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# HAEMETOLOGICAL CHANGES IS FISH EXPOSED TO RAW DISTILLERY WASTE

Zoology

**D. Saroja** Department of Zoology, Presidency College, Chennai-600005
**M. Abdul Rahim*** Department of Zoology, Presidency College, Chennai-600005 *Corresponding Author
**V. Indra** Assistant Professor, Department of Zoology, Presidency College, Chennai-600005
**S. Seetharaman** Department of Zoology, Presidency College, Chennai-600005
**E. Venkadachalam** Department of Zoology, Presidency College, Chennai-600005

## ABSTRACT

The present study has under taken to explore the toxic effect in aquatic organisms especially fresh water fish *Cyprinus carpio* are exposed to alcoholic distillery effluent. The discharge of industrial effluent causes water pollution due to the presence of dissolved organic and inorganic material which present in the industrial waste water without proper treatment cause changes in the physical, chemical and biological characteristic of water such as color, temperature, turbidity BOD, COD etc. The untreated effluents, deteriorate water quality day by day which affects aquatic biota and production. Hence the present study has taken to find the impact of alcoholic distillery effluents on hematological parameters such as Red Blood Cell (RBC), White Blood Cell (WBC), Hemoglobin (Hb), and Packed Cell Volume (PVC) in *Cyprinus carpio (Linn*.). The hematological parameter RBC 2.52 x $10^6$/ $mm^3$, WBC 3.115x$10^3$mm , Hb 6.362 x$10^3$mm and PVC 16.845 x$10^3$mm recognized in fish very much valuable tools for maintaining and function of fish healthy life.

## KEYWORDS

Haematology, *Cyprinus carpio*, Haemoglobin (Hb), Red Blood Cell (RBC), White blood cell (WBC) and Packed Cell Volume (PCV)

## INTRODUCTION

Industrial toxicants generally bind more strongly than to the other chemicals so displacing them from their normal sites through they also exerts toxic effects by binding to the other sites, among these alcoholic and distillery effluent has different chemical characteristic from the familiar toxic effects by binding to the other sites, familiar toxic components and prefers oxygen sites exclusively (Sharma *et al*., 1982).

Blood constituents have been well established for many of the higher vertebrates as diagnostic tools in human and animal medicine. Finney, 1978 changes in blood parameters are often quick respond environmental or physiological attractions further more they are easily measurable and provide an integrated measure of the physiological states of organism. Das and Nanda, 1986 emphasized the need for the study on haematological physiological variables because of their relationship with respiration (HB & related factors) defense mechanisms (leucocyte values) and energetic (Metabolite levels).

Significant elevation or reduction in hematological values of fishes exposed to different environmental toxicants have been reported by Finney, 1964 stated that any kind of stress not resulting in gross changes and mortality produces certain changes in the hematology of fishes in responses stressing agents are indicators of stressful state in fish and they are useful in deciding when to initiate prophylactic measures in fish culture.

Hematological techniques including measurement of hemocrit erythrocyte and leukocyte counts have proved valuable for fishery biologist in assessing the health of fish and in monitoring stress responses (Talapatra and Banerjee, 2007) several species of fish are susceptible to the affects of industrial effluents as reflected in the blood changes including anemia and alterations in erythrocyte morphology. Farkas *et al*., 2002 stated that estimation of haemoglobin can be used as an index of anemia and fluid volume disturbance.

The charges in the blood parameters would be a high of fish physiological response against environmental stresses (Vosyliene, 1996). It has been illustrated that the blood variables are indicators of stress and give information on the physiological response of fish to a changing external environment (Hrubec *et al*., 2001)

## MATERIALS METHOD

The fresh water carp *Cyprinus carpio* were collected from fish form near Poondy, Thiruvallur district, brought to the laboratory, average length is 7.5 cm and average weight is 8.0g. The fishes were kept for two weeks to acclimatize in laboratory conditions and fed with food pellets maintained at 30± 2$^0$ C with water exchange.

### Collection of effluent water and experimental design

The raw alcoholic distillery effluent collected EID Parry, Nellicuppam in Caddalore district, Tamil Nadu, India. The 20 liters of water samples were collected from the mouth of outlet of the industry in polypropylene container. The physic-chemical parameter of the sample were estimated APHA (2009).Two groups of fishes were used for control and experiment. The control fishes were maintained in normal chlorine free water with aeration and second group of fishes were grown in effluent water 7, 14, 21 & 28 days of exposure.

### Hematological parameters analyze

The blood sample were collected by direct heart puncturing using sterile disposable plastic syringe with a 22-gauge needle (Walia *et al*., 2013). For an anticoagulant heparine sodium (1%) was used (Svobodova *et al*. 2003). To separate the plasma samples were centrifuged at 3000rpm for 15 minutes and stored at 4$^0$C. The blood sample were subjected to haematological analysis for RBC, WBC Hb and PVC (Shakoori *et al*., 1976).

## RESULTS

The result obtained for the haemotological parameter such as RBC, WBC, HB and PCV are illustrates in (Table1-4 and Fig .1- 4). Fish exposed to various concentration *i.e* 0.7, 1.4, 2.1 and 2.8 % of distillery effluent of sub lethal concentration ie for 28, 21, 14 and 7 days respectively and are tabulated in (1-4). In general the haemotological parameters showed significant changes in the fish exposed to effluent sample.

The Haematological parameters significantly changed when the fish exposed to the effluent comparing to the control group. The total RBC, WBC, Hb and PVC in the present study observation is reported significant difference in fish exposed to distillery effluent.

In the present study various abnormalities were observed in the blood cells *i.e* sequential corresponding to the concentration of the toxicant and duration of the exposure time on 7$^{th}$& 14$^{th}$ days *i.e* immature RBC & hyperemia. Degenerated red cells (DR) were very common during 7, 14, 21 & 28 days of exposure periods. The fresh water fish *Cyprinus carpio* exposed to various concentrations (0.7, 1.4, 2.1 and 2.8) of contaminated raw alcoholic distillery effluent waste water showed prominent reduction of erythrocytes. The mean average of RBC was illustrated in the (table.1 and fig.1), and was found lowest (2.52 x $10^6$/ $mm^{3)}$ on 28$^{th}$ day for the 0.7% treatment. The analysis of variance showed a highly significant ($p<0.01$) decrease in RBC count when fish exposed with respect to days and in concentration except at concentrations 1.4 & 2.1% (Table 1& Fig.1)

It was noticed that during 7th day WBC count increased gradually in all the concentrations like 0.7, 1.4, 2.1 and 2.8 %. In 7.0% concentrations of the pollutant, the increase of leukocyte count noted greater stress effect at higher concentration and time duration. The White blood cells of *Cyprinus carpio* exposed to sublethal Concentration of alcoholic distillery effluent in the expect group count was 6.2 x $10^3$ per cubic mm of blood. The WBC Count decreased to 3.115x$10^3$mm on 28th day for of 2.8% treatment. But it was found that at 0.7% the WBC was found to be increased than control.

In the case of Hb steep decline was observed throughout the study period. The analysis of variance day wise & Concentration wise, showed highly significant variation (P<0.01) from the day 7th to 28th day of exposure period.

The PVC values of *Cyprinus carpio* exposed to distillery effluent to sub lethal concentration were decreased 16.845 x$10^3$mm, throughout the study period (7,14, 21&28%) at it was significant at (P<0.01) level.

## DISCUSSION

Abdel-Moneim *et al*., 2008 some observation is reported the significant difference in fish exposed to dye stuff and chemical effluent water. In the present study indicated that, fish exposed to alcoholic effluents reduced counting the rate of RBC, WBC, Hb and PVC. The decrease in haemoglobin count result may impair oxygen supply slow metabolic rate and less production of energy (Ahmed *et al*., 1995; Atamanalp and Yanik, 2003). The WBC reduced hypersensitivity, decrease in PVC shows that shrinkage of cells in the blood (Ahmed *et al*., 1995).

The oxygen transfer from gills to blood and causes more stress due to the effluent effects. The haematological parameters have been recognized as a valuable tools for fish, monitoring health, closely related to response to fish environment and biological factors (Vutkuru, 2005). Haemotological parameters are easier to measure and can provide integrated measure of physiological states of fishes. Blood is involved respiration, defense mechanisms and movements of nutrients and metabolites. Larsson *et al*., 1985 studied the histopathological changes in hepatopancreas of *Channa gachua* chemically exposed to bioassay of $Hg_2Cl_4$ and sodium pentachlorophenol. Singh and Reddy, 1990 observed histological changes in gills and intestine of Barbus stigma after distillery effluents exposure Lehmann *et al*., 1976. This study on hematological changes in fish serves as an effective tool is the diagnosis of the extent of environment pollution and also the abiotic fish diseases Satyanarayan *et al*., 2003.

Hypoxia, anemia and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient $O_2$ unless other wise compensated by erythropoiesis or suitable physiological adjustments. Decreased availability of $O_2$ generally causes increased synthesis of hemoglobin, release of blood cells from storage sites and enhanced erythropoiesis Sharaf *et al*., 2010. Mulitple form of Hb allows fish to adjust more efficiently to physiological stress decrease in RBC count and Hb content of fish from polluted sewage fed pond due to the inhibition of aerobic glycolysis curtailing of Iron & Hb via the lowered energy status is fly. Lehmann *et al*., 1976 Banerjee V. and Banerjee (1998) have suggested that heavy metal exposure decrease the total RBC count & Hb content due to impaired interstitial absorption of Iron.

Blood is a very sensitive tissue that is affected by environmental changes, so by the current comparative research related to the hematological and biochemical profile of the blood of fish *Cyprinus carpio* with reference to sublethal concentration with pollutant can cause changes is the hematological parameters (Shivaknmar, 2005) decrease and increase is the RBCs count may be due to chronic exposure of heavy metals in the *Cynipus Sp* was observed by Bela Zutshi 2010 & Gill & Sreedevi *et al*., 1992. Significant reduction of RBC in fish exposed to cd & other parameters such as MCV, MCH & CHC are indicatory of anemia. The concentration of blood parameters were significantly elevated when the fish *Cyprinus carpio* exposed to heavy metal Cu, Cd & Cr by Vinodhini and Narayanan, (2009) suggested that the presence of toxic metals in aquatic environment has strong influence on the hematological parameters is the common carp *Cyprinus carpio*. The increase in leucocyte number is all the experimental fishes after 14 & 21 days of pollutant treatment is probable for the removal of cellular debris of necrosis tissue at quicker rate as reported by (Duthie and Tort, 1985)

In the present investigation it is seen that during 7th day WBC count increased gradually is all concentrations. In 0.7of in % concentrations of the pollutant, the increase of 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 concentration and time duration. It is an agreement with the report that the increase in WBC is stressed animals is a protective response to stress (Samuel, 1986).

Leucocytes are involved in the immunological response (Bela Zutshi, *et al*., 2010) & Palanisamy *et al*., (2011) have reported that the change in leucocyte count with initial increase and subsequent decrease is due to the concentration of toxic pollutant and duration of explosive. The reduction of WBC count is the experimental group may be due to the release of epinephrine during stress and weakening of the immune system. Similar observations were made by (Svobodova *et al*., 1997). The reduction of hemoglobin affects the $O_2$ binding capacity (Vinodhini and Narayanan, 2009). The reduction of hemoglobin due to toxicants lead to significant decrease reduction in $O_2$ binding capacity of types (Svobodova *et al*., 1997) suggested that toxic substances exposure also decreased the RBC, Hb % & PCV % due to impaired intestinal absorption of Iron. The changes of RBC and abnormalities were observed in the present study fish exposed to alcoholic distillery effluent.

**Table 1: Total RBC Count $10^6$/ mm3 *Cyprinus carpio* exposed to different concentrations of alcoholic distillery waste water**

| Concentration | 7 days | 14 days | 21 days | 28 days | Mean |
|---|---|---|---|---|---|
| **Control** | 3.526 | 3.499 | 3.48 | 3.401 | 3.476 |
| **0.7** | 3.486 | 3.410 | 3.21 | 3.198 | 3.326 |
| **1.4** | 3.398 | 3.319 | 3.00 | 3.059 | 3.194 |
| **2.1** | 3.189 | 3.101 | 2.96 | 2.910 | 3.040 |
| **2.8** | 3.101 | 3.001 | 2.52 | 2.010 | 2.658 |
| **Mean** | 3.34 | 3.266 | 3.042 | 2.915 | 3.139 |

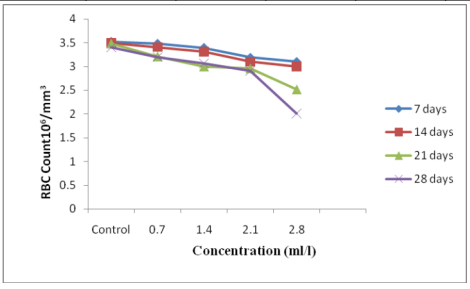


**Figure 1: Total RBC Count $10^6$/ $mm^3$ *Cyprinus carpio* exposed to different concentrations and days of alcoholic distillery waste water**

Conc & Day ** P<0.01 level of significance

**Table 2: Total WBC count $10^4$ /$mm^3$ in *Cyprinus carpio* exposed to different concentrations and days of alcoholic industrial waste water.**

| Concentration | 7 days | 14 days | 21 days | 28 days | Mean |
|---|---|---|---|---|---|
| **Control** | 6.385 | 5.245 | 6.128 | 6.219 | 6.244 |
| **0.7** | 6.910 | 6.000 | 5.988 | 5.215 | 5.828 |
| **1.4** | 6.007 | 5.939 | 5.105 | 4.589 | 5.410 |
| **2.1** | 5.289 | 4.600 | 4.619 | 3.898 | 3.761 |
| **2.8** | 5.214 | 4.026 | 4.001 | 3.115 | 3.271 |
| **Mean** | 5.881 | 5.362 | 5.168 | 4.607 | 4.903 |

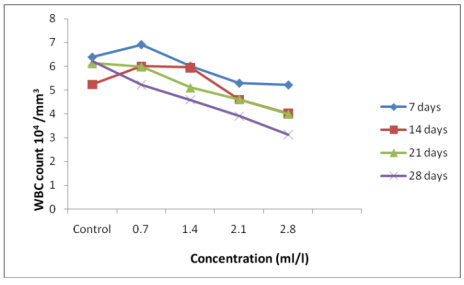


**Figure 2: Total WBC count $10^4$ /$mm^3$ in *Cyprinus carpio* exposed to different concentrations and days of alcoholic industrial waste water.**

Conc & Day ** P<0.01 level of significance

**Table 3: Total Hb (g/100 ml) in *Cyprinus carpio* exposed to different concentrations of alcoholic distillery effluent**

| Concentration | 7 days | 14 days | 21 days | 28 days | Mean |
|---|---|---|---|---|---|
| Control | 9.729 | 9.601 | 9.500 | 9.488 | 9.5795 |
| 0.7 | 9.421 | 9.219 | 9.123 | 8.993 | 9.2395 |
| 1.4 | 8.593 | 8.488 | 8.241 | 8.128 | 8.3887 |
| 2.1 | 7.972 | 7.693 | 7.56 | 7.006 | 7.6275 |
| 2.8 | 6.815 | 6.215 | 6.00 | 5.818 | 6.362 |
| Mean | 8.506 | 8.2432 | 8.0848 | 7.8866 | 41.197 |

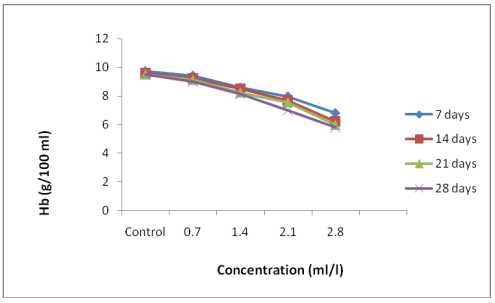


**Table 3: Total Hb (g/100 ml) in *Cyprinus carpio* exposed to different concentrations and days of alcoholic distillery effluent**

Conc & Day ** P<0.01 level of significance

**Table 4: Total Packed cell volume (%) in *Cyrinus carpio* exposed concentration and days distillery effluent**

| Concentration | 7 days | 14 days | 21 days | 28 days | Mean |
|---|---|---|---|---|---|
| Control | 28.650 | 28.614 | 28.518 | 28.535 | 28.579 |
| 0.7 | 27.560 | 26.599 | 23.967 | 22.932 | 25.265 |
| 1.4 | 24.381 | 21.786 | 19.949 | 18.997 | 21.2782 |
| 2.1 | 21.093 | 18.943 | 16.369 | 14.387 | 17.698 |
| 2.8 | 19.964 | 17.489 | 15.989 | 13.938 | 16.845 |
| Mean | 24.33 | 22.6862 | 20.9584 | 19.7578 | 21.933 |

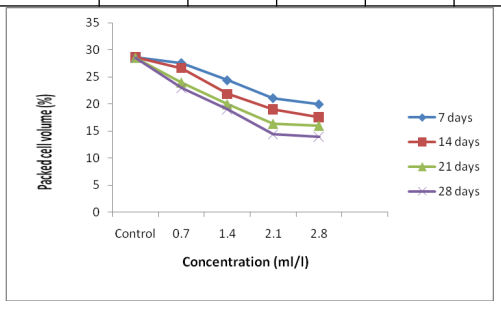


**Figure 4: Total Packed cell volume (%) in *Cyrinus carpio* exposed concentration and days distillery effluent**

Conc & Day ** P<0.01 level of significance

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