CHAPTER-5
CHAPTER-5: HAEMATOLOGICAL RESPONSE OF
A. testudineus ON EXPOSURE TO
OPME

5.1 INTRODUCTION:

Haematological parameters are widely used for clinical laboratory diagnosis of pathology and diseases of animal and men. Recently piscine haematology has been increasingly given significance in assessing the physiological response of fishes to pollutants (Homechowdhury, 1988). It is a tool for knowing fish health in response to environmental pollution. Fish haematology is fragmentary and confusing as there is wide intra and inter specific variation in the blood profile of fishes. Therefore it is necessary to study the normal blood parameters of a fishes before processing to study the alteration due to toxic substance. Keeping this in view some important blood parameters of A. testudineus was undertaken. The changes in fish haematology are reflected in various physiological changes like oxygen consumption and metabolism which ultimately result in the death of the fish (Vijayaram et al., 1988). There are many works which establish that stress caused by various factors like nutritional deficiencies, parasites, pesticide, herbicides, industrial wastes and metal pollutant bring about alterations in the normal haematology of a fish. Iwama et al. (1986) and Pellitero et al. (1987) have reported various
changes in blood parameters of fishes due to bacterial or viral infection. Homechowdhury (1988) reported changes in blood profile of fish due to mahua oil toxicity. Effect of pesticides (Mahajan and Dhee, 1979; Srivastava and pandey, 1986; chakravarty and Banerjee 1989) and metals (Christensen et al. 1972; Lewisa 1978; Mirhra et al. 1979; Homechowdhury 1988; Sen 1991; Behera, 1997) on fish blood parameters have been studied in many fish species. The present work is aimed to study the effect of sublethal concentration of OPME on the haematological parameters of *Anabas testudineus*.

### 5.2. MATERIALS AND METHODS:

*A. testudineus* is a fast growing hardy fish which can survive under adverse condition. It can also be easily maintained in the laboratory. As such it is a very good test animal. Healthy species of *A. testudineus* (Weighing 20-22 g and 10-12 cm in length) were collected and aclimatised to the laboratory condition for a fortnight. They were fed with commercial fish food. As the LC$_{50}$ has been worked out to be 63.09% for *A. testudineus* to OPME, four sublethal concentration (5, 10, 20 & 40%) were proposed in forty litre capacity aquaria. One aquarium was filled with deep well water (0%) which is to serve as the control.

To all the five aquaria ten number of fishes were released for 30 days. The OPME effluent concentrations were renewed after every alternate day to maintain the desired concentration & to avoid excess deposition of excretory materials. No mortality occurred either in
experimental or in control groups. On the termination of the exposure period blood was drawn from the gill region with the help of heparinized needles & stocked in heparin coated glass vials. Haematological parameters like haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC) and packed cell volume (PCV) were determined. All the haematological parameters were done by the standard methods described by Dacie & Lewis (1975). The absolute value like mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC) and Mean cell volume (MCV) were done by using the following formulae.

\[
\text{MCH (Mean cell haemoglobin)} = \frac{\text{Hb in g.} / 1000 \text{ ml. of blood}}{\text{TEC in million} / \text{mm}^3} 
\]

It is expressed in picogram (pg)

\[
\text{MCHC (Mean cell haemoglobin concentration)} = \frac{\text{Hb in g/}100 \text{ ml. of blood}}{\text{PCV/}100 \text{ ml.}} \times 100
\]

It is expressed in percentage (%)

\[
\text{MCV (Mean of cell volume)} = \frac{\text{PCV/}1000 \text{ ml. of blood}}{\text{TEC in million} / \text{mm}^3}
\]

It is expressed in cubic micron (\(\mu^3\))

5.3 RESULTS:

The results obtained are summarized in Table 8-14. It was observed that in control group (0%) of fishes, Hb, TEC, TLC, PCV, MCH, MCHC & MCV were 12.59 ± 0.08 g/dl, 2.595 ± 0.078 million /
mm³, 15.71 + 0.81 thousand / mm³, 45.4 + 1.02 %, 48.52 + 1.34 picogram, 27.74 + 0.76 % and 176.74 + 7.3 micron³ respectively. After the fishes were exposed to different concentration (5, 10, 20, & 40%) of OPME for 30 days there was a significant (P< 0.001) alteration in all the above haematological parameters.

There was a gradual depletion in the haemoglobin level from control to higher concentration. Percentage of decrease was 7.3, 48.53, 62.58 & 65.76% in fishes to 5, 10, 20, & 40% of OPME respectively. The difference in haemoglobin concentration between (among) fishes of different concent was significant (F= 16296.8, P < 0.001)

TEC showed difference of 10.82% in 5%, 31.29% in 10%, 69.82% in 20% and 73.06% in 40% concentration OPME treated fish the difference in TEC number was also significant (F=2505.454, p < 0.001) and was positively correlated to haemoglobin concentration (r=+ 0.95)

The packed cell volume declines by 3.52% in 5%, 25.66% in 10%, 45.37% in 20% & 57.04% OPME exposed test fishes. Analysis of variance of above data shows significant difference (F= 1528.84, P< 0.001) between fishes exposed to different concentrations.

Mean cell haemoglobin concentration showed 3.96% decreased in 5% effluent treated fish followed by 30.78 % in 10%, 31.5% in 20% and 20.11% in 40 % concentration of OPME. The difference in MCHC value between fishes exposed to different concentration was significant
However the difference between the MCHC of fishes of 10% & 20% was insignificant (LSD = 0.7495).

There was a marked and significant elevation (p < 0.001) of TLC, MCH & MCV from their respective control values. TLC showed 4.3, 128.7, 180.45 & 237.42% increased in fishes exposed to 5, 10, 20, & 40% concentration of OPME. Fishers analysis of variance (ANOVA) justify the difference (F = 1682.08, P < 0.001) except between the control and 5% group fish (LSD = 1.158).

Mean corpuscular haemoglobin showed 3.91% increased in 5% OPME treated fish followed by 25.12% decreased in 10% concentration. However it further showed increase from control value (23.94% in 20% & 27.08% in 40%). The difference in above MCH value was significant (F = 409.81, P < 0.001).

There was 7.13, 7.31, 79.28 & 57.94% increase in mean cell volume (MCV) of fishes exposed to 5, 10, 20 & 40% concentration of OPME respectively. The difference in MCV value was significant (F = 316.76, P < 0.001). However the MCV of fishes of 5 & 10% do not differ significantly (LSD = 10.177).
Table 8. Changes in haemoglobin concentration (g%) of *A. testudineus* on exposure to paper mill effluent

<table>
<thead>
<tr>
<th></th>
<th>0%</th>
<th>5%</th>
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<th>20%</th>
<th>40%</th>
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<tr>
<td>Mean</td>
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<tr>
<td>S.D</td>
<td>0.08</td>
<td></td>
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<td></td>
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<tr>
<td>Change in %</td>
<td>-7.3</td>
<td>-48.53</td>
<td>-62.58</td>
<td>-65.76</td>
<td></td>
</tr>
</tbody>
</table>

- * Significant (P< 0.001) from control
- * - indicate decrease
Figure-2. CHANGES IN HAEMOGLOBIN CONCENTRATION (g %) OF *A. testudineus* ON EXPOSURE TO PAPER MILL EFFLUENT

![Graph showing changes in haemoglobin concentration (g %) of *A. testudineus* on exposure to paper mill effluent.](image-url)
Table 9. Changes in total erythrocyte count (x 10\(^6\) mm\(^{-3}\)) of *A. testudineus* on exposure to paper mill effluent.

<table>
<thead>
<tr>
<th>O%</th>
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<td>2.62</td>
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<td>0.7</td>
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<tr>
<td>2.63</td>
<td>2.36</td>
<td>1.85</td>
<td>0.76</td>
<td>0.69</td>
</tr>
<tr>
<td>2.65</td>
<td>2.34</td>
<td>1.7</td>
<td>0.78</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean</td>
<td>2.595</td>
<td>2.314(^a)</td>
<td>1.783(^a)</td>
<td>0.783(^a)</td>
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SD 0.078 0.046 0.069 0.022 0.013

Change in % -10.83 -31.29 -69.82 -73.06

\(^a\) significant at (p<0.001) from control

- indicates decrease
Figure-3. CHANGES IN TOTAL ERYTHROCYTE COUN (X 10^6 mm^3) OF A. testudineus ON EXPOSURE TO PAPER MILL EFFLUENT
Table-10. Alteration in total leucocyte count (x $10^3$ mm$^{-3}$) of *A.testudineus* exposed to paper mill effluent

<table>
<thead>
<tr>
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</thead>
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<td>Mean</td>
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<td>Change in %</td>
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<td>+128.7</td>
<td>+180.</td>
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* indicates increase

a significant at (p<0.001) from control
Figure 4. ALTERATION IN THE TOTAL LEUCOCYTE COUNT ($10^3$ mm.$^{-3}$) OF *A. testudineus* EXPOSED TO PAPER MILL EFFLUENT.

![Graph showing alteration in total leucocyte count of A. testudineus exposed to paper mill effluent.](image-url)
Table-11. Changes in packed cell volume (%) of *A.testudineus* on exposure to paper mill effluent

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<td>43.5</td>
<td>34.0</td>
<td>24.5</td>
<td>18.5</td>
</tr>
</tbody>
</table>

Mean 45.4 43.8$^a$ 33.75$^a$ 24.8$^a$ 19.5$^a$

SD 1.02 0.71 0.81 0.71 1.16

Change in % -3.52 -25.66 -45.37 -57.07

$^a$ significant at (p<0.001) from control

- indicates decrease
Figure 5  CHANGES IN PACKED CELL VOLUME(%) OF *A. testudineus* ON EXPOSURE TO PAPER MILL EFFLUENT

![Graph showing changes in packed cell volume of A. testudineus on exposure to paper mill effluent.](image-url)
Table-12. Mean cell haemoglobin (%) alteration in *A.testudineus* exposed to paper mill effluent.

<table>
<thead>
<tr>
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<td>Change in %</td>
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<td>-25.12</td>
<td>+23.94</td>
<td>+27.08</td>
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*a* significant at (*p*<0.001) from control

*+* indicates increase

*-* indicates decrease
Figure 6. MEAN CELL HAEMOGLOBIN (%) ALTERATION IN A. testudineus ON EXPOSURE TO PAPER MILL EFFLUENT
Table-13. Changes in mean cell haemoglobin concentration (pg) of *A.testudineus* on exposure to paper mill effluent.

<table>
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<td>0.5</td>
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<tr>
<td>Change in %</td>
<td>-3.96</td>
<td>-30.78</td>
<td>-31.5</td>
<td>-20.11</td>
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</table>

a significant at (p<0.001) from control
b do not differ significantly
- indicates decrease
Figure-7. CHANGES IN MEAN CELL HAEMOGLOBIN CONCENTRATION (pg) OF *A. testudineus* ON EXPOSURE TO PAPER MILL EFFLUENT

![Graph showing changes in mean cell haemoglobin concentration (pg) of A. testudineus on exposure to paper mill effluent.](image)
Table- 14. Changes in mean cell volume ($\mu^3$) of *A. testudineus* exposed to paper mill effluent.

<table>
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<td>Mean</td>
<td>176.74</td>
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<td>189.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>316.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>279.16&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>SD</td>
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<td>4.81</td>
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* a significant at (p<0.001) from control
* b do not differ significantly
* + indicates increase
Figure 8. MEAN CELL VOLUME ($\mu^3$) OF A. testudineus ON EXPOSURE TO PAPER MILL EFFLUENT
5.4 DISCUSSIONS:

The above finding clearly indicates that there was a positive correlation among the Hb, TEC & PCV decline, which refers to acute anaemia. Similar decrease in Hb %, TEC and PCV was also reported in many fishes under the stress of metals like mercury, cadmium, riger, zinc, copper, and lead (Panigrahi and Mishra, 1978; Srivastava and Mishra, 1987; Goel and Maya, 1986; Banerjee and Kumari, 1988; Sen et al, 1991; Behera, 1997). This may be attributed to the deficiency of iron and its decreased utilization in haemoglobin synthesis (Yadav et al., 1993). The decreased in above parameters may have been caused due to decreased erythropoetic activity or increased destruction of red cells (Sastri & Gupta, 1994). Decreased in iron uptake may be related to decreased iron uptaken by the intestinal villi & mucosa resulting in defective absorption. Chakravarti & Vanerjee (1989) described decrease in Hb and they attributed it to poor oxygen transport caused by the damage or due to increased accumulation of CO2.

Increased in MCV & MCH and decreased in Hb indicates that the anaemia is of macrocytic type. Increased in MCV values may be considered as an index of RBC destruction (Sailaja & Naidu, 1989). MCHC shows corelationship with TEC, PCV & Hb.

Increased in TLC indicates leucopoesis. This elevation may be related to an adaptive response to the new environment of fish against the
toxicant in the environment. Adak (1995) obtained similar results in zinc & arsenic treated *Channa punctatus*. Goel and Gupta (1988), Garg et al. (1989), Nanda and Behera (1996) and Nanda & Pradhan (1997) also obtained similar result in different fish species treated with various metals. This they attribute to dysfunctioning of haemopoetic tissue along with disleucopoiesis and liver dysfunction.

Thus in the present investigation it was noted that haemotological values altered. These may be related to necrotic damage in the gill epithelium and precipitation of mucous in the gill which would cause hypoxia. OPME contains various heavy metals as well as high alkaline organic load due to more chlorine and sulphate which might have brought about the above alterations. A comparative histochemical study of the erythropoetic tissues of fishes exposed to OPME in relation to concentration as well as duration of time may help to understand the real causes of above alterations.