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Genotoxic responses of *Oreochromis niloticus* to oil-contaminated water

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ABSTRACT

Moragoda Ela is an open canal connected with three cross drains that serve critical functions in the Galle Municipal area drainage system. However, the canal is under severe pollution stress due to numerous illegal discharge points of wastewater from commercial establishments adjoining the canal and contaminating water with polycyclic aromatic hydrocarbons (PAHs). The study aims to assess the genotoxic effects induced by these xenobiotic compounds with aquatic genetic biomarkers. Genotoxic alterations induced by these pollutants were investigated based on the micronucleus assay and erythrocytes nuclear abnormality assays in Tilapia fish (*Oreochromis niloticus*) exposed to this water for 10 and 28 days, respectively. A standard control experiment setup containing dechlorinated ground well water was set up to monitor deviation. Fish exposed to the water had significantly higher ($p<0.05$) nuclear abnormalities compared to the control fish exposed for the specified period. After exposure of the fish to the Moragoda Ela water for 10 and 28 days, a significant ($p<0.05$) increase in the genotoxic capacity of the water was evidenced. The study further revealed a time-dependent genotoxic effect on fish erythrocytes. Limited availability of affordable techniques to determine the different PAHs in Morgoda Ela water was a constraint during the study to obtain a comprehensive conclusion.

Keywords: Erythrocytes nuclear abnormalities, Genotoxicity, Tilapia fish.

INTRODUCTION

Anthropogenic waste has been deposited in large quantities in aquatic ecosystems. Petroleum products are among the most environmentally toxicologically important pollutants often associated with human activities (Pacheco and Santos, 2001). In developing countries, 90-95% of all sewage and wastes are dumped into the surface water of freshwater ecosystems in untreated conditions (UNFPA, 2001). Considerable contamination in freshwater ecosystems by petroleum hydrocarbons results from industry and municipal waste discharges. As a result, highly hazardous aromatic hydrocarbons may find their way into rivers and other aquatic environments, posing significant pollution concerns. As a result, it is critical to monitor aquatic ecosystems, particularly in areas where chemical contamination is a problem (Silva et al., 2003).

Aquatic animals have been often used in different assays to evaluate and estimate surface water pollutants (Koeman et al., 1977). Because they can metabolize, concentrate, and retain various toxins, fish are good subjects for detecting possible mutagenic and carcinogenic effects of contaminants in water. This makes them an excellent model organism for possible testing toxins that may impact people (Ali et al., 2008). Generally, changes in fish will mirror the changes in the environment because of their sensitivity to low concentrations of genotoxins (Al-Sabti and Metcalfe, 1995). In addition, fish will accumulate even higher amounts of genotoxins when feeding on other organisms as they are in higher ranks in ecological food chains, which have consumed the pollutant, thereby being an indicator of the shape of the whole ecosystem (Ali et al., 2008).

Definition for a biomarker can be derived as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioural changes) related to exposure to or toxic effects of environmental chemicals (Peakall and Walker, 1994). Genotoxicity is a deleterious action, which affects a cell's genetic material changing its integrity and function (Luch, 2005). Genotoxic activity in fish has been evaluated using a variety of endpoints. Micronuclei assays and another erythrocyte nuclear abnormality (ENA) assays are the most common and promising biomarkers for assessing environmental genotoxicity in fishes (Barsienė et al., 2006).

Moragoda Ela is an open canal connected with three cross drains that serve critical functions in the Galle Municipal area drainage system. It plays a significant role in conveying surface drainage and floodwaters. However, there are numerous illegal discharge points of wasted water from residences, commercial establishments (vehicle service stations, fuel filling stations) and warehouses located adjoining the canal subjected to contaminate water with polycyclic aromatic hydrocarbons (PAHs) (Kanthi and Jayaweera, 2021). After exposure, this study was designed to investigate the genotoxicity/ genotoxic alterations induced by water from the ecologically stressed Moragoda Ela in freshwater fish, *Oreochromis niloticus*.

METHODOLOGY

Fish management and exposure to Moragoda Ela water: Healthy juvenile of 72 with average length (105.10±6.86 mm) and average weight (26.76±5.706 g) of initial size were utilized for the experiment obtained from the Udawalawa fish breeding centre, governed by the National Aquaculture Development Authority of Sri Lanka. Fishes were acclimatized under laboratory conditions for 15 days prior to exposure. The water in the aquaria was changed daily, and fish were fed twice a

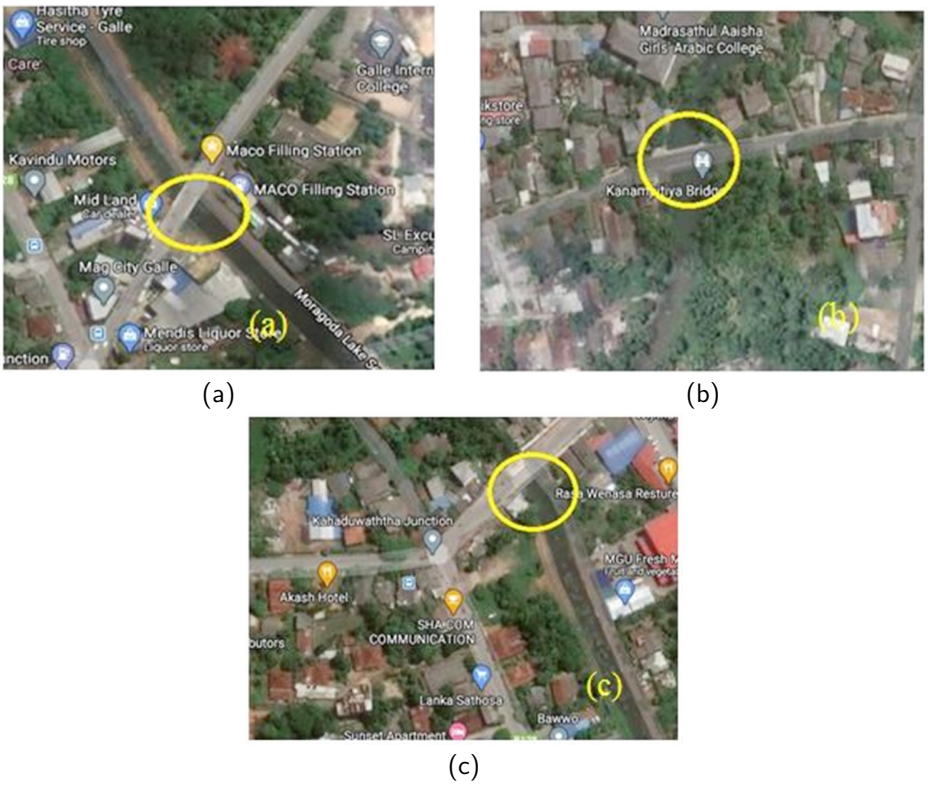

Figure 1: Water sampling sites of Moragoda Ela cross, (a) Site 1; Beligaha bridge area, (b) Site 2; Kanampitiya bridge area, (c) Site 3; Kahaduwattha bridge area.

day with the commercial feed diet. During acclimatization, everything was maintained as the actual experiment except for the exposure. The control water was dechlorinated groundwater of drinking – water quality obtained away from the Moragoda canal. The locations of the water sampling sites of Moragoda Ela for exposure (Figure 1) were selected according to the research findings from previous literature (Kanthi and Jayaweera, 2021). The fish were divided into three groups for the experiment, with 36 fish in control with two replicates (Group I, 18 fish per replicate) and 18 fish in Group II and III. Group I served as the primary control and was maintained under normal control water conditions for the same periods of the experiment. The experimental Groups II (2 replicates, nine fish in each) and III (2 replicates, nine fish in each) were exposed to Moragoda Ela water for 10 and 28 days. Physico-chemical properties of the water of control and exposed groups were determined by standard methods prescribed by APHA (1998). Nuclear abnormality tests were carried out after the exposure period, micronuclei test and other erythrocytes, nuclear abnormality tests were carried out.

Micronucleus test and other ENA test: Five fish from replicate (10 fish per group) were taken to prepare blood smears. For the blood collection, randomly selected fish were anaesthetized by using benzocaine solution. Blood samples were obtained by caudal vein puncture (4 slides per fish), directly smeared on the slide, and air-dried overnight. The air-dried blood smears were fixed using Absolute methyl alcohol (Methanol) before the staining of the smears. Then slides were stained with 10% Giemsa solution for 60 minutes. Then slides were rinsed with distilled water and air-dried at room temperature overnight. Counting of micronucleus and ENA types were done according to Ivanova et al., 2016; on each slide, areas with a uniform spread in monolayer without overlapping cells were targeted for count ENA types. Twenty fields were analyzed (Total – 100 fields per fish) at a magnification of 1000x, chosen by systematic random sampling method. The total number of analyzed erythrocytes per fish was around 10000, depending on the density of cells distribution on the smear. Micronucleus and ENA types classified according to Barsiene et al. (2006) and Cavas et al. (2003). Round or ovoid-shaped non-refractory particles with colour and structure similar to chromatin, with a diameter 1/3–1/20 of the main nucleus and detached from it (Barsienė et al., 2006) and on the same optical plane (l-Sabti and Metcalfe, 1995) were interpreted as Micronuclei (MN) (Figure 2). Nuclear buds (NB), Fragmented apoptotic cells (FA) and Bi nucleated cells (BN) were scored as interpreted in Figure 2. Blebbed nuclei (BL), Lobbed nuclei (LB) and Notched nuclei (NT) were interpreted and scored as altered nuclei (AN) (Cavas et al., 2003). The mean frequency of MN, binucleated, fragmented-apoptotic cells, nuclear buds and altered nuclei and standard deviations were evaluated per 5000 erythrocytes per fish.

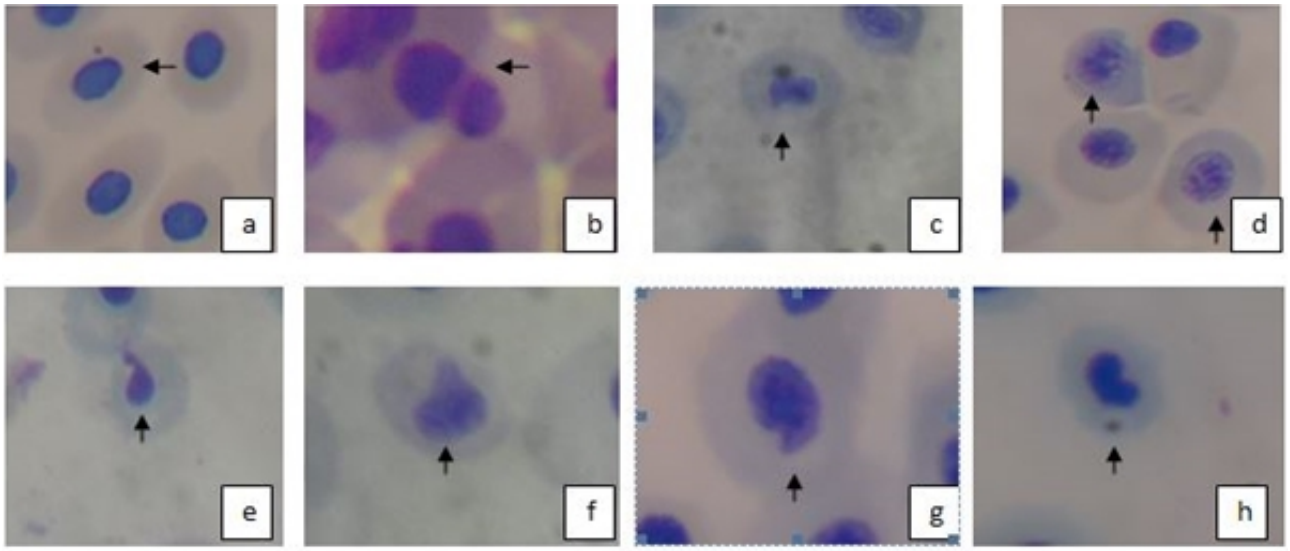

Figure 2: Photomicrography (1000×) of the erythrocytes of *Oreochromis niloticus* with abnormalities; (a) Micronuclei, (b) Bi nucleated cell, (c) and (f) Nuclear buds (e), (g), and (h) Altered nuclei and (d), show Fragmented apoptotic cells (Black arrow)

Table 1: Physico-chemical properties of the water of control group and Moragoda Ela water

Parameter	**Temperature** [^oC]	**pH**	**Salinity** [0/00 Cl]	**Dissolved Oxygen** [mg/L]
Water in control group	29.456±0.14	6.896±0.01	0.159±0.02	4.2±0.40
Moragoda Ela water	29.004±0.13	5.547±0.15	0.233±0.40	3.8±0.23

Table 2: Frequency of Erythrocyte nuclear abnormalities in peripheral blood of *Oreochromis niloticus* after exposure to Moragoda Ela water for 10 and 28 days, respectively (Mean ± SD calculated from 10 fish per group)

	ENA type				
Group	**MN**	**NB**	**BN**	**FA**	**AN**
Control	0^a	0^a	0^a	0.90 ± 1.10^a	8.20 ± 6.82^a
Group 01	4.20 ± 2.89^b	56.60 ± 21.00^b	3.70 ± 2.54^b	207.00 ± 69.85^b	559.10 ± 248.19^b
Group 02	5.50 ± 2.71^b	212.70 ± 63.17^b	4.00 ± 2.49^b	83.30 ± 29.96^c	912.30 ± 129.52^c
P-value	0.001	0.001	0.001	0.001	0.001

MN: Micronuclei NB: Nuclear buds BN: Bi nucleated cell FA: Fragmented apoptotic cell AN: Altered nuclei
Results from Mann-Whitney U test for comparison between pairwise are indicated by the superscript letter (a, b, c), where shared letter within a column indicate homogeneity

Statistical analysis: The statistical analysis was carried out using SPSS (ver. 16) statistical package. Non- parametric Kruskal-Wallis test was used to compare mean frequencies of erythrocyte abnormalities among three experimental groups, and the nonparametric Mann–Whitney U test was used for pairwise analysis.

RESULTS AND DISCUSSION

Table 1 shows the physicochemical properties of the water of the control group and the Moragoda Ela water prior to the experiment. There was no significant difference ($p>0.05$) between control and Moragoda Ela water from different sampling points for water quality parameters. However, slight surface oil luminescence of layer was observed in Moragoda Ela water samples due to discharge outputs from nearby fuel stations and vehicle service stations.

Micronuclei (MN), nuclear buds (NB), binucleated cells (BN), fragmented apoptotic cells (FA) and altered nuclei (AN) were observed as different erythrocyte nuclear abnormalities in exposure groups (Figure 2). FA and AN types were observed in the control group too, but in very few frequencies (< 10ENA per 10000 cells) compared with Group I and II. Altered nuclei were the most frequent ENA type which enumerated in high frequency in all groups. According to the results from the Kruskal-Wallis test, there was a significant difference ($p < 0.05$) of frequencies of all nuclear abnormalities over the exposure period among three experimental groups (Table 2). When considering the results from Mann-Whitney U test for the pairwise testing between control and Group I and Group II, there was a significant difference ($P<0.05$) for

the frequencies of all nuclear abnormality types and pairwise results between Group I and II showed a significant difference only for frequencies of FA and AN abnormality types (Table 2).

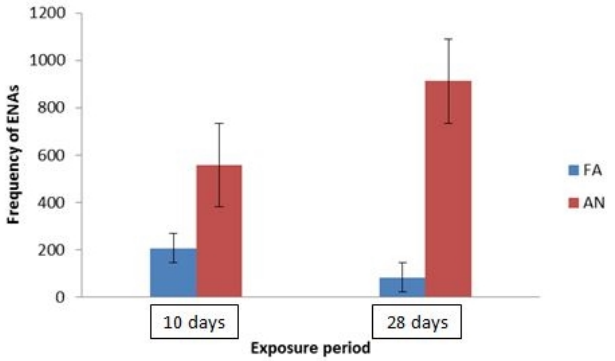

Figure 3: The indication of mean frequencies of FA and AN abnormality types after 10days and 28 days exposure periods (Mean±SE calculated from 10 fish per group)

According to Figure 3 there is an induction of frequency of altered nuclei and deduction of fragmented apoptotic cells within exposure period with significant difference between two exposure groups with Moragoda Ela water. Water pollution is a significant concern that affects the structure and functions of aquatic ecosystems in direct or indirect ways. Crude oil is a significant pollutant of aquatic ecosystems, which cause toxicity in the aquatic environment due to its highly toxic components such as polyaromatic hydrocarbons. The water-soluble fraction of crude oil creates hazardous or even lethal effects on aquatic organisms, and through the process of bioaccumulation and biomagnification in food chains, it also affects the higher vertebrates, including humans (l-Sabti and Metcalfe, 1995). As the previous study revealed, Moragoda Ela water contains genotoxicants that can induce abnormalities in fish erythrocytes, evidenced by the formation of different ENA types during the exposure period (10 and 28 days). The two periods of exposure showed significantly different effects (erythrocyte nuclear abnormalities) between the groups. This suggests a time-dependent effect, as earlier reported by Alink et al (2007), Kanthi et al (2015) and Obiakor et al (2010).

Genotoxicity effects in erythrocytes in peripheral blood of different marine and freshwater fishes due to PAHs have been investigated by researchers (Ayllon, and Garcia-Vazquez, 2000; Barsiene and Barsyte, 2000; Barsiene et al., 2006; Bolognesi et al., 2006; Barsiene and Andreikėnaitėb, 2007) through ENA biomarker. Sensitive biomarker micronuclei and other ENA tests are used to evaluate organisms' genotoxic effects due to xenobiotic contaminants in aquatic ecosystems. When assessing aquatic pollution, fish serve as useful genetic models for evaluating pollution in aquatic ecosystems (Mitchell and Kennedy, 1992; Park and Erstfeld, 1997). The present study exhibits the genotoxic effects of Moragoda Ela water contaminated of various discharges of PAHs on erythrocytes of *Oreochromis niloticus* peripheral blood. According to the results obtained, there was a significant induction of nuclear abnormalities in peripheral blood exposed to Moragoda Ela water. The presence of micronuclei and induction of nuclear buds, fragmented apoptotic cells, binucleated cells, and altered nuclei have been considered a reliable approach in assessing the genotoxic effects of water contaminated with PAHs.

There was a significant induction of micronuclei, nuclear buds, fragmented apoptotic cells, binucleated cells and altered nuclei in Moragoda Ela water-treated groups compared to the control group. Micronuclei, nuclear buds and binucleated cells were absent within the control period, but there was some insignificant account of altered nuclei and fragmented apoptotic cells in the control group. This insignificant frequency of altered nuclei in the control group compared to that of nuclear abnormalities in the treated group resulted as the frequency of altered nuclei is a combination of blebbed nuclei, notched nuclei, and lobed notched nuclei and kidney-shaped nuclei. These results support the selection of nuclear abnormalities tests in fish erythrocytes as a sensitive model for screening the mutagenic activity of water-born chemical compounds under laboratory conditions (Pacheco and Santos, 1996). The inducement of ENAs by the Moragoda Ela water portrays its irreversible DNA damaging potential. There is a need to identify the toxicants causing this damage to the biological cells. According to previous studies, Moragoda Ela has been an active site of effluent and municipal discharges contaminated with different kinds of PAHs (Kanthi and Jayaweera, 2021). However, further detailed analysis is necessary to identify the various fractions of PAHs in Moragoda Ela water.

When considering the genotoxic effect of Moragoda Ela water contemplation with exposure time, the present study recorded an induction of frequency of altered nuclei and deduction of fragmented apoptotic cells within the exposure period with significant difference between two exposure groups. Genotoxic effects increase with increased exposure, and it cannot be excluded that long-term exposure to low concentrations of genotoxicants in surface water leads to significant genotoxicity in cells of fish and other aquatic organisms (Alink et al., 2007). However, the mechanism of its occurrence remains unknown, but certain organic genotoxicants can accumulate in freshwater organisms (Obiakor, 2010). Micic et al. (2002) discussed that such a type of deduction of fragmented apoptotic cells within exposure time is recognized as one of the main ways to eliminate abnormal cells from the bloodstream.

The current investigation has revealed that the Moragoda Ela water may have genotoxic consequences, posing a risk to the human population that depends on it. Because the identification and actual presence of these genotoxic-inducing substances in the water are un-

known, studies on the chemical condition of Moragoda Ela should be carried out to anticipate future pollution and avoid possible human genotoxicity.

CONCLUSION

According to the findings of this study, water contaminated with xenobiotic substances causes genotoxic effects in fish erythrocytes. The effect was time-dependent, with significant changes in various erythrocyte nuclear abnormalities occurring throughout the exposure period.

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