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Analysis of Short and Long-Term Impacts of Petrochemical Effluents on Cattla Cattla Fish on Neelambur Pond, Coimbatore, Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study aimed to assess the impact of petrochemical effluent on fish blood, examining both short-term and long-term exposure periods to evaluate toxicity stress symptoms in the rapidly evolving petroleum industry. Various biochemical and hematological parameters, along with enzymatic changes, were analyzed by exposing different organs of freshwater fish, *C. catla*, to the petrochemical effluent. The results indicated a significant decrease in hemoglobin (Hb) content, red

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blood cells, packed cell volume, and mean corpuscular hemoglobin (MCH) values. Conversely, there was a notable increase in white blood cells (WBC) during the exposure periods compared to the control. Hematological parameters, including Hb, RBC, mean corpuscular volume (MCV), and MCH, exhibited fluctuating results, with a consistent decrease observed in both short- term (24, 48, 72, and 96 hours) and long-term (10, 20, and 30 days) exposure periods, suggesting the occurrence of anemia in the exposed fishes due to petrochemical effluent. In the biochemical analysis, exposed fish showed a significant reduction in protein, carbohydrate, and lipid content across all organs, accompanied by increased enzyme activity observed in all organs of freshwater fish, *C. catla*. Consequently, this survey is instrumental in predicting potential risks to the population and aquatic system.

Keywords: Petrochemical effluent; biochemical; anti-oxidant; fish.

1. INTRODUCTION

Industrial effluents are a significant contributor to water pollution, containing suspended solids, pesticides, organic and inorganic substances, along with various toxic metal compounds. These pollutants are present at levels that have the potential to impact the quality of receiving waters and pose a threat to the aquatic ecosystem [1]. Numerous industries in India release their effluents into inland water bodies, directly or indirectly affecting nearby rivers. It has been observed that nearly all industries are non-compliant with regulations regarding effluent discharge, resulting in pollution of nearby freshwater bodies and agricultural land [2]. Aquatic organisms possess the ability to accumulate and concentrate heavy metals up to a certain threshold. Nevertheless, when the concentration and toxicity of heavy metals surpass the tolerance levels of these organisms, it can lead to severe toxic effects on their associated indicators and impact their life activities. Simultaneously, this exposure may induce genetic mutations or variations, contributing to alterations in species diversity and survival rates [3].

Wastewater from industries involved in crude oil extraction and the production of fuels, lubricants, and other petroleum-based goods is known as petroleum refinery effluent (PRE) [4]. These effluents comprise a range of pollutants, such as hydrocarbons, ammonia, heavy metals, sulphides, and phenols [5], [6]. Contamination by heavy metals is recognised as a detrimental factor to the health of fish, primarily impacting their growth performance, survival, and reproductive capabilities. The intake of heavy metals through the food chain by human populations is a global concern, extensively documented worldwide [7]. These metals can significantly affect crucial functions and reproduction in fish, weaken the immune system, and induce pathological changes. Consequently, fish serve as bio-indicators, playing a vital role in monitoring heavy metal pollution. The presence of harmful and destructive metals in freshwater bodies poses a threat to aquatic species, constituting an ecological issue [8]. Various toxicity endpoints, such as oxidative stress, reduced liver detoxification ability, diminished stomach digestion, impaired reproductive performance, *microbiota dysbiosis* in the gut, and alterations in genetic coding and gene expression in offspring, have been assessed to evaluate the harmful effects of effluents on fish [9].

India, endowed with an extensive coastline, witnesses a substantial consumption of marine fish among its population, whereas lakes and rivers serve as the primary sources for most freshwater fish [10]. Numerous studies have investigated the concentrations of heavy metals in commonly consumed freshwater and marine fish [11-14]. The influence of Sago effluent on *Catla catla*, a freshwater fish, was observed by [15]. Similarly,[16] reported comparable findings regarding the impact of sago industry effluent on *Labeo rohita*. Documented the effects of tannery effluent on *Catla catla*, a freshwater fish [17]. Noted the toxic impact of dyeing effluent on *Catla catla*[18]. In the case of *Cirrhinus mrigala*, [19] made similar observations in the context of sago effluent. Additionally, [20] conducted a study on the impact of distillery effluent on *Labeo rohita*.

Many bioassays have been conducted to monitor and evaluate the toxicity of wastewater from domestic and various industrial sources [21]. Blood variables in the diverse habitats of fish serve as indicators of metabolic status under physiological stress [22]. [23] Observed a reduction in haematocrit, Hb, MCH, and bidirectional fluctuations in RBC count in *Clarias*

gariepinus when exposed to metal finishing effluent. Exposure to tannery effluent led to a significant reduction in RBC, Hb, and HCT in *Channa punctatus*, resulting in an anemic condition [24].

Histopathological alterations have been widely utilised as biomarkers to assess the health of fish exposed to toxicants in both field and laboratory studies. One significant advantage of histopathological biomarkers in environmental assessment studies is the examination of target organs [25]. Similar studies on fish exposed to different industrial effluents have been conducted previously [26–27]. The environmental impacts of petroleum refinery effluents (PRE) have been evaluated through toxicity tests and field surveys [28, 29], indicating adverse effects of PREs on aquatic organisms [30].

In this current investigation, we assess the physico-chemical parameters of the water in Neelambur Pond across various sampling stations. We examine the sub-lethal impacts of short- and long-term exposure to petrochemical effluent on the biochemical, haematological, and enzymological conditions of the common carp, *Catla catla*. Finally, we compare the effectiveness of specific microorganisms in mitigating the pollution load present in petrochemical effluent.

2. MATERIALS AND METHODS

2.1 Study Area

The Neelambur pond is situated at a latitude of 11º 03’ 29” N and a longitude of 77º 03’ 29” E. It has a catchment area of 75.47 square miles and water spread area of 0.668 square kilometers. The pond covers an area of approximately 334 acres with a depth of 14.60 feet, which can be increased to a depth of 24.76 feet by water inflow. The maximum flood discharge capacity of the pond is 2777.69 cubic feet per second. The Noyyal River feeds into the Odderpalayam pond, which is then connected to the Irugur pond. From Odderpalayam, the river flows towards Sengulam, where it receives domestic sewage from the surrounding areas. It continues for about 1½ kilometre and reaches Ravathur, where it receives effluents from a petroleum company. After travelling a distance of 3 kilometres, it arrives at Kulathur, where it collects sewage and additional effluents. Finally, after another 1½ kilometres, it reaches the Neelambur pond. The water in this pond serves various purposes, including washing, bathing, and drinking for cattle, as well as irrigation and fish farming.

For the study, the research area was divided into four sampling stations, and water samples were collected and analysed for several key physical and chemical water quality parameters. These parameters include temperature, pH level, electrical conductivity, total solids, total dissolved solids, total alkalinity, total hardness, dissolved oxygen content, biological oxygen demand, chemical oxygen demand, chloride concentration, sulphate levels, and phosphate levels. Fig. 1 provides a visual representation of the study area and the described locations in the water system.

2.2 Physico- Chemical Analysis of Neelambur Pond Water

Water samples were acquired from four specific locations within Neelambur Pond in Coimbatore, utilising plastic containers to maintain sample integrity. The collected samples were promptly transported to the laboratory and stored at a refrigeration temperature of 4°C. The evaluation of the physico-chemical properties of these water samples followed established protocols outlined in the standard methodologies recommended by [31]. These widely acknowledged methods formed the basis for the thorough analysis of the water samples.

2.3 Impact Studies of Petrochemical Effluent on Fresh Water Fish, *Catla Catla*

2.3.1 Procurement and acclimatization of fishes

For the experimental study, *Catla catla,* a type of freshwater fish, was chosen as the test specimen. Healthy *Catla catla* specimens were obtained from the "Tamil Nadu Fisheries Development Corporation Ltd. Aliyar, Pollachi." These fish were subjected to a 15-day acclimatisation process under laboratory conditions at room temperature. Throughout the acclimatisation period, the fish were provided with a regular diet consisting of a conventional mixture of rice bran and oil cake in a 1:1 ratio. However, feeding was discontinued one day prior to the commencement of the experiment.

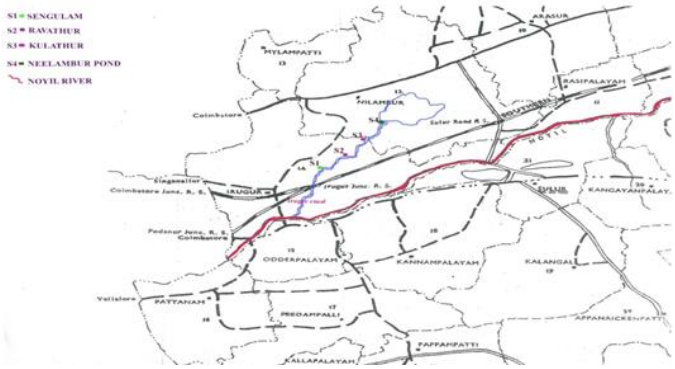

Fig. 1. Area Map – Course of Neelambur Pond with Different Sampling Stations

2.4 Bioassay

Fish of uniform size were selected for the study, and the static bioassay method was employed. To conduct the experiments, various concentrations of petrochemical effluent were prepared, with a pilot study being conducted to identify the concentration range that resulted in mortality rates ranging from 10 to 90 percent. Subsequently, ten fish from the stock were exposed to each of these different concentrations of the petrochemical effluent, while a control group was run concurrently. The mortality rates were recorded at 12-hour intervals, and any deceased fish were promptly removed from the experiment. The bioassay experiments for the petrochemical effluent were carried out separately, and the LC50 values for a 96-hour period were determined using probit analysis, following the methodology established by [32]. The petrochemical effluent was exposed to a sub lethal concentration of 1/10th of the 96-hour LC_{50} value for both short-term and long-term periods.

2.5 Toxicity Studies

Fishes were divided into three groups, each group consisted of 20 fishes.

Group I - Control fishes
Group II Fishes exposed to short term duration of petrochemical effluent
Group III Fishes exposed to long term duration of petrochemical effluent

At the conclusion of the designated exposure period, all fish from the three experimental groups were humanely euthanized. Tissues, including gill, liver, kidney, and muscle, were carefully isolated from the fish specimens, and utilized for subsequent biochemical and enzymological analyses.

2.6 Biochemical Analysis of Tissue Sample

2.6.1 Preparation of sample

To prepare the tissue samples, 100 mg of isolated tissues, namely gill, liver, kidney, and muscle, were homogenized using 1 ml of a 0.9 percent sodium chloride solution. Subsequently, 1 ml of a 5 percent trichloroacetic acid solution was added to all four samples, and the resulting mixtures were centrifuged at 3000 rpm for 10 minutes. The supernatant obtained from this process was used for carbohydrate estimation, following the anthrone method developed by [33]. The residue left after the centrifugation step was dissolved in 1 ml of a 0.1 N sodium hydroxide solution and utilized for protein estimation, employing the method established by [34]. For lipid estimation, the method of [35] was applied. This involved grinding 100 mg of the gill, liver, kidney, and muscle tissues with 5 ml of a chloroform-methanol mixture, followed by a subsequent centrifugation step.

2.7 Hematological Analysis

The blood samples were collected from the caudal vein of both control and treated fish. Hemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Packed Cell Volume (PCV) were assessed using the protocols outlined by [36], [37]. The anticoagulant used for the estimation of MCV, MCH, and PCV was heparin liquid powder (5,000 I.U).

2.8 Enzyme analysis of Tissue Sample

The activities of enzymes such as Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT), Lactate dehydrogenase (LDH) were investigated in the

gill, liver, kidney, and muscle tissues of both control and experimental fish.

2.8.1 Preparation of samples for enzyme assay

Tissues including gill, liver, kidney, and muscle were isolated from both the control and experimental fish. Each tissue sample, weighing 100 mg, was carefully weighed, and subsequently homogenized with 2.5 ml of a 0.25 M sucrose solution under ice-cold conditions, following the method outlined by [38].

2.9 Statistical Analysis

The biochemical composition, hematological changes, and enzyme levels of *Catla catla* fish were subjected to statistical analysis employing the student't' test. The data obtained from various biological treatment methods were analyzed using a two-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Physico-Chemical Characteristics of Neelambur Pond Water During Summer Season

The water temperature ranged between 32.00ºC and 35.00ºC at most stations in Neelambur Pond. The water quality at all sampling stations was found to be alkaline, with pH values ranging between 7.50 (S3) and 7.10 (S1). The maximum dissolved oxygen (DO) was recorded at S4 (2.30 mg/l), followed by S3 (0.08 mg/l), S2 (0.06 mg/l), and S1, with a minimum DO of 0.02 mg/l. In the case of sulphate was maximum being observed in S_4 (124.00 mgl^{-1}) followed by S_3 (82.00 mgl^{-1}) and S_1 (81.00 mgl^{-1}), While S_2 showed a minimum of 80.10 mgl^{-1}. Maximum phosphate content was recorded at S_1 (2.90 mgl^{-1}), while all the other stations (S_4, S_3 and S_2) showed a minimum level (2.15 mgl^{-1}, 2.10 mgl^{-1} and 2.00 mgl^{-1} respectively). Cadmium levels were detected in stations S1, S2, and S3, with the maximum concentration observed in S2 (0.45 mgl^{-1}), while the minimum was noted in S3 and S1 (0.39 mgl^{-1} and 0.08 mgl^{-1}, respectively). Cadmium was not present in S4. The highest lead content was recorded in S2 (0.92 mgl^{-1}), followed by S3 and S1 (0.87 mgl^{-1} and 0.76 mgl-1, respectively). Lead was absent in S4. Maximum zinc concentration was observed in S1 (0.23 mg/l), and the minimum was noted in S2 (0.09 mg/l), with no zinc present in S3 and S4. Cobalt was found in S1 and S3, with the highest cobalt content observed in S1 (0.17 mgl-1) and the minimum in S2 (0.07 mgl^{-1}). Cobalt was not present in S3 and S4 (Table 1). Similar findings were also reported by [39].

3.2 Evolving Short and Long-Term Exposure of Petrochemical Effluent on Fresh Water Fish, *Catla catla* (Bioassay)

The mortality of *Catla catla* increased in correlation with rising concentrations of petrochemical effluent, while no mortality was observed in the control group. Table 2 presents the 96-hour LC_{50} values for the toxicity of petrochemical effluent *to Catla catla*. The calculated 96-hour LC_{50} value for petrochemical effluent, with a 95 percent confidence limit, was determined to be 27.20 percent, with a lower limit of 26.45 and an upper limit of 27.26. These

Table 1. Physico chemical characteristics of neelambur pond at different sampling stations during summer season

Parameters	Station 1	Station 2	Station 3	Station 4
Temperature ºC	34.00±1.00	35.00±0.63	34.00±0.50	32.00±0.80
pH	7.10±1.00	7.30±0.03	7.50±0.20	7.40±0.57
Total Alkalinity	7.90±1.10	7.20±0.15	8.90±0.10	7.80±1.00
Total Hardness	3.12±0.12	2.50±0.25	2.30±0.10	2.09±0.04
Dissolved Oxygen	0.02±0.01	0.06±0.01	0.08±0.01	2.30±0.10
Chloride	5.85±0.10	5.73±0.01	6.20±2.00	6.43±1.12
Sulphate	81.00±1.00	80.10±2.00	82.00±2.00	124.00±1.00
Phosphate	2.90±0.05	2.00±0.01	2.10±1.00	2.15±0.36
Cadmium	0.08±0.01	0.45±0.05	0.39±0.01	BDL
Lead	0.76±0.26	0.92±0.02	0.87±0.01	BDL
Zinc	0.23±0.04	0.09±0.01	BDL	BDL
Cobalt	0.17±0.12	0.07±0.01	BDL	BDL

All values are in mgl^{-1} except pH and EC (mmhos/cm).

Table2. Tolerance of *Catla catla* to petrochemical effluent

Sample	96 hours LC_{50} in (%) concentration	95% Confidence		Probit Equation	Chi-square
		Lower limit	Upper limit		
Petrochemical effluent	27.20	26.45	27.26	Y=15.36+7.47	-9.66

Table 3a. Changes in the protein content in the tissues of *Catla catla* on short term exposure

Sample (mg/g wet tissue)	Exposure periods				
	Control	24 hrs	48 hrs	72 hrs	96 hrs
Gill	1.77±0.11	1.45±0.44	1.35±0.22	1.00±0.07	0.90±0.02
't'		1.56 NS	3.79**	12.65**	17.12**
% change		-18.07	-23.72	-43.50	-49.15
Liver	1.88±0.06	1.64±0.03	1.55±0.20	1.12±0.11	0.65±0.22
't'		7.58**	3.43**	13.33**	11.94**
% change		-12.76	-17.55	-40.42	-65.42
Kidney	1.55±0.22	1.12±0.25	1.10±0.23	1.05±0.20	1.00±0.09
't'		2.86**	3.10**	3.70**	5.10**
% change		-27.74	-29.03	-32.25	-35.48
Muscle	2.10±0.26	1.70±0.28	1.66±0.30	1.58±0.47	1.43±0.22
't'		2.28 NS	2.44**	2.13 NS	4.30**
% change		-19.04	-20.95	-24.76	-31.90

values indicate that petrochemical effluent exhibits high toxicity to *Catla catla*. Furthermore, the calculated sub-lethal concentration value (equivalent to 1/10th of the 96-hour LC_{50}) for petrochemical effluent was found to be 2.72 percent. Similarly, toxicants contained in industrial effluents have been reported to be toxic, depending on the dose and exposure duration [40], and they can impart serious damage to aquatic life [41]. There have been several reported cases of fish mortality due to the discharge of industrial effluents from several industries into the receiving water bodies [42], [43]. The pollutants that build up in the food chain are responsible for the adverse effects and, finally, the death of aquatic organisms [44]. That is why the preferred way to evaluate the ecological influence of toxic compounds is mortality or bioassay experiments in general, as studied by [45].

3.3 Effect of Petrochemical Effluent on Biochemical Composition of *Catla catla* in Short Term and Long-Term Exposure Periods

3.3.1Total protein content

The amount of protein estimated in different tissues of the fish, *Catla catla,* subjected to short-term (24, 48, 72, and 96 hours) and long-term (10, 20, and 30 days) exposures is presented in Tables 3a and Table 3b. The study found that fish exposed to petrochemical effluent had varying levels of protein content in their gill tissue and liver tissue after various exposure times. Short- term exposure resulted in protein levels of 1.45, 1.35, 1.00, and 0.90 mg/g, while long-term exposure resulted in protein levels of 0.85, 0.76, and 0.55 mg/g. The liver tissue protein content was 1.64 mg/g, 1.55 mg/g, 1.12 mg/g, and 0.65 mg/g, while long-term exposure levels were 0.23, 0.17, and 0.10 mg/g. Kidney recorded 1.12 mg/g, 1.10 mg/g, 1.05 mg/g, and 1.00 mg/g of protein in fish exposed to short-term exposure to petrochemical effluent for 24, 48, 72, and 96 hours, respectively. 0.98 mg/g, 0.80 mg/g, and 0.76 mg/g were recorded in the kidneys of fish exposed to long-term exposure to petrochemical effluent for 10, 20, and 30 days. The mean control value was 1.55 mg/g. The muscle protein levels in the fish that were subjected to short- and long-term exposure to petrochemical effluent were 1.70 mg/g, 1.66 mg/g, 1.58 mg/g, 1.43 mg/g, and 1.37, 1.22, and 1.05 mg/g, respectively. The mean control value is 2.10 mg/g.

3.3.2 Total Carbohydrate content

The amount of carbohydrate in the tissues estimated after exposing the fish to short-term and long-term exposure periods of the

petrochemical effluent is presented in Tables 3a and Table 3b. The gills of the fish exposed to 2.72 percent petrochemical effluent for 24, 48, 72, and 96 hours were found to contain 12.55 mg/g, 11.00 mg/g, 10.80 mg/g, and 10.55 mg/g of carbohydrate. In the case of long-term exposed fishes, the values were 10.20 mg/g, 9.80 mg/g, and 8.87 mg/g after 10, 20, and 30 days, respectively. The fish maintained as controls were found to contain a mean of 12.80 mg/g in their gill tissue. Liver tissue was found to contain 17.00 mg/g, 16.50 mg/g, 16.00 mg/g, and 15.90 mg/g of carbohydrate in 24, 48, 72, and 96-hour exposures at a 2.72 percent concentration of petrochemical effluent. Under treatment of effluent for 10, 20, and 30-day exposures, the values were 15.40 mg/g, 15.00 mg/g, and 14.20 mg/g, respectively. The mean carbohydrate content in the liver of the control was 17.90 mg/g. 15.33 mg/g, 14.09 mg/g, 14.00 mg/g, and 13.70 mg/g of carbohydrate were found in the kidney tissue of 24, 48, 72, and 96-hour-treated fish. The values of carbohydrate estimated in the fish exposed to long-term periods in 2.72 percent petrochemical effluent were 12.50 mg/g, 11.08 mg/g, and 10.90 mg/g in their kidney tissue. The mean control value was 15.70 mg/g. The mean carbohydrate content in the muscle of the control fish was 14.80 mg/g. The amount of carbohydrate in the fish exposed to 24, 48, 72, and 96 hours of 2.72 percent petrochemical effluent was 14.20 mg/g, 14.00 mg/g, 13.80 mg/g, and 13.55 mg/g of carbohydrate, respectively. The amounts of carbohydrate in long-term treatment were 13.34 mg/g, 12.81 mg/g, and 11.00 mg/g under 10, 20, and 30 days of exposure at a 2.72 percent petrochemical effluent concentration.

Table 3b. Changes in the protein content in the tissues of *Catla catla* on long term exposure

Sample(mg/g wet tissue)	Exposure periods		
	10 days	20 days	30 days
Gill	0.85±0.04	0.76±0.01	0.55±0.03
't'	17.08**	20.20**	23.69**
% change	-51.97	-57.06	-68.92
Liver	0.23±0.04	0.17±0.12	0.10±0.03
't'	46.66**	27.03**	56.28**
% change	-87.76	-90.95	-94.68
Kidney	0.98±0.20	0.80±0.02	0.76±0.26
't'	4.21**	7.52**	5.07**
% change	-36.77	-48.38	-50.96
Muscle	1.37±0.25	1.22±0.52	1.05±0.56
't'	4.42**	3.35**	3.72**
% change	-34.76	-41.90	-50.00

Values are mean ± SD, n=5, Figs. in parenthesis are percentage decrease over control.
*** - Significant at one per cent level; *- Significant at five per cent level; NS- Non significant.*

Table 4a. Changes in the carbohydrate content in the tissues of *Catla catla* on short term exposure

Sample (mg/g wet tissue)	Exposure periods				
	Control	24 hrs	48 hrs	72 hrs	96 hrs
Gill	12.80 ± 0.03	12.55±0.07	11.00±1.58	10.80±0.11	10.55±0.06
't'		6.56**	2.54**	38.49**	71.15**
% change		-1.95	-14.06	-15.62	-17.57
Liver	17.90±0.04	17.00±0.31	16.50±0.11	16.00±0.44	15.90±0.34
't'		5.93**	25.99**	9.54**	12.73**
% change		-5.02	-7.82	-10.61	-11.17
Kidney	15.70±0.31	15.33±0.09	14.09±1.21	14.00±0.47	13.70±0.12
't'		2.50**	2.86**	6.66**	13.25**
% change		-2.35	-10.25	-10.82	-12.73
Muscle	14.80±0.11	14.20±0.22	14.00±1.58	13.80±0.42	13.55±0.23
't'		5.42**	1.12^{NS}	5.07**	10.67**
% change		-4.05	-5.40	-10.40	-8.44

Table 4b. Changes in the carbohydrate content in the tissues of *Catla catla* on long term exposure

Sample (mg/g wet tissue)	Exposure periods		
	10 days	20 days	30 days
Gill	10.20±0.15	9.80±0.12	8.87±1.39
't'	36.05**	51.44**	6.55**
% change	-20.31	-23.43	-31.87
Liver	15.40±0.41	15.00±0.36	14.20±3.16
't'	13.50**	17.68**	2.61**
% change	-13.96	-16.20	-20.67
Kidney	12.50±0.07	11.08±0.12	10.90±1.58
't'	21.95**	30.33**	6.65**
% change	-20.38	-29.42	-30.57
Muscle	13.34±0.06	12.81±0.14	11.00±1.81
't'	25.61**	24.68**	2.21 NS
% change	-9.86	-13.44	-25.67

Values are mean ± SD, n=5, Figs. in parenthesis are percentage decrease over control.
*** - Significant at one per cent level; *- Significant at five per cent level; NS- Nonsignificant.*

3.3.3 Total Lipid content

The amount of lipid in the tissues estimated after exposing the fishes to short term and long-term exposure periods of the effluent are presented in Table 5a and5 b. The Lipid content in the gill tissue of fishes exposed to short term exposure periods in terms of 24, 48, 72 and 96 hours were 15.70 mg/g, 15.00 mg/g, 14.92 mg/g and 14.80 mg/g respectively. The fishes exposed to long term periods of 10, 20 and 30 days in 2.72 per cent effluent contained 14.70 mg/g, 14.22 mg/g and 13.52 mg/g lipid in their gill respectively against an average of 16.80 mg/g in the control. Liver tissue was found to contain 11.95 mg/g, 11.90 mg/g, 11.30 mg/g and 11.17 mg/g of lipid in short term exposure periods of 24, 48, 72 and 96 hours. The fishes subjected to long term periods were found to contain 11.09, 10.00 and 9.87 mg/g of lipid. The mean control value was 12.53 mg/g. Kidney recorded 14.98 mg/g in the control fishes. The fishes exposed for short term periods were found to contain 14.73, 14.30, 14.11, and 13.98 mg/g of lipid. However, those exposed to longer durations contained 13.23, 12.78 and 12.55 mg/g. The control fishes were found to contain 14.98 mg/g of lipid in their kidney. The amount of lipid in the muscle tissue were 15.00, 15.08, 14.10 and 13.85 mg/g in the fishes exposed to 2.72 per cent effluent after 24, 48, 72 and 96 hour exposure periods. However, the fishes exposed to longer durations were found to contain 13.88, 13.45 and 12.00 mg/g of lipid. The control fishes were found to contain 15.90 mg/g of lipid in their muscles.

In the current investigation, a reduction in protein, carbohydrate, and lipid content was observed across all organs in *Catla catla* fish subjected to both short-term and long-term exposure. Similar findings were reported by [46], who noted a significant decrease in protein, carbohydrate, and lipid levels in the muscle, liver, and intestine of *Cyprinus carpio* when exposed to sublethal concentrations of textile mill effluent. [47] documented changes in the protein and lipid content of the intestine, liver, and gonads in freshwater murrel, *Channa punctatus* (Bloch), following exposure to lead. The significant decrease in total protein content suggests that effluent treatment-induced stress triggers proteolysis. Stress-induced acceleration of protein metabolism in humans and animals has been reported [48]. The decline in protein levels may be attributed to stress in fish, as proteins are likely to undergo hydrolysis and oxidation through the tricarboxylic acid (TCA) cycle to meet the heightened demand for energy caused by stress [49]. The observed alterations in tissue protein in the present study indicate disruptions in physiological activities. Similar findings have been reported, that the reduction in tissue proteins can arise from factors such as a compromised or diminished protein synthesis rate, their consumption in cellular repair and organization processes, and a decline in the absorption of amino acids into the polypeptide chain [50]. The decline in protein fractions within the liver, brain, and kidney may be attributed to their breakdown and potential utilization for metabolic purposes.

As per [51], the reduced levels of carbohydrate constituents in tissues of animals exposed to toxicants could be attributed to the prevalence of hypoxic conditions induced by pollutant stress.

Table 5a. Changes in the lipid content in the tissues of *Catla catla* on short term exposure

Sample (mg/g wet tissue)	Exposure periods				
	Control	24 hrs	48 hrs	72 hrs	96 hrs
Gill	16.80±3.95	15.70±1.62	15.00±1.29	14.92±1.20	14.80±0.31
't'		1.24 NS	2.34**	2.55**	3.83**
% change		-6.54	-10.71	-11.19	-11.90
Liver	12.53±0.07	11.95±0.37	11.90±0.56	11.30±2.46	11.17±0.75
't'		3.34**	2.45**	1.11 NS	3.98**
% change		-4.62	-5.02	-9.81	-10.85
Kidney	14.98±0.37	14.73±0.04	14.30±0.52	14.11±0.28	13.98±0.30
't'		1.46**	2.90**	4.10**	4.62**
% change		-1.66	-4.53	-5.80	-6.67
Muscle	15.90±0.31	15.00±0.36	15.08±0.12	14.10±0.53	13.85±1.59
't'		4.17**	5.38**	6.45**	2.65**
% change		-5.66	-5.15	-11.32	-12.13

Table 5b. Changes in the lipid content in the tissues of *Catla catla* on long term exposure

Sample (mg/g wet tissue)	Exposure periods		
	10 days	20 days	30 days
Gill	14.70±1.18	14.22±0.06	13.52±0.04
't'	2.87**	1.45**	1.85**
% change	-12.50	-15.35	-19.52
Liver	11.09±1.01	10.00±1.69	9.87±2.48
't'	3.17**	3.34**	2.39**
% change	-11.49	-20.19	-21.22
Kidney	13.23±0.36	12.78±0.44	12.55±0.86
't'	7.44**	8.43**	5.72**
% change	-11.68	-14.68	-16.22
Muscle	13.88±0.69	13.45±0.85	12.00±1.61
't'	5.91**	6.01**	5.30**
% change	-12.70	-15.40	-24.52.

Values are mean ± SD, n=5, Figs. in parenthesis are percentage decrease over control.
*** - Significant at one per cent level; *- Significant at five per cent level ; NS - Non significant.*

The decline in carbohydrate content within the muscle, intestine, and brain might be a consequence of glucose utilization to meet the heightened energy demands imposed by the severe anaerobic stress associated with mercury intoxication [52]. Another potential factor contributing to tissue depletion could be the impairment of glycogen synthesis.

3.4 Effect of Petrochemical Effluent On Haematological Changes In The Freshwater Fish, *Catla Catla* Under Short And Long-Term Exposure Periods

The analysis of haematological parameters in fish plays a crucial role for biologists in evaluating the overall health and diverse physiological responses of fish under the influence of environmental stressors. In the investigation of *Catla catla*, various hematological parameters, including red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb) content, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and packed cell volume (PCV), were scrutinised following exposure to petrochemical effluent. This examination spanned both short-term durations (24, 48, 72, and 96 hours) and extended to long-term periods (10, 20, and 30 days).

Exposure to petrochemical effluent significantly influenced the red blood cell (RBC) count in fish, showing a noteworthy decrease ($P < 0.05$) compared to the control group in both short-term and long-term exposures. The results of RBC content for both the treated and control fish are presented in Fig. 2. This finding aligns with similar observations reported by [53] and studies by [54] and [55]. These studies suggested that

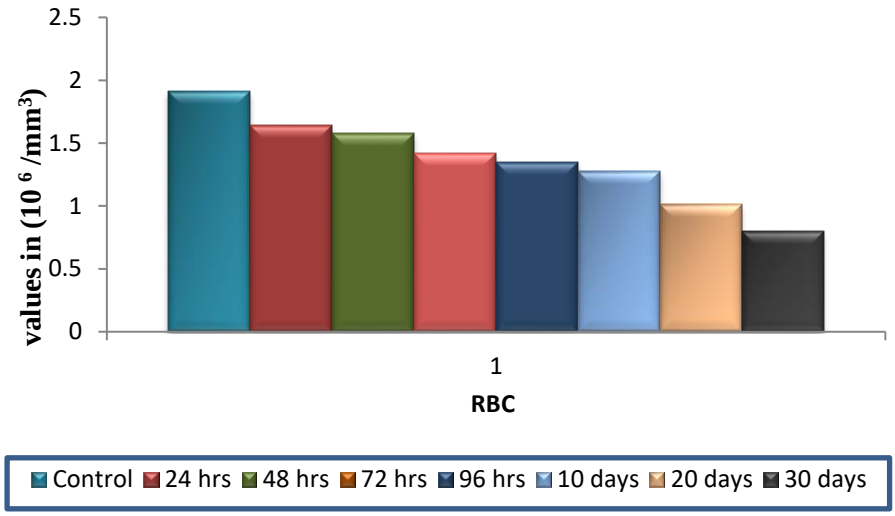

Fig. 2. Changes in Red Blood Cells

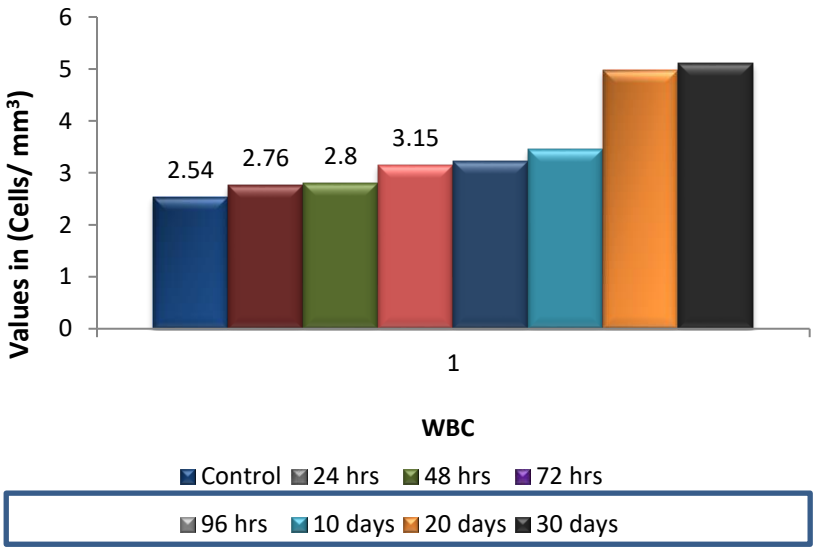

Fig. 3. Changes in White Blood Cells

heavy metals, such as Hg, Cd, Cr, Cu, Zn, As, Ni, and Pb, present in tannery effluent and paper mill effluent, may alter hemoglobin levels by decreasing their affinity for oxygen binding capacity. This alteration renders erythrocytes more fragile and permeable, leading to cell swelling, deformation, and damage.

In both short-term and long-term exposures to petrochemical effluent, the fish demonstrated a significant increase in white blood cell (WBC) count compared to the control group ($P < 0.05$). The extent of this increase in WBC count varied depending on the duration of exposure. The results of WBC content for both the treated and control fish are detailed in Fig. 3. These findings emphasize the impact of petrochemical effluent on fish WBC counts, with longer exposure durations resulting in more pronounced increases. The observed change in leukocyte count is likely attributed to immunological reactions aimed at producing antibodies to cope with the stress induced by pollutants. The heightened WBC count in this study may be correlated with an increased production of antibodies, serving as a defense mechanism for the survival and recovery of fish exposed to sub lethal concentrations of toxicants present in the test media. Similar observations were reported in *Heteropneustes* fossilis due to manganese poisoning [56], copper sulphate and potassium dichromate-induced toxicity in *Channa punctatus*

[57], as well as pulp and paper mill effluent toxicity in *Cyprinus carpio* as reported by [58].

Exposure to petrochemical effluent significantly influenced the red blood cell (RBC) count in fish, showing a noteworthy decrease ($P < 0.05$) compared to the control group in both short-term and long-term exposures. The results of RBC content for both the treated and control fish are presented in Fig. 2. This finding aligns with similar observations reported by [53] and studies by [54] and [55]. These studies suggested that heavy metals, such as Hg, Cd, Cr, Cu, Zn, As, Ni, and Pb, present in tannery effluent and paper mill effluent, may alter hemoglobin levels by decreasing their affinity for oxygen binding capacity. This alteration renders erythrocytes more fragile and permeable, leading to cell swelling, deformation, and damage.

In the present study, the assessment of hemoglobin (Hb) content in fish exposed to petrochemical effluent at various durations revealed a significant decrease ($P < 0.05$) compared to the control group. The extent of this reduction in Hb content varied depending on the exposure duration. Fig. 4, visually illustrate the results of Hb content for both the treated and control fish. These findings underscore the detrimental effects of petrochemical effluent on fish Hb content, with longer exposure durations leading to more pronounced decreases. Moreover, mean corpuscular hemoglobin (MCH) in the blood of fish exposed to petrochemical effluent exhibited a significant decrease ($P < 0.05$) compared to the control group for both short and long exposure periods. The mean values of these results are depicted in Fig. 5 Similarly, packed cell volume (PCV) in the blood of fish exposed to petrochemical effluent showed a significant decrease ($P < 0.05$) compared to the control group for both short and long exposure periods, with mean values illustrated in Fig. 6.

Exposure to petrochemical effluent significantly influenced the red blood cell (RBC) count in fish, showing a noteworthy decrease ($P < 0.05$) compared to the control group in both short-term and long-term exposures. The results of RBC content for both the treated and control fish are presented in Fig. 2. This finding aligns with similar observations reported by [50] and studies by [53] and [54]. These studies suggested that heavy metals, such as Hg, Cd, Cr, Cu, Zn, As, Ni, and Pb, present in tannery effluent and paper mill effluent, may alter hemoglobin levels by decreasing their affinity for oxygen binding capacity. This alteration renders erythrocytes more fragile and permeable, leading to cell swelling, deformation, and damage.

Previous studies by [59] reported reductions in haematocrit, Hb, and mean corpuscular volume (MCV) of Nile tilapia exposed to a polluted environment under laboratory conditions. Harmful effects on animals and fish exposed to pollution were also documented by [60]. MCV

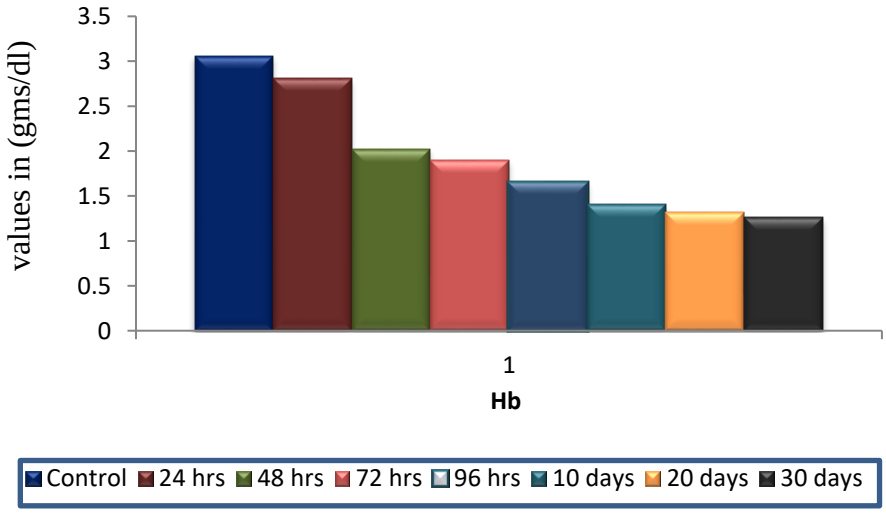

Fig. 4. Changes in hemoglobin

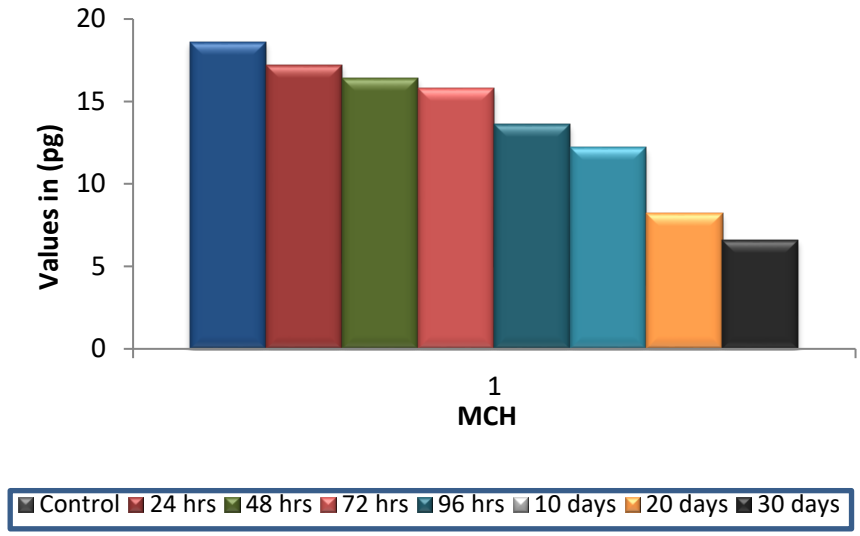

Fig. 5. Changes in mean corpuscular hemoglobin

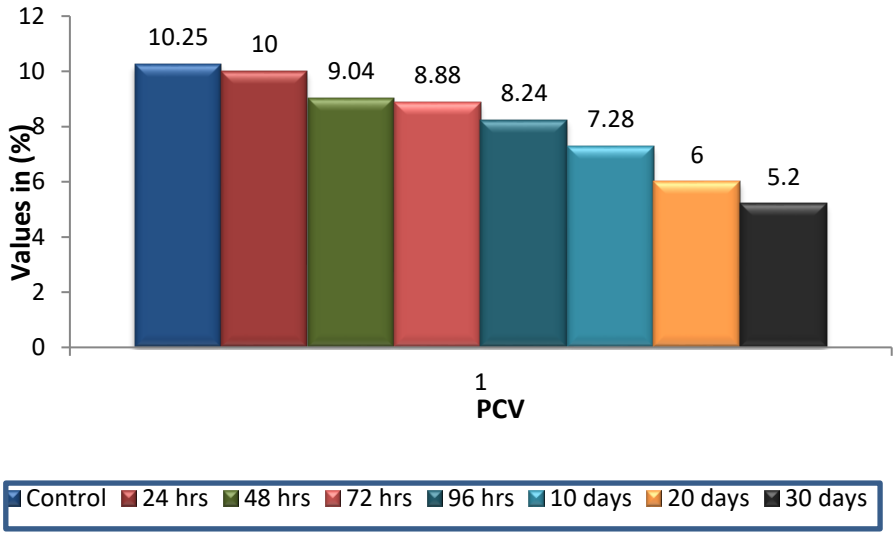

Fig. 6. Changes in packed cell volume

and MCH, along with mean corpuscular hemoglobin concentration (MCHC), displayed appreciable decreases in exposed fish, indicating hypochromic microcytic nemia. The reduction in PCV may be associated with reduced cell counts and hemoglobin concentration, a phenomenon also observed in *Colisa fasciatus* due to exposure to zinc sulfate [61]. Additionally, [54] reported decreased MCV, MCH, and MCHC values in fish exposed to tannery effluent.

Exposure to petrochemical effluent significantly influenced the red blood cell (RBC) count in fish, showing a noteworthy decrease ($P < 0.05$) compared to the control group in both short-term and long-term exposures. The results of RBC content for both the treated and control fish are presented in Fig. 2. This finding aligns with similar observations reported by [53] and studies by [54] and [55]. These studies suggested that heavy metals, such as Hg, Cd, Cr, Cu, Zn, As, Ni, and Pb, present in tannery effluent and paper mill effluent, may alter hemoglobin levels by decreasing their affinity for oxygen binding capacity. This alteration renders erythrocytes more fragile and permeable, leading to cell swelling, deformation, and damage.

3.5 Glutamate Pyruvate Transaminase (GPT)

The activity levels of the enzyme GPT estimated in different tissues under different exposures are presented in figs. 7 and 7a**.** The gill tissue was found to contain 9.85 IU/L of GPT in the control fish. In groups exposed to a 2.72% petrochemical effluent concentration for varying durations, fish subjected to shorter periods (24, 48, 72, and 96 hours) exhibited GPT (Glutamate Pyruvate Transaminase) activity levels of 11.08, 12.20, 13.05, and 14.08 IU/L, respectively. For longer exposure periods of 10, 20, and 30 days, GPT activity levels were 15.10, 16.40, and 17.00 IU/L. In liver tissues, GPT activity levels after short-term exposures (24, 48, 72, and 96 hours) were 35.88, 36.24, 36.79, and 37.00 IU/L, respectively, while long-term exposures (10, 20, and 30 days) recorded levels of 37.28, 38.28, and 39.00 IU/L. The mean control value for liver tissue was approximately 35.00 IU/L. In kidney tissues, GPT activity levels after shorter exposure periods were 33.72, 34.14, 34.50, and 35.12 IU/L, while long-term exposures resulted in levels of 36.20, 37.00, and 38.40 IU/L. The mean control value for kidney tissue was 33.44 IU/L. GPT activity levels in muscle tissue after short-term exposure were 21.37, 22.56, 23.81, and 24.12 IU/L, and for long-term exposures, the levels were 25.24, 26.48, and 27.38 IU/L, with a mean control value of 20.13 IU/L. [62] reported that GOT and GPT are two key enzymes known for their role in the utilisation of protein and carbohydrates. Any change in the protein and carbohydrate metabolism causes a change in GOT and GPT. [63] Observed that ATP, as a membrane-bound enzyme, plays a key role in the active transport system and is highly sensitive to mercury compounds. The heightened GPT activity observed could suggest an anaerobic character in the fish's carbohydrate metabolism, potentially facilitating the fulfillment of escalated energy requirements during extended toxic stress, as proposed by [64]. Conversely, the elevated GPT activity may signify an augmented pace of proteolysis within the tissue, as highlighted by [65].

3.6 Lactate Dehydrogenase (LDH)

The level of LDH activity in various tissues of the fish, *Catla catla,* estimated in the present study after short-term and long-term exposures at 2.72 percent petrochemical effluent concentration is presented in figs. 8a and 8b. In the control group, the gill tissues of *Catla catla* fish exhibited an LDH level of approximately 50.75 IU/L, when subjected to short-term exposure (24, 48, 72, and 96 hours) to 2.72% petrochemical effluent,

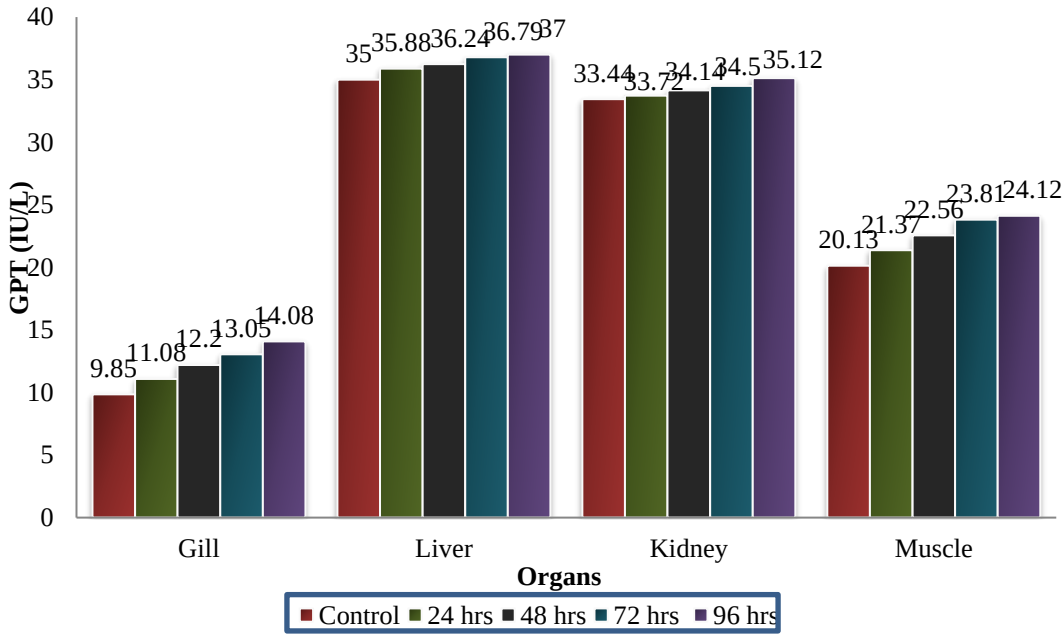

Fig. 7a. Level of GPT (IU/L) in the fish, *Catla catla* exposed petrochemical effluent in short term durations

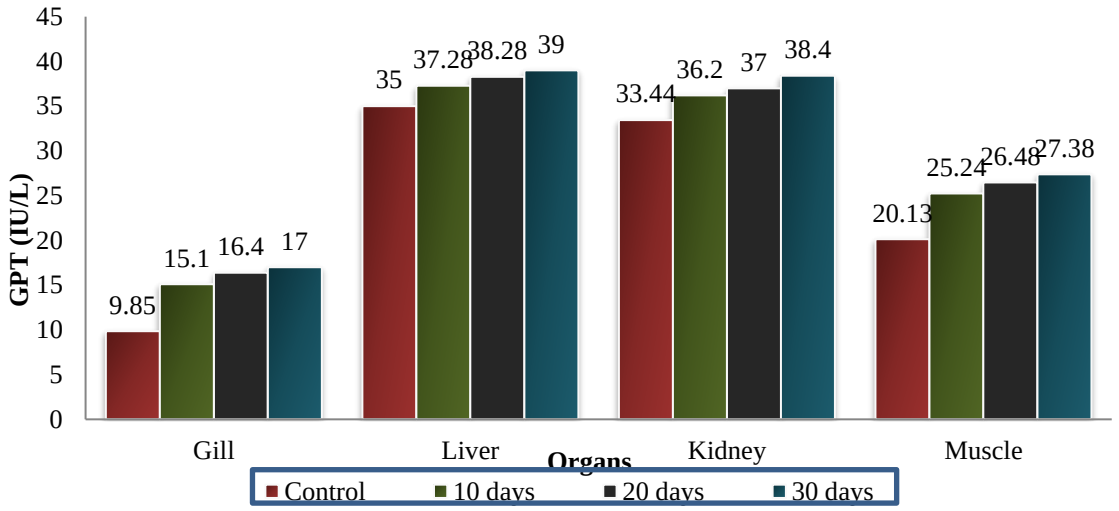

Fig. 7b. Level of GPT (IU/L) in the fish, *Catla catla* exposed petrochemical effluent in long term durations.

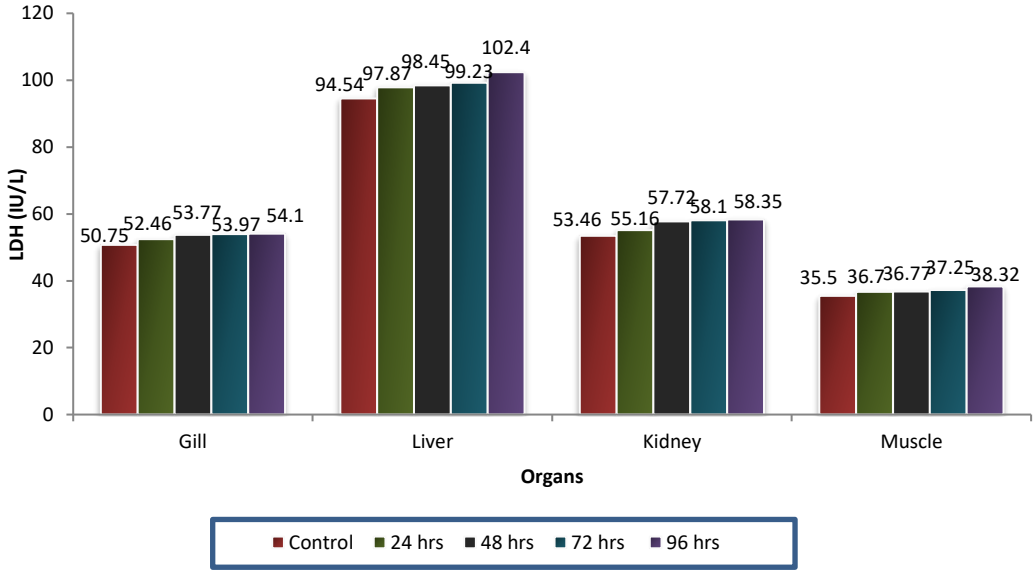

Fig. 8a. Level of LDH (IU/L) in the fish, *Catla catla* exposed petrochemical effluent in short term durations

LDH levels in the gill tissues were measured at 52.46, 53.77, 53.97, and 54.10 IU/L, respectively. Long-term exposure (10, 20, and 30 days) resulted in LDH levels of 55.16, 57.72, and 58.09 IU/L. In the liver tissues of the control fish, LDH activity was recorded at 94.54 IU/L, while short-term exposure produced levels of 97.87, 98.45, 99.23, and 102.40 IU/L. Long-term exposure showed LDH levels of 104.38, 105.10, and 107.10 IU/L. The kidney tissues of the control fish had an LDH level of 53.46 IU/L, increasing to 55.16, 57.72, 58.10, and 58.35 IU/L during short-term exposure and reaching 58.80, 60.02, and 76.27 IU/L during long-term exposure. Muscle tissue LDH levels in the short-term treatment were noted as 36.70, 36.77, 37.25, and 38.32 IU/L, and for long-term exposure, the levels were 38.68, 39.14, and 40.76 IU/L, compared to the control group's 35.50 IU/L of LDH in muscle tissue. [66] Top of Form reported on the brain LDH level in *Channa punctatus* on exposure to hexa chlorocyclo hexane for 15 days. According to [67], increased LDH activity has been reported in different tissues of the liver, muscle, intestine,

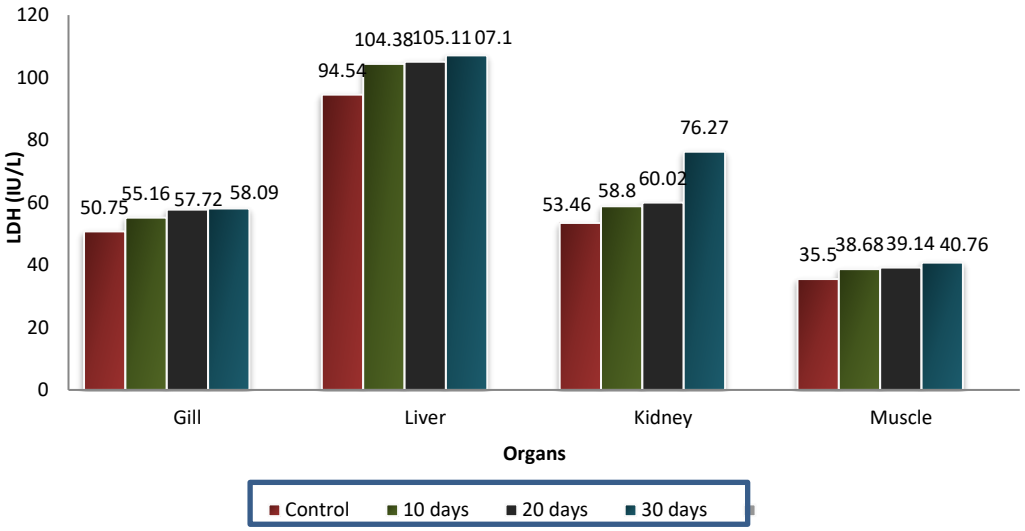

Fig. 8b. Level of LDH (IU/L) in the fish, *Catla catla* exposed petrochemical effluent in long term durations

kidney, gill, and brain of *Channa punctatus when* exposed to low and high concentrations of phenyl mercuric acetate for short- and long-term exposure.

The observed increase in LDH activity in fish subjected to effluent exposure in the current study may indicate the activation of an alternate energy pathway during stress, specifically involving the rapid conversion of lactate to pyruvate and subsequently to glucose. Similar findings were documented in other fish species exposed to phenol pollution by [68], [69] and [70]. also reported comparable observations in *Clarias gariepinus* exposed to arsenic. Lactate dehydrogenase functions as an anaerobic enzyme in the conversion of pyruvate to lactate within the Embden Meyerhoff pathway. This cytoplasmic enzyme is closely associated with cellular metabolic activity, serving as a crucial link between the glycolytic pathway and the tricarboxylic acid cycle. Its activity tends to increase during strenuous muscle exercise, as elucidated by [71].

4. CONCLUSION

Exposure to the effluent led to a significant reduction in hemoglobin (Hb) content, red blood cells, packed cell volume, and mean corpuscular hemoglobin (MCH) values. In contrast, there was a noticeable increase in white blood cells (WBC) during the exposure periods compared to the control. Haematological parameters, such as Hb, RBC, mean corpuscular volume (MCV), and MCH, displayed fluctuating results, with a consistent decrease observed in both short-term (24, 48, 72, and 96 hours) and long-term (10, 20, and 30 days) exposure periods. The decline in haematological parameters indicates the development of anaemia in the exposed fish due to petrochemical effluent. In the biochemical analysis, exposed fish exhibited a significant reduction in protein, carbohydrate, and lipid content across all organs. These parameters serve as valuable indicators for the early detection of xenobiotic processes and their impacts, enabling the implementation of corrective measures before irreversible damage occurs to aquatic organisms and their communities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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