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Municipal Garbage-Induced Micronuclei and Associated Nuclear Abnormalities in *Heteropneustes fossilis* from Deepor Beel, a Ramsar Site of Assam, Northeast India

Nibedita Talukdar*
Faculty of Science (Zoology), Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti-781026, Assam, India
***Corresponding Author:** nibedita.talukdar@adtu.in

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ABSTRACT
The present study investigated the genotoxic effects of solid waste on *Heteropneustes fossilis* collected from two distinct sites of Deepor Beel, Assam. Site 1 is located near the Guwahati Municipal Corporation (GMC) garbage dumping area in Boragaon; while Site 2 is near the Rani-Garbhanga reserve forest area, relatively free from anthropogenic disturbances. The study was done by using micronucleus assay following the standard protocol. The analysis showed that *Heteropneustes fossilis* from Site I exhibited a significantly higher frequency of micronuclei and other nuclear abnormalities, including bilobed, notched, and blebbed nuclei, compared to those from Site II. The results highlight the adverse impact of garbage dumping on Deepor Beel which is a Ramsar site. The micronucleus assay in this study proved to be an effective tool for ecotoxicological monitoring highlighting the need for detailed investigations to protect the only Ramsar site of Assam, Northeast India.

INTRODUCTION

Management of waste material is one of the biggest environmental problems in urban areas. Urbanization and population growth increase municipal wastes that are finally dumped in empty lands usually at the outskirts of the city **(Ramesh *et al*., 2017)**. Discharge of effluents into the drains, open land and/or natural water bodies results in a large number of environmental problems which primarily form a threat to biodiversity **(Massar, 2012; Lozenka *et al*., 2016)**.

Deepor Beel is the only Ramsar site of Assam, North East India, which is situated along the southern banks of the Brahmaputra River basin at southern bank of Brahmaputra River. It is 10km away from Guwahati City of Kamrup District. It is a large fresh water lake and serves as the largest storm water storage basin. The bird sanctuary, Deepor Beel, is also designated as Wetlands of International Importance under the Ramsar Convention on wetlands, 1971 **(Saikia, 2005; Basistha, 2016)**. The beel is providing shelter to a large number of biodiversity. The natural resources of the Deepor

are the only source of livelihood of most of the local people of fourteen neighboring villages.

The Guwahati Municipal Corporation (GMC) solid waste open dumping site is 15 years old and has been operational since 6th June, 2006. This garbage dump is located in Boragaon in the fringe area of Deepor Beel. Around 300 metric tons of solid wastes are generated by the city per day and dumped at this site **(Gohain & Bordoloi, 2013)**. The garbage dump contains unsegregated, unsorted, non- biodegradable and toxic wastes of the city. The dump remains uncovered and there is no proper scientific method for the disposal of the solid waste. As a result, solid wastes and run off from the garbage dump are continuously discharging into the water, and the quality of water of this wetland has gradually degraded causing threats to the biodiversity **(Saikia, 2005)**.

Genotoxic parameters are important tools for the assessment of the health status of aquatic environment and inhabitant fish exposed to deleterious pollutants in an acute or chronic way **(Talukdar *et al.*, 2016)**. Micronuclei assay is one of the simplest and quickest methods to evaluate genotoxic effects in different organism **(Campana *et al.*, 2003; Bolognesi *et al.*, 2004)**. Micronuclei appear like small nuclei that arise during mitosis when a chromosome or fragment of chromosome fails to move with one of two daughter nuclei **(Udroiu, 2006)**. **Carrasco *et al.* (1990)** first explained the development of nuclear abnormalities in fish erythrocytes. Nuclear abnormalities such as blebbed, lobbed, binucleated cells and notched nuclei are also used to monitor genotoxicity.

Several studies on physico- chemical parameters of water of Deepor Beel have been conducted **(Roy & Kalita, 2011; Gohain & Bordoloi, 2013)**. In our previous study, higher concentrations of heavy metals were recorded in water samples of the garbage dumping site of Deepor Beel **(Talukdar *et al.*, 2021)**. To the best of our knowledge, no study exists on the genotoxic effects of solid waste disposal on fish species of the Deepor Beel. In this context, the present study aimed to investigate the induction of micronuclei and other nuclear abnormalities in *Heteropneustes fossilis* caused by toxic substances of solid waste dumped into this site of Deepor Beel, as well as conducting a comparison using a reference site without any anthropogenic stress.

MATERIALS AND METHODS

The protocols of the experimentation were reviewed and approved by Institutional Animal Ethics Committee (IAEC), Assam down town University, Assam, India (Reference number: AdtU/Ethics/stdnt-lett/2022/290). The experiments were carried out in accordance with the IAEC guidelines.

Study sites and collection of fish samples

Live specimens of *Heteropneustes fossilis* with 70- 72g in weight and 20- 21.5cm in length (N=40) were collected from two different sites of Deepor Beel in November, 2023. Site 1 was located near Guwahati Municipal Corporation (GMC) garbage dump, Boragaon Area (S_1; 26°8'26.02"N and 91°39'58.03"E; garbage dump contaminated site). Site 2 lies near Rani-Garbhanga reserve forest area (S_2; 26°6'28.21"N and 91°38'6.07"E; free from anthropogenic disturbances) and is far away from the main road and villages. The latter site was considered as a reference (control) site.

Micronucleus assay

The genotoxicity study was done in erythrocytes by using micronucleus assay following the standard method of **Schmidt (1975)**. A total of 3000 erythrocytes per fish were examined under a Leica make bright field microscope (DFC295) at 100X magnification and frequency of micronuclei (MN) and other nuclear abnormalities (NA) were scored. Micronuclei were considered as small inclusions of nuclear material inside erythrocytic cytoplasm. Criteria for identification were round or oval in shape, with a flat and well-defined outline, coloration similar to that of the main nucleus and a size from 1/3 to 1/20 in relation to that of the main nucleus **(Al-Sabti & Metcalfe, 1995)**. Nuclear abnormalities were identified as described by **Carrasco *et al.* (1990)**.

Statistical analysis

All results were expressed as Mean ± SEM (Standard Error of Mean) using MS Office Excel (version 2007). The differences of micronuclei and nuclear abnormalities between two sites were analyzed using one-way ANOVA analysis (SPSS version 16.0). The difference was considered the levels of significance at $P< 0.05$.

RESULTS

1. Induction of micronuclei in collected fish *Heteropneustes fossilis*

The frequency of micronuclei and other nuclear abnormalities in erythrocytes of *Heteropneustes fossilis* of two sites; S1 and S2 are shown in Table (1) and Fig. (1).

Table 1. Frequency of micronuclei and other nuclear abnormalities in blood erythrocytes of *Heteropneustes fossilis* collected from garbage dumping site (S_1) and reference site (S_2) of Deepor Beel

Site	MN (%)	NA (%)
Site 1 (S_1)	$3.53 \pm 0.67^{*}$	$14.33 \pm 1.22^{*}$
Site 2 (S_2)	0.40 ± 0.008	0.91 ± 0.05

(n= 20). All values are expressed in Mean ± SEM). Superscript (*) shows statistical significance ($P < 0.05$) and represents significance difference between frequency of micronuclei and other nuclear abnormalities in fish of two sites. (MN-micronuclei, NA-other nuclear abnormalities).

Significantly increased frequencies of micronuclei (1a) and other nuclear abnormalities were observed in collected fish of garbage dumping site (S1), as compared to reference site (S2). The frequency of micronuclei in S1 increased to 3.53% while the frequency of micronuclei at the reference site was 0.4%.

2. Induction of nuclear abnormalities in collected fish *Heteropneustes fossilis*

Moreover, a significant increase in frequency of nuclear abnormalities was observed at garbage dumping site (S1) compared to reference site (S2). The frequency of nuclear abnormalities was found to be 0.91% in fish of S2 while at S1, significantly higher frequency of nuclear abnormalities was observed (14.33%). The observed nuclear abnormalities were bilobed nuclei, notched nuclei, and blebbed nuclei (Fig. 1b-d).

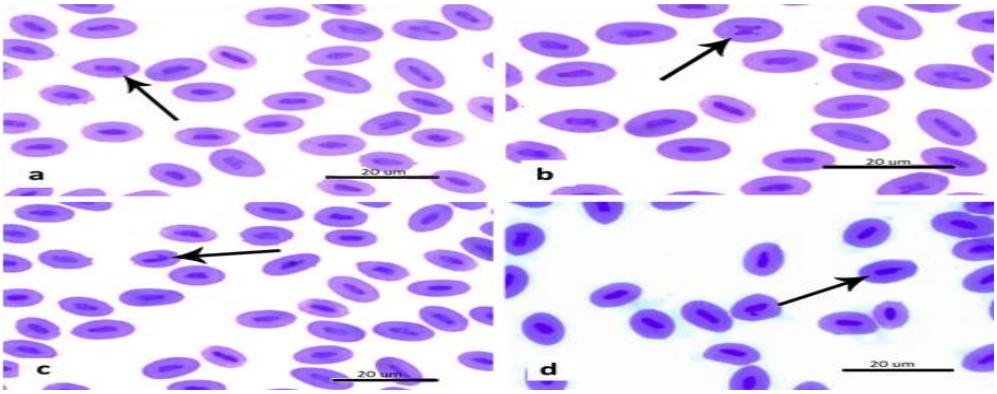

Fig. 1. Photomicrograph showing erythrocyte with micronuclei and other nuclear abnormalities: (a) Micronuclei; (b) Bilobed nuclei; (c) Notched nuclei; (d) Blebbed nuclei**.**

DISCUSSION

As a consequence of the municipal garbage dump, toxic materials are released to the water of Deepor Beel in huge quantities. The induction of micronuclei and other nuclear abnormalities are indicators of genotoxicity of a xenobiotic agent having clastogenic (chromosome breaking; DNA target) or aneugenic effects (chromosome number; mostly non-DNA target) **(Talukdar, 2016)**. Chromosomal damage represented through the formation of micronuclei results from incorrect or inefficient DNA repair and/ or from the presence of metals around the mitotic apparatus. The micronuclei represent an index of accumulated genotoxic agents and are expressed during cell division **(Kligerman, 1982)**. Micronucleus formation was also detected in fish sampled from estuarine areas impacted by several source of anthropogenic activities derived from industries, agriculture, sewage and harbour activities. In fish, since the damages of genetic material due to clastogenic agents are highly significant, it is possible to monitor the environmental health status through micronucleus assay concerning pollution with

clastogenic action **(Normann *et al*., 2008)**. The current study is supported by earlier investigations involving experimental studies in the laboratory and analytical approaches in fish samples collected from polluted water bodies **(Normann *et al*., 2002; Fillion *et al*., 2006; Ali *et al*., 2008)**.

In the present study, the micronucleus assay used in *Heteropneustes fossilis* represented an effective tool for assessment of genotoxic effect of toxic substances of garbage dump. The continuous discharge of toxic substances from the garbage dump into Deepor Beel makes it difficult to identify all the toxic substances that are contributing in genotoxicity in *Heteropneustes fossilis*. But the recorded data of higher concentrations of heavy metals in surface water and ground water **(Gohain & Bordoloi, 2013; Talukdar *et al*., 2021)** of garbage dumping site have proven that heavy metals are certainly contributing to genotoxicity.

CONCLUSION

The findings of the present study revealed the usefulness of genotoxicity biomarkers in monitoring the contamination of environmental pollutants in aquatic ecosystem. The Deepor Beel is receiving continuous flow of untreated effluents from the garbage dump for more than one decade which has created a major threat to Deepor Beel and its biodiversity. Our study highlights the urgent need of a close investigation on this environmental problem to save this Ramsar site, Deepor Beel.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this article.

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