

ORIGINAL ARTICLE

Alteration in Tissue Biochemical Parameters of Untreated Distillery Effluent Exposed Fish, *Mystus vittatus* (Bloch)

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ABSTRACT

Biochemical parameters serve as effective tools for evaluating the impact of anthropogenic influences on the health and physiological condition of fishes, particularly in response to environmental stressors. Therefore, the present investigation has been carried out to study the chronic impact of sublethal concentration of raw distillery effluent on the biochemical parameters of different vital tissues of *Mystus vittatus*. The result revealed that on long-term exposure of sublethal concentration of distillery effluent biomolecules such as glycogen, lipid, nucleic acids, and protein significantly decreased with increased free amino-acids in gill, liver, muscles and kidney of test fish. The trends of alterations in the level of different biomolecules in different tissues were different although it depends on concentrations of effluent and duration of exposure. The order of percent decrease of glycogen in tissues was Kidney > liver > gill > muscle; the percent decrease of total protein content in effluent exposed fish was found in the order of kidney > gill > muscle > liver; the percent decrease of lipid content effluent exposed fish was found to be in the order of liver > kidney > muscle > gill; the percent decrease of DNA was Gill > muscle > kidney > liver; the percent decrease of RNA was Liver > muscle > gill > kidney whereas the order of percent increase in the amino acids content in effluent exposed fish was kidney > liver > muscle > gill. Declined glycogen, protein and lipid levels in effluent fish was due to stress and impairment in metabolic process of fish.

Keywords: Biomolecules, Vital tissues, *Mystus vittatus*, Distillery effluent.

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INTRODUCTION

Industrial effluents alter the chemical composition of a natural aquatic environment, it typically causes changes in the biochemical, pathological, and behavioural characteristics of the inhabitants, particularly fish [1]. Effluents discharged from distilleries, sugar factories, tanneries factories, pulp and paper mills, etc., contain substantial amounts of toxic chemicals that cause death of organisms. The chemical pollutants present in the industrial effluent, which enter into the aquatic systems, affect the food chain through bioaccumulation.

Distillery industries are the agro-based industries characterized by high levels of both organic and inorganic components, resulting in the generation of potent wastes that pose challenges for disposal. These industries produce 90 000 liters of alcohol and discharges about 1.2 million litres of effluents daily, producing 15 times more waste than the actual product. Ethanol produces as a byproduct in the distilleries, create a significant depletion of natural and human resources. The raw effluent or spent wash contains trace amount of dark brown pigment called melanoidins. The spent wash, being acidic and rich in organic and inorganic salts, exhibits elevated electrical conductivity (EC). The presence of high levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and various organic compounds such as phenols, lignin, and oil and greases in the spent wash poses a potential threat to aquatic biota. The dark color of the effluents obstructs sunlight, hindering photosynthesis and proving detrimental to aquatic life. Contaminated water undergoes natural decomposition when pollution levels are within safe concentrations. However, when these levels exceed the safe limits, industrial effluents induce alterations in physico-chemical properties of aquatic ecosystems, thereby affect the inhabitants [2,3].

Biochemical parameters serve as effective tools for evaluating the impact of anthropogenic influences on the health and physiological condition of fishes, particularly in response to environmental stressors. The close connection between the circulatory system of fish and their external environment makes these parameters valuable indicators. The effects of external stressors and toxic substances on exposed fish can be detected through a clinical assessment of fish physiology. The body components, such as proteins, carbohydrates, and lipids, play crucial roles in body construction and energy production. Their involvement in major physiological events makes the assessment of these parameters a diagnostic tool for determining the physiological phases of organisms [4]. The accumulation and biotransformation of the toxicants in various tissues of fish under toxic stress conditions can be determined through the metabolism of biomolecules particularly glycogen, protein, lipid and nucleic acids [5].

Fishes are much sensitive to changing aquatic environment and play an important role in monitoring of water pollution so they are considered as good bioindicator [6]. The gill, liver, muscles and kidney play crucial role in vital functions such as respiration, detoxification, metabolic activities, and ultrafunction, serves as a valuable approach in environmental monitoring. Thus, the main objective of this study was to analyze the impact of distillery effluents and their impact on biochemical parameters of these tissues of freshwater catfish, *Mystus vittatus*.

MATERIAL AND METHODS

The freshwater catfish, *M. vittatus* were collected from local fresh water bodies in and around of Balrampur and acclimatized to laboratory conditions for 15 days. The healthy fishes of uniform length (8.7 ± 0.20 cm) and weight (8.5 ± 1 g) were selected. The test fishes were exposed to two sub-lethal concentrations i.e. 2.5% or 1/10 of 96h LC₅₀ and 5.0 % 1/5th of 96h LC₅₀ of untreated distillery effluent along with control group for 10, 20 and 30 days. After every exposure period the fishes were sacrificed for biochemical studies of gills, liver, kidney and muscles tissues. These tissues were weighted and minced with 5 ml of 5% TCA (Tri Chloro Acetic acid) and homogenates with the help of homogenizer. The homogenates were carefully collected and centrifuged for 15 minutes at 3000 rpm. The supernatants were used for the analysis of glycogen, total protein, total lipids, amino acids and nucleic acids by the standard methods [8-11].

RESULTS AND DISCUSSION

Biologically active substances including carbohydrates, proteins, and lipids are impacted by toxicants or pollutants. During the exposure of toxicants or pollutants even at sublethal concentration detoxifying phase, animals undergo significant metabolic stress [12]. The alterations in biochemical parameters of tissue such as Gill, Liver, muscles and kidney of fish, *M. vittatus* exposed to sublethal concentrations of untreated distillery effluent are presented in Table 1 to 6. T- value of biochemical parameters showed in the parentheses of table. The potency of toxicity of the distillery effluent differs in relation to tissues, concentrations of effluents and duration of exposure. In the present study except amino acids a significant decline in the amount of biochemical constituents like glycogen, protein, lipid and nucleic acids in liver, muscles, gill and kidney of *Mystus vittatus* in untreated distillery effluent stressed condition when compared to that of control fish.

Glycogen

The glycogen content was decreased significantly ($P < 0.05$) in the tissues such as gill, liver, muscles and kidney of untreated distillery effluent exposed fish, *M. vittatus*. The percentage depletion of tissue glycogen was gradually increased from initial to 30 days of exposure period. The per cent decrease of glycogen content in untreated distillery effluent exposed fish, *M. vittatus* was found to be in the order of Kidney > liver > gill > muscle (Table 1). The decrease in glycogen content of the tissues, gill, liver, muscles and kidney in untreated distillery effluent exposed fishes can be due to its enhanced utilization as an immediate source to meet energy demands under effluent stress condition. It could possibly be because of anoxic or hypoxic environments, which typically increases the consumption of glycogen [13]. Anoxia or hypoxia elevates the intake of carbohydrates, which causes respiratory stress in organisms, even at a sublethal level, requiring them to spend more energy [14].

Similarly, the decreases in glycogen content in tissues of freshwater fishes exposed to various industrial effluents were observed by various workers [15-21]. This may have occurred as a result of rapid glycogenolysis, suppression of glycogenesis via transferase depression and glycogen phosphorylase activation [19]. The marked glycogenolysis observed in the present investigation is the result of chronic exposure of untreated distillery effluent may be attributed to a stress-induced elevation in circulating catecholamines [22]. The decrease in the glycogen content during the present study indicates breakdown of storage glycogen or due to increased glycogenolysis to meet the excess energy demand by the

respective tissue as a consequence of stress caused by toxicant present in the untreated distillery effluents. Decreased corticosteroid hormone levels in the adrenal gland may have had an impact on synthesis of glycogen in the liver and muscles, which in turn may have decreased the concentration of glycogen in these tissues. The mechanism underlying the depletion of glycogen might be attributed to increased glycogenolysis and suppression of glycogenesis via the release of adrenal catecholamines in response to stress.

Table 1: Effects of sublethal concentrations of untreated distillery effluent on tissue glycogen (mg/g wet tissue) of *M. vittatus* at different period of exposure.

Tissue	Group	Exposure periods in days		
		10	20	30
Gill	Control	5.25±0.01	5.30±0.02	5.30±0.02
	2.5% of LC ₅₀	5.37±0.01(-16.971)	4.93±0.12(5.589)	4.09±0.03(53.57)
	5.0% of LC ₅₀	4.76±0.04(17.892)	4.12±0.03(57.040)	3.96±0.02(79.608)
Liver	Control	23.21±0.02	23.12±0.05	24.18±0.05
	2.5% of LC ₅₀	20.11±0.06(263.447)	19.22±0.12(60.533)	18.21±0.06(145.778)
	5.0% of LC ₅₀	16.92±0.06(147.575)	15.37±0.08(122.794)	13.93±0.39(46.534)
Muscles	Control	14.77±0.05	14.40±0.08	14.50±0.10
	2.5% of LC ₅₀	13.11±0.01(84.785)	12.33±0.10(25.534)	11.07±0.05(-1.042)
	5.0% of LC ₅₀	11.33±0.05(-58.448)	10.31±0.08(-12.902)	9.22±0.06(3.847)
Kidney	Control	3.84±0.05	3.80±0.06	3.79±0.02
	2.5% of LC ₅₀	3.06±0.03(12.819)	2.73±0.05(22.125)	2.14±0.03(66.200)
	5.0% of LC ₅₀	2.58±0.05(49.544)	2.14±0.03(56.101)	1.85±0.02(163.413)
All values were Significant at P< 0.05;		t-value in parentheses		

Protein

Proteins play a vital role by supplying energy in metabolic pathways and biochemical reactions. Proteins are the most crucial energy source to avoid during prolonged or chronic periods of stress [12]. The protein content was decreased significantly (P<0.05) in the tissues such as gill, liver, muscles and kidney of untreated distillery effluent exposed fish, *M. vittatus*. The percentage depletion of tissue protein was gradually increased from initial to 30 days of exposure period. The decreased total protein content of tissues in untreated distillery effluent exposed fish, *M. vittatus* was found to be in the order of kidney > gill > muscle > liver (Table 2).

Table 2: Effects of sublethal concentrations of untreated distillery effluent on tissue protein (mg/g wet tissue) of *M. vittatus* at different period of exposure.

Tissue	Group	Exposure periods in days		
		10	20	30
Gill	Control	18.88±0.06	18.80±0.12	18.60±0.31
	2.5% of LC ₅₀	15.85±0.06(105.537)	13.74±0.05(138.653)	12.21±0.03(44.317)
	5.0% of LC ₅₀	11.07±0.03(170.556)	10.20±0.04(122.754)	8.76±0.16(127.890)
Liver	Control	30.09±0.13	30.10±0.10	30.28±0.12
	2.5% of LC ₅₀	27.75±0.07(23.126)	26.58±0.08(87.196)	22.87±0.12(154.704)
	5.0% of LC ₅₀	25.09±0.50(15.657)	24.04±0.54(27.635)	19.36±0.21(233.682)
Muscles	Control	44.44±0.16	44.74±0.30	45.48±0.28
	2.5% of LC ₅₀	38.46±0.44(30.383)	33.35±0.28(150.881)	28.93±0.97(44.846)
	5.0% of LC ₅₀	35.25±0.40(75.437)	30.16±0.08(81.756)	23.13±0.05(138.440)
Kidney	Control	7.23±0.02	7.18±0.03	7.19±0.03
	2.5% of LC ₅₀	5.21±0.02(100.096)	4.22±0.04(111.475)	3.14±0.03(112.849)
	5.0% of LC ₅₀	2.91±0.03(228.747)	2.33±0.11(71.295)	1.60±0.03(266.738)
All values were Significant at P< 0.05;		t-value in parentheses		

Similar depletion in protein content in the tissues has been also observed in various industrial effluents exposed fishes by various researchers [15-21]. The reduction in protein content in different tissues of industrial effluent exposed fish may be attributed to stress caused by xenobiotics present in the effluent which ultimately resulted in proteolysis. During stress condition fish need more energy to overcome the stress, since fishes have less amount of carbohydrate so next alternative source of energy is protein to meet increased demand of energy during stress condition [23]. Protein depletion leads to proteolysis processes and utilization of its degenerated products for increased metabolism in order to meet the energy requirements [12].

In the present study the depletion of total protein content in the tissues may be due to breakdown of protein into free amino acid. The decrease in protein level observed in the present investigation might be attributed to both their breakdown and potential metabolic purposes. The depletion in protein content in experimental fishes may be due to decreased protein biosynthesis due to higher affinity of metal compounds present in untreated distillery effluent towards different amino acids residues of proteins, which is considered as the premier biochemical parameter for early indication of stress. Another reason of protein depletion in tissues of experimental fish was the utilization of storage protein in the formation of mucoproteins, a constituent of mucous. It was also observed in the current investigation that to counter the toxic effect of effluent, experimental fish secrete more mucous in comparison to control. Thus, decrease in tissue protein of fishes exposed to various pollutants is a physiological strategy played by the animal to adapt itself to the changing environmental conditions. Thus, the depletion of protein fraction may have been due to their degradation and possible utilization for metabolic purposes. Depletion in the protein level in different tissues/organs of experimental fishes was found under the stress of various toxicants or pollutants.

Amino acids

The amino acids content was increased significantly ($P < 0.05$) in the tissues such as gill, liver, muscles and kidney of untreated distillery effluent exposed fish, *M. vittatus*. The percentage elevation of tissue glycogen was gradually increased from initial to 30 days of exposure period. The per cent increase in the amino acids content over the control fish was found to be in the order of kidney > liver > muscle > gill (Table 3).

Table 3: Effects of sublethal concentrations of untreated distillery effluent on tissue amino acids (mg/g wet tissue) of *M. vittatus* at different period of exposure.

Tissue	Group	Exposure periods in days		
		10	20	30
Gill	Control	1.94±0.01	1.95±0.02	1.95±0.01
	2.5% of LC ₅₀	2.09±0.02(-11.523)	2.16±0.01(-11.502)	2.45±0.06(-14.335)
	5.0% of LC ₅₀	2.63±0.03(-43.639)	2.79±0.06(-24.770)	3.09±0.06(-31.882)
Liver	Control	2.11±0.01	2.12±0.01	2.10±0.01
	2.5% of LC ₅₀	2.50±0.01(-31.391)	2.65±0.04(-22.924)	2.92±0.04(-44.092)
	5.0% of LC ₅₀	2.62±0.07(-12.309)	2.89±0.05(-22.718)	3.20±0.02(-50.697)
Muscles	Control	0.75±0.01	0.75±0.01	0.75±0.01
	2.5% of LC ₅₀	0.85±0.04(-6.205)	0.88±0.02(-10.502)	0.92±0.02(-26.623)
	5.0% of LC ₅₀	0.99±0.01(-22.220)	1.04±0.01(-38.333)	1.14±0.01(-27.366)
Kidney	Control	1.13±0.01	1.14±0.01	1.13±0.02
	2.5% of LC ₅₀	1.40±0.01(-46.765)	1.68±0.04(-28.093)	1.92±0.01(-69.678)
	5.0% of LC ₅₀	1.60±0.05(-20.444)	1.80±0.02(-53.889)	2.12±0.01(-206.282)

Similar increase in free amino acid level was found in tissues of freshwater fishes exposed to industrial effluent [24,25]. This elevation in free amino acids level in the tissues indicates stepped up the activities of proteases and aminotransferases along with the fixation of ammonia into keto acid [22,26] or due to depletion of reserved glycogen so that they can try to yield metabolic energy by gluconeogenesis process [27]. Another reason of elevation of free amino acids content was due to less use of amino acids in protein biosynthesis because stress conditions induce the transamination process [28] and their use to maintain the acid-base balance [29]. In the current investigation, the significant elevation in free amino acids level along with significant depletion in protein content in the tissues of distillery effluent exposed fishes, *Mystus vittatus* was due to increased proteolysis and an inverse relationship between protein and amino acids in the tissues to meet the extra demand of energy requisites in addition to the carbohydrate and fat during stress conditions [6, 25]. Therefore, under stress condition to fulfill the increased energy demand, freshwater fishes may degrade storage protein to augment the available energy supply, thus increasing the free amino acids level in these tissues.

Lipid

Lipids are the important component of the cell membranes and are essential for normal cellular permeability. It also plays an important physiological role for storage of energy and vitamins [30]. In the present study lipid content was decreased significantly ($P < 0.05$) in the tissues such as gill, liver, muscles and kidney of untreated distillery effluent exposed fish, *M. vittatus*. The percentage depletion of tissue

lipid content was gradually increased from initial to 30 days of exposure period. The per cent decrease of lipid content in untreated distillery effluent exposed fish, *M. vittatus* was found to be in the order of Liver > kidney > muscle > gill (Table 4). Similarly, the decrease in tissues lipid content were observed in influent exposed fishes were observed by various workers [15-17,19,21,31-33]. The decrease in lipid content in these tissues can be due to their active mobilization towards the blood and/or tissue metabolism or might be due to the utilization of stored lipid as a source of energy to meet the additional demand of energy to conduct regular metabolic activity under stress condition. Loss of lipids observed in the present study may be due to inhibiting the synthesis of lipid and the mobilization of the stored lipid, either through β oxidation or through a gradual unsaturation of lipid molecules [34]. Due to less cholesterol content in fresh water fishes, the lipid content in the tissues depleted during stress condition [35]. Thus, the depletion of lipid in the tissues may have been due to inhibition of lipid synthesis and increased utilization of stored lipid as a source of supplementary energy to conduct regular metabolic activity under stress condition.

Table 4: Effects of sublethal concentrations of untreated distillery effluent on tissue lipid (mg/g wet tissue) of *M. vittatus* at different period of exposure.

Tissue	Group	Exposure periods in days		
		10	20	30
Gill	Control	21.05±0.01	21.15±0.02	21.04±0.01
	2.5% of LC ₅₀	19.30±0.09(40.610)	18.87±0.07(7.804)	17.65±0.07(94.799)
	5.0% of LC ₅₀	17.87±0.11(62.361)	16.35±0.05(117.295)	14.80±0.03(347.109)
Liver	Control	41.70±0.99	43.36±0.11	43.26±0.03
	2.5% of LC ₅₀	38.35±0.14(5.981)	35.16±0.06(90.831)	32.46±0.55(40.416)
	5.0% of LC ₅₀	33.08±0.12(18.435)	31.30±0.10(167.714)	29.36±0.43(67.019)
Muscles	Control	24.48±0.01	24.40±0.04	24.83±0.48
	2.5% of LC ₅₀	21.31±0.07(93.682)	20.58±0.04(97.163)	19.16±0.03(634.433)
	5.0% of LC ₅₀	18.13±0.01(449.013)	17.89±0.05(531.539)	17.55±0.09(127.726)
Kidney	Control	15.14±0.17	15.10±0.01	15.07±0.01
	2.5% of LC ₅₀	14.27±0.05(24.463)	13.87±0.05(44.981)	12.43±0.05(30.204)
	5.0% of LC ₅₀	12.68±0.06(66.233)	11.38±0.08(103.367)	10.49±0.23(38.189)

Nucleic acids

Nucleic acid indices are sensitive to changes in an organism's particular growth rate [36] and they are utilized to identify early indicators of stress physiology in relation to its habitat [37]. The nucleic acid (DNA and RNA) content was decreased significantly ($P < 0.05$) in the tissues of untreated distillery effluent exposed fish, *M. vittatus*. The percentage depletion of tissue nucleic acid was gradually increased from initial to 30 days of exposure period. The per cent decrease of DNA content in untreated distillery effluent exposed fish, *M. vittatus* was found to be in the order of Gill > muscle > kidney > liver whereas the RNA content was decreased to be in the order of Liver > muscle > gill > kidney (Table 5). Similarly, the decrease in tissues nucleic acid contents were observed in effluent exposed fishes by some researchers [36-39]. This decrease in nucleic (both DNA and RNA) content of the tissues of distillery effluent exposed fishes can be due to genotoxic action of xenobiotics present in the untreated distillery effluent that decreases the mitotic index and disturbed the process of cell division or due to inhibitory action of xenobiotics on biosynthesis of nucleic acids or by apoptosis due to focal necrosis [37]. The decrease in nucleic acids contents in these tissues was the indication of malfunction and degenerative changes in the vital organs of stressed fish [40]. The significant decrease in both protein and nucleic acids as observed in the present investigation would suggest that the toxicants or metals present in the industrial effluent impaired the process of protein synthesis in the tissues of fishes exposed to untreated distillery effluents. Since DNA serves as a primer in RNA polymerase and DNA replication reactions, inhibitions of this enzyme prevent the synthesis of nucleic acids by interfering with RNA transcription and DNA replication activities. The tissues' protein content may decrease if RNA synthesis is inhibited at transcription-stage, as RNA synthesis is essential to protein synthesis. Because of this, the toxicant found in untreated distillery effluent may act as an inhibitor of DNA synthesis, reducing transcription and the amount of RNA in tissues. The overall outcome shows that the spent wash or untreated distillery effluent interferes with DNA synthesis, which may have an impact on RNA synthesis. Therefore, changes in the content of nucleic acids have an impact on the production of proteins.

Table 5: Effects of sublethal concentrations of untreated distillery effluent on tissue nucleic acids (mg/g wet tissue) of *M. vittatus* at different period of exposure.

Tissue	Group	Exposure periods in days		
		10	20	30
DNA				
Gill	Control	7.12±0.01	7.12±0.01	7.12±0.02
	2.5% of LC ₅₀	6.70±0.02(48.893)	6.46±0.05(19.198)	6.36±0.03(28.504)
	5.0% of LC ₅₀	6.11±0.01(97.257)	5.82±0.06(19.198)	6.36±0.03(28.504)
Liver	Control	2.52±0.02	2.52±0.03	2.52±0.02
	2.5% of LC ₅₀	2.41±0.01(7.028)	2.18±0.02(30.442)	2.15±0.21(18.233)
	5.0% of LC ₅₀	2.20±0.01(27.305)	2.11±0.01(26.958)	2.14±0.03(13.578)
Muscles	Control	4.73±0.01	4.80±0.02	4.75±0.02
	2.5% of LC ₅₀	4.33±0.01(63.180)	4.05±0.03(100.333)	3.53±0.06(29.589)
	5.0% of LC ₅₀	3.59±0.03(52.307)	3.16±0.03(57.257)	2.59±0.04(58.075)
Kidney	Control	8.77±0.03	8.82±0.02	8.65±0.21
	2.5% of LC ₅₀	8.49±0.02(17.186)	8.30±0.01(34.661)	8.15±0.04(4.170)
	5.0% of LC ₅₀	8.21±0.01(48.034)	7.84±0.03(81.916)	7.15±0.02(12.847)
RNA				
Gill	Control	7.02±0.01	7.09±0.03	7.05±0.02
	2.5% of LC ₅₀	6.54±0.28(23.422)	6.47±0.03(0.03)	6.07±0.04(45.639)
	5.0% of LC ₅₀	6.04±0.03(21.794)	5.72±0.02(51.175)	5.21±0.06(46.457)
Liver	Control	0.915±0.002	0.917±0.003	0.917±0.002
	2.5% of LC ₅₀	0.837±0.01(23.737)	0.768±0.03(8.650)	0.676±0.003(145.027)
	5.0% of LC ₅₀	0.692±0.002(96.031)	0.612±0.002(169.868)	0.572±0.002(202.590)
Muscles	Control	3.36±0.03	3.48±0.01	3.39±0.04
	2.5% of LC ₅₀	3.20±0.01(7.325)	3.11±0.01(32.043)	2.94±0.06(16.617)
	5.0% of LC ₅₀	3.06±0.01(12.041)	2.81±0.02(31.250)	2.46±0.06(28.299)
Kidney	Control	4.78±0.03	4.83±0.02	4.79±0.02
	2.5% of LC ₅₀	4.63±0.02(5.738)	4.46±0.04(12.004)	4.19±0.02(125.858)
	5.0% of LC ₅₀	4.32±0.02(21.419)	4.12±0.21(56.028)	3.91±0.03(79.599)

CONCLUSIONS

The alterations in the biochemical set up of fish during exposure to industrial effluent imposes stress on fishes which leads to rapid mobilization of these energy yielding biomolecules to yield excess energy required by the stressed animals to compensate the heavy physical exercise in the form of erratic and rapid movements under stress condition caused by toxicant present in the effluent.

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CONFLICT OF INTEREST

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