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# BIOCHEMICAL ALTERNATIONS INDUCED BY TEXTILE INDUSTRIAL EFFLUENT ON COMMON CARP *CYPRINUS CARPIO*

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**ABSTRACT**

The aim of the present investigation was to study physic-chemical analysis of textile dye effluent and the biochemical parameters in the finger lings of Cyprinus carpio exposed to sub lethal concentrations of 72 and 96 hr of textile dyeing effluent for 30 days period. In the present study, total protein, total free amino acids, glycogen and glucose were observed in, gills, liver, kidney and muscle tissues of Cyprinus carpio. The study showed that the level of total protein was decreased and total free amino acids was increased and simultaneously the level of glycogen was decreased and glucose was increased in the gills, liver , kidney and muscle tissue of Cyprinus carpio. The resulted parameters were analysed statistically and were found to be significant at $P < 0.05$ level.



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## INTRODUCTION

Industrial pollution is a global concern .The rapid industrialization is one of the major causes of water pollution. The discharge of untreated and partially treated waste water from various industries like chemicals , pesticides , fertilizer , pulp and paper mill , sugar and distillery , tannery and textile processing etc., have polluted the aquatic bodies drastically. The metal and other pollutants dispersed through effluents are very stable in the environment for longer periods resulting in bio-magnification. Fishes are sensitive to contaminations of water pollutants that may significantly damage certain physiological and bio chemical process when they enter the organs of these animals (Nemcsok, 1987). The toxicity bio assay is a biological tool used to evaluate the risk of pollutants and it gives an global answer for chemical agents dissolved in industrial and domestic effluents (Parthipan, 2014), as they effect the activity of biologically active molecules in aquatic living organism. Fish is one of the most sensitive animals as bio –indicators (Dhanapakium, 2001). Environment and chemical stress can interfere with physiological and bio chemical functions such as growth, development, circulatory and reproduction system in fish. Estimation of total protein and amino acids contents of various tissues are considered as important factors for toxicological studies (Tilak, 2007), since the food value of fish is directly dependent on their protein content and , the contamination by the toxic substance might reduce their nutritive value. The carbohydrate metabolism also disturbed when fishes were exposed to environmental stress condition. Glucose is one of the most important bio-chemical substances which gives immediate energy to organisms .Glucose has been shown to be a sensitive bio - chemical indicator of environmental stress for chemical pollutants. Hence , the present study was under taken related to the effect of textile dye effluent on bio chemical changes of common carp, *Cyprinus carpio* var, Communis is totally wanting.

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## MATERIALS AND METHODS

For the present study , the fish finger lings of *Cyprnius carpio* , (7.9 ± 2.0 cm , length ) were purchased from the Tamil Nadu Government Fisheries Farm , Mettur ,Mettur Dam .Tamil Nadu ,India. The finger lings selected from the stocks for the experiments were of 7.0 to 8.5 cm in length and weighed 6.0 to 9.0 gm and were free from any structural and behavioral clinical symptoms .These fish thus selected were pre-treated with potassium permanganate solution (1 %) and considered as apparently ‘’normal ’’.

From these observations the sub lethal, medium lethal and lethal concentrations were calculated.

**Collection of tissues for bio-chemical studies:** For bio-chemical studies , adequately acclimated finger lings *Cyprinus carpio* were divided into 3 groups of 20 each .Fish of group 1 was reared in river water and treated as control. Experimental animal belonging to groups 2 and 3 were exposed into two different ( 72 and 96 hrs ) sub lethal concentrations for 30 days .The test animals were fed daily by prepared feed and the test media changed daily . After 30 days of exposure the fish were dissected out and entire liver was care fully removed.

| S.No | Parameters | Values |
|---|---|---|
| 1 | Colour | Reddish Brown |
| 2 | $P^H$ | 8.9±0.2 |
| 3 | Electrical Conductivity | 33800mmhos/ cm |
| 4 | Total Solids | 7500 |
| 5 | Total Dissolved Solids | 5920 |
| 6 | Total Suspended Solids | 1580 |
| 7 | Chloride | 2185 |
| 8 | Total Hardness | 560 |
| 9 | Carbonate | 112.0 |
| 10 | Bicarbonate | 962.0 |
| 11 | Alkalinity | 6400 |
| 12 | Dissolved Oxygen | 1.232 |
| 13 | Dissolved Carbon dioxide | 44.0 |
| 14 | BiologicalOxygen Demand(BOD) | 1015 |
| 15 | Chemical OxygenDemand(COD) | 2800 |
| 16 | Sodium | 1969 |
| 17 | Potassium | 20 |
| 18 | Calcium | 5.34 |
| 19 | Chromium | 1.822 |
| 20 | Sulphate | 486 |
| 21 | Phenol | 1.28 |
| 22 | Copper | 0.354 |
| 23 | Mangaanese | 0.021 |
| 24 | Lead | 0.052 |
| 25 | Nitrate | 632.0 |

**Table 2. The level of Glycogen content in the different tissues of *Cypriuns carpio* exposed to 72 and 96 hr sub- lethal concentration of textile dye effluent (value are in percentage) on 30 days exposure**

| Name of tissue | Control | Textile dye effluent sub-lethal concentration | | | |
|---|---|---|---|---|---|
| | | (72h) | Percentage | (96h) | Percentage |
| Gill | 0.495±0.002 | 0.236±0.012 | -52.3*** | 0.150±0.010 | -69.69*** |
| Liver | 3.275±0.009 | 2.249±0.007 | -31.32** | 3.110±0.015 | -5.38NS |
| Muscle | 0.395±0.004 | 0.149±0.009 | -62.27*** | 0.318±0.013 | -77.21*** |
| Kidney | 0.195±0.008 | 0.135±0.015 | -30.76*** | 0.228±0.007 | -16.92* |

The values are the mean of 10 samples. *P < 0.05 **P < .001 ***P < 0.001 NS. Not significant ± S.D10 animals.

**Collection of textile dyeing effluent:** Textile dye effluent was collected alternate day in a 5 liters plastic can from the discharge point into the river Cauvery at Komarapalayam , Namakkal District , Tamil Nadu , India and transported to the laboratory and kept in a refrigerator for the physico-chemical analysis and for other experimental studies.

**Physico-chemical analysis:** The physico-chemical analysis were carried out by following the standard methods (Apha, 2005).

**Experimental design:** For survival studies, the effluent was diluted to different concentrations considering the original effluent as 100 % , the effluent was diluted to 10 to 100 % with dechlorinated tap water. Different concentrations of textile dye effluent were taken in plastic aquaria (60 cm L X 45cm B X 20 cm H) .The volume of the medium in plastic aquaria was maintained as 20 liters .In each concentration was observed at regular intervals from 24 hrs up to 120 hrs exposure and survival was expressed in terms of percentage.

A piece of muscle below the dorsal fin, the entire kidney, and gills were also removed in the same way. The tissues were weighed accurately (100 grams of tissues) by a electrical monopan balance to the nearest milligram for the bio-chemical analysis. Parameters were estimated using standard methods. The studied results are tabulated and calculated. The mean and statistical significance of the data was assessed through student ‘ t’ test at $P < 0.05$ and $P < 0.05$. level significant.

## RESULTS & DISCUSSIONS

Physico-chemical results were recorded and presented in the Table.1.The raw effluent contained high amount of TDS and TSS, less dissolved oxygen content and high BOD and COD , Carbonate, bi-carbonate and also contained high amount of some of metals.

### Bio-chemical parameters

**Glycogen:** The glycogen was found to be decreased in studied tissues of *Cyprinus carpio* exposed to textile dye effluent at

**Table 3. The level of glucose content in the different tissues of finger lings *Cyprinus carpio* exposed to 72 and 96 hr sub-lethal concentration of textile dye effluent (value are in percentage ) on 30 days exposure**

| Name of tissue | Control | (72h) sub lethal concentration of textile dye effluent | Percentage | (96h) sub lethal of textile dye | Percentage |
|---|---|---|---|---|---|
| Gill | 158.25±1.15 | 363.66±0.28 | 48** | 271.94±2.29 | 56.19*** |
| Liver | 112.86±0.91 | 361.63±0.22 | 65.20*** | 125.84±1.28 | 68.79*** |
| Muscle | 137.86±0.84 | 363.66±0.28 | 59.7*** | 148.25±1.31 | 62.58*** |
| Kidney | 168.47±1.21 | 278.41±1.25 | 38.18** | 172.11±1.28 | 39.488** |

The values are the mean of 10 samples.
*P < 0.05 **P < .001 ***P < 0.001 NS. Not significant ± S.D10 animals.

**Table.4. The level of protein content in the different tissues of fingerlings , *Cyprinus carpio* exposed to 72 and 96 hr sub-lethal concentration of textile dye effluent (value are in percentage ) on 30 days exposure**

| Name of tissue | Control | (72h) | Percentage | (96h) | Percentage |
|---|---|---|---|---|---|
| Gill | 24.95±4.310 | 20.53±4.56 | -17.71* | 17.28±2.51 | -30.7*** |
| Liver | 13.60±2.28 | 14.78±3.82 | -8.67NS | 13.16±1.652 | -3.23NS |
| Muscle | 19.26±5.768 | 13.67±5.52 | -29.02** | 12.48±3.82 | -35.53*** |
| Kidney | 17.56±2.56 | 21.40±2.16 | -21.86** | 14.78±3.98 | -15.83* |

The values are the mean of 10 samples.
*P < 0.05 **P < .001 ***P < 0.001 NS. Not significant ± S.D10 animals.

**Table 5.The level of Amino acid content in the different tissues of finger lings *Cyprinus carpio* exposed to 72 and 96 hr sub-lethal concentration of textile dye effluent (value are in percentage ) on 30 days exposure**

| Name of tissue | Control | (72) | Percentage | (96) | Percentage |
|---|---|---|---|---|---|
| Gill | 638.21±0.26 | 710.84±0.41 | 11.38 | 896.0±0.26 | 40.39 |
| Liver | 640.72±0.39 | 721.84±0.82 | 12.66 | 908.65±0.18 | 41.81 |
| Muscle | 642.76±0.32 | 825.01±0.51 | 28.35 | 898.05±0.31 | 39.71 |
| Kidney | 641.62±0.19 | 721.25±0.81 | 12.41 | 810.11±0.26 | 26.26 |

The values are the mean of 10 samples.
*P < 0.05 **P < .001 ***P < 0.001 NS. Not significant ± S.D10 animals.

**Table 6. The level of lipid content in the different tissues of finger lings *Cyprinus carpio* exposed to 72 and 96 hr sub-lethal concentrations of textile dye effluent (value are in percentage ) on 30 days exposure**

| Name of tissue | Control | (72) | Percentage | (96) | Percentage |
|---|---|---|---|---|---|
| Gill | 0.485±0.412 | 0.432±0.032 | 10.92* | 0.368±0.041 | 24.12** |
| Liver | 0.673±0.035 | 0.663±0.051 | 1.48NS | 0.652±0.63 | 3.12NS |
| Muscle | 0.425±0.25 | 0.253±0.043 | 40.7*** | 0.196±0.025 | 53.88*** |
| Kidney | 0.532±0.014 | 0.376±0.031 | 29.32** | 0.218±0.037 | 59.02*** |

The values are the mean of 10 samples.
*P < 0.05 **P < .001 ***P < 0.001 NS. Not significant ± S.D10 animals.

two sub lethal concentrations (Table.2).The glycogen has decreased more significantly in muscle tissues (77.21%) followed by gills ,(-69.69 %) and less significant in the kidney tissues compared to control at P < 0.05 level of significance. The reduction on glycogen content was higher in $Lc_{50}$ 96 hr exposed tissues of fingerlings *Cypriuns carpio* except liver tissues which was statistically non-significant.

**Glucose:** The total content of glucose in treated fingerlings for 30 days exposure in 72 & 96 hrs sub –lethal concentrations of textile effluent are presented in the table. There was increased level of glucose notified in all tissues but level of glucose observed in liver (68.79%) was highly significant at 0.1% than in muscle (62.58%) and gill (56.19%) followed by kidney (39.48%) which was found to be statically significant at P < 0.01 level. The fish treated with 72 hr sub lethal concentration of textile effluents also showed more or less similar results.

**PROTEIN:** In 72 and 96 hr sub –lethal concentrations of textile effluent exposed finger lings *Cyprinus carpio* showed a decreased level of protein contents in all four studied tissues. The decreases was more pronounced in 96 hrs sub lethal concentration of treated muscle (-35.53%) tissue followed by gill (-30.7%) and kidney (-15.83%).

A decreased level of total protein content in liver (3.23%) noticed was minimum compared to control in both treated concentration and this was found to be non-significant in both treatments. But, the reduced level of total protein content noticed in gill and kidney tissues was higher in 72 hrs sub lethal concentration of textile effluent treated *Cyprinus carpio* at various significant level.

**Amino Acids:** The results of amino acid was increased in studied four tissues of finger lings *Cyprinus carpio* treated at 72 , 96 hr and sub –lethal concentrations of textile dye effluent .The increased level was highly significant in gill tissue (40.39%) followed by liver (41.81) and muscle (39.71%) and less significant was found in kidney (26.26%) compared to control in 96 hr sub lethal concentration. But, comparatively the level of percent increases were less in 72 hrs sub-lethal concentration of treated tissues of *Cyprinus carpio* compared to 96 hrs sub-lethal concentration and which were found to be significant at various level.

**Lipid:** The total lipid content at 72 & 96 hr sub lethal concentrations in textile dye effluent treated tissues of *Cyprinus carpio* treated with was presented in the table .5 . A decreased level of lipid content was observed in the treated fish tissues which were found to be statistically significant (P

< 0.001) in kidney and muscle tissues at 96 hrs treatment compared to control after 30 days exposure. Depletion percentage of lipid content was higher in kidney (59.02%) and muscle (53.88%) tissues while it was less significant (P < 0.05) (23.12%) in gill tissues of fish compared to control. There was non-significant depletion of lipid level was calculated in liver (1.48 % and 3.12 %) tissues in both 72 and 96 hrs treatments as compared to control respectively. However, the minimum rate of total lipid content was higher in 96 hrs treated tissues of fish than to 72 hrs treated tissues compared to control. The Bio assay tests have proved as an essential tool to evaluate chemical toxicity there by much attention has been drawn on it during last few decades. Acute toxicity is caused by a relatively large dose of chemicals. The onset of symptom is sudden and the intensity of effects rises rapidly and may result in death of organisms. The acute toxicity tests are more widely used methods for determining the toxicity range of textile dye effluent. The final action of toxicity is due to metabolism and accumulation of the residues in fish tissues as reported (Mcleay, 1975). The residues are accumulated in different tissues, causing toxicity to the fish which ultimately results in bio magnification through the food chain. Thus present study was confirmed with the work of (Dhanapakium, 2007). In the present observation showed , depletion in glycogen content in both (72 and 96 hrs) sub lethal concentrations of textile dying effluent. The finding can be correlated with the work of (Bakthavathsalm, 1982). The observed decreased level in different tissues of glycogen may be due to glycolysis for production of energy to overcome toxic effect of the effluents. Similar decreased level of glycogen has also been noticed (Bhattacharya, 1978), and that depletion in glycogen may be attributed to its utilization to meet high energy demand created by stress of effluents. This could have happened by rapid glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of transferase (Susan, 1999a).

In the present study, next to muscle the depletion of glycogen content level in gill tissues might be due to a possible glycogenolysis, resulting in anaerobic glycolysis to cope up with the adverse condition. The stress full situation mainly disturbs the rate of carbohydrate metabolism through the level of glycogen and glucose content in toxicant exposed animals. Glycogen a reserve energy source decreased during entire textile dyeing effluent treatment in both sublethal concentrations treated *Cyprinus carpio* . A fall in glycogen levels in the tissues indicate the possibility of glycogenolysis. It may be due to hypoxia, since hypoxia increases carbohydrate consumption . The similar results were observed in *Thalmile crenata, Anabastes tudiennues* and *Anabass candens* when exposed to copper, lead nitrate and mercuric chloride respectively (Bhattacharya, 1978). A marked decreased in liver glycogen indicates an extensive utilization of energy store, perhaps, this stepped up utilization is to meet the extra demands of energy necessitated by the quick and brisk movement, which the animal shows in its behavoural response under the heavy metal which are present in the textile effluent influence. The marked decrease in the liver glycogen reserves in *Cyprinus carpio*, consequent on textile effluent treatment may be due to glycogenolysis since a decrease in hepatic glycogen suggests an enhanced glycogenolysis (Dhanapakium, 1995). The depletion of liver glycogen content is high at 72 hr sub-lethal concentration compared to control and to the 96 hr sub lethal concentration and concomitant increase with increase in during the exposure period. The results suggest that the excess amount of metal accumulated in the finger ling's *Cyprinus carpio* body through the gill ventilation. In the present experiment an elevation of sugar observed in all the studied tissues of effluent exposed fingerlings, *Cyprinus carpio* . This could be attributed to the physiological stress caused by textile dyeing effluent. Similar increases in role blood glucose concentration were reported in fish exposed to different concentrations of phenol (Couch, 1975), reported similar hyperglycemia in the fresh water fish, *Orechromis mossambicus* exposed to sub-lethal concentration of phenol. Further, the present work was confirmed with the report in the fish, *Labeo rohita* exposed to metal and metal mixture. Reported that the increase in blood sugar level contributes an active flux of metabolites (Dhanapakium, 2001). The elevated blood glucose levels reflect an increase in this rate of transportation of glucose probably from the liver to muscle where high energy demand was meet due to brisk and erratic movements. When fish absorb little oxygen from the environment, the respiratory metabolism is depressed and therefore stored intra cellular glycogen is utilized. Under such conditions, the hyper glycaemia hormone is released for the degradation of glucose. This glucose leaks in to the blood causing hyper glycaemia (Mary chanravathy, 1996).

**Protein:** In present investigation there was decrease in protein content in all the tissues of *Cyprinus carpio* treated in 72 and 96 hrs sub lethal concentration of textile dye effluent and this level of decreases was highly significant in gill tissue compared to control and lesser in other tissues. The higher decreased level was noticed in gill and kidney tissues at 72 hr sub-concentration and this was due to stress the significant decrease in total protein content indicates was due to, stress. The effluent treatment induces proteolysis. Further, protein decrease may also be due to stress in fish as protein is likely to undergo hydrolysis and oxidation through TCA cycle to meet the increased demand for energy caused by the stress (Sathyanarayan, 2005). The alteration in the tissue protein, noticed in the present investigation in sub lethal concentrations of 72 and 96 hr. suggested disturbance in the physiological activity. The present results was supported when the fish exposed to nickel and copper (Susan, 1999).

Stated that protein can be expected to be involved in the compensatory mechanisms of stressed organisms. In the present experiment, higher level of protein decreased was noticed in muscle tissues compared to other tissues after exposure to 72 and 96 hr and the present observations may be due to reduced protein synthesis, increased proteolysis and also due to utilization for metabolic processes under textile effluent toxicity. The present study was confirmed with observed in fish exposed to toxicant. This decrease of total protein resulted in the present work might be due to the intestine proteolysis as a result of activity of lysosome enzymes leading to depletion of both tissues and circulating protein as has been suggested by in *Cyprinus carpio* exposed to copper and zinc mixtures (Maruthi, 2000). This inference gets further support by the that attributed the decline in protein content to an alteration in the nucleic acids in the *H.fossilis* exposed to sub lethal concentrations of aldrin. The lesser reduction in protein content was noticed in the present work may be due to increase in synthesis of detoxication enzymes as suggested by (Muley, 2007). It also act as sensitive index of toxicant and consistently renews its own intrinsic protein which have a very high turnover rate (Apha, 2005). A greater amount of protein is synthesized by the liver which are needed

repair of damaged all organelle and tissue regeneration. Furthermore, a compensatory production of enzyme lost as a result of tissues damage or to meet the increased demand to detoxify the heavy metal proteins are needed. Essential metal always function in combination with organic molecule mostly with protein, either bound to metal protein or loosely bound metal protein complex.

**AMINOACID:** In the present work, decreased level of protein along with an increased levels of free amino acids in might indicates an increased catabolism of protein and decreased. Increased in amino acid may be due to increased protease activity which is reflected in the decreased in protein content and increase in the level of amino acids. Similar to the present study report was also given by (Sathyanarayan, 2005), with effect of chromium on protein metabolism of fresh water mussel*, Lamelliderus margillalus*. Further the present results was correlated with work of Kewaljaiswal *et al.* (1991) who studied in prawn exposed to pollutants. The observed increases in free amino acid level may be attributed to increased proteolysis or their decreased incorporation in to protein. A similar increased in aminoacids and transaminase . Increase in the free amino acid level correlates with depletion of protein and glycogen. Hence, the increase in the free amino acid may be partly for gluconeogenesis through transamination and transamination reactions, to supply the necessary keto acids, to act as precursors for the maintenance of carbohydrate metabolism to meet the energy demand during stress condition (Desai, 2001).

**Lipid:** In the present study, all studied tissues of treated *Cyprinus carpus* showed more decrease in the lipid content with much decrease in muscle tissues after 30$^{th}$day exposure. The results of this investigation clearly showed that both sub lethal sub-lethal concentration (72 & 96) are capable of including significant alternations in lipid content in the tissue of *Cyprinus carpio***.** Similar decreased level upto -33.64% in industrial effluents exposed fish, *Labeo rohita,* was also reported (20). The present investigation was concordance with the work , observed a decrement of lipid content in different tissues (liver, intestine, muscle, brain and gill) of fish, *Rasborad aniconius* to pulp and paper mill effluents (Haniffa,. 1991), reported a decline in lipid content in *Oreochromis mossabicus* exposed to textile mill effluent and this result was strengthen the present study. In the present observations, decreased total lipids were observed in muscle, gills liver and kidney tissues were similar with of *Sarotherodon mossambicus* after exposure to sub-lethal concentration of methyl parathion (25) noted decreased total lipid content in the liver tissues of *Cyprinus carpio* and *Tilapia mossambicus* respectively after exposure to acidic water. The lipid decreased content in tissues studied in the present study may also be due to drop in the production of NADPH which of lipid, as glucose-6-phosphate dehydrogenase and malic enzyme exhibited decreased actives in the liver of effluent exposed fish. It can also be pointed out that the excess activity of enzyme in gill and liver, due to many result in the utilization of the lipid [. Also the accumulation of organic acid and in organic compounds which are present in the effluent, in the brain and the kidney may cause the disintegration of nerve cells, cortex of kidney.

**Conclusion**

To sum up from the observations made in this investigation, it may be pointed out that the changes obtained on the biological parameter on the fish treated with textile effluents. The biochemical analysis of different parameters conducted in this study like, total protein, glycogen, total lipid were decreased whereas amino acids and glucose were in increased These changes have already been discussed in the earlier text. It is therefore, needless to say, that the textile effluents have factors which induced changes which go against wellbeing of the aquatic organism like fish and therefore, they become potent abiotic pathogens adversely affecting the aquatic biosphere. Hence the study of toxicological bioassay all the more become a necessity to assess the water quality. From the study on biological, respiratory and metabolic factors suggest that ultimately the fish resorts to hyperglycaemia, which was noticed in the tissues as an adaptive change. As against this, negative changes were recorded tissues, with respect to total glycogen, (hypoglycaemia) and total protein (hypoproteinemia) in contrast increase total free amino acid, degenerative changes. The results of the present study suggests that the reason for the difference in the action of effluent is not clear, but the fingerlings of *Cyprinus carpio* showed some metabolic defence mechanisms for these chemicals present in the effluent. This kind of changes in biochemical observed in this study as a whole reveal that the textile effluents pose more problems of pollution. Hence, the environmental awareness becomes more necessary, since fish forms delicious component of human food and further it is poor man's dish. It may be concluded that effluents from textile effluent factories presently evaluated causes lethal effects, proper treatment of effluents is a necessary prerequisite for safe disposal into the aquatic system (or) environment.

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