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Evaluation of heavy metal and genotoxicity status of mud fish obtained from Asa River using RAPD Marker

Abubakar M.I.[1]*, Omosanya T.A.[1], Alabi A.T.[1]., Coker O.M.[2], Folorunso L.A.[2], Aminu M.O.[2], Abdulraheem I.[3], and Abdulsalami S.A.[4]

[1]Department of Fisheries and Aquaculture, Kwara State University, Malete, P.M.B1530, Nigeria
[2]Department of Aquaculture and Fisheries, University of Ilorin. Kwara State, P.M.B1515, Nigeria
[3]Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Ogun State, P.M.B5025, Nigeria
[4]Department of Fisheries, Aquaculture and Wildlife, University of Abuja, Nigeria

***Corresponding author, Email address: abubakar.im@unilorin.edu.ng*

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Abstract: A study was carried out on the evaluation of heavy metals and genotoxicity status of mud fish obtained from the Asa River, Kwara State. One hundred and twenty (120) fish samples were collected with the assistance of local fishermen from three sampling sites along the Asa River at Ologuso, Dangote, and Laduba, respectively. The fish samples were sacrificed and carefully dissected to remove their organs for analysis. Water samples were collected and analyzed for the heavy metal concentration present in them using a BUCK Scientific Atomic Absorption Spectrophotometer (ACCUSYS 230). Mean values of the concentration of specific heavy metals were determined using One-way Analysis of Variance (ANOVA), and the significance differences among means were compared using the Turkey HSD posttest. DNA (RAPD), a powerful PCR-based techniques with the modified Cetyltrimethyl ammonium bromide (CTAB) method, were used to determine DNA status and mutations in the fish samples. The results obtained revealed a significant difference ($p<0.05$) among the three sampled sites. Sampling site B had the highest level of Cd concentration (0.047±0.010mg/l) while Sampling site C had the least (0.02±0.006mg/l). Sampling site A had the highest Pb and Fe (0.208±0.005mg/l) and (1.541±0.025mg/l) concentrations, respectively. There was no significant difference ($p<0.05$) in the level of Fe in the three locations. The results obtained showed that the heavy metal concentrations from the three sampling sites exceeded the limits recommended by the WHO, USEPA, and FAO, respectively. High genetic diversity (0.7778) was also observed among fish species from the three sampled locations. The polymorphic value (0.7438) obtained justified the efficiency of RAPD marker as a means of determining genotoxicity in fish species.

1. Introduction

The World Health Organization (WHO, 2011) observed that water is important to sustain life and is a substance that must be available to all in adequate, safe and reachable form. Water pollution is the introduction of unwanted and harmful substances into the aquatic ecosystem **(**Alrumman *et al.*, 2016: Alaqarbeh *et al.*, 2022). The deliberate discharge and accidental release of harmful chemical compounds into the aquatic environment can disturb the structure and function of the natural ecosystem (Cavas *and* Gozukura, 2005). One of the problems affecting the wellbeing of aquatic organisms is the exposure of their surroundings to several hazards which could be chemical, physical or biological

contaminating agents such as heavy metals, aromatic polycyclic hydrocarbons and various agricultural chemicals (Deepak *et al.*, 2021). WHO, (2011) described heavy metals as non-biodegradable ubiquitous toxic elements in the environment that are harmful when it exceeded the threshold limits. Kinuthia *et al.*, (2020) stated that heavy metals can be introduced through natural phenomena such as runoff from the environment, volcanic eruption, or landslide or anthropogenic activities such as disposal of untreated waste, effluent containing chemicals from industrial or agricultural sites, mining crude oil spills, domestic wastes, and several other toxic by-products into the water bodies respectively. They are usually in form of persistent organic pollutants (POPs) because they quickly amass in the body and are leisurely digested in and excreted from aquatic animals (Abugu *et al.*, 2013*)*. Alrumman *et al.,(*2016) observed that cadmium, lead, mercury, zinc, copper, Nickel, cobalt, molybdenum, chromium, tin, titanium and iron are the most commonly found heavy metals in aquatic organisms especially fishes due to their bio-accumulative tendencies in water.

Fish are affected mainly by heavy metals pollution than any other aquatic organisms because they live, feed, and occupy the top of the trophic level and are constantly exposed to toxic substances containing heavy metals (Karim *et al.*, 2013; Abdel-Baki *et al.*, 2011). These pollutants are found to biologically accumulate in fish because they are readily taken up by various body parts, such as body scales, gills, digestive tract, liver, and muscles (Cordeli *et al.*, 2023; Mahmood and Al Khafaji, 2016; Squadrone *et al.*, 2013). Abdel-Baki *et al.*, (2011) stated that the rate of bio-accumulation of heavy metals in aquatic organisms depends on several factors such as; the organisms that digest metals, specific concentrations of metal in a marine habitat, concentration of heavy metals in surrounding soil sediments, as well as the feeding habits of the organism. *Clarias gariepinus* is one of the most popularly consumed, widely accepted, and highly valued freshwater culturable fish species in Nigeria and sub-Saharan Africa, owing to its ease of breeding in captivity, toughness to a wide range of adverse water quality conditions, and fast growth (Ogamba et al., 2016). It is rich in protein, vitamins, a healthy fatty acid with low cholesterol, and minerals, which are healthy for consumption and have the capability of reducing the risk of heart diseases and stroke (Alrumman *et al.*, 2016). Thus, its importance as food for human consumption is widely explored. Fish species are observed to be a perfect model for sensing the occurrence of genotoxicity as well as genotoxic toxins in aquatic ecosystems (Aich *et al.*, 2015) because they are very sensitive to small quantities of metals within the water body, are abundant, and also live in some different habitats (Bukola *et al.*, 2015; Karim *et al.*, 2019; Nasri *et al.*, 2024). The uptake of these metals may be followed by their metabolism which may result in more toxic by-products. For example, mercury can be converted into very high toxic methyl-mercury by the microbial action which in turn is taken up by fish species (Bukola *et al.*, 2015). Genotoxicity is the ability of hazardous substances to destroy the integrity of the genetic materials or information in cells (DNA/RNA), (Kaur and Tarang-shah, 2018). Genotoxins are chemicals or agents that cause DNA or chromosomal damage (Kaur and Tarang-shah, 2018). They have been reported to cause mutations by forming strong covalent bonds with DNA resulting in the formation of DNA adducts, preventing accurate replication. Genotoxins affecting germ cells (sperm and egg cells) can pass genetic changes down to descendants and have been implicated to be against sustainable development portraying them as significant factors in congenital abnormalities which account for 589,000 deaths in humans annually (Kaur and Tarang-shah, 2018).

Asa River is one of the major rivers used for domestic and agriculture purposes in Ilorin metropolis, the capital of Kwara State, Nigeria. However, Asa-River is being polluted by domestic wastes, agricultural wastes, and chemicals discharged from many industries located along its course

(Ogundiran *et al.*, 2014) Yet there is no published literature available on the evaluation of heavy metal-induced genotoxicity in fishes collected from Asa River, therefore the aim of this study was to evaluate the heavy metal and genotoxicity status of mud fish from Asa River in Kwara State, Nigeria using RAPD Marker

2. Materials and Methods

2.1 Ethical Approval

The experimental protocol and procedures used in this study were approved by Department of Aquaculture and Fisheries, University of Ilorin. Ilorin, Nigeria: Ethical Review Committee (Protocol Identification Code: DERC/AQF/204; DERC Approved Number: DERC/AQF/2221/2224) and conform with the "Guide to the care and use of Animals in Research and Teaching (Ethical Principles for Medical Research of World Medical Association Declaration of Helsinki).

2.2 Study site

The study was carried out along Asa River as shown in **Figure 1**. The study was carried out along River Asa in Asa Local Government Area (LGA), Kwara State, North-Central Nigeria. The river originates from Oyo State and flows through Asa LGA towards South-North of Ilorin direction between longitudes 4°30' to 4°37' N and latitudes 8°27' to 8°32' Eforming a dividing boundary between Eastern and Western Ilorin, surface area of 302 hectares and a maximum depth of 14m (Oladimeji *et a*l., 2016). Industries such as Global Soap and Detergent, Bottling Company, Tuyil Pharmaceuticals and Nigeria Bottling Company are located along theriver side. There are considerable artisanal fishery, rain-fed and irrigation farming along River Asa and its tributaries in Ilorin metropolis such as Laduba, Dangote, Ologuso Kajola, Elega, Egbejila, Osin-Asa, Olokonro, Isale-Asa and Iyadundu. The fishing activities are usually carried out by the traditional fishing methods such as canoes with paddles, fishing nets, hooks, traps and more recently few motorized canoes have been introduced. Three sampling locations (n=2), Ologuso, Dangote, and Laduba were selected as indicated in the map (**Figure 1**). These locations were selected because there are lots of anthropogenic activities resulting in waste disposal directly into the river daily as indicated in **Table 1.**

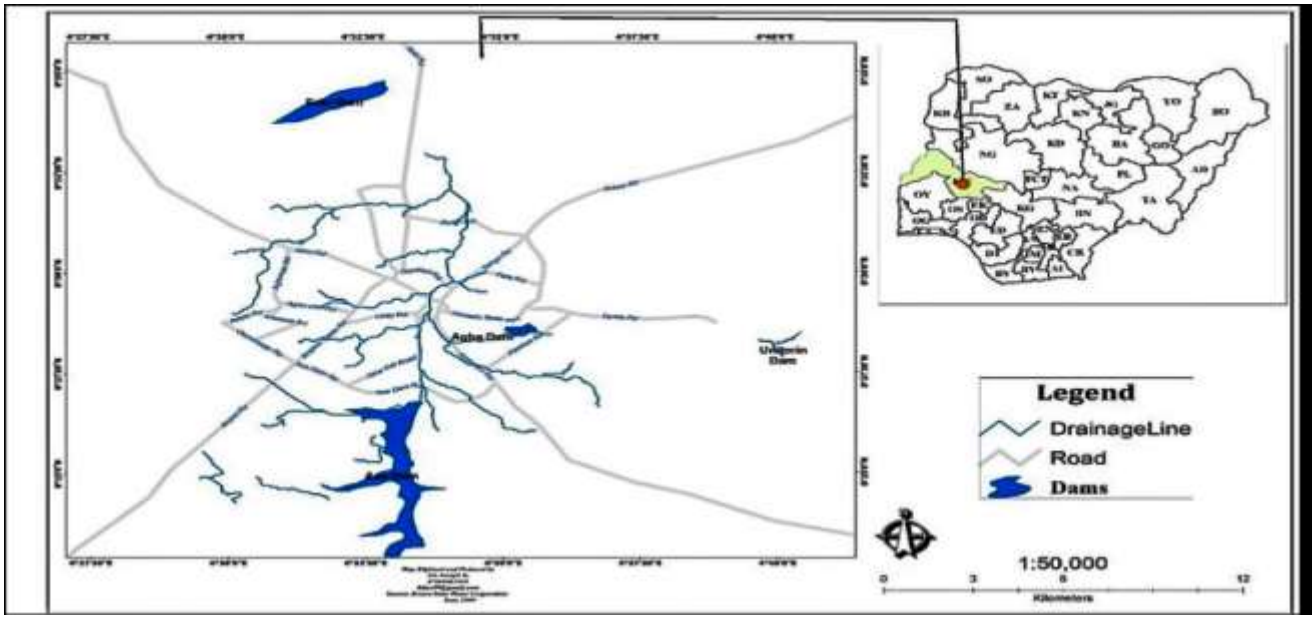

Fig. 1: Map of Ilorin showing Asa River. Source: (Research gate)

Table1: Predominant activities in permanent sites

Site	Location	Predominant activities
A	Ologuso	Fish farming, poultry, farming activities, effluent discharge location.
B	Dangote	Fish farming, Cement production, granite production.
C	Laduba	Fishing Activities

2.3 Sample Collection and Preparation

6 Live Mud fishes (*Clarias gariepinus and Heterobranchus spp*) of medium size (28-37cm) and average weight (400-900g) were caught with Gora trap. They were collected from three sampling areas (n=3) along Asa-river with the help of Local fishermen and were identified using the manual of Idodo-Umeh (2008) and standard fish taxonomy protocols. The samples were transported to the Central Laboratory, University of Ilorin in plastic containers of 25 L capacity filled with water to two-third volume. Fishes were acclimatized to laboratory conditions in a big plastic aquarium with dechlorinated water and enclosed with a net to prevent fish from escaping and to ensure adequate ventilation as described by Abubakar *et al.,* (2019).

2.4 Water Sample and Collection

Waters from Asa River at three sampling points were collected with Water sampler (Von Dorn water sampler) within the study area. At every sampling point, the Von Dorn water sampler was thoroughly washed with the water sample before its collection. Samples for heavy metals were stored in the pre-washed (1-liter keg) and fixed to a pH < 2 by the addition of 2ml grade concentration HNO_3. All samples were properly labeled and kept in ice chest in cooler prior to transporting to the laboratory for analysis (Salaudeen *et al*., 2016).

2.5 Heavy Metal Analysis of water samples

100 mL of each of the water sample from the three sampling areas were transferred into beakers containing 10 mL of concentrated HNO_3. The samples were digested and the solutions were used for heavy metal analysis (APHA, 1990). Cadmium, Iron, and Lead in the water samples from the three sampling stations were analyzed using a BUCK scientific (ACCUSYS 230) Atomic Absorption Spectrophotometer (AAS). The AAS was standardized using stock standard solutions from the respective metals.

2.6. Blood Collection

Blood samples were collected from the fish samples for genotoxicity test through caudal venous puncture using the methods of Abubakar *et al*., (2016) as follows: The fishes were anesthetized with clove oil, methanol, and distilled water in a ratio of 2.5:5:1 respectively. Needle was inserted under the skin of the ventral midline of the caudal peduncle of an anesthetized fish and then eased towards the vertebral column until the base of the column was reached and a fraction of the blood sample was withdrawn.

2.7. Extraction of genome Dna and Purification

Total nucleic was extracted using a modified CTAB (Cetyltrimethyl ammonium bromide) extraction protocol (Wilson and Walker, 2005): 1ml offish blood was centrifuge at 500rpm for 5 minutes to precipitate the cells. Supernatant was discarded and 1ml CTAB extraction buffer (2% CTAB powder) (w/v), 100mM Tris-HCl, 20mM EDTA, 1.4M NaCl and 0.2% β-Mercaptoethanol (v/v) which were added before their use were vortex followed by incubation at 60^{o}C for 10mins. Tubes were placed on ice and equal volume of Phenol, Chloroform and Iso-amyl alcohol at 25:24:1 were added followed by vortex and centrifuged at 12000g for 10mins. 450µl of the supernatant was collected into a new sterile tube to which 300µl of cold Isopropanol was then added, mixed gently and incubated for 1hr at -20^{o}c. Samples were later centrifuged at 12000rpm for 10mins to sediment the nucleic acid. The Supernatant was decanted gently to ensure that the pellets were not disturbed. The Pellets were washed with 500µl of 70% ethanol to remove salt and contaminants. The products were centrifuged at 12000rpm for 5mins. This was repeated twice to dissolve the DNA completely. Ethanol was then decanted while the DNA was air dried at room temperature until no trace of ethanol was seen in the tube. Pellets were then suspended in 50µl TE buffer treated with EDPC and stored till further use.

2.8 RAPD- PCR Electrophoresis Amplification and Gel

Using the RAPD- PCR program (Kalendar, 2007). PCR was set up to detect the extent of diversity in the 6fish samples collected from three different locations. Reaction cocktail (2.5 µl of 5x GoTaq colourless reaction buffer, 0.75 µl of 25mM MgCl2, 0.25 µl of 10 mM of dNTPs mix, 0.25 µl of 10 pmol (OPB-08) TCCACACGG primers and 0.3units of Taq DNA polymerase Promega, USA) made up to 42 µl with sterile distilled water 2µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermal-cycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by denaturation at 94°C for 15 sec, annealing at 50°C -60°C (with a temperature increase by 1°C after every 5 cycles) and a final termination at 72°C for 10 mins and chilled at 4^{o}C.

2.9 Data Analysis

The data obtained from heavy metal were managed with Microsoft office Excel 2003. Data were statistically analyzed with one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 16.0 for window. Statistical significance of difference among means was compared using Turkey HSD Post test at 95% confidential unit. The genetic relationship among fish samples was estimated by constructing a dendrogram cum the Unweighted Pair Group Method (UPGMA) using the Mega6 software.

Genetic diversity parameters such as major allele frequency, gene diversity and polymorphic information content were generated using the power marker software. Clear and distinct amplification products were scored as ‘1’ for presence and ‘0’ for absence of bands. Bands of the same mobility were scored as identical. The genetic similarities were calculated using Jaccard’s similarity coefficient (Jaccard, 1908). The similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973). The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Sneath and Sokal, 1973).

3. Result and Discussion

3.1Heavy Metal Analysis

The concentration of heavy metals in the water samples from the three (3) sampling units are presented in the **Table 2** below:

Table 2: Mean concentrations of heavy metals in water samples from the three locations

Heavy metal	Location A (Ologuso)	Location B (Dangote)	Location C (Laduba)
Cadmium	0.022 ± 0.009^{b}	0.047 ± 0.010^{a}	0.020 ± 0.006^{b}
Iron	1.54 ± 0.025^{a}	0.35 ± 0.001^{c}	0.008 ± 0.003^{c}
Lead	0.208 ± 0.005^{a}	0.15 ± 0.017^{a}	0.069 ± 0.035^{a}

Means of parameters with the same superscripts along the column are not significantly different at $p<0.05$

The result shows that location B (Dangote) had the highest level of cadmium with a mean value of 0.047±0.010mg/l while Location C (Laduba) had the least level of cadmium concentration with a mean value of 0.002±0.006mg/l. There is no significant difference in cadmium concentration between Location A and C, but location B varied significantly. Lead had the highest mean concentration of 0.208±0.005mg/l in sample A while location C had the lowest (0.069±0.035mg/l). The mean values of lead concentrations in Location B were also higher than that of the Location C. For Iron, Location A had the highest mean value (1.541±0.025) while Location C had the lowest mean value (0.01±0.003). Mean value of Iron from the Location A (1.54 ± 0.025mg/l) is also greater than that of Location C (0.008 ± 0.003mg/l).

Table 3: Comparison of data obtained with recommended standard limits

	Loc A	**Loc B**	**Loc C**	**WHO**	**USEPA**	**FAO**
Cadmium (Mg/l)	0.022±0.009	0.047±0.010	0.020±0.006	0.003	0.05	0.003
Lead (Mg/l)	0.208±0.005	0.115±0.017	0.060±0.035	0.01	0.015	0.01
Iron (Mg/l)	1.541±0.025	0.035±0.001	0.01± 0.003	0.3	0.3	0.3

WHO- World Health Organization, USEPA- United States Environmental Protection Agency, FAO- Food and Agricultural Organization

Mean values for the Cadmium concentrations at the three sampled locations (A, B and C) exceeded both WHO and USEPA limits greatly, highlighting the importance of monitoring and managing heavy metal pollution in these areas. Mean values for the Lead also exceeded the WHO and USEPA limits in all the three sampled locations emphasizing the urgent need for comprehensive intervention strategies to address the pollution problem in those areas. Iron concentration at location A significantly exceeds both WHO and USEPA limits. Thus, indicating severe contamination while at locations B and C, the concentrations are much lower suggesting a relatively clean condition. Despite their lower concentrations, there still exist a significant difference from the standard limits suggesting the importance of monitoring and reducing heavy metals from the areas.

3.2. Molecular Analysis

Phenogram of Primer OPB-08

PCR amplification with RAPD (Random Amplified Polymorphic DNA) primer (OPB-08) showed clear and well-differentiated band patterns of DNA samples extracted by the DNA extraction protocol (**Plate 1**). Electrophoresis separation of DNA extracted by the present protocol showed intense bands very close to the gel wells signifying high degree of purity and intact DNA. It is known that the presence of smear could be a sign of degradation of the extracted DNA which easily affects the quality of the subsequent molecular application results (Devi *et al*., 2013).DNA samples extracted by the present protocol were assessed for successful PCR amplification with RAPD primer(OPB-08).The presence of clear and well-differentiated band patterns (Plate.1) reflects the efficiency of the protocol to produce genomic DNA with high purity suitable for molecular studies based on PCR techniques (Devi *et al*., 2013).

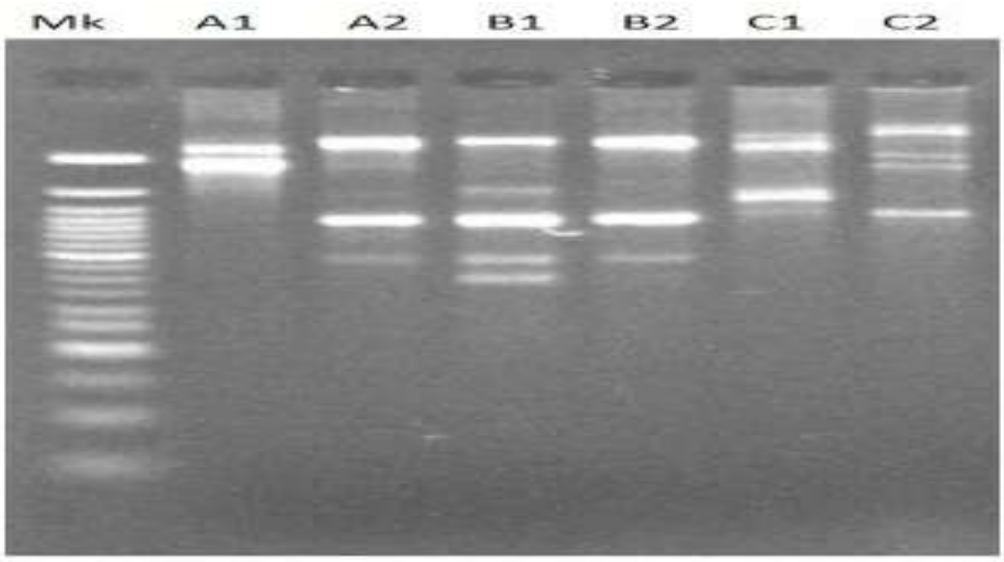

Plate 1: PCR amplification profile pattern obtained using primer OPB-08 (TCCACACGG) with analysis in agarose gel electrophoresis of six fish samples collected from the three locations along Asa River.

NB: (MK) referred to size marker-100bp DNA ladder.

A1, A2, B1, B2, C1 and C2: stand for the six fish samples from the three different locations

3.3 *Polymorphism statistics analysis*

Calculation of the polymorphic information content (PIC, a measure of discriminatory ability) based on the results obtained from RAPD markers using Nagy *et al*., (2012) and Amyousefi *et al*., (2018). There are two measurements of the quality of a polymorphism as a genetic marker: Heterozygosity (H) and polymorphic information content (PIC). The maximum value of H and PIC for binary data is 0.5, because two alleles per locus are assumed, and both are influenced by the number and frequency of the alleles. For Co-dominate markers, these values vary between 0 and 1 (Blanc *et al*., 2005).

The results showed that: the PIC value provides an estimate of the discriminatory power of a locus taking into consideration; not only the number of alleles but also the relative frequencies of those alleles. PIC values vary from 0 (monomorphic) to 1 (very highly discriminative with many alleles in

equal frequencies). iMEC is calculated as a basic measure of polymorphism indices for individual markers. More details of the basic measures of polymorphism indices for the primers are given in **Table 4.**

The Major Allele Frequency, Sample size, number of observable samples, Allele Number, Availability, Gene Diversity, and PIC (polymorphic information content) of the RAPD marker, are presented in **Table 4**.

Table 4: Major Allele Frequency, Allele No, Gene Diversity and PIC of the OPB-08 Random primer used in the genotyping of 120 fish samples.

Marker	Major Allele freq	Sample size	No. of obs	Allele no	Availability	Gene Diversity	PIC
PRIMER	0.3333	6.0000	6.0000	5.0000	1.0000	0.7778	0.7438

The higher the values of the polymorphic information content, the higher the genetic diversity. This indicates high heterogeneity among the sampled fish.

3.4 Dendrogram

The use of UPGMA method with arithmetic mean produced the dendrogram which clearly separated the genotypes to two clusters (A and B) **Figure 1**. Cluster A reveals that B_1, C_1, B_2, and C_2 have similar features while Cluster B indicates the similarities that exist between A_1 and A_2.

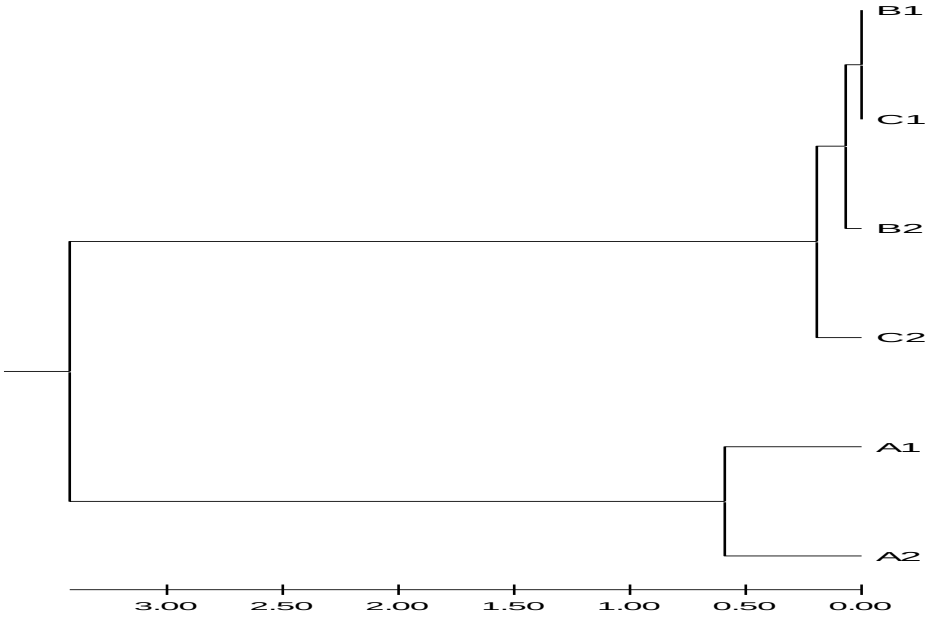

Fig 1: The dendrogram cluster tree was generated with UPGMA cluster analysis using Jaccard's similarity coefficients obtained from RAPD markers for the six fish species samples from three locations.

From the graph, all the visible bands were scored. The scoreable bands were subjected to cluster analysis, which generated the dendrogram. The banding pattern of the genotype was determined by the strong and consistent bands shared among the fish samples from the three sites.

3.5 Discussion

The results of this study revealed that the concentrations of heavy metals such as Pb, Fe, and Cd were generally high in sampling sites A and C (Ologuso and Laduba). The mean values obtained heavy metal concentrations could be attributed to anthropogenic activities going on around the sampling sites and which might be due to their exposure to domestic, agricultural, and industrial influences. This is in line with the study of Salaudeen *et al.* (2016) whose report on heavy metals concentrations in Asa-river water over two seasons from four different locations showed higher concentrations in all sampling points. Similar observations were made on the levels of heavy metals in Ureje Dam in Ado Ekiti by Adefemi *et al.*, (2014). Heavy metal concentrations in the river water at all three sampling sites were found to be Fe > Pb > Cd, Pb > Cd > Fe, Pb > Cd > Fe. This is in agreement with the work of Abdel-Moati *et al*. (2010), who observed that heavy metals in natural water might be attributed to the increased cover of the aquatic and higher plants, which absorb metals from water sediment, huge amounts of raw sewage, and agricultural and industrial wastewater discharged into the river. Farming activities are also found to contribute to the levels of some metals in surface water through run-off. The high level of Iron and Lead content in site "A" might be due to the effluent discharged by industries, fishing, and agricultural activities that release higher concentrations of iron into the water body. It can also be due to industrial and anthropogenic activities, geological characteristics of the areas surrounding the river, urbanization, and agricultural activities (Wang and Wang, 2021) (El and Joner 2018). Site B had the highest cadmium concentration. This could be related to the cement and granite production activities being carried out in the area (Salaudeen *et al.*, 2016). Cadmium in water may also arise from industrial discharges and mining wastes. It is widely used in metal plating. Chemically, cadmium is very similar to zinc, and these two metals frequently undergo geochemical processes together (Salaudeen *et al.*, 2016). The lowest mean value concentration of all the tested heavy metals was found at location C. This was supported by the works of Oladimeji *et al.*, (2016) who affirmed that Laduba had the least concentrations of lead and iron in their study. This may be due to the absence of industry discharging effluent in the location as only artisanal fishing activities was found to take place along the river course. Cadmium evaluation impacts on the gills of young Gambusia showed damage of gill morphology and increasing number and form of chloride cells (Adam *et al*., 2019). Adam *et al*., (2019), indicated that an acute exposure to heavy metals (mix Cu, Cd, Zn), resulted in the degradations of various components of proteins and lipids in the gill tissue of rainbow trout (*Oncorhynchus mykiss*) in freshwater. Exposure for 15 and 30 days in *Oreochromis niloticus* showed that the gill exhibited severe hyperplasia and proliferation of chloride cells as well as the significant length shortening and reducing of the secondary gill lamellae (Mekkawy et al., 2023). In this study, the concentration of heavy metals was compared with World health Organization (WHO, 2011), United State Environmental Protection Agency (USEPA, 2000) and Food and Agricultural organization (FAO, 2001) standard limits to ascertain the level of toxicity of the heavy metals in water sample. Mean values of all the heavy metals in this study exceeded the standard limit, thus, making Asa River heavily polluted. This is supported by the work of Ogundiran, et al., (2014) who reported that Asa River water is grossly polluted with the level of pollution decreasing downstream, which was found to be much higher during the dry season and relatively lower during the peak of rainy season. The concentration levels of metals in three Ekiti State dams (Ureje, Egbe, and Ero dam) during the dry season shows that Fe, Pb & Cd in water were above the recommended limit set by WHO and similar

regulatory bodies, there by indicating that the waters of the dams may not be suitable for drinking nor the fish safe for human consumption (Olugbemide and Owolabi; Olufemi, 2019).

According to the study of Salaudeen *et al.,* (2016), all metals with the exception of copper showed higher concentrations in Asa-river during the dry season and with the exception of Cu and Zn, all the metal concentrations in water were above the World Health Organization (WHO) and Federal Ministry of Environment (FME) stipulated values for drinking purpose. The study of Olatunji *et al.,* (2011) also confirmed that the downstream Asa River segment is indeed polluted. The results of Oladimeji *et al.,* (2016) revealed that heavy metals (pb^{2},fe^{2+},or fe^{3+}) content of water samples in Asa-Osin and lower-Asa-river settlements exceeded the established and recommended rate by USEPA, (2000) and WHO (2011). The observed values for Lead (Pb), Iron (Fe) in Laduba according Oladimeji *et al.*, (2016) were within the ambient permissible level recommended by WHO (2011); USEPA, (2000). However, in this study, the lead concentration slightly exceeded the standard limit while iron level was within the recommended standard limits of WHO (2001), by USEPA, (2000) and FAO(2011).The genotypic result in which cluster A had 4 genotypes (B_1, C_1, B_2, C_2) while cluster B had 2 genotypes (A_1 and A_2) (plate 2) with a single cluster of 33.33 per cent similarity (Table 4). This is similar to study of khan *et al.*,, (2013) who observed 43 percent similarity on genetic diversity in common Bean using Molecular Marker. The sequences of RAPD fragments of primer OPB-08 reflect a high degree of polymorphism. The genetic similarity between samples from the sampling sites was found to be high in C_1, B_1, C_2, B_2 revealing that pair genetic distance shows high similarities. This might be due to the difference in the heavy metals composition at different sampling sites. Location A (Ologuso) with high mean values of Cadmium and Iron reflects high heterozygosity (gene diversity). The heterozygosity values of 0.7778 among the fish samples (**Table 4**) reveals substantial genetic variation within the studied population. This finding aligns with the study of Yin, *et al.,* (2020) whose study indicated high gene diversity in *Salvia miltiorrhiza* populations across different regions of China using RAPD and ISSR markers, Otudjehuh and Abdeelah (2006) assessed genetic diversity in *Eucalyptus grandis* population using RAPD markers observed significant gene diversity across different provenances in Brazil. It also aligns with the study of Owolabi *et al.,* (2021) *on* genetic diversity of *Clarias gariepinus* populations in rivers of southwest Nigeria that showed high gene diversity values reflecting the species adaptation potential and genetic variability. It also agreed with the work of Shittu *et al.,* (2020) who assessed genetic diversity in *Heterobranchus bidorsalis* population across Nigerian rivers, thus, emphasizing the role of RAPD markers in revealing population structure and toxic level of heavy metals in organism.

The PIC value (0.7438) obtained confirmed the significant of Random Amplified Polymorphic DNA (RAPD) to be an effective marker in detecting genetic variability in line with the works of Muhammed *et al.,* (2018) who highlighted high PIC values and marker utility for assessing genetic variation and population differentiation. Fayanda *et al.* (2019) evaluated genetic diversity in *Chrysichthys nigrodigitatus* populations across southwestern Nigerian rivers, demonstrating informative PIC values and the utility of RAPD markers for genetic differentiation studies and Adesalu *et al.* (2018) investigated genetic diversity in *Labeosene galensis* population across rivers in Osun state, Nigeria, highlighting the importance of RAPD markers in assessing genetic variability and informing conservation strategies. The study's results of high gene diversity and informative PIC values are consistent with the findings across different plant species, highlighting the robustness and applicability of RAPD markers in gene diversity studies. The dendrogram differentiated the populations into two clusters. The samples in the two locations exhibited a high level of genetic diversity. The diversity is evident among and within the studied populations as estimated by RAPD-PCR technique (Hendrick,

1992). Most of the RAPD polymorphism is shown to be presumably due to a substitution in the gene (Zimmer et al., 1991). The DNA standardization extraction protocol presented here is crucial for assessing fish genotoxicity. Therefore, it is of great value in the study of molecular analysis in fish samples.

Conclusion

The study concluded that heavy metal concentrations from the three sampling sites exceeded the limit recommended by the WHO, USEPA, and FAO, respectively. The high level of iron and lead contents in site A were due to the effluent discharged by industries, fish farming and agricultural activities that released high contents of iron and lead into the water body. The sequence of RAPD frequency of primer OPB-08 reflected a high degree of polymorphism. The PIC values confirmed the significance of Random Amplified Polymorphic DNA (RAPD) as an effective marker in detecting variability.

Conflict of interest

The authors declare no conflict of interest in writing and publishing this manuscript.

Author contribution: M.I. Abubakar initiated the research and wrote the manuscripts. Omosanya and Alabi, worked on sample collection. Folorunso, Aminu, Coker, Somrat and Abdulraheem worked on the statistical analysis.

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