GENOTOXIC ASSESSMENT OF *Oreochromis niloticus* and *Coptodon guineensis* ISOLATED FROM TWO HEAVY METAL CONTAMINATED RIVERS IN LAGOS STATE

*ADEBESIN, O.A., J.C. IGWE, V.N. MARTINS, O.E. EJUETUEYIN, O.O. ADESUYI AND N.N. MOKOLO
Department of Cell Biology and Genetics, University of Lagos, Akoka, Nigeria
*Corresponding Author: oadebesin@unilag.edu.ng, +2348023919330, 0000-0002-2544-261X

ABSTRACT
Accumulation of heavy metals in water bodies contaminated by industrial effluents directly or indirectly poses health risk to both aquatic organisms and humans, culminating in genetic disorders and various organ diseases. This study was conducted across three locations in Lagos State, Nigeria: Maya River, Erikorodo, Ikorodu, Osborne Foreshore Lagoon, Ikoyi and a fish farm at the Nigerian Institute for Oceanography and Marine Research (NIOMR), Badore. Genotoxic assessment of the flesh and histopathology of the liver samples harvested from Oreochromis niloticus and Coptodon guineensis collected from the study sites were undertaken and physicochemical analyses of the water and sediment samples were conducted using atomic absorption spectrophotometer. Results showed that olive tail moment for wild Oreochromis niloticus from Maya River and wild Coptodon guineensis from Osborne foreshore lagoon was 13.50 and 5.54 respectively compared to 4.6 and 5.32 of the same species from the farm. ANOVA showed statistical significance in DNA damage, with p-values 0.012 for Oreochromis niloticus. High concentrations of Iron and Zinc, along with the highest levels of heavy metals, were detected in Maya River which suggests that aquatic animals sourced from these sites may pose health challenges if consumed.
Keywords: *Genotoxicity, Heavy metals, Single-cell gel electrophoresis, DNA damage*

INTRODUCTION

Heavy metals are considered to be one of the main sources of the pollutants in the environment. They have significant toxic effects on its ecological and measurable quantities (Kim and Kim, 2020). The metals having high densities, atomic weights and numbers are called heavy metals. They are naturally present in environmental and biological samples of different composition and origins (Gumpu *et al.*, 2015; Patle *et al.*, 2022).

Some heavy metals like mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), iron (Fe), copper (Cu), cobalt (Co), vanadium (V), zinc (Zn), manganese (Mn), nickel (Ni), and lead (Pb) are essential for biochemicals and physiological functions (Abdulhatab *et al.,* 2020). The main sources of heavy metals as environmental pollutants include industrial and agriculture waste, mining activities, tailings, occupational exposure and paints (Patle *et al.*, 2022).

In several parts of the world, average concentrations of Cr, Mn, Fe, Co, Ni, As, and Cd in surface water bodies exceed the maximum allowed values for drinking water (Kumar *et al.*, 2021; Prasad *et al.*, 2022). Heavy metals are not biodegradable, hence, the reason they tend to bioaccumulate, which causes an increase in their concentration overtime in living organisms (Zamora-Ledezma *et al.,* 2021).

Aquatic environments, particularly rivers and sea are the ultimate recipients of contaminants and heavy metals (Shah, 2021). Slight changes in the quality of the environment's physicochemical properties can harm the normal physiology of aquatic organisms especially fish which are highly sensitive to such changes (Lakra and Nagpure, 2009; Akter *et al.,* 2021). They impact aquatic ecosystems directly by entering into the living organisms leading to toxicity or indirectly by altering the food web (Capillo *et al.*, 2018; Merola *et al.*, 2021; Shiry *et al.*, 2021). Bioavailability and the absorption of heavy metals depend on many factors including heavy metals concentration, duration of exposure, interaction with other metals, the age and size of the fish, mechanisms for detoxification, metabolic processes, feeding behavior and the physicochemical parameters of the environment (Kovendan and Vincent, 2013; Delahaut *et al.*, 2020).

Fish are the ideal organisms for studies in toxicology and toxicogenomics (Liu *et al.*, 2022). They are also at the top of the aquatic food web (Aliko *et al.*, 2019; Burgos-Aceves *et al.*, 2019; Sula *et al.*, 2020). Fish are suitable bioindicators for aquatic ecosystems as they are highly sensitive to environmental alterations as they readily metabolize, detoxify and accumulate heavy metals in their bodies (Abdel-Baki *et al.*, 2011). They respond similarly to how higher vertebrates respond to toxicants which help in heavy metal toxicity tests that can possibly be mutagenic, carcinogenic and teratogenic to humans (Yilmaz *et al.*, 2004; Shahjahan *et al.*, 2022).

MATERIALS AND METHODS

STUDY SITE

The study was conducted with samples from three selected locations, namely Maya River, Erikorodo, Ikorodu, Lagos state, Nigeria (6.6457669˚N longitude and 3.5537778˚E latitude), Osborne Foreshore Lagoon, Ikoyi, Eti-Osa, Lagos state, Nigeria (6.4621713˚N longitude and 3.4254933˚E latitude) and fish farm, Biotechnology Department, Nigerian Institute for Oceanography and Marine Research (NIOMR), Badore out station, Eti-Osa, Lagos State, Nigeria (Plate 1).

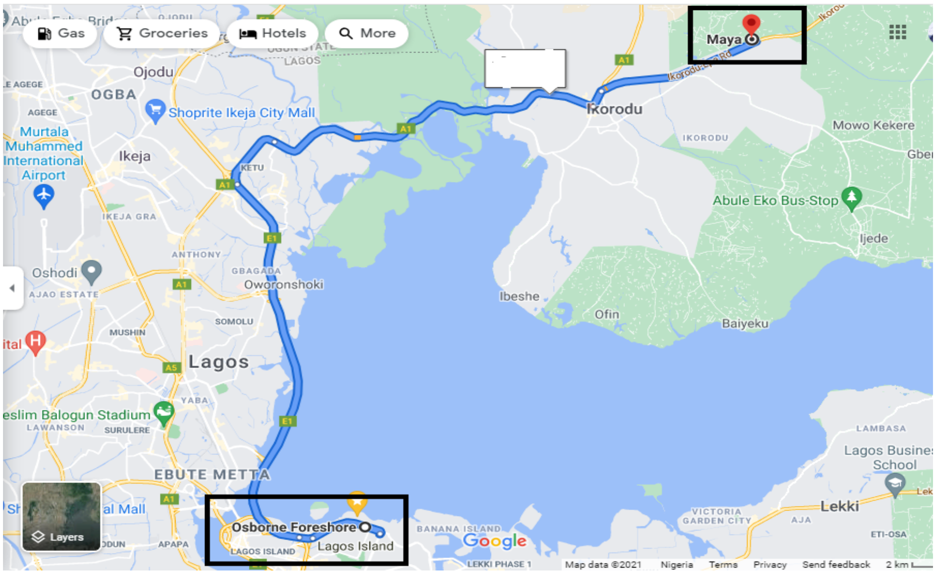

Plate 1: Map of the Sampling Sites

SAMPLE COLLECTION

Fish samples were collected by local fishermen from three different sampling points and transported in a cold chamber to the molecular laboratory, Cell Biology and Genetics department, University of Lagos, Akoka for analysis. Water samples were collected using labeled brown sterile bottles while sediment samples were collected in unused black sterile containers, tagged and sealed. Water and sediment samples were then transported in a cold chamber to chemistry laboratory, Chemistry department, Faculty of Science, University of Lagos, Akoka for analysis. Five samples of *Oreochromis niloticus* were collected from Maya River, Erikorodo, five samples of *Coptodon guineensis* were collected Osborne Foreshore Lagoon, and five samples each of *Oreochromis niloticus* and *Coptodon guineensis* were sourced from the fish farm at NIOMR, which served as the reference site.

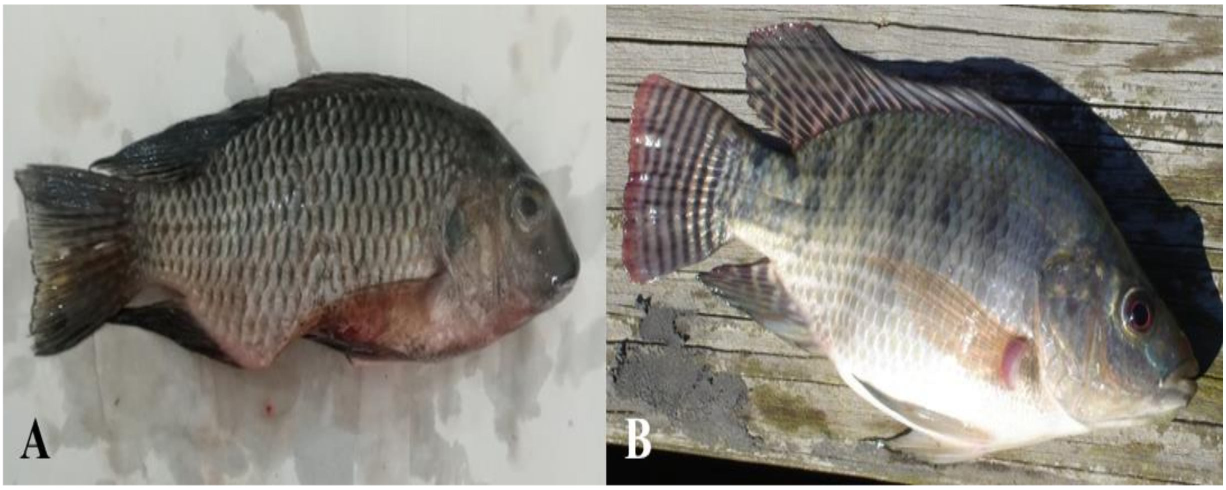

Plate 1: A-*Coptodon guineensis* **B-*Oreochromis niloticus***

Sample Extraction

Upon collection of the fish samples, their wet weight and total body length were measured. The fishes were immobilized and sacrificed by dissection through the mouth to harvest the liver and through the sides to cut out the flesh for analyses. The flesh samples were transferred into sterile bottles, labeled and stored on ice and transported for comet assay, while the liver was washed, blotted, weighed and preserved in Bouin's fluid for histopathology.

Physico-Chemical Analysis

Physico-chemical analyses were conducted on both sediment and water samples from each sampling point. Concentrations of heavy metals in the water and soil samples were measured using atomic absorption spectrophotometry (Model, Perkin-Elmer A Analyst 200). Physico-chemical assessment of surface water samples for electrical conductivity, pH, dissolved oxygen (DO), total solids (TS) and total dissolved solids (TDS) were measured using the Horiba U50G Multi water sampler. Standard methods (APHA-AWWA-WEF 2005) were used to measure other physicochemical characteristics like acidity, alkalinity and so on. Wrinkler's method was used in measuring biological oxygen demand; by comparing dissolved oxygen content before and after incubation for a 5 day period at 20°C. The HACH COD reactor (Model DRB200) was used in the determination of chemical oxygen demand. Physicochemical assessments of soil samples included the measurements of Total organic carbon (TOC) and Total organic matter (TOM). Argentometric method 4500-C1-B was used to measure chloride levels in water. Nitrates (NO_3^-), phosphates (PO_4^-) and sulphates (SO_4^-) levels were measured colorimetrically and determination of heavy metals concentration was measured using atomic absorption spectrophotometer.

Single Cell Gel Electrophoresis

Alkaline comet assay was used to assess DNA damage as described by Singh et al. (1988) with slight modifications. All steps of the comet assay were performed under dim-light conditions to prevent additional DNA damage. Chilled phosphate buffered saline was used to wash freshly collected flesh tissue samples after which it was digested using trypsin. The obtained cells were then suspended in 0.5 % low melting point agarose (LMPA), overlaid on slides precoated with a thin layer of 1 % normal-melting agarose, then a third layer of 1 % LMPA was applied and allowed to solidify after which the slides were submerged in lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Trizma base, 0.2 mM NaOH, 1 % Triton X-100, and DMSO [pH 10]) for 24 hours at 4 ˚C to lyse cells the immobilized cells in situ, thereafter the slides were transferred and submerged in electrophoresis buffer (300 mM NaOH, 1 mM EDTA [pH 13]) for 30 mins to facilitate DNA unwinding. This was followed by 30 mins of electrophoresis at 25 V and 300 mA current in the same buffer. After electrophoresis, slides were transferred into a neutralizing buffer (pH 7.5), dried, stained with Giemsa stain, viewed under a microscope and acquired digital images loaded onto Casplab_1.2.3b software for analysis.

Histopathological Analysis

Stored liver samples were cross-examined for any observable lesion after which the liver tissues were prepared and sectioned using the technique employed by Avwioro (2011). Briefly, the liver tissues removed from Biouin's fluid were placed in 10% formal saline for 30 minutes, washed, cut and placed inside well-labeled tissue embedding cassettes before processing the tissue using a 24 hours automated tissue processor. The automated tissue processor includes glass containers and electric metal containers with paraffin wax in them. They are all controlled thermostatically. After processing, an automatic tissue embedding centre is used to incorporate the samples into paraffin blocks. The cooled embedded tissues trimmed to centrally place the specimen with a margin of 2-3mm, were sectioned at 4µm using a rotary microtome. The sections were floated in 20% alcohol and attached to the microtome blocks for fine cutting, floated out in warm water (35°C), mounted on slides, left to drain and placed on a hot plate for proper adhesion of tissue to the slide. The prepared slides were stained with hematoxylin and counterstained with eosin in order to display the tissues' overall structure. Each slide was then mounted on the stage, observed under a light microscope (Leica MD-500, Leica, Microsystems. Germany), examined for changes in the structure of exposed tissues when compared to the reference and the digital images acquired.

Statistical Analysis

Statistical analysis was done using Microsoft excel 2016 and GraphPad prism software 8.0, using analysis of variance (ANOVA), significant at $P < 0.05$. Data expressed as mean ± sem (standard error of mean). DNA damage image analyses were done using Casplab_1.2.3b.

RESULTS

Mean Weight

The mean weight of each experimental group (Table 1) was taken before sacrificing the experimental animals with farm bred species weighing significantly more than wild species.

Table 1: Weight of fishes

Species	weight(g)
Oreochromis niloticus (farm)	263.72 ± 9.11*
Oreochromis niloticus (wild)	236.14 ± 4.72
Coptodon guineensis (farm)	256.60 ± 6.40*
Coptodon guineensis (wild)	229.00 ± 2.90

Values with asterix (*) differ significantly ($p<0.05$) between farm (control) and river (wild) specimens within species

Heavy Metals Concentration in Water and Sediments

The concentrations of nutrients and other physico-chemical parameters in the water samples from the sampling sites as compared to the standards set by the Federal Ministry of Environment (FME), Federal Republic of Nigeria are shown in Table 2.

Table 2: Physio-chemical Parameters of Water Samples from the Study Sites

Physicochemical	Osborne	Ikorodu	FME Values (2019)
Temp (^{0}C)	29.53	29.31	20-33
pH	7.02	6.5	6.5-8.5
ORP (mV)	-27	-57	300-500
Conductivity (mS/cm)	0.839	0.37	-
Turbidity (NTU)	0	259	1
DO (mg/l)	2.51	0.89	6
% DO	33.1	11.6	-
TDS (g/l)	0.307	0.24	-
Salinity (ppt)	0.41	0.18	-
Acidity (mg/L)	116	44	-
Alkalinity (mg/L)	148	16	-
Chloride (mg/L)	249.92	119.96	600
Hardness (mg/L)	670	380	
TS (g/L)	0.345	0.362	
TSS (g/L)	0.038	0.122	NS

The concentration of heavy metals in the water samples from the sampling sites is compared to the standards set by the Federal Ministry of Environment (FME), Federal Republic of Nigeria is shown in Table 3. Copper levels were elevated in both water bodies, with the Maya River showing a higher concentration.

Table 3: Heavy Metals analysis of Water Samples from the Study Sites

Heavy Metals (mg/L)	Osborne	Ikorodu	FME Values (2019)
Cadmium (Cd)	0.009	0.025	0.005
Cobalt (Co)	0.0005	0.0004	-
Chromium (Cr)	0.015	0.005	<1
Iron (Fe)	0.008	0.128	1
Arsenic (As)	BDL	BDL	0.05
Lead (Pb)	0.004	0.002	0.01
Zinc (Zn)	0.014	0.01	0.03
Mercury (Hg)	BDL	BDL	0.001
Copper (Cu)	**0.428**	**1.689**	0.001
Manganese (Mn)	0.018	0.009	5

BDL – Below detection level

The concentration of physico-chemical parameters in the sediment samples from the sampling sites are shown in Table 4.

Table 4: Physicochemical Analysis of Sediment Samples from the Study Sites

Physicochemical	Osborne	Ikorodu
pH	6.92	6.01
Conductivity(Us/cm)	657	245
Organic Carbon (%)	0.7	3.3
Total Organic Carbon (%)	0.91	4.29

The concentration of heavy metals notably Iron, Zinc and Copper were higher in sediment samples from Maya River (Table 5) while Lead was more abundant in Osborne Lagoon.

Table 5: Heavy metals Analysis of Sediment Samples from the Study Sites

Heavy Metals (mg/kg)	Osborne	Ikorodu	FME Values (2019)
Cadmium (Cd)	0.55	0.4	0.1
Cobalt (Co)	BDL	BDL	0.5
Chromium (Cr)	0.175	0.1	1.0
Iron (Fe)	**13.72**	**24.62**	**100**
Arsenic (As)	BDL	BDL	0.05
Lead (Pb)	**0.175**	0.075	0.1
Zinc (Zn)	**4.05**	**16.25**	**10**
Copper (Cu)	**0.425**	**0.45**	**1.0**
Mercury (Hg)	BDL	BDL	0.001
Manganese (Mn)	0.631	0.922	0.5

BDL – Below detection level

Single-Cell Gel Electrophoresis

Single-cell gel electrophoresis performed on fish samples revealed the presence of DNA damage. Plate 2a presents a micrograph of normal cells showing minimal to no comet formation which indicates no DNA damage while Plate 2b presents a micrograph showing comet formation indicative of DNA damage (Arrows pointing at comets) in the cells of the fish.

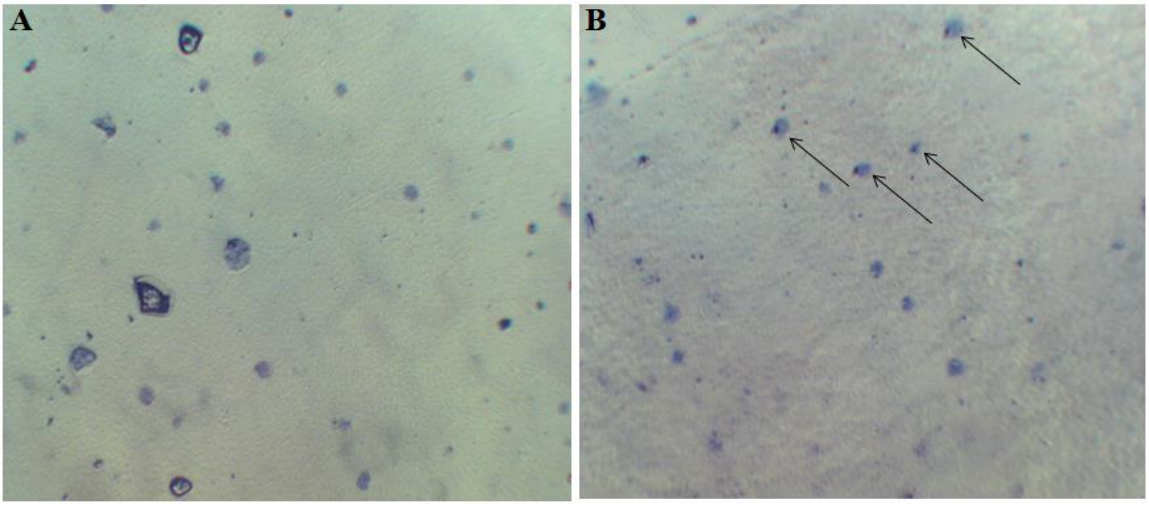

Plate 2: Micrographs of Single Gel Electrophoresis

The extent of DNA damage in *Oreochromis nilocticus* and *Coptodon guineensis* was assessed and expressed as % tail DNA and tail length as shown in Table 6 below. Results showed that there was significantly higher DNA damage in wild *Oreochromis niloticus* compared to their farm bred counterpart while *Coptodon guineensis* and their farm bred counterparts had no significant differences.

Table 6: Extent of DNA Damage In Flesh (100 Cells) of *Oreochromis niloticus* and *Coptodon guineensis* Expressed as % Tail DNA (±SEM)

SPECIE	Tail Length (um)		% Tail DNA	
	Farm	Wild	Farm	Wild
Oreochromis niloticus	05.43 ± 0.33	08.33 ± 0.50*	32.01 ± 1.99	41.53 ± 1.68*
Coptodon guineensis	09.05 ± 0.65	08.90 ± 0.96	37.58 ± 2.15	38.95 ± 2.72

Values with asterix (*) differ significantly (p<0.05) between control and river specimens within species

The analysis of the Olive Tail Moment in *Oreochromis niloticus* and *Coptodon guineensis* obtained from wild and farm environments conducted to assess the occurrence of DNA damage revealed (Figure 2) which was most significant in wild *Oreochromis niloticus*.

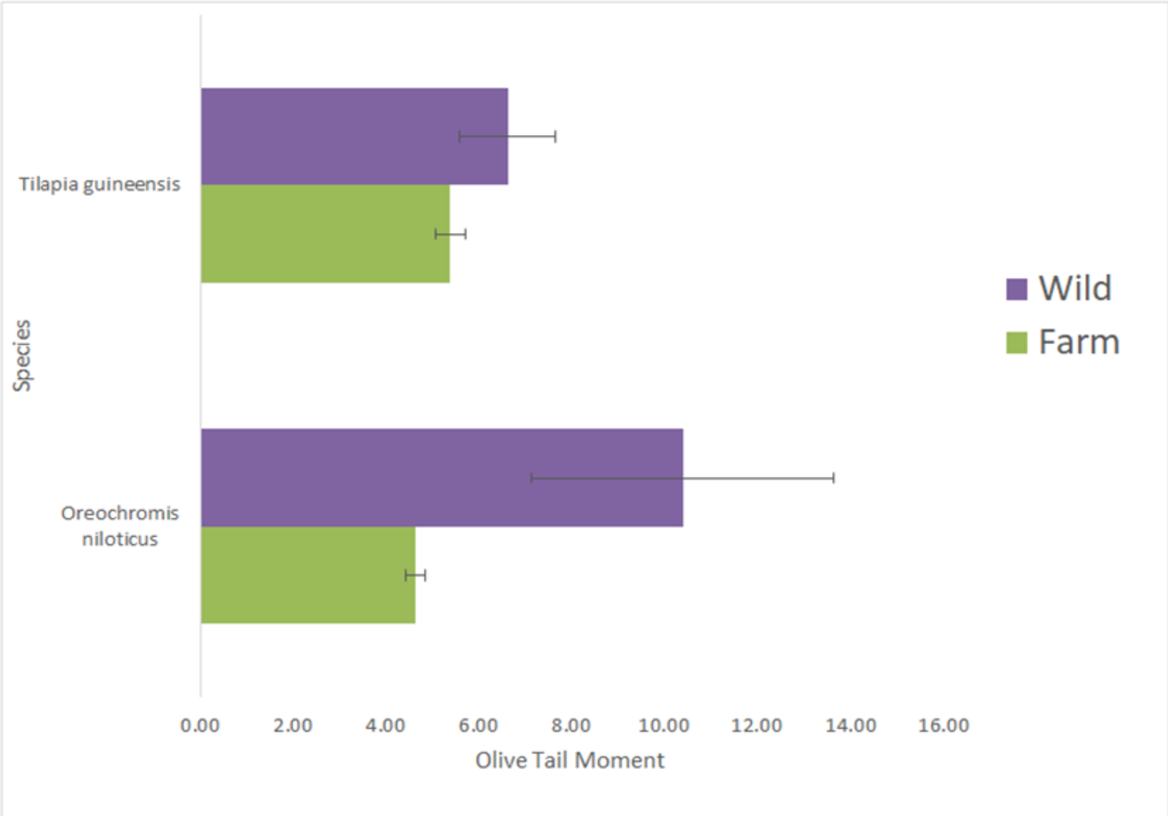

Figure 2: Genotoxic Assessment of Sampled Fishes

Histopathological Analysis

Photomicrographs (Plate 3) of histological sections of liver tissues of the fish samples compared to the reference.

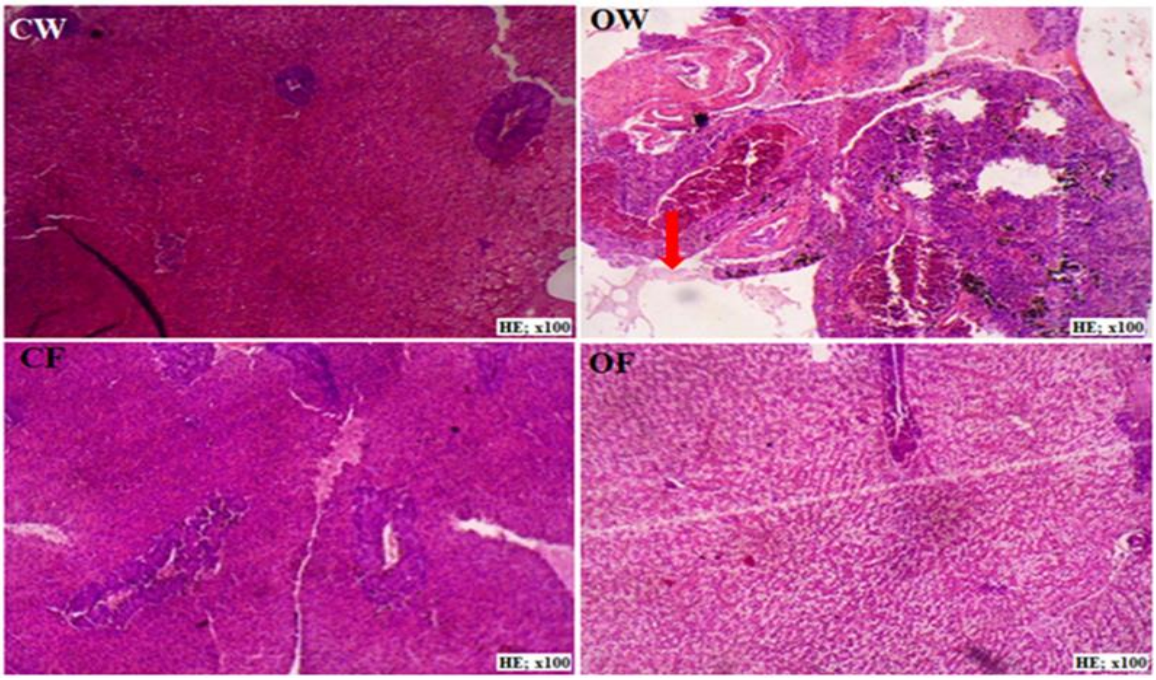

Plate 3. Histological sections of liver tissue of samples fishes from CW (*Coptodon* Wild), OW (*Oreochromis* Wild), CF(*Coptodon* Farm) and OF(*Oreochromis* Farm) groups

A: CW (*Coptodon* Wild) group shows parallel radially arranged plates of hepatocytes with Central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. No abnormalities are seen (NORMAL LIVER). Haematoxylin and Eosin Stain X 100.

B: OW (*Oreochromis* Wild) group shows parallel radially arranged plates of hepatocytes, Congested blood vessels are also seen (VASCULAR CONGESTION-Red arrow). Haematoxylin and Eosin Stain X 100

C: CF (*Coptodon* Farm) group shows parallel radially arranged plates of hepatocytes with Central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells

No abnormalities are seen (NORMAL LIVER). Haematoxylin and Eosin Stain X 100

D: OF(*Oreochromis* Farm) group shows parallel radially arranged plates of hepatocytes with Central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells

No abnormalities are seen (NORMAL LIVER). Haematoxylin and Eosin Stain X 100

DISCUSSION

The Single Cell Gel Electrophoresis (SCGE) also known as the comet assay was used to analyze the extent of DNA strand breaks in the cells of the fish species studied. The level of DNA strand breaks was observed to be higher in the cells of wild fish samples compared to the cells of samples from the farm which served as control, signifying DNA damage in the cells of the wild fish samples. This observation is supported by the work of Ostling and Johnson, 1984 as revised by Sing et al. 1988 and the work of Jiang et al. 2023.

At high pH, DNA strands separate more easily when breaks in DNA strands are present and the rate of separation is the index of DNA breakage. This occurs due to damaged DNA (loop with breaks) or cleaved DNA fragments that migrate faster during electrophoresis forming a "comet tail" while undamaged DNA containing the nucleoid body remains intact forming a “comet head”. During electrophoresis, genotoxic insult is unveiled via DNA strand breaks, as the damaged DNA moves at a rate different from that of the undamaged.

The analysis of Tables 2, 3, 4, 5 and 6 revealed the presence of heavy metals in the water and sediment samples along with their role as contributors of DNA damage in *Oreochromis niloticus* and *Coptodon guineensis*. The concentrations of these metals especially iron (Fe) and zinc (Zn) was very high in Maya, Ikorodu, exceeding the recommended limits. These high levels of heavy metals concentrations reflect DNA damage in fish species from the sample sites as revealed by the comet assay results that showed higher tail DNA percentages and olive tail moments in wild fish compared to their control group from the farm species.

Elevated heavy metals concentrations can generate reactive oxygen species (ROS) which causes oxidative stress that damages cellular components including DNA thereby contributing to genotoxic effects in fish (Kocadal *et al.,* 2020). Histopathological analyses revealed abnormalities in the liver of the fish from the sample sites which indicated biochemical disruptions causing heavy metal bioaccumulation. Since heavy metals are non-biodegradable making them a persistent and continuous threat to DNA integrity which results in mutagenesis, carcinogenesis and impaired reproductive capacity (Madhu *et al.,* 2022).

The extent of DNA damage is assessed using the amount of migrated DNA. Specifically the percentage of DNA present in the tail during single cell electrophoresis (comet assay). This percentage is directly proportional to the extent of DNA damage that occurs in a particular cell. Analysis of the olive tail moment revealed that the level of DNA strand breaks observed were significantly higher in the cells of *Oreochromis niloticus* samples collected from Maya River when compared to the DNA strand breaks observed in the cells of *Coptodon guineensis* samples collected from Osborne Foreshore lagoon.

These observed differences are believed to be associated with higher levels of water pollution caused by the actions of factory workers in the Maya River area leading to the ingestion of genotoxins by the inhabiting fishes compared to lower DNA damage in fish samples from Osborne Foreshore lagoon due to the reduced level of pollution. Histological sections of the liver tissues of the fishes from both reference sites and Osborne Foreshore lagoon showed no significant differences in the arrangement of their hepatic cells. However, histological sections from the liver tissues of the fishes collected from Maya River, Ikorodu showed significant differences, including the presence of congested blood vessels around the hepatic cells of *Oreochromis niloticus*. This finding suggests potential heavy metal exposure impact on the liver cells which could lead to liver damage. These observations further indicate that the two factories located near Maya River may be sources of pollutant via toxic effluents discharged from these factories into the river. The ingestion of these pollutants by aquatic animals in the river, can cause deleterious effects on their health, particularly the liver.

Assessment of physico-chemical characteristics of both water and sediment samples from both study sites showed significantly high levels of Iron (Fe) and Zinc (Zn) in the water and sediment samples from Maya, Ikorodu site compared to the water and sediment samples from Osborne Foreshore lagoon. The increased turbidity levels in water samples from Maya River, Ikorodu may impact the economic (fishing) and recreational use of this water body adversely. The results from this study establish a very high level of water pollution in the Maya River, as a result of the actions of the industries predominantly present in that area that constantly discharge untreated or inadequately treated effluent wastes into adjourning water bodies. These effluent wastes may contain carcinogenic and mutagenic genotoxins, which can be bioaccumulated in the tissues of the inhabiting aquatic lives through their gills and/or skin.

Consumption of these fishes by humans exposes them to the danger of developing cancer as these accumulated genotoxins which would mostly be heavy metals, are quite persistent, not easily degraded and can be transferred to the consumers. It is very important to implement measures that would help stop these industrialists from further damaging the ecosystem via discharge of untreated or inadequately treated industrial effluents into the river bodies. Constant and intensive sensitization is needed to educate factory workers, farmers, fishermen and residents on the dangers their actions pose to the environment and human health in particular. These initiatives would help protect public health and preserve aquatic ecosystems.

CONCLUSION

The study demonstrated the harmful impact caused by heavy metal pollution from industrial effluents on aquatic organisms, focusing on *Oreochromis niloticus* and *Coptodon guineensis* in Lagos State, Nigeria. The findings revealed high concentrations of heavy metals, especially iron and zinc, which were detected in water and sediment of Maya River, Ikorodu at higher concentration levels.

The comet assay revealed substantial DNA damage in wild fish populations compared to farmed controls, demonstrating the genotoxic effects of contaminated water which poses great threat not only to the aquatic ecosystem, but also to human health through contaminated fish consumption. Histopathological analyses revealed liver abnormalities in fish from heavily polluted areas, confirming the adverse physiological effects of heavy metal exposure.

These findings emphasized the urgent need for stricter enforcement of regulations which would help in limiting industrial discharges into water bodies and raise public awareness regarding the health risks associated with consuming fishes sourced from contaminated water bodies. By establishing clear connections between heavy metal pollution, DNA damage, and potential health risks, this research provides valuable insights for improving environmental management and advocating sustainable practices to protect both aquatic ecosystems and human health. Future studies should focus on long-term monitoring of heavy metal levels and its effects on aquatic species, as well as developing effective remediation strategies to restore ecosystem health in order to safeguard both aquatic life and human health.

AUTHORS CONTRIBUTIONS

OAA designed the study and edited the manuscript; JCI and OEE experimented and collected the data; OOA and NNM analysed the data; VNM wrote the draft of the manuscript.

REFERENCES

Abuhatab, S., El-Qanni, A., Al-Qalaq, H., Hmoudah, M. and Al-Zerei, W. (2020). Effective adsorptive removal of Zn^{2+}, Cu^{2+}, and Cr^{3+} heavy metals from aqueous solutions using silica-based embedded with NiO and MgO nanoparticles. *Journal of Environmental Management*, *268*, 110713. https://doi.org/10.1016/j.jenvman.2020.110713

Abdel-Baki, A.S., Dkhil, M.A. and Al-Quraishy, S., (2011). Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. *African Journal of Biotechnology*, *10*(13), 2541-2547. https://doi.org/10.5897/AJB10.1772

Akter, S., Jahan, N., Rohani, M.F., Akter, Y. and Shahjahan, M., (2021). Chromium supplementation in diet enhances growth and feed utilization of striped catfish (Pangasianodon hypophthalmus). *Biological trace element research*, *1*, 1-9. https://doi.org/10.1007/s12011-021-02608-2

Aliko, V., Mehmeti, E., Qirjo, M. and Faggio, C., (2019). 'Drink and sleep like a fish': goldfish as a behavior model to study pharmaceutical effects in freshwater ecosystems. *Journal of biological research-bollettino della Societa Italiana di Biologia Sperimentale*, 92(1). https://doi.org/10.4081/jbr.2019.7939

Avwioro, G. (2011). Histochemical uses of haematoxylin—a review. *Jpcs*, *1*(5), 24-34.

Burgos-Aceves, M.A., Lionetti, L. and Faggio, C., (2019). Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. *Science of the total environment*, *670*, 1170-1183. https://doi.org/10.1016/j.scitotenv.2019.03.275

Capillo, G., Silvestro, S., Sanfilippo, M., Fiorino, E., Giangrosso, G., Ferrantelli, V., Vazzana, I. and Faggio, C., 2018. Assessment of electrolytes and metals profile of the Faro Lake (Capo Peloro Lagoon, Sicily, Italy) and its impact on Mytilus galloprovincialis. *Chemistry & biodiversity*, *15*(5), 1800044. https://doi.org/10.1002/cbdv.201800044

Delahaut, V., Rašković, B., Salvado, M.S., Bervoets, L., Blust, R. and De Boeck, G., (2020). Toxicity and bioaccumulation of Cadmium, Copper and Zinc in a direct comparison at equitoxic concentrations in common carp (Cyprinus carpio) juveniles. *PLoS One*, *15*(4), e0220485. https://doi.org/10.1371/journal.pone.0220485

Gumpu, M.B., Sethuraman, S., Krishnan, U.M. and Rayappan, J.B.B., (2015). A review on detection of heavy metal ions in water–an electrochemical approach. *Sensors and actuators B: chemical*, *213*, 515-533. https://doi.org/10.1016/j.snb.2015.02.122

Hemmaphan, S. and Bordeerat, N.K., (2022). Genotoxic effects of lead and their impact on the expression of DNA repair genes. *International journal of environmental research and public health*, *19*(7), 4307. https://doi.org/10.3390/ijerph19074307

Jiang, N., Naz, S., Ma, Y., Ullah, Q., Khan, M. Z., Wang, J., Lu, X., Luosang, D.Z., Tabassum, S., Chatha, A.M.M. and Basang, W. D. (2023). An overview of comet assay application for detecting DNA damage in aquatic animals. *Agriculture*, *13*(3), 623.https://doi.org/10.3390/agriculture13030623

Kim, Y. and Kim, J., (2020). Bioinspired thiol functionalized carbon dots for rapid detection of lead (II) ions in human serum. *Optical Materials*, *99*, 109514. https://doi.org/10.1016/j.optmat.2019.109514

Kocadal, K., Alkas, F. B., Battal, D. and Saygi, S. (2020). Cellular pathologies and genotoxic effects arising secondary to heavy metal exposure: A review. *Human & Experimental Toxicology*, *39*(1), 3-13. https://doi.org/10.1177/0960327119874439

Kovendan, K., Vincent, S., Janarthanan, S. and Saravanan, M., 2013. Expression of metallothionein in liver and kidney of freshwater fish Cyprinus carpio var. communis (Linn) exposed to arsenic trioxide. *American Journal of Scientific and Industrial Research*, *4*(1), 1-10.

Kumar, S., Islam, A.R.M.T., Hasanuzzaman, M., Salam, R., Khan, R. and Islam, M.S., (2021). Preliminary assessment of heavy metals in surface water and sediment in Nakuvadra-Rakiraki River, Fiji using indexical and chemometric approaches. *Journal of environmental management*, *298*, 113517. https://doi.org/10.1016/j.jenvman.2021.113517

Lakra, W.S. and Nagpure, N.S., (2009). Genotoxicological studies in fishes: a review. *Indian Journal of Animal Sciences*, *79*(1), 93-97.

Liu, Y., Chen, L., Yu, J., Ye, L., Hu, H., Wang, J., and Wu, B., (2022). Advances in single-cell toxicogenomics in environmental toxicology. *Environmental Science & Technology*, *56*(16), 11132-11145. https://doi.org/10.1021/acs.est.2c01098

Madhu, N. R., Sarkar, B., Slama, P., Jha, N. K., Ghorai, S. K., Jana, S. K., Govindasamy, K., Massanyi, P., Lukac, N., Kumar, D., Kalita, J.C., Kesari, K.K. and Roychoudhury, S. (2022). Effect of environmental stressors, xenobiotics, and oxidative stress on male reproductive and sexual health. In *Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility Volume II* (pp. 33-58). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-12966-7_3

Merola, C., Bisegna, A., Angelozzi, G., Conte, A., Abete, M.C., Stella, C., Pederiva, S., Faggio, C., Riganelli, N. and Perugini, M., (2021). Study of heavy metals pollution and vitellogenin levels in brown trout (Salmo trutta trutta) wild fish populations. *Applied Sciences*, *11*(11), 4965. https://doi.org/10.3390/app11114965

Patle, A., Kurrey, R., Deb, M.K., Patle, T.K., Sinha, D. and Shrivas, K., (2022). Analytical approaches on some selected toxic heavy metals in the environment and their socio-environmental impacts: A meticulous review. *Journal of the Indian Chemical Society*, *99*(9), 100545. https://doi.org/10.1016/j.jics.2022.100545

Prasad, M., Aswal, R.S., Joshi, A., Kumar, G.A. and Ramola, R.C., (2022). A systematic study on occurrence, risk estimation and health implications of heavy metals in potable water from different sources of Garhwal Himalaya, India. *Scientific Reports*, *12*(1), 20419. https://doi.org/10.1038/s41598-022-24925-9

Shah, S. B., (2021). Heavy metals in the marine environment—an overview. *Heavy metals in Scleractinian corals*, (pp: 1-26). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-73613-2_1

Shahjahan, M., Taslima, K., Rahman, M.S., Al-Emran, M., Alam, S.I. and Faggio, C., 2022. Effects of heavy metals on fish physiology–a review. *Chemosphere*, *300*, 134519. https://doi.org/10.1016/j.chemosphere.2022.134519

Shiry, N., Derakhshesh, N., Gholamhosseini, A., Pouladi, M. and Faggio, C., (2021). Heavy metal concentrations in Cynoglossus arel (bloch & schneider, 1801) and sediment in the chabahar bay, Iran. *International Journal of Environmental Research*, *15*(5), 773-784. https://doi.org/10.1007/s41742-021-00352-y

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell. Research*. *175*, 184-191. https://doi.org/10.1016/0014-4827(88)90265-0

Sula, E., Aliko, V., Barceló, D. and Faggio, C., 2020. Combined effects of moderate hypoxia, pesticides and PCBs upon crucian carp fish, Carassius carassius, from a freshwater lake-in situ ecophysiological approach. *Aquatic Toxicology*, *228*, 105644. https://doi.org/10.1016/j.aquatox.2020.105644

Yilmaz, O. and Rickard, S., 2004. Blind separation of speech mixtures via time-frequency masking. *IEEE Transactions on signal processing*, 52(7), 1830-1847. https://doi.org/https://doi.org/10.1109/TSP.2004.828896

Zamora-Ledezma, C., Negrete-Bolagay, D., Figueroa, F., Zamora-Ledezma, E., Ni, M., Alexis, F. and Guerrero, V.H., (2021). Heavy metal water pollution: A fresh look about hazards, novel and conventional remediation methods. *Environmental Technology & Innovation*, *22*, 101504. https://doi.org/10.1016/j.eti.2021.101504