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RESEARCH ARTICLE

Study of the Genotoxic Effects of Polluted Water of Certain Ponds of Narsinghpur in the R.B.Cs. of the Fish *Labeo rohita*, by Applying Micronucleus Assay

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ABSTRACT

Agricultural pests and ecto-parasites in animals/poultry are controlled by spraying pesticides/insecticides which include organophosphates, carbamates and organochlorines. These all are extensively used in India. The chronic use and excessive doses of pesticides become part of food chain leading to a series of hematological, biochemical, reproductive, histopathological and genotoxicological, mutagenic and carcinogenic changes in the body. This research deals with the genotoxicological and mutagenic alterations rendered by pollutants in fishes.

Water samples were collected from the study pond aiming to physio-chemical parameters. Pond water presented high level of pollutants all the year around. Fishes were collected from the polluted pond and their blood samples collected for micronucleus assay.

Micronucleus assay were applied to study the genotoxic and mutagenic effects in R.B.Cs. of fish. In the present studies we found formation of micronuclei, binucleated and multinucleated cells, pyknotic nucleus, intercellular bridge, sticky chromosomes accumulation in the cells proving genotoxicity.

Key words- genotoxic, micronucleus, chlorinated, phosphorylated, bio indicator.

INTRODUCTION

The present studies are to determine the genotoxic effects of polluted water on the fish R.B.Cs. The fish selected for following studies is a teleost fish named as Labeo rohita. This fish is cultured in the selected ponds. The ponds in which fish is cultured are surrounded with fields used for crop culture and for the production of vegetables.

In these fields for protecting crops from insect/pests, and for increasing crop production formers used various chlorinated and phosphorylated insecticides/pesticides and fertilizers. These chemicals and their remnants invade ponds when it rains. Flow off from the fields enters directly into the ponds affecting living organisms. Higher level of BOD results in depletion of available oxygen for aquatic organisms, while high amount of COD in effluent is toxic to biological life (Victoria A. Oriacu *et al*).

The micronucleus assay is a simple and sensitive assay for evaluation of genotoxic properties of various agents. Since teleost erythrocytes are nucleated, micronucleus assay provides information about the measure of clastogenic activities (Nagpure *et al*, 2007). In fishes chromosomes are small in size but large in numbers. Thus micronucleus in fishes could be smaller in size. The formation of micronucleus depends on the rate of proliferation of the cells, which in turn depends on fish species, environmental conditions and target tissues.

The micronuclei are small, extra nuclear bodies that are formed during mitosis from acentric chromosomal fragments, or whole chromosomes that are not included in daughter nucleus. Thus, micronucleus may contain a fragment of chromosome or it may contain a whole body of chromosome that is unable to travel to the spindle pole during anaphase.

MATERIALS AND METHODS

Water samples are collected from the ponds and physio-chemical properties of sample water are determined using standard analytical methods.

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Fishes were captured from the four selected polluted ponds. Blood were collected by the method described below and micronucleus assay was performed using the method described below:

- **Collection of blood samples**: Blood were collected by caudal puncture. For this purpose plastic syringes of 1 ml capacity and needle (1.0 inch, 22 gauge) were used. The blood is directly drawn from caudal vein. Mixture of dry ammonium oxalate and potassium oxalate in the ratio of 3:2 is used as blood anti-coagulant.
- **Preparation of MNi slides:**
  - **1-** A thin layer of blood is smeared on precleaned glass slides with the help of glass slide.
  - **2-** Slides are air dried over night at room temperature in a dust free moisture free environment.
  - **3-** Slides are then fixed by dipping in absolute methanol for 10 minutes.
  - **4-** Slides are then air dried for 1 hour.
  - **5-** Then the slide are stained with 10% Giemsa stain for 30 minutes.
  - **6-** Then they are washed for 3 times with double distilled water, so as to remove every Giemsa particle.
  - **7-** Slides are dried overnight in dust and moisture free environment.
  - **8-** Then the slides are mounted with DPX to make them permanent.
  - **9-** These permanent blood slides are observed under the microscope with the help of the eye piece 10X and the objective lance of 100X. A drop of immersion oil is used.
  - **10-** In the blood samples collected from different fishes of the different ponds, mean values of the MNi produced were obtained. For this purpose values of the MNi produced per 100 RBCs were observed and tabulated in frequency table. Obtained mean values of MNi produced in the fish RBCs collected from all the four ponds were compared to obtained highest frequency of MNi production within the fishes.

Calculation:

$$\text{Mean\%} = \frac{\text{No. cells find with micronucleus}}{\text{Total no. of cells observed}}$$

RESULTS AND DISCUSSION

The result of the physio-chemical analysis of water revealed higher values of most parameters (D.O., B.O.D., C.O.D., Nitrates, Phosphates, Potassium, Chlorides, Fluorides, Iron, Sulfates, Alkalinity, Turbidity, Hardness, pH , and Temperature.) than the standards set by Environmental Protection Agency (**Table 1**).The values obtained for D.O. are showing hyperoxic conditions of ponds. Values obtained for B.O.D. and C.O.D. are showing high level of biological oxygen demand and chemical oxygen demand. Values obtained for dissolved salts like nitrates, phosphates, potassium, chlorides, fluorides, iron and sulfates is also not satisfactory. The alkalinity, turbidity and hardness of pond water are also not matching the standards of Environmental Protection Agency (Table 1).

Chlorinated compounds are known to increase acidic conditions (pH below 7) while the phosphorylated compounds are known to increase alkaline conditions (pH above 7) of waters (Russel E. Train, 1979.). Wide range of pH change also causes alterations in blood parameters (Mahdi Ghanbari & Jami, 2010).

The pH value of pond I and II are 6.9333 and 6.8333 respectively, showing acidic nature of water, while that of pond III and IV are 8.375, and 9.1666 respectively, showing alkaline conditions (Table 1).

(**Fig 1**) shows normal RBCs. and nucleus in the blood obtained from normal fish *L. rohita.*

Insecticides/pesticides have been reported to lead to DNA damage which appears in the form of micronucleus formation, chromosome aberrations and mitotic aberrations (Sankar *et al.,* 2010; Hussain *et al*., 2011, 2012).Micronucleus appearance in the cytoplasm is considered as biomarker of DNA damage (Saleh & Sarhan, 2007). Micronuclei are of same color, refraction and texture to that of nucleus and appear as separate small nuclei having size of 1/10 in length and 1/3 in diameter of the main nucleus.

The erythrocyte micronucleus test is a preferred bioindicator of environmental mutagenicity. During micronuclei analyses, some authors have observed the occurrence of other nuclear abnormalities, suggesting that they must also be taken into consideration as potential indicators of cytotoxicity (Tolbert *et al*., 1991, 1992; Fenech *et al*., 1999) . In fish, these nuclear alterations have been reported after exposure to chemical agents or

polluted waters (Cavas and Ergene-Gozukara, 2003; Da Silva Souza and Fontanetti, 2006).

Fishes collected from pond I showing the formation of Micro-Nucleus (MN), Inter Cellular Bridge (ICB), Pycnotic Nucleus (PN) and Sticky Chromosomes (SC) in RBCs (**Fig 2**). (**Fig 3**) showing the formation of Micro-Nuclei (MN), Pycnotic Nuclei (PN), and Sticky Chromosomes (SC) in the RBCs of the fish collected from pond II. Fishes collected from pond III showing the formation of large number of Micro-Nuclei (MN), Pycnotic Nuclei (PN), and sticky Chromosomes (SC) in RBCs (**Fig 4**). (**Fig 5**) showing formation of Micro-Nucleus (MN), Pycnotic Nucleus (PN), Inter Cellular Bridge (ICB), and in the centre of the photograph is the bulk of sticky chromosomes in the RBCs of the fish collected from pond IV.
The mean % values of micronucleus formation in the blood of *Labeo rohita* are shown in (**Table 2**).

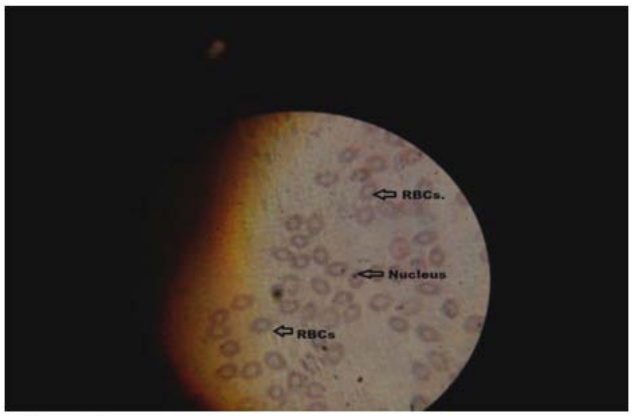

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Figure 1: Photomicrograph of blood of *Labeo rohita* (control), showing normal RBCs. and their nucleus, at 1000 X magnification.

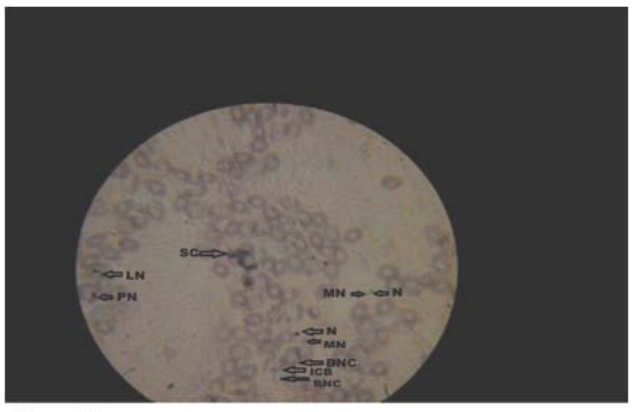

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Figure 2: Photomicrograph of blood of *Labeo rohita* collected from pond I, showing, sticky chromosomes (SC), lobed nucleus (LN), pyknotic nucleus (PN), nucleus (N), micro nucleus (MN),inter cellular bridge (ICB), binucleated cells (BNC), at 1000 X magnification.

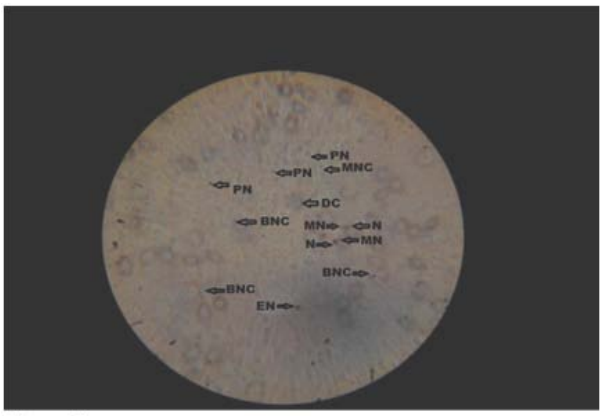

Figure 3: Photomicrograph of blood of *Labeo rohita* collected from pond II, showing, nucleus (N), micronucleus (MN), enlarged nucleus (EN), pyknotic nucleus (PN), binucleated cells (BNC), multinucleated cells (MNC), destroyed cells (DC), at 1000 X magnification

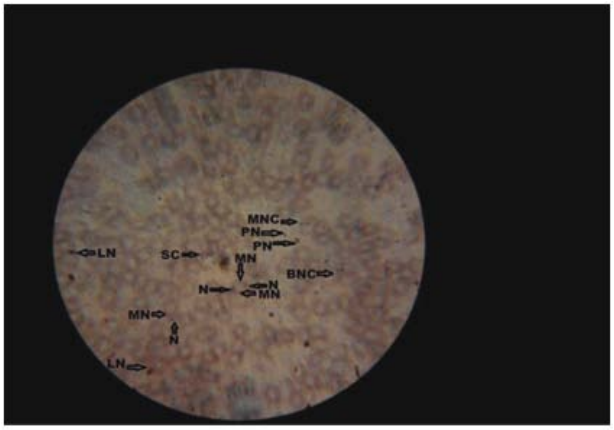

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Figure 4: Photomicrograph of blood of *Labeo rohita* collected from pond III, showing, nucleus (N), micro nucleus (MN), binucleated cells (BNC), multi nucleated cells (MNC), pyknotic nucleus (PN), destroyed cells (DC), at 1000 X magnification

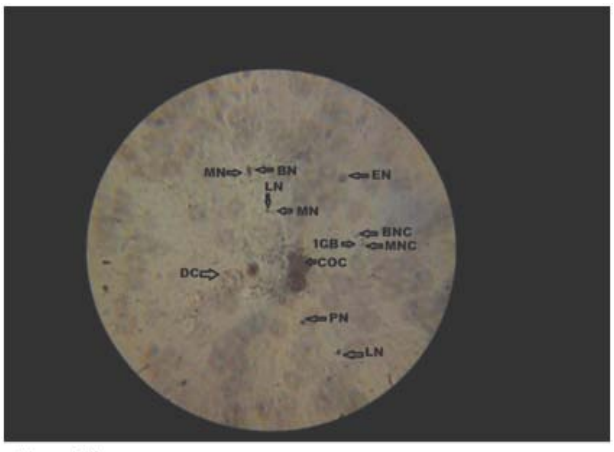

Figure- 125

Figure 5: Photomicrograph of blood of *Labeo rohita* collected from pond IV, showing, branched nucleus (BN), micro nucleus (MN), lobed nucleus (LN), pyknotic nucleus (PN), enlarged nucleus (EN), bi-nucleated cells (BNC), multinucleated cells (MNC), chromatin oozed out of cell (COC), inter cellular bridge (ICB), destroyed cells (DC), at 1000 X magnification

Table 1: Showing permitted values and obtained values of different physiochemical parameters in gm/l and temperature in ^{0} C

S No	Parameters	Permitted value	Obtained values			
			Pond I	Pond II	Pond III	Pond IV
1	D.O.	5-10	13.7166	13.4416	12.4916	9.7583

2	B.O.D.	6.00	10.575	10.6333	10.375	10.5666
3	C.O.D.	6.00	10.5083	10.425	10.375	10.1666
4	Nitrates	45	69.8333	71.8333	62.8333	96.3333
5	Phosphates	0.05	1.00	0.9416	0.925	0.9333
6	Potassium	<20	45.833	50.6666	29.4166	27.50
7	Chlorides	200	326.25	317.50	228.6666	206.0833
8	Fluorides	0.5	0.175	0.375	0.0	0.05
9	Iron	0.1	0.0583	0.0583	0.075	0.333
10	Sulfate	200	246.6666	239.1666	254.1666	264.1666
11	Alkalinity	200	215.8333	234.1666	434.1666	459.1666
12	Turbidity	10	78	71.8333	76.1666	78.0833
13	Hardness	200	483.3333	632.50	992.50	687.50
14	pH	6.5-7.5	6.9333	6.8333	8.375	9.1666
15	Temperature	13-22 C.	17.0833	16.0416	14.6666	15.00

Table 2: Frequency of MNi in R.B.Cs. of *L. rohita*

S No	No. of RBCs observed/ slide	No. of RBCs with MNi	Total	Mean%
1	400	10	10	2.50
2	400	12	12	3.00
3	400	20	20	5.00
4	400	12	12	3.00
5	400	3	3	0.75
6	400	12	12	3.00
7	400	21	21	5.25
8	400	24	24	6.00
9	400	18	18	4.50
10	400	40	40	10.00

CONCLUSION

The results obtained from this study indicate that the water of selected 4 ponds is highly polluted and it has genotoxic potential. Nuclear anomalies and formation of micronucleus in blood erythrocytes of the fish L. rohita were identified as good genotoxic biomarker.

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