme mediated bio defence mechanisms of the fish. Which pose a serious threat to human beings by secondary poisoning through food chain.

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IV. Table

Table 1. Changes in the protein content (mg/g) in the gills, liver, kidney and muscle of *Oreochromis mossambicus* exposed to electroplating effluent for different periods

Organs	**Durations**							
	Control	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**7 days**	**14days**	**21days**
Gill **'t' value** **%**	10.7±0.80	1.19±0.53 6.29** -46.87	1.01±0.67 8.15* -54.91	0.24±0.52 12.89* -89.28	0.20±0.33 13.12* -91.07	0.19±0.83 16.24* -91.51	0.12±1.13 19.11* -94.64	0.09±1.44 21.65* -95.98
Liver **'t' value** **%**	17.7±0.48	1.08±0.21 8.24** -36.84	0.97±0.45 10.64** -43.27	0.90±0.34 11.57** -47.36	0.84±0.41 14.31** -50.87	0.73±0.61 15.13** -57.30	0.67±0.71 17.42** -60.81	0.60±0.67 19.32** -64.91
Kidney **'t' value** **%**	10.5±0.35	0.81±0.11 8.27** -28.94	0.71±0.33 11.18** -37.71	0.69±0.42 12.17** -39.47	0.60±0.27 15.83** -47.36	0.53±0.47 16.28** -53.50	0.47±0.83 18.39** -58.77	0.43±0.93 19.85** -62.28
Muscle **'t' value** **%**	27.1±0.79	2.0±0.42 4.33** -8.25	1.69±0.62 7.51** -22.47	1.49±0.45 4.24** -31.65	1.32±0.72 5.75** -39.44	1.07±0.78 7.16** -50.91	0.40±0.99 10.52** -81.65	0.18±1.09 11.46** -91.74

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

Table 2. Changes in the carbohydrate content (mg/g) in the gills, liver, kidney and muscle of *Oreochromis mossambicus* exposed to electroplating effluent for different periods

Organs	**Durations**							
	Control	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**7 days**	**14days**	**21days**
Gill **'t' value** **%**	2.24±0.8	8.1±0.53 12.29** -24.29	7.9±0.67 13.15* -26.16	6.7±0.52 13.89* -37.38	6.3±0.33 13.92* -41.12	5.0±0.83 14.24* -53.27	4.2±1.13 15.11* -60.74	3.4±1.44 16.65* -68.22
Liver **'t' value** **%**	1.71±0.42	12.1±0.21 19.24** -31.63	7.52±0.45 21.64** -57.51	6.4±0.34 22.57** -63.84	5.2±0.41 24.31** -70.62	4.3±0.61 25.13** -75.70	3.6±0.71 26.42** -79.66	2.0±0.67 27.32** -88.70
Kidney **'t' value** **%**	1.14±0.35	8.9±0.11 9.27** -15.23	7.6±0.33 11.18** -27.61	7.0±0.42 11.87** -33.33	6.2±0.27 12.83** -40.95	5.1±0.47 14.28** -51.42	3.2±0.83 15.39** -69.52	2.4±0.93 15.85** -77.14
Muscle **'t' value** **%**	2.81±0.79	23.7±0.42 17.33** -12.54	20.7±0.62 21.51** -23.61	20.7±0.45 21.24** -23.61	19.0±0.72 22.75** -29.88	18.4±0.78 23.16** -32.10	10.1±0.99 31.52** -62.73	9.3±1.09 32.46** -65.68

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

Table 3. Changes in the lipid content (mg/g) in the gills, liver, kidney and muscle of *Oreochromis mossambicus* exposed to electroplating effluent for different periods

Organs	Durations							
	Control	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**7 days**	**14days**	**21days**
Gill **'t' value** **%**	21.5±0.80	16.4±0.53 11.29** -23.72	10.6±0.67 18.15* -50.69	10.1±0.52 18.89* -53.02	7.8±0.33 21.12* -63.72	7.4±0.83 21.24* -65.58	6.4±1.13 23.11* -70.23	5.3±1.44 24.65* -75.34
Liver **'t' value** **%**	16.4±0.42	14.6±0.21 8.24** -10.97	12.5±0.45 10.64** -23.78	11.1±0.34 11.57** -32.31	8.10±0.41 14.31** -50.60	7.3±0.61 15.13** -55.48	6.71±0.71 16.42** -59.08	6.2±0.67 17.32** -62.19
Kidney **'t' value** **%**	10.8±0.35	7.6±0.11 9.27** -29.53	7.21±0.33 9.98** -33.24	5.4±0.42 12.17** -50	4.3±0.27 13.83** -60.18	3.2±0.47 14.28** -70.37	2.0±0.83 14.89** -81.48	1.7±0.93 15.85** -84.25
Muscle **'t' value** **%**	20.5±0.79	17.8±0.42 14.33** -13.17	17.0±0.62 14.51** -17.07	9.7±0.45 24.24** -52.68	8.6±0.72 25.75** -58.04	7.2±0.78 27.16** -64.87	6.4±0.99 27.52** -68.78	5.3±1.09 28.46** -74.14

Values are mean± SD, n=5, Figures in parenthesis decrease over control.
*- significant at 5% (t<0.05) **-significant at 1% (t<0.01) NS- Non Significant

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