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Toxicity Evaluation of Handloom Textile Effluent on the Hematological Parameters of Oreochromis mossambicus (Peters, 1852) Fingerlings

Sourav Debnath[1], Sangram Keshari Rout[2], Soumalika Guha[3], Ankit Sarkar[4]

[1] Ph.D scholar in Life Sciences, Department of Aquatic Environment Management, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal-700094, India

[2] Professor, Department of Aquatic Environment Management, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal-700094, India

[3,4] Ph.D scholar, Department of Aquatic Environment Management, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal-700094, India

Abstract

Alteration of various hematological parameters were studied in *Oreochromis mossambicus* after 90 days exposure of Handloom textile effluent at different concentrations i.e. T0 -T5 (0% control, 4.356%, 5.445%, 7.26%, 10.89% and 21.78%). The effluent was collected from handloom factory cluster of Santipur, Nadia region. Concentration ranges was finalized according to $1/10^{th}$, $1/8^{th}$, $1/6^{th}$, $1/4^{th}$, $1/2^{nd}$ of 96 hrs LC_{50} value (43%) for this experimental setup. The studied hematological parameters include total erythrocytes count (RBC), total leucocytes count (WBC), Packed Cell Volume (PCV), Hemoglobin concentration (Hb) and Mean Corpuscular Hemoglobin Concentration (MCHC). In this experiment, it is evident that the quantity of RBC, Hemoglobin and MCHC values are decreasing from T0 to T5. But at the same time, it is also found that PCV and WBC values increased onwards T3 even after an initial decrease.

Keywords: Handloom textile effluent, *Oreochromis mossambicus*, Hematological parameters and LC_{50} test

1. Introduction

The exclusiveness of West Bengal, India is associated with its culture, food and few indigenous small-scale industries etc. Among them, Handloom textile sector is the potential as well as profitable industry in rural side. West Bengal has a very small power loom base with barely 5930 power looms working in small clumps in the districts of Nadia, Howrah, Kolkata and North 24-Parganas and produces around 4510 million M^2 of cloth. West Bengal handloom products like Tangail, Dhaniakhali, Santipuri, Murshidabadi, etc have evolved as a national brand attraction with geographical recognition. State also has a well-built 'Bengal Handloom brand' in both domestic and international market and creates organized brand promoting activities (WB report, 2019).

The handloom sectors as well as overall textile industries engender large volumes of wastewater daily. The wastewater with organic and inorganic substances is definitely the reason for causing the pollution of the environment if discharged non-selectively. Ranaghat, Fulia and Shantipur are three suburban towns that are famous for the numerous handloom textile factories operating in Nadia district (West Bengal, India) and producing magnificent varieties of handloom clothes for many years. But the ejection of effluent from these factories into the next door to water bodies and also river Churni become a silent-killer of aquatic environment of that particular zone (Sanyal *et al.*, 2018). Ultimately, such water could affect the purity of drinking water and intimidates to public health on the side of consumers. So it becomes very much relevant as well as discussing topic in the field of ecotoxicology (Uwidia *et al.*, 2013).

Fishes are very much prone to be sensitive to water contamination. When such contaminants through effluents enter into the organ system of fishes it can directly affect and alter the hematological parameters of the organisms. Due to increased consciousness of worldwide environmental issues, there has been a great fascination in ecologically friendly, wet processing textile techniques in recent years (Padama *et al.*, 2006). The present assessment is launched to identify systematically the impact of handloom textile dyeing industry effluents on some of the hematological parameters in freshwater fish *O. mossambicus*. A number of hematological indices such as haematocrit (Hct), haemoglobin (Hb), RBCs and so on are used to study the utility status of the oxygen carrying capacity of the blood stream and have been used as indicators of pollution (Shah and Altindog, 2004, Soni*et al.*, 2006, Seriani *et al.*, 2011, Mgbenka and Oluah, 2003). Characterization of such wastewaters is precondition for the experiment of pollution potential and mandatory treatment options.

2. Material and methods

2.1 Procurement of selected fishes

The selected experimental fish, *O. mossambicus* advanced fingerlings were procured from Bengal Fish Hatchery of Sonarpur, Kolkata and transported to the wet laboratory, Faculty of fishery sciences, Kolkata with proper arrangement. The fishes were acclimatized for two weeks and fed with commercial feed composed with 30% crude protein at a rate of 3% body weight per day.

2.2 Source and collection of Handloom Textile Effluents

For this study, the raw effluent was collected from the textile factory of Santipur region, Nadia, West Bengal. After the collection, the effluent was carried out to Experimental site and kept in to the five rectangular FRP tanks of 500L capacity.

2.3 Experimental design

With the objective of evaluating the effects of Handloom Textile Effluents on fresh water fishes *O. mossambicus*, the present experiment was conducted for the period of 90 days in Toxicology Laboratory of the Department of Aquatic Environment Management at Faculty of Fishery Sciences, Chakgaria, Kolkata, India.

2.4 Acute toxicity study

The median lethal concentration (LC_{50}) of Handloom textile effluent on *O. mossambicus* was performed in a static renewal system using different concentrations of test solution including control (25%, 30%, 35%, 40%, and 45% along with control 0%). The experiment was conducted in triplicate in 60 L glass tank. A total of one hundred eighty acclimatized fishes with average weight 15 ± 1 gm (mean± SD) were randomly selected and distributed in different experimental groups keeping 10 fishes in each tank. Cumulative mortality of fishes was studied at every 24 h and the median lethal concentration was finalized using Finney's probit analysis method during the experiment. The LC_{50} value of handloom textile effluent on *O. mossambicus* was calculated as 43%.

2.5 Chronic toxicity study

During the chronic toxicity experiment one hundred fifty acclimatized *O. mossambicus* with average weight 15 ± 1 gm (mean± SD) were collected randomly and distributed in experimental tanks arranged in six experimental groups viz. T0 (control), T1 (4.356%), T2 (5.445%), T3 (7.26%), T4 (10.89%), T5 (21.78%) in triplicate using completely randomized design (CRD). The test was performed for 90 days and the fishes were fed with commercial feed containing 30% crude protein at a rate of 3% body weight regularly and the left-over feed was removed at a day interval using syphoning pipe. The water quality parameters were observed and blood samples were collected from each experimental group on 1st, 30th, 60th and 90th day of experiment.

2.6 Collection of blood for hematological analysis

On respective sampling day two fishes were selected from each experimental group and anesthetized by clove oil at a dose of 50 $\mu L\ L^{-1}$ to lower the stress. Blood was withdrawn from the caudal vein of the fish by medical syringe cleaned by EDTA. The collected blood sample was shifted to EDTA coated vials and properly mixed to avert clotting.

2.7 Hematological analysis

- Total erythrocyte count (TEC) and total leukocyte count (TLC) were studied by taking 20 µl of blood sample and stirred with RBC or WBC diluting fluid (Himedia, India) in a properly clean vial. The calculated TEC and TLC values are expressed as numbers per cubic millimetre of blood sample.

  Total erythrocyte count per cubic millimetre of blood sample was calculated using the following formula:

$$\mathbf{TEC\ (10^6/mm^3) = \frac{N \times Dilution}{Depth\ of\ fluid \times Area\ counted}}$$

$$\mathbf{= N \times 200 / 0.2 \times 0.1}$$

$$\mathbf{= N \times 10000}$$

Where, N is total erythrocytes counted in 5 squares of the haemocytometer. 0.2 is the area counted and 0.1 is depth of fluid.

$$\mathbf{TLC\ (10^3/mm) = \frac{N \times\ Dilution}{Depth\ of\ fluid \times\ Area\ counted}}$$

$$\mathbf{= N \times 200 / 4 \times 0.1}$$

$$\mathbf{= N \times 500}$$

Where, N denotes total leukocyte counted in 4 squares of the haemocytometer.

- The hemoglobin (Hb) level of blood was measured following the Cyanmethemoglobin method. Drabkins Fluid was used in this method. 20 µl Blood was stirred with 5 ml of Drabkin's working solution. The absorbance was estimated using a spectrophotometer at 540 nm wavelength. The hemoglobin concentration was then by using the following formula:

$$\mathbf{Haemoglobin\ Content\ (g/dl) = \frac{\{OD\ (T)\ \times\ 251\ \times\ 60\}}{OD(S)} \times 1000}$$

Where, OD (T) = Absorbance of test OD (S) = Absorbance of standard

- Packed cell volume (PCV) was calculated by capillary action of blood sample into micro haematocrit tubes. Micro haematocrit reader's PCV value is expressed as percentage.

$$\mathbf{PCV} = \left(\frac{\textbf{Height of RBC column}}{\textbf{total height of the column}}\right) \times \mathbf{100}\ (\%)$$

- The average haemoglobin concentration within the given red blood cells is called MCHC value. The MCHC expressed as the amount of haemoglobin (%) in red blood cells is calculated as follows,

$$\mathbf{MCHC} = \frac{\textbf{Hemoglobin conc.}\ \%}{\textbf{PCV as fraction}}$$

2.8 **Statistical analysis**

Two way analysis of variance (ANOVA) test using the statistical package SPSS software and Pearson's correlation test were used to compare the observations.

3. Results and Discussion

3.1 Haematological analysis of blood sample:

3.1.1 Red Blood Corpuscles (RBC) :

The *O. mossambicus* exposed to various concentrations of handloom textile effluent contaminated wastewater showed a prominent reduction of RBC with respect of increasing pollution due to wastewater. The mean value of RBC count was found maximum in blood sample 2.95 ± 0.07 in control tank at the 1st day. Minimum value was 2.26 ± 0.24 at 30th day in T3 treatment. According to Pearson's correlation test, the RBC count showed significant variance as well as it showed striking difference among different exposure days among various concentrations. The analysis of variance showed a highly significant ($P<0.05$) decrease in RBC count in exposed fishes with respect to days and concentration. Reduction of RBC value due to toxicant exposure has been reported by Chowdhury *et al*. (2004). Even a low level of industrial effluent exposed for a period of time can reduce the erythrocyte production (Abernatty *et al*., 2003). Another studies on Tilapia and Carp fish also reported the occurrence of variation in total erythrocyte count (Mishra *et al*., 2020; Amte *et al*., 2013).

3.1.2 WBC count:

Leucocytes count is essential for studying the lethal and sub lethal effects in fish caused by toxic effluents. Fig. 2 shows the alteration of Total Erythrocyte Count (TEC) or WBC according to different concentrations of effluent among 30, 60 and 90 days exposure. The variations in TEC are statistically significant ($P<0.05$) after 30 and 60 days of exposure but in initial days it varies insignificantly ($P>0.05$). Maximum value of WBC in blood sample was 8.5 ± 0.44 found in T5 effluent treatment at the 1st day. Minimum value was at 90th day in this particular concentration. Among various concentrations, the WBC count showed significant variance as well as it showed striking difference among different exposure days. Significant increase in the leucocyte (WBCs) count resulted in leucocytosis in the fish exposed to effluent samples which is an immunological response for the adaptation to cope up with the stressful condition due to the effluents. It was evident that textile effluent causes the variation in WBC count in *Oreochromis mossambicus* and results the maximum value at higher concentration (Amte *et al.*, 2013). It is in agreement with the report that the increase in WBC in stressed animals is a protective response to stress (Akinrotimi *et al*., 2012). This WBC's increase due to effluent might be recovered by production of antibodies.

3.1.3 Hemoglobin:

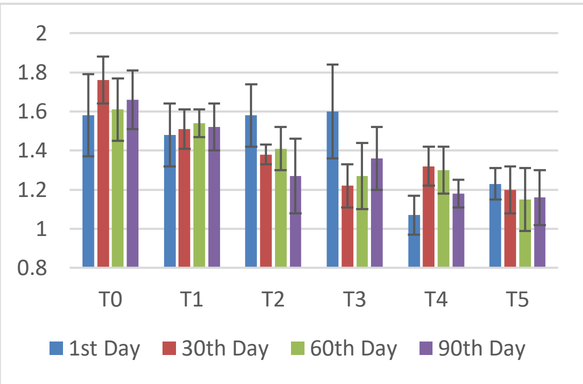

Figure 1. Variation of RBC(10^6/mm^3) along the different experimental days in different concentration of Handloom textile effluent.

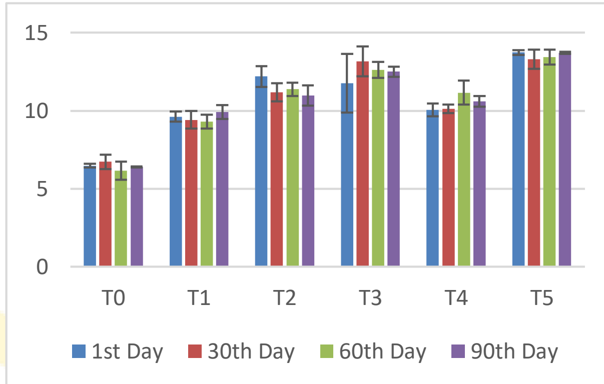

Figure 2. Variation of WBC(10^6/mm^3) along the different experimental days in different concentration of Handloom textile effluent.

The values are statistically significant ($P<0.05$) at 5, 15 and 25 % effluent concentrations after 30, 60 and 90 days exposure. Maximum value of Hemoglobin in blood sample was found 9.72 ± 0.26 in control tank at the 30th day. Minimum value was 3.22 ± 0.34 at 1st day in T5 concentration. Among various concentrations, the RBC count showed significant variance as well as it showed striking difference among different exposure days. The values decrease along concentrations and also varied day wise. According to Mishra *et al. (*2020), paper mill effluent was also responsible for the decrease of hemoglobin percentage in tilapia fish. Hemoglobin was found to be depleted in fish due to Nickel and it may be due to the erythrocytes reduction (Ololade and Oginni, 2010).

3.1.4 PCV value:

Packed Cell Volume (PCV) is definitely another key parameter for detecting the hematological alteration in fish sample. In this study, Maximum value of PCV in blood sample was 28.6 ± 0.06 found in control tank at the 1st day. Minimum value was 10.05 ± 0.06 at 90th day in T5 concentration. Among various concentrations, the RBC count showed significant variance as well as it showed striking difference among different exposure days. PCV after exposure to different concentrations of effluent is shown in fig.4. The reduction in PCV correlated with reduced cell counts and hemoglobin concentration. Javed *et al.* (2016) reported that hematological alteration was also found in *C. punctatus* due to thermal power plant effluent. Decreased PCV value was also experimented in *Sarothedon melanotheron* exposed to industrial effluent (Nte*et al*., 2011). Similar reduction in haematocrit value was found in *Clarias gariepinus* exposed to effluent from metal finishing company (Adakole, 2012).

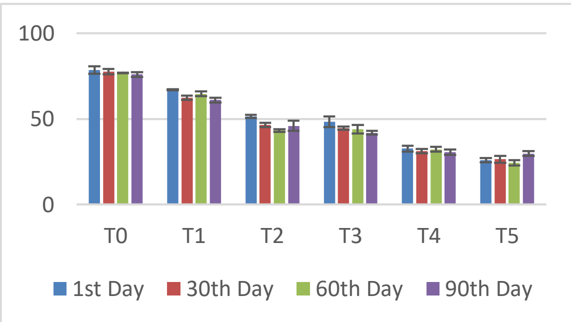

Figure 3. Variation of Hemoglobin along the different experimental days in different concentration of Handloom textile effluent.

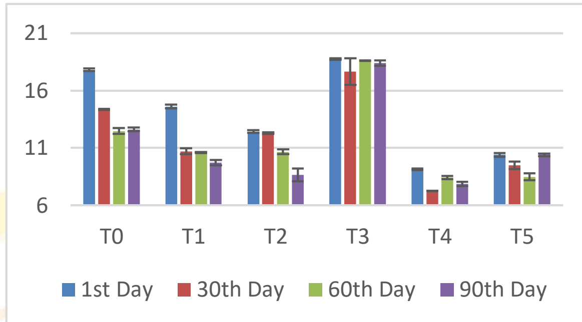

Figure 4. Variation of PCV (%) along the different experimental days in different concentration of Handloom textile effluent.

3.1.5 MCHC value:

The hematological index such as MCHC indicated noticeable variation in *O. mossambicus* exposed to different concentrations of effluent contaminated water (Fig.5). Mean corpuscular hemoglobin concentration (MCHC) of all effluent concentrations in comparison to control is in a decreasing trend. Maximum value of MCHC in blood sample was found 34.54 ± 0.16 in control tank at the 30th day. Minimum value was 25.59 ± 0.18 at 60th day in T5 treatment concentration. Among various concentrations, the RBC count showed significant variance as well as it showed striking difference among different exposure days. Another report from Amte *et al*. (2013) showed that textile effluent can reduce the MCHC value in *O. mossambicus*. This hematogical parameter was used to study the swelling of number of RBC by analyzing MCHC values (decreasing) or increased MCHC indicates shrinkage of RBC. According to the present study, it can be concluded that the variation in MCHC values represent the status of fish health due to anemia (low numbers of hemoglobin carried by RBC).

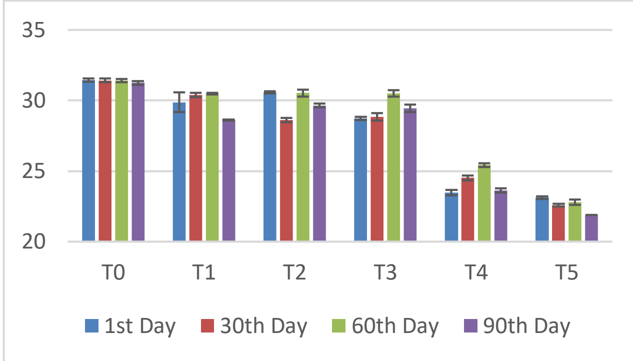

Figure 5. Variation of MCHC (gm.cc/ml) along the different experimental days in different concentration of Handloom textile effluent.

4. Conclusion

In view of the above findings it is observed that handloom textile effluent of various concentrations plays a pivotal role to cause aquatic pollution. Contaminants or ingredients in this type of textile effluent collected from rural part of Bengal may be the cause for the pollution which is eventually giving a threat to aquatic animals i.e. fishes as well as human. In this study, it can be concluded that *O. mossambicus* is one of those fishes which is sensitive to the toxic ingredients present in handloom textile effluent. In this research finding, it is clearly seen that T5 or highest concentration of untreated effluent caused alteration of blood parameters which denotes a threat for the immunity as well as sustainability of *O. mossambicus.* The lowest treatment group (T1) also showed minute but confirmatory presence of alteration. So it also suggests the need to improve the effluent treatment process to lower the concentration of untreated effluent when it would be discharged into waterbodies . Hematological alterations may be effectively used as a potential biomarker of handloom textile effluent toxicity at their various concentrations, at which either mortality was nil or very low to the freshwater fish in the field of environmental bio monitoring.

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