Assessment of Water Quality Using Haematological indices as Biomarkers

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ABSTRACT
The validity of various blood parameters as indicators of aquatic pollution in the Swiss albino mice, (Mus musculus) is discussed. The aim of this study was to assess the toxic stress of pollution of Chambal River at Nagda (M.P. India) in two different habitats in Swiss albino mice Mus musculus. Water from two different habitats of Chambal River (upstream and downstream) were collected during winter months (2012) and chemical parameters assessed. The COD, BOD, TSS, TS and TDS were much higher than the standard quality in station 2 (downstream) which may be due to sewage and discharge of industrial pollutants as this station receives greater agricultural, industrial and domestic wastes than the first one. Analysis of red blood cell count, haematocrit, hemoglobin concentration and white blood cell count and biochemical parameters in Mus musculus were carried out. Individuals of Group II (downstream) had lower blood indices than I. Statistical analysis revealed that differences in hematological and biochemical parameters between two habitats. It is clear from the results that the water quality induces changes in the values of many secondary hematological parameters. The hematological indices of MCHC, MCH and MCV were also followed similar trend indicating that the Chambal River is contaminated which induced several haematological alternations. Plasma glucose levels decreased in the mice of Station 2. The study showed that the environmental conditions significantly impacted the health status of the animal. It is suggested that the above parameters can be conveniently employed as health monitoring tools and changes in the water quality.

1. INTRODUCTION
Fresh water resources all over the world are threatened not only by over exploitation and poor management but also by ecological degradation. The main source of freshwater pollution can be attributed to discharge of untreated waste, dumping of industrial effluent, and run-off from agricultural fields. Industrial growth, urbanization and the increasing use of synthetic organic substances have serious and adverse impacts on freshwater bodies. Heavy metals and other xenobiotics are transported by blood and get accumulated in various tissues. Therefore, haematological indices are considered as patho physiological indicators of the body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants[1]. Biomarkers in different animals have been used within environmental monitoring programs to estimate the degradation of aquatic ecosystems[2,3]. Hematological changes in animals including human may be used for assessing the effects of contaminants, because blood parameters respond to low doses of pollutants [4]. Hematological indices are common diagnostic tools used by clinicians and other health professionals in evaluating the current health status of an individual[5]. Biomarkers in different animals have been used within environmental monitoring programs to estimate the degradation of aquatic ecosystems[2,3]. Hematological changes in animals including human may be used for assessing the effects of contaminants, because blood parameters respond to low doses of pollutants [5].

With this background the present study evaluated the quality of the water of Chambal River at Nagda (M.P) in winter through the analysis of physical-chemical parameters of the water, and
the analysis of blood parameters in of Mus musculus.

2. MATERIALS AND METHODS

Study area
Chambal River in Nagda (Western Madhya Pradesh) is very close to tropic of cancer at 23°27'N and 75°25' and 517 meters above MSL. More than one lakh of residents in and around the Nagda rely on water from Chambal River for public and industrial use. Waste after coming from the factory complex runs in a channel for about 3 km and joins River Chambal near Juna Nagda. River Chambal receives water from different Industries and municipal sewage.

Description of Study stations

Station 1:
This station is located at upstream at Nayan village. Human activities are reduced here. This station was taken as the reference station (control) owing to the absence of effluent discharge coming into the River from industries.

Station 2:
This station is located near Mukteswar temple near Juna Nagda and located in downstream. The discharges of industrial complex and domestic municipal waste are drained into this station. It is about 4 km away from station 1.

The surface water samples from upstream and downstream were collected in winter (December 2012) in glass bottles at about 10 cm below the surface. All collected water samples were refrigerated at 4°C, transported to the laboratory and various parameters like pH, COD, BOD, and DO, EC, TDS, TSS and hardness were analyzed according to APHA[6].

Experimental model:
For the present experiment 20 healthy six months old Swiss albino male mice Mus musculus of 6-8 weeks old weighing (22-26±gm) were procured from the veterinary college, Mhow. They were After acclimatization for two weeks under standard laboratory conditions (photoperiod and temperature) mice were divided into 2 groups. Group (I) represented the control group was fed on diet free from any other additions with normal drinking water from station 1 and Group (II) was given same diet with water from station 2 as drinking water. Blood sample was collected into sterilized vials at the end of 14 days exposure containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant and processed immediately for haematological analysis. All analyses were performed on pooled blood samples.

Hematological studies:
Neubauer chamber hemocytometer was used in the calculation of Erythrocytes (RBC) and (leucocytes) WBC. Ammonium heparinized capillary tubes (Fisher scientific co.) were used to measure the haematocrit. Hemoglobin concentration was measured by the cyanmethaemoglobin method using a commercially available kit (Span, India). Red blood indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and means corpuscular haemoglobin concentration (MCHC) were calculated using standard formula given by Dacie and Lewis[7]. Total plasma protein and plasma glucose concentration were determined using commercial kits (Siemens Diagnostics, Ltd, India).

3. RESULTS AND DISCUSSION

A summary of physic-chemical parameters obtained in Chambal River from both stations are shown (Table1).

The water quality parameters in the various treatments level (Table 1) indicated that there were significant differences (p > 0.001) in the value of all parameters studies except temperature.

Conductivity is a measure of the ability of water to pass an electrical current. Conductivity in water is affected by the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions (ions that carry a negative charge) or sodium, magnesium, calcium, iron, and aluminum cations. The EC values were found high in station 2, may be due to dissolved salts present in the effluent. Inorganic dissolved solids are essential ingredients for aquatic life. They regulate the flow of water in and out of organisms’ cells and are building blocks of the molecules necessary for life. A high concentration of dissolved solids, however, can cause water balance problems for aquatic organisms and decrease dissolved oxygen level.

TDS value of the sample from station II is much higher than the permissible limits. Higher turbidity in station 2 reduces the amount of light that can penetrate the water, which reduces photosynthesis and DO production. Therefore dissolved oxygen was found to less in station 2. The influx of industrial effluents significantly leads to increase in total dissolved solids in station...
2. The presence of aquatic plants in a stream and flow of water also affects the dissolved oxygen concentration. Green plants release oxygen into the water during photosynthesis. In this study we found poor vegetation in station 2. As a consequence low DO was observed. As this study conducted during winter the water in the River was stationary hence no flow was observed which may further decreased the DO. Water hardness is based on major-ion chemistry concentrations. Results clearly indicate that water samples from station II were showed high concentrations of total hardness. The high concentration of total hardness in water samples may be due to dissolution of ions from sediment rocks, seepage and run off from soil.

BOD (Biochemical oxygen demand) is the amount of oxygen required by micro-organisms to degrade the organic matter. The BOD values depend on the dissolved organic matter in the waste water samples. More the organic matter more the demand of oxygen by microbes to degrade it. Whereas in COD (Chemical Oxygen Demand) use of strong chemical agent (such as potassium dichromate) is done to degrade both the organic as well as inorganic matter present in the wastewater samples. Also, COD values are always higher than the BOD values. Because COD includes both biodegradable and non-biodegradable substances whereas BOD contains only bio-degradable. In this study it was observed that BOD and COD values in station 2 were higher than the standard limits. In addition, poor vegetation, high levels of TDS and suspended solids in decreased the DO content and consequently high BOD and COD was observed in station 2.

Hematological studies:
Haematological assessment is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of animal exposed to contaminants. The haematological changes produced by the effects of exposure of polluted and non polluted water showed a significant different in all the parameters (Table 2). It is clear from the results haemoglobin, haematocrit, plasma glucose and RBC values decreased significantly (P>0.01) in the mice from station 2 (downstream). The hematological indices of MCHC, MCH and MCV were also followed similar trend.

Water quality parameters are an indicator of toxicants effects in the organisms. In present study, a decrease in the haemoglobin, RBC and HCT in the mice of GroupII was observed. The reduction in RBC, Hb and PCV consequently led to reduction in oxygen carrying capacity of blood, resulting to anaemic condition. The anaemic status may be as a result of inhibition of erythropoesis and haemosynthesis consequent to an increase in the rate erythrocytes destruction in haemopoietic organs.

The red cell indices define the size and Hb content of the RBC and consist of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), and the mean corpuscular hemoglobin (MCH). The red cell indices define the size and Hb content of the RBC and consist of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), and the mean corpuscular hemoglobin (MCH). MCV refers to the average size of the RBCs constituting the sample. Our results clearly indicate that MCV and MCH decreased significantly (P>0.01) in the mice of station II. It may be either due to hypoxia or microcytic anaemia. The development of this condition probably an adaptive response through the influx of immature erythrocytes from the hematopoietic tissues to the peripheral blood to make up the reduced erythrocytes number and decreased hemoglobin concentration.

The MCHC measures the average concentration of Hb in the RBCs. It is most valuable in monitoring therapy for anemia because the two most accurate hematologic determinations (Hb and Hct) are used in its calculation. Our results clearly indicate that there was a significant decrease in MCHC in the mice of Group II. The significant decrease in the MCHC in this study was an indication of erythrocytes swelling and/or due to a decrease in hemoglobin synthesis. Prolonged reduction in hemoglobin content was harmful and affects the oxygen transport in the blood. Consequently hypoxia and degeneration of RBC could be attributed as pathological conditions.

Decreased heme synthesis in bone marrow, increased rate of destruction or reduction in the rate of formation of RBCs, and increased erythrocyte lipid peroxidation could be the possible reasons for such reduced hematological levels.
Table 1: Summary of physico chemical parameters of Chambal River for two different stations

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Station I</th>
<th>Station II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temperature, °C</td>
<td>22.1±0.8</td>
<td>22.4±0.2 NS</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>7.1±0.2</td>
<td>9.4±0.6**</td>
</tr>
<tr>
<td>3</td>
<td>COD mg/l</td>
<td>12.1±0.4</td>
<td>38.1±1.1</td>
</tr>
<tr>
<td>4</td>
<td>BOD mg/l</td>
<td>1.6±0.02</td>
<td>54.1±3.4**</td>
</tr>
<tr>
<td>5</td>
<td>TSS mg/l</td>
<td>16.1±1.1</td>
<td>136.3±8.9</td>
</tr>
<tr>
<td>6</td>
<td>Total Dissolved solids (TDS mg/l)</td>
<td>110.10±.8</td>
<td>389.15±.13.4</td>
</tr>
<tr>
<td>7</td>
<td>Electrical conductivity, (u mho/cm)</td>
<td>85±7.3</td>
<td>290±12.6.</td>
</tr>
<tr>
<td>8</td>
<td>Hardness, mg/l</td>
<td>200±12.4</td>
<td>982±213.4</td>
</tr>
<tr>
<td>9</td>
<td>Dissolved oxygen, ml/l</td>
<td>7.2±0.5</td>
<td>4.8±0.31**</td>
</tr>
</tbody>
</table>

NS= Not significant, * P> 0.5, ** P> 0.01 significant

Table 2: Summary of different haematological and biochemical indices of Mus musculus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Station I</th>
<th>Station II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematocrit%</td>
<td>34.1±1.4</td>
<td>22.3±0.06 NS</td>
</tr>
<tr>
<td>Hb.gm/100ml</td>
<td>14.4±0.69</td>
<td>8.90±0.5**</td>
</tr>
<tr>
<td>RBC (million/ml)</td>
<td>2.59±0.06</td>
<td>2.12±0.07*</td>
</tr>
<tr>
<td>WBC/10/L</td>
<td>4.4±0.11</td>
<td>5.11±0.21</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>34.1±4.1</td>
<td>22.11±3.4**</td>
</tr>
<tr>
<td>MCV (ug)</td>
<td>238.1±10.1</td>
<td>203.1±13.1*</td>
</tr>
<tr>
<td>MCH(g)</td>
<td>92.1±13.1</td>
<td>64.23±10.11*</td>
</tr>
<tr>
<td>Plasma protein gm/L</td>
<td>3.40±0.2</td>
<td>2.0±0.44*</td>
</tr>
<tr>
<td>Plasma glucose mg/L</td>
<td>244.1±6.72</td>
<td>190.1±6.71**</td>
</tr>
</tbody>
</table>

* P> 0.5, ** P> 0.01 significant

4. CONCLUSION

The present investigation revealed that exposure to Chambal affects the physiology of the mice by reducing blood indices. The assessment of the effects of contaminated water on blood indices of mice is of great importance for a possible prediction of such effects on humans. The use of a group of biomarkers covering different levels of biological organization provides knowledge about at what level pollutants interact with the body and at what level the body is more susceptible to the action of pollutants. The results of studies such as the present one are essential to the design of effective strategies targeting the rehabilitation of biodiversity in aquatic ecosystems. Therefore, care should be taken in discharging the effluent into water bodies so as to minimize its impact on aquatic organism.

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