Haematological changes in fish *Oreochromismossambicus* (Peters) exposed to the wastewater collected from Vattakayal near industrial area Chavara, Kollam (Dt)

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Abstract

Thequality of water and the wellbeing of fishes are interconnected and directly proportional. The fluctuations in any of the parameters severely affect the dwelling organisms, especially fishes. Fish live in close contact with their environment, and are more susceptible to physical and chemical changes in the environment which may be reflected in their blood constituents. The present study is to analyse the haematological parameters in the fish (*Oreochromismossambicus*) exposed to different concentrations of effluent contaminated wastewater collected form Vattakayal near industrial area. Change observed in the count of RBC, WBC and Hb.Morphological changes in the RBC were observed on the 1stand 20th days of the experiment include Mild hypochromic , Degenerated red cell, Immature red cells, Moderate hypochromic, Enlarged nucleus, Binuleated lymphocytes, Anisopoikilocytic red cells, Binucleated red cells, Vacuole in cytoplasm, Severe hypochromia, Reactive lymphocytes.

Key words: *Oreochromismossambicus* (Peters), hematology, Waste water

Introduction

Addition of unwanted substances into the water bodies cause changes in the physical, chemical and biological characteristics of the aquatic system which lead to ecological imbalance.The industrial effluents contribute a lot to water pollution forming a threat to aquatic plants and animals (Ramona *et at*., 2001).A greater part of the pollutants exhibit biomagnification and bioaccumulation capabilities with a broad spectrum of impacts, and stresses on aquatic organisms (Censi*et al*.,2006).The pollution leads to a steady decline in the aquatic flora and fauna, particularly fishes. Wedemeyer (1996) reported that the fishes are more susceptible to stress than many other animals because of their intimate dependence upon their surrounding environment.

The hematological studies in fishes have assumed greater importance because these parameters were used as an efficient and sensitive index to monitor the physiological and pathological changes induced by natural or anthropogenic factors such as bacterial or fungal infection or pollution of water resources (Blaxhall, 1972). The toxicants are stressors which are accumulated in the fish through the food chain or absorption through the general body surface and severely affect the life supporting system at molecular and biochemical levels. The Pollutants generally produce relatively quick changes in hematological characteristics of fish (Johansen *et al*., 1994; and Rizkalla*et al*., 1999). The present study is toanalyse the haematological parameters in the fish (*Oreochromismossambicus*) exposed to different concentrations of effluent contaminated wastewater from station 11 (Plate I).

Materials and methods

Contaminated water samples

The effluent contaminated water sample was collected during premonsoon from station 11 (Plate I). The collected samples were stored in the refrigerator.

Experimental Animal

The fish selected for the present study was *Oreochromismossambicus* (Peters) belonging to the family Cichlidae. It can inhabit both in freshwater and saline water (Uchida *et al*.,2000). *Oreochromismossambicus* was selected for the study because of its easy availability, hardy nature, rapid growth rate and tolerance to varied environmental salinity (Pullin, 1991).

Experimental Protocol

Healthy fishes with active movements were considered for the experimentation. The test fishes were starved for 24hrs prior to and during the 96 hrs test period when the fishes were exposed to different concentrations of effluent contaminated wastewater to determine 96 hrs LC_{50} values. The test was carried out in 90 x 60 x 30cm rectangular tanks with ten healthy fishes in triplicate. Controls were also maintained. The test medium was changed every day in order to remove the metabolic waste (Ammonia). The lethal concentration of effluent contaminated water for 50% fish death (LC_{50} - 96 hrsexp) was calculated using the computerized programme, SPSS ver. 10 for Finney's probit analysis. The leathel concentration was calculated as 2.4%. Based on the LC_{50} value, four different sub lethal concentrations (0.15%, 0.3%, 0.6% and 1.2%) of the contaminated water were prepared.

Healthy fishes irrespective of sex of uniform body weight (30 ± 5 gm) and body length (12 ± 1.4 cm) were selected for the experimental study. The fishes were divided into five groups: F1, F2, F3 , F4 and control group (C), with ten fishes in each group and maintained without feed for 12 hours before the exposure to effluent. The fishes in F1, F2, F3 , F4 groups were experimentally exposed to sub lethal concentrations 0.15%, 0.3%, 0.6% and 1.2% respectively, under controlled conditions in aquarium water. The fifth group (C) served as control, and these fishes were maintained in chlorine free tap water without effluent. Three sets of experiments for each group were also conducted and the test was performed by the semistatic (renewal) method in which the exposure medium was changed every 24 hrs to maintain toxicant strength and level of dissolved oxygen as well as minimizing the ammonia excretion levels during this experiment (Kori-Siakpere, 1995). The total period of exposure was 20 days. From the test and control group fishes, blood samples were collected at 1st day, 5th day, 10th day and 20th day of experimental duration and various hematological analysis were done to find out the changes in fishes.

Hematological analysis

RBC count was done with a Neubauer crystalline counting chamber as described by Sohn and Henry (1969). WBC count was determined following the procedure described by Hunter and Bomford (1963). The haemoglobin content was estimated by acid haematin method (Sahli, 1982).

Blood Smear

The blood smear was prepared over grease free clean and dried slides. The slides were stained with Wright's stain (Hesser, 1960) and morphological changes in the RBC of control and experimental fishes were observed. Photographs were taken using OLYMPUS CH 20i microscope with Olympus E420 camera.

Statistical Analysis

The data obtained were tabulated, graphically represented and subjected to statistical analysis using the computerized programme, SPSS ver.19. Analysis of variance (ANOVA) was carried out to test the level of significance of variation between control and experimental mean. The pair wise comparison of mean values were done using Duncan Multiple Range Test (DMRT) (Duncan, 1955).

Results and Discussion

Erythrocyte (RBC) Count

The *Oreochromismossambicus* exposed to various concentrations (0.15%, 0.3%, 0.6% & 1.2%) of effluent contaminated wastewater showed a prominent reduction of RBC with respect of increasing pollution due to wastewater. The mean value of RBC count (Table 1) was found lowest (2.18 x10^6/mm^3)on the 20th day for the 1.2% treatment. The analysis of variance showed a highly significant ($P<0.01$) decrease in RBC count in exposed fishes with respect to days and concentration. Duncan multiple range tests showed significant variation of erythrocytes between days and in concentration except at concentration 0.3% and 0.6% (Table 2 and 3).

In the present investigation the RBC count in *Oreochromismossambicus* reduced significantly when the fish was exposed to the selected toxicant at various concentrations and time duration. In majority of vertebrates, including fishes, the erythropoetic activity is regulated by erythropoietin produced in the kidney (Gordon *et al*., 1967). Reduction in the RBC due to toxicant exposure has been reported by Chrowdhury*et al*. (2004). The decrease in RBC in the present investigation, might be resulted from the inhibition of RBC production or due to the accumulation of effluents in the gill region causing damage in the structure of the gill resulting in haemolysis, as reported by Nte*et al*. (2011).

White Blood Cell (WBC) Count

Table 4 show the Leucocyte (WBC) count of *Oreochromismossambicus* exposed to various concentration of wastewater (0.15%, 0.3%, 0.6% and 1.20%) for different days (1st day, 5th day, 10 th day and 20th day) of treatment. The WBC count decreased to 2.26 10^4/mm^3 on 20th day for of 1.2% treatment. But it was found that at concentration at 0.15% the WBC count showed an increasing trend from the control

Table 1 Total RBC count (*10^6/ mm^3*) in *Oreochromismossambicus*exposed to different concentrations of industrial waste water

Concentration	1 Day	5 Days	10 Days	20 Days	Mean
Control	2.877	2.877	2.883	2.873	2.878
0.15%	2.643	2.600	2.500	2.443	2.547
0.30%	2.583	2.500	2.450	2.400	2.483
0.60%	2.553	2.493	2.440	2.333	2.455
1.2%	2.540	2.430	2.293	2.180	2.361
Mean	2.639	2.580	2.513	2.446	2.545

Day** $P<0.01$ Significant
Concentration** $P<0.01$ Significant

Table 2 Duncan (Day wise)

Day	1	2	3	4
1 Day				2.639
5 Days			2.580	
10 Days		2.513		
20 Days	2.446			

Table 3 Duncan test (Concentration wise)

Concentration	1	2	3	4
Control				2.878
0.15%			2.547	
0.30%		2.483		
0.60%		2.455		
1.2%	2.361			

Table 4 Total WBC count ($10^4/mm^3$) in *Oreochromismossambicus*exposed to different concentrations of industrial wastewater

Concentration	1 Day	5 Days	10 Days	20 Days	Mean
Control	6.243	6.243	6.253	6.213	6.238
0.15%	6.680	6.780	6.440	5.150	6.263
0.30%	7.180	7.240	4.840	4.270	5.883
0.60%	7.770	3.660	3.500	3.440	4.593
1.2%	8.180	3.750	3.180	2.260	4.343
Mean	7.211	5.535	4.843	4.267	5.464

Day** P<0.01 Significant
Concentration** P<0.01 Significant

Table 5 Duncan (Day wise)

Day	1	2	3
1 Day			7.211
5 Days		5.535	
10 Days	4.843	4.843	
20 Days	4.267		

Table 6 Duncan test (Concentration wise)

Concentration	1	2
Control		6.238
0.15%		6.263
0.30%		5.883
0.60%	4.593	
1.2%	4.343	

value to a mean value of 6.44 $10^4/mm^3$ at the 10 thday. Similarly at concentration 0.3% an increase upto 7.24 $10^4/mm^3$ by 5th day and at concentrations 0.6% and 1.2 % an increase was observed even on the 1st day, and during the remaining days significant decrease was noticed. In all cases on the 1st day the Leucocytes count indicated an increase from 6.68 $10^4/mm^3$ to 8.18 $10^4/mm^{3,}$ but on the 5th, 10th and 20th days the leucocyte count decreases with the increase in concentration of effluent contaminated wastewater. The univariate analysis (ANOVA) of WBC showed that it was highly significant (P<0.01) day wise and concentration wise. Duncan test (day wise) where the variations are grouped into three subsets in which the Ist day showed significant variation from all other days, highest mean value obtained was 7.211 $10^4/mm^3$. In concentration wise multiple range tests no significant variation was noticed between the concentrations 0.15% and 0.3% and between 0.6% and 1.2% (Table 5 and 6).

In the present investigation it is seen that during the 1st day the WBC count increased gradually in all concentrations. In 0.15% and in 0.3% concentrations of the pollutant, the increase of leucocytes were observed upto 10th day and 5th day respectively and after that a steep decline. The increase in leucocyte count noted is a response of animal to adapt to the stress condition in the beginning, and the subsequent decline in leucocytes count indicates the weakening of the immune system due to greater stress effect at higher concentrations and time duration Leucocytes are involved in the immunological response (Santhakumar, 1999). Johansson-Sjobeck& Larsson (1978) and Ruparella*et al*. (1990) have reported that the change in leucocyte count with initial increase and subsequent decrease is due to the concentration of heavy metal and duration of exposure. The reduction in WBC count of the experimental group may be due to the release of epinephrine during stress and weakening of the immune system. Similar observations were made by Witeska (2003) and Al-Ghanim (2012).

Estimation of Haemoglobin Content

The variation in the hemoglobin content in the experimental fish is given in Table 7. It was observed that there was a steep decline in the level of haemoglobin content for fish exposed to various concentrations during the study period. The analysis of variance, day wise and concentration wise, showed highly significant variation (P<0.01) from 1st day to 20th day of treatment. Duncan multiple range tests showed a significant

Table 7 Total Haemoglobin (*g/100 ml*) in *Oreochromismossambicus*exposed to different concentrations of industrial wastewater

Concentration	1 Day	5 Days	10 Days	20 Days	Mean
Control	9.433	9.500	9.433	9.367	9.433
0.15%	9.000	8.333	7.433	6.200	7.742
0.30%	6.833	6.133	6.067	5.133	6.042
0.60%	5.633	4.833	4.800	4.700	4.992
1.2%	5.567	4.767	4.600	4.433	4.842
Mean	7.293	6.713	6.467	5.967	6.610

Day** P<0.01 Significant
Concentration** P<0.01 Significant

Table 8 Duncan test (Day wise)

Day	1	2	3
1 Day			7.293
5 Days		6.713	
10 Days		6.467	
20 Days	5.967		

Table 9 Duncan test (Concentration wise)

Concentration	1	2	3	4
Control				9.433
0.15%			7.742	
0.30%		6.042		
0.60%	4.992			
1.2%	4.842			

variation in day wise (except between 5th and 10th days) and concentration wise (except between 0.6% and 1.2%) (Table 8 and 9).

The reduction of haemoglobin affects the oxygen binding capacity (Bonga, 1997; Ruane*et al*., 1999) and it also indicates anaemic condition in fish which may be due to the stress related haemolysis (Panigrahi and Mishra, 1978andAdeyemo, 2005). Similarly there observed the reduction of Hb due to heavy metal stress (Goel*et al*., 1985). Das et *al*. (2006) also reported the same in three Indian carps after exposure to acidic and alkaline pH. Depletion in haemoglobin was noticed in fish exposed to Nickel in laboratory condition and it may be due to the lyses of erythrocytes (Ololade and Oginni, 2010). The above findings supported the present observation that the reduction of haemoglobin due to toxicants lead to significant decrease in haemoglobin, finally reduction in oxygen binding capacity of fishes.

Morphological changes in RBC

The changes occurred in the blood cells were sequential corresponding to the concentration of the toxicant and duration of the exposure time. Mild hypochromic (**MH**) condition was observed on the 1st and 5th days. Degenerated red cell (**DR**) was very common by 1st, 5th, and 20th days of experiment. The immature red cells (**IR**) were noticed during 1st, 10th and 20th days of treatment. Moderate hypochromic (**MOH**) condition and RBC with enlarged nucleus (**EN**) were noticed by the 5th, 10th and 20th days. Binuleated lymphocytes (**BL**) Anisopoikilocytic red cells (**APC**), binucleated red cells (**BR**) were observed on 10th and 20th days. Vacuole in cytoplasm (**V**), severe hypochromia(**SH**), reactive lymphocytes (**RL**) were common on the 20th day of the experiment (Plate I, and II)

Thus, in the present study various abnonmalities were observed in erythrocytes of the fish exposed to the toxicant. The immature red cells and hypochromia frequently observed corroborate with the study on the effects of pollution on *Gobiusniger* (Katalay and Parlak, 2004).

The fish exposed to different environmental pollution had changed the morphology of blood cells due to toxic effects (Kalashnikova, 1976). In *Oreochromisniloticus* exposed to lead, the percentage of immature erythrocyte count and binucleated erythrocytes were found increased (Kosai*et al*., 2011). Similarly the exposure of fish to ultra violet radiation (320-400nm) resulted micronuclei and binuclear erythrocytes in *Clariasgariepinus* (Sayed *et al*., 2007). The occurrence of vacuole in the cytoplasm of erythrocytes and changes in the nucleus were observed in *Gambusiaaffinis* for 0.1 ppm and 1.0 ppm Cu and Cd concentrations (Guner*et al*., 2011).

Conclusion

The haematological and histological studies were carried out in the fish (*Oreochromismossambicus*) exposed to the wastewater from the industry (Chapter 4). The effluent contaminated wastewater used for the experiment was collected from station S11. The LC_{50} 96 hrs was calculated using statistical programme (SPSS Ver. 19). The sub-lethal concentrations were used

to study the haematological and histological changes in the fish on 1st, 5th, 10th and 20th days of the experiment. The results showed that in the exposed fish, the RBC count reduced significantly at various concentrations and time durations. But an increase in the WBC count was observed in the first day in all concentrations and in 0.15% and 0.3% the increase was up to 10th and 5th day respectively, and after that a steep decline. Haemoglobin content declined significantly in the experimental animal in day wise and concentration wise.Morphological changes in the RBC were observed on the 1stand 20th days of the experiment. There was noticeable changes in the RBC corresponding to each concentration and time duration compared to the control.

PLATE-I

Morphological changes in the blood corpuscles of fish exposed to different concentrations of effluent contaminated wastewater for Day-1 (x1000).

a) Control

b) 0.15% c) 0.3%

d) 0.6% e) 1.2%

MH - Mild hypochromia
IR - Immature red cells
DR - Degenerated red cells

PLATE-IV

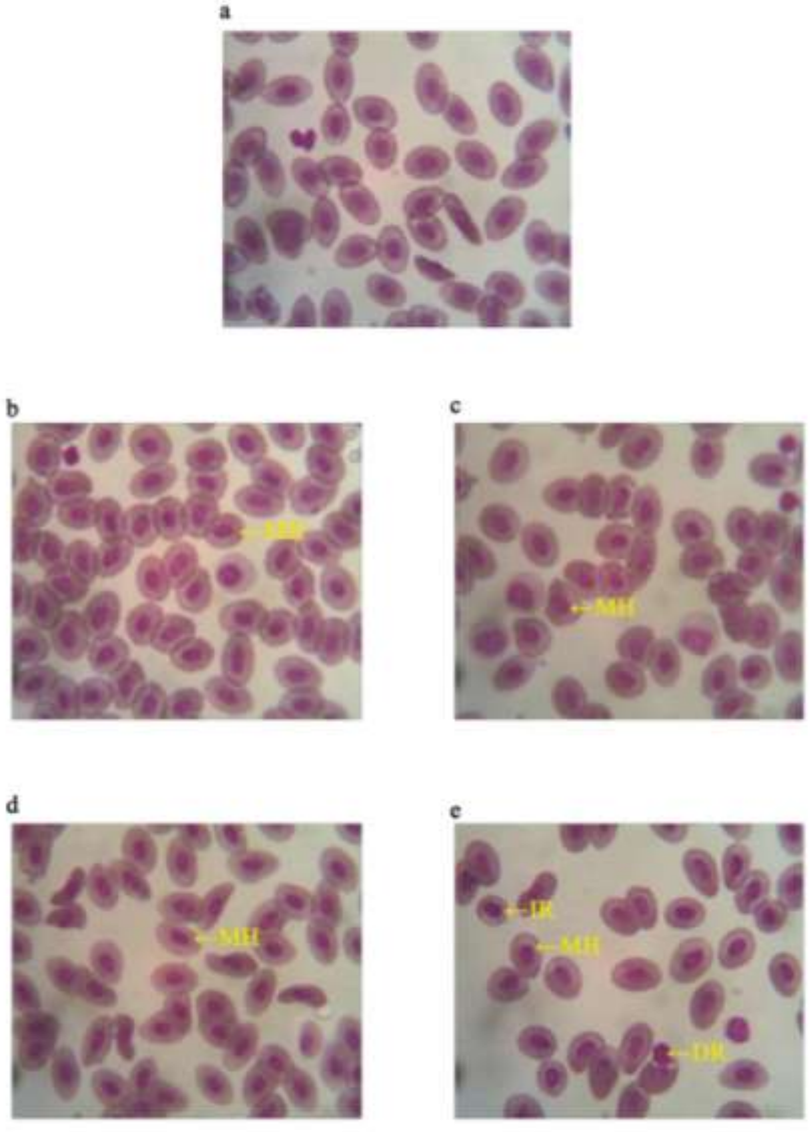


PLATE-II

Morphological changes in the blood corpuscles of fish exposed to different concentrations of effluent contaminated wastewater for Day-20 (x1000).

a) Control

b) 0.15% c) 0.3%

d) 0.6% e) 1.2%

BR	- Binucleated red cells	V	- Vacuole in cytoplasm
DR	-Degenerated red cells	BL	- Binuleated lymphocytes
MOH	-Moderate hypopchromia	IR	- Immature red cells
APC	-Anisopoikilocytic red cells	SH	- Severe hypochromia
EN	- RBC with enlarged nuclei	RL	- Reactive lymphocytes

PLATE-VII

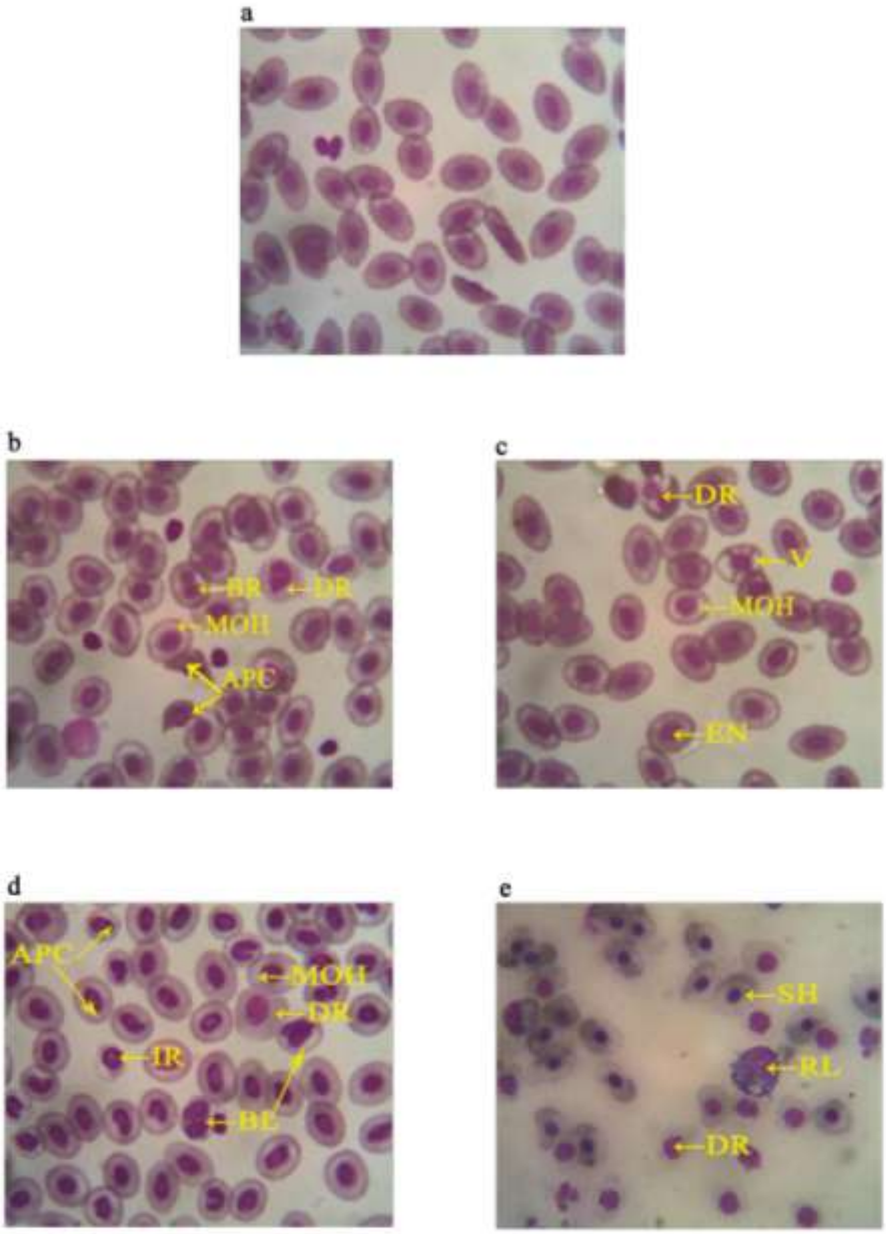
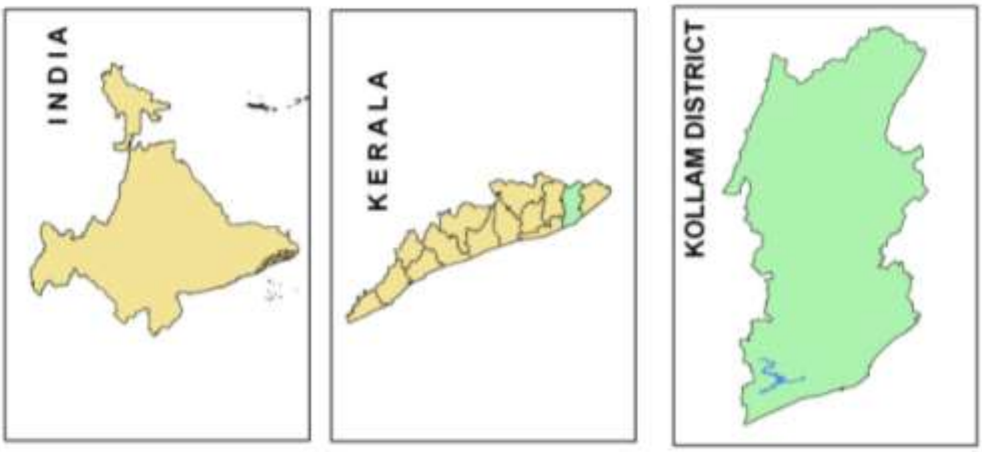


PLATE-III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