Original Research Article

SHORT AND LONG-TERM EFFECTS OF PHYTOCHEMICAL EFFLUENT EXPOSURE ON BIOCHEMICAL AND ANTIOXIDANT ENZYMATIC ACTIVITIES IN *CATLA CATLA* FISH ON NEELAMBUR POND, COIMBATORE, TAMILNADU

Abstract

The petroleum industry stands out as one of the most rapidly evolving sectors, with projections indicating even more accelerated growth in the years ahead. This study was conducted to assess the impact of petrochemical effluent on fish blood, focusing on short-term and long-term exposure periods to evaluate toxicity stress symptoms. Various biochemical, hematological parameters, and enzymatic changes were analyzed by exposing different organs of freshwater fish, *C. catla*, to the petrochemical effluent. The exposure of fish to the effluent resulted in a significant decrease in hemoglobin (Hb) content, red 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. The decline in hematological parameters suggests anaemia 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, along with 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].

Wastewaters from industries involved in crude oil extraction and the production of fuels, lubricants, and other petroleum-based goods are known as petroleum refinery effluents (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 recognized 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 to 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 fishes 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 utilized 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 fishes 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 water in Neelambur pond across various sampling stations. We examine the sublethal impacts of short and long-term exposure to petrochemical effluent on the biochemical, haematological, 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 a 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 kilometers and reaches Ravathur, where it receives effluents from a petroleum company. After traveling a distance of 3 kilometers, it arrives at Kulathur, where it collects sewage and additional effluents. Finally, after another 1½ kilometers, it reaches the Neelambur pond. The water in this pond serves various purposes, including washing, bathing, 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 analyzed 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, sulfate levels, and phosphate levels. **Figure.1** provides a visual representation of the study area and the described locations in the water system.

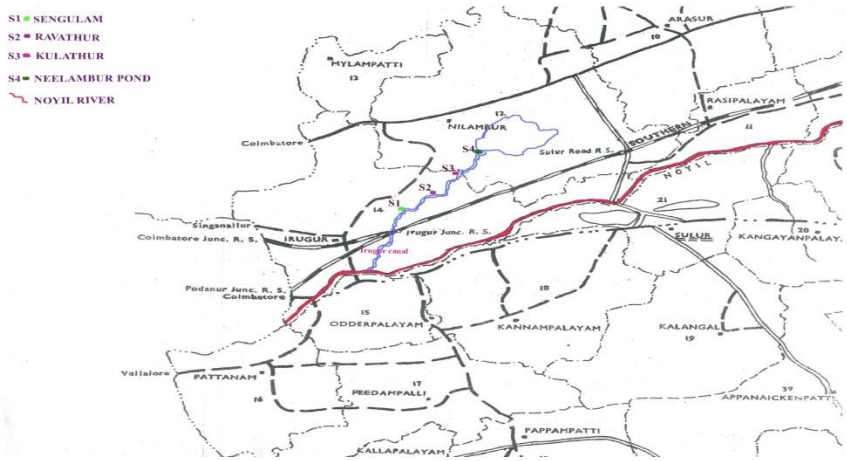

Figure 1. Area Map – Course of Neelambur Pond with Different Sampling Stations

2.2 Physico- Chemical Analysis of Neelambur Pond Water

Water samples were acquired from four specific locations within Neelambur Pond in Coimbatore, utilizing 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 acclimatization process under laboratory conditions at room temperature, as indicated in Plate 1.Throughout the acclimatization 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.

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]. For both short-term and long-term exposure periods to the petrochemical

effluent, a sublethal concentration equivalent to 1/10th of the 96-hour LC50 value was utilized.

2.5 Toxicity studies

Fishes were divided into three groups, each group consisted of 20 fishes (Plate 2).

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 Haematological 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 mgl-1), followed by S3 (0.08 mgl-1), S2 (0.06 mgl-1), and S1 with the minimum DO of 0.02 mgl-1.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 mgl-1), and the minimum was noted in S2 (0.09 mgl-1), 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 I).Similar findings were also reported by [39].

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^{o}C	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).

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 values indicate that petrochemical effluent exhibits high toxicity to *Catla catla*. Furthermore, the calculated sublethal 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 the 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 build up in the food chain are responsible for the adverse effects and finally 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].

Table 2 : 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

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 exposures (10, 20 and 30 days) are presented in Table 3 a and Table 3 b.The study found that fishes exposed to petrochemical effluent had varying levels of protein content in their gill tissueand 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 1.64mg/g, 1.55mg/g, 1.12mg/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 fishes exposed to short term exposure of 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

kidney of fishes exposed to long term exposure of petrochemical effluent for 10, 20 and 30 days. The mean control value was 1.55 mg/g.The muscle protein levels in the fishes that were subjected to short term and long term of exposure 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.

TABLE 3 a: 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 't' % change	1.77±0.11	1.45±0.44 1.56 ^{NS} -18.07	1.35±0.22 3.79** -23.72	1.00±0.07 12.65** -43.50	0.90±0.02 17.12** -49.15
Liver 't' % change	1.88±0.06	1.64±0.03 7.58** -12.76	1.55±0.20 3.43** -17.55	1.12±0.11 13.33** -40.42	0.65±0.22 11.94** -65.42
Kidney 't' % change	1.55±0.22	1.12±0.25 2.86** -27.74	1.10±0.23 3.10** -29.03	1.05±0.20 3.70** -32.25	1.00±0.09 5.10** -35.48
Muscle 't' % change	2.10±0.26	1.70±0.28 2.28 ^{NS} -19.04	1.66±0.30 2.44** -20.95	1.58±0.47 2.13 ^{NS} -24.76	1.43±0.22 4.30** -31.90

TABLE 3 b: 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 't' % change	0.85±0.04 17.08** -51.97	0.76±0.01 20.20** -57.06	0.55±0.03 23.69** -68.92
Liver 't' % change	0.23±0.04 46.66** -87.76	0.17±0.12 27.03** -90.95	0.10±0.03 56.28** -94.68
Kidney 't'	0.98±0.20 4.21**	0.80±0.02 7.52**	0.76±0.26 5.07**

% change	-36.77	-48.38	-50.96
Muscle 't' % change	1.37±0.25 4.42** -34.76	1.22±0.52 3.35** -41.90	1.05±0.56 3.72** -50.00

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

3.3.2 Total Carbohydrate content

The amount of carbohydrate in the tissues estimated after exposing the fishes to short term and long-term exposure periods of the petrochemical effluent are presented in Table 3 a and Table 3b.The gill of the fishes exposed to 2.72 per cent petrochemical effluent for 24, 48, 72 and 96 hours was 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 fishes maintained as control 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, 72and 96-hours exposures in 2.72 per cent concentration of petrochemical effluent. Under treatment of effluent for 10, 20 and 30-days 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 hours treated fishes. Values of carbohydrate estimated in the fishes exposed to long term periods in 2.72 per cent petrochemical effluent were 12.50 mg/g, 11.08 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 fishes exposed to 24, 48, 72 and 96 hours in 2.72 per cent petrochemical effluent were 14.20 mg/g, 14.00 mg/g, 13.80 mg/g and 13.55 mg/g of carbohydrate respectively. The amount of carbohydrate in long term treatment were 13.34 mg/g, 12.81 mg/g and11.00 mg/g under 10, 20 and 30days exposure in 2.72 per cent petrochemical effluent concentration.

Table 4 a: 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 't' % change	12.80 ± 0.03	12.55±0.07 6.56** -1.95	11.00±1.58 2.54** -14.06	10.80±0.11 38.49** -15.62	10.55±0.06 71.15** -17.57
Liver 't' % change	17.90±0.04	17.00±0.31 5.93** -5.02	16.50±0.11 25.99** -7.82	16.00±0.44 9.54** -10.61	15.90±0.34 12.73** -11.17
Kidney 't' % change	15.70±0.31	15.33±0.09 2.50** -2.35	14.09±1.21 2.86** -10.25	14.00±0.47 6.66** -10.82	13.70±0.12 13.25** -12.73
Muscle 't' % change	14.80±0.11	14.20±0.22 5.42** -4.05	14.00±1.58 1.12^{NS} -5.40	13.80±0.42 5.07** -10.40	13.55±0.23 10.67** -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 't' % change	10.20±0.15 36.05** -20.31	9.80±0.12 51.44** -23.43	8.87±1.39 6.55** -31.87
Liver 't' % change	15.40±0.41 13.50** -13.96	15.00±0.36 17.68** -16.20	14.20±3.16 2.61** -20.67
Kidney 't' % change	12.50±0.07 21.95** -20.38	11.08±0.12 30.33** -29.42	10.90±1.58 6.65** -30.57
Muscle 't' % change	13.34±0.06 25.61** -9.86	12.81±0.14 24.68** -13.44	11.00±1.81 2.21^{NS} -25.67

Values are mean ± SD, n=5, Figures 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 5 a and 5 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.

Table 5 a: 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 't' % change	16.80±3.95	15.70±1.62 1.24 NS -6.54	15.00±1.29 2.34** -10.71	14.92±1.20 2.55** -11.19	14.80±0.31 3.83** -11.90
Liver 't' % change	12.53±0.07	11.95±0.37 3.34** -4.62	11.90±0.56 2.45** -5.02	11.30±2.46 1.11 NS -9.81	11.17±0.75 3.98** -10.85
Kidney 't' % change	14.98±0.37	14.73±0.04 1.46** -1.66	14.30±0.52 2.90** -4.53	14.11±0.28 4.10** -5.80	13.98±0.30 4.62** -6.67
Muscle 't' % change	15.90±0.31	15.00±0.36 4.17** -5.66	15.08±0.12 5.38** -5.15	14.10±0.53 6.45** -11.32	13.85±1.59 2.65** -12.13

Table 5 b: 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 't' % change	14.70±1.18 2.87** -12.50	14.22±0.06 1.45** -15.35	13.52±0.04 1.85** -19.52
Liver 't' % change	11.09±1.01 3.17** -11.49	10.00±1.69 3.34** -20.19	9.87±2.48 2.39** -21.22
Kidney 't' % change	13.23±0.36 7.44** -11.68	12.78±0.44 8.43** -14.68	12.55±0.86 5.72** -16.22
Muscle 't' % change	13.88±0.69 5.91** -12.70	13.45±0.85 6.01** -15.40	12.00±1.61 5.30** -24.52.

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

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 hematological 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 Corpuscles (RBC), White Blood Corpuscles (WBC), Hemoglobin (Hb) content, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Packed Cell Volume (PCV), were scrutinized 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 **Figure 2** This finding aligns with similar observations reported by [50] and studies by [51] and [52]. 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 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 **Figure 3**. These findings emphasize the impact of petrochemical effluent on fish WBC counts, with more prolonged exposure durations resulting in more pronounced increases.The observed change in leucocyte 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 sublethal concentrations of toxicants present in the test media. Similar observations were reported in Heteropneustes fossilis due to manganese poisoning [53], copper sulfate and potassium dichromate-induced

toxicity in *Channa punctatus* [54], as well as pulp and paper mill effluent toxicity in *Cyprinus carpio* as reported by [55].

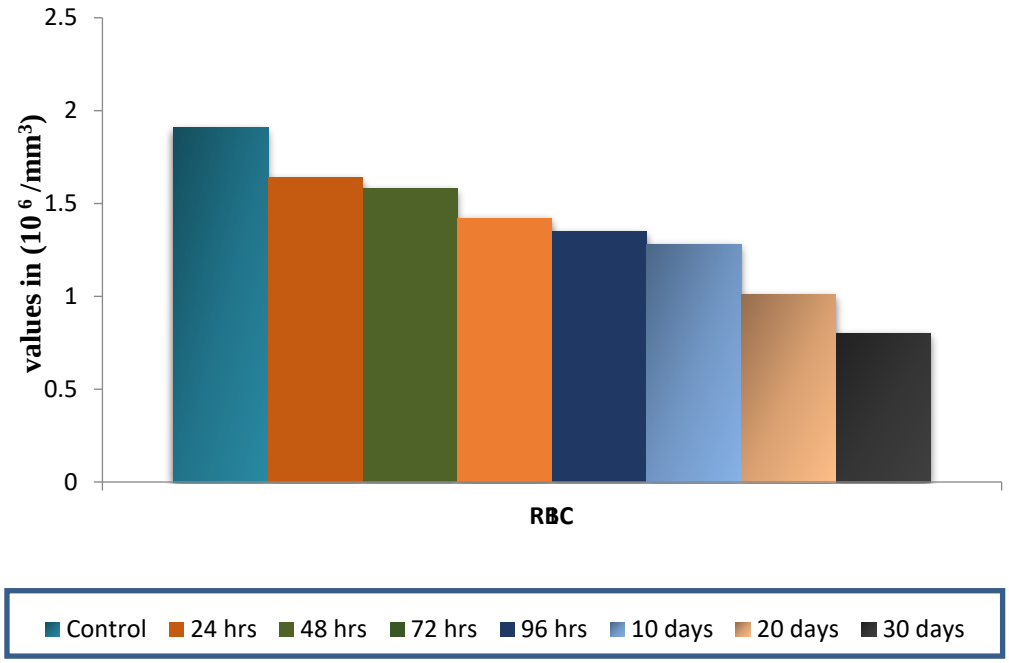

Figure 2: Changes in Red Blood Cells

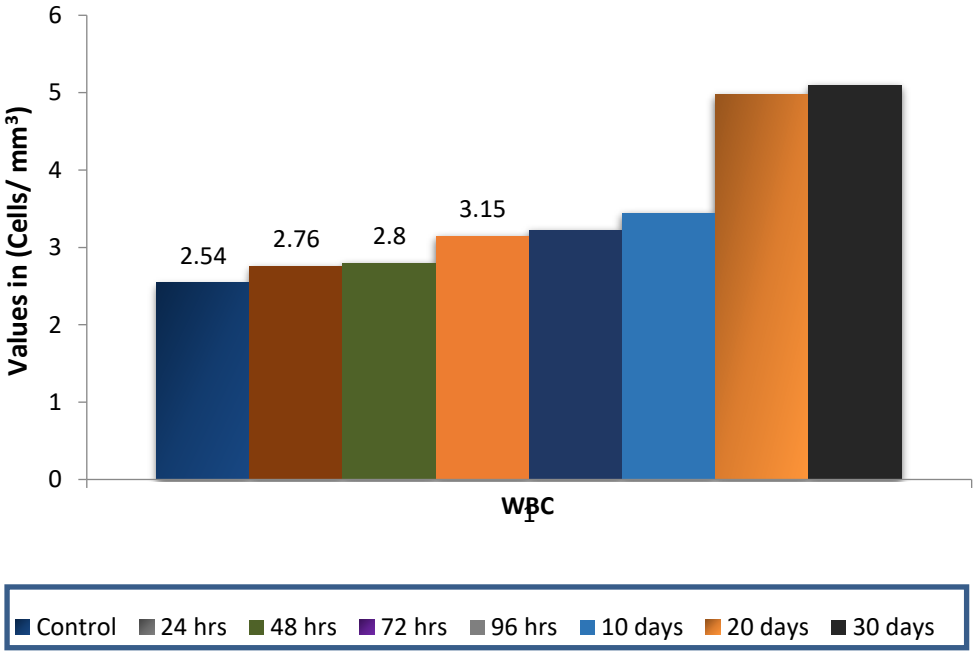

Figure 3: Changes in White Blood Cells

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. **Figure 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 **Figure 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 valuesillustrated in **Figure 6**.

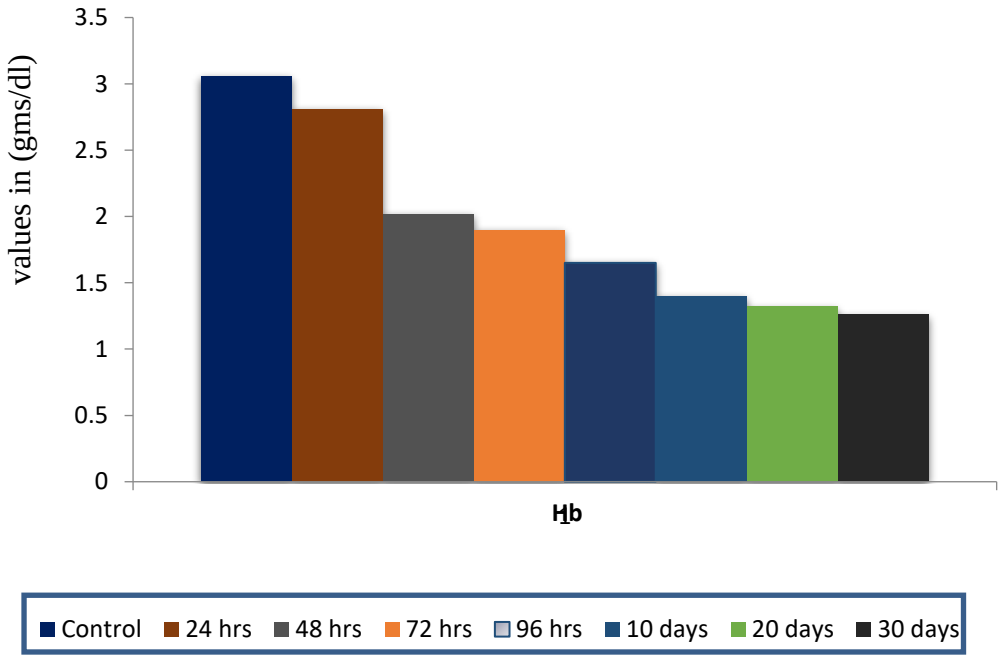

Figure4: Changes in haemoglobin

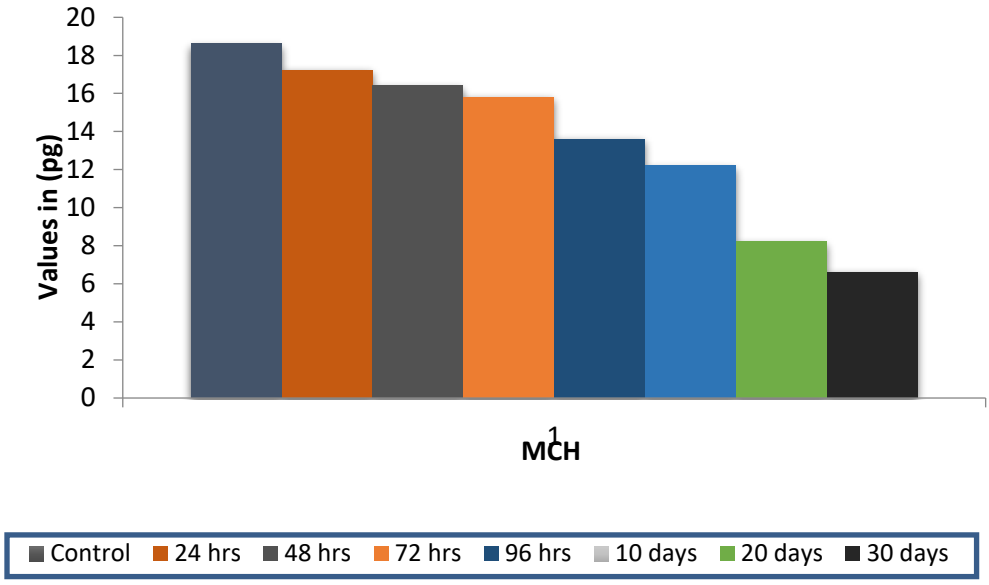

Figure 5: Changes in mean corpuscular haemoglobin

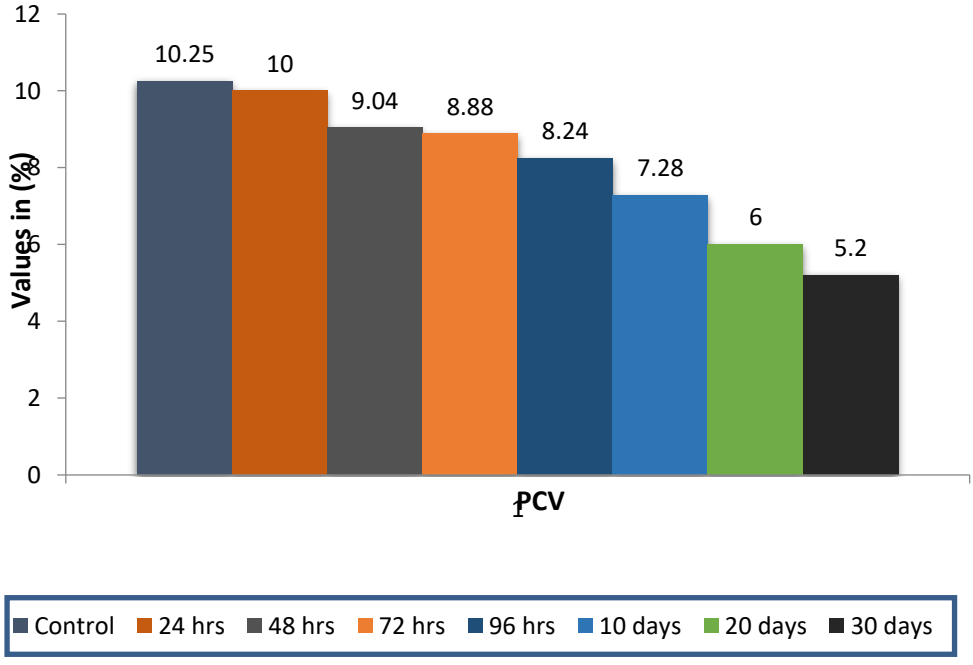

Figure 6: Changes in packed cell volume

Previous studies by [56] 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 [57]. MCV and MCH, along with mean corpuscular hemoglobin concentration (MCHC), displayed appreciable decreases in exposed fishes, indicating hypochromic microcytic

anemia. 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 [58]. Additionally, [52] reported decreased MCV, MCH, and MCHC values in fish exposed to tannery effluent.

3.5 Glutamate Pyruvate Transaminase (GPT)

The activity levels of the enzyme GPT estimated in different tissues under different exposures are presented in the **figure 7 and 7 a**.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. [59] reported that the GOT and GPT are two key enzymes known for their role in the utilization of protein and carbohydrate. Any change in the protein and carbohydrate metabolism causes change in GOT and GPT. [60] observed that the ATP as a membrane bound enzyme plays a key role in the active transport system and is highly sensitive to mercury compounds.

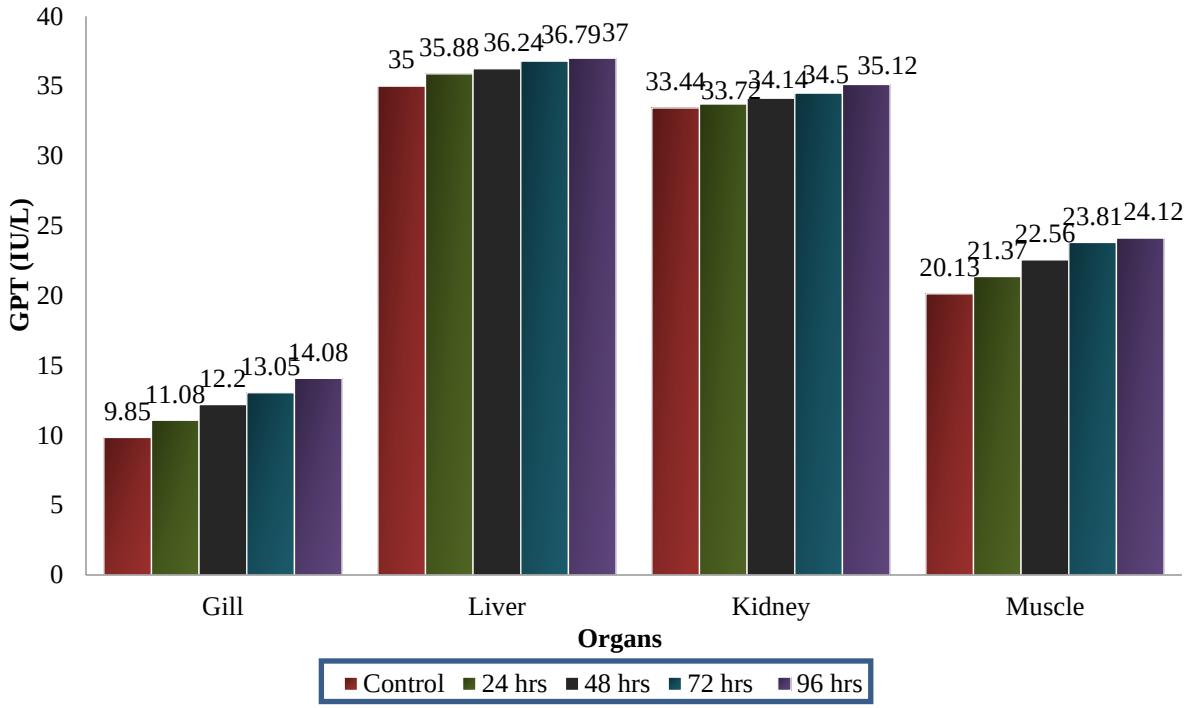

Figure 7a. Level of GPT (IU/L) in the fish, *catlacatla*exposed petrochemical effluent in short term durations.

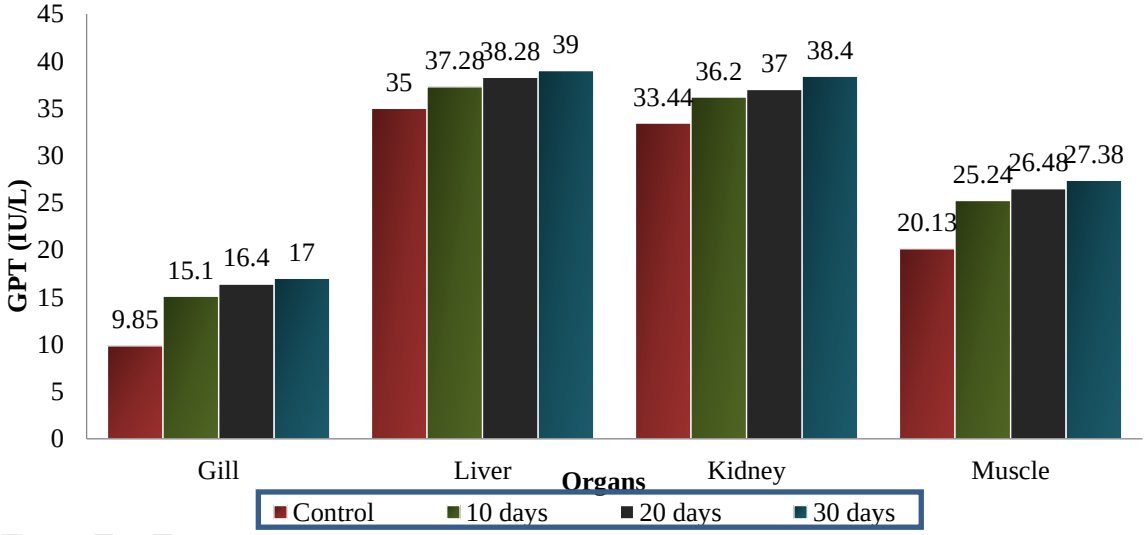

Figure 7b. Level of GPT (IU/L) in the fish, *catlacatla*exposed petrochemical effluent in long term durations.

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 in 2.72 per cent petrochemical effluent concentration are presented in **figure 8a and 8 b**.In the control group, gill tissues of *Catla*

*catla*fish exhibited an LDH (Lactate Dehydrogenase) level of approximately 50.75 IU/L. When subjected to short-term exposure (24, 48, 72, and 96 hours) to 2.72% petrochemical effluent, 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. [61] reported on the brain LDH level in *Channa punctatus* on exposure to Hexachlorocyclohexane for 15 days.According to [62] increased LDH activity has been reported in different tissues of liver, muscle, intestine, kidney, gill and brain of *Channa punctatus*,when exposed to low and high concentration of phenyl mercuric acetate for short and long-term exposure.

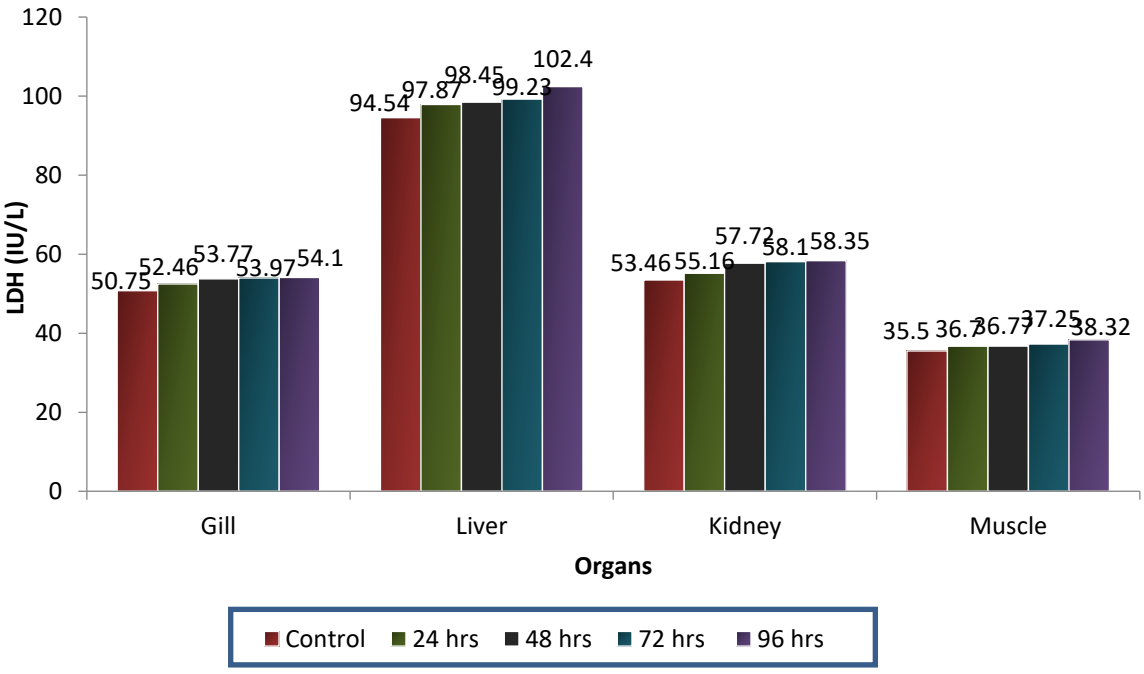

Figure 8a. Level of LDH (IU/L) in the fish, *catlacatla*exposed petrochemical effluent in short term durations.

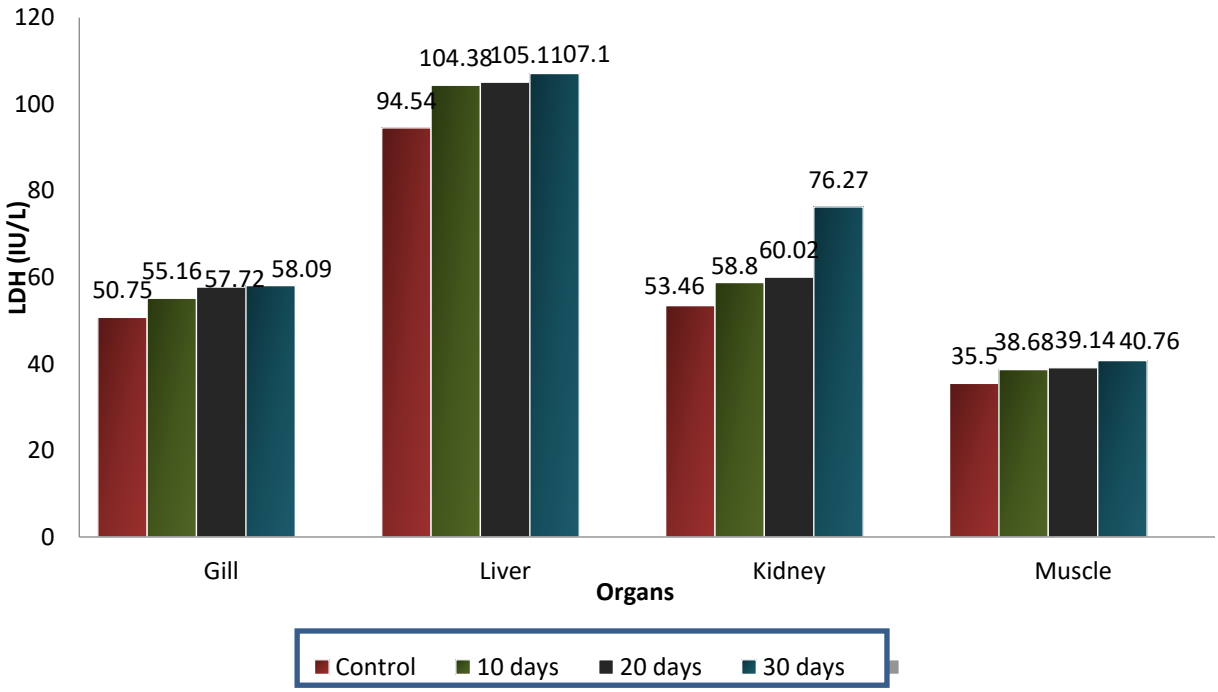

Figure 8b. Level of LDH (IU/L) in the fish, *catlacatla*exposed petrochemical effluent in long term durations.

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. Hematological 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 hematological parameters indicates the development of anemia 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.

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