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# Study of Changes in Genetic Diversities and Hepatic Histological Structures of Two Ichthyofauna as a Consequence of Water Pollution in Two Different Areas of West Bengal

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**ABSTRACT**

Water pollution affects biodiversity harshly. An attempt was made to find out the effect of aquatic pollution on the tissues of the fish, taking two different types of carps from two different areas of West Bengal (Barrackpore in North 24 Parganas and Raiganj in North Dinajpur). The carps used were *Labeo rohita* (major carp) and *Labeo bata* (minor carp) and were sampled from two different ponds within 1 km of each locality. The physicochemical parameters of the respective ponds were estimated from which the fishes were collected and comparisons were made. Liver tissues were taken from the fishes for histological and molecular analysis. From histological studies, it was found that there were some irregularities in the liver tissue structures which might be due to water pollution. DNA isolation from fish liver followed by gel electrophoresis was done to check whether water pollution affect the integrity of the extracted genomic DNA of the fish samples.

***Keywords*:** *Labeo rohita, Labeo bata,* Water pollution, ichthyofauna, physicochemical parameters, fish liver, histopathology, DNA gel electrophoresis

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## 1. INTRODUCTION

In recent years, water pollution is one of the major environmental concerns, which implies several threats to biodiversity. Water is synonymous to life and is a primary driving force for major physical, chemical and biological changes all over the world. Water resources are declining day by day at a faster rate due to rapid urbanization and population load. Deterioration of the water quality is now a global problem (Mahananda *et al*., 2010). From ancient times the rain water is being stored in small water body like ponds in most of the area in India. Several anthropogenic activities and interventions on pond as well as water bodies ultimately deteriorate the water quality, including accumulation of toxic chemicals and sediments, shrinkage of catchment area, loss of aesthetic value and biodiversity of the aquatic body. The accumulation of various kinds of pollutants and nutrients through the domestic sewage, municipal effluents, and agricultural runoff into the ponds leads to changes in the physico-chemical characteristics of fresh water.

These physico-chemical parameters help us assess the quality of water and provide information regarding its being suitable for drinking and aquaculture. Fish is one of the most important constituent of the aquatic fauna. It provides a good source of animal protein in diet for humans and is widely used as a model organism for different biological studies and researches. Aquaculture is thus gaining importance day by day at national and international level. A good number of pesticides and fertilizers are used in aquaculture for eradication of enemies of fish as well as for growth of plankton, the natural food of herbivorous fishes. However, the assessment of the ecotoxicological hazards caused by pesticides and fertilizers to ecosystems is based on the data on the toxicity and effects to non-target organisms. Fishes are among the group of non-target aquatic organisms. Histological changes provide a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (Bernet *et al*., 1999). The organs where changes can be adequately observed are the liver, kidney, gills etc in the fish (Bernet *et al*., 1999). In the present study, the histopathology of the liver of *Labeo rohita* and *Labeo bata* has been studied. The histology of the liver varies among species, but there are general features that are found in the majority of species. The hepatic structure normally varies (and considerably) in direct relationship to gender, age, available food - especially with regard to glycogen and fat content, or temperature, and with endocrine influences strongly connected to the environmentally regulated breeding conditions (Genten *et al*., 2009). Moreover, another objective of the study was to determine whether the integrity of DNA is by any way affected by the aquatic pollution.

## 2. MATERIAL AND METHODS

Water samples were collected from the two ponds of Barrackpore area and are referred as S1 and S2 and two ponds from Raiganj in the North Dinajpur area which are referred to as S3 and S4 (Fig. 1). Samples were collected in glass containers previously cleaned by distilled water. During sampling, water taken from a depth of 30 centimeter below the surface of each pond and from four sites. The samples were labeled and transported to the laboratory, stored at 4 °C in the refrigerator for analysis of selected parameters including temperature, pH, dissolved oxygen, dissolved free carbon dioxide, total hardness and total alkalinity using different methods. Temperature was measured by digital thermometer; pH was measured by portable pH

meter and the rest were calculated by chemical methods (APHA, 1995; Michael, 1984). To study the hepatic histology, liver tissues were dissected out and cut into small pieces for preservation in Bouin's fixative for 18 hours. It is followed by dehydration, sectioning (Microtome machine used Leica RM 2125RTS) and lastly routine staining by Haematoxylin and Eosin stain and were observed under a compound light microscope with high resolution and eventually photographed with a digital camera attached to the microscope. Fish samples were collected from previously mentioned water bodies. Genomic DNA was isolated from the liver of the fishes collected from two water bodies. The genomic DNA extraction was carried out following the method of Shiozawal *et al*., 1992 and Adriane *et al*., 2003. The extracted genomic DNA was run in 0.8% agarose gel (prestained with 0.5µg/ml ethidium bromide) and electrophoresis was carried out at constant voltage and current (Horizontal Gel Apparatus GeNei Catalogue No. 106470GB), and followed by visualization of the gel in UV transilluminator and photographs were taken with a digital camera.

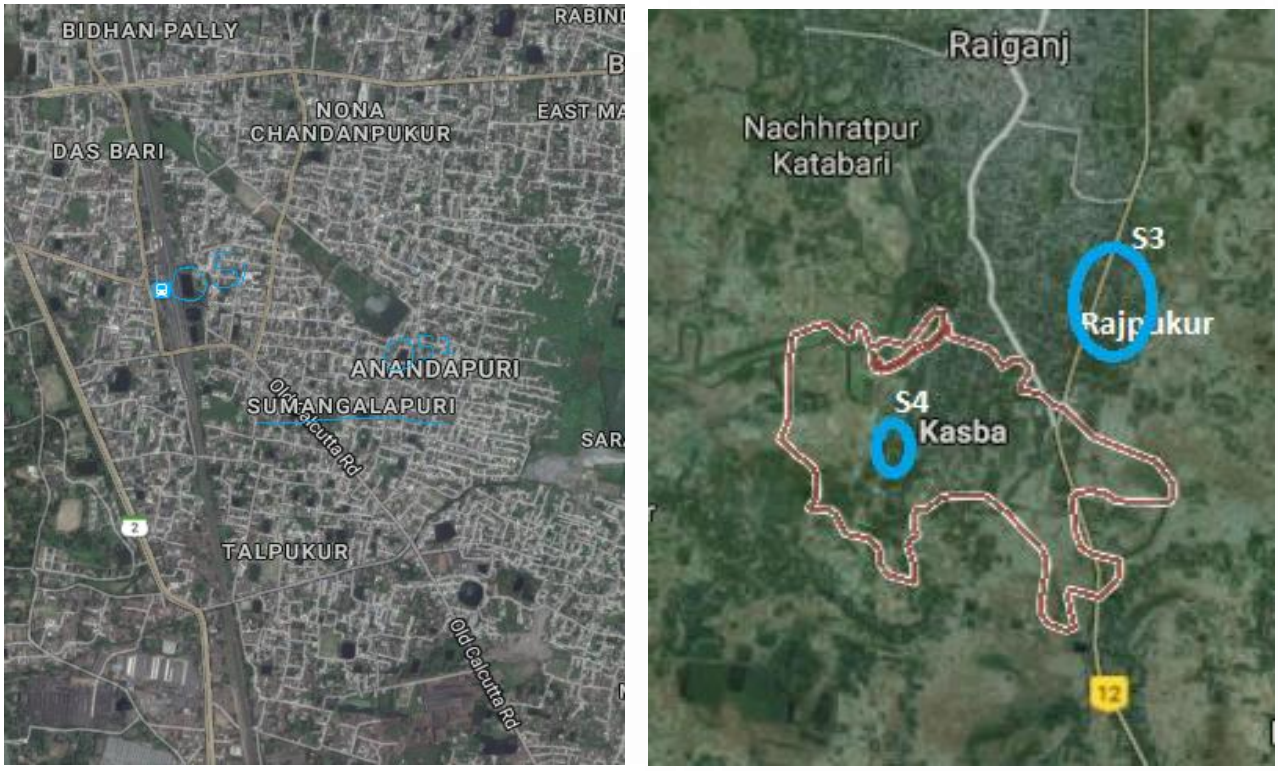


**Fig. 1.** Map showing two collection spots. S1 = Barrackpore railway side pond, S2 = Barrackpore Andandapuri (Sumangalapuri) pond, S3 = Rajpukur Raiganj, S4 = Kasba Raiganj

## 3. RESULT AND DISCUSSION

Data on the physicochemical parameters of water are shown in Table 1. It was found that the pond in Site 2 (S2) and Site 4 (S4) are polluted as it has high level of biological oxygen

demand (BOD) and low level of dissolved oxygen (DO) compared to the Site 1 (S1) and Site 3 (S3). The greenish colour of the water is due to the presence of green algae and cyanobacteria. The hardness of the water is also high compared to Site 1, suggesting the presence of carbonate and bicarbonate in the water due to the regular household runoffs by local inhabitants. BOD is highest in case of Site 4 and algal bloom was evident.

**Table 1.** The physico-chemical parameters of various ponds (mean value)

| **Parameters** | **Sampling sites** | | | | **Tolerance limit for freshwater fish culture Standards- BIS** |
|---|---|---|---|---|---|
| | **S1** | **S2** | **S3** | **S4** | |
| pH | 8.1 | 6.4 | 7.5 | 7.9 | 6.5 – 8.5 |
| Total alkalinity (mg/l) | 210 | 240 | 256 | 400 | 100-300 |
| Hardness (mg/l) | 146 | 264 | 152 | 356 | 80-150 |
| Dissolved Oxygen (mg/l) | 4.6 | 3.8 | 5.2 | 3.2 | 4 (minimum) |
| Biochemical Oxygen Demand (mg/l) | 1.8 | 2.2 | 1.0 | 2.5 | |
| Free carbon dioxide (mg/l) | 2 | 6 | 1.7 | 1.2 | 12 (at sunrise) [maximum] |
| Colour | Grayish | Greenish | Clear | Greenish | |
| Temperature (°C) [average] | 25.77 | 26.43 | 21 | 21.2 | 2-35 (range) |
| S1 = Barrackpore railway side pond, S2 = Barrackpore Andandapuri (Sumangalapuri) pond, S3 = Rajpukur Raiganj, S4 = Kasba in Raiganj | | | | | |

The histological sections are presented in Fig. 2. The histological sections of the liver of *Labeo rohita* and *Labeo bata* collected from the Site 1, has normal hepatocytes (Fig. 2a, 2c, 2e and 2f). But the fishes collected from Site 2, necrosis of hepatocytes and large void area was observed in section of *Labeo rohita* (Fig. 2b); and necrosis and cirrhosis of hepatocytes was observed in liver section of *Labeo bata* (Fig. 2d). Thus, it is evident from the histopathological studies that the water pollution might be an important factor for the histological tissue changes in the liver of the fishes.

The integrity of the genomic DNA of the fishes collected from the Site 2 was greatly affected due to water pollution compared to the less polluted Site 1 (Fig. 3). The genomic DNA is found to form smear (Fig 3b) in the agarose gel compared to the clear visible band in the samples collected from pollution free Site 1 (Fig 3a). The smear formation of the DNA extracted from the liver of the fish collected from polluted water body (Site 2) may be due to the fact of necrosis that occurs in the hepatocytes due to pollution.

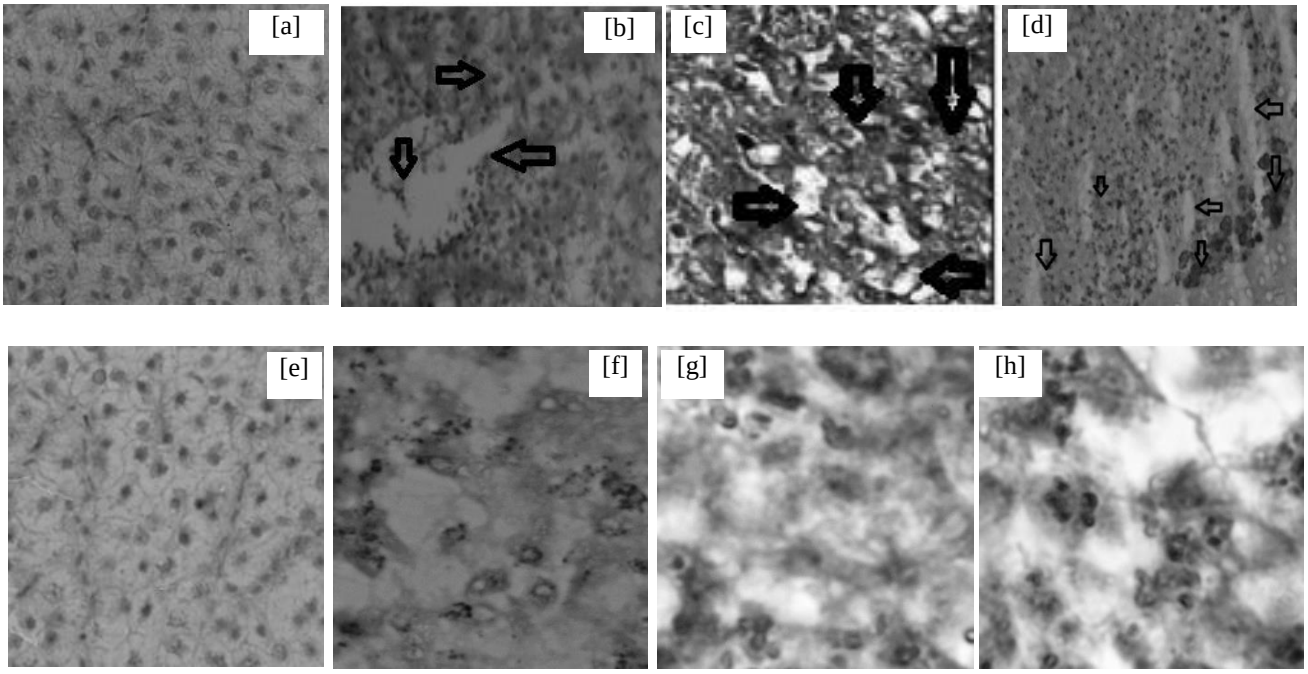


**Fig. 2.** Histological sections of fish liver from different location, **a.** *Labeo rohita* collected from S1 having normal hepatocytes, **b.** *Labeo rohita* collected from S2 with huge space and necrotic hepatocytes, **c.** *Labeo bata* collected from S1 showing normal hepatocytes, **d.** *Labeo bata* collected from S2showing abnormality in the hepatocytes, **e.** *Labeo rohita* collected from S3 having normal hepatocyte, **f.** *Labeo rohita* collected from S4 showing necrosis in hepatocyte, **g.** *Labeo bata* collected from S3 having ablesion and pyknosis in the hepatocytes , **h** .*Labeo bata* collected from S4 having void space in the hepatocytes.

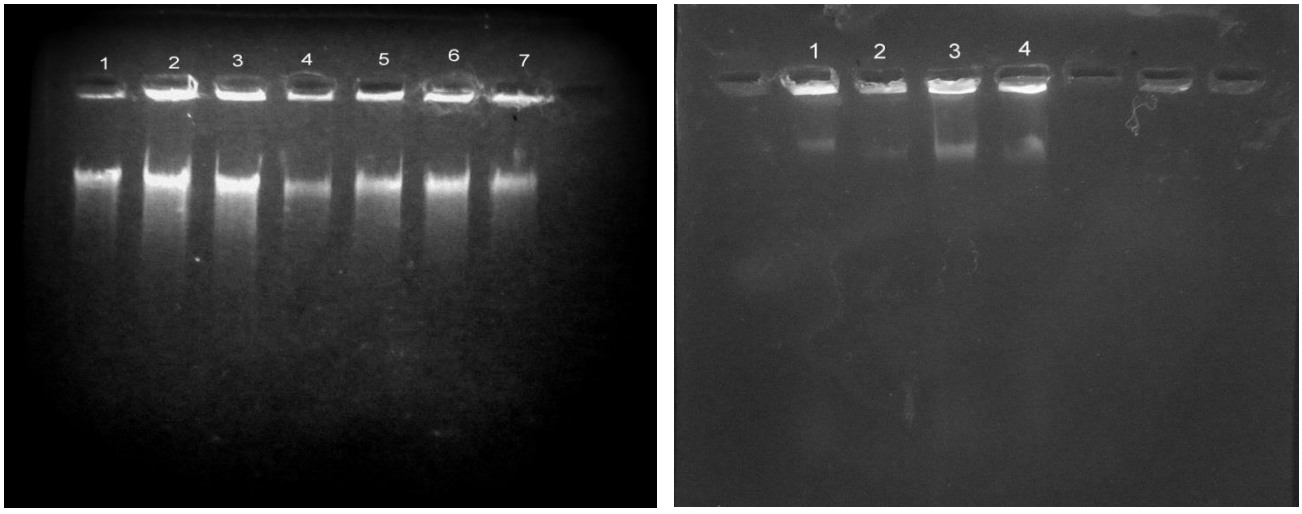


**Fig. 3.** 0.8% agarose gel of extracted DNA from fish liver of different locations, **[a]** DNA gel electrophoresis of extracted DNA from liver of Labeo rohita of S1 (Lane -1), S2 (lane-2,3), S3 (lane-4,5), S4 (6,7) **[b]** DNA gel electrophoresis of extracted DNA from liver of Labeo bata from S1 (lane 4), S2 (lane-3), S3 (lane-1), S4 (lane 2)

## 4. CONCLUSIONS

Pollution is the introduction of contaminants into the natural environment that causes adverse change. Pollutants can be either foreign substances or naturally occurring contaminants. The adverse effect of aquatic pollutants can be well determined by observing the changes in the histopathology of the liver of the two most commonly found and consumed carps. The physicochemical parameters of the water of the two ponds clearly show that differences in the water quality of the aquatic bodies are responsible for the abnormal changes in fishes like the necrosis, cirrhosis and pyknosis of the hepatocytes. Since liver is the primary organ for detoxification of organic xenobiotics therefore any toxic by-products tend to accumulate in it and the organ suffers harmful effects (Hawkes, 1980). Hence, observation of the histological section of the liver can prove very useful indicator as any toxic effect will be reflected by the abnormalities of the liver cells. The smear formation of the extracted genomic DNA in the agarose gel is greatly related to the water pollution that affects the hepatocytes of the fish liver. Therefore, it can be concluded that pollution is related to abnormality of the hepatocytes in the fish. Moreover, a comparative molecular analysis like RAPD can be carried out with the fishes from different water bodies covering a large region, to further clarify and establish the relation of pollution with the tissue abnormalities in the aquatic organisms and the relation between same and the different species of fishes.

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