ASSESSMENT OF TOXIC STRESS OF WATER POLLUTION ON HAEMATOLOGICAL INDICES

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Abstract: The present study was aimed to assess the pollution stress on Chambal River at Nagda (M.P.India) in Swiss albino mice *Mus musculus*. Two sampling stations were chosen along the river corresponding to the locations were notable industrial and municipal waste effluent discharges into the river. Water from upstream and downstream was collected during winter months (2012) and various chemical parameters assessed. The EC, COD, BOD, TSS, TS and TDS were much higher than the standard limits in station 2 (downstream). Haematological analysis of different parameters in *Mus musculus* were also carried out. The mice of Group II (downstream) had lower blood indices significantly than I. Statistical analysis shown significant differences in both water chemistry and blood indices.

The higher limits of water quality indicators in downstream may be due to municipal sewage and discharge of industrial pollutants as this station receives greater agricultural, industrial and domestic wastes. Results of the present investigation also shown that the water quality induced changes in the values of both primary and secondary hematological indices. Both primary indices such as RBCs number and Haematocrit value was reduced in the mice treated with polluted water. The secondary blood indices of MCHC, MCH and MCV were also followed similar trend and caused hypoxia and hypoxemia. 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 other possible reasons for such results.

The study showed that the water quality of Chambal River at Nagda significantly affected the health status of the animal. It is advocated that haematological indices can be used as health monitoring tools and changes in the water quality. We hope that the results of this study will assist the officials of the industries and regulatory authorities to the design the effective strategies for the rehabilitation of biodiversity in aquatic ecosystems.

Keywords: Chambal River**,** haematological indices, pollution, toxic stress.

Introduction: The major cause of water pollution in developing countries is domestic and industrial waste that is directly released into streams or ponds without treatment. These wastes mainly contain variety types of pollutants including heavy metals [1], [2]. The change in the water chemistry due to contamination can adversely affect all levels of food chain of an ecosystem [3]. Consequently the contaminated surface water can also affect the health of both animals and humans. Degradation of water quality or depletion of water resources and loss of aquatic biodiversity are well-known aspects of the environmental scenery. Hence it requires urgent attention at global and national level [1]. The present study has been carried out at Nagda (western Madhya Pradesh) where there are about 10 (large and small) industries. Since 1952 these have industries been engaged in the manufacture, production, and processing of fibres, chemicals and dyes, cotton and synthetic yarn, and engineering products. Most of them use various inorganic and organic chemicals as raw materials and discharge their waste effluents directly into the streams. The pollutants present these effluents enter in the body and through blood circulation they get accumulated in the body tissues. They also enter into the food chain and cause various health disorders to aquatic organisms including man [4].Therefore, blood indices can be considered as biomarkers of the pollution influenced stress therefore they can be employed as an important in diagnosing toll for the assessment of the structural and functional status of an animal.

From 2007 to till today, the local Government and pollution control board at Nagda, continuously received and receiving a lot of complaints related to the water quality of the Chambal River at Nagda and nearby villages as the people suffering from various health disorders. Therefore, the present study evaluated the quality of the water of Chambal River at Nagda (M.P) in winter 2012 and correlated the parameters with blood indices in a mice Mus musculus.

Materials and Methods

Study Area. About Nagda: Nagda is an Industrial town and very close to tropic of cancer at 23'27N and 75'25 and 517 meters above MSL. It is a city and municipality in Ujjain district in the Indian state of Madhya Pradesh. (Fig. 1) and is situated in the bank of Chambal River.

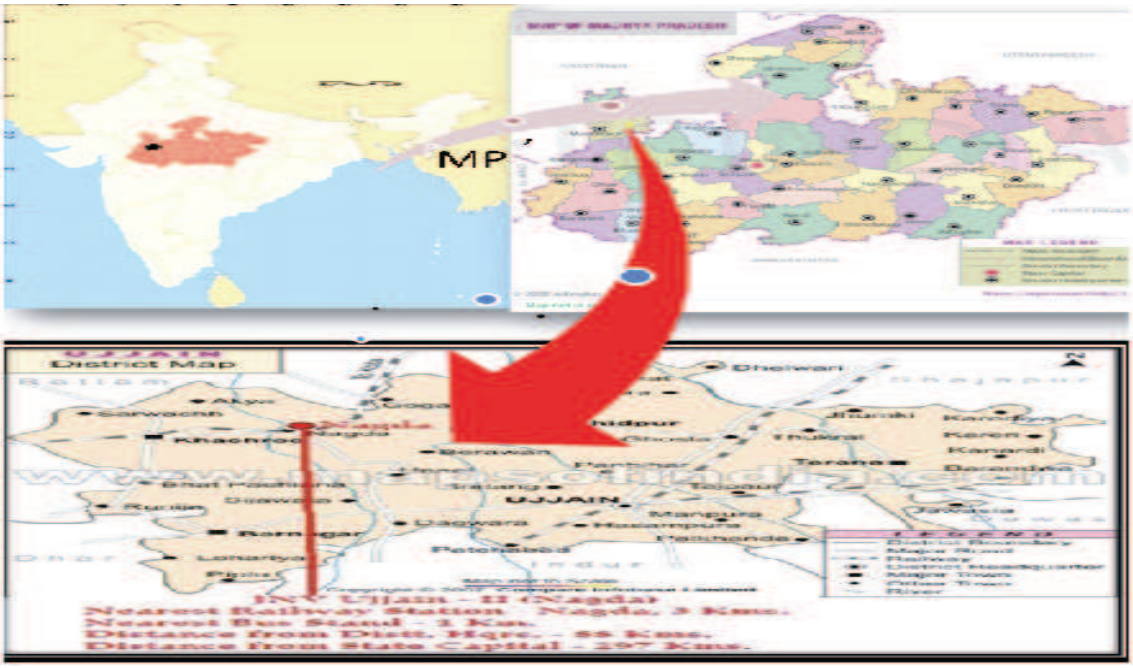

Fig.1. Showing Study area at Nagda, Ujjain.M.P.

This constituency has fast growth both in population and industrial development. Consequently there is an increase the sewage and effluent discharge. The Chambal River at this place receives many inputs both municipal and industrial effluent which may cause deterioration of water quality of the river.

Study areas:

Station 1: This site is situated at upstream of Chambal River, at Nayan village and taken as control. This station was taken as the reference station (control) due to the absence of human activities and effluent discharge coming into the River from industries and municipality.

Station 2: This site is located in downstream near Mukteswar temple at Juna Nagda. The discharges of both 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) and were refrigerated at 4 ºC. Various water quality parameters like pH, COD, BOD, and DO, EC, TDS, TSS and hardness were analyzed according to APHA[5].

Experimental model: For the present investigation 20 healthy six months old Swiss albino male mice *Mus musculus* of 6-8 weeks old weighing (22-26±gm) were obtained from the veterinary college, Mhow. After acclimatization for two weeks mice were divided into 2 groups .Group (I) is control one and fed on diet free from any other additions with normal drinking water from station (1). Group (II) was given same diet but with water from station 2 as drinking water. Blood sample was collected into sterilized vials at the end of 14 days and processed for blood analysis.

Hematological studies: Neubauer chamber hemocytometer was used in the calculation of Erythrocytes (RBC) and (leucocytes) WBC. Haematocrit was measured using ammonium heparinized capillary tubes (Fisher scientific co.). Hemoglobin concentration was measured by using a commercially available kit (Span, India). Secondary blood indices of RBC 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[6].Total plasma protein and glucose concentration were determined using commercial kits (Siemens Diagnostics, Ltd, India).

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

The water quality parameters of Chambal River at Nagda shown significant ($p > 0.001$) differences in both upstream and downstream except temperature.

Electrical conductivity (EC) is a measure of the ability of water to pass an electrical current. It is affected by the presence of inorganic dissolved solids. The values of EC at station 2 may be due to dissolved salts present in the effluent. But they are essential elements for aquatic life and can regulate the flow of water in and out of organisms. However a high concentration of dissolved solids can cause water

imbalance and decrease dissolved oxygen level [3].
We also found higher TDS value of the sample from station II is than the permissible limits. The entry of both municipal and industrial effluents significantly leads to increase in total dissolved solids in station 2. As a consequence dissolved oxygen (DO) was also reduced 2. The presence of aquatic plants in the River also reflects the percentage of dissolved oxygen concentration. Because green plants release oxygen into the water during photosynthesis. But we observed poor vegetation in this segment of the River. As this study was conducted during winter the water in the River was stationary hence no flow was observed which may further decreased the DO concentration. Results clearly point out that water samples from station II were illustrated high concentrations of total hardness 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. It is a measure of the oxygen consumed by the micro organism to decompose the waste. Hence, BOD values are depending on the dissolved organic matter in the waste of water samples. While 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 found that BOD and COD values in station 2 at downstream were higher than the standard limits. Besides, 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.

Table1: Physico chemical parameters of Chambal River for two different stations

S. No	Parameter	Station I	Station II
1	Temperature, C^o	22.1±.8	22.4±0.2NS
2	pH	7.1±.0.2	9.4±.0.6**
3	COD mg/l	12.1±.0.4	38.1±.1.1
4	BOD mg/l	1.0±.0.02	54.1±.3.4**
5	TSS mg/l	16.1±.1.1	136.3±.8.9
6	Total Dissolved solids (TDS mg/l)	110.10±.8.6	389.15±.13.4
7	Electrical conductivity, (u mho/cm)	85±.7.3	290±12.6.
8	Hardness, mg/l	200±.12.4	982±23.4.
9	Dissolved oxygen, ml/l	7.2±.0.5	4.8±.0.31**

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

Hematological studies: Red blood cell indices are measurements that explain the size and oxygen carrying protein (hemoglobin) content of erythrocytes. The indices reflect the health status of the organism hence used to help in the differential diagnosis of anemia and whole health status of the organism[4].
They also help in diagnosing the structural and functional status of animal exposed to contaminants. In the present investigation mice exposed to the polluted and non polluted water showed a significant different in all the parameters (**Table 2**). Results reveal that haemoglobin, haematocrit, plasma glucose and RBC values decreased significantly (P.0.>01) in the mice treated with polluted water of downstream. The secondary indices like MCHC, MCH and MCV were also followed similar trend.
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 erythropoeiesis and haemosynthesis consequent to an increase in the rate erythrocytes destruction in haemopoietic organs [8].
The secondary indices of RBC provide the information of the size and haemoglobin content. Our results clearly shown that MCV and MCH decreased significantly (P>0.01) in the mice treated with polluted water (station II). It may be due to reduced RBCs and haemoglobin in the treated mice which resulted Hypoxemia (low oxygen in blood) and hypoxia (low oxygen in your tissues). Without oxygen other organs like brain and heart get damaged which

is a dangerous for any organism[7]. It may be due to the entry of immature erythrocytes from bone marrow and other the hematopoietic tissues into the blood circulation to fulfill the requirement of RBCs number and decreased hemoglobin concentration[8]. The MCHC is a measure of the concentration of hemoglobin in a given volume of packed erythrocytes. This is most valuable tool in diagnosis of anemia because it is calculated by dividing the hemoglobin by the haematocrit. In the present investigation we found a significant decrease in MCHC in the mice of Group II. The decrease in the MCHC in the mice treated with polluted water of downstream was an indication of abnormal erythrocytes and/or due to a decrease in hemoglobin synthesis. Moreover the prolonged experiment (14days) reduced both RBCs and hemoglobin content in the mice. Consequently hypoxia and degeneration of RBC could be recognized as pathological conditions [9].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 other possible reasons for such results [10].

Table2: Effect of Polluted water on Haematological indices of Mus musculus

Parameter	Station I	Station II
Haematocrit%	34.1±1.4	22.3±.068**
Hb.gm/100ml	14.4±0.69	8.90±0.5**
RBC(million/ul)	2.59±0.06	2.12±0.07*
WBC/10/L	4.4±0.11	5.11±0.21
MCHC (%)	34.1±4.1	22.11±3.4**
MCV (ug)	238.1±10.1	203.1±13.1*
MCH(g)	92.1±13.1	64.23±10.11*

* P> 0.5, ** P> 0.01 significant

Conclusion: The present study discovered that exposure to polluted water can affect the physiology of the mice by reducing various blood indices. This type of investigation in mice is of great importance for a possible prediction of such effects on other organisms including human. The use of such biomarkers provide 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 current investigation are essential to the design the effective strategies for policy makers targeting the rehabilitation of biodiversity in aquatic ecosystems.

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