7. Introduction

Fish haematology has a good scope in the understanding normal and altered physiology, ecological relations and even phylogenetic kinships of fishes. Piscine haematology by virtue of its academic interest and its applied values in understanding the physiological state of fishes and monitoring the health status of fishes under natural, experimental and cultural conditions have been surveyed during the past many years, attracting tremendous scientific attention all over the world (Blaxhall, 1972; Hickey, 1976; Munkittrick and Leatherland, 1983). The uncompromising assiduous efforts of fish haematologists over the past many years have paid due dividends; information on fish blood has piled up to such gargantuan heights that it is today incredulous that fish haematology was once a much overlooked, rather an almost unattended aspects of fishery and fish toxicological science. The fish blood is an important tissue of the body which performs most of the vital activities of the life (Saxena and Sharma, 1979). Haematological techniques, including total erythrocyte and leucocyte counts and measurement of haematocrit have been proved valuable for fish biologists in assessing the health of fish (Hesser, 1960; Blaxhall, 1972; Bell and Margolis, 1976; Hickey 1976).
The effluent discharge standards based upon conventional bioassay do not necessarily ensure that the fish populations will not be subjected to stress (Haniffa et al., 1986). Changes occurring in the haematological characteristics of fishes have been found to provide a sensitive parameter in assessing the health of fish and to arrive at permissible discharge level (Nair et al., 1984). Because of the sensitivity, reliability and rapidity of the haematological response and its apparent involvement in disease resistance (Watson et al., 1956; Corbel, 1975), leucocrit (Mc Leay and Gordon, 1977) and microhaematocrit (Snieszko, 1960) procedures have been developed. While assessing the physiological effect of aquatic pollutants on fish life, it is necessary to take into account any pathological change occurring in the blood, which will impair the respiratory physiology of the fish. The present investigation describes studies carried out to determine the effects of treated sugar mill effluent on haematological variables. This study was undertaken to find whether this blood cell response is indeed a generalized stress response for these fish and to ascertain whether changes in blood cell counts and morphometry could be used as a tool to detect levels of pollutants not acutely toxic to fish.

7.2 Materials and methods

7.2.1 Experimentation

_C. striatus_ fingerlings collected from local ponds were maintained at CARE for 20 days prior to testing. During this time fish were held under natural illumination at a low population density (50 / tank) in cement tank (4 x 2 x 1.5 ft (1.28m^3)) dechlorinated well water. Fish were fed two times (8 A.M and 16 P.M) daily with moist chicken intestine. At the time of starting the fish measured 3.92 ± 0.32 cm fork length and weighed 2.9 ± 0.35g. After the acclimatization, 50 fish were placed in each culture tank (400 l capacity) filled with two sub-lethal concentration effluents excluding control viz (0%) control, 1/3 of 96 hr LC₅₀ (30.5%) and 2/3 of LC₅₀ 96 hrs (61%) of anaerobically UASB treated oil separator tank effluent. Once in three days, fresh effluent was supplied and the amount was maintained daily. The fish were fed on fresh moist chicken intestine _ad libitum_ twice a day. This experiment was continued for 60 days. During the culture period fish were weighed after 15, 35, 55 and 75 days. For haematology caudal peduncle of the test fish was dried, cleaned with 95% alcohol and the blood severed from the ventral aorta were collected in a glass vial containing few drops of 10%
Ethylene diamino tetra-acetic acid (EDTA). Contents in the vial were mixed gently. The randomly collected blood was used for the estimation of total erythrocyte count (RBC) and total leucocyte count (WBC), erythrocyte sedimentation rate (ESR) Haematocrit (Ht), Haemoglobin (Hb) and mean corpuscular haemoglobin (MCH). For differential blood counting (WBC) blood smear was made directly from the caudal region (Dubowski; 1962).

7.2.2 Total counts of Erythrocytes and Leucocytes

Erythrocytes (EC)

Erythrocyte and Leucocyte counts were determined according to procedures described by Mc Leay (1973), and Shaw and Bernard (1930). Using the following composition, the Hayem’s solution was prepared for erythrocyte count: 2.5 g Na₂SO₄, 7 H₂O, 1g NaCl and 0.25g HgCl₂ were dissolved in 100 ml of distilled water at the pH range of 7 - 7.4. Blood was drawn in a dry erythrocyte pipette to the 0.5 graduation and Hayem’s solution drawn to the 101 mark. The pipette was then shaken well for a minute. Immediately after shaking, the counting chamber was filled with mixture, free from air bubbles (dilution 1: 200). Counting was made under 400 times magnification, after 30 seconds of filling. The EC were counted in five large squares (1 large square = 16 small squares), and the number of small squares being 80 in 1/400 mm². All cells lying inside the group squares and also the EC lying to the left and below the demarcation line were counted. Calculation of the number of EC was done according to the following equation.

\[
EC \text{ mm}^3 = \frac{\text{Total No.of Erythrocytes} \times 400 \times 10 \times 200}{80}
\]

Leucocyte count (LC)

Leucocyte (LC) count was made by mixing the solution A and B. Solution A was prepared by dissolving 0.025g of neutral red, 0.9g of NaCl in 100 ml distilled water with 7-7.4 pH. Solution B was prepared by dissolving 0.012g of crystal violet, 3.8g of sodium citrate and 0.4 ml of formalin in 100 ml of distilled water with 7-7.4 pH. The solutions were prepared freshly and filtered before use. The blood was drawn up to the 0.5 mark in the leucocyte pipette. Solution A was drawn in until the bulb was about half filled and solution B was then drawn into
the 101 mark, after shaking. The counting chamber was filled with the mixtures so as to be free
from air bubble and counting was done in the large squares which were present at the four
angular points of the Neubauer counting chamber and were demarcated by triple lines (1 mm²).
The number of leucocytes was calculated from the following equation.

\[
LC / \text{mm}^3 = \frac{\text{Number of leucocytes in four squares of 1 mm}^2}{4 \times 1/10 \times 1/200}
\]

\[
LC / \text{mm}^3 = \frac{\text{LC in 4 mm}^2 \times 2,000}{4} = \text{LC in 4 mm}^2 \times 500
\]

7.2.3 Differential leucocyte count

For the preparation of blood smear a fresh drop of blood was placed on a degreased slide (kept for 2 days in chromsulfuric acid, thoroughly rinsed with tap water, wiped with a clean linen cloth and kept in alcohol and benzene 1:1) May - Grunwald, Giemsa, pappenheim (combined May - Grunwald - Giemsa staining) of pH 6.8 (Hallmann, 1966; Henning, 1966; Romeis, 1968) were used for smear preparation. Fixed and fresh air-dried smears were flooded with 20 - 30 drops (0.5-0.8 ml) of May-Grunwald solution for about 3 minutes. Equal quantity of distilled water was added for 1 minute. By decanting and without rinsing, staining with dilute Giemsa solution (10 drops in 10 ml of distilled water) was made for 15-20 minutes and the slide was flushed vigorously with distilled water. Microphotographical observations for the differential blood count were made on dried and stained blood smears under the microscope (10 x 40 and 10 x 100 magnification). Cytomorphology and cytometry of representative blood cell types were studies based on pappenheim stained smears. For cytometry, a calibrated oculometer and 10 x 100 magnification were used; 50 cells of each type were measured for length and width of cell and nucleus. Photomicrographs of representative cell types were also prepared using binocular microscope (Hertel & Reuss, Optic, West Germany).
7.2.4 Haematological variables

7.2.4.1 Erythrocyte Sedimentation Rate (ESR)

Blood was drawn in a corning glass capillary tube measuring 15cm in length with 1mm inner diameter and calibrated in mm. Blood was filled upto 10 cm by capillary action and one end was sealed and placed in a vertical position for an hour without disturbance. The sedimentation rate was read off after one hour. After an hour the length of the clear plasma column at the top was measured in mm.

7.2.4.2 Haematocrit value (Ht)

The blood was drawn into a corning glass capillary as used for ESR test. Both the openings of the pipette were closed with rubber stoppers and was centrifuged for three minutes in 3000 rpm. After centrifuging, the capillary tubes were placed on a reading device and the volume was read off. The Ht was expressed as the percentage fraction of blood cells in the total volume.

\[
Ht = \text{Total height of the blood column (100%)} - \text{Plasma Volume (\%)}
\]

7.2.4.3 Estimation of Haemoglobin (Hb)

Shali's haemometer was used for the determination of haemoglobin content by acid haematin method. The shali's pipette was filled with blood slightly above the '20' mark; the pipette was wiped with a soft absorbent tissue paper to remove excess blood and the volume was adjusted exactly to '20' mark. The blood was expelled into a haemometer tube containing 0.1 N hydrochloric acid up to the '20' mark. The contents were mixed by gently drawing in and expelling the solution 3 to 5 times. The contents were again mixed using a glass stirrer and allowed to stand for ten minutes. This mixture was diluted with distilled water by adding drop by drop, and stirred thoroughly, using the glass stirrer after the addition of each drop and the colour of the solution was matched with that of the standard provided in the haemometer. The amount of the Hb in the sample was directly read in g% from the graduated tube (Samuel, 1978).
7.2.4.4 Determination of mean corpuscular haemoglobin concentration (MCHC)

The mean corpuscular haemoglobin concentration in g% (g / 100ml) for 100 ml erythrocytes was calculated by the following formula

\[
\text{MCHC} = \frac{\text{Haemoglobin g} \% \times 100}{\text{Haematocrit volume} \%} \times \frac{\text{g}}{100 \text{ ml}}
\]

7.2.4.5 Determination of mean corpuscular haemoglobin (MCH)

It was the average haemoglobin content of a single red cell in micrograms, and was determined by the following formula

\[
\text{MCH (pg)} = \frac{\text{Haemoglobin g} \% \times 10}{\text{Erythrocyte count (millions /mm}^3)} \times \frac{\text{pg}}{} \]

7.2.4.6 Determination of Mean Corpuscular Volume of individual erythrocytes (MCV)

The mean volume of the individual erythrocyte can be calculated from the following formula.

\[
\text{MCV} = \frac{\text{Haematocrit value} \times 10}{\text{Number of erythrocytes (million / mm}^3)} \times 3\text{ m}^3
\]

7.2.4.7 Statistical Analysis

Multiple regression analysis test, standard deviation, standard error and students 't' tests, as detailed by Zar (1984) were followed.
7. 3 Results

7. 3. 1 Total cell counts

Total Erythrocyte count (TEC)

Exposure to effluent and control (tap water) produced increase in values in the peripheral variable of TECs. The elevation was dose and time dependent and more pronounced in individuals exposed to the highest concentrations at highest exposure time Table 7.1. In control group the TEC value was increased from $2.36 \times 10^6$ mm$^3$ to $2.88 \times 10^6$ mm$^3$ in 20 days exposure, $3.56 \times 10^6$ mm$^3$ in 40 days exposure and $4.19 \times 10^6$ mm$^3$ in 60 days exposure. The TEC value increased to $3.24 \times 10^6$, $3.92 \times 10^6$ and $4.3 \times 10^6$ mm$^3$ in 30.5% effluent concentration, after 20, 40 and 60 days of exposure. 61% effluent caused a rapid increase of TEC value as $3.41$, $4.27$ and $4.85 \times 10^6$ mm$^3$ in 20, 40 and 60 days of exposure. Multiple regression analysis showed that the concentrations of effluent ($t = 2.74; d.f = 24; P = 0.012$) and days of exposure ($t = 6.10; d.f = 24; P = 0.00$) had significant impact on TEC. The regression of log TEC ($Y$) on log concentrations ($x_1$) and log days ($x_2$) may be fitted in the following equation as

$$\hat{Y} = 0.5810 + 0.0278x_1 + 0.2535x_2$$

Total Leucocyte Count (TLC)

Table 7.1 reports the changes observed in total number of WBCs in C. striatus due to effluent concentrations and duration of exposure. Increase in WBCs was from $2.85 \times 10^4$/ mm$^3$ (control) to $3.38 \times 10^4$ mm$^3$, $3.65 \times 10^4$ to $3.85 \times 10^4$/ mm$^3$ and $4.44 \times 10^4$ to $4.61 \times 10^4$ mm$^3$ in the fish exposed for 20, 40, 60, days in 30.5% effluent concentrations. The same increased to $3.71 \times 10^4$, $4.14 \times 10^4$ and $4.87 \times 10^4$/ mm$^3$ in fish exposed for 20, 40 and 60 days at 61% effluent concentration respectively. The increased TLC was found to be dose and time dependent. Multiple regression analysis of TLC on effluent concentrations and days of exposure showed significant positive effect ($t = 5.55; d.f = 24; P = 0.0$ and $t = 10.0; d.f = 24; P = 0.0$). The regression of log TLC ($Y$) on log days ($x_1$) and log concentrations ($x_2$) may be presented as

$$\hat{Y} = 0.4801 + 0.03751 x_1 + 0.2771 x_2$$
Table 7.1  Peripheral corpuscular haemogram of *Channa Striatus* exposed to 30.5 and 61% treated sugar mill effluent for 20, 40 and 60 days.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Haematological Variables</th>
<th>Control</th>
<th>20 day</th>
<th>40 day</th>
<th>60 day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>30.5%</td>
<td>61%</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>TEC x 10^6 mm^3</td>
<td>2.36±0.31</td>
<td>2.88±0.45</td>
<td>3.24±0.57</td>
<td>3.41±0.60</td>
</tr>
<tr>
<td>2</td>
<td>TLC x 10^4 mm^3</td>
<td>2.32±0.19</td>
<td>2.85±0.22</td>
<td>3.38±0.144</td>
<td>3.71±0.11</td>
</tr>
<tr>
<td>i</td>
<td>Lymphocytes (%)</td>
<td>72.3±0.57</td>
<td>72±2.0</td>
<td>71.3±0.57</td>
<td>63.6±3.2</td>
</tr>
<tr>
<td>ii</td>
<td>Monocyte (%)</td>
<td>13.6±1.5</td>
<td>14.3±1.5</td>
<td>13.6±1.5</td>
<td>19.3±3.2</td>
</tr>
<tr>
<td>iii</td>
<td>Eosinophil (%)</td>
<td>4±1.0</td>
<td>3.33±0.57</td>
<td>4.66±0.57</td>
<td>5.33±1.2</td>
</tr>
<tr>
<td>iv</td>
<td>Neutrophil (%)</td>
<td>7±1.0</td>
<td>7±1.0</td>
<td>7.66±1.2</td>
<td>10±1.0</td>
</tr>
<tr>
<td>v</td>
<td>Basophil (%)</td>
<td>3±1.0</td>
<td>3.3±0.57</td>
<td>2.66±0.57</td>
<td>1.7±0.57</td>
</tr>
<tr>
<td>3</td>
<td>TTC x 10^6 mm^3</td>
<td>1.95±0.24</td>
<td>2.49±0.49</td>
<td>2.13±0.39</td>
<td>3.08±0.52</td>
</tr>
<tr>
<td>4</td>
<td>Hb (g %)</td>
<td>9.78±1.82</td>
<td>10.7±1.25</td>
<td>11.17±0.75</td>
<td>12.59±2.7</td>
</tr>
<tr>
<td>5</td>
<td>Ht (%)</td>
<td>34.87±1.42</td>
<td>36.6±1.6</td>
<td>37.2±0.96</td>
<td>38.57±2.9</td>
</tr>
<tr>
<td>6</td>
<td>ESR (mm/hr)</td>
<td>7.5±0.7</td>
<td>7.3±0.38</td>
<td>7.1±1.1</td>
<td>7.2±0.82</td>
</tr>
<tr>
<td>7</td>
<td>MCV (μm^3)</td>
<td>149±14.8</td>
<td>129.4±15.1</td>
<td>117.2±17.7</td>
<td>114.4±12</td>
</tr>
<tr>
<td>8</td>
<td>MCH (pg)</td>
<td>41.27±2.3</td>
<td>37.3±1.5</td>
<td>34.95±3.8</td>
<td>36.7±1.3</td>
</tr>
<tr>
<td>9</td>
<td>MCHC (g/100 ml)</td>
<td>27.9±4.1</td>
<td>29.15±2.18</td>
<td>29.95±1.25</td>
<td>32.4±4.46</td>
</tr>
</tbody>
</table>

Each value is an average of five observations and ± indicates standard deviation.
Total Thrombocyte Count

Table 7.1 shows the changes observed in total number of thrombocytes in *C. striatus* due to effluent concentrations and duration of exposure. Increase in TTCs was from $2.49 \times 10^4/\text{mm}^3$ (control) to $2.13 \times 10^4/\text{mm}^3$, $2.9 \times 2.84 \times 10^4/\text{mm}^3$ and $2.63 \times 3.16 \times 10^4/\text{mm}^3$ for 20, 40 and 60 days in 30.5% effluent concentrations. The same increased to $3.08 \times 10^4/\text{mm}^3$, $3.44 \times 10^4/\text{mm}^3$ and $4.02 \times 10^4/\text{mm}^3$ for 20, 40 and 60 days in 61% effluent concentrations. The effluent concentration ($t = 2.01$; d.f = 24; $P = 0.055$) and days of exposures were found to exert significant positive effect on total thrombocyte count. The regression of log TTC ($Y$) on log concentrations ($x_1$) and log days ($x_2$) of exposure may be presented as

$$Y = 0.6766 + 0.0339 x_1 + 0.1664 x_2$$

7.3.2 Differential cell count

Lymphocytes

Table 7.1 shows the percentage of lymphocytes in the blood of *C. striatus* exposed to different concentrations of treated sugar mill effluent for various durations. The control fish had 72%, 72.3% and 72% lymphocytes and the same declined to 71.3%, 67% and 69% in the fish exposed to 30.5% effluent for 20, 40 and 60 days. It declined to 63.6%, 62.3% and 60% in 61% effluent after 20, 40 and 60 days of exposure respectively. Among the criterion factors analysed for influence on lymphocytes level, effluent concentrations ($t = -5.68$; d.f = 24; $P = 0.00$) and exposure time ($t = -1.43$; d.f = 24; $P = 0.166$) had significant influence on the lymphocytes counts. The regression of log lymphocyte count ($Y$) on log concentrations ($x_1$) and log exposure time ($x_2$) may be presented as

$$Y = 4.4063 - 0.0285 x_1 - 0.0293 x_2$$

Monocyte

Increase in percentage of monocytes was observed in both the experimental concentrations. This elevation was due to concentrations of effluent and days of exposure. Control groups showed 14.3%, 14.3% and 15.3% of monocytes after 20, 40 and 60 days of exposure respectively. A small reduction of monocyte percentage was noticed (13.6%) in 30.5% effluent concentration after 20 days. Monocytes increased to 16.3 and 16.6% in 30.5% effluent after 40 and 60 days; and increased to 19.3, 17.3 and 18.6% in 61% effluent
concentration after 20, 40 and 60 days of exposure (Table 7.1). Multiple regression analysis of monocyte as a function of two criterion factors revealed the positive influence of effluent concentrations \((t = 3.61; \text{d.f} = 24; P = 0.001)\) and duration of exposure \((t = 1.48; \text{d.f} = 24; P = 0.15)\). The regression equation of log monocyte count \((Y)\) on log effluent concentrations \((x_1)\) and log days of exposure \((x_2)\) may be presented as

\[
\hat{Y} = 2.4862 + 0.0412x_1 + 0.0691x_2
\]

**Eosinophil**

Increase in effluent concentration and exposure period enhanced the percentage of eosinophil as seen in Table 7.1. In the control, the percentage was 3.33, 3.33 and 4.0 after 20, 40 and 60 exposure days and it increased to 4.66%, 5.0% and 5.33%; 5.33%, 7.3% and 6.66% in 30.5% and 61% effluent concentrations after 20, 40 and 60 days of exposure (Table 7.1). Multiple regression analysis of eosinophil showed significant positive influence of effluent concentrations \((t = 6.89; \text{d.f} = 24; P = 0.0)\) and duration of exposure \((t = 2.25; \text{d.f} = 24; P = 0.034)\). The regression equation of log eosinophil \((Y)\) on log effluent concentration \((x_1)\) and log exposure days \((x_2)\) may be presented as

\[
\hat{Y} = 0.977 + 0.1066x_1 + 0.1429x_2
\]

**Neutrophil**

Table 7.1 shows the change in the percentage of neutrophil after exposure to effluent. The neutrophil percentage increased from 7 to 7.66, 7.66 to 9.66 and 6.33 to 6.66 % in 30.5% effluent after 20, 40 and 60 days of exposure. Further it increased to 10.0%, 10.6% and 11.3% in 61% effluent after 20, 40 and 60 days of exposure. Multiple regression analysis of neutrophil level showed significant positive influence of effluent concentration \((t = 4.37; \text{d.f} = 24; P = 0.002)\); but exposure duration failed to exert any significant influence on neutrophil content \((t = -0.08, \text{d.f} = 24, P = 0.936)\). The regression equation of log neutrophil \((Y)\) on log effluent concentrations \((x_1)\) and log exposure days \((x_2)\) may be fitted in the equation as

\[
\hat{Y} = 2.075 + 0.0727x_1 - 0.0055x_2
\]
Basophil

Table 7.1 shows the change in percentage of basophil in the blood of *C. striatus* exposed to different concentrations of effluent for various durations. The control fish showed 3.3%, 2.3% and 2.3% of basophils after 20, 40 and 60 days and it declined to 2.66% and 2.3% in 30.5% effluent concentrations after 20, 40 and 60 exposure days. But in 61% concentration, the basophil percentage increased to 3.3% after 60 exposure days. Multiple regression analysis of basophil showed significant negative influence of effluent concentration (t = -1.12; d.f = 24; P = 0.273) and days of exposure failed to exert any significant influence on basophil levels (overall P = 0.59). The regression equation of log basophil (Y) on log effluent concentration (x_1) and exposure days (x_2) may be presented as

\[ \hat{Y} = 1.2167 + 0.0274x_1 + 0.01893x_2 \]

7.3.3 Haematological variables

7.3.3.1 Haemoglobin

As shown in Table 7.1, haemoglobin content increased from 10.7 g% to 11.17 g%, 11.22 g% to 11.87 g% and 13.68 g% to 14.2 g% in the fish exposed to 30.5% effluent after 20, 40 and 60 days. The same increased to 12.59 g%, 12.67 g% and 16.2 g% in the fish exposed to 61% effluent after 20, 40 and 60 days of exposure (Table 7.1). Multiple regression analysis of haemoglobin content as a function of two criterion factors revealed the significant positive influence of effluent concentrations (t = 2.19; d.f = 24, P = 0.039) and days of exposure (t = 3.84; d.f = 24; P = 0.001). The partial regression of log haemoglobin (Y) on log effluent concentration (x_1) and log exposure days (x_2) may be fitted in the equation as

\[ \hat{Y} = 1.8128 + 0.02776x_1 + 0.1998x_2 \]

7.3.3.2 Haematocrit

Table 7.1 shows elevation in haematocrit values with the increase in exposure time and effluent concentration. The haematocrit value was 36.6%, 39.0% and 47.0% in the control group after 20, 40 and 60 days. It increased to 37.2%, 41.5% and 48.3%; 38.57%, 43.6% and 50.0% in the fish exposed to 30.5% and 61% effluent concentrations after 20, 40 and 60 days. Significant positive effect of effluent concentrations (t = 2.42; d.f = 24; P = 0.023) and exposure
days \( t = 8.72; \) \( d.f = 24; P = 0.000 \) on haematocrit was observed on multiple regression analysis. The partial regression of log haematocrit \( (Y) \) on log effluent concentration \( (x_1) \) and log exposure days \( (x_2) \) may be fitted in the equation as
\[
\hat{Y} = 2.9091 + 0.0153 x_1 + 0.2255 x_2
\]

7.3.3.3 Erythrocyte Sedimentation Rate (ESR)

The ESR gradually decreased with the increase in effluent concentration and duration of exposure (Table 7.1). It was 7.3, 7.5 and 6.5 mm/hr in the control fish after 20, 40 and 60 exposure days and it declined to 7.1, 6.7 and 6.07 mm/hr; 7.2, 6.4 and 5.5 mm/hr in the fish exposed to 305% and 61% effluent after 20, 40 and 60 exposure days. Significant negative impact of effluent concentrations \( (t = -2.07; d.f = 24; P = 0.049) \) and days of exposure \( (t = -3.09; d.f = 24; P = 0.005) \) on ESR were observed in multiple regression. The partial regression of log ESR \((Y)\) on log concentration \((x_1)\) and log duration \((x_2)\) may be fitted in the equation as
\[
\hat{Y} = 2.5935 - 0.02258 x_1 - 0.0238 x_2
\]

7.3.3.4 Mean Corpuscular Volume (MCV)

Table 7.1 reports the changes in MCV of the control and the effluent exposed fish. Effluent concentration and duration of exposure caused a decrease in MCV. Reduction of MCV was observed in the control also i.e., 129.4, 110.0 and 112.3 \( \mu m^3 \) after 20, 40 and 60 days and it further decreased to 117.0, 107.8 and 112.8 \( \mu m^3 \); 114.4, 103.4 and 103.1 \( \mu m^3 \) in the fish exposed to 30.5% and 61% effluent concentrations after 20, 40 and 60 days of exposure. Significant negative effect of effluent concentration \( (t = -1.91; d.f = 24; P = 0.068) \) and duration of exposure \( (t = -2.21, d.f = 24; P = 0.037) \) on MCV was observed on multiple regression. The partial regression of MCV \((Y)\) on log effluent concentration \((x_1)\) and log duration of exposure \((x_2)\) may be presented as
\[
\hat{Y} = 5.1209 - 0.0248 x_1 - 0.0969 x_2
\]

7.3.3.5 Mean Corpuscular Haemoglobin (MCH)

MCH showed an exposure duration dependent decrease from 37.3 to 34.95, 31.41 to 30.9 and 32.65 to 33.05 pg in \( C. striatus \) exposed to 30.5% effluent after 20, 40 and 60 days.
The same decreased to 36.7, 29.85 and 33.4 pg in the fish exposed to 61% effluent after 20, 40 and 60 days (Table 7.1). Multiple regression analysis of MCH as a function of two critical factors revealed, the significant negative relationship that prevailed between MCH and days of exposure ($t = -2.39; \text{d.f} = 24; P = 0.025$). Among the two variables, effluent concentration failed to exert significant negative influence ($t = -0.48; \text{d.f} = 24; P = 0.637$). Further the interaction of exposure period and concentration of effluent had a significant positive impact ($P = 0.0526$). The partial regression of log MCH ($Y$) on effluent concentration ($x_1$) and duration of exposure ($x_2$) may be fitted in equation

$$\hat{Y} = 3.92 - 0.0051 x_1 - 0.1036 x_2$$

7.3.3.6 Mean Corpuscular Haemoglobin concentration (MCHC)

Except for the test individuals exposed for 40 and 60 days in 30.5% effluent, others showed an increase in MCHC level. The increased MCHC was dose dependent. (Table 7.1). In control the MCHC was 29.15, 28.6 and 30.56 g / 100 ml after 20, 40 and 60 days of exposure and it increased to 29.95 and 32.4; 29.55 and 32.36 g / 100 ml in 30.5% and 61% effluent concentration after 20 and 60 days of exposure respectively. Multiple regression analysis of MCHC showed significant positive influence of effluent concentrations ($t = 1.06; \text{d.f} = 24; P = 0.298$) and days of exposure failed to exert any significant influence on MCHC ($t = -0.01; \text{d.f} = 24; P = 0.99$). Further the interaction of effluent concentration and exposure period had a positive impact of greater significance ($P = 0.643$). The regression equation of log MCHC on log effluent concentration ($x_1$) and log duration of exposure ($x_2$) may be presented as

$$\hat{Y} = 3.4102 + 0.00865 x_1 - 0.000361 x_2$$

7.3.4 Cytomorphology and Cytopathology of Blood cells

But for some minor difference in cell/nuclear dimension, the different cellular elements in the peripheral blood of Channa striatus was very similar in cytomorphological and tinctorial properties.

Cell types in peripheral blood

The general cytomorphology of the cell types in the peripheral blood was presented in Table 7.2. The principal cell types observed in the peripheral blood were erythrocytes,
Table 7.2 Cytometry of cells in peripheral blood of control *C. striatus* (values are in μm). Pappenheim stained dry smear.

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Days</th>
<th>Location</th>
<th>Cytometric Parameter</th>
<th>Erythrocyte</th>
<th>Thrombocyte</th>
<th>Leucoyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lymphocytes</td>
<td>monocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entire Cell</td>
<td>Range Size</td>
<td>9.7 - 13.2</td>
<td>7.5 - 11.3</td>
<td>14.3 - 17.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.47±0.92</td>
<td>9.67±0.75</td>
<td>9.76±0.85</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>Nucleus</td>
<td>Range Size</td>
<td>3.26-5.41</td>
<td>4.6±7.2</td>
<td>7.9-9.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.47±0.38</td>
<td>5.56±0.34</td>
<td>5.8±0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entire Cell</td>
<td>Range Size</td>
<td>9.4-13.7</td>
<td>7.2-11.4</td>
<td>13.6-17.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.67±0.89</td>
<td>9.77±0.76</td>
<td>9.69±0.81</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Nucleus</td>
<td>Range Size</td>
<td>3.15-5.82</td>
<td>4.4-7.51</td>
<td>7.6-9.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.5±0.32</td>
<td>5.77±0.24</td>
<td>5.96±0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entire Cell</td>
<td>Range Size</td>
<td>9.1-14.2</td>
<td>7.4-11.6</td>
<td>14.5-17.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.61±0.86</td>
<td>9.8±0.69</td>
<td>9.9±0.82</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>Nucleus</td>
<td>Range Size</td>
<td>9.2-13.9</td>
<td>7.6-12.1</td>
<td>143-17.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.69±0.23</td>
<td>5.64±0.31</td>
<td>6.15±0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entire Cell</td>
<td>Range Size</td>
<td>9.2-13.9</td>
<td>7.6-12.1</td>
<td>14.3-17.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.06±0.55</td>
<td>10.11±0.43</td>
<td>10.07±0.73</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>Nucleus</td>
<td>Range Size</td>
<td>3.21-6.42</td>
<td>4.3-7.8</td>
<td>7.6-9.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.92±0.32</td>
<td>5.89±0.21</td>
<td>6.53±0.21</td>
</tr>
</tbody>
</table>

Each value is an average of five observations and ± indicates standard deviation.
Fig. 7.1  Normal blood smear of *Channa striatus* to show erythrocytes, thrombocytes, monocytes and lymphocytes. ca x 400.

Fig. 7.2  Normal blood smear of fish to show erythrocytes, monocytes and monoblast. ca x 1000.

Fig. 7.3  Normal blood smear of fish to show mature erythrocytes and lymphocytes. ca x 1000.

Fig. 7.4  Normal blood smear of fish to show spherical thrombocytes and macrophage. ca x 1000.

Fig. 7.5  Normal blood smear of fish to show elongated thrombocytes, neutrophil, lymphocytes and erythrocyte. ca x 1000.

Fig. 7.6  Blood smear of *C. striatus* exposed to 30.5% concentration for 60 days to show erythrocytes and macrophages. ca x 1000.

Fig. 7.7  Blood smear of fish exposed to 30.5% concentration for 60 days to show spiked thrombocytes and erythrocytes. ca x 1000.

Fig. 7.8  Blood smear of fish exposed to 30.5% concentration for 60 days to show monocyte and neutrophil. ca x 1000.
thrombocytes, monocytes, neutrophils, eosinophils and basophils. Immature stages of erythrocytes namely proerythroblasts, acidophilic erythroblasts and acidophilic proerythroblasts were occasionally encountered. In the peripheral blood of *C. striatus* exposed to 30.5% and 61% effluent, haemoblasts, the stem cells, were present though in very few numbers.

**Erythrocytes**

In the control setup, most dominant blood cell type in the peripheral blood was subspherical to oval with smooth cell margin. The cytoplasm was devoid of cytoplasmic condensations and vacuoles and stains homogeneously pale-pink. The shape of the cell was subspherical and some time elliptical. The average size of the entire cell of erythrocytes was 11.47 µm at the beginning of experiment and 12.06 µm after 60 days. The size of the erythrocyte nucleus was 4.47 µm at the beginning and 4.92 µm after 60 days (Fig. 7.1 - 7.8). The fish exposed in 30.5% effluent was found to harbour no abnormality in the shape of erythrocyte. The size of erythrocyte was 11.69 µm and 12.07 µm at the 20 days of exposure end of experiment (60 days). In the nucleus also more or less the size was same (4.67 µm and 5.12 µm). The highest concentration of 61% effluent caused visible changes in cell types of exposed fish. The erythrocytes were swollen. The nucleus was enlarged and was circular and sometimes a pseudo structure (Fig. 7.9, 7.10 and 7.11). Further changes lead to the formation of large, vacuolated very faintly stained nucleus (Fig. 7.14 and 7.15). Some of the erythrocytes had undergone morphological changes wherein only nuclei were visible. In the treated fish, the hypertrophied erythrocytes had weak cell membranes which were not able to withstand the force of surface tension and the erythrocytes broke releasing the nuclei. The nuclei in the pathological specimens did not have the same basophilia as the nuclei of healthy erythrocytes; this should be due to increasing effluent concentration. The entire cell size of the erythrocyte was also reduced to 11.11 and 12.15 µm after 20 and 60 days of exposure. The nucleus size of erythrocyte also got reduced to 4.36 µm 5.32 µm after 20 and 60 days of exposure respectively (Table 7.2).

Multiple regression analysis of the size of erythrocytes in the entire cell showed significant positive influence of days of exposure (t = 1.29; d.f = 27; P = 0.208) whereas concentrations failed to exert any significant influence on erythrocyte cell size. Further the
Table 7.3  Cytometry of cells in peripheral blood of *C. striatus* exposed to 31.5 and 61% of treated sugarmill effluent (values are in μm).  Pappenheim stained dry smear.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Effluent concentration (%)</th>
<th>Days</th>
<th>Location</th>
<th>Cytometric Parameter</th>
<th>Erythrocyte</th>
<th>Thrombocyte</th>
<th>Leucocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lymphocytes</td>
</tr>
<tr>
<td>1</td>
<td>30.5</td>
<td>20</td>
<td>Entire Cell</td>
<td>Range</td>
<td>9.1-14.0</td>
<td>7.2-11.9</td>
<td>7.2-11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nucleus</td>
<td>Size</td>
<td>11.69±0.94</td>
<td>9.51±0.18</td>
<td>9.73±0.26</td>
</tr>
<tr>
<td>2</td>
<td>30.5</td>
<td>40</td>
<td>Entire Cell</td>
<td>Range</td>
<td>9.4-14.9</td>
<td>7.0-11.3</td>
<td>7.3-11.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nucleus</td>
<td>Size</td>
<td>12.21±0.41</td>
<td>9.35±0.71</td>
<td>10.10±0.74</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>60</td>
<td>Entire Cell</td>
<td>Range</td>
<td>9.2-14.7</td>
<td>7.1-11.6</td>
<td>7.3-12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nucleus</td>
<td>Size</td>
<td>12.07±0.60</td>
<td>9.19±0.19</td>
<td>9.92±0.35</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>20</td>
<td>Entire Cell</td>
<td>Range</td>
<td>8.8-14.3</td>
<td>7.0-11.8</td>
<td>6.9-11.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nucleus</td>
<td>Size</td>
<td>11.11±0.85</td>
<td>7.8±2.26</td>
<td>9.35±1.5</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>40</td>
<td>Entire Cell</td>
<td>Range</td>
<td>8.9-14.6</td>
<td>6.7-11.4</td>
<td>7.0-11.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nucleus</td>
<td>Size</td>
<td>11.38±0.56</td>
<td>8.8±0.55</td>
<td>9.59±0.63</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>60</td>
<td>Entire Cell</td>
<td>Range</td>
<td>8.7-15.2</td>
<td>6.8-11.7</td>
<td>6.8-11.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nucleus</td>
<td>Size</td>
<td>12.15±0.43</td>
<td>8.68±0.42</td>
<td>9.25±0.42</td>
</tr>
</tbody>
</table>

Each value is an average of five observations and ± indicates standard deviation.
interaction of exposure period and concentration of effluent had positive impact of greater significance ($P = 0.4814$). The regression equation of log erythrocyte entire cell size ($Y$) on log exposure days ($x_1$) and log effluent concentration ($x_2$) may be presented as

$$\hat{Y} = 2.5064 + 0.01263 x_1 - 0.00203 x_2$$

Multiple regression analysis of the size of erythrocyte nucleus showed significant positive influence on days of exposure ($t = 2.47$; d.f $= 27$; $P = 0.0199$) and concentrations ($t = 0.97$; d.f $= 27$; $P = 0.3417$). The partial regression equation may be presented as

$$\hat{Y} = 1.6641 + 0.0251 x_1 + 0.00601 x_2$$

**Thrombocyte**

These cells were highly variable in their shape and size between control and fish exposed to $2/3$ (61%) concentration. Their shape ranged from round to subspherical, oval to elongate or irregular. The oblong or elongated from (Fig. 7.2, 7.4 and 7.5) were the most numerous type and they were the largest among the thrombocytes in circulation in control and fish exposed to 30.5% effluent. They usually occurred solitarily, but occasionally tended to aggregate in small groups. Shape of the nucleus conformed with that of the cell nucleus and was centrally placed. The cytoplasm lacking granules and vacuoles formed an even entire rim around the nucleus. The nucleus stained uniformly deep-purple; chromatin was heavily condensed into a dense homogenous mass. The ovoid and spiked forms were very few in number and distributed solitarily. The long nucleus forms were very rare and usually occur in clusters. The size of thrombocytes in control and exposed was more or less same. The size of entire cell was 9.67 µm and 10.11 µm in control at initial period and after 60 days; 9.51 and 9.19 µm in fish exposed to 30.5% effluent after 20 and 60 days and the size of nucleus was 5.77 µm and 5.89 µm in control and 5.28 µm and 5.61 µm in fish exposed to 30.5% effluent after 20 and 60 days.

The thrombocytes shape changed to irregular and cloudy in the fish exposed to 61% effluent concentration (Fig. 7.12, 7.14) The spherical or ovoid shape was changed to triangular with high density of cytoplasm (Fig. 7.16). Thrombocytes showed size reduction when compared to the control. The entire cell size reduced to 7.8 µm and 6.8 µm after 20 and
60 days of exposure time. The nucleus size was also reduced to 5.26 μm and 4.94 μm after 20 and 60 days of exposure. The reducing size of cells were dose and duration dependent (Table 7.2).

Among the two criterion factors analysed, exposure duration was found to exert positive significant impact \( t = 0.98; \) \( d.f = 27; \) \( P = 0.3365 \) and concentration was found to exert significant negative influence \( t = -2.86; \) \( d.f = 27; \) \( P = 0.008 \) on thrombocyte cell size. The regression equation of log thrombocyte size \( (Y) \) on log days \( (x_1) \) and log concentration \( (x_2) \) may be presented as

\[
\hat{Y} = 2.3343 + 0.01999 x_1 - 0.03577 x_2
\]

Multiple regression analysis of thrombocyte nucleus size as a function of two factors revealed that a significant positive influence prevailed between thrombocyte nucleus size \( (Y) \). Days of exposure \( (x_1) \) \( t = 0.58; \) \( d.f = 27; \) \( P = 0.568 \) and concentration \( (x_2) \) exerted significant negative influence \( t = -2.90; \) \( d.f = 27; \) \( P = 0.0074 \). The partial regression equation may be presented as

\[
\hat{Y} = 1.8919 + 0.0059 x_1 - 0.01828 x_2
\]

**Leucocytes**

The leucocyte population in the peripheral blood was composed of five major cell types; lymphocytes, monocytes, neutrophils, eosinophils and basophils.

**Lymphocyte**

Lymphocytes were the most common leucocytes constituting about 74% of the total leucocyte population. Their morphology was highly variable. Varying in shape from roughly round, subspherical or oval, lymphocytes were provided with cytoplasmic extensions in both control and fish exposed to 30.5% effluent. Fish exposed to 61% effluent did not show any considerable change in the shape and size of the lymphocytes. The lymphocyte size was 9.69 μm and 10.07 μm in the control, 9.73 μm and 9.92 μm in the fish exposed to 30.5% effluent; 9.35 μm and 9.25 μm in the fish exposed to 61% effluent after 20 and 60 days. The size of lymphocyte nucleus was 5.8 μm and 6.53 μm; 6.16 and 6.64 μm; 5.69 μm and 6.07 μm in the control and the fish exposed to 30.5% and 61% effluent concentration after 20 and 40 days.
Fig. 7.9  Blood smear of *C. striatus* exposed to 61% concentration for 60 days to show irregular erythrocytes, basophil granulocyte. ca x 400.

Fig. 7.10  Blood smear of fish exposed to 61% concentration for 60 days to show irregular erythrocytes, neutrophils and macrophages. ca x 1000.

Fig. 7.11  Blood smear of fish exposed to 61% concentration for 60 days to show clumped erythrocytes. ca x 1000.

Fig. 7.12  Blood smear of fish exposed to 61% concentration for 40 days to show spherical and elongated thrombocytes. ca x 1000.

Fig. 7.13  Blood smear of fish exposed to 61% concentration for 40 days to show irregular thrombocytes, proerythrocyte monocyte and erythrocyte. ca x 1000.

Fig. 7.14  Blood smear of fish exposed to 61% concentration for 60 days to show clumped erythrocytes and proerythrocytes. ca x 1000.

Fig. 7.15  Blood smear of fish exposed to 61% concentration for 60 days to show abnormal nucleus in erythrocytes and irregular cytoplasmic region. ca x 1000.

Fig. 7.16  Blood smear of fish exposed to 61% concentration for 60 days to show abnormal triangular erythrocytes with reduced nucleus. ca x 1000.
The lymphocytes of fish exposed to 61% effluent were more or less circular blood cells with nuclei occupying almost completely the entire space. These are generally smaller than the RBC and their nuclei were intensely basiphil. There were a few hypertrophied small lymphocytes in the micrograph (7.11 and 7.14) and in some places, two hypertrophied small lymphocytes with the vacuoles have been seen. In the same fish the large lymphocytes had became much larger due to inhibition of the serum caused by loss of selective permeability of the plasma membrane.

Significant negative impact of effluent concentrations \( (x_2) \) \( (t = 0.85; d.f = 27; P = 0.4) \) on lymphocyte entire all size \( (Y) \) was observed. The days of exposure \( (x_1) \) failed to exert any significant influence on lymphocyte cell size \( (t = 0.55; d.f = 27; P = 0.589) \). The regression equation may be presented as

\[
\hat{Y} = 2.3648 + 0.0049 x_1 - 0.00467 x_2
\]

Among the two variables tested in multiple regression analysis of lymphocyte nucleus size \( (Y) \), significant positive influence was exerted by days of exposure \( (x_1) \) \( (t = 2.48; d.f = 27; P = 0.0198) \) whereas concentration \( (x_2) \) failed to exert any influence on the thrombocyte nucleus size. The regression equation may be presented as

\[
\hat{Y} = 1.8973 + 0.0248 x_1 - 0.0009 x_2
\]

Monocyte

Monocytes were much less frequent than neutrophils in the peripheral blood. They were larger than the neutrophils and were usually subspherical cells in \( C. striatus \) of control and exposed fish. They contain a few reddish - pink granules and a few small vacuoles. Cytoplasmic condensations occasionally imparted a rough or granular appearance to the cytoplasm (Fig. 7.7, 7.2 and 7.8). Cytoplasmic condensations were sparse in the fish exposed to 61% effluent and they stained purple. In the fish exposed to 61% effluent showed that the monocyte has a slightly irregular outline (Fig. 7.13). Monocytes of the three groups of fish slightly differed in size. The largest monocytes were observed in those exposed to 30.5% effluent (mean size 16.25 \( \mu \)m and 15.44 \( \mu \)m), followed by control (15.55 \( \mu \)m and 15.58 \( \mu \)m) and 61% effluent (15.9 \( \mu \)m and 13.79 \( \mu \)m) exposed fish after 20 and 60 days of exposure (Table 7.2 and 7.3).
Among the two variables analysed in multiple regression, monocyte entire cell \((Y)\), exerted significant negative influence by days of exposure \((x_1)\) \((t = -1.38; \text{d.f} = 27; P = 0.1786)\) whereas the concentration \((x_2)\) failed to influence the monocyte entire cell size. The regression equation may be presented as

\[^{\hat{Y}} = 2.8450 - 0.01228 x_1 - 0.00293 x_2\]

Significant negative impact of concentration \((x_2)\) \((t = -0.93; \text{d.f} = 27; P = 0.362)\) was confirmed on monocyte nucleus size \((Y)\) and days of exposure \((x_1)\) failed to exert only significant impact on monocyte nucleus size. The partial regression equation may be presented as

\[^{\hat{Y}} = 2.2959 - 0.00328 x_1 - 0.00536 x_2\]

**Neutrophil**

Mature neutrophil was large and subspherical with smooth cell margin. The diffuse, fine granulation imparted a smooth ground-glass-appearance to the cytoplasm (Fig. 7.5 and 7.8). Cytoplasm occasionally harbours one or two small vacuoles in the fish exposed to high concentration. Fig. 7.10 shows a group of neutrophils in the effluent exposed fish (61%) and a neutrophils in the group had undergone swelling of the nucleus and there was cytoplasmic basiphilia probably due to diffusion of basiphil material from the nucleus. The size of the neutrophil (entire cell) of control, fish exposed in 30.5% and 61% effluent were 12.22 \(\mu m\) and 11.68 \(\mu m\); 11.45 \(\mu m\) and 11.53 \(\mu m\); 11.17 \(\mu m\) and 10.89 \(\mu m\) after 20 and 60 days of exposure. The nucleus size was 7.25 \(\mu m\) and 6.9 \(\mu m\); 6.83 \(\mu m\) and 7.47 \(\mu m\); 6.52 \(\mu m\) and 6.38 \(\mu m\) in the control and those exposed to 30.5% and 61% effluent after 20 and 60 days of exposure (Table 7.2 and 7.3).

Significant negative impact of days of exposure \((x_1)\) \((t = -1.00; \text{d.f} = 27; P = 0.3279)\) and concentration of effluent \((x_2)\) \((t = -2.71; \text{d.f} = 27; P = 0.0116)\) on neutrophil cell size \((Y)\) was observed on multiple regression analysis. The partial regression equation may be presented as

\[^{\hat{Y}} = 2.5843 - 0.00623 x_1 - 0.01125 x_2\]

Significant negative influence was noticed for effluent concentration \((x_2)\) \((t = -1.24; \text{d.f} = 27; P = 0.2260)\). But days of exposure \((x_1)\) failed to influence the neutrophil nucleus size \((Y)\) on multiple regression analysis. The regression equation may be presented as

\[^{\hat{Y}} = 2.0891 - 0.000022 x_1 - 0.00717 x_2\]
Eosinophil and Basophil

These granulocytes were the least frequent cells observed in the peripheral blood. They constituted only 3 - 10% of the total leucocytes population (Table 7.1) They were small in size and also the most irregular shaped cells provided with blunt cytoplasmic extensions. Cytoplasm was finely granulated and highly vacuolated. The nucleus was comparatively small, oval or irregular and either centrally placed or eccentric in position. There was no pathological changes observed between control and effluent (30.5%) exposed fish. The basophil granulocyte had undergone extreme hypertrophy due to exposure to effluent and the concentration had affected the permeability of the plasma membrane. In some cells the nucleus was disturbed and its contents were released into the cell.

The size of the eosinophilic granule (entire cell) was 8.04 μm and 7.39 μm (control); 7.53 μm and 7.7 μm (30.5% effluent); and 7.15 μm and 6.76 μm (61% effluent) after 20 and 60 days respectively. The nucleus size was 5.11 μm and 4.63 μm in the control and 5.03 μm and 4.72 μm in the fish exposed to 30.5% effluent and 4.79 μm and 4.23 μm in 61% effluent exposed fish (Table 7.2 and 7.3). With regard to basophil, the size of cell was 11.65 and 11.25 μm in control, 11.2 and 11.11 μm in the fish exposed in 30.5% effluent and 10.88 and 10.35 μm in the fish exposed to 61% effluent respectively after 20 and 60 days. The biggest size was observed in the control followed by 30.5% and 61% effluent exposed fish. The nucleus size was 5.17 and 4.93 μm in control; 5.16 and 5.95 μm in the fish exposed to 30.5% effluent; and 5.05 and 4.84 μm in the fish exposed to 61% effluent respectively after 20 and 60 days (Table 7.2 and 7.3). The size of nucleus increased in the fish exposed to 30.5% effluent.

Multiple regression analysis of cell size of eosinophil (Y) showed significant negative influence of days of exposure (x₁) (t = -1.07; d.f = 27; P = 0.2926) and concentration of effluent (x₂) (t = -2.57; d.f. = 27; P = 0.0161). The regression equation may be presented as

\[ \hat{Y} = 2.1941 - 0.00907x_1 - 0.01328x_2 \]

Among the two variables treated in multiple regression analysis of eosinophil nucleus size (Y), significant negative influence was exerted by days of exposure (x₁) (t = -1.34; d.f = 27;
\[ P = 0.1918 \) and effluent concentrations \((x_2)\) \(( t = -1.00; \text{d.f}=27; P = 0.1918)\). The partial regression equation is
\[
\hat{Y} = 1.8087 - 0.01358x_1 - 0.00623x_2
\]

Multiple regression analysis of basophil entire cell size \((Y)\) as a function of two factors revealed the significant negative relationship prevailing between basophil cell size and days of exposure \((x_1)\) \(( t = - 0.94; \text{df}=27; P = 0.3549)\) and concentration \((x_2)\) \(( t = -2.19; \text{d.f}=27; P = 0.0370)\). The partial regression may be fitted in the equation as
\[
\hat{Y} = 2.5477 - 0.007x_1 - 0.0099x_2
\]

Significant positive impact of effluent concentration \((x_2)\) \(( t= 0.87; \text{d.f}=27; P = 0.3891)\) on basophil nucleus size was observed on multiple regression. The exposure days \((x_1)\) failed to exert any significant impact on basophil nucleus size. The regression equation may be presented as
\[
\hat{Y} = 1.8182 - 0.00318x_1 + 0.00727x_2.
\]

7.4 Discussion

The increase in effluent concentrations and the duration of exposure resulted in a number of negative effects such as increase in RBCs, WBCs, haemoglobin, haematocrit and a decrease in erythrocyte sedimentation rate, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. The increase in RBCs' total counts during the experiment probably reflects a stress mediated haemo concentrations due to diuresis, similar to that reported previously by Hurn (1969); Swift and Lloyd (1974) and McLeay (1975). The marked increase in WBC - T counts during exposure of fish to stressful conditions for 20, 40 and 60 days is consistent with reported increases in both leucocyte and thrombocyte counts of C. striatus exposed to stressful situation. Circulating white blood cells in the fingerlings of C. striatus are predominantly lymphocytes, which in vertebrates are susceptible to lysis by cortico-steroids (Bennett et.al., 1972). A comparative account of haematological results obtained by several authors are presented in Table 7.4.
Mean values of RBC and WBC counts in effluent exposed *C. striatus* increased at 20, 40 and 60 days of exposure. After the exposure, number of circulating lymphocytes decreased, whereas number of other leucocyte cell types did not show any decrease. In the present investigation an increase in haemoglobin and haematocrit value was observed. Whereas Howard and Walden (1967) observed a reduction in haematocrit values of fish exposed to pulp mill effluents.

Table 7.4 Comparision of haematological variables with other effluents

<table>
<thead>
<tr>
<th>Effluent Type</th>
<th>RBC 10 /mm3</th>
<th>WBC 10 /mm3</th>
<th>Hb (g%)</th>
<th>Ht (%)</th>
<th>MCV (µm)</th>
<th>MCH (pg)</th>
<th>MCH %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kraft pulp</td>
<td>C 0.69</td>
<td>7</td>
<td>-</td>
<td>21.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>McLeay 1973</td>
</tr>
<tr>
<td></td>
<td>E 0.92</td>
<td>6.2</td>
<td>-</td>
<td>24.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Titanium Dioxide</td>
<td>C 3.91</td>
<td>14</td>
<td>15.5</td>
<td>45.7</td>
<td>117</td>
<td>39.6</td>
<td>33.9</td>
<td>Nair et al 1984</td>
</tr>
<tr>
<td></td>
<td>E 4.37</td>
<td>32</td>
<td>18.5</td>
<td>48.1</td>
<td>110</td>
<td>42.1</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>Textile Mill</td>
<td>C 3.9</td>
<td>25.6</td>
<td>9.2</td>
<td>30</td>
<td>75</td>
<td>24.1</td>
<td>32.1</td>
<td>Murugesan and Haniffa 1985</td>
</tr>
<tr>
<td></td>
<td>E 2.5</td>
<td>26.9</td>
<td>8</td>
<td>26</td>
<td>101</td>
<td>32.1</td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>Distillery</td>
<td>C 0.53</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Haniffa and Porchelvi 1985</td>
</tr>
<tr>
<td></td>
<td>E 2.7</td>
<td>52</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E 2.39</td>
<td>1.25</td>
<td>12.39</td>
<td>-</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sugar Mill</td>
<td>C 4.19</td>
<td>4.44</td>
<td>13.68</td>
<td>47</td>
<td>112.3</td>
<td>32.6</td>
<td>27.9</td>
<td>Present Investigation</td>
</tr>
<tr>
<td></td>
<td>E 4.85</td>
<td>4.89</td>
<td>16.2</td>
<td>50</td>
<td>103</td>
<td>30.6</td>
<td>32.4</td>
<td></td>
</tr>
</tbody>
</table>

McLeay (1973) stated that *O. kisutch* erythropoisis was stimulated when exposed to pulp mill effluent. This was due to the elevated demands for oxygen and carbondioxide transport in the polluted medium. He has also reported that erythropoiesis could also be stimulated by an increased fragility or rate of destruction of circulating erythrocytes. In the present investigation too, this was confirmed. In the present investigation, the elevation in the number of RBCs and WBCs was accompanied by a reduction of erythrocyte sedimentation rate, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration.
In the present study exposure to effluent caused a decrease in lymphocytes and basophil granulocyte and an increase in monocyte, eosinophil, and neutrophil granulocytes. Mc Leay (1973)suggested that the decline in the number of lymphocytes in the effluent exposed fish may be due to stress mediated increase in corticosteroids secretion by the internal tissues. According to Ellis (1976) and Johnson-Sjobeck and Larson (1978) stimulated lymphopoiesis and release of lymphocytes from lymphomyeloid tissue resulted in an increase in WBC count. Besides increase in the number of blood cells, there was an elevation of haemoglobin in *P. flesus* exposed to titanium dioxide effluent also showed elevated values of haematocrit and haemoglobin. Such enhancements in Ht and Hb was also observed in *H. fossilis* exposed to textile mill effluent (Haniffa et.al., 1986). Nair et.al., (1984) suggested that the haematological disturbances are haemopoietic or erythrocytic mobilization response to hypoxaemia induced by acid stress. In the present investigation RBC cellular swellings were observed. Holeton and Randall (1967) also noted that when fishes were exposed to hypoxis environment, there was an increase in Ht level, mainly because of the cellular swelling and not due to increase in RBC number. Singh and Singh (1982), reported that the increase in Ht value in the fish exposed to toxic medium might have resulted due to a higher PCO₂ in the blood and subsequent enlargement of erythrocytes. Cytopathological studies in the effluent exposed (61%) *C. striatus* showed remarkable enlargement of the erythrocytes. The changes of red blood cell indices are in accordance with the changes observed for RBCs, haemoglobin and haematocrit.

The decrease in basophil, observed in the blood cells could be as; the granular endoplasmic reticulum with its ribosomes is responsible for the basophil of the cytoplasm; in the effluent treated cells, the endoplasmic reticulum is very much affected and the ribosomes may not be synthesized; this results in the decline in basophils. The vacuolation of erythrocytes and leucocytes observed in the treated fish (61%) may be accounted as follows. As stated by Cameron (1964), early damage to cells and consequent autolysis are recognizable by the formation of vacuoles. Necrosis of the cell results in the loss of permeability characteristics of the membrane; the cell continuous to swell through "water logging". Further serious disturbances in water and electrolyte movements between cell and environment are said to take place. Srivastawa *et.al.,* (1985) observed nuclear and cellular hypertrophy and bursting of erythrocytes of *Heteropneustes fossilis* exposed to textile mill effluent.

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As may be inferred from the literature, mature erythrocytes and thrombocytes form ubiquitous components of the peripheral blood of telosts. It is also noticeable that not all leucocyte types except lymphocytes, are universally represented in telosts. Even if present in a species, certain leucocyte types need not necessarily be present in circulation. Thus macrophages in *Pleuronectes platessa* are restricted to the haemopoietic organs (Ellis; 1976). Size of mature erythrocytes of fishes is correlated with several factors such as body length, body weight, sex, habit, physiological adaptations, habitat, seasons etc. (Yadav and Benerjee, 1985). Banerjee (1986), noted differences in the size of erythrocytes of the same species of fish from two different localities as the result of some physiological adjustments by the fish to meet the ambient ecological conditions. In the present investigation the erythrocyte size has reduced slightly in the fish exposed to high concentration (61%). Physiologically, small erythrocytes are more efficient than large ones in gaseous exchange (Hartman and Lassler, 1964). Tinctorial affinity of the cytoplasm of mature erythrocytes of teleosts is acidophilic; it is because of the high content of haemoglobin and an admixture of a large quantity of carbonic anhydrase, in addition to other acidophilic compounds (Rybak, 1968).

The morphotypes of thrombocytes are generally classifiable into specific categories such as spiked, spindle shaped, oval to elongated, and the so called 'lone nucleus' forms. In the present investigation the size of the thrombocyte was reduced remarkably in the fish exposed to high concentration (61%). The observed shape was mostly oval to elongated in normal cells and irregular in the fish exposed to high concentration.

But for the variation in the size of the cell, the morphology of the lymphocyte is strikingly similar throughout the vertebrate kingdom (Ellis, 1977). Weinreb (1963), showed that variation in the amount of cytoplasm and the tendency to pseudopod formation in lymphocytes resulted in a range of size. The spherical shaped neutrophils were changed into sub-spherical shape in the fish exposed to high concentration. Tinctorial affinity of neutrophil cytoplasm exhibited only minor inter- and intraspecific differences. In the present study it ranged from colourless to pale-blue or some time pale-pink, in the fish exposed to high concentration. The
eosinophil and basophil populations were very low. The eosinophil was the smallest cell among the leucocytes. The size of the nucleus both in RBCs and WBCs, varied from control to fish exposed to effluent. Since sugar mill effluent contained high organic matter like suspended solids etc. (in untreated or highly concentrated effluents only); it could be responsible for the cytomorphological and cytopathological changes in *C. striatus*. 