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Flux of tissue substrates in *Danio rerio* exposed to raw tannery effluent

Sivakumar P.[1], Kanagappan M.[2] and Sam Manohar Das S.[3]

[1]Research Scholar, Department of Zoology, Scott Christian College (Autonomous), Nagercoil, India.
[2]Associate Professor, Department of Zoology, Scott Christian College (Autonomous), Nagercoil, India.
[3]Associate Professor, Department of Zoology,Scott Christian College (Autonomous), Nagercoil, India.

Corresponding author: capeparam@rediffmail.com

Abstract -The present study in raw tannery effluent is to investigate the biochemical parameters in the gill, muscle and liver of *Danio rerio*. The study is conducted to determine the carbohydrate, protein and lipid content in the muscle, liver and gills of *Danio rerio* exposed to sublethal concentration of tannery effluent for 45 and 90 days. The results showed significant decrease in the carbohydrate, protein and lipid content levels in exposed fish, *Danio rerio* after 45 and 90 days exposure when compared with control, it was concluded that the effect of the presence of toxic substance in the tannery effluent on different organs act differently depending on the duration of exposure time.

Key Words: Tannery effluent, Muscle, Gill, Liver, Protein, Lipid, Carbohydrate, *Danio rerio.*

1. INTRODUCTION

Industrial effluent or waste is a great menace not only to the health of human race but also to the entire living organism all over the world. Industrial effluent mainly contributes to aquatic pollution containing a vast array of toxic substances including heavy metals [1, 2]. It leads to alteration in physical, chemical and biochemical properties of water bodies as well as environment.

The industrial wastes generally contain high quantities of dissolved and suspended solids, organic and inorganic chemicals, high BOD and COD, oil and grease, besides toxic metals which cause deleterious effects on the freshwater fish when discharged in to water bodies. The problem of environmental pollution and its deleterious effects on aquatic biota, including fish is receiving focus during the last few decades [3, 4].

Tannery effluent is one of the hazardous pollutants of the industry and the effluents are ranked as the highest pollutants among all industrial wastes [5, 6].Through the excessive organic load present in tannery waste the oxygen content of the waters is depleted and leads to the death of fish and other aquatic animals. These effluents contain toxic chemicals such as sulfides, chromium salts and other substances, including heavy toxic trace metals that turn tannery effluents into noxious wastewaters [7, 8].

The untreated effluent containing chromium from the tanneries are discharged into fresh water bodies affected the aquatic organisms. Chromium is one of the highly toxic heavy metal to aquatic fauna. Heavy metals concentration in the tissues of fish enters into human beings through food chain and causes potential health hazards sometimes lethal [9].

Fish population is generally considered to be very sensitive to all kinds of environmental stressors to which they are exposed. Gills, liver and muscle are the primary target organs. Effects of tannery effluents on the muscle and liver glycogen in fish *Sarotherodon mossambicus* [10]. The biochemical changes occurring in the body give early indication of stress [11]. During the stress an organism needs sufficient energy which is supplied from reserve materials like, protein, lipid and glycogen. If the stress is mild, then only stored glycogen is used as a source of energy, but when the stress is strong, then the energy stored in lipid and protein will be used. Proteins are highly sensitive to heavy metals and are one of the earliest indicators of heavy metals poisoning. The impairment in protein synthesis due to heavy metal stress was reported by many investigators [12, 13]. Measurement of total protein provides an insight on the biochemical mechanism of metabolism under stressful conditions [14].

Considering the above facts, the present study is aimed to assess the effect of industrial effluents on the biochemical composition of gill, liver and muscle of the fish *Danio rerio*.

2. MATERIALS AND METHODS

2.1 Collection of Sample

The test fish *Danio rerio* (*D.rerio*) of length 4.7 ± 1.0 cm and 3.9 ± 1gm of body weight were collected from Nagercoil J.J. aqua farm in Kanyakumari district and acclimated to the laboratory conditions for 30 days. The medium was changed once in two days and no mortality of fishes was recorded during the period of investigation.

2.2 Collection of Effluent

The tannery effluent was collected from a tannery at Ambur, Vellore District in Tamil Nadu. Only the raw effluent was used for the study. Various parameters of the tannery effluent and the water sample from the laboratory were also analyzed.

2.3 Bio-chemical Analysis

2.3.1 Estimation of total proteins

About 50mg of muscle, liver and gill were dissected out from the fish were homogenized in 5ml of 25% of trichloroacetic acid buffer, precipitated with 10ml of 80% ethanol and centrifuge at 1000rpm for 15 min. The tissue sample was homogenized and was treated with ethanol. The precipitate was dissolved in 10ml of 1N NaOH solution and was used for total protein estimation. The results were expressed as mg protein/g wet tissue. Total protein content of the selected tissues was estimated by the method of Lowry using bovine serum albumin as standard [15].

2.3.2 Estimation of carbohydrates

Sulphuric acid (66% [v/v]) containing 50mg of anthrone and 1gm of thiourea were prepared fresh for every experiment to determine the total carbohydrates present in the sample. Standard stock glucose solution was prepared by dissolving 100mg of glucose in 100mg of standard benzoic acid. The samples were de proteinzed with 80% ethanol and centrifuged at 1000g for 10min. The clear supernatant was used for the carbohydrate estimation following the method of Roe using anthrone reagent [16].

2.3.3Estimation of Lipids

50mg of wet tissue was homogenated with 1 to 5 ml of chloroform with tissue homogenizer. It was centrifuged at 1000rpm for 10 min. The supernatant was collected and evaporated to dryness. To this, 3ml of potassium dichromate was added and read at 620nm. The dichromate reagent was prepared by dissolving 2gms of potassium dichromate in 100ml of concentrated sulphuric acid [17].

3. RESULT

3.1 Total Protein content

The protein levels in Muscle, gill and liver of *D. rerio* exposed to sub-lethal concentration of tannery effluent showed significant decrease when compared to control fish. The decrease in muscle, gill and liver *D. rerio* protein levels were more pronounced at 90 days of exposure periods (Table :1). The total protein concentration of muscle, gill and liver was 38,18 and 23 mg/100mg wet tissue for 45 days and it decrease from control fishes and 33, 15 and 20 mg/100mg wet tissues showing a significant decrease from control at the end of 90 days exposure respectively. The mean difference were statistically significant at $P \leq 0.05$ level. (Fig. 1).

3.2 Total Lipid content

During sublethal exposure of tannery effluent, total lipid level in serum significantly decreased in experimental fish than the control (Table:2). The total lipid concentration of Muscle, gill and liver was 45,34 and 27 mg/100mg wet tissues and it decrease from control fishes exposed for 45 days and 38, 23 and 22 mg/100mg wet tissue showing a significant decrease from control at the end of 90 days exposure respectively. The mean difference between control and experimental groups were statistically significant at $P \leq 0.05$ level. (Fig. 2).

3.3 Total Carbohydrate content

The total carbohydrate concentration of Muscle, gill and liver of the exposed fishes was 29, 24 and 26 mg/100mg wet tissues and it decrease from control in fishes exposed for 45 days and 27, 20 and 23 mg/100mg wet tissues showing a significant decrease from control at the end of 90 days exposure respectively (Table:3). The mean difference between control and experimental groups were statistically significant at $P \leq 0.05$ level. (Fig.3).

Table -1 : Protein level in selected tissues of *D. rerio* exposed to untreated tannery effluent (mg/100mg wet weight of tissue)

Days in Exposure / Different Tissues	Muscle	Gill	Liver
Control	41 ± 1.4	20 ± 1.5	25 ± 2.0
45 days	38 ± 1.6 (-7.3%)*	18 ± 2.0 (-10%)	23 ± 2.0 (-8%)
90 days	33 ± 2.2 (-19.5%)*	15 ± 3.2 (-25%)*	20 ± 2.9 (-20%)*

* Significant difference ($p \leq 0.05$)

[Each value indicate the mean (x±SD) of five estimations]

Table - 2 : Lipid content in selected tissues of *D. rerio* exposed to untreated tannery effluent (mg/100mg wet weight of tissue)

Days in Exposure / Different Tissues	Muscle	Gill	Liver
Control	49 ± 2.5	40 ± 2.2	30 ± 2.5
45 days	45 ± 1.9 (-8.2%)	34 ± 1.5 (-15%)*	27 ± 1.6 (-10%)*
90 days	38 ± 1.5 (-22.5%)*	23 ± 1.8 (-42.5%)*	22 ± 1.4 (-26.6%)*

* Significant difference ($p \leq 0.05$)
[Each value indicate the mean (x±SD) of five estimations]

Table -3 : Carbohydrate content in selected tissues of *D. rerio* exposed to untreated tannery effluent (mg/100mg wet weight of tissue)

Days in Exposure / Different Tissues	Muscle	Gill	Liver
Control	30 ± 1.9	30 ± 1.0	30 ± 1.0
45 days	29 ± 1.6 (-3.3%)	24 ± 1.6 (-20%)*	26 ± 1.4 (-13.3%)*
90 days	27 ± 3.5 (-10%)*	20 ± 1.5 (-33.3%)*	23 ± 1.8 (-23.3%)*

* Significant difference ($p \leq 0.05$)
[Each value indicate the mean (x±SD) of five estimations]

Figure 1: Changes in the protein level in selected tissues of *D. rerio* exposed to untreated tannery effluent (mg/100mg wet weight of tissue)

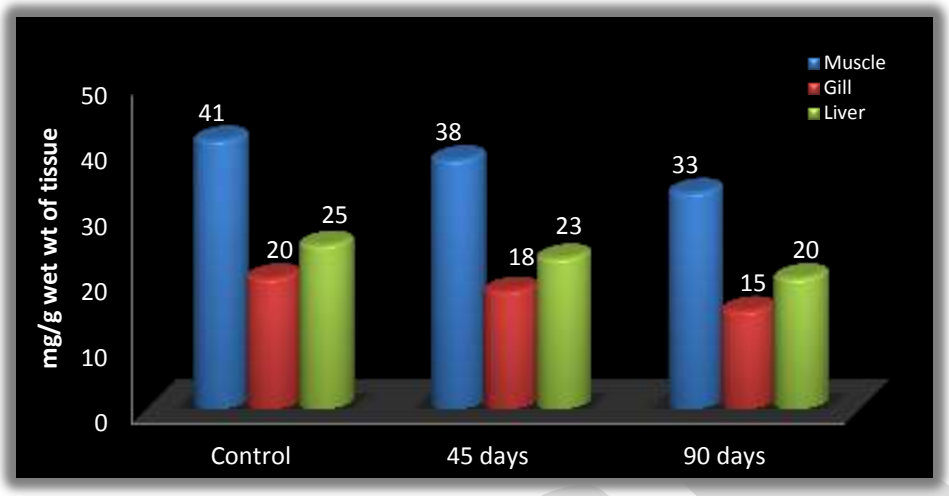


Figure 2: Changes in the lipid content in selected tissues of *D. rerio* exposed to untreated tannery effluent (mg/100mg wet weight of tissue)

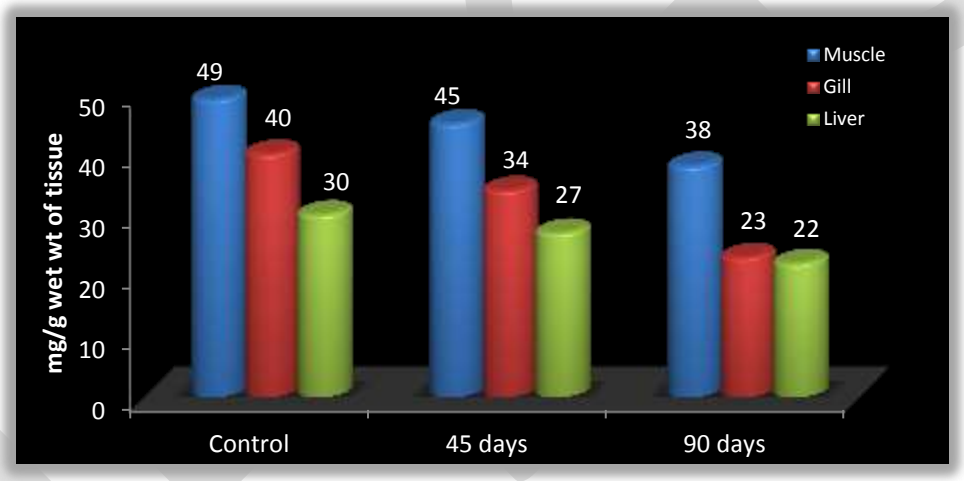
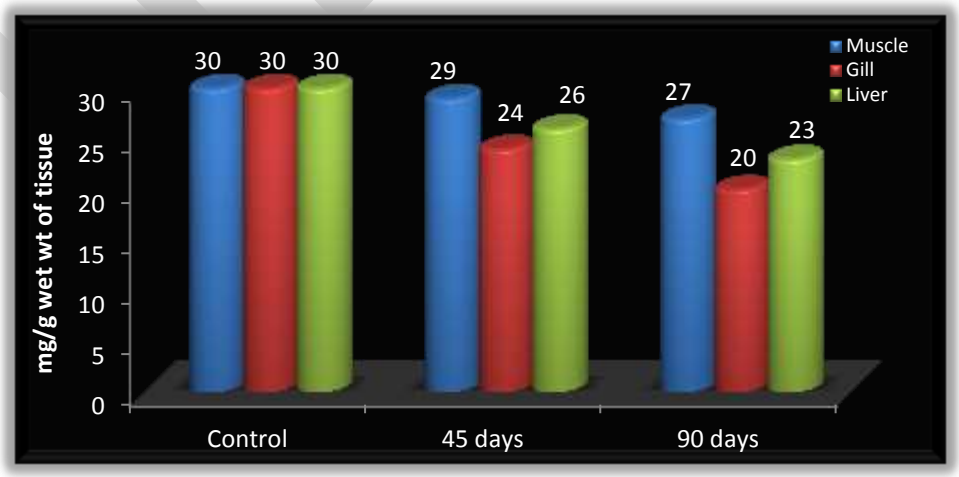


Figure 3: Changes in the carbohydrate content in selected tissues of *D. rerio* exposed to untreated tannery effluent (mg/100mg wet weight of tissue)



4. DISCUSSION

The observations from the present study showed that, the tannery effluent at sublethal concentrations altered the biochemical composition (Carbohydrate, Protein and Lipid) of the various organs of test fish, due to utilization of biochemical energy to counteract the toxic stress caused due to heavy metals present in effluents.

Heavy metal poisoning induced physiological and biochemical changes in the liver of an animal can be regarded as an index for the identification of pollutant stress [18]. The depletion of protein level induces diversification of energy to meet the impending energy demands during the toxic stress. The reduction in tissue proteins reflects a prior increased energy cost of homeostasis, tissue repair and detoxification under toxic stress. It is also possible that when an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands. Hence depletion in protein level is observed [19].

The decrease in total protein level in liver and muscle of *Channa punctatus* exposed to monocrotophos for 15, 30 and 60 days [20]. A significant decrease was reported in the protein content in almost all tissues in *Channa punctatus* when exposed to sublethal and lethal concentration of fenvalerate [21]. The decrease in protein content in rainbow trout (*Oncarhynchus mykiss) was* due to contaminated environment condition [22].

The reduction in the glycogen levels in the tissues of fry of common carp, *Cyprinus carpio* (Linn) was reported [23]. This may be due to generalized disturbances in carbohydrate consumption [24]. The reduction in protein, glycogen and lipid in tissues of freshwater fish *Labeo rohita* induced by heavy metals from electroplating industry [25]. Decrease in the level of tissue protein may be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress [26].

Biochemical changes occurred in *Cyprinus carpio* in response to nickel and lead [27]. Effects of mercury and cadmium on proteins and enzymes in *Oreochromis mossambicus* [28]. Effects of copper and mercury on the glycogen and protein contents of liver and muscle of the fish *Macrones gulio* [29].

Decreased content of the carbohydrate was probably due to glycogenolysis and utilization of glucose to meet increased metabolic cost [30]. Decreased in carbohydrate content might indicate an immediate utilization to meet the excess demand of energy under toxic stress of tannery effluent. This condition happened by rapid glycogenolysis and inhibitions of glycogenesis through activation of glycogen phosphorylase and depletion of glycogen transferase respectively or through stress induced increase in catacholamines.

Carbohydrate depletion is more prevalent under hypoxic conditions due to toxic stress [31,32]. The decline in protein showed, an intensive proteolysis which in turn could contribute to increase of free amino acids to enter into TCA cycle as keto acids thus supporting the view of Jha [33]. The level of protein, carbohydrates and lipid contents were gradually decreased in the fish *Oreochromis mossambicus* reared at the sublethal concentration of textile dye effluent [34].

Decrease in total lipid content might be due to utilization of lipids during the toxic stress[35] and Insecticides are found to reduce the concentration of lipids in the tissues of fishes [36,37]. The endogenous fat in animal is found to be the only source of energy during prolonged stress. Thus, the reduced level of total lipids in the blood of the species under study is the indicative of the utilization of the same to meet the energy demand during the stress caused by the tannery effluent.

Decrease in protein might be due to inhibition of protein synthesis or increase in the rate of degradation of amino acids [38,39] which may be entered into tricarboxylic acid (TCA) cycle through aminotransferases, probably to cope up with high energy demands in order to meet the stress condition. The fall in protein level during exposure might be due to increased catabolism and decreased anabolism of proteins.

The biochemical substances, such as proteins, carbohydrate and lipid play a role in the tissue construction and energy production. In the present investigation protein, carbohydrate and lipid contents in different tissues of *D.rerio*, which were exposed to sublethal (4.5ppm) concentration of raw tannery effluent showed decrease protein, carbohydrate and lipid contents in muscle, gill, and liver at 45 and 90 days of exposure. The present observations revealed that the decline in the protein, lipid and carbohydrate level in different tissues was directly proportional to the exposure days.

5. CONCLUSION

In the present study, it was observed when the muscle, gill and liver of *D.rerio* were kept in sublethal concentration of raw tannery effluent showed decrease in protein, carbohydrates and lipid contents under different period of exposure.

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