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Aquatic Genetic Biomarkers of Exposure and Effect in Catfish (*Clarias gariepinus*, Burchell, 1822)

[1]M.O. Obiakor, [2]C.D. Ezeonyejiaku, [3]C.O. Ezenwelu and [3]G.C. Ugochukwu

[1]Department of Environmental Management,
Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria
[2]Department of Zoology, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria
[3]Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka,
Anambra State, Nigeria & Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria

Abstract: River Oyi has been under drastic pollution stress of its water and requires extensive monitoring using sensitive biomarkers. Genotoxic alterations induced by River Oyi were investigated based on the karyomorphological analysis and micronucleus assay in *Clarias gariepinus* exposed to its water for 10 and 28 days, respectively. A standard control experiment containing groundwater of drinking quality was set up to monitor deviation. Fish exposed to the water had significantly higher ($P<0.05$) number of chromosomal aberrations and micronuclei compared to the control fish exposed at the specified period. After exposure of the fish to the River Oyi water for 28 days, significant ($P<0.05$) increase in the genotoxic capacity of the water was evidenced. The study further revealed dose and time response relationship and effect. River Oyi being a major tributary of the Anambra River, which serves large human population of the state, needs to be continuously monitored on sustainable basis to avert possible human genotoxicity and loss of aquatic biodiversity.

Key words: Genotoxicity · Micronucleus · Fish · River Oyi · Biomonitoring · Chromosomal aberrations · Dose-time response relationships

INTRODUCTION

Aquatic animals have often been used in assay to evaluate surface water [1, 2]. Substances displaying mutagenic, teratogenic and carcinogenic potentials are easily evaluated because of high sensitivity of these organisms to these pollutants on low concentrations [3-5]. Many toxic and potentially toxic chemical substances, some of which are of natural origin and others due to human activities are released into the fresh water ecosystem daily. It is difficult to practice even elementary hygiene without sufficient quantities of water free of these contaminants [6]. As such, it is necessary to protect the water sources themselves from faecal, agricultural and industrial contaminations (pollutants). In developing countries, 90 to 95 percent of all sewage and 70 percent of all industrial wastes are dumped untreated into surface water [6]. Due to the increasing environmental exposure to these agents, the need for monitoring terrestrial and aquatic ecosystems, especially in regions compromised by chemical pollution is paramount [7-10].

Genotoxicity is a deleterious action, which affects a cell's genetic material affecting its integrity [11, 12]. Several genotoxic substances are known to be mutagenic and carcinogenic, specifically those capable of causing genetic mutation and of contributing to the development of human tumors or cancers [13-18]. These genotoxicants have been reported to cause mutations because they form strong covalent bonds with deoxyribonucleic acid (DNA), resulting in the formation of DNA adducts preventing accurate replication [19, 20]. Genotoxins affecting germ cells (sperm and egg cells) can pass genetic changes down to descendants (Hartwell *et al.,* 2000) and have been implicated to be against sustainable development principles by WHO [11, 21], portraying them as significant factors in congenital anomalies, which account for 589,000 deaths annually.

Corresponding Author: Obiakor, Maximilian Obinna, c/o Ezeonyejiaku, Chigozie, D., Department of Zoology, Nnamdi Azikiwe University, P.M.B 5025, Awka, Anambra State, Nigeria.
Tel: +234(0)8038989932; E-mail: maxiugobiks@yahoo.com.

Biomarkers are biological responses to environmental chemicals at the individual level or below demonstrating departure from normal status [22, 23]. Biomarker responses may be at the molecular, cellular or 'whole organism' level. An important thing to emphasize about biomarkers is that they represent measurements of effects (Biomarkers of effect), which can be related to the presence of particular levels of environmental chemical (Biomarkers of exposure); they provide a means of interpreting environmental levels of pollutants in biological terms. Fish are excellent subjects for the study of the mutagenic and carcinogenic potential of contaminants present in water. This is so because they can metabolize, concentrate and store waterborne pollutants [24, 25].

Micronucleus (MN) assay is an ideal monitoring system that uses aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. Research reports maintained that it can be applicable to freshwater and marine fishes and that gill cells are more sensitive than the hematopoietic cells to micronucleus inducing agents [26]. Micronucleus formation in freshwater and marine fish is a function of water pollution. Kligerman [27] demonstrated that fish inhabiting polluted waters have higher frequencies of micronuclei. The micronuclei frequencies may vary according to the season, the kind of pollution involved and the species of fish. In laboratory tests involving fish, several substances have been shown to have genotoxic potential [28, 29]. An advantage of environmental monitoring based on genotoxicity studies, is that they reveal a measure of sublethal effects of xenobiotics in biological systems both *in vitro* and *in vivo*.

River Oyi, a tributary of Anambra River has been an active site of municipal and industrial wastewater effluent discharge including solid wastes. By and large, this river genotoxic potentials have been considered relatively high and thus, not rigorously monitored. It is to this effect that the study was designed to investigate the genotoxicity/ genotoxic alterations induced by water from the ecologically stressed River Oyi in freshwater fish, *Clarias gariepinus* (Burchell, 1822) after exposure.

MATERIALS AND METHODS

Fish Management and Exposure to River Oyi Water: Healthy specimens of 72 fish were procured from the local Otuocha market and treated with 0.05% $KMnO_4$ solution for 4 mins to clear any dermal infection. The fish were transported to the laboratory in plastic containers, so that they could be exposed to River Oyi water. Fishes were acclimated under laboratory conditions for 15 days prior to exposure and maintained under normal day- night light duration in order to prevent stress. The water in the aquaria was changed daily and fish were fed trice a day with the commercial feed diet. Therefore, the fish stocks were shielded from full impact of pollutants. The control water was natural groundwater of drinking-water quality. This water, which was not treated with chlorine or any other disinfectant, was aerated and rapidly filtered before distribution. Physico-chemical properties of the holding water during acclimation and that of Oyi River were determined by Standard APHA Methods [30] (Table 1). Every effort was made to provide healthy condition for fish and no mortality occurred during this period.

For the experiment, the fish were divided into three groups with 36 fish in the control (Group I) and 18 fish in Group II and III. Group I served as the primary control and was maintained under normal conditions of control water for the same periods of the experiment. The experimental Groups II and III were exposed to River Oyi water for 10 and 28 days, respectively. After the exposure period, two tests were carried out; chromosomal aberration and micronucleus tests.

Karyomorphological Analysis: Chromosomal Aberration Test: After the periods of exposure, 10 fish of all the groups were injected with 0.5% colchicine intraperitoneally 4hrs prior to dissection to arrest the metaphase stage as described by Okonkwo and Obiakor [31]. The fish were anesthetized with ethylene glycol and the kidneys and gill arches were dissected out for study. The kidneys and gill arches were subjected to chromosomal analysis according to the method described by Klingerman *et al.* [32]. More than 100 well-spread metaphase plates were analyzed for chromosomal aberrations at a magnification of X1500, under oil immersion for all the groups.

Micronucleus Test: Blood samples were obtained by caudal vein puncture using a heparinized syringe from 8 fish of all the groups. Micronucleus test was embarked upon as described by Obiakor *et al.* [33]. Microscopic slides were prepared for each fish. Scoring of micronuclei was carried out at X1000 oil immersion lens adopting the criteria of Fenech *et al.* [34]. Mean micronucleus (MN) frequencies and standard deviation were expressed as the number of MN per 5000 erythrocytes examined for each exposure period.

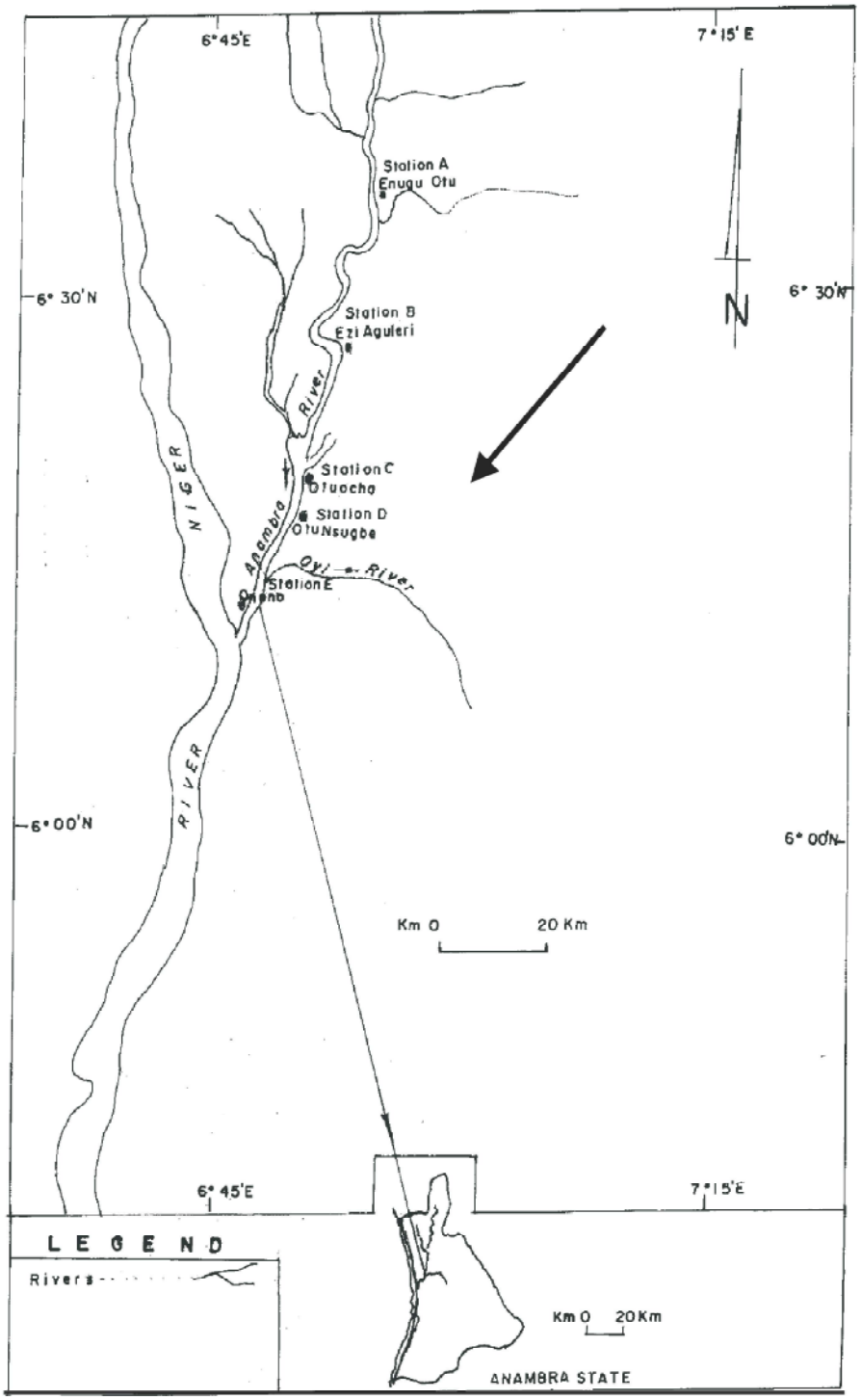

Fig. 1: Map of Anambra River showing River Oyi as its tributary (arrow) Source: Obiakor [36]

Statistics: Differences between groups were analyzed using the Student's *t*- test with an alpha error or limit of significance of 0.05 (i.e. $P<0.05$).

RESULTS

Table 1 shows the physico-chemical properties of the holding and River Oyi water prior to the experiment.

Table 1: Physico-chemical properties of the holding and River Oyi water

	Temperature (°C)	pH	Hardness (Mg/l)	Dissolved oxygen (Mg/l)
Holding water	27.2 ± 0.08	6.3 ±0.08	10.0 ±0.0	3.9 ±0.12
River Oyi water	27.1 ±0.08	6.2 ±0.08	10.0 ±0.0	3.7 ±0.94

Table 2: Frequency of chromosomal aberrations induced in fish gill and kidney cells of *Clarias gariepinus* (Burchell, 1822) after exposure to River Oyi water for 10 and 28 days, respectively

Group	Evaluated metaphase number	CB	F	AF	RC	Total number of aberrations	Occurrence (%)
I	103	3	3	1	0	7	6.25
II	120	17	19	7	1	44	39.29*
III	131	21	29	8	3	61	54.46**

CB: Chromosome break; F: Fragment; AF: Acentric fragment; RC: Ring chromosome.

* = Significantly different (P<0.05), compared to the control.

** = Significantly higher (P<0.05), comparing Group II and III.

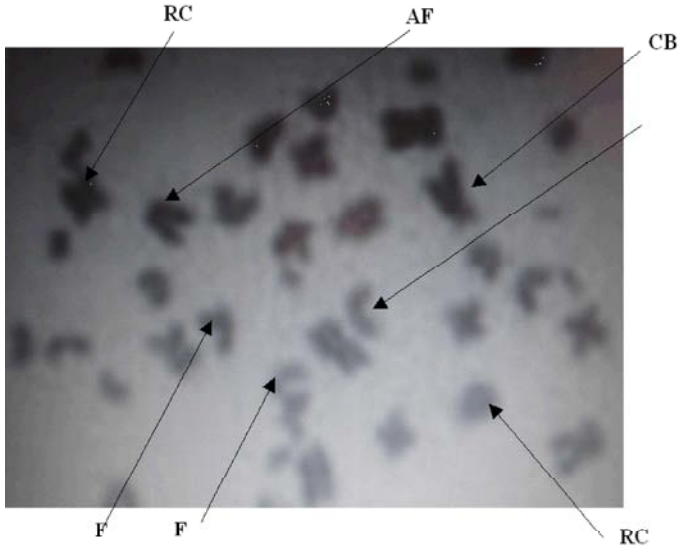

Fig. 2: Metaphase spread with arrows showing the observed chromosomal aberrations in gill cells of *Clarias gariepinus*, Burchell 1822) after exposure to River Oyi water for 10 and 28 days, respectively; CB: Chromosome break; F: Fragment; AF: Acentric fragment; RC: Ring chromosome.

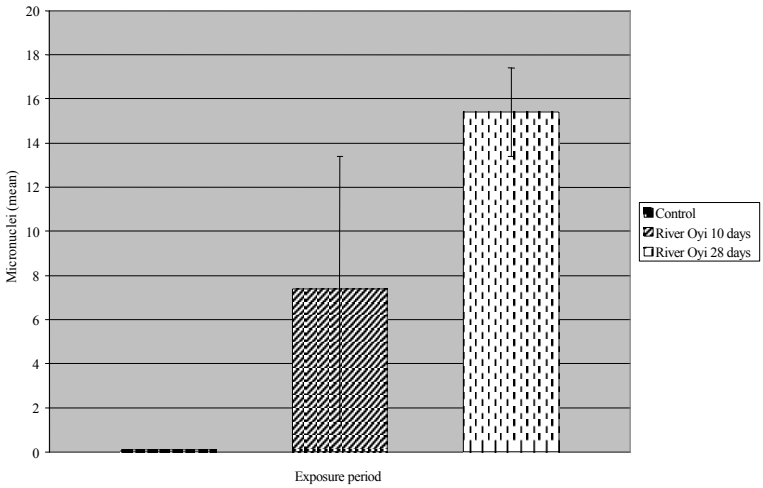

Fig. 3: Relative abundance of the micronuclei formed in the blood of *Clarias gariepinus* (Burchell, 1822) at 10 and 28 days of exposure to River Oyi water. Bar shows mean ± S.E.M.

The chromosomal aberrations observed in the fish species (Group I-III) exposed to the River Oyi water and that of the control for 10 and 28 days, respectively are shown in Table 2 and Figure 2. The experimental Group II and III were higher ($P<0.05$) in the induced chromosomal aberrations than Group I (primary control). Comparison between Group II and III revealed statistical difference, with Group III of 28 days exposure being higher ($P<0.05$).

The relative abundance of the micronuclei formed in the blood of the test fish species at different exposure periods is shown in Figure 3. Differences ($P<0.05$) were observed between the control and the two groups, Group II and III, respectively.

DISCUSSION

As the present study revealed, River Oyi water contains genotoxicants that are able to induce chromosomal aberrations and irreversible DNA damage evidenced by the micronuclei formation during the periods of exposure (10 and 28 days). The two periods of exposure showed significantly different effects (chromosomal aberrations and micronuclei) between the groups. This suggests dose and time dependent effects as earlier reported by Alink *et al.* [35].

Okonkwo and Obiakor [31] had earlier documented the diploid chromosome number and karyotype of *Clarias gariepinus* (Burchell, 1822) from the Anambra River and came up with the fact that the species possesses 56 chromosomes (28 pairs). Our findings on the diploid number of the same species are consistent with that report. Follow-up and confirmatory karyological analysis of similar species from the River Oyi also attests to the same report. This karyological similarity could be explained by the geographic relationship and proximity between the two rivers, as River Oyi discharges into the Anambra River, which enable free intermingling of the respective biodiversities. Support hypothesis explaining the observed factual similarity could also be that we may have sampled migrant *Clarias gariepinus* from the Anambra River in the Oyi River.

Observations in the current study revealed chromosomal aberrations such as chromosome break, fragment, acentric fragement and ring chromosome. The mechanism behind these cytogenetic abnormalities induced by the River Oyi water may involve disruption of cell division [31], DNA synthesis and repair. Consequently, Alink *et al.* [35] have reported sister chromatid exchange (SCE) in Eastern mudminnow (*Umbra pygmaea* L.) after exposure to surface water of River Rhine used for drinking in The Netherlands.

The inducement of micronuclei by the River Oyi water portrays its irreversible DNA damaging potential. There is need to identify the toxicants causing this damage to the biological cells. Generally, the compounds causing these effects (chromosomal aberrations and micronuclei) are unknown. River Oyi has been an active site of effluent and municipal discharges of unknown origins, concentrations and transport pathways for decades now. Obiakor [36], reporting on the material loading of the Anambra River, ecologically implicated River Oyi to be the major contributor of its pollution status since no rigorous and coordinated monitoring exercise has been initiated to reduce the pollution stress of the river.

As shown in this eco-survey, exposure for 28 days revealed high ($P<0.05$) damage frequency in the genetic system of the test fish species. 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 marked genotoxicity in cells of fish and other aquatic organisms [35]. However, the mechanism of its occurrence remains unknown but certain organic genotoxicants can accumulate in freshwater organisms. Bioconcentration factors (BCF) have been reported by Casserly *et al.* [37], Lu *et al.* [38], Mailhot [39] and effects detected using number of biomarkers [40, 17].

The present study has shown the possible genotoxic effects of the River Oyi water and as such poses a threat to the dependent human population. Studies should be carried out on the chemical state of River Oyi since the identity and actual presence of these genotoxic-inducing agents in the water are not known in order to forestall further pollution and avert possible human genotoxicity, which is capable affecting the genetic materials of the future population.

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