

Effect of Season on Genotoxicity and Heavy Metal Accumulation in *Oreochromis niloticus* and *Clarias* gariepinus from the Lower River Niger, Nigeria

*EJIKEME, C.E.¹, AYOOLA, S.O.¹AND AKINOLA, M.O.² ¹Department of Marine Science, University of Lagos. ²Department of Cell Biology and Genetics, University of Lagos. **Corresponding Author* E-mail: ejikemecharity@gmail.com

Abstract

The level of heavy metal concentration and genotoxicity was assessed in both Oreochromis niloticus and Clarias gariepinus from the Niger River, Nigeria. Fish samples were collected both during the dry and wet season between April, 2017 and September, 2018. Aluminium (Al), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni), Zinc (Zn), Mercury (Hg), Arsenic (As), and Lead (Pb) concentrations in fish samples were also analysed using Inductively Coupled Plasma Optical Emission Spectrometroscopy (ICP-OES). Result showed higher accumulation of heavy metals in both fish tissues in the wet season than the dry season. The highest accumulated heavy metal in the fish sample was Fe during the dry seasons [(Fe (O. niloticus) = $64.28\pm12.93 \text{ mg/g}$, Fe(C. gariepinus)] = $96.06\pm58.36 \text{ mg/g}$ and wet season [Fe(O. niloticus) = $67.42\pm14.11 \text{ mg/g}, Fe (C. gariepinus)] = 99.16\pm59.36 \text{ mg/g}).$ Heavy metal accumulation evaluated followed the trend Fe > Al > Cd > Zn > Pb > Cu > As > Ni > Cr > Hg for the dry season Al > Zn > Pb > Cu > Cr > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cd > As > Hg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry season and Fe > Al > Lg for the dry seaso Cr > Cd > Hg > As for the wet season in C. gariepinus. Most of these heavy metals were beyond international permissible limits. The four polymorphic RAPD markers used in the study showed more polymorphisms in C. gariepinus than O. niloticus in both the dry and wet season. There was no difference in the polymorphic bands between dry and wet season in O. niloticus while more polymorphic bands were observed in wet season (50 bands) than dry season (47 bands) tissues of C. gariepinus. The result indicated a lower Genomic template stability (GST) in C. gariepinus (dry = 2.70, wet = 5.41) than O. niloticus (dry = 13.16, wet = 13.16) when compared in both seasons. This study revealed the level of toxic metal accumulation and genotoxic potentials of these fishes in the food chain of locals consuming them. Drastic measures are of high necessity in order to protect both the aquatics and potential consumers in the area.

Keywords: Heavy metal, RAPD, Genotoxic, Polymorphism, fish.

Introduction

The contamination of the aquatic ecosystem by pollutants has become a topical issue of

considerable concern in many parts of the world and has led many researchers to investigate their occurrence, distribution, toxicity to aquatic organisms and concentrations in several

ecosystems (Ademoroti, 1996). Over the past few decades, the contamination of freshwater with heavy metal has become a matter of concern (Izuchukwu et al., 2017). Freshwater fishes tend to accumulate heavy metals in their organs more than marine fishes (Montazer and Ali, 2018) because freshwater fishes tend to lose salts and gain water and are more exposed and vulnerable to heavy metal pollution (Kallel et al., 2017). River Niger, the third longest river in Africa and the fourteenth longest in the world, is not just nature's gift but has the potential to generate power, promote national integration, engender productivity and create jobs, business opportunities and livelihood for millions of Nigerians. Over the last decades, lower River Niger environment has witnessed rapid urbanization; agricultural practices and industrial development leading to increase in pollution of the water body. These have triggered some serious concerns for the safety of the environment and final consumers of its biota.

Globally, rivers and sea bodies are becoming receptacles for chemicals, organic and heavy metal pollutants. The contamination of natural waters by heavy metals depreciates aquatic biota and poses considerable environmental risks and concerns (Akan et al., 2012). Heavy metals have been a major and serious threat to human health and ecosystems integrity (Ogbomida et al., 2018; Ezemonye et al., 2019). This occurrence of heavy metal contaminants in excess of natural loads has become a problem of increasing concern (Abdelaiz et al., 2019). Heavy metals are specifically important due to their toxicity and ability to bio accumulate in aquatic biomes (Ambedkar and Muniyan, 2011). Heavy metals can be incorporated into food chains and absorbed by aquatic organisms to a level that might affect their physiological state (Alipour and Banagar, 2018). Heavy metal concentrations in aquatic organisms depict the past as well as the current pollution load in the environment in which the organism lives (Beheary et al., 2015). Toxicity from heavy metals is of major environmental and health concern worldwide, as they bio accumulate along the food chain and may pose health risk to consumers (Afshan et al., 2014; Salarizadeh and Kavousi, 2015). Various metals are accumulated

in fish body in different amounts due to differences resulting from different affinity of metals to fish tissues, different uptake, deposition and excretion rates. Heavy metal bioaccumulation by fish and subsequent distribution in organs is greatly interspecific. In addition, many factors can influence metal uptake like sex, age, size, reproductive cycle, swimming pattern, feeding behaviour, and geographical locations (Kallel *et al.*, 2017).

Fish, being major components of most aquatic habitats, are largely used for the assessment of aquatic environment quality and are accepted as bio-indicators of environmental pollution (Borkovic et al., 2008). Fish, as a bio indicator species, plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The analyses of water or sediment samples, however, are subject to a variety of shortcomings, in that the methods do not allow for the estimation of the quantity of the metal which is biologically available (Etim et al., 1991). C. gariepinus and O. niloticus are commonly used in ecotoxicology studies since they are readily available; have high consumer acceptability; high resistance to poor water quality; amenability to laboratory testing, economic importance and are excellent piscine model for toxicological studies.

The effects of heavy metal pollutants on organisms have been determined using several methods and techniques which include gene expression of metallothionein, micronucleus assays, comet assays and molecular marker techniques (Juhari, 2014). However, the development of molecular technology has provided suitable tools for DNA analysis in the field of genotoxicology (Tanee *et al.*, 2016).

Several markers have been used in toxicological studies; however, random amplified polymorphic DNA (RAPD) has been mostly used to detect various types of DNA damage and mutations (point mutations, rearrangements, and small deletions or insertions). Furthermore, RAPD banding profiles can be scored for genomic template stability (GTS) analysis to detect changes in DNA. This technique has been successfully applied to the study of DNA damage and mutation caused by heavy metals in both plants and animals (Juhari, 2014). Hence, this study to elucidated the genotoxic effect of



Figure 1: Lower River Niger showing the sampling stations **Source:** Geo-informatics and Surveying Department, University of Lagos

some heavy metals in two fish species, *O. niloticus* and *Clarias gariepinus* using RAPD markers.

Materials and Methods

Description of Study Area

This study was conducted in twelve georeferenced sampling sites along lower River Niger, in the south eastern part of Nigeria (Figure 1). The geographical coordinates of the sampling stations were obtained using Geographical Position System (German 72 model) with position accuracy less than 5m.

Collection of sampled fishes

Sampled fish species (*O. niloticus* and *C. gariepinus*) were collected between the months of April, 2017 and September, 2018. Fish samples were caught in a trap net and identified using taxonomic keys (Olasebikan and Raji, 1998) and

storage for heavy metal analysis was according to APHA (2005). They were frozen in refrigerator in the University of Uyo Fisheries Laboratory for further analysis. Cultured fish used as control were obtained from a local fish farm in Onitsha, Nigeria.

Heavy metal evaluation in tissues of fishes

Fish tissues (flesh) digestion was carried out following the procedures described by European / International Organization for Standardisation PN-EN ISO13657 (2006) in which all digested samplers were put inside glass containers. Heavy metals analysis of digested samples was done using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES Optima 2000 DV, PerkinElmer Inc. Shelton, CT, USA) guidelines according to PN-EN ISO11885 (2009). Heavy metals analysed were Aluminum (Al), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni), Zinc (Zn), Mercury (Hg), Arsenic (As) and Lead (Pb). All glassware used for trace element analysis were thoroughly cleaned by soaking in detergent followed by soaking in 10% nitric acid for 24 hours and finally rinsed with distilled water several times prior to use.

Genomic DNA Isolation

DNA was extracted and prepared from blood samples of four per species using Blood-Animal-Plant DNA preparation kit (Jena Biosci., Germany) following manufacturer's instructions. The quality and quantity of extracted DNA samples was verified using nanodrop spectrophotometer (Thermo Fisher Sci., USA). The concentrations of extracted DNA samples were adjusted to $20 \text{ ng/} \mu \text{L}$ for PCR amplification.

PCR amplification

Ten RAPD primers were screened on four fish per species that were randomly selected, from which four highly polymorphic RAPD primers (Table 1) were selected for the study. A reaction mixture of 20 µL comprising of 4 µL master mix (Solis BioDyne 5X FIREPol[®] PCR Master Mix Ready to Load), 0.5 µL oligo primers, 2 µL DNA sample and 13.5 µL nuclease free water were used in the study. RAPD amplification was done following the thermal cycling conditions; Initial denaturation of 90 °C for 5 min, then 40 cycles of 95 °C for 1 min, 30 °C for 1 min, 72 °C for 2 min and a final extension of 72 °C for 10 min. Amplicons were then separated in 1.5% (w/v) agarose using 0.5x TBE (Tris Borate EDTA) buffer stained with Ethidium bromide. The setup was run for 1 hour at 80 v. Separated were visualized using ultraviolent light from a trans-illuminator.

Analysis of Molecular Data

RAPD data analysis comparing the PCR products profile of the control with fish samples collected from lower River Niger was carried out. Consistent amplified RAPD products (75% i.e. bands that appeared in three of the four sample replicates per group treated) were scored in the study. Bands were scored 1 for present and 0 for absence of bands. Presence, absence and intensity of bands in relation to control bands were also considered in the study. Genomic template stability (%) was calculated as follows:

GTS = 100 - (100a/n)

Where a means the average number of the differences in DNA profiles, *n* means the number of bands that were selected through control profiles in DNA (Cekic *et al.*, 2017).

Results

Evaluation of heavy metal in tissues of sampled Fish species

Heavy metal analysis of tissues of both O. niloticus and C. gariepinus showed that metal accumulation was higher in the wet season than the dry season, although not significantly different (p > 0.05) for all metals evaluated (Table 1). The highest metal accumulated was iron in both fish species studied for both dry and wet seasons. Accumulation of mercury was least (0.15mg/g) in O. niloticus in the dry season, while cadmium was the least (0.04mg/g) in C. gariepinus. However, during the wet season, mercury was least accumulated in O. niloticus (0.21mg/g) and arsenic was least accumulated in C. gariepinus (0.14 mg/g). More heavy metal accumulation was observed in the wet season than the dry season in both fish species evaluated. The distribution of heavy metal accumulation in the fishes evaluated followed the trend Fe > Al > Cd > Zn > Pb > Cu > As > Ni > Cr >Hg for the dry season and Fe > Al > Zn > Cu > Pb >Cr > Ni > Cd > As > Hg for the wet season in O. niloticus; Fe>Al>Zn>Pb>Cu>Cr>Ni>Cd> As > Hg for the dry season and Fe > Al > Zn > Pb > Cu > Ni > Cr > Cd > Hg > as for the wet season in C. gariepinus.

Heavy metals	O. nile	oticus	С. д	FAO/WHO	
(mg/g)	Dry Season	Wet Season	Dry Season	Wet Season	mg/kg
Aluminium	16.40±5.30	18.98±5.06	15.53±2.78	18.40 ± 3.49	-
Cadmium	1.32 ± 2.46	0.28±0.33	$0.04{\pm}0.05$	0.41 ± 0.61	0.2
Chromium	0.77 ± 0.22	1.03 ± 0.57	1.05 ± 0.31	0.44 ± 0.40	0.05
Copper	1.14 ± 0.56	2.68 ± 2.90	1.08 ± 0.38	1.15 ± 0.38	2.0
Iron	64.28±12.93	67.42±14.11	96.06±58.36	99.16±59.36	-
Nickel	0.32 ± 0.25	0.43 ± 0.22	0.67 ± 0.26	0.48 ± 0.31	0.6
Zinc	5.46 ± 1.68	6.96 ± 2.08	7.47±2.63	9.50±3.56	30
Mercury	0.15 ± 0.17	0.21±0.24	0.35 ± 0.44	0.18 ± 0.28	0.5
Arsenic	0.22 ± 0.28	0.24±0.31	0.11±0.15	$0.14{\pm}0.18$	1.0
Lead	1.41±0.58	2.14±0.33	1.25 ± 0.78	2.36±0.47	2.0

 Table 1: Heavy metal concentration accumulation in fish (O. niloticus and C. gariepinus) tissues during study

Values represents mean ± standard deviation; FAO/WHO (2003; 2011)

RAPD marker performance in the study

Four polymorphic RAPD markers were used to assess the level of genotoxic effect of nickel, mercury, lead and zinc metals in *O. niloticus* and *C. gariepinus* in the study. The number of loci generated by RAPD markers used ranges from 13 (OPAE-01) to 18 (RAPD-4) for *O. niloticus*

samples and 15 (RAPD-1) to 20 (OPAE-01) loci for *C. gariepinus* samples (Table 2). In *O. niloticus* samples, all RAPD markers gave a 100% polymorphism between samples evaluated with two unique loci (397 bp in OPAE-01 and 2288 bp in RAPD-4) observed (Table 2). The unique loci were associated with the control (397 bp) and zinc



Figure 2: RAPD profile for RAPD-4 marker in *O. niloticus* and *C. gariepinus*; M: DNA ladder; -ve; negative control; 1-4: replicated *O. niloticus*; 5: Control for *O. niloticus*; 6-9: replicated *C. gariepinus*; 10: Non treated *C. gariepinus*

treated sample (2288 bp). In *C. gariepinus* samples, percentage polymorphism ranged between 80% (RAPD-1) and 100% (OPC-04) with four unique loci (2083 and 1614 bp in OPAE-01, 833 bp in OPC-04 and 1686 bp in RAPD-1) observed (Table 3). These unique loci were associated with mercury treatment (2083 bp), lead treatment (1614 bp) and control (833 and 1686 bp). Overall, OPAE-01 gave a total of 22 loci which were polymorphic while both RAPD-1 and RAPD-4 gave 18 polymorphic loci in the study. OPAE-01 gave the highest number of unique loci while the other RAPD markers gave one each unique locus among samples evaluated in the study (Table 4).

Effect of heavy metals on the RAPD profile of *O. niloticus* and *C. gariepinus*

Figure 2 shows a typical RAPD profile for RAPD-4 marker in *O. niloticus* and *C. gariepinus*. RAPD profiles varied between heavy metal in both control fishes and between fish species evaluated (Table 5 and 6). A total of 38 bands were observed in *O. niloticus* control fish group. There was appearance of 2 bands for nickel treated group, 8 bands for mercury treated group, 11 bands for lead treated group and 12 bands for zinc treated group. Normal band disappearance was highest in the nickel treated group and least in the lead treated group. Polymorphism was higher as observed in the nickel treated group compared to the lead treated

Table 2: Sequence and amplified band size range of RAPD markers used in the study

Primers	Sequences $(5' - 3')$	Size range (bp)
OPAE-01	TGAGGGCCGT	2083 - 229
OPC-04	CCGCATCTAC	2039 - 497
RAPD-1	GCCAGATCAG	1985 - 438
RAPD-4	TGCTCTGCCC	2288 - 302

			Polymorph	ic bands		
Fish Species	Primers	Monomorphic bands	Unique bands	Non- unique bands	Total bands	%Polymorphism
s	OPAE-01	0	1	12	13	100.00
ticu	OPC-04	0	0	17	17	100.00
niloticus	RAPD-1	0	0	16	16	100.00
0. 1	RAPD-4	0	1	17	18	100.00
sm	OPAE-01	2	2	16	20	90.00
epir	OPC-04	0	1	17	18	100.00
gariepinus	RAPD-1	3	1	11	15	80.00
C. 8	RAPD-4	2	0	14	16	87.50

Table 3: Performance of RAPD markers in O. niloticus and C. gariepinus

Table 4: Performance of RAPD markers in O. niloticus and C. gariepinus

	Monomorphic	Polymorp	hic bands		
Primers	bands	Unique	Non-unique	Total bands	%Polymorphism
	Danus	bands	bands		
OPAE-01	0	3	19	22	100.00
OPC-04	0	1	18	19	100.00
RAPD-1	0	1	17	18	100.00
RAPD-4	0	1	17	18	100.00

group which showed the least polymorphism for *O*. *niloticus* (Table 5).

On the other hand, a total of 37 bands were observed in *C. gariepinus* control fish group. There was appearance of 15 bands for nickel treated group, 22 bands for mercury treated group, 14 bands for lead treated group and 24 bands for zinc treated group. Normal band disappearance was highest the zinc treated group and least in the mercury treated group. Polymorphism was higher as observed in the zinc treated group as compared to the nickel treated group which showed the least polymorphism (Table 6).

Effect of heavy metals on the RAPD profile of *O. niloticus* and *C. gariepinus*

There was not much variations observed in the RAPD profiles of wild *O. niloticus* between dry and wet seasons sampled tissues evaluated (Table 5). There was appearance of 2 bands for dry season and 3 bands for wet season in wild *O.*

niloticus evaluated. Normal band disappearance was highest in dry season tissue (b=31). However, polymorphism was the same in both seasons (a + b = 33) when compared (Table 4).

On the other hand, there was appearance of 15 and 13 bands from dry and wet season tissues of wild *C. gariepinus* evaluated (Table 6). Normal band disappearance was highest in wet season tissue (b = 24). Polymorphism was in proximity between the dry and wet season (dry season = 36, wet season = 36) when compared (Table 6).

Effect of heavy metals on genomic template stability (GTS) in *O. niloticus* and *C. gariepinus*

Estimation of GTS showed varied genome stability response among the sampled fishes in dry and wet seasons (Figure 3). In wild *O. niloticus*, GTS was 13.16% for both the dry and wet season. On the other hand, GTS in wild *C. gariepinus* was 2.70% and 5.41% for dry and wet seasons when compared to the controls (Figure 3).

Primers	Control	Dry Season				Wet Season			
	Control	a	b	c	d	a	b	c	d
OPAE-01	6	2	4	2	0	1	5	1	1
OPC-04	12	1	8	3	0	3	9	2	1
RAPD-1	9	0	9	0	0	1	8	1	0
RAPD-4	11	1	8	1	2	0	8	1	1
Total	38	2	31	6	2	3	30	5	3
a + b		33				33			
a+b+c+d		41				41			

Table 5: RAPD pattern changes induced by heavy metal treatments in O. niloticus

a: appearance of new bands; b: disappearance of control bands; c: decrease control band intensity; d: increased control band intensity; a+b: polymorphic bands; a+b+c+d: varied bands

Table 6: RAPD pattern changes induced by heavy metal treatments in C. gariepinus

Primers	Control Dry Season					Wet Season				
	Control	a	b	c	d	a	b	с	d	
OPAE-01	13	3	5	2	1	1	8	4	1	
OPC-04	6	6	1	3	2	3	3	2	1	
RAPD-1	10	2	10	0	0	3	8	1	1	
RAPD-4	8	4	5	1	2	6	4	3	1	
Total	37	15	21	6	5	13	23	10	4	
a + b	36					36				
a+b+c+d	47						50			

a: appearance of new bands; b: disappearance of control bands; c: decrease control band intensity; d: increased control band intensity; a+b: polymorphic bands; a+b+c+d: varied bands



Figure 3: Genomic template stability in wild species of O. niloticus and C. gariepinus

Discussion

In this study, the trend in the heavy metal accumulation in the tissues of both O. niloticus and C. gariepinus reflects variations in heavy metal concentrations of River Niger between the two seasons. The seasonal variations observed in the studies corroborate the report of Ibrahim et al., (2018), who also observed seasonal variations in the heavy metal concentrations in both O. niloticus and C. gariepinus. Factors that could contribute to the seasonal heavy metal accumulation in tissues of these fish species include the fish's regulatory ability, behaviour and feeding habits (Baldisserotto et al., 2005). So also, the chemical nature of the metals, ionic strength and pH are co-contributing variables in the accumulation process (Okoro et al., 2016).

More heavy metal accumulation was observed in the wet season than the dry season in both fish species evaluated. The differences in heavy metal accumulation pattern in sampled fish species in this study corroborates with reports from Okoro *et al.*, (2016) who also observed variation in heavy metal accumulation between *Coptodon zillii*, *Oreochromis niloticus*, *Sarotherodon galilaeus* and *C. gariepinus*. For the dry season, Al, Cd, Cu, As and Pb were observed more in *O. niloticus* than *C. gariepinus* while the reverse was observed for Cr, Fe, Ni, Zn, and Hg. On the other hand, during the wet season evaluations, *O. niloticus* was observed to harbour more Al, Cr, Cu, Zn and As while *C. gariepinus* harboured more Cd, Fe, Ni, Hg and Pb. These were similar to reports by Sani (2011). These differences may be attributed to both the body structure (presence of scales which may act as a barrier to heavy metal accumulation) and physiology of the individual fish species examined.

Heavy metals accumulations in *O. niloticus* were greater during the wet season than dry season except for Cd which showed more accumulation during the dry season than wet season. The seasonal heavy metal accumulation difference in *O. niloticus* corroborates results from Ibrahim *et al.*, (2018) in Lake Njuwa, Adamawa State except for Cr which was reported to be higher in the dry season than wet season in the tissues of *O. niloticus*.

Similarly, there was more heavy metal accumulation in the tissues of *C. gariepinus* in the wet season than the dry season except for Ni and Hg which were more during the dry season. The more Ni and Hg in the tissues of *C. gariepinus* during the dry season was contrary to reports from Ibrahim *et al.*, (2018) which reported more Ni in the wet season for *C. gariepinus* from Lake Njuwa, Adamawa State.

The high concentrations of heavy metals during the wet seasons can be attributed to the increases in surface run-off. Large amount of pesticides from farms containing heavy metals are brought to the surface during heavy rainfall. This leads to accumulations of materials in the water bodies during the run-off especially chemical fertilizers containing the heavy metals (Ni, Pb, Cu). However, several previous studies found that mean heavy metal concentrations in fish were higher in the dry season (Oguzie, 2003; Obasohen and Eguavoen, 2008). This was attributed to high temperature, which increases the activity, ventilation, metabolic rate and feeding seasons (Nussey et al., 2000), while the low heavy metal concentrations in the wet season might be due to the dilution of metal level associated with heavy rains as reported by Obasohan and Eguavoen (2008). Observation from this study can also result from the effluent discharge from industrial activities around the water body which can contribute immensely to the high amount of heavy metals in the water body.

The use of RAPD assay in genotoxicity studies has increased due to its rapid, nonradioactive nature, applicability to any organism and its potential to detect a wide range of DNA damage and mutations including point mutations and large rearrangements (Zhang et al., 2017). Studies have reported that the RAPD assay is more sensitive than classic test such as the comet and micronucleus assay since RAPD analysis is capable of detecting temporary DNA changes at lower concentrations of pollutants (Atienzar et al., 2006; Zhou et al., 2011; Zhang et al., 2017). An important characteristic of a good marker is polymorphism between related individuals. The level of polymorphism reflects the resolving power of a marker. In the study, all markers selected for the study were >80% polymorphic in both fish species making it suitable for the molecular study. Similar response was observed in the works of Danish et al., (2012).

Observing banding patterns in relation to a control (unpolluted) sampled groups have been reported in literature to also be a reliable means of assessing genotoxic impact of heavy metals in animal studies (Zhang *et al.*, 2017). The study showed significant differences in RAPD profile in *O. niloticus* from the dry and wet season when compared to the control with respect to band intensity, disappearance of normal bands, and appearance of new bands of amplified DNA. Bands disappear when the primer fails to bind to a certain site on the DNA template that was altered

by the genotoxic substance, while new bands appear when some sites on the DNA become accessible to the primer after structural change by the genotoxic agent (Swaileh *et al.*, 2013). As for the variation of band intensity, oxidative DNA damage and DNA-protein cross-links might block *Taq* polymerase processing and decrease the intensity of some specific RAPD bands, while DNA modifications enhancing the pairing activity of the primer with the DNA template might be a possible explanation for the increase in intensity of some RAPD bands (Lee *et al.*, 2007).

Band disappearance is induced mainly by genomic rearrangements, decreased point mutation, DNA damage in primer binding sites, and interaction of DNA polymerase in the test organism with damaged DNA, as suggested by Liu *et al.*, (2005). The differences in band patterns of the fishes when compared to the controlled fishes are the result of induced mutation, DNA damage, or DNA alteration. The presence and absence of bands in the products amplified with the primers used in this study gave a clear indication of their ability of the test for DNA damage in test species (Kumar *et al.*, 2015).

Similar results were reported by Zhang *et al.*, (2017) who observed that the RAPD profile of the fishes per season clearly differs from their control counterparts and exhibit distinct changes between seasons.

The GTS is a percentage value that reflects PCR amplification profile changes of the test sample relative to the control (Zhang *et al.*, 2017). Previous studies have however confirmed that the GTS was a sensitive parameter to reflect the changes in RAPD profiles induced by pollutants (Zhou *et al.*, 2011; Aksakal and Esim, 2015).

Although, significantly reduced when compared to the control fish, GTS in *O. niloticus* did not differ between both seasons while GTS was higher in wet season than dry season in *C. gariepinus*. The decrease in genomic DNA stability in *C. gariepinus* may be the result of band disappearance and appearance of new bands (Atienzar and Jha, 2006).

Conclusion

The study showed more heavy metal accumulation in fish tissues more than WHO permissible limits signifying potential toxicity if these fishes are consumed by final consumers. The study revealed the level of evaluated seasonal heavy metals accumulation in the tissues of *O. niloticus* and *C. gariepinus*. Although, more heavy metals were found in the tissues of fishes in the wet season than the dry season, *C. gariepinus* was observed to accumulate more heavy metals and show more DNA damage than *O. niloticus* in both seasons. These findings necessitate constant biomonitoring of lower River Niger to ensure good human health and ecosystems integrity.

Conflict of Interest

The authors declare that no conflict of interest exists.

References

Abdelaiz, D., Abdelali, E., Gaidoumi, I., Chaouni, B., Taleb, A. and Abdelhak, K. (2019). Characterization and Quantification of heavy Metals in Oued Sebou Sediments. *The Scientific World Journal*, 9.

https://doi.org/10.1155/2019/7496576.

- Ademoroti, C.M. (1996). *Environmental Chemistry and Toxicology*. Foludex Press Ltd. Ibadan. 215pp.
- Afshan, S., Ali, S., Ameen, U.S., Farid, M., Bharwana S.A., Hannan F., Ahmad R. (2014). Effect of different heavy metal pollution on fish. *Research Journal of Chemical and Environmental Sciences*. 2(1): 74-79.
- Akan, J.C., Mohmoud, S., Yikala, B.S., Ogugbuaja, VO. (2012). Bioaccumulation of some heavy metals in fish samples from River Benue in Vinikilang, Adamawa State, Nigeria. *American Journal of Analytical Chemistry*. 3 (11): 727-736.
- Aksakal, O., and Esim, N. (2015). Evaluation of arsenic trioxide genotoxicity in wheat seedlings using oxidative system and RAPD assays. *Environmental Science and Pollution Research*. 22(9):7120-7128.
- Alipour, H., and Banagar, G. (2018). Health risk assessment of selected heavy metals in some edible fishes from Gorgan Bay. Iran. *Iranian Journal of Fisheries Sciences*, 17(1):21–34.
- Ambedkar, G., and Muniyan M. (2011). Bioaccumulation of some heavy metals in the

selected five freshwater fish from Kollidam River, Tamilnadu, India. *Archive of Applied Science Research*. 3 (3): 261-264.

- Atienzar, F.A., Conradi, M., Evenden, A.J., Jha, A.N, Depledge, M.H. (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a]pyrene. *Environmental Chemistry*. 18: 2275–2282.
- Atienzar, F.A., and Jha, A.N. (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutation Research*. 613(2–3):76–102.
- Baldisserotto, B.M.J., Chowdhury, Wood, C.M. (2005). Effects of Dietary Calcium and Cadmium on Cadmium Accumulation, Calcium and Cadmium Uptake from the Water, and their Interactions in Juvenile Rainbow Trout. *Aquatic Toxicology* 72(1): 99-117.
- Beheary, M.S., Abu-Almaaty A.H., El-matary F.A. (2015). Genetic polymorphism and changes in the concentration of heavy metals in catfish *Clarias gariepinus* from different sites as a result of environmental contamination. *Advances in Environmental Biology*. 9(18): 20-32.
- Borkovic, S.S., Pavlovic, S.Z., Kovacevic, T.B., Štajn, A.Š., Petrović, V.M., Saičić, Z.S. (2008). Antioxidant defence enzyme activities in hepatopancreas, gills and muscle of spiny cheek crayfish (*Orconectes limosus*) from the River Danube. *Comparative Biochemisty and Physiology: Toxicology and Pharmacology*, 147 (1): 122-128.
- Cekic, F.O., Ekinci, S., Inal, M.S., Unal, D. (2017). Silver nanoparticles induced genotoxicity and oxidative stress in tomato plants. *Turkish Journal of Biological Zone*. 41: 700-707.
- Danish, M., Singh, I.J., Giri, P., Singh, C.P. (2012). Molecular characterization of two population of catfish *Clarias batrachus L*. using random amplified polymorphism DNA (RAPD) markers. *African Journal of Biotechnology*. 11(77): 14217-14226.
- Etim, L., Akpan, E.R. and Muller, P. (1991). Temporal trends in heavy metal concentrations in the Clam, *Egeria radiata* (Bivalvia: *Tellinacea Donacidae*) from the Cross River, Nigeria. *Review of Tropical Hydrobiology*, 24(4): 327-333.

- Ezemonye, L., Adebayo, P.O., Enuneku, A.A., Tongo, I., Ogbomida, E. (2019). Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin River, Nigeria. *Elsevier Toxicology Reports* 6(1): 1-9.
- FAO/WHO (1989). Evaluation of certain food additives and the contaminants mercury, lead and cadmium, WHO Technical Report, Series No. 505.
- FAO/WHO (2011). Food Standards Programme Codex Committee on Contaminants in Foods Fifth Session: The Hague, The Netherlands, 21 -25 March 2011.
- Food and Agriculture Organization (FAO) (2003). Heavy Metal Regulations – Faolex. Legal Notice no. 66/2003.

http://faolex.fao.org/docs/pdf/eri42405.pdf.

Hamilton, P.B., Rolshausen, G., Uren Webster T.M., Tyler C.R. (2017). Adaptive capabilities and fitness consequences associated with pollution exposure in fish. Phil. Trans. R. Soc. B 372: 20160042.

http://dx.doi.org/10.1098/rstb.2016.0042

- Ibrahim, D., Ibrahim, A.S., Paul, E.D., Umar, M., Zannah, U.A.S. (2018). Determination of some heavy metal contents in tilapia and catfish species in Lake Njuwam, Adamawa State, Nigeria. *Journal of Applied Science and Environmental Management*. 22(8): 1159-1165.
- Izuchukwu, U.I., Okeke, D.O., Okpashi, V.E. (2017). Determination of heavy metals in fish tissues, water and sediment from the Onitsha Segment of the River Niger Anambra State Nigeria. J. Environmental Analytical Toxicology, 7(5): 507. DOI: 10.4172/2161-0525.1000507.
- Jarah, S., Zhou, B., Abdullah, R., Lu, Y., Yu, W. (2019). Urbanization and urban sprawl issues in city structure: A case of the Sulaymaniah Iraqi Kurdistan Region. *Sustainability*, 11(2), 485. doi:10.3390/su11020485
- Juhari, E.L. (2014). Metallothionein gene expression and genotoxic effects of heavy metals on Oreochromis sp. Master of Biotechnology. Institute of Biological Sciences. University of Malaya, Kuala Lumpur. 70 p.
- Kallel, A., Ksibi, M., Dhia, H.B., Khélifi, N. (2017). Recent advances in Environmental Science

from the Euro-Mediterranean and surrounding Regions. In Proceedings of the Euro-Mediterranean Conference for Environmental Integration (EMCEI-1), Springer: Tunisia.

- Kumar, P., Kumar, R., Nagpure, N.S., Nautiya, P., Kushwaha, B., Nwani, C.D., Lakra, W.S. (2015). In vivo assessment of DNA damage in *Cyprinus carpio* after exposure to potassium dichromate using RAPD. *Turkish Journal of Veterinary and Animal Sciences*. 39: 121-127.
- Liu, W., Li, P.J., Qi, X.M, Zhou, Q.X., Zheng L., Sun T.H., Yang Y.S. (2005). DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. *Chemosphere*. 61: 158–167.
- Montazer, M.I., and Ali, S. (2018). Determination of Heavy Metals in Freshwater Fishes of the Tigris River in Baghdad. *Fishes communication*.
- Nussey, G., Van, Vuren, J.H.J., Du, Preez, H.H. (2000). Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga S. Africa. *Water SA*. 26 (2): 269-284.
- Obasohan, E.E., Eguavoen, O.I. (2008). Seasonal variations of bioaccumulation of heavy metals in a freshwater fish (*Erpetoichthys calabaricus*) from Ogba River, Benim City, Nigeria. *African Journal of Gen. Agriculture*. 4:153–163.
- Ogbomida, E.T., Nakayama, S., Bortey-Sam, N., Oroszlany, B., Tongo, I., Enuneku, A. A., Ogbeide, O., Ainerua, M.O., Ishizuka M. (2018). Accumulation patterns and risk assessment of metals and metalloid in muscle and offal of freerange chickens, cattle and goat in Benin City, Nigeria. *Ecotoxicol. Environ. Saf.*, 151:98-108.
- Oguzie, F.A. (2003). Heavy metals in fish, water and effluents of lower Ikpoba River in Benim City, Nigeria. *Pakistan Journal of Science and Industrial Research*. 46(3):156–160.
- Okoro, H.K., Iyiola, O.A., Simon, I., Oladipo, S.O. (2016). Determination of heavy metal genotoxicity and their accumulation pattern in different fish organs of selected fish species collected from the Asa River, Ilorin, Kwara State. *Journal of Applied Science and Environmental Management*. 20(3): 735-745.
- Olaosebikan, B.D., and Raji, A. (1998). Field guide to Nigerian freshwater fishes. Federal College of Freshwater Fisheries Technology, New Bussa,

Nigeria. 105 - 106pp.

- PN-EN 13657 (2006). Characterization of waste. Digestion for subsequent determination of aqua regia soluble portion of elements. CEN, Brussels
- PN-EN ISO 11885 (2009). Water quality determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES). CEN, Brussels.
- Salarizadeh S., and Kavousi R. (2015). Application of rapid amplified polymorphic DNA (RAPD) to detect the genotoxic effect of cadmium on two Iranian ecotypes of cumin (*Cuminum cyminum*). Journal of Cell and Molecular Research. 7(1): 38-46.
- Sani, U. (2011). Determination of some heavy metal concentration in the tissues of Tilapia and catfishes. *Biokemistri*. 23(2): 73-80.
- Savva, D., Castellani, S., Mattei, N., Renzoni, A., Walker, C.H. (1994). The use of PCR and DNA fingerprints to detect the genotoxic effects of environmental chemicals. In: Proceedings of the 6th International Conference on Environmental Contamination. Delphi, Greece: CEP. pp. 105-110.

- Swaileh, K.M., Barakat, S.O., Hussein, R.M. (2013). RAPD assessment of in vivo induced genotoxicity of raw and treated wastewater to albino rat. *Bulletin* of Environmental Contamination and Toxicology 90(5):621–625.
- Tanee, T., Sudmoon, R., Thamsenanupap, P., Chaveerach, A. (2016). Effect of cadmium on DNA changes in *Ipomea aquatica* Forssk. *Poland Journal of Environmental Studies*. 25(1):311-315.
- Uysal, K., Köse, E., Bülbül, M., Dönmez, M., Erdoğan Y., Koyun M., Özmal, F. (2008). The comparison of heavy metal accumulation ratios of some fish species in Enne Dame Lake (Kütahya/Turkey). *Environmental Monitoring and Assessment*, 157(1-4), 355–362.
- Zhang, H.C., Liu, T.Y., Shi, C.Y., Chen, G.W., Liu, D.Z. (2017). Genotoxicity evaluation of an urban river on freshwater planarian by RAPD assay. *Bulletin of Environmental Contamination and Toxicology*. 5p doi: 10.1007/s00128-016-2027-9.
- Zhou, L., Li, J., Lin, X., Al-Rasheid, K.A.S. (2011). Use of RAPD to detect DNA damage induced by nitrofurazone in marine ciliate, *Euplotes vannus* (Protozoa, Ciliophora). *Aquatic Toxicology* 103 (3-4):225-232.



www.theajfarm.com

EJIKEME, C.E., AYOOLA, S.OAND AKINOLA, M.O. African Journal of Fisheries and Aquatic Resources Management Volume 4, 2019 ISSN: 2672-4197 (Prints) ISSN: 2672-4200 (Online) Pp 9-20