INDUCTION OF MICRONUCLEI ABNORMALITIES IN WILD POPULATION OF *Sarotherodon melanotheron* ALONG A CONTAMINATION GRADIENT OF A TROPICAL LAGOON ECOSYSTEM

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ABSTRACT
The urgent need for more sensitive indicators in biomonitoring of aquatic pollution prompted the assessment of potential changes in water quality and micronuclei induction in the erythrocytes of Sarotherodon Melanotheron from the Lagos Lagoon using standard methods and procedures. Physicochemical analysis of surface water revealed varying concentrations of measured parameters with relatively higher levels in the dry season. The obtained levels from the evaluation of lagoon surface water quality did not exceed the Nigerian Federal Ministry of Environment limits set for environmental protection and the condition factor of fish used for the study were within optimum range. The results of the micronuclei test revealed possible nuclear alterations as observed by higher ratio of immature erythrocytes. Although there was no significant difference in the observed immature cells, relatively higher mean values for immature cells (219.0± 98.31) was recorded in the dry season compared to the wet season (89.33 ± 2.03), suggesting potential haemolytic anaemia during the dry season. Significantly ($p < 0.05$) higher mean values of micro-nucleated cells (99.67 ± 12.84) were also recorded in erythrocytes of fish during the dry month of May. From the results, the importance of using the micronuclei frequency (MN) and other nuclear abnormalities in socioeconomically important fish species to provide in situ biological indicators of the effects of anthropogenic activities in coastal waters cannot be overemphasized.

Keywords: Micronuclei, Fish erythrocytes, Black Jaw Tilapia, Lagos lagoon, Genotoxicity

INTRODUCTION

The expansion and unregulated anthropogenic perturbations of highly productive estuaries and lagoon ecosystems are perhaps one of the most serious threats to Nigerian coastal waters. The consistent and sometimes severe pressure on one of such socioeconomically important ecosystems, the Lagos Lagoon complex, has been attributed to the diverse forms of human activities emanating from the surrounding city centers (Amaeze *et al.,* 2012). Consequently, a number of studies have been carried out basedon chemical monitoring of surface waters and theassessmentof bioticcommunities for pathology and bioaccumulationof non-biodegradable compounds toillustratethe rise in pollution within the Lagoon (Amaeze *et al.,* 2014) over the years.However,investigation of genotoxic potentialhas become necessary inthe monitoring of pollution inhighly impacted aquatic ecosystems due to the continued production and release of toxic and hazardous substances.

Micronucleus test, one of the most popular and promisingtest of environmental genotoxicity has served as an index ofcytogenetic damage (Fenech *et al.,* 2003). As demonstrated by several researchers, it is a sensitive biomarker of genotoxicity and for testing the pollution of aquatic ecosystems due to easy sampling, technical feasibility and high sensitivity (Ayllon and Garcia-Vazquez, 2000; Bolognesi and Hayashi, 2011; Carrasco *et al.,* 1990; Heddle *et al.,* 1991). Micronuclei is cytoplasmic chromatic masses with the appearance of small nuclei that arise from chromosome fragments or intact whole chromosomes lagging behind in the anaphase stage of cell division. The occurrence of micronuclei has been adopted as a good substitute for the chromosomal assay; and based on the frequency of micronuclei, a monitoring system for potential genotoxicty of an agent has been proposed (Al-Sabti and Metcafe, 1995; Guha and Khuda-Bukhsh, 2002).In addition, micronuclei records reflect action of clastogenic or aneugenic compounds in tissues with actively dividing cells (Heddle *et al.,* 1991).According to Heddle *et al.,*(1991), their presence in cells is a reflection of structural and/or numerical chromosomal aberration arising during mitosis. However, in fish erythrocyte,micronuclei (MN) and Nuclear Abnormalities (NAs) may appear spontaneously and their frequencies may be season-dependent (Bolognesi *et al.,* 2006).

Over the years, many in-vivo and in-situ studies have been carried out on aquatic invertebrates and fishes to investigate genotoxic effects on aquatic organisms. Fish provide a suitable model for monitoring aquatic genotoxicity and wastewater quality as a result of their ability to metabolize xenobiotics and accumulate

pollutants (Grisolia and Starling, 2000). Both organic and inorganic compounds including toxic metals have been shown to be chromosomal genotoxicants eliciting invivoformation of erythrocytic micronuclei (MN) in fish (Fenech, 2000; Sanchez-Galan *et al.,* 1999).Hence, chromosomal and cytogenetic studies on fish have received considerable attention in recent years, (Galetti *et al.,* 2000; Okonkwo and Obiakor, 2010; Ozouf-Costaz and Foresti 1992). The application of the micronuclei test has also been adapted to both *ex situ* lab conditions (Al-Sabti 1995, Belpaeme *et al.,* 1996) and *in situ* bio-monitoring (Odeigah and Osanyipeju 1995, Porto *et al.,* 2005). Sensitivity of fish species towards the induction of erythrocytic MN and other nuclear abnormalities has been reported in both laboratory and field studies (Akinrotimi *et al.*,2007; Adeyemo, 2007; Bolognesi and Hayashi, 2011; Gabriel *et al.,* 2004).

The black jaw tilapia, *Sarotherodon melanotheron*, is a pale (variable light blue, orange, golden yellow) cichlid whose common name refers to the dark pigmentation usually (but not always) concentrated on the underside of the head (the chin) in adult animals. In the wild, they feed on benthic diatomsand filamentous algae. Despite the numerous investigations in literature on aquatic pollution in the Lagos lagoon complex, limited number of studies emphasises on the mutagenic hazard and safety of edible fish exposed to organic and inorganic pollutants in this ecosystem.Since seafood is constantly relied upon as a source of protein and economic power to the public, it becomes imperative to highlight the integrity of the water quality and edible fish following the enormous anthropogenic activities in and around the Lagos lagoon.The analysis of environmental genotoxicity provides early warning signals of adverse long-term effects of the contamination (Rybakovas *et al.,* 2009). Hence, this study was carried out to assess water quality parameters andto determine the environmental risk of anthropogenic contamination to wild population of this socioeconomically important ichthyofauna in the Lagos lagoon using micronuclei abnormalities in the erythrocytes of *Sarotherodon melanotheron*.

2.0 MATERIALS AND METHODS

2.1 Description of Study Sites

The Lagos lagoon lies between longitude 3^{o} 23and 3^{o} 43''E and between latitude 6^{o} 22and 6^{o} 38''N. It forms part of an intricate system of water ways made up of lagoons and creeks that are found along the coast of Nigeria from the Republic of Benin border to the Niger Delta (Fig 1). Samples of *Sarotherodon melanotheron* obtained from three sites along the coast of and western axis of the Lagos lagoon

between latitude 6^o 31, 27.94'' N and longitude 3^o 23, 58.32''E (Bariga),latitude 6^o 30, 52.74''N and 3^o 24, 14.40''E (behind the University of Lagos Vice Chancellor's lodge) and between 6^o 29, 52.71''N and 3^o 23, 46.26''E (Okobaba).

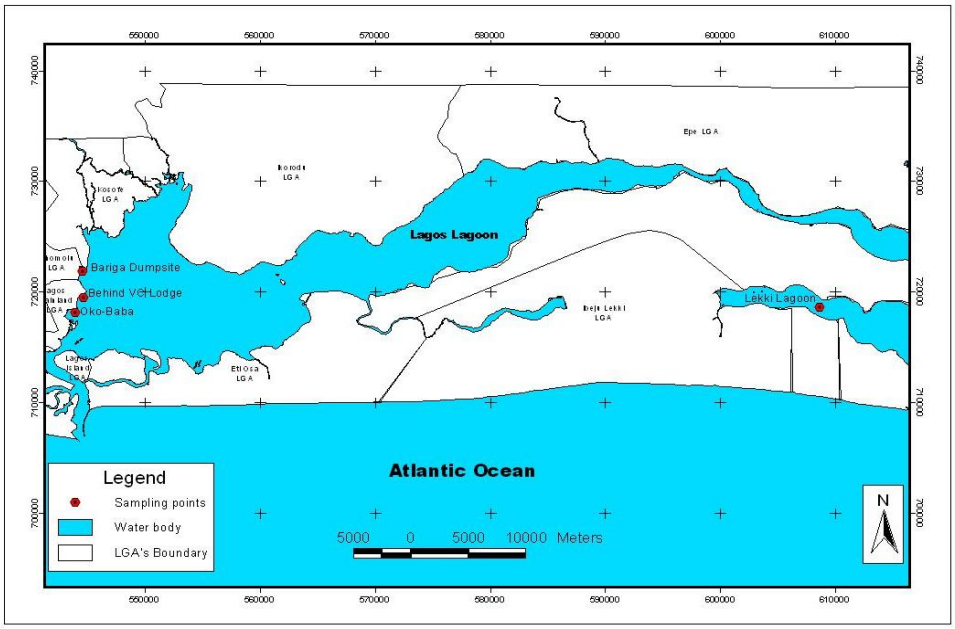

Fig 1: Lagos Lagoon complex showing sampling sites

In addition to designated study locations on the Lagos lagoon, complementary fish samples were obtained from locations on the Lekki lagoon as a control for comparative evaluation of the pollution and stress status of both ecosystems. The Lekki lagoon situated in Lagos state, Nigeria has a surface area of about 247km^{2}and a depth of about 6.4m at low tide. Lekki lagoon lies between longitude 4^0 00' and 4^0 15' and latitude 6^0 25' and 6^0 37'N. However, most parts of the lagoon are generally shallow and less than 3.0m deep. The Lagoon opens into the sea via the Lagos lagoon and harbor.

2.2 Collection of Water Samples and Physicochemical Analysis

2.2.1 Insitu Determination of Physicochemical parameters ofSurface Water samples

Water samples collected using one litre plastic bottles were preserved in a cool dry place and transported to the Aquatic Toxicology and EcophysiologyLaboratorylocated in the Department of Marine Sciences, University of Lagos for further processing and analysis. pH, Conductivity and

Total dissolved solid of surface water samples weredetermined in-situ using HANNA's water proof PH/EC/TDS meter ((Model; HI1991301). The turbidity of the water sample was measured using HACH DR 2000 direct reading spectrophotometer method 8237. The temperature was determined by means of simple mercury in glass thermometer calibrated in degrees centigrade (^{0}C) while the salinity of the water samples was determined using a portable salinometer. Water samples for heavy metals analysis (50 mls each) were acidified with 2 drops of concentrated HNO_3 (APHA, 2005) before transporting cool in icepacks to the laboratory for further processing and analysis.

2.2.2 Laboratory Determination of Chemical parameters in Water samples

Chemical oxygen demand (COD), biochemical oxygen demand (BOD)andtotal suspended solids (TDS) were determined according the American Public Health Association standardized methods (APHA, 2005) while alkalinity and sulphate were determined by titration method. Dissolved oxygen was measured by using the Gritting Oxygen Meter (Model 40) while Nitrate-nitrogen and phosphate-phosphorus were determined by the Colorimetric method. Total metal (Fe, Pb, Zn, Ni and Co) contents in surface water samples were measured by flame atomic absorption spectrometry (AAS 2000 Series Bulk Atomic Absorption Spectrophotometer) according to the method described by Farounbi *et al.*,(2007).

2.3 Collection of Fish samples, *Sarotherodon Melanotheron* from the Wild

Samples of *Sarotherodon melanotheron* were obtained from the wildfor a six-month periodusing gill and cast nets of 1-3cm diameter (Ayoola and Kuton, 2009). The range of length and weight of examined fishes from both Lagoons was 13.5 – 21.5cm and 2.5g and 142.5g respectively (Table 1).The condition factor (K), an indication of changes in the food reserves stored in the muscles and the well-being of individual fish was calculated thus according to the method of (Le Cren, 1951);

$$K = \frac{\text{Total body weight x } 100}{(\text{Total length})^3}$$

2.4 Collection of Blood Samples and Micronuclei Test (MN)

Blood samples obtained from a puncture to the caudal vein of examined fish samples using heparinised syringes were immediately smeared onto clean glass slides, air dried overnight, and then fixed with 5% absolute methanol for 15 min. Each slide was stained with 5% Giemsa solution for 20 min. The erythrocytes for each *Sarotherodon melanotheron* specimen were identified, counted and scored

microscopically under 1000× magnification.Differential cells counting and Micronucleus induction test was carried out at the laboratory located in the Department of Cell Biology and Genetics of the University of Lagos. The micronuclei test was performed according to the methods of Grisolia and Cordeiro (2000) with some minor modifications. The main criteria for scoring the micronucleus (MN) were based on those of Al-Sabti and Matcalfe (1995). The frequencies of micronuclei induction were calculated for each individual/slide of *Sarotherodon melanotheron*and expressed in percentage as per 1,000 cells.

2.5 STATISTICAL ANALYSIS

Data analysis was performed using computer statistical package SPSS 10.0 and graphical representations were drawn using Microsoft Excel software and represented asmean, range (upper and lower limit of several values). One-way analysis of variance (ANOVA) was used to test for statistical differences in the means of the scored cells recorded during the study period.Pearsoncorrelation coefficient wasalsoused to compare the relationship between the cell counts and chemical parameters (heavy metals) of water.

3.0 RESULTS

3.1 Physicochemical Parameter of Lagos Lagoon Surface Water

Temporal variations in the physicochemical characteristics of surface water samples from the Lagos lagoon during the study period is presented in Table 1. Surface water temperature ranged from 25.3 - 31.9 °C and 25 - 27.2 °C during the dry (February - April) and wet (May-July) months respectively. Ph ranged from 6.00 - 7.40 while turbidity varied from 0.04-0.13 FTU. Conductivity fluctuated between 468 – 45770NSem^{-1}with the highest mean conductivity value of 5004.67NSem^{-1}recorded in the wet months. Alkalinity which varied from 124 – 20mg/L recorded the highest mean value of 104.03mg/L in the drymonths. Salinity exhibiteda marked seasonal variation with the highest mean value of 18.46‰ recorded in the dry season.Measurable variations were observed for examined nutrient parameters with relatively higher mean values also recorded in the dry months. Sulphate (SO_4^{2-}) concentrations ranged from 0.10 - 12.80mg/L while nitrate (NO_3) values which ranged between 0.03.2 - 8.62mg/L recorded an average value of 6.06 mg/L in dry months and 0.88mg/L in the wet months. Meanwhile, phosphate (PO_4)levels ranged between 0.02 – 1.52 mg/L. Dissolved oxygen concentration ranged from 4.1 - 5.93 mg/L and 25.3 - 31.9 mg/L with a mean value of 4.42 mg/L and 5.12mg/L for the dry and wet season respectively. BOD values range between 1.0 - 5.0 mg/L with a higher mean value of 3.22 mg/L

in the dry monthsas compared to the wet months (1.37 mg/L). Chemical oxygen demand (COD) ranged between 5.0 – 13 mg/L with highest mean value recorded in the dry months (9.61 mg/ L). Notable differences were observed for Total suspended solids (TSS) and total dissolved solids (TDS) during the study periodwith higher mean values for both parameters also recorded in the dry months. The obtained TSS values ranged from 4.00 -83.00 mg/L while TDSranged from 102 – 381mg/L.

Table 1: Summary of Physicochemical parameters of the Lagos lagoon water surface water during the dry and wet months (February to July)

Parameters			**MEAN**	
	MIN	**MAX**	**DRY**	**WET**
pH	6.00	7.40	6.99	6.48
Turbidity (FTU)	0.03	0.13	0.08	0.04
Conductivity (NSem-1)	468.00	45770.00	34646.67	5004.67
Alkalinity (mg/L)	20.00	124.00	104.03	25.11
Salinity (ppt)	1.00	21.89	18.46	6.62
SO_4^{2} (mg/L)	0.10	12.80	8.43	1.12
NO_3 (mg/L)	0.03	8.62	6.06	0.88
PO_4 (mg/L)	0.02	1.52	0.90	0.15
DO (mg/L)	4.00	5.93	5.12	4.42
Water temperature (^oC)	25.00	31.90	27.90	26.13
BOD(mg/L)	1.00	5.00	3.22	1.67
COD(mg/L)	5.00	13.00	9.00	9.61
TSS(mg/L)	4.00	83.00	65.56	10.00
TDS(mg/L)	102.00	381.00	287.22	191.33
Fe (mg/L)	0.09	18.14	14.50	3.20
Pb (mg/L)	0.01	0.07	0.06	0.02
Zn(mg/L)	4.70	78.00	58.10	12.24
Ni (mg/L)	0.00	0.16	0.08	0.01
Co (mg/L)	0.00	0.00	0.00	0.00

3.1.1 Concentrations of Trace Metals in Lagos Lagoon surface water

Generally, examined trace metals were detected in low but measurable concentrations in Lagoon surface water (Table 1.)Leadranged from 0.01-0.07 mg/L insurface water with amean concentration of 0.06 mg/L and 0.02mg/L in

the dry and wet season respectively.Slightly lower levels which range from 0.00-0.16mg/L and a mean concentration of 0.08mg/Lwere recorded for Ni during the study.Meanwhile Co levels were below detection limit used in this study. As compared to other trace metals, relatively higher mean values of 58.10mg/Lwas recorded for Zn in the dry season than the wet season (12.24mg/L).

3.2 Condition Factor (K) of *Sarotherodon melanotheron*

The results of the condition factor (K) of *Sarotherodon melanotheron* obtained from the Lagos lagoon for the present study is shown in Table 2. The condition factor calculated for *Sarotherodon melanotheron* using the length-weight relationship showed that the mean k-value was 1.55. The mean length and weight of examined fishes ranged from 13.5-21.5cm and 2.5- 142.5grespectively.

Table 2: Morphometric parameters and condition factor values for *Sarotherodon melanotheron* from the Lagos and Lekki lagoon

Month	Length (cm)	Weight (g)	Condition Factor (K)
February	13.5	2.5	0.10
March	16.5	88.3	1.97
May	18	90.1	1.54
June	21.5	142.5	1.43
July	20	120.3	1.50
Mean	17.9	88.74	1.55

3.3 Genotoxic Indices of Stress in *Sarotherodon melanotheron*

There was a wide variation in the proportion of micronuclei cells observed in the erythrocytes of *Sarotherodon melanotheron* from the study locations. The result shows relatively higher mean value of immature erythrocytes in the dry months than the wet months (Table 3).Observed mean total immature erythrocytes ranged from 89.33 ±2.03 in July to 219.0 ± 78.31 in May. Irregular and inconsistent number of micro nucleated cells which varied from 5.67 ± 2.33 - 99.67 ± 12.84 was observed in examined fish samples throughout the study period. However, the highest mean number of micro nucleated cells was recorded in fish samples caught in the month of May while the lowest was recorded in the month of February.Further analysis of the data using One-way ANOVA showed that the scored micro nucleated and immature cells to detect damaged chromosomes (Fig. 2) in examined *Sarotherodon melanotheron* samples was also significantly different ($p < 0.05$)inthe month of May.

Table 3: Frequency of micronuclei and immature cell in peripheral erythrocytes of *Sarotherodon melanotheron* from the Lagos lagoon

Sampling Month	Total no of cells scored	Micro nucleated cells (mean ± SEM)	Immature cells (mean ± SEM)
February	10,696	5.667 ± 2.333	93.33 ± 33.41
March	16,131	86.33 ± 32.13	173.3 ± 45.08
May	14,933	99.67 ± 12.84	219.0 ± 78.31
June	12.119	60.67 ± 3.383	91.00 ± 3.786
July	11,283	28.67 ± 4.372	89.33 ± 2.028

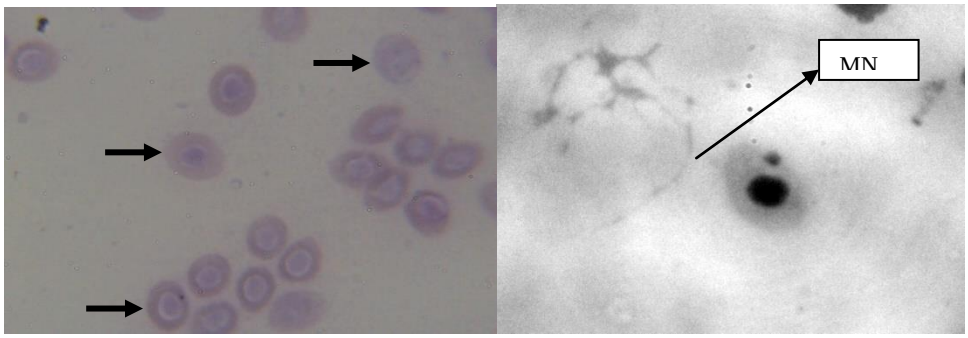

Fig.2: Photomicrograph of Immature, mature and micro nucleated cells (MN) in erythrocyte of *Sarotherodon melanotheron*

4.0 DISCUSSION

Increasing pollution from unregulated anthropogenic activities may affect the physicochemical characteristicsand stability of aquatic bodies as well as cause developmental and genotoxic changes in aquaticinhabitants. Almost all examine water quality parameters were higher in dry season (February – April) than in the wetSeason (May – July). This obtained results is similar to the report of Onyema *et al.*, (2010) for the physicochemical characteristics of the Barbeach. This may be attributed to seasonal variation which caused influx of fresh water in the system from rainfall which diluted the water body. The mean water temperature of 27.90°C and 26.13°C recorded during the dry and wet season respectively was in accordance with the range (20-30°C) suggested by the Federal Ministry of Environment (FEMv, 1999)for optimum physiological state of fish. Dissolved oxygen (DO) required for the metabolism of aerobic organisms which depends highly in part on temperature is often used as an indicator of water quality. In the present study dissolved oxygen (DO) levels fell within optimum range for survival of fish in warm water. This observation also reflected in the mean values of BOD and COD which were within the range for water suggested by Federal

ministry of Environment (FEMv, 1999).On the other hand, the marked seasonal variationexhibited by salinity with highest mean valuerecorded in the dry season suggest influences of precipitation and tidal mixing.

In biomonitoring ofaquatic ecosystem health, gross health indices such as the condition factor CF, have been accepted as integrative indicators of general fish condition to provide information on the ability of animals to tolerate environmental stresses. Though the CF might be quite general, non-specific and does not give information of specific responses to toxic substances in the media (Linde-Arias *et al.,* 2008), studies have shown that it is a valuable tool that indicates the impact of environmental alterations on fish performance (Barton, *et al.,* 2002) and the general effects of pollution in fish (Van der Oost, *et al.,* 2003). The low CF values for fishes obtained in the dry month of February compared to other monthsmaybe indicative of the impairedconditions and the higher environmental stress affecting the fish.

Changes of erythrocyticnuclei have also been increasingly used to evaluate genotoxiceffects of different compoundsincluding heavy metals in aquatic ecosystem.As reported by Francoise *et al.,* (2011), the steady increase in emission ofheavy metals in natural waters due to various industrial andagricultural activities have led to the damage in geneticmaterials,causing further genotoxic effects in exposed organisms including fish.Furthermore, a direct relationship between the MN frequencies and heavy metal concentrations in water, although with differential species sensitivity have reported in literature (Al-Sabati, 1995, Sanchez-Galan *et al.,* 2001).In the present study, Fe, Zn and Pb in surface water were found to exceed the permissible level of 0.03, 5, 0.05 and 0.02 mg/L respectively recommended by WHO (2003) and United State Environmental Protection Agency (USEPA, 2003) for surface water. Generally, variations in levels of examined heavy metals in this study could be attributed to the quality and quantity of thedrainage water as well as a variety of interacting environmentalfactors.

Over the years, fish micronuclei assayrepresents a sensitive means of measuring genotoxic activity in the laboratory and field studies.However, genotoxic damage mainly depends on the type of pollutant involved and fish species exposed to that pollutant (Ali *et al*., 2008). In this study, a clear seasonal variation was observed in the frequency of micronuclei and nuclear abnormality. Higher levels of immature cells were observed in the dry months suggesting the presence of hemolytic anemia. In a related study, Osman *et al.,* (2011) detected six nuclear

lesions (NL) beside micronuclei (MN) in the blood of Nile tilapia *Oreochromis niloticus* and African catfish *Clariasgariepinus* collected from the whole course of the river Nile. According to their report, higher incidences of MN and NL were found in the blood of fish collected from the heavily polluted areas. A significant increase in the frequency of MN in specimens from rivers contaminated with chemical substances was also reported by Sanchez-Galan *et al.,* (1998) for *Salmo trutta* and for *Zacco platypus* by Hayashi *et al,*(1998). Similarly, Grisolia and Starling (2001) showed a significant increase in the number of micronuclei in specimens of *Cyprinuscarpio*, *Oreochromis niloticus*, and *Tilapia rendali* at the Paranoa lake (Brazil) affected by the discharge of municipal waste waters. In another study, Al-Sabti (1994) demonstrated an increased frequency of micronuclei (MN) in erythrocytes of Prussian carp from Slovenian rivers contaminated by sewage pollutant and chromium II and IV from the leather industry. The results of the present study therefore indicated that wild population of *Sarotherodon melanotheron* in Lagos and Lekki lagoons are exposed to contaminants with genotoxic potentials and demonstrated the importance of using MN frequency and other nuclear abnormalities in different socioeconomically important fish species to provide *in situ* biologicalindicators for biomonitoring anthropogenic impacts on coastal waters. However, further studies are needed to better elucidate the relationship between heavy metals and genotoxicity on edible fishery resources such as *Sarotherodon melanotheron* so as toprovide supportfor further studies inseverely impacted areas of the region.

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