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Genotoxic potentials of some selected heavy metals exposure on *Clarias gariepinus* (Burchell, 1822) and *Oreochromis niloticus* (Linnaeus, 1758) using RAPD-PCR technique

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ABSTRACT

Fish health and the aquatic ecosystem are strongly interwoven and interrelated. The aquatic ecosystem receives a range of anthropogenic chemicals which have toxicological or lethal health effects on the aquatic animals. The aim of this study was to determine the level of induced mutation and genomic stability of different sub-lethal doses (25%, 50% and 75% LC_{50}) of heavy metal [Nickel (Ni), Mercury (Hg), Lead (Pb)and Zinc (Zn)] in Clarias gariepinus (Burchell, 1822) and Oreochromis niloticus (Linnaeus, 1758) using RAPD-PCR technology over a period of 21 days. Four highly polymorphic RAPD markers were evaluated on isolated DNA from both heavy metal exposed fishes and control fishes after exposure. Observation of RAPD profiles in C. gariepinus revealed more damaging effect as sub lethal doses increased with zinc (a + b = 44 bands) > mercury (a + b = 41 bands) > nickel (a + b = 37 bands) > lead (a + b = 35 bands) than with nickel (a + b = 37 bands) > zinc (a + b = 34 bands) > lead (a + b = 32 bands) > mercury (a + b = 31 bands) for O. niloticus when compare to the control groups. Although, the genomic stability template decreased sub lethal heavy metal doses, higher stability was observed in O. niloticus (GTSPb = 26.32% > GTSHg = 18.42% > GTSZn = 10.53% > GTSNi = 2.63%) than in C. gariepinus (GTS_{Pb} = 5.41% > GTS_{Ni} = 0.0% > GTS_{Hg} = -10.81% > GTS_{Zn} = -18.92%). The results obtained showed differential variation in heavy metal induced genetic mutation and genomic stabilty in C. gariepinus and O. niloticus. These observable differences might be due to the physiological structure of the fish species evaluated. This study also confirms that RAPD–PCR technology is a useful tools in detecting the genotoxic effect of heavy metals in aquatic organisms but due to low reproducibility of RAPD results, it is recommended that this technology should be used along with other molecular techniques in fish genotoxicity studies.

Introduction

Contamination of the aquatic ecosystem by heavy metals has become a serious issue of concern globally and there has been prompt investigation on their occurrence, distribution, toxicity to aquatic organisms and ecosystems (Sankar *et al.*, 2006; Abdelaiz *et al.*, 2019; Sarong *et al.*, 2013, 2015). They become incorporated into food chains through absorption by aquatic organisms to a level that might affect not only their physiological state but complete removal of these heavy metals upon gaining entrance into the environment becomes difficult (Osman, 2014; Alipour and Banagar, (2018). Fishes are one of the main aquatic organisms in the food chain and may accumulate large amounts of certain metals above the levels in the aquatic environment from food, water, and sediments (Ambedkar and Muniyan, 2011; Bawuro *et al.*, 2018). Fish is high protein, but accumulation of heavy metals is of concern as edible fishes may be a health risk, especially for consumers (Alipour *et al.*, 2014).

Toxicity test were used to examine the genotoxic impacts of heavy metals on fish (Salem *et al.*, 2014), and recently, more sensitive and selective assays through advances in molecular biology have been developed. Molecular techniques with major

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significance are mainly PCR-based molecular methods such as Polymerase Chain Reaction (PCR), Real-Time Polymerase Chain Reaction (RT-PCR), Random Amplified Polymorphic DNA (RAPD). Nested PCR and Multiplex Polymerase Chain Reaction (multiplexPCR), etc (Ador *et al.,* 2022). Rapid Amplified Polymorphic DNA (RAPD)have been extensively used in this regard (Tajik-Esmaelili *et al.*, 2017). RAPD is a powerful PCR-based technique, anchored on random genomic segment amplification, able to detect DNA damage and mutations in organisms and fish inclusive (Rocco *et al.,* 2014).

Kumar *et al.* (2015) noted that a comparison between untreated and treated genomes shows RAPD analysis can be successfully used to evaluate environmental pollutant effect on the structure of DNA in living organisms. The alteration of a single nucleotide in a 10-base RAPD primer leads to marked changes in the RAPD profile of a given template (Rocco *et al.,* 2014; Shekhawat *et al.,* 2019). It is reasoned that nucleotide changes in the genomic DNA should have the same effect on RAPD profile as those observed for changes within the primer. Theoretically, therefore, single base-change mutations in genomic DNA could possibly be detected by this method (Enan, 2006). Though, RAPDs have low reproducibility and result can be difficult to interpret, among several PCR-based tools, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) is a simple, robust, rapid and cost-effective tool (ReshmaRaj and Das, 2021). Therefore, highly standardized laboratory protocols are required because of their sensitivity to reaction conditions (Ador *et al.,* 2022; Stefanska *et al.,* 2022).The quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions greatly influence the outcome of RAPD. Also, to ensure reproducibility, purified and high molecular weight DNA is required for RAPD analyses (ReshmaRaj and Das, 2021).

Hence, this study was aimed to investigate the potential genotoxic effect of heavy metals pollution in two commonly consumed fish species, *Clarias gariepinus* and *Oreochromis niloticus* using RAPD-PCR technology. These species are the two popular freshwater fish worldwide (Aderolu *et al.*, 2017; Marimuthu *et al.*, 2019).

Materials and Methods

Experimental fish

A total of one hundred and twelve fishes were purchase from a local fish farm in Lagos, Nigeria, comprising fifty-six (56) each of healthy adult of *C. gariepinus* (with mean ± SD weight and length of 658.4 ± 24.66 g and 36.2 ± 2.17cm) and *O. niloticus* (mean ± SD weight and length of 458.5 ± 3.14 g and 34.3 ± 2.62cm) were used for the study. The specimens were acclimatized to the laboratory conditions in Marine sciences laboratory, University of Lagos Nigeria for 14 days under a 12-h photoperiod and fed to satiation before the commencement of the experiment with 2 mm Blue crown feed (35% crude protein). After acclimatizing for two weeks, the fish were weighed using a digital scale (Salter 1073BKDR).

Estimation of sub lethal concentrations and *in vivo* exposure

A preliminary toxicity range-finding test was carried out to determine approximate range by selecting the concentrations of the test metal solution, nickel, mercury, lead and iron, for the definite test following standard methods USEPA (2002). Stock solutions for the definite test were prepared in double-distilled water. The experiment was carried out in triplicate under normal day/night illumination to obtain the 96-h LC_{50} value of the test chemical for the test species. Fish mortality was recorded as 10%, 20%, 50%, 70%, 80%, and 100% at 96 h after exposure for respective concentrations of the test chemical. The 96-h LC_{50} value of the test chemical was determined using probit analysis, as described by Hamilton *et al.* (1977).

Using the 96-h LC_{50}, sub lethal test concentrations (75% of LC_{50}) of heavy metals were calculated for the *in vivo* experiments for *C. gariepinus* (Ni = 0.032mg/L, Hg = 0.32mg/L, Pb = 0.035mg/L, Zn = 0.627mg/L) and *O. niloticus* (Ni = 0.008 mg/L, Hg = 0.016 mg/L, Pb = 0.006 mg/L, Zn = 0.598 mg/L).Seven fish replicates per metal concentration treatment was set for each fish species with control. This study was conducted under semi-static test conditions following OECD Guideline (2019). The specimens were exposed to the test concentrations continuously for 21 days. Blood samples were collected at the same time intervals by puncturing the caudal vein with a heparinized syringe after exposure duration and processed for RAPD- PCR analysis.

Genomic DNA isolation

DNA extracted was prepared from blood samples of four sampled fishes of each species per treatment using Blood-Animal-Plant DNA preparation kit (Jena Biosci., Germany). 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 ng/µL for PCR amplification.

RAPD-PCR amplification and gel electrophoresis

Ten RAPD primers were screened on randomly selected four fish samples of each species from which four highly polymorphic (70% polymorphism) RAPD primers (Table 1) were selected for the study. A reaction mixture of 20 µL comprising of 4 µL master mix (Solis BioDyne 5X Hot FIREPol® PCR Master Mix Ready to Load with BSA and 12.5 Mm $MgCl_2$), 0.5 µL oligo primers, 2 µL DNA sample and 13.5 µL nuclease free water were used in the study. RAPD amplifications was done using thermal cycler (PTC-100 Peltier Thermal Cycler, USA) following the thermal cycling conditions and parameters: 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 standard (Carl Roth, GmbH+CoKG (Germany) using 0.5x TBE (Tris Borate EDTA) buffer stained with Ethidium bromide (0.5 µg mL^{-1}). The setup was run for 1 hour at 80 V and visualized using ultraviolent light from a trans-illuminator.

Estimate of genomic DNA template stability (GTS %)

Amplified RAPD products were score 1 for present of band and 0 for absent of bands. Bands with ≥ 75% consistency among replicated samples were evaluated in the study. The RAPD data profiles generated from heavy metal treated samples were compared with profile for control sampled fishes. Genomic template stability (%) was calculated using the method proposed by Cekic *et al.* (2017).

Table 1. Sequence and amplified band size range of RAPD markers used in the study.

Primers Codes	Sequences (5′ – 3′)	Size range (bp)	PIC
OPAE-01	TGAGGGCCGT	229 – 2083	0.786
OPC-04	CCGCATCTAC	497 – 2039	0.747
RAPD-1	GCCAGATCAG	438 – 1985	0.661
RAPD-4	TGCTCTGCCC	302 – 2288	0.634

PIC: polymorphic information content

Results

RAPD marker performance in the study

In the present study, four polymorphic RAPD markers were used to evaluate the level of genotoxic effect of nickel, mercury, lead and zinc metals in *C. gariepinus* and *O. niloticus*. The markers generated variable band sizes, 229 – 2083 bp were observed using OPAE-01, 497 – 2039 bp for OPC-04, 438 – 1985 bp for RAPD-1 and RAPD-4 gave band sizes between 302 – 2288 bp. The highest discriminating marker amongst the RAPDs used was OPAE-01 with PIC value of 0.786 while the least was RAPD-4 with PIC value of 0.634 (Table 1).

RAPD profile evaluation of selected heavy metal sub lethal dose treatments in *C. gariepinus*

Observation of RAPD profiles varied between heavy metal sub lethal dose treatments against both control fishes and between fish species evaluated. A total of 37 bands were observed in *C. gariepinus* control group. For nickel sub lethal dose treatments in *C. gariepinus*, there was appearance of 12 bands in both S1 and S2 treated groups while 15 bands appeared in S3 treated group. Normal band disappearance increased with sub lethal dose increase. It was highest in sub lethal dose S3 treated group (b = 22) and least in the sub lethal S1 treated group (b = 19) (Table 2). In mercury sub lethal dose treatments in *C. gariepinus*, there was an increase in band appearance with increased sub lethal doses (S1 = 12, S2 = 16, S3 = 20). Normal band disappearance was 18 in S1, 23 in S2 and 21 in S3 sub lethal dose groups for mercury treatments in *C. gariepinus*. For lead sub lethal dose treatments in *C. gariepinus*, there was an increase in band appearance with increased sub lethal doses (S1 = 10, S2 = 11, S3 = 14) while normal band disappearance was 22 in S2, 21 in both S1 and S3 sub lethal dose groups. For zinc sub lethal dose treatments in *C. gariepinus*, there was appearance of 17 bands in S1, 24 bands in S2 and 20 bands in S3 sub lethal dose treated groups. However, normal band disappearance was 19 bands in S2, 16 bands in S1 and 24 bands in S3 sub lethal dose groups for zinc treatments. The highest polymorphic bands were observed in zinc treatments in all sub lethal doses when compared with other metals evaluated in the study (Table 2).

RAPD profile evaluation of selected heavy metal sub lethal dose treatments in *O. niloticus*

Observation of RAPD profiles also varied between sub lethal heavy metal treatments in *O. niloticus*. A total of 38 bands were observed in *O. niloticus* control group. For nickel sub lethal dose treatments, there was decline in band appearance with sub lethal dose increase (S1 = 10 bands, S2 = 9 bands, S3 = 2 bands) while band disappearance increased with sub lethal dose increase (S1 =13 bands, S2 = 26 bands, S3 = 35 bands). In mercury sub lethal dose treatments, 8, 11 and 8 bands new bands were observed in S1, S2 and S3 treated groups

respectively while control band disappearance increased with sub lethal dose increase (S1 = 18 bands, S2 = 20 bands, S3 = 23 bands). Likewise, in lead sub lethal dose treatments, S1, S2 and S3 treated groups showed 9, 16 and 10 new bands respectively that were not in the control group. Control band disappearance in sub lethal dose treatment group S1, S2 and S3 were 19, 16 and 21 bands respectively. Similarly, in zinc sub lethal dose treatments, there were 8, 4 and 12 new bands in S1, S2 and S3 treated groups respectively compared to the control bands, while control band disappearance in sub lethal dose treatment group S1, S2 and S3 were 16, 28 and 22 bands respectively. The study showed nickel gave the highest polymorphic bands in all sub lethal doses except S1 in which zinc treatment gave 24 polymorphic bands compared to 23 polymorphic bands in nickel (Table 3).

Genomic Template Stability (GTS)

Estimation of GST yielded by RAPD markers in *O. niloticus* and *C. gariepinus* is presented in Table 4. GTS showed varied genome stability response between fishes among treatments when compared to the control groups. In *C. gariepinus*, GTS was highest in lead treated group (GTS_{Pb} = 5.41%). Both mercury and zinc treated *C. gariepinus* gave the lowest GTS (GTS_{Hg} = -0.10.81; GTS_{Zn} =-18.92%) in the study when compared to the control. On the other hand, GTS was lowest in nickel treated group (GTS_{Ni} = 2.63%) while it was highest in lead treated group (GTS_{Pb} = 26.32%) as observed in *O. niloticus* sampled groups (Table 4).

Table 2. RAPD profile variations induced by heavy metal treatments in *C. garipienus*.

Marker	Control	S1				S2				S3			
		a	b	c	d	a	b	c	d	a	b	c	d
Nickel													
OPAE-01	13	3	8	3	1	4	6	0	0	2	7	1	0
OPC-04	6	4	4	0	2	2	4	0	2	4	6	0	0
RAPD-1	10	4	4	0	2	3	5	1	3	4	6	1	0
RAPD-4	8	1	3	1	2	3	5	1	3	5	3	2	0
Total	37	12	19	4	7	12	20	2	8	15	22	4	0
a + b		31				32				37			
Mercury													
OPAE-01	13	2	6	0	4	3	9	3	0	4	5	2	0
OPC-04	6	3	3	0	1	4	5	0	0	8	4	2	0
RAPD-1	10	5	4	1	2	6	4	2	3	4	7	0	0
RAPD-4	8	2	5	2	3	3	5	1	0	4	5	1	0
Total	37	12	18	3	10	16	23	6	3	20	21	5	0
a + b		30				39				41			
Lead													
OPAE-01	13	2	7	0	0	0	8	2	0	3	6	3	0
OPC-04	6	3	3	0	0	2	5	1	0	4	5	1	1
RAPD-1	10	1	8	1	0	7	3	1	0	4	6	1	2
RAPD-4	8	4	3	0	0	2	6	1	2	3	4	1	3
Total	37	10	21	1	0	11	22	5	2	14	21	6	6
a + b		31				33				35			
Zinc													
OPAE-01	13	4	4	0	1	5	7	0	1	3	10	0	1
OPC-04	6	3	6	1	0	9	0	2	1	9	4	1	0
RAPD-1	10	4	5	1	1	4	3	0	1	3	5	2	0
RAPD-4	8	6	4	0	1	6	6	1	3	5	5	2	0
Total	37	17	19	2	3	24	16	3	6	20	24	5	1
a + b		36				40				44			

a: appearance of new bands; b: disappearance of control bands; c: decrease control band intensity; d: increased control band intensity; a + b: polymorphic bands; S1: 25% sublethal dose; S2: 50% sublethal dose; S3: 75% sublethal dose

Table 3. RAPD profile variations induced by heavy metal treatments in *O. niloticus*.

Marker	Control	S1				S2				S3			
		a	b	c	d	a	b	c	d	a	b	c	d
Nickel													
OPAE-01	6	1	5	0	0	0	6	0	1	0	6	0	0
OPC-04	12	2	5	0	1	5	5	0	0	0	12	0	0
RAPD-1	9	5	3	0	1	3	7	0	0	0	9	0	0
RAPD-4	11	2	0	1	2	1	8	0	0	2	8	1	0
Total	38	10	13	1	4	9	26	0	1	2	35	1	0
a + b		23				35				37			
Mercury													
OPAE-01	6	2	3	1	2	2	3	1	3	0	6	0	0
OPC-04	12	4	4	1	1	4	6	2	0	0	8	2	1
RAPD-1	9	0	4	0	0	1	6	0	0	4	5	0	0
RAPD-4	11	2	7	0	0	4	5	0	1	4	4	1	2
Total	38	8	18	2	3	11	20	3	4	8	23	3	3
a + b		26				31				31			
Lead													
OPAE-01	6	2	4	0	1	3	2	1	2	1	5	1	3
OPC-04	12	1	7	0	1	5	6	1	1	1	7	2	0
RAPD-1	9	3	3	1	1	1	5	0	1	5	4	0	0
RAPD-4	11	3	5	0	1	4	3	0	2	3	5	1	1
Total	38	9	19	1	4	16	16	2	6	10	21	4	4
a + b		28				29				32			
Zinc													
OPAE-01	6	3	0	1	1	1	4	2	2	0	6	0	0
OPC-04	12	2	3	1	0	1	8	1	1	3	8	1	0
RAPD-1	9	2	4	1	0	2	7	0	0	6	4	1	1
RAPD-4	11	1	9	1	2	0	9	0	3	3	4	1	0
Total	38	8	16	4	3	4	28	3	6	12	22	3	1
a + b		24				32				34			

a: appearance of new bands; b: disappearance of control bands; c: decrease control band intensity; d: increased control band intensity; a + b: polymorphic bands; S1: 25% sublethal dose; S2: 50% sublethal dose; S3: 75% sublethal dose

Table 4. Estimate Genomic Template Stability (%) Yielded by RAPD Markers in *O. niloticus* and *C. gariepinus* Exposed to Heavy Metal.

Treatments	*C. gariepinus*	*O. niloticus*
Control	100.00%	100.00%
Nickel	0	2.63%
Mercury	-10.81%	18.42%
Lead	5.41%	26.32%
Zinc	-18.92%	10.53%

The study showed a decline in GTS with increase sub lethal doses for all evaluated heavy metals in both fish species, with higher GTS decline rate in *C. gariepinus* when compared to *O. niloticus*. In *C. gariepinus*, both mercury and zinc showed a negative stability with both S2 and S3 dose treatments (Figure 1). In *O. niloticus*, the least GTS was observed in nickel sub lethal dose S3 treatment (GTS_{Ni} = 2.83) followed by zinc sub lethal dose S3 treatment (GTS = 10.53) (Figure 2). GTS when compared for both fishes revealed more genomic stability in *O. niloticus* than in C. *gariepinus*.

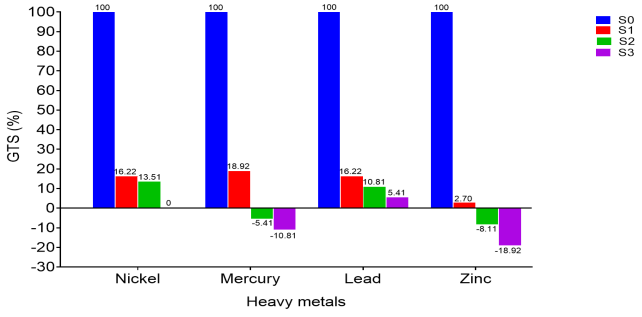

Figure 1. GTS of *C. gariepinus* in evaluated heavy metal sublethal doses.

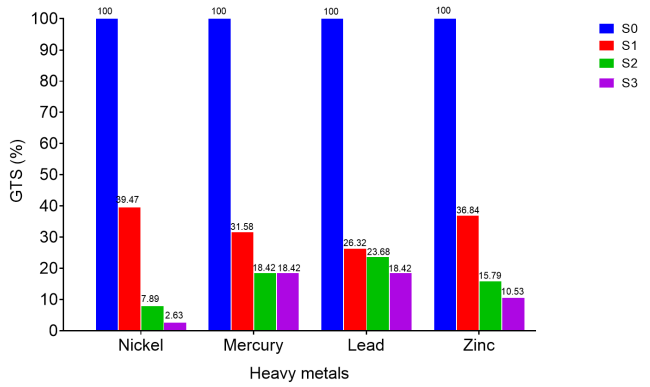

Figure 2. GTS of *O. niloticus* in evaluated heavy metal sublethal doses.

Discussion

Heavy metal pollution in aquatic environment and subsequent uptake in the food chains by aquatic organisms is a major global concern, as accumulation of heavy metals by aquatic organisms, fishes inclusive, may result in many detrimental consequences. These include, decline in aquatic productivity and reproductive capacities, morphological abnormalities, neurophysiological disturbances, genetic alteration of the cell (mutation), teratogenesis and carcinogenesis, abnormal enzymatic activities, to mention a few as reported by Obaroh *et al.* (2015). The accumulation of these metals may also flow to the consumers which may ultimately affect the health of man, depending on the level of accumulation or heavy metal type. Understanding the nature of heavy metal induced effects at the molecular level could provide the required information to proffer options for mitigations when such event arises. RAPD-PCR, amongst other molecular marker technologies, have been used, due to its sensitivity, not requiring fore knowledge of the organism's genome sequence, cost effective to mention but a few, to identify and understand heavy metal effects on the DNA. Several studies have reported that the RAPD assay ability of detecting temporary DNA changes at lower concentrations of pollutants (Atienzar *et al.,* 2006; Zhou *et al.,* 2011; Zhang *et al.,* 2017).

Variable banding patterns were observed in relation to the control samples between fishes and between heavy metal sub lethal doses in this study. Such differences which includes, presence of additional bands, absence of control bands, increased and decreased band intensities relating to genotoxic impact of heavy metals have documented in literature in plant and animals' studies (Enan, 2006; Zhang *et al.,* 2017). Significant differences in RAPD profile in both *C. gariepinus* and *O. niloticus* were observed for the evaluated sub lethal heavy metal doses when compared to the control, depicting the level of DNA damage in the fishes with respect to type and dose of heavy metal used. Atienzar and Jha (2006) further reported that the variation of band intensity may be as a result of oxidative DNA damage, mutations and/or complex chromosomal rearrangements and DNA-protein cross-links from blocked *Taq* polymerase processes while modifications in the DNA enhances the pairing activity of the primer with the DNA template might be a possible explanation for the increase in intensity of some RAPD bands and/or appearances of new bands. Other causes of band disappearance include genomic rearrangements and decreased point mutation, as indicated by Liu *et al.* (2005). The differences in band patterns observed in the treated 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 the ability of the test for DNA damage in test species (Kumar *et al.,* 2015).

OPAE-01 and RAPD-1 were observed to detect more polymorphism in *C. gariepinus* than *O. niloticus* in all the heavy metal treatments while vice versa for OPC-04 and RAPD-4 with all heavy metal treatments in the study. This varied resolving ability of the markers might be due to the DNA templates in the fish species. Enan (2006) stated RAPD yield pattern is dependent on and reflect the DNA sequence template of the organism used.

The study revealed a decline in GTS with increase in sublethal doses of heavy metals in both fishes. Similar results have been reported in previous studies (Osman *et al.,* 2012; Kumar *et al.,* 2015; Zhang *et al.,* 2017). Kumar *et al.* (2015) stated that decrease in genomic DNA stability and integrity may be as a result of band disappearance and appearance of new bands.

Conclusion

The study revealed more damaging effect in *C. gariepinus* than *O. niloticus* with increase in sub lethal heavy metal doses while *C. gariepinus* showed a higher genomic instability with both zinc and mercury at higher sublethal doses, genomic instability was highest in *O. niloticus* treated with nickel when compared to control groups. These differential responses between the fish species with sub lethal heavy metal doses used in the study might be related to the fishes' physiological structure. *C. gariepinus* is a scale-less cartilaginous fish and thus, the easy

assimilation and penetration of these heavy metals into the blood stream while *O. niloticus* is a scaled bony fish. The outer scales help to reduce the rate at which heavy metals are assimilated into the blood stream. An important characteristic of a good marker is polymorphism between related individuals. The level of polymorphism reflects the resolving power of a marker. In this study, all selected markers were above 70% polymorphic in both fish species making it suitable for the molecular study. This study also confirmed RAPD as a useful and powerful PCR-based technique capable of detecting DNA damage and mutations in fish. RAPD-PCR technology is useful tool in examining and estimating genomic variation in genotoxic studies.

References

Abdelaiz, D., E. Abdelali, I. Gaidoumi, B. Chaouni, A. Taleb, K. Abdelhak. 2019. Characterization and quantification of heavy metals in oued sebou sediments. The Scientific World Journal, 9: 7496576.

Aderolue, A.Z., M.O. Lawal, L.I. Okoronkwo, F.O. Awobajo, F.I. Jesuniyi. 2017. Dietary effect of *Cissus populnea* and *Securidaca longepedunculata* aqueous leave extracts on reproductive, haematological and biochemical parameters of African catfish, *Clarias gariepinus* (Burchell, 1822) broodstocks. Aceh Journal of Animal Science, 2(1): 1-11.

Ador, M.A.A., S. Haque, S. I. Paul, J. Chakma, R. Ehsan, A. Rahman. (2022). Potential application of PCR based molecular methods in fish pathogen identification: A Review. Aquaculture Studies, 22(1): AQUAST621.

Aksakal, O., N. Esim. 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., G. Banagar. 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., M. Muniyan. 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., A.N. JhA. 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.

Bawuro, A.A., R.B. Voegborlo, A.A. Adimado. 2018. Bioaccumulation of heavy metals in some tissues of fish in Lake Geriyo, Adamawa State, Nigeria. Journal of Environmental and Public Health, 2018: 1-7

Enan, M.R. 2006. Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnology and Applied Biochemistry, 43:147-154.

Kumar, P., R. Kumar, N.S. Nagpure, P. Nautiyal. 2015. In vivo assessment of DNA damage in *Cyprinus carpio* after exposure to dichromate using RAPD. Turkish Journal of Veterinary and Animal Sciences, 39: 121-127.

Liu, W., P.J. Li, X.M. Qi, Q.X. Zhou, I. Zheng, T.H. Sun, Y.S. and Yang. 2005. DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere, 61: 158–167.

Marimuthu, K., H. Palaniandy, Z. A. Muchlisin. 2019. Effect of different water pH on hatching and survival rates of African catfish *Clarias gariepinus* (Pisces: Clariidae). Aceh Journal of Animal Science (2019) 4(2): 80-88.

Obaroh, I.O., U. Abubakar, M.A. Haruna, M.C. Elinge. 2015. Evaluation of some heavy metals concentration in River Argungu. Journal of Fisheries and Aquatic Science, 10(6): 581-586.

OECD. 2019. Guidelines for the testing of chemicals: Test Guideline No. 203 Fish, Acute Toxicity Testing.

Osman, A.G. 2012. Biomarkers in Nile Tilapia *Oreochromis niloticus niloticus* (Linnaeus, 1758) to assess the Impacts of River Nile Pollution: Bioaccumulation, Biochemical and Tissues Biomarkers. Journal of Environmental Protection, 3: 966-977.

Osman, A.G. 2014. Genotoxicity tests and their contributions in aquatic environmental research. Journal of Environmental Protection, 5: 1391-1399.

ReshmaRaj, S., D.N. Das. 2021. Advances in Animal Genomics. Academic Press, New York.

Rocco, L., I.V. Valentino, G. Scapigliati, V. Stingo. 2014. RAPD-PCR analysis for molecular characterization and genotoxic studies of a new marine fish cell line derived from *Dicentrarchus labrax*. Cytotechnology, 66 (3): 383–393.

Salem, Z.B., N. Capelli, E. Grisey, P.E. Baurand, H. Ayadi. 2014. First evidence of fish genotoxicity induced by heavy metals from landfill leachates: the advantage of using the RAPD PCR technique. Ecotoxicology and Environmental Safety, 101: 90-96.

Sankar, T.V., A.A. Zynudheen, R. Anandan, P.G. Viswanathan-Nair. 2006. Distribution of organochlorine pesticides and heavy metal residues in fish and shellfish from Calicut Region, Kerala, India. Chemosphere, 65: 583-590.

Sarong, M. A., A.L. Mawardi, M. Adlim, Z.A. Muchlisin. 2013. Cadmium concentration in three species of freshwater fishes from Keuretoe River, Northern Aceh, Indonesia. AACL Bioflux, 6(5):486-491.

Sarong, M.A., C. Jihan, Z.A. Muchlisin, N. Fadli, S. Sugianto. 2015. Cadmium, lead and zinc contamination on the oyster *Crassostrea gigas* muscle harvested from the estuary of Lamnyong River, Banda Aceh City, Indonesia. AACL Bioflux, 8(1):1-6.

Shekhawat, S.S., A. Gaurav, B. Joseph, H. Kumar, N. Kumar. 2019. Random amplified polymorphic DNA-based molecular heterogeneity analysis of Salmonella enterica isolates from foods of animal origin. Veterinary World, 12(1): 146.

Stefanska, I., E. Kwiecien, M. Górzynska, A. Salamaszynska-Guz, M. Rzewuska. 2022. RAPD-PCR-based fingerprinting method as a tool for epidemiological analysis of *Trueperella pyogenes* infections. Pathogens, 11: 562.

Tajik-Esmaelili, S., A. Majd, S. Irian, M. Nabiuni, F. Ghahremaninejad. 2017. Assessment of DNA damage using random amplified polymorphic DNA in vegetative stage bean (*Phaseolus vulgaris* L.) grown under a low frequency electromagnetic field. Applied Ecology and Environmental Research, 15(4): 729-739

USEPA. 2002. calculating upper confidence limits for exposure point concentrations at hazardous waste sites. OSWER 9285.6-10. Washington, D.C.: United States Environmental Protection Agency, Office of Research and Development.

Zhang, H-C., T.Y.Liu, C.Y. Shi, G.W. Chen, D.Z. Liu. 2017. Genotoxicity evaluation of an urban river on freshwater planarian by RAPD assay. Bulletin of Environmental Contamination and Toxicology, 98: 484-488.