Heavy metals are ubiquitous in the biosphere, where they occur as part of the natural background of Chemicals. Anthropogenic activities have also introduced substantial amounts of them into the environment by mobilization from their natural insoluble deposits or environmental sinks. Heavy metals are wide spread pollutants of great concern as they are non-degradable and thus persistent. Haematology indicates have different sensitivities of various environmental factors and chemicals. In fish, changes in these parameters and their peculiarities depend upon the concentrations of heavy metals and the duration of exposure. The alterations in the hematologic indices of freshwater fish exposed to Cr(VI) are well documented, and the metal is reported to induce a decrease in most blood parameters investigated. Hexavalent chromium compounds are carcinogenic and are used in chromium planting cement and paint production industries, presenting high potential for contamination of aquatic environment. Soluble chromium is extremely pernicious and shows the mutagenic effects, due to strong oxidizing nature. The weight reduction in the spleen of Tilapia (*Oreochromis niloticus*) due to decrease of lymphocyte and leukocyte counts. Haematological parameters examined PCV, haemoglobin, TEC, TLC and ESR which all are decreased due to proportional to the increase in the Portland cement powder in solution.
INTRODUCTION:
Tanning industry contributes significantly towards exports, employment generation and occupies an important role in the Indian economy while on the other hand, tannery wastes are ranked as the highest pollutants among all the industrial wastes. The effects of sub lethal concentrations of Portland cement powder in solution on some haematological parameters of the Nile tilapia (*Oreochromis niloticus*) mean weight 8.20±0.25g was investigated using static bioassay system for 70 days. The sub lethal concentrations used were 19.60, 9.80, 4.90, 2.45, 1.23 and 0.00 (control) mg/L. There were significant differences (p<0.05) in the water quality parameters monitored. However, temperature did not show any significant variation (p>0.05) in test tanks and the control.[1] Tilapia species is one of the mostly popular fresh water fish consumed in several countries. They are mainly lacustrine fish that are well adapted to enclosed water. Tilapia exhibits high tolerance to adverse environmental condition. Portland cement is composed of tricalcium aluminate, tricalcium aluminoferrite, Belite/dicalcium silicate, Alite/tricalcium silicate, sodium oxide, potassium oxide, and gypsum. The dry powder is obtained by grinding the clinker to which gypsum is added to control the settling processes.[7] Studies on the impact of cement dust on surrounding vegetation showed continuous decrease in the growth rate, diversity and productivity of the flora and fauna.[11] Haematological analysis provides a quick screening method for the assessment of the health status of the fish thus its variables are now in use when clinical diagnosis of fish physiology is applied to determine the effects of external stressors.[17]

MATERIAL AND METHODS: Both male and female fingerling of Nile Tilapia used had 8.20±0.25g in average weight, 6-9cm in length and were obtained from rock water fish farms. The fishes were kept in large plastic aquaria whose capacity is 30L and supplied with well–aerated dechlorinate municipal water and acclimatized for 10 days. In this period the fish were fed with pelleted reference diet at eight to sixteen hours. A preliminary acute toxicant concentration Portland cement powder solution is added. Fish were weighted and anesthetized with 1:15 benzocaine solution after being captured. Approximately 1ml of blood was extracted from the caudal vein, using a heparinised syringe.[2] Blood samples are taken from control and experimental fish at the end of the 70 days exposure period. The blood samples are used for the determination of haematological parameters PCV, Hgb, TEC, TLC, and ESR.[9] The blood glucose level was positively correlated with weight and length, whereas total protein was positively correlated with haemoglobin. The erythrocyte count was positively correlated with the haemoglobin and negatively correlated with MCV and MHC.

STATISTICAL EVALUATION: For the survival tests, Statistical Analysis of data was carried out with SPSS statistical package program. A value of p<0.05 was considered to be significant. For the accumulation tests, the experiments were repeated four times and only the arithmetic mean of the four experiments at each concentration was taken to express the result.[3]
BLOOD COLLECTION: Blood collected from the caudal peduncle with the use of 5ml syringe and needle that has been treated with anti–coagulant such as heparin to prevent clotting into small sampling bottles containing Ethylene diamine tetra–acetic acid (ETDA).[18]

PACKED CELL VOLUME (PCV): Blood were collected into micro haematocrit heparinised tube which was sealed with critical at one end. The sample tubes were then centrifuged for 5 minutes at 12000 rpm using Hawksley microhematocrit centrifuge. The haematocrit values were read on a microhematocrit reader. A mean of two readings was recorded as percentage for the fish haematocrit.[18]

HAEMOLOGICAL EXAMINATION: The measurement of haemoglobin was performed by using Hb Sahli (Sahli’s Haemoglobinometer). The blood sample was obtained by using Sahli’s pipette up to 20mm (the line of Sahli’s pipette) and added in 0.1HCl solution up-to number 2 (the yellow line boundary). Blood sample was inserted in Sahli’s tube and waited until turning blackish brown. Then the aqua distillate was added till it corresponded with the colour of the two tubes of Hb –Sahli. The haemoglobin value was read in unit g/dl.[19]
TOTAL ERYTHROCYTE COUNT (TEC) AND TOTAL LEUKOCYTE COUNTING (TLC) BY USING HAEMATOCYTOMETER: The data of erythrocyte and leukocyte cells of Nile Tilapia which was obtained by manual calculation by using hand Haematocytometer or Neubauer’s chamber. It is necessary to dilute the blood sample or blood specimen using one of the RBC diluting fluids (heyem’s or formalin citrate diluting fluid). Neubauer’s chamber is a specialized thick glass slide used to count the eukaryotic cell suspension. Haematocytometer has a size of 30x70x 4mm. We measured easily TLC and TEC with the help of Neubauer’s chamber.

TLC calculations: Count the cells in the Neubauer chamber. These are counted in the four large corners labelled as WBC and if the number is Y. One large area is 1×1mm, and the depth is 0.1 mm. Total area counted in 4 large squares=4×1×0.1=0.4μL (4/10). Y×10/4 is the WBC in the cell in 1μL. Now dilution is 1:20. Number of WBC in 1μL=Y×10×20/4=Y×50=Total WBC count. Total TLC=counted cells(Y)×50=TLC/cm.

TEC calculation: After counting the cells under the microscope, we know the No of RBC in 5 squares of the central square. Let’s consider it as ‘N’ no. of cells. Now, the volume of the fluid inside the camber is the product of Area and depth of the Hemocytometer/Neubauer’s chamber. The central area is the 1 sq. Mm which is divided into 25 parts so the area is 25 squares=1 sq.mm. Out of these 25 squares, the RBCs are counted in 5 squares. So the Area of the 5 small squares is=5/25 i.e 1/5. The depth of the Haemocytometer is 0.1 mm as described above in a short description of Haemocytometer. Now apply the following formula to get the total RBC count = N×Dilution / Area×Depth

N×200(or 100 as the dilution is made) / (1/5×0.1). Total RBC count = N×10000 / cube mm.

ESR calculation: An erythrocyte sedimentation rate (ESR) is a type of blood test that measures how quickly erythrocytes settle at the bottom of test tube that contains a blood sample. Normally red blood cells settle relatively slowly.

RESULTS AND DISCUSSION:

Table:1 Water quality parameters for sub lethal bioassay of Portland cement on fingerlings of the Oreochromis niloticus during the 70 days exposure period.[1]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Portland cement concentration (mg/L) [Values are shown in mean±SE]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>0.00</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21.26±0.13</td>
</tr>
<tr>
<td>pH</td>
<td>7.25±0.01</td>
</tr>
<tr>
<td>Free Carbon (IV)oxide(mg/L)</td>
<td>1.67±0.05</td>
</tr>
<tr>
<td>Dissolved oxygen(mg/L)</td>
<td>6.10±0.13</td>
</tr>
<tr>
<td>Total Alkalinity(mg/L)</td>
<td>7.67±0.39</td>
</tr>
</tbody>
</table>

The results of water quality parameter (Table:1) revealed the temperature showed no variation in all tanks recording 21.26°C including control tank. pH was in the range of 7.42-7.90; the highest pH value is found in highest toxicant concentration (19.60). Dissolved Oxygen (DO) content decreases as the Portland cement toxicant concentration increases in the range of 5.42 to 3.16mg/L while the control tank recorded DO value of 6.10 mg/L. Free carbon (IV) oxide and total alkalinity values varied significantly (p<0.05) with increase in toxicant concentration, such that the highest toxicant concentration (19.60mg/L) had the highest carbon(IV) oxide and total alkalinity values of 2.40 and 30.20mg/L, while the least free carbon(IV)oxide and total alkalinity values (1.67 and 7.67mg/L) were recorded in the control tank (0.00mg/L).[1]
The summary of the mean values of haematological parameters is as represented (Table: 2). Changes in haematological values occur in relation to the physiological stress, decrease and toxic environmental conditions.\[^9\] PCV is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen-carrying component of the blood.\[^6\] Haematological components have been developed for evaluation of fish health conditions. As a matter of fact, blood serves as the most convenient indicator of the general condition of the animal body. Subsequently, haematological studies are promising tools for investigating physiological changes caused by environmental pollutants.\[^5,10\] PCV, Hgb, TEC, TLC, and ESR values decrease with the increase of conc. PCPS. The significant decrease in the PVC in this study could be attributed to gill damage and/or impaired osmoregulation causing anaemia and haemodilution.\[^13\] Hgb is oxygen-carrying component in the blood of fish and concentration can be used as a good indicator of anaemia. Decreased haemoglobin following metal exposure usually result in haemodilution which has been regarded as a mechanism that reduce the concentration of the toxicant in the circulatory system. The decrease in the Hgb of Heteroclarias and Clarias gariepinus exposed to sub lethal concentrations of cadmium and cassava mill effluent respectively.\[^14\] TEC is indirectly proportional to the toxicant of PCPS. Causes of this reduction of circulating erythrocytes of stressed fish has been attributed to aggregation of RBCs in damaged the gills. The WBC of the blood respond to various stressed including infection of the chemical irritants. So, decrease of TLC may be as the result of bio concentration of the test toxicant in the kidney and liver.\[^16\] The decrease of ESR could be damaged gills and impaired osmoregulation during the sub-lethal exposure of the fish to PCPS.\[^4\]

**CONCLUSION:**

The measuring of haematological parameters, which are used in this study, has provided valuable information. The employment of haematological techniques has provided valuable knowledge for fishery biologists in the assessment of fish health and in monitoring stress responses. We assume that variation in values of blood indices may be a defensive mechanism against the toxicity of heavy metals through stimulation of erythrocytes. Some changes were noted in gill, liver, kidney, brain and muscle of Tilapia when exposed to sub lethal concentrations of PCPS. For these reasons we can observe Tilapia is affected by damage the kidney, degeneration of tubular epithelium and edema, necrosis etc. In addition, results provided evidence that haematological parameters can be sensitive indicator of aquatic pollution.\[^18\]

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