International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Assessment of Genotoxicity in Two Fish Species from East Kolkata Wetlands: Impacts and Insights amidst Shifting Ecological Dynamics

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Abstract: ***The present study was examined genotoxicity especially micronucleation (MN) and nuclear abnormalities (NAs) in the peripheral erythrocytes of two selected fish species (Labeo bata and Oreochromis sp.). These fishes were collected from two sites viz. Bhojerhat as site A and Chowbhagaas site B of East Kolkata wetlands (EKW). In the fishes (just died) blood drawn from heart, smeared, and stained with Giemsa followed by scoring of percentage frequency of MN and NAs. In the present study, MNand NAs frequency (%) for both L. bata and Oreochromis sp., were higher in the site B without significant change when compared to site A. It is concluded that the genotoxicity through MN and NAs test in these studied fish did not show genotoxic effect as mutation risk, which may be due to COVID - 19 pandemic followed by lockdown. It is suggested in future to evaluate the physico - chemical properties and the concentration of metal (loids) in the river water and sediment of EKW.***

Keywords: Genotoxicity, Peripheral erythrocytes, MN and NA test, Fish species, East Kolkata Wetlands

1. Introduction

East Kolkata Wetlands (EKW) is comprised of 12, 741 hectares of land declared as Ramsar site in November 2002 have been delivering environmental benefits worth $38.54 million. It has supported a livelihood of 1.5 lakh (Approx) inhabitants directly. [1]

EKW is situated in the eastern fringes of Kolkata city approximately between latitude 22^{o} 25’ to 22^{o} 40’ North and longitude 88^{o} 22’ and 88^{o} 55’ east. This area is composed of “Sewage fed Fisheries” and/or “Bheries”, covering around 12, 000 ha in area. [2, 3]

Metal pollution and its hazardous effect play a critical role in polluting aquatic systems leading to problems in aquatic animals and, subsequently, human health. [4] (Saravi et al., 2009). In the freshwater aquaculture systems, toxic metals are potentially accumulated in sediments, water, and aquatic organisms and subsequently transferred to human beings through the food chain; however, a metal determination is mainly monitored in the water, sediments, and biota. [5, 6]

The metals have tendency to accumulate in the vital organs of aquatic organisms. [3, 7 - 12]Water pollution by heavy metals is a matter of great concern and causes genotoxicity in fish especially micronucleation (MN) and abnormal nucleation (NAs) in the red blood cells. [13 - 19]In the recent study, few investigators estimated metals in the water and sediment of fishponds of EKW [3, 12] but the genotoxicity screening is lacking.

In the present study, the genotoxicity especially micronucleation and abnormal nucleation in the peripheral erythrocytes was examined in the selected fish species inhabiting the fishponds of EKW.

2. Materials and Methods

Selection of study sites
The selection of study sites especially in Bhojerhat as site A (Latitude = 22°30' N and Longitude = 88°32' E) and Chowbhagaas site B (Latitude = 22°31' N and Longitude = 88°25' E) market, the fishes are sourced from East Kolkata Wetland (sewage - fed fishponds), The Google Earth image of study sites is exhibited in Fig 1.

Figure 1: Google Earth image of study sites, A = Bhojerhat; B = Chowbhaga (Source: Google Earth)

Selection of fish species
In the present study, two types of fish species were selected. One is Bata fish (*Labeo bata*) and other is Lylantica

(*Oreochromis* sp.) as per maximum availability in the study sites.

Study period

This study was conducted for the period of six months in the year 2022.

Genotoxicity study especially MN and NAs test

The protocol was followed as per earlier study by Talapatra & Banerjee. [14] In each fish 2000 erythrocytes were counted separately the peripheral red blood cells for *L. bata* and *Oreochromis* sp. The frequencies of MN and NAs in peripheral red blood cells were detected under Binocular microscope (OLYMPUS) using a 400X lens. Different types of NAs such as blebbed nucleus (BLN), binucleus (BN), notch nucleus (NN), lobed nucleus (LN), dumbbell shaped nucleus (DSN), retracted nucleus (RN), nuclear Karyolysis (NC), Vacuolated cytoplasm (VC) and fragmented nucleus (FN) were studied and scored separately. Frequencies (%) of MN and NA were expressed per 1000 cells.

Statistical analysis

All the data were expressed as mean ± standard deviation (M ± SD) and the comparative analysis was performed by using student 't' test in the PAST tool as developed by Hammer et al. [20]The p value at <0.05 is considered as significant.

3. Results

Fig 1 exhibits the microphotographs (400x magnification) of MN and NAs in the peripheral red blood cells of *Labeo bata*.

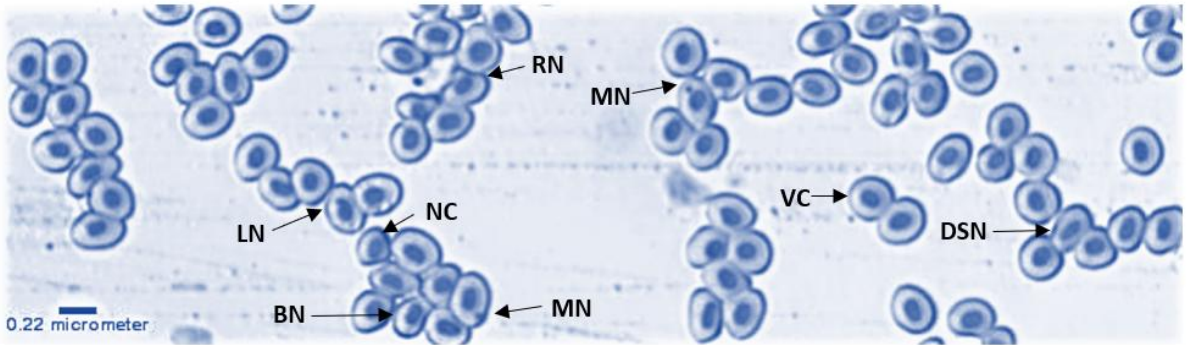

Figure 1: Microphotographs (400x magnification) of MN and NAs in the peripheral red blood cells of *Labeo bata (*MN = Micronucleus; NA = Nuclear abnormalities; LN = Lobed nuclei, VC = Vacuolated cytoplasm; NC = Nuclear cariolysis; DSN = Dumble shaped nuclei; RN = Retracted nuclei; BN = Binuclei)

Fig 2 exhibits the frequency (%) of MN and NAs in the peripheral red blood cells of *Labeo bata*. In the present study for *L. bata*, MN frequency was higher in the site B without significant change. The NAs such as LN, BN, DSN, VC, RN, RN and NC values were also increased in the site B without significant change when compared to site A.

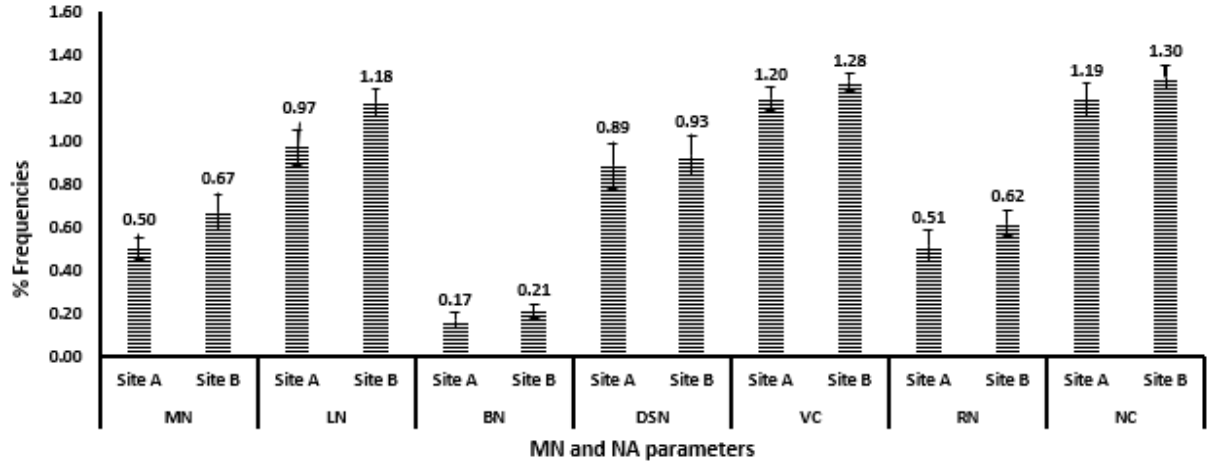

Figure 2: MN and NAs frequency (%) in the peripheral red blood cells of *Labeo bata*

Fig 3 depicts the microphotographs (400x magnification) of MN and NAs in the peripheral red blood cells of *Oreochromis* sp.

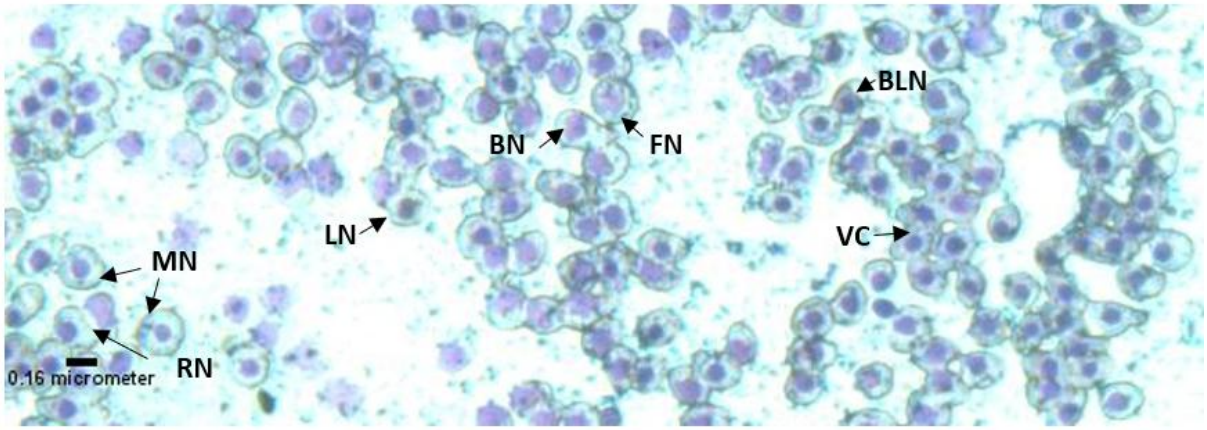

Figure 3: Microphotographs (400x magnification) of MN and NA in the peripheral red blood cells of *Oreochromis* sp. (MN = Micronucleus; NA = Nuclear abnormalities; LN = Lobed nuclei, BLN = Blebbed nucleus; VC = Vacuolated cytoplasm; FN = Fragmented nucleus; RN = Retracted nuclei; BN = Binuclei)

Fig 4 exhibits the frequency (%) of MN and NAs in the peripheral red blood cells of *Oreochromis* sp. In the present study for *Oreochromis* sp., MN frequency was higher in the site B without significant change. The NAs such as LN, BN, DSN, VC, RN, RN and NC values were also increased in the site B without significant change when compared to site A.

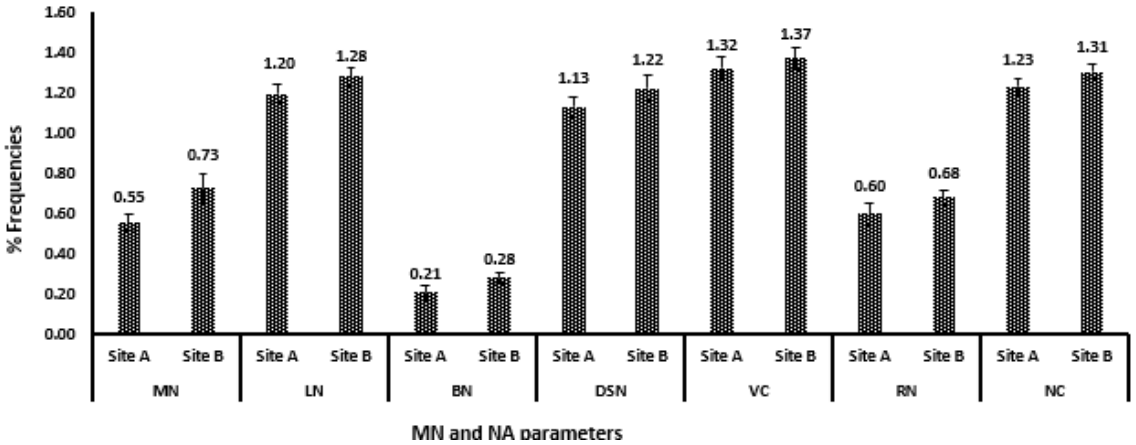

Figure 4: MN and NA frequency (%) in the peripheral red blood cells of *Oreochromis* sp.

4. Discussion

The genotoxicity study of two fish species inhabitingin the EKW has indicated that there isno mutation risk because lower values (% frequency) of MN and NAs in the peripheral red blood cells of studied fish.

In an earlier study, Talapatra and Banerjee [14] reported that significantly higher ($P<0.001$) frequencies of MN and NAs were observed in the blood cells when compared to negative as well as the laboratory control group of fishes. The study was found the genotoxicity in red blood cells of fish (*L. bata*) inhabited fishponds of EKW. In the recent study by Dutta et al. [12] reported that before 2019 the levels of toxic elements were higher while Kumar et al. [3] revealed that the bioaccumulation of several metals in muscle tissues are within the safe level as per prescribed limit. Moreover, the metal contamination in other vital organs viz. liver, gill, kidney, and brain were also observed below the toxic level. The present study is agreement with the study of Kumar et al. [3]that lower rate of accumulation and this study did not show alarming genotoxic effects as mutagenic risk of these two fish species when studied during post COVID - 19 period because COVID - 19 lockdown helps to protect the environmental degradation especially in the aquatic ecosystems.

5. Conclusion

It is concluded that the studies of genotoxicity especially induction of micronuclei and nuclear abnormalities in the peripheral erythrocytes of these two inhabiting fish species in the EKW were observed less risk of mutation. This lower risk may be due to without exposure of genotoxins during COVID - 19 period followed by lockdown. It is suggested in future to evaluate the physico - chemical properties and the concentration of metal (loids) in the river water and sediment of EKW.

Acknowledgement
Authors are thankful to the fish catchers.

Conflict of interest
Authors do not have any conflict of interest.

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