# **Original Research Article**

# Changes in Haematological Parameters of Freshwater Air-breathing Teleost Channa punctata, Bloch on Exposure to Refinery Effluents

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Abstract: The contamination of the aquatic ecosystem from the effluents discharged from various industries is an exaggerating ecological problem now-a-day. These effluents have posed a serious threat due to their toxic characteristics on the aquatic flora and fauna especially on fishes which may ultimately affect human health as fish is a proteinaceous staple food. Haematological parameters are considered as stress indicators and are useful for assessing the health of fish exposed to changing environment. The aimwas to study the impact of the refinery effluents on the haematological parameters of *Channa punctata* Bloch. A total of 80 fishes were divided into control and experimental groups. The control group was kept in tap water and experimental groups were exposed to two different concentration 25% and 50% of the refinery effluents. The experiment was conducted for 7 days. The cytomorphological changes of the blood cell components were taken into account besides the haematological parameters. The gross morphology of the blood cell component were in time and dose dependent manner. During experimental period, total leucocyte count, Mean Corpuscular Volume and Mean Corpuscular Haemoglobin were gradually increased whereas erythrocyte count, haemoglobin, haemocrit, Mean Corpuscular Haemoglobin Concentration showed depletion in its level. From the present study, it is evident that refinery effluents might have potential to cause haematotoxicity in fishes if released without proper treatment.

Key words: Channa punctata Bloch, Haematotoxicity, Pollution, Refinery effluents.

## Introduction

An effluent is an outflowing of water or gas from a factory, farm, commercial establishment or residential areas to a natural water body. Industrial effluent is any wastewater generated by an industrial activity which includes any material that is rendered useless during a manufacturing process. The rate of increase in production of industrial goods and services, coupled with the diverse sophistication in technology has led to tremendous increase in waste and other by-products. Pollution of the aquatic environment is an outcome of different industrial sources or anthropogenic activities. Rapid industrialization and alarming rise of population has been continuously contributing in enhancing water pollution (Ganguly, 2013). Deterioration of aquatic ecosystems is a major global problem since the past two decades. In India, pollution of the surface of the water bodies by chemical contamination from industrial effluents as well as domestic sewage had flourished within a short period of time. A huge volume of complex wastes is generated by different industries such as sugar and fertilizer factories, textile, petrochemical,vegetable oil factories, pharmaceuticals, oil refineries, pulp and paper industries etc. These industrial effluents and waste product may produce toxic substances by chemical decomposition which have the potential to cause deleterious effects to the aquatic biota (Muthuswamy and Jayabalan, 2001). Different findings have documented the effects of these effluents like the impact of tannery effluent on Labeo rohita, Hamilton (Praveena et al., 2013), toxicological effect of selenium on Heteropneustes fossilis Bloch (Srivastava et al., 2011). Exposure to sublethal concentration (2.6 mg/l) of a triterpene based piscicidal glycoside of Aesculus indicas howed neurotoxicity in fish after prolonged poisoning (Bhatt, 1992). The wastewater discharged from industries possess capability to induce oxidative stress as well as contain endocrine disrupting substances that can affect the heath of aquatic biota (Mnkandla et al., 2016). Petroleum wastewater from the refineries contains high levels of pollutants and is characterized by presence of large quantities of oil products and chemicals like oil and greases, phenols, sulphides, ammonia, suspended solids, cyanides, nitrogen compounds and certain heavy metals like chromium, iron, nickel, copper, molybdenum etc. (Wake, 2005). The presence of heavy metals in the effluents can have harmful effects on the environment (Somnath, 2002). Further, discharging of the effluents into freshwater system decreases the dissolved oxygen (D.O) level thereby causing heavy mortality rate in fish with the interference of fish respiratory metabolism (Hingoroni et al., 1979), serious impairment in metabolic, physiological and structural processes of the aquatic organisms. Subsequently, the aquatic biota including fishes faces exposure to the pollutants (Shalaby et al, 2007). The toxic compounds present in these pollutants are known to induce haematological, physiological, genotoxic, carcinogenic, immunotoxic alterations (Jagetia and Aruna, 1997; Tsutsui et al., 1997; Taysse et al., 1995; Abo-Hegab et al., 1990; Assem et al., 1992) and its lipophilic nature facilitates a high bioaccumulation rate along the food chain. Fish present in the polluted environment displayed a higher risk of developing lymphocystis and liver damage leading to liver neoplasia (Austin 1999). In a similar study it was found that the polluted waters of the Lower Lake of Bhopal were responsiblefor

occurrence of tumours (fibromas) in catfish (Heteropneustes fossilis) (Qureshi and Prasad, 1995). The heavy metals present in petrochemical discharges are prominent factors resulting in altered metabolism of aquatic organisms. It was revealed by studies that exposure to copper resulted in coagulation of the mucus layer of the gills, which inhibited oxygen transport and caused respiratory stress (Westfall 1945) or reduced the number of lymphocytes and granulocytes in the blood, leading to reduced phagocytosis (Mushiake et al. 1985). The assessment of haematological parameters function as a good indicator of abnormalities in fish metabolism or biochemical changes induced by stress or pollutants (Singh et al., 2010). Using the haematological values for evaluating sub lethal concentrations of environmental pollutants and also for possible genotoxicity is highly considered (Blaxhall 1972). The present situation and the worsen environmental status have called for serious caution while fulfilling our social needs on Earth. Therefore, the natural resources around us should be explored in a reasonable way to fulfill our daily needs. At the same time, it should be taken care of that these environmental resources are not exposed to any deleterious effects.

It is therefore the main objective of this research to provide some valuable information on the status of the refinery effluent although treated and also the level of impact on the fishes if any. Under this contemplated background, the present study is an attempt to investigate the impact of stressful environment created by the refinery effluents discharged from Guwahati Refinery, Noonmati, Guwahati on the haematological parameters of the food fish, *Channa punctata* Bloch following exposure to sub-acute concentration of the refinery effluents for 7 days. It is expected that the result of the study will bring to light on the extent of environmental pollution from the refinery effluents and its impact on aquatic life.

# Materials and methods Site selection

The Guwahati Refinery is situated at greater area of Noonmati covering an area of about 350.98 sq.km having geographical coordinates with latitude and longitude  $26^{\circ}11'$  N  $91^{\circ}48'$  E/

26.18° N 91.80° E. The site selected for collection of effluents was from the channel coming out from the refinery near Sector-III, Noonmati, Guwahati.

#### Sample Collection

Sample was collected at 15 days interval from January to March. The effluent discharged from the Guwahati Refinery was collected in 5-10 litres closed gallons from the origin point of the effluent channel near Sector –III of Guwahati refinery. The sample is bought into laboratory and kept for further analysis.

# Test animal and acclimatization

*Channa punctata,* Bloch is selected as the model in terms of having accessory respiratory organ, harmless, easy in handling, availability and also cost effectiveness.

#### Systematic position of Channa punctata, Bloch

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Channiformes
Family	-	Channidae
Genus	-	Channa
Species	-	Channa punctata, Bloch.

#### Acclimatization

A total of 80 live, healthy *Channa punctata*, Bloch. fishes (weight: 30-32 gm, length :12-14 cm) were collected from the local markets of Guwahati. The fishes were wiped with cotton soaked in KMnO<sub>4</sub> and then washed with a diluted solution of KMnO<sub>4</sub> for removal of bacterial infections. The fishes are then rinsed in fresh water and acclimatized under normal laboratory conditions in glass aquaria (46cm× 18cm× 18cm) and buckets of 20 litres capacity having 15 litres of aerated water for 5 days before commencement of the experiment. During acclimatization and exposure period, both control and experimental fishes were fed on fish foods vita flakes and *ad libitum* and natural foods i.e. earthworms daily and the water of both control and experimental aquaria was changed after feeding in every alternative day.

## Evaluation of dose

Study design involves repeated administration usually on daily basis or 4-5 times per week.  $LC_{50}$  is done by probit analysis. The

method (Litchfield and Wilcoxon, 1949) were used to calculate 24, 48, 72, 96h  $LC_{50}$  values at 95% confidence limits. The presumably harmless (safe) concentration of refinery effluent was estimated by the formula of (Hart *et al.*, 1945). Further, in control group, mortality was determined by Abbott's formula (1925)-

Corrected mortality = % Test mortality - % Control mortality X 100 100-% Control mortality

#### Experimental design

Live healthy fishes after acclimatization were divided into control and experimental groups. Fishes in group of 20 were kept in aquaria containing tap water alone and another 2 groups of 30 fishes each are kept in experimental aquaria exposed to collected refinery effluents diluted in tap water in the ratio of 1:3 (25%) and 1:1(50%) for 7 days. During the exposure period, both experimental and control group received the natural photoperiod (13.25  $\pm$  1.17hr) and maximum ambient temperature (19.50  $\pm$  3.68 p C) condition inside the laboratory. The experiment was repeated for two replicas to verify the effects of the effluents at reaching firm conclusion.

# Material and methods Collection of blood

Blood samples were collected separately from 5 live fishes belonging to each groupfrom caudal artery by puncturing the caudal peduncle or by cardiac puncture method with 22 gauze needles after narcotizing and immediately stored in vials containing EDTA.

#### Blood smear preparation

A drop of blood is placed on 5 clean microscopic slides for every sample and blood smears were prepared and stained with Wright stain for DLC (Differential Leucocyte Count) and cytomorphological studies.

#### Methods for haematological parameters

The haematological parameters were studied using the standard methods. The total RBC and WBC counts were made by improved Neubaurer hemocytometers and hemoglobin content was determined by Sahlin-Hellige method. Packed Cell Volume (PCV) was measured by the Wintrobe method. RBC related indices viz Mean Corpuscular Volume (MCV), Mean Corpuscular Heamoglobin (MCH), Mean Corspuscular Haemoglobin Concentration (MCHC) were determined by using standardformula (Baker and Silverton, 1985). Morphological studies of the blood cells are carried out in a zigzag fashion for 100 cells.

#### Statistical Analysis

All measurements were performed in average of three replicates. The data obtained were analyzed by two-way ANOVA using SPSS software (ver. 21).

## Results

## Cytomorphology of blood cells

The analysis of the blood cell morphology components revealed various grade of alterations and abnormalities. (Fig. I&II)

# Cytomorphology of RBC

The erythrocytes showed various changes in the shapes all throughout the experimental period in effluent treated group fishes (Fig. I). The total percentage of the abnormal shapes were progressively increased in significantmanner in both the concentration with corresponding decrease of normal oval or elliptical shaped cells with advancement of treatment. (Table 1).

## Cytomorphology of WBC

No significant changes in morphology of WBC occur was observed during the exposure period.

## Cytomorphology of Platelets

Scattered platelets were seen during exposure to low dose and highly aggregated platelets where prominent in the high dose group at later days of exposure. (Fig. II)

# Numerical studies of blood cell components Total RBC Count (million cells/µl)

The total erythrocyte count has showed a decreasing trend on exposure to refinery effluents. The decrease in erythrocytes in the air breathing fish during exposure to the effluents indicates an inhibited production of red blood cells. The development of erythropenia may be due to interference with haemopoiesis. The TEC decrease is higher in higher dose of effluent treated groupas compared to the lower dose. The decrease in TEC is represented in Table 2.

# Hemoglobin Content (g/dl)

Significant reduction in the hemoglobin content of the fish is observed on exposure to the refinery effluents. The results are represented in Table 3.



Fig. 1. A: Oval shaped RBC of control group. B,C,D,E,F,G,H: Morphological changes observed in RBC in treated groups (25%, 50%). OS-Oval Symmetry, MRF-Money Roll Formation, VC-Vacuolated Cytoplasm, SC-Stomatocyte, EC-Echinocyte, MD-Membrane Disintegration, CD-Cell Disappearance, TD-Tear Drop, TG-Toxic Granules, HCN-Hyperchromic Nucleus.



Fig. 2. A: Platelets in Control Group. B,C: Morphological changes observed in Platelets in treated groups (25%, 50%). PA: Platelet Aggregation.

**Table 1.** Percentage (%) of Morphological alteration of fish erythrocyte exposed to two different doses (25%, 50%) of refinery effluents (Mean  $\pm$  SEM). n= 5,\*p<0.01). \* denotes significant difference between control and treated groups.

Animal Group	Days	Oval shaped	Oval shaped	Membrane	Ring shaped	Microcyte	Echinocyte	Spherocytes	Teardrop
		(OS)	Asymmetry	disintegration					
			(OSA)	(MD)					
Control		98 ± 0.06%	$0.6 \pm 0.02\%$	$0.7 \pm 0.02\%$	$0.7 \pm 0.006\%$	_	_	_	_
Refinery	0	-	-	-	-	-	-	-	-
effluent treated	1	97.2 ± 0.02%	$0.70\pm~0.02\%$	$0.83 \pm 0.01\%$	$0.7 \pm 0.01\%$	_	$0.6 \pm 0.01\%$	_	_
(25%)	3	96.5 ± 0.01%	$0.77 \pm 0.01\%^*$	$0.85 \pm 0.06\%$	$0.80 \pm 0.03\%$	$0.1 \pm 0.02\%$	$0.62 \pm 0.01\%$	$0.3 \pm 0.01\%$	$0.2 \pm 0.06\%$
	5	$96.1 \pm 0.06\%^*$	$0.8 \pm 0.06\%^*$	0.79± 0.01%	$0.84 \pm 0.06\%^*$	$0.17 \pm 0.02\%$	$0.71 \pm 0.06\%$	$0.5 \pm 0.02\%$	$0.2 \pm 0.01\%$
	7	95.6 ± 0.02%	$0.83 \pm 0.04\%$	$0.87 \pm 0.02\%$	$0.88 \pm 0.02\%^*$	$0.2 \pm 0.06\%$	$0.75 \pm 0.02\%$	$0.7 \pm 0.01\%$	$0.3 \pm 0.01\%$
Refinery	0	-	-	-	-	-			
effluent treated	1	$97.28 \pm 0.02\%$	$0.71 \pm 0.02\%$	$0.82 \pm 0.03\%$	$0.73 \pm 0.01\%$	$0.1 \pm 0.01\%$	$0.3 \pm 0.03\%$	_	_
(50%)	3	$96.79 \pm 0.06\%$	$0.79 \pm 0.01\%^*$	$0.81 \pm 0.02\%$	$0.79 \pm 0.06\%$	$0.2 \pm 0.04\%$	$0.32 \pm 0.01\%$	$0.1 \pm 0.01\%$	$0.2 \pm 0.03\%$
	5	$96.43 \pm 0.01\%^*$	$0.82 \pm 0.06\%^*$	$0.83 \pm 0.04\%$	$0.84 \pm 0.02\%^*$	$0.25 \pm 0.2\%$	$0.38 \pm 0.02\%$	$0.2 \pm 0.03\%$	$0.25 \pm 0.01\%$
	7	95.84 ± 0.06%*	$0.85 \pm 0.03\%^*$	$0.86 \pm 0.01\%$	$0.85 \pm 0.04\%^*$	$0.4 \pm 0.06\%$	$0.5 \pm 0.04\%$	$0.28 \pm 0.01\%$	$0.42 \pm 0.02\%$

**Table 2.** TEC (million cells/ µl) of *Channa punctata*, Bloch exposed to two different concentration (25%,50%) of Refinery effluent (Mean ± SEM). n=5, \*p<0.001\*denotes significant difference between control and treated groups.

Animal Group	Days	TEC (million cells/µl)
Control		$5.23 \pm 0.07$
Refinery effluents	0	-
treated (25%)		
	1	$4.9 \pm 0.07$
	3	$3.69 \pm 0.06^{**}$
	5	$3.21 \pm 0.04^{**}$
	7	$2.31 \pm 0.05^{**}$
Refinery effluents	0	-
treated (50%)		
	1	$4.8 \pm 0.08$
	3	$3.48 \pm 0.06^{**}$
	5	$3.11 \pm 0.05^{**}$
	7	$2.15 \pm 0.04^{**}$

#### Packed Cell Volume (PCV) (%)

The present study has showed a significant decrease in PCV (%). This might be due to a slight hypoxia during exposure to the effluents. The results are represented in Table 3.

Mean Corpuscular Volume (MCV)(fl), Mean Corpuscular Haemoglobin (MCH) (pg), Mean Corpuscular Haemoglobin Concentration(MCHC) (%)

The erythrocytes constant MCV, MCH, MCHC offer relationship on size, form and the hemoglobin constants of erythrocytes. The alterations in the haematological indices i.e. significantly (p<0.001) between groups (p<0.001) between days increase in MCV and decrease in MCH and MCHC in the present study may be due to defense against the toxic effect of the refinery effluents. The results are tabulated in Table 3.

Animal Group	Days	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Control		10.12±0.07	31±0.10	59.27±0.16	19.84 ±0.1	32.64 ± 0.17
Refinery effluents treated (25%)	0	-	-	-	-	-
	1	9.8 ± 0.08	30.9±0.11	60.17±0.11	$20 \pm 0.13$	$31.7 \pm 0.11$
	3	$8.7 \pm 0.07^{*}$	30.6±0.07	64.23±0.12*	22.5 ±0.11	$28.4 \pm 0.15^*$
	5	$7.8 \pm 0.05^{*}$	$29 \pm 0.09$	$68.5 \pm 0.1^{**}$	26.2 ±0.12**	$26.8 \pm 0.1^{*}$
	7	$7 \pm 0.1^{**}$	28.1±0.11*	$70 \pm 0.13^{**}$	30.3 ±0.11**	$24.9 \pm 0.2^{**}$
Refinery effluents treated (50%)	0	-	-	-	-	-
	1	$9 \pm 0.07$	30.8±0.12	$61.3 \pm 0.46$	22.6 ±0.12	$31.2 \pm 0.12$
	3	$8.1 \pm 0.072$	30.2±0.08	$65.5 \pm 1.2^*$	25.62±0.11*	$27.8 \pm 0.2^{*}$
	5	$7.3 \pm 0.1^{*}$	28.5±0.11*	$68.7 \pm 2.31^{**}$	$28.5 \pm 0.1^{**}$	$25.6 \pm 0.11^{**}$
	7	$6.3 \pm 0.08^{*}$	27.9±0.12*	$71.2 \pm 0.76^{**}$	$29.3 \pm 0.11^{**}$	$22.6 \pm 0.13^{**}$

**Table 3.** Hb (g/dl), PCV (%), MCV (fl), MCH (pg), MCHC (%) of *Channa punctata* exposed to two different concentration (25%,50%) of Refinery effluent (Mean± SEM). n= 5, \*p<0.01\*denotes significant difference between control and treated groups.

**Table 4.** TLC (million cells/ $\mu$ l) of *Channa punctata* Bloch exposed to different concentration of Refinery effluent (Mean ± SEM). n= 5, \* p<0.01 \*denotes significant difference between control and treated groups.

Animal Group	Days	TLC (million cells/µl)
Control		6.25 ± 0.3
Refinery effluents	0	-
treated (25%)		
	1	6.3± 0.1
	3	$6.7 \pm 0.2$
	5	$6.8 \pm 0.1$
	7	$7.2 \pm 0.11^{**}$
Refinery effluents	0	-
treated (50%)		
	1	$6.67 \pm 0.11$
	3	$6.83 \pm 0.4$
	5	7.29 ± 0.3**
	7	8.18 ± 0.2**

## Total Leucocyte Count (million cells/ µl)

The TLC in the present study marked a significant increase with the increase in the exposure period. The increase in TLC may be due to some infections in the body or a defense mechanism induced by the toxicity of the effluent water. The results are represented in Table 4.

#### Differential Leucocyte Count (DLC) (%)

Differential Leucocyte Count (DLC) done from the blood smear prepared revealed that as the exposure period increased the neutrophils showed a declining trend whereas the lymphocytes increased indicating that there is some abnormalities or infections or entry of some toxic substances into the body. The DLC (%) observed under microscope are tabulated in Table 5.

 Table 5. DLC (%) of Channa punctata, Bloch exposed to two different concentration (25%,50%) of Refinery effluent (Mean ± SEM). n= 5, \*p<0.001\*denotes</th>

 significant difference between control and treated groups.

Animal Group	Days	Neutrophil (%)	Lymphocyte (%)	Basophil (%)	Eosinophil (%)	Monocyte (%)	
Control		$55.8 \pm 0.4$	$28.2 \pm 0.3$	$2.6 \pm 0.2$	5.30 ±1.21	8.2 ± 0.3	
Refinery effluents	1	$54.1 \pm 0.2$	$30.8 \pm 0.1$	$3 \pm 0.4$	$4.9 \pm 1.1$	$8 \pm 0.17$	
treated (25%)	3	$51 \pm 0.17^*$	$32.5 \pm 0.23$	3.8 ±0.65	$4.7 \pm 1.12$	$7 \pm 0.2$	
	5	$50 \pm 0.3^*$	$34.3 \pm 0.17^*$	3.7 ±0.14**	$4.4 \pm 1.17$	$6.8 \pm 0.11^{**}$	
	7	$49 \pm 0.4^{*}$	$37.8 \pm 0.2^*$	$4 \pm 0.2^{**}$	$4 \pm 1.19^{*}$	$6.5 \pm 0.3^*$	
Refinery effluents	1	54 ± 0.16	$29.7 \pm 0.3$	3.3 ±0.13	$4.7 \pm 0.9$	$7.8 \pm 0.4$	
treated (50%)	3	47.5 ±0.13**	$34.3 \pm 0.1^{**}$	3.9 ±0.21**	4.5 ± 1.1	$6.8 \pm 0.27^{**}$	
	5	$40.7 \pm 0.2^{**}$	$40.8 \pm 0.25^{**}$	4.2 ±0.10**	$4 \pm 1.3^{*}$	$6.3 \pm 0.13^{**}$	
	7	$36 \pm 0.4^{**}$	$45.3 \pm 0.1^{**}$	4.9 ±0.17**	$3.7 \pm 0.8^{*}$	$6.1 \pm 0.17^{**}$	

#### Discussion

Piscine haematology can be regarded as a bio-indicator for assessing the health and general condition of the animal subjected to stressful environment (Docon et al., 2010). It has been established by many investigators that the toxic substances usually penetrate the fish through their gills. The low dissolved oxygen, high BOD in the effluent water causes the fishes to pass more water over the gills to meet their oxygen demand inside the body. This leads to rapid penetration of the toxic substances through gills which immediately get mixed with the circulating blood (Noorjahan, 2011). It is important to assess the cytotoxicity of the environmental pollutants on aquatic organisms. Many studies have shown that different insecticides present in the aquatic environment can cause abnormal effects on the blood components in fishes (Narendra et al., 1993). The morphological alterations detected in the erythrocytes serves as an indicator of cytotoxicity. In the present experimental observation, erythrocytes showed asymmetry in their shape, membrane disintegration, toxic granule deposition in the cytoplasm, vacuolated cytoplasm, cytoplasmic droplets formation, microcyte, echinocyte, stomatocyte, tear drop cells, spherocytes. Some of the cells showed a ring like appearance and foamy cytoplasm and projection of the cytoplasmic processes are also observed. Moreover, during the latter period of exposure the nucleus of the erythrocytes showed hyperchromic bizzary nuclear pattern, the nucleus of some cells got completely degenerated. The inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction might lead to anaemia. These findings are supported by the study of Komarovskiy (1969) who showed that after exposure to pesticides based on triazines the fishes showed decrease in haemoglobin volume and erythrocyte count. In the present study, TEC and haemoglobin concentration of the fish showed a gradual decrease after exposure to refinery effluents. Many previous studieshave also found similar kind of observartions (Misra et al., 2020; Mishra and Poddar, 2013; Kumar et al., 2019). Decrease in erythrocytes in the air breathing fish during exposure to the effluents indicates an inhibited production of red blood cells. The development of erythropenia may be due to interference with haemopoiesis.

The significant reduction in TEC count coupled with low hemoglobin content may be due to the destructive action of the toxicants present in the effluent water. This is supported by the findings carried out on altered haematological parameters of Heteropneustes fossilis exposed to toxicants present in river water (Gupta and Chandra, 2013). The decrease in the hemoglobin content indicates that the fish is not able to supply sufficient oxygen to the tissues. Prolonged reduction in the hemoglobin content is hazardous to the oxygen transport and the degeneration of the erythrocytes may be an indication of pathological changes in the fishes exposed to refinery effluents. These might also induce oxidative stress in fishes which are exposed to hypoxic condition triggered by contaminants (Livingstone, 2003). The elevated values were found for the red cell indices MCV, MCH whereas MCHC showed a decreasing trend. These indices allow the determination of the morphological anaemia. MCV indicates the status of the size of the Red blood cell and reflects abnormal cell division during erythropoiesis. The increase in MCV may be attributed to the swelling of erythrocytes as a result of hypoxic condition or macrocytic anaemia or osmotic stress. The reduction in MCHC concentration alongwith increase of MCV might be due to underlying macrocytic anaemia (Rao et al., 1985). The significant decrease in MCHC in the present study indicates swelling of erythrocytes or reduction in hemoglobin synthesis. These findings are similar to that found in an earlier study (Praveena et al., 2013). Total Leucocyte count (TLC) was increased in the fishes exposed to refinery effluents and it indicates increase in antibody production which help the fishes to defend its body exposed to toxicant. A study conducted also depicted elevated level of TLC in fish exposed to pesticides (James and Sampath, 1996; Gupta and Chandra, 2013). Higher TLC reflects damage due to infection in the tissues or some form of physical stress or lymphocytosis or leukemia (Banerjee et al., 2010).

Therefore, the present study has revealed that refinery effluents although treated renders a toxicological impact to the aquatic flora and fauna which may be due to combined effect of the interaction of the toxic substances in the effluent water as well as the alteration of the physicochemical condition of the water.

#### Conclusion

The analysis of the finding revealed that the induction of the toxicological stress in fishes exposed to the Refinery effluents can pose a perilous threat to the consumers of such hydrobionts from the standing water bodies polluted with this Refinery effluents. So, it can be concluded that refinery effluent has potential to cause haematotoxicity in fishes. It is evident from the study that the exposure of the fish Channa punctata Bloch. to refinery effluents lead to marked changes in the behavior of fishes and also induced cytotoxicity and impact on the different haematological parameters of the fish. Hence from the study it can be concluded that although proper measures for treatment of effluents from the refinery are claimed to be taken but the results indicate that the treatment might not have reached to the desired extent. So, it is necessary that the complete treatment of the waste water should be undertaken before it is discharged into the nearby aquatic ecosystems. Moreover, the information from the reliable sources about the death of fishes in river Brahmaputra might be because of the toxicological impact of these effluents. If the treatment is not proper then the toxic components of these effluents will persist in the ecosystem and through food chain get accumulated into the humans, which can cause deleterious effects in the near future.

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