JCBPS; Section B; May 2017 – July 2017, Vol. 7, No. 3; 865-875, E- ISSN: 2249 –1929

[DOI: https://doi.org/10.24214/jcbps.B.7.3.86575.]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

Available online at www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT

Research Article

A study of *Cyprinus carpio* exposed to detergent and its Haematological analysis

Perumal Karthiga[1,3], Subramaniam Kamalesu[2], Athanas Anish Babu[1] and Mala Parthiban[3*]

[1]Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

[2]Department of Chemistry, Vivekanandha College of Arts and Sciences for Women, Tiruchengode, Tamilnadu, India.

[3]Department of Zoology, Govindammal Aditanar College for Women, Tiruchendur, Tamilnadu, India.

Received: 04 July 2017**; Revised:** 12 July 2017; **Accepted:** 22 July 2017

Abstract: Now a days detergent have become a public nuisance due to its wider use because unlike soap they were neither soluble nor biodegradable that once put into water they are tended to remain there resisting conversion into less complex and more soluble substances, they also tended to create foam in the water. The reason is detergent is a surface active ingredient; it will lowers the surface tension. Waste water having detergent in them, when allowed to flow in the inland water system bring out extensive change in physical parameters of water such as pH, salinity, alkalinity as well as there is a change in blood parameters. These parameters show increased value due to the involvement of the detergent. This makes us to think over this topic and made us to do work on the toxicity of the detergent on the blood parameters and cytomorphology of the fish *Cyprinus carpio*.

Keywords: *Cyprinus carpio*, Blood parameters, cytomorphology, detergent

1. INTRODUCTION

Water is the one of the most precious elements of life on planet. Earth is the only planet of solar system that is supporting life because of the presence of water. It is critical for satisfying the basic human needs like food production, health energy and maintenance of regional and global eco system. Water is the commonest molecular component of earth. Water will be the only natural resources to touch all aspects of human civilization. Only 1% of total water on this planet is available for consumption[1]. The contamination of fresh waters with a large variety of chemicals released from domestic, industrial and other man - made activities usage resulting environmental pollution which has become not only a national but a global problem. Pesticides are one among them and the most potentially harmful chemicals of the environment in an unplanned manner of indiscriminate usage. While, many destructive chemicals have been discarded into the environment as waste. Detergents have been intentionally release over vast area of our country. Among the various pollutants, detergents play an important role because, their use is unavoidable. Earlier works have pointed out that the use of detergents have got potentialities of changing the aquatic as well as creating damage to the eco system. These chemicals are hazardous to the natural flora and fauna of the aquatic ecosystems, which is the largest component of the global biodiversity. Though they have contributed considerably to the welfare of humans, their adverse effects on non-target organisms are alarming. Fish form an important class of organisms on the basis of their use as nutritive food and are also a useful as indicator of pollution. It is necessary to monitor certain clinical parameters in order to determine the lethal and sub lethal concentrations of pesticides as pollutants on the physiology of fish.

In current science most of the water bodies are dumped with house hold wastes, include soaps and detergents, where detergents are the complex mixture of surface active agents. This house hold waste constitute minimum of 400 million pounds of waste and are discharged into aquatic environment annually. The detergents are harmful and they can cause acute toxicity to fresh water fishes[2] indiscriminate discharge of detergent into aquatic bodies to deterioration of the aquatic environment. Besides agriculture run of containing fertilizers and bio-cidal agro chemicals are also drained into water bodies, they will interact synergistically or antagonistically with aquatic fauna. Waste water having detergent in them, when allowed to flow into inland water system bring extensive changes in the physicochemical parameters of the water such as pH, salinity, alkalinity and severe depletion in the dissolve oxygen content. As a result, yield and productivity of such aquatic system are drastically altered [3].

Adverse effect of detergent to some aquatic organism, have been well documented [4]. The prime property of detergent is, they are surface active and they concentrate at surface and interface absorption. In commercial detergents, the lipophilic and hydrophilic balance is adjusted so, that all agents will be soluble in the medium. In the aquatic environmental live organism provide convenient full time monitoring pollution as one can volute the pollution status of the wetland by analysis of population structure of the organism[5]. The quality assessment of water can made by using the biological indices which can be calculated from the species composition of the sample, the diversity of the species and their distribution pattern.

Hematological parameters may serve as valuable piece of work since these parameters help to assess the health status as well as the environmental quality of piscine hematology, by virtual of its academic

interest, in understanding physiological state of fishes and in monitoring the health status of fishes under natural, experimental cultural condition. The study has tremendous scientific attention all over the world [6-8]. Numerous physiological bio-chemical, histochemical changes in blood and tissues of affected fishes have been reported [9].

Blood parameters of fresh water fishes have been studied by a number of workers. The foundation of the work on the hematological of fishes were laid down in earlier 20th century, studied respiratory function of the blood in fishes investigated hemoglobin concentration of blood in fishes[10]. In India worked out hematological of genus channa[11]. Some done a considerable amount of look on hematology of fishes have been accumulated [12-14]. Use of haematological parameters as indicators of stress can provide valuable information concerning the physiological reaction of fish in a changing environment and it provides an ideal tool for toxicological studies. Hematological indices are very important parameters for the evaluation of fish physiological status, the changes of which depend on fish species, age, cycle of sexual maturity of spawners and diseases. Based on the works, we are interested to study the blood parameters with relation to detergent concentration. So, our present work deals with hematological observation in the blood of a fresh water fish ***Cyprinus carpio*** exposed to different concentration of detergent.

2. MATERIALS AND METHODS

Detergent used in the present study was purchased from market at Thoothukudi, Tamil nadu, India.

2.1. Stock preparation: 1% of detergent was prepared in deionized water, which served as stock solution. From the stock, varying concentration of the detergent were made up by adding sufficient quantity of distilled water. Following concentration of detergent solution from 0.004%, 0.005%, 0.006%, 0.007% and 0.008% were prepared.

2.2. Collection and maintenance: The fishes were collected from local fresh water sources in and around Tiruchendur and they were acclimated to laboratory condition. During the period of acclimatization the fishes were fed with pelleted feed. The water was changed once in two days.

2.3. Peripheral Blood studies: Analysis of the peripheral blood was an earned out in apparently healthy fishes, and also in experimental fishes, exposed to lethal concentrations of detergent. Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Hemoglobin Content (HBC), Mean Corpuscular Volume (MCV) and Packed Cell Volume (PCV) were determined[15, 16].

2.4. Collection of blood sample: The fishes were caught very gently in a small dip net, one at time, avoiding as much stress as possible and immediately stunted by a sharp blow on the head. Caught the dip of tail and then sharp blow on the head. Suddenly for few minutes it becomes unconscious. During that particular period use the sharp knife on the grill region after discarding the first drop, the freely oozing blood was collected in a small bottle containing sufficient quantity of anticoagulation (ethylene diamine tetra acetic acid/2 ml blood). The blood was thoroughly mixed anticoagulation using a thin, blund glass rod, during the process of collection. Collection of blood was stopped as soon as the blood oozing from the gill shows the sign of clotting. Blood samples accidentally contaminated with body fluid or urine or water etc., were discarded. Samples for analysis were prepared immediately after collecting the blood. Anticoagulation blood was used for analyzing of all the blood.

2.5. Total Erythrocyte and Leucocyte Count (TEC, TLC): Enumeration of erythrocyte was done with a haemocytometer with improved Neubauer ruling (Weber and sons, England). Blood was diluted 200 times in the standard RBC pipette with haeme's fluid coloured light but blue with methylene blue. The number of erythrocytes in million/ mm^3 of the blood were calculated by following formula. Enumeration of Leucocyte was done along with total count of erythrocytes.

$$RBC = \frac{\text{No of cells counted x 400 x blood dilution}}{\text{No of small aquares counted}}$$

2.6. Haemoglobin Content (Hbc): The conversional sahli's aid hematin method had to be employed for estimating the haemoglobin content. Fill the haemometer tube to the level of lowest graduated mark with 0.1 ns HCl. Blood is taken from the fish. The capillary pipette is wiped outside without touching the tip place the tip of the pipette at the bottom of haemometer containing the acid. Gently blow the contains of the pipette into the acid. Rinse the pipette in acid solution and place the tube in stand. Make sure that the scales on the tube are on the sides so that we cannot read. Dilute the acidified blood with distilled water drop wise and thoroughly until the colour of the blood matches the colour of the standard. Make sure that the scale on the sick. Wait for minutes and then the tube is rotated so that the graduation is visible. Read the haemoglobin value on scales of the tube at the level of the fluid, record the result and compare.

2.7. Packed Cell Volume (PCV): Haematocrit or packed cell volume (PCV) was measured by micro haematocrit method. Non-heparinished micro haematocrit tube (MICO) and the micro haematocrit centrifuge were used. Sealing wax was used for sealing the capillaries haematocrit was directly read with the micro haematocrit reader.

2.8. Mean Corpuscular Volume (MCV):

$$\text{Mean Corpuscular Volume} = \frac{\text{Haematocrit x 10}}{\text{Erythrocyte}}$$

2.9. Cytomorphology of Blood Cells: Cytomorphology of the cells was studied by the conventional smear technique, fresh non- anti-coagulated blood was used for preparing blood smears. Air dried, thin blood smears were fixed in methanol for a minimum period of ten minutes and stained by Leishman's stained. Cytomorphology of the blood cell types were studied based on the stained smear.

2.10. Statistical Analysis: Result obtained in the foregoing experiment having subjected to the following statistical analysis.

Standard deviation

$$S.D. = \sqrt{\frac{\sum d^2}{n-1}}$$

Where, d is the derivation of each score from mean and n is the total number of samples.

Standard Error (S.E.)

$$S.E. = \frac{S.D}{\sqrt{n-1}}$$

Students't' Test

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{S.E_1 - S.E_2}}$$

Where, $\overline{X_1}$ is the mean control, $\overline{X_2}$ is the mean experimental, $S.E_1$is the Standard error of control and $S.E_2$ is the Standard error of experimental.

Correlation co-efficient

The simple correlation coefficient 'r' was determined from the formula

$$r = \frac{\sum X.Y}{\sqrt{\sum X^2 . \sum Y^2}}$$

Regression Analysis

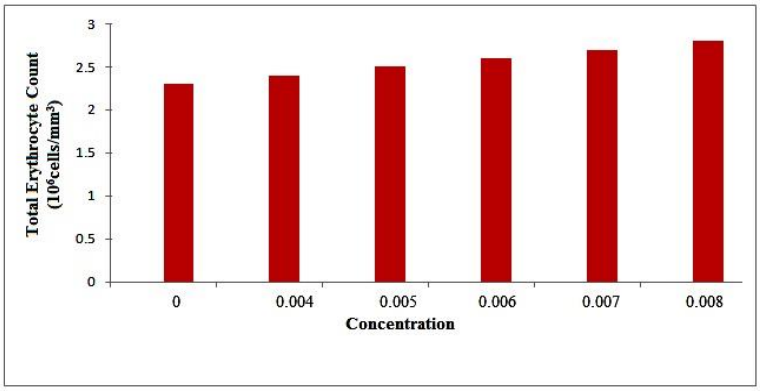
Regression analysis carried out with the two objectives. To analyse the separate and total constituent of one or two independent variables to the variation of a dependent variable. To develop predictive equation for the observed relation all the regression equation are computed using the least square method.

The basic formula is Y= a + bx

Where Y is the dependent variable, X is the independent variable, a is intercept on Y and b is the slope. Where, the observed relation Y and X deviated from linearity value, one of the variable was transformed into common logarithm (log_{10}).

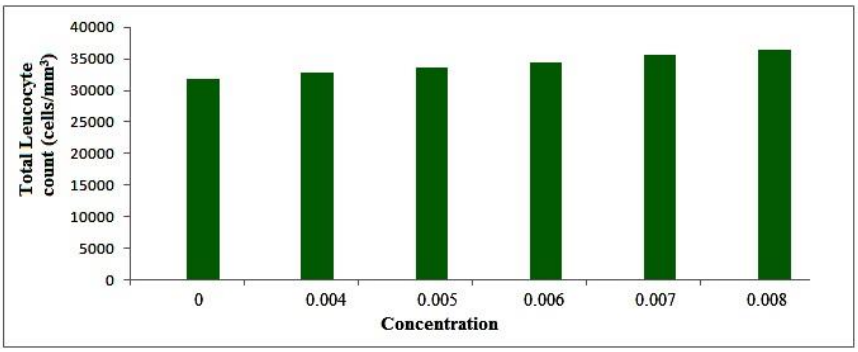
3. RESULTS

3.1. Total erythrocyte count (TEC): Graph 1 represents the total erythrocyte count (TEC) in *Cyprinus carpio* exposed to sub lethal concentrations of detergent. A careful observation of the table shows that TEC tends to show an increase in relation to concentration and also to days of exposure. TEC ranges from 2.3 × 10^6 cells/mm^3 to 2.8 ×10^6/mm^3.



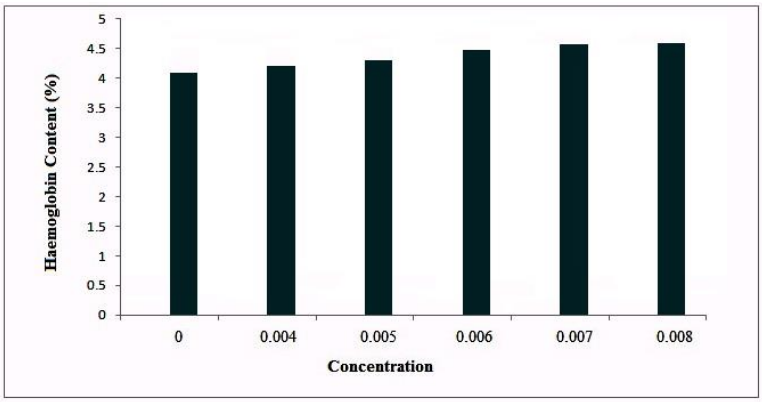
Graph. 1: Erytrocyte count exposed to various concertration of detergent

3.2. Total Leucocyte Count (TLC): The present study reveals that there that there is an increase in TLC with the increase in the concentration of the test solutions. The data obtained are shown in the **graph 2**.



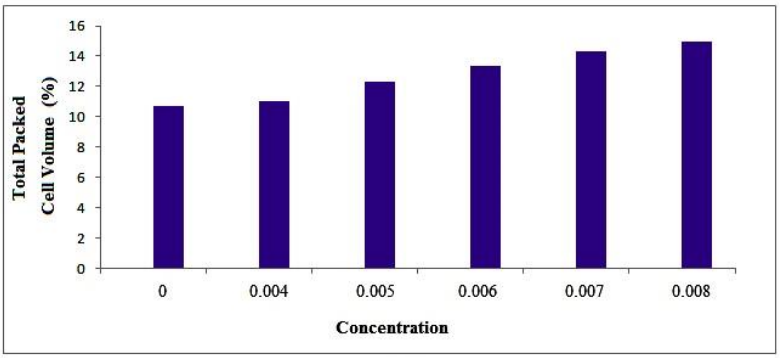
Graph .2: Total Leucocyte count exposed to various concertration of detergent

3.3. Haemoglobin content (Hbc): The haemoglobin content of *Cyprinus carpio* estimated in relation to sublethal doses of detergent are graphically depicted in **graph 3**. The HB content was 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6 repectively, detergent showed a dose dependent increase in the Hb content up to 30 days, in various concentrations.



Graph. 3: Haemoglobin content exposed to various concertration of detergent

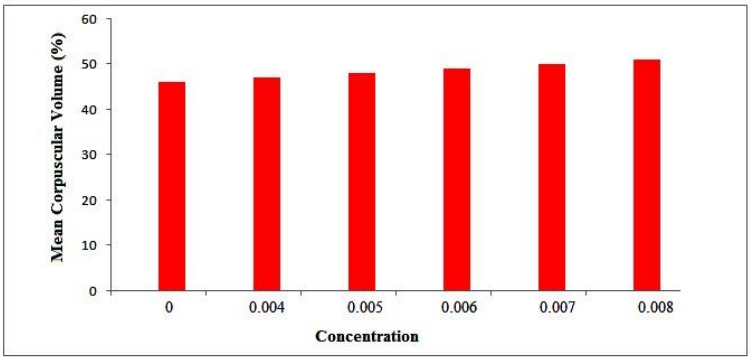
3.4. Packed Cell Volume (PCV): Packed cell volume or Hematocrit estimated is presented in **graph 4.** On various concentration like 0.004, 0.005, 0.006, 0.007 and 0.008% of detergent respectively. An increasing, trend was observed, when the concentration get increased.



Graph .4: Total Cell Volume exposed to various concertration of detergent

Due to increasing, statistical analysis of the data is presented here.

3.5. Mean Corpuscular Volume (MCV): Mean Corpuscular Volume (MCV) was calculated and expressed in cubic meisons and reported **graph 5**. The general trend seemed to be increases of the MCV values in sub lethal doses of detergent. However, there was a significant increase.



Graph. 5: Mean Corpuscular Volume exposed to various concertration of detergent

3.6. Cytomorphology of cell treated with detergent: The blood cell photographs were taken and to an extent we are able to note some changes in the RBC, we are able to note some changes in the RBC.

4. DISCUSSION

The indiscriminate discharge of detergents as a result of house hold activities leads to the deterioration of the aquatic environment, in turn water pollution, and results in the incidences of mortality to some extent and may adversely affect fish life. The blood has an inevitable role in all immunological system. Whenever there is any change in the environmental condition the animal will try to adapt its self to survive in the polluted water. Because toxicity adapt the fishes through changes in haematology and increase the survival values. The response of the organism due to changing environmental condition is reflected in the haematological parameters. Therefore this variation help to screen the healthy state of fish submitted to the exposed toxicants[17-19].

Altered environment create stress which in turn alters the physiological conditions which may be found in the number of RBC, WBC, or in the percentage of Hb and etc. considering here the results of our study we have exposed our study animal, a fresh water fish, *Cyprinus carpio* to the detergent detergent and observed the changes in the haematological paramers.

The findings of the present investigation reveal that detergent induces significant changes in erythrocyte, leucocyte number, haemoglobin percentage and PCV, MCV values. The detergents event at a sub lethal concentration resulted in increase in erythrocyte count, leucocyte count and haemoglobin concentration of 30 days exposed fishes which are in agreement with earlier works [20], so there is a correlation between the period of exposure and percentage increase in the blood parameters. The significant increase in RBC number and haemoglobin concentration could be ascribed to enhanced erythropoiesis which is trygerred as a typical stress response [21] and then the results suggest that the haemolysed erythrocytes might have

caused the elevated count. The same reason may be assigned to the haemopoietic responses recoded in the present study. Thus the rise in TEC may be due to stress caused the detergent which might have influenced the rapid mobilization of RBC from the haemopoietic tissue probably this may be due to transport of higher amount of oxygen particularly to withstand pollutant induced stress conditions (**Fig. 1).**

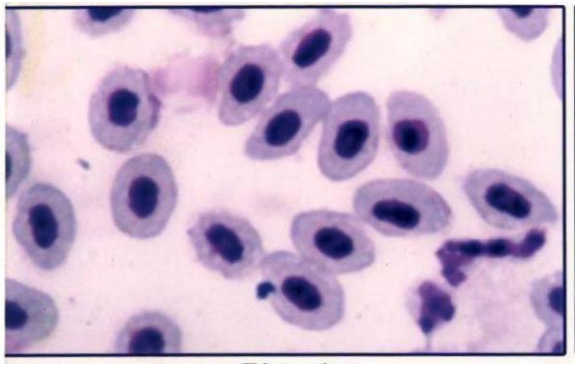


Fig. 1: Normal blood cells of C.Carpio showing normal RBC and platelets (Giemsa stain 100X)

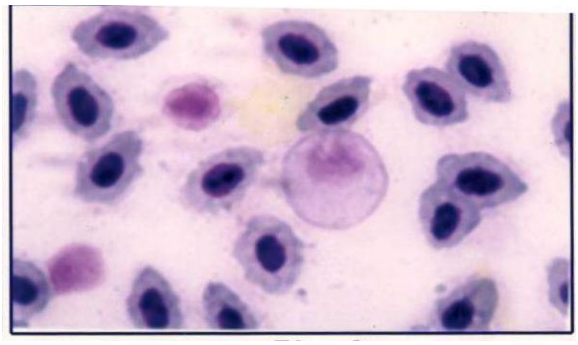


Fig. 2: Blood smear of C.Carpio exposed to detergents for 30 days; note the enlarged WBC and deformed RBC (Giemsa stain 100X)

In our study an increase in TLC has been observed. WBC count increase in the detergent exposed fishes for all the periods of exposure was also reported **(Fig. 2).**

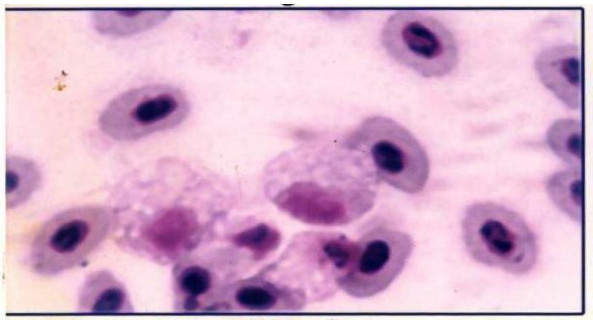


Fig. 3: Blood smear of C.Carpio exposed to detergents for 30 days; note the cytological degeneration in enlarged WBC (Giemsa stain 100X)

Increased haemoglobin content due to exposure to detergent indicates polycythemia in **(Fig. 3)** same as reported [22]. Differential change in the haemotocrit (PCV) value showed increasing trend this findings or in agreement with the reported work[23]. Our increased PCV values may be considered as combined effect of erythrocyte swelling and compensative mechanism of the fish to increase the oxygen carrying capacity of the blood **(Fig. 4).** And elicited value was also observed in mean corpuscular value (MCV) in *Cryprinus carpio* exposed to detergent suggest macrocytic anemia. Increased MCV indicates increase in Lymphocytes population and slow onset of macrocytic anemia condition due to pollutant stress [24].

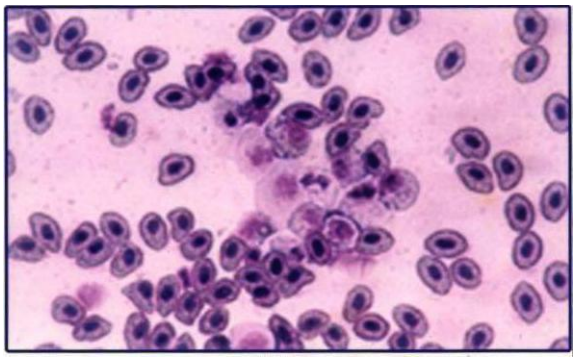


Fig. 4: Blood smear of C.Carpio exposed to detergents for 30 days; note the deformed RBC (Giemsa stain 100X)

The increase in the number of white blood cells may play a part in the immunological defends system during exposure to detergent and appears to be associated with increased circulatory levels of granulocytes which are known to respond for phagocytosis. These suggest the development of a certain degree of tolerance during pesticide stress condition. Taking the TLC immunological mechanism stimulates leukocytosis in response to stressors. Blood smears of the control and treated were observed. In the control fish, the RBC were oval in shape with elliptical nuclei. The changes in the shape of the RBC from oval to round with elongated nuclei and the cell with altered margin were observed this abnormality noted in our study[25]. This indicates that the blood shows immediate response to the changes in the external environment which can be recognized by the abnormal shape.

Stress usually causes release of ACTH and this effect the change in the WBC. This indicates the increase defense mechanism during stress. This increase may attributed as a adaptive value of fishes. Leukocytosis is stimulated in response to stressors. Finally we concluded chronic exposure of fishes to detergent adapt the fishes through changes in haemotology and increase the survival value.

5. CONCLUSIONS

Detergent has some injurious effect on fresh water fish *Cyprinus carpio*. Haematological parameters such as Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Hemoglobin Content (HBC), Mean Corpuscular Volume (MCV), Packed Cell Volume (PCV), are influenced by detergents. TEC showed increased trend high may be a reason for Hb content increased trend which may be a reason for Hb content increase. Erythrocyte count lead to erythropoiseis and polycythemia, this erythrocyte also aids in

the TLC elicitation and PVC, This is one of the mechanism for the stress compensation to detergent pollution which in turn adversely affect the biotic life of the water especially the fish and the fish in turn compensating by altering its physiological responses.

REFERENCES

1. B.X. Innocent, P. Martin, Haematological indices as bioindicators of pollution-A study on fresh water crab paratelphus Hydrodromous (HERBST) exposed of cyperguard. Indian J. Envt. Studies 2003; 7(1): 41-45.
2. K.L. Carthy, D. Arthur, Biological effect of oil pollution Littoral Communities. Proc. Symp. 1968.
3. S. Jeyasurya, M.A. Subramaniam, Vanadaraj. Effects of detergents on the oxygen consumption of the cat fish Mystus Vittatus. J. Ecobiol. 1991; 3(3): 217-220.
4. P.D. Abel, Toxicity of detergents to fish and aquatic invertebrates. J. Fish Biol. 1974; 6: 279-296.
5. M.N. Madhyastha, R.R. Nayak, Preliminary studies on the effect of a detergent point on the blood cells of Rashora danicoius. Proc. All India symp. Environ. Bio.unl.Kerala,India 1979.
6. P.C. Blaxhall, Some blood parameters of the Rainbow Trout: the Kemp Loop variety. J. Fish Biol. 1973; 4:593-604.
7. C.K. Hickey, Fish Haematology: its use and significance. N. Y. Fish Game J. 1976; 23: 170-175.
8. K.R. Munkittrick, L. Leather L and JF. Haematocrit values in feral gold fish carassius auratus L., as indicators of the health of population. J.Fish.Biol 1983; 23:153-162.
9. M. Katz, G.L. Pederson, M. Yoshinaka, D. Sjolseth, Effect of pollution on fish life. J. Water Pollutant Control feed. 1969; 41: 994-1016.
10. F.G. Hall, J.E. Gray, The influence of Asphyxiation on the Blood Constituents of marine fish. J. Biol. Chem. 1929; 67: 549-554.
11. A.N. Yadav, S.N. Sharma, G. Guha, B.R. B.R. Singh, Blood parameters of two hill stream habitats in relation to their habitat. Hydrobiology 1978; 60: 75-59.
12. V Pradhan, A study of blood of a few Indian fishes. I. Hameoglobin Proc. Ind. Acad.Sci. 1961; 54: 251-256.
13. A.K. Srinivastava, Srivastava. Effects of chlordecore on the gonads of fresh water catfish hetecopneustus fossils. Bull-environm contam,toxicol. 1994; 53:186-191.
14. S.N. Bhatt, H.R. Singh, Haematalogical Studies on Hill Stream Fish Schizothorax. Int. J. Acad. Ichthyol. 1981; 2(1): 13-15.
15. E.F. Hesser , Methods for Routine Fish Hematology, the Progressive Fish-Culturist. 1960; 22(4): 164-171.
16. P.C. Blaxhall, Some blood parameters of the Rainbow Trout: the Kemp Loop variety. J. Fish Biol. 1973; 4: 593-604

17. R.J. Reberts, Fish pathology, Bailliere Tindull London 1989.
18. B.N. Pandey, P.K Chrubey, B.J.Munsh, Studies on a fresh water air breathing silwoid fish on blood components heteropreutes fossils (Bloch)in relation to body weight-folia, haematal Leipzing. Biol. 1976; 103: 116.
19. G.R. Shukla, R.R. Sharma, A.K. Pandey, Histopathological alterations in fish tissues induced by pesticides toxicity Aquacult. 2001; 2(1): 31-43.
20. B. Zutshi, S.G.R. Prasad, R. Nagaraja, Alteration in hematology of Labeo rohita under stress of pollution from Lakes of Bangalore, Karnataka, India. Environ Monit Assess. 2010; 168: 11–19.
21. T.S. Gill, J.C. Pant, 1981, effect of sub lethal concentrations of mercury in teleost panties Conchonius Biochemical and Haematological responses, Indian J. Exp. Biol. 19,571-573.
22. D.N. Roy, Haematological response of trivalent chemium poisoning in India teleost cat fish heteropteustes fossils. Indian.J.Environ and Ecoplan. 2001; 5(3): 607-616.
23. Kumari, Munni, G. Kumari Perils of Environmental pollution by pesticides: An assessment through leucocytic and haemostatic response of a fresh water fish, Clarias Batrachas Him, J. Env. 2001; 3: 36-43.
24. A.K. Gupta, P. Muni, Kumar, Rajana. Enzymological study on the effects of aldein on a fresh water Teleost Fish, Notopterus notopterus, J. Notion. 1997; 9: 9-12.
25. Madhyastha MN, Nayak RR. Preliminary studies on the effect of a detergent point on the blood cells of Rashora danicoius. Proc. All India symp. Environ. Bio.unl.Kerala,India 1979.

Corresponding author: Mala Parthiban,

Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

On line publication Date: 22.7.2017