EFFECTS OF GROUNDWATER ON THE HEMATOLOGY OF *POECILIA RETICULATA* AFTER LONG TERM EXPOSURE IN MICRO COSM

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ABSTRACT

The Guppy fish, *Poecilia reticulata* (Peters), was exposed for 180 days to groundwater (pH 8.0, EC 1700 μmho Cm⁻¹, TDS 1517 mg L⁻¹, Cl⁻ = 400 mg L⁻¹, TA = 800mg L⁻¹, Pb = 0.4mg L⁻¹, Cu = 3.84mg L⁻¹, and Zn =11.88 mg L⁻¹) of Sanganer Industrial Area, Jaipur, Rajasthan (India) known world over for textile printing. Fish mortality was found nil but there was significant reduction in their Red Blood Cell Count (2.7%). Macrocyclic condition was observed in RBCs along with morphological abnormalities (poikilocytosis). Sensitivity of hematological parameters to the groundwater contamination was in order of: Poikilocytosis > RBC Count > Anisocytosis. The present investigation suggests mild contamination of groundwater of Sanganer industrial area by textile wastewaters.

INTRODUCTION

Increasing contamination of the water bodies all over the world during last few decades has shifted considerable pressure on the groundwater resources but their contamination by industrial wastewaters is a cause of greater concern. Textile industries in Rajasthan rank first in terms of pollution in the environment.

Sanganer is about 16 km far from Jaipur, Rajasthan (India) is famous for its traditional textile printing units. These industries discharge large quantities of dye wastewaters having higher concentrations of COD, BOD, TH, TDS, EC and heavy metals like Pb, Cu, Cr, Fe, Cd etc. which cause environmental degradation (Sharma et al. 2009, Murugesan et al. 2013). The continuous release of the wastewater from industries and sandy nature of soil of the study area allows maximum percolation of pollutants in the water table contaminating groundwater.

In our earlier study we reported physico-chemical characteristics of groundwater at various sites in this area and found one site (Madrampura) to be more contaminated than the other sites (Ojha et al. 2013), and therefore, it has been selected for risk assessment on animal model.

Fish bioassay is extensively used in toxicity studies. It is likely that there may be no signs of mortality and no distinctive behavioral changes yet toxicity may be inferred on account of hematological responses (Sharma et al. 2009). Earlier studies suggested fish RBCs to be an ideal indicator of pollutant toxicity i.e. sensitive in general rather being responsive to specialized class and concentration. Fish commonly used in general toxicity assessments are *Cyprinus carpio*, *Heteropeustis fossilis*, *Clarias lazera*, *Oncorhynchus mykiss*, *Gambusia affinis*, *Poecilia reticulata* etc. to see the hematological response of wastewaters released from different industries such as detergents (Sandbacka et al. 2000, Jobling et al. 2004), distillery (Ramakritinan et al. 2005), textile printing (Sharma et al. 2003, Sharma et al. 2006, Soni et al. 2006, Sharma et al. 2007, Sharma et al. 2009, Poornima et al. 2011) and pesticide (Parma et al. 2007). These authors have reported adverse effects in fish of the wastewater at different concentrations.

The aim of this study, therefore, was to investigate the effects of long term exposure (180 days) of groundwater on behavior, general health and hematological parameters in fish *Poecilia reticulata*.

MATERIALS AND METHODS

Study area and Sample Collection

Sanganer town (26°48’-26°51’N latitude and 75°46’-75°50’E longitudes) is 16 km south of Jaipur city, Rajasthan. Its total
area is about 635.5 km², of which only 12.9 km² is urban settlement, while the rest forms the rural area. Sanganer is world famous for its unique textile printing i.e. Bandhej, Block, Screen printing etc.

The groundwater samples used during the present study were collected from Madrampura, Sanganer, Jaipur (Fig. 1) because of higher contamination (Ojha et al. 2013). The physico-chemical analysis of the groundwater samples was carried out using standard method (APHA 1989).

**Fish Bioassay**

Experiments were conducted as per the guidelines of the Institutional Ethical committee, in the Zoology Department, University of Rajasthan, Jaipur. To study the effect of groundwater on behavioral and hematological parameter for 180 days, freshwater fish *Poecilia reticulata* collected from a tank in the Botany Department were acclimatized and maintained in plastic buckets (Size = 15L) at 26±2°C for 14 days in the greenhouse as described by Sharma et al. (2012). Bucket water was kept oxygen saturated using good amount of a submerged hydrophyte *Ceratophyllum demersum*. Mortality during the period of acclimatization was not recorded.

20 fishes per bucket were gently introduced filled in with potable groundwater of the University campus (control)/groundwater collected from the study area. Two replicates were made each for control and experimental groups. Evaporative losses were made up and waters in control and treatment sets were replaced at fortnight intervals, as described elsewhere (Sharma et al. 2003).

![Schematic diagram of study area](image)

*Fig. 1. Schematic diagram of study area (Madrampura, Sanganer Industrial Area) Jaipur, Rajasthan*
Test fishes were observed three times in a day to note behavioral change and mortality. Autopsy of surviving fish was done after their 180 days exposure to draw blood from gills for RBC count (Dacie and Lewis 1982) and blood smear preparation (Lee et al. 1993). RBC measurements were made by an occulometer standardized with a micrometer scale as parallel magnification (10x X 100x). The percentage of abnormal RBCs was calculated by observing approximately 200 RBCs in 20 microscopic fields (10x X 100x) using oil immersion.

**Statistical analysis**

The SYSTAT 5.05 was used for statistical analysis. Results are expressed as Mean±SEM. Two tail t-test was used to analyze the data with the level of statistical significance at P<0.05.

**RESULTS**

**Physicochemical characteristics of groundwater**

Physico-chemical characteristics of the groundwater of Madrampura (Sanganer) are shown in Table 1. The levels of EC, TA, TDS and lead were little higher than the limits set by the Bureau of Indian Standard, New Delhi (BIS, 1999).

**Behavior and Mortality**

Behavioural changes such as vigorous movements, imbalanced swimming, darkening of body, pronounced secretion of mucus and other stress marker conditions were totally absent in both control and treated fishes. Indeed, control and treated fishes showed normal swimming movements and chased feed—a sign of good health. To sum up, treated fishes were as normal as control.

**General Health**

Length, width and weight of control fish were almost similar to treated fish suggesting no adverse effect on general health of fish in the treatment (Table 2).

**RBC Count and Anisocytosis**

RBCs were normochromic in control as well as in treated group having a distinct centrally placed nucleus, which was more or less oval in outline and granular in view. Compared with control, RBC counts decreased significantly in the treated fish after 180 days of exposure to groundwater of Madrampura, Sanganer (Table 3). The diameter of RBC and nucleus however, increased significantly (Table 3).

**Poikilocytosis**

Compared with control, percentage of morphological abnormalities in RBCs was significantly higher in the treated fishes (Fig.2).

| Table 1. Physico-chemical characteristics of groundwaters of University campus and Madrampura, Sanganer |
|----------------------------------|--------|--------|--------|--------|--------|--------|--------|
| **Group**                        | **Parameter** | **pH** | **TDS (mg L⁻¹)** | **EC (µmho Cm⁻¹)** | **TH (mg L⁻¹)** | **TA (mg L⁻¹)** | **Cl⁻ (mg L⁻¹)** | **Pb** |
| University Groundwater  |                  | 7.2    | 500    | 400    | 156    | 300    | 130    | -     |
| Madrampura Groundwater        |                  | 8.0    | 1517   | 1700   | 189    | 800    | 400    | 0.40  |
| BIS, Indian standards (IS 10500:1991) |                  | 6.5-8.5| 500    | 500    | 300    | 300    | 250    | 0.05  |
| **Remark**                     |                  | Normal | High  | High  | High  | High  | High  | High  |

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<thead>
<tr>
<th>Table 2. Fish health in control and treatment after 180 days exposure</th>
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<tr>
<td><strong>Length (mm)</strong></td>
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<tr>
<td>Control</td>
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<td>Treatment</td>
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Table 3. Effects of groundwaters on RBC counts and anisocytosis in *Poecilia* after 180 days exposure

<table>
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<th>Parameters</th>
<th>Control</th>
<th>Treatment</th>
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<tr>
<td>RBC Count ($\times 10^4$ mm$^{-3}$)</td>
<td>26.6±0.29</td>
<td>25.9±0.24(-2.7%)*</td>
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<tr>
<td>RBC diameter ((\mu)m)</td>
<td>6.63±0.12</td>
<td>6.93±0.09(+4.52%)</td>
</tr>
<tr>
<td>Nucleus diameter ((\mu)m)</td>
<td>3.15±0.07</td>
<td>3.31±0.08(+5.07%)</td>
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Fig. 2. Poikilocytosis in RBCs of control and treatment

**DISCUSSION**

The behavioral pattern is probably the first warning system to denote the state of the health of an organism without sacrificing the individual. The groundwater of industrial area did not affect fish behavior possibly because of very low level of pollution, and therefore, general health and hematological parameters were used as indicators to measure stress response to exogenous and endogenous changes in fish. The groundwater of industrial area had neither adverse effect on fish health nor caused their death (Table 2).

RBCs are highly sensitive to environmental toxicants. The presence of contaminants such as heavy metals in the groundwater of industrial area not only decreased RBC count significantly but also affected their morphology (Table 3, Fig. 2). Macrocyclic condition was observed in RBCs along with increase in tendency of morphological abnormalities (poikilocytosis).

The reduction in RBC counts ascribed to suppression of erythropoesis has been reported in textile wastewater exposed fish (Goel et al. 1981, Sharma et al. 2003, 2006, 2009, Poornima et al. 2011, Murugesan et al. 2013). Both anisocytosis and poikilocytosis were also observed in textile wastewater exposed fish possibly on account of increase in RBC membrane fluidity (Soni et al. 2006, Sharma et al. 2009). Such morphological aberrations may adversely affect oxygen carrying capacity of RBCs in the blood and so, overall metabolism of the pollutant exposed organism.

The cytotoxicity of dyes, heavy metals and textile wastewaters to erythrocytes in terms of reduction in their counts (Goel et al. 1981, Kurde and Singh 1995, Sharma et al. 2003) and poikilocytosis (Sharma et al. 2007) is well documented and so, they may serve as an important tool for monitoring environmental contamination.

Present study has thus revealed the mild toxic effects of groundwater on the three indices of blood (RBC count, anisocytosis and poikilocytosis) in *Poecilia reticulata*. The sensitivity of the hematological parameters to pollutants in the groundwater was in order of Poikilocytosis > RBC Count > Anisocytosis. Thus, Poikilocytosis was found the most sensitive parameter for measuring toxicity of groundwater in fish. This proves potential of fish bioassay to detect groundwater contamination. Due to lesser cost on the experiment, it can be concluded that the fish bioassay is an cost effective method to check toxicity of groundwater.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


