Journal of Advances in Medical and Pharmaceutical Sciences

Volume 26, Issue 10, Page 20-39, 2024; Article no.JAMPS.117884
ISSN: 2394-1111

Histological Based Environmental Monitoring of the Lower Nun River: Using the Histo-morphometry of Kidney, Gills, Liver, and Testes of *Coptodon zillii*

Allison Theodore Athanasius [a*] and Oguon Timipa Richard [b]

[a] *Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria.*
[b] *Department of Anatomy, Basic Medical Sciences, Bayelsa Medical University, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jamps/2024/v26i10718

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117884

Original Research Article

Received: 19/04/2024
Accepted: 22/06/2024
Published: 01/10/2024

ABSTRACT

It is an ecotoxicological study aimed at using chemical and bio-monitoring methods to pollution status of the Lower Nun River (LNR) basin using the histo-morphometry of the Liver, Kidney, Gills, and the Testes of *Coptodon zillii*. Based on literature review on the environmental burdens of the LNR, the following Target Chemicals (TC) were chosen for the study: Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni) and Lead (Pb). The study involved the sampling of water, sediment

---

**Corresponding author: E-mail: drtheodoreallison@gmail.com;*

Cite as: *Athanasius, Allison Theodore, and Oguon Timipa Richard. 2024. "Histological Based Environmental Monitoring of the Lower Nun River: Using the Histo-Morphometry of Kidney, Gills, Liver, and Testes of Coptodon Zillii". Journal of Advances in Medical and Pharmaceutical Sciences 26 (10):20-39. https://doi.org/10.9734/jamps/2024/v26i10718.*

and feral fish from two stations along the LNR, Peremabiri (PER) and Igbomotoru(IGB) – Experimental sites.Water and sediment samples were analyzed to ascertain the physico-chemical concentrations of potential contaminants, while the harvest fish organs of liver, kidney, gills and testes were subjected to histological study to ascertain the exposure effect. Control fishes were harvested from African Aqua-culture Centre (ARAC) – Reference Site. Analyzed sediment and water values were used to evaluate Environmental Water Quality Index (EWQI) and sediment quality, while the fish organs were analyzes and used for pollution certification and stratification. Study results showed that EWQI of PER was poor (41.9); sediment quality showed that Cr, CU and Pb were above the Maximum Allowable Toxicant Concentration (MATC) for fresh water body. Fish from IGB showed worse lesion prevalence and organ histopathologic Index (Iorg) when compared to PER, with ARAC having the best outcome. The environmental pollution status (EPS) LNR was normal at PER and was slightly polluted at IGB. The study gave credence to the use of histology as a biomarker to assess sublethal effects of environmental stressors, and in determination of the pollution status.

Keywords: Histology; lower nun river; bio-monitoring; chemical monitoring; ecotoxicology; fish and aquatic ecosystem.

1. INTRODUCTION

“Niger Delta region is an ecologically threatened geographical region, in the South-South geo-political region of Nigeria. The region consists of swamp forest, mangrove forest and coastal landforms. The Niger Delta swamp forest is a tropical moist forest eco-region, and the second largest in Africa” [1]. Niger Delta has an extensive mangrove forests which serve a critical role in regional ecological biodiversity, and support subsistence gathering practices, and market-based income opportunities. The coastal landform consist of a low lying depositional plain characterized by extensive estuarine lagoons and mangrove swamps fronted beach-ridge barriers.

“The Niger delta area is well-endowed with both renewable and non-renewable natural resources. The major non-renewable resources include the fossil fuels, crude oil and natural gas, and constructional materials (sand and gravel). The major renewable natural resources include a wide variety of economically important timber species and non-timber forest resources; a wide variety of wildlife; and a rich aquatic life” [2].

“Beyond the legitimate crude oil activities by Multi-National Oil Companies (MNOC), the region in the past years has become a hub of illegal crude oil refining, with processing of stolen crude from Oil company’s pipelines” [3]. “Crude oil related crimes have continued to rise in the Niger Delta despite efforts by the nation’s security agencies, to curb the menace” [4]. “Makeshift refineries, usually hidden in oil-soaked clearings, support tens of thousands of people locally” [3,5,6]. Government military task forces have been working assiduously to locate and destroy illegal refining camps, as well as locally built sea going boats, often referred to as "Cotonou" boats, and wooden barges loaded with stolen crude and other illegally refined products in the region.

Beyond the political delineation of Niger Delta, an environmentally relevant scientific border of Niger Delta was determined in a study by Niger Delta Environmental Survey (NDES) in 1995. A study that was midwifed by Shell Development Company (SPDC) in conjunction with Nigerian National Petroleum Company (NNPC), Elf Petroleum Nigeria Ltd (EPNL) and Nigerian Agip Oil Company (NAOC), and in partnership with other reputable international organizations[Imperial College Centre for Environmental Technology (ICCET), The World Conservative Monitoring Centre (WCMC) Cambridge, The World Wild Life Fund (WWF), Swedish Environmental Research Council (SERC) Stockholm and British Field Studies Council (BFSC)] which used the methodologies of Geographic Information System (GIS) Mapping, Surface Geological Mapping and Photogrammetry, and Geophysical exploration. The scientific Niger Delta area was thereby determined as the geographical drainage basin of the following estuaries [2]:

- Northern Niger Delta border is located at about (5°33²49"N, 6°31'38"E) in Delta State, where the River Niger bifurcates into the Forcados River and Nun River. The upper reaches of Forcados River and Nun

River drain the northern basin of Delta State.

- Western Niger Delta border is delineated along the Benin River estuary (5°44'11°N, 5°03"E) in Delta State. This estuary drains the western basin of Delta State and the southernbasin of Edo State.
- Eastern Niger Delta border is delineated along the lower reaches of Imo River estuary (4°27'16"N, 7°35'27"E) in Rivers State. The estuary drains the north-easternbasin of Rivers State and the western basin of Akwa-Ibom State
- Central Niger Delta region is in Bayelsa State. It is drained by the lower reaches of the Nun River which runs eastward and empties into the ocean.
- Southern Niger Delta border is delimited by a vast Niger Delta coastline, which stretches between two notable landmark inlets (western and eastern inlets) used in delineating the Bight of Bonny from the Gulf of Guinea in the Atlantic Ocean. The western-inlets are marked by Forcados River estuary at Oporoza in Delta State, and eastern-inlet is marked by the Imo River estuary at Opobo in Rivers State.

This index study identifies drainage aquatic basins suitable for the study of the environmental burdens or activities around potential impacted communities. Hence the LNR, was chosen for the ecotoxicologicaly evaluation of the PER and IGB ecosystem.

The LNR is very notorious for illegal refining and bunkering of crude oil. Operatives of the anti-illegal oil bunkering troop of the Joint Task Force (JTF) in collaboration with Oil and Gas Task Force, on illegal oil bunkering of the Southern Ijaw Local Government Area (SILGA) Council, of Bayelsa State, have destroyed illegal oil refinery camps at the PER and IGB axis of the LNR [4].

Peremabiri (PER) and Igbomotoru (IGB) are oil host community, situated along the lower reaches of the LNR. Many industrial and agricultural projects are fast becoming a prominent feature of the banks of the lower reaches of Nun River. These include a commercial rice farm at PER and other crude oil flow station along the banks of the many tributaries of the Nun River.

Shell Petroleum Development Company, (SPDC) is operating crude oil flow station, with gas flare channels and numerous pipelines along the stretch of the LNR banks at Oporoma (Nun River Flow Station) and Diebu (Diebu Creek Flow Station). The Nigeria Agip Oil Company (NAOC) is also operating one of the largest swamp crude oil exploration in the world along the banks of the LNR tributaries, with gas flare point at Ogboinbiri and Tebedaba; two communities that are a few kilometers away from PER and IGB.

Crude oil and gas produced from these flow stations are transported via pipelines to gas processing terminals or to crude oil exporting terminal at the coast of Atlantic ocean. These pipelines transverses fishing rivers and farming land across various communities in the LNR basin. These pipelines are often prone to natural damage or sabotage along its route. Oil pipelines are often times vandalized by oil bearing communities to register a protest, and in many occasions by criminal oil bunkers for illegal crude oil refining.

The connecting creeks between PER and IGB of the LNR became notorious for illicit oil activities which led to visible land, water and atmospheric changes. There was visible presence of black soot along the water ways. Travellers on open sea crafts or boat were often drenched with black soot while travelling through these creeks. This sad turn of event was gradually turning the lush vegetation of the troubled mangrove swamp of Bayelsa into a brown field [4]. Agip was once forced to suspend her crude oil production activity in Bayelsa State, due to the activities of vandals and oil theft [4].

The Nun River is a one of the most important river systems in the Niger Delta providing nursery and breeding grounds for a large variety of fish [7,8]. Inhabitants of the LNR are traditionally fishers. Fishing in the river is intense, but catch per unit effort is reducing over the time due environmental degradation activities in the LNR basin [8].

Most studies of the LNR where based on assessing contamination of the ecosystem; which includes sediment quality [9,10], water quality [11-13], hydrodynamics of pollutants [14] bio-diversity [11], biological contaminants [15,16] and bioaccumulation of xenobiotics in sentinel aquatic organisms [10,12,17]. There is a study gap on the bioavailability and bioactivity or effect of observed contaminants on biological organism [18,19], which defines environmental pollution .

Histology based ecotoxicological assessment tools are therefore used as complimentary environmental tool to establish or vitiate any biological alterations that resident organisms might have been exposed to to needed as a complement to chemical analysis.

The incorporation of an effective suite of bioassays and histological biomarkers of exposure can provide insights into the causality of any higher-level adverse effects that may be observed [19]. During recent decades, many studies of oil spills in European waters and elsewhere have clearly demonstrated the potential and usefulness of applying biological-effect techniques in oil spill impact assessments, particularly concerning sublethal and long-term impacts at lower levels of biological organization, and monitoring the efficacy of remediation [20-38].

The study used environmental chemical monitoring procedures to ascertain contamination, which was integrated into environmental bio-monitoring procedures to ascertain pollution. All pollutants are contaminants, but not all contaminants are pollutants [37] Contamination is the presence of any substance above the allowable level for that environment. Hence contamination is simply the presence of a substance where it should not be or at concentrations above background [39] Pollution is contamination that can result in adverse biological effect to resident communities [19,39] Pollution is therefore the evidence of alterations in biological structures, that usually lead to biochemical and physiological change of a living organism exposed to environmental substances [59-61].

Scientifically establishing environmental pollution cannot be done solely on the basis of chemical analysis because such analysis provides no information on bioactivity of environmental chemical [39] their effective on the biosphere or their toxicity and ecotoxicity on biological organism. A holistic evaluation of anthropogenic agents effect on any ecosystem; which is the energy exchange between the living and non-living components of geographical space, should therefore incorporate scientific tools that can measure environmental exposure effect [62-65]. Environmental exposure effect-based assessment tools are integral in establishing the distortion of a natural ecosystem by the introduction of an anthropogenic agent and its effect or impact on the living organism of that geographical space. Effect-based environmental studies assesses toxicity in laboratory exposure studies and ecotoxicity in feral or field exposure scenario where the environmental components are studied in its spatio-temporal form [54-56]. Ecotoxicity and toxicity are results of alterations in biological system, which forms the yardstick for measurement and stratification of pollution. Hence, this study is meant to combine physico-chemical study of the aquatic environment with field and laboratory histological studies to ascertain the impact of anthropogenic activities on the pollution status of the LNR [57,58].

Target organs such as liver, testes, kidney and gills, have a tendency to accumulate high levels of heavy metals [40]. Heavy metals undergo metabolic activation that induces a cellular change in these target organs. Tissue lesions and apoptosis that arise from bioaccumulation, along with infections, diseases and parasites stimulate necrotic alterations in these organs with an inflammatory defensive reaction which are observed as exposure effects and used in the certification and stratification [19,41].

2. MATERIALS AND METHODS

2.1 Study Area

Experimental Area: The Nun River is an estuary of River Niger in Nigeria. It has its upper reaches (Upper Nun River) in Delta State and its lower reaches (lower Nun River – LNR) in Bayelsa State. The Nun River flows through sparsely settled zones of freshwater and mangrove swamps and coastal sand ridges before completing its 100-mile (160-km) easterly course to the Bight of Bonny, at Akassa in Brass Local Government Area of Bayelsa State [42]. In 1963, petroleum was discovered along the LNR (about 89 km upstream). In 1965 the Trans-Niger Pipeline was completed, and oil from the LNR fields was piped to Rumuekpe, where there is a link with the Trans-Niger line. At the turn of the 21st century, however, control of Nigeria's oil wealth at the LNR and elsewhere was an issue that caused increasing violence [40]. Two sample stations of Peremabiri (PER) and Igbomotoru (IGB) was selected for this study. The two communities are just a few kilometers apart and are host to multinational oil exploratory activities. PER is also a host to an industrial rice farm and an area noted for illegal oil refining activities [4]. IGB is an area notorious for illegal oil bunkering and refining activities [4].

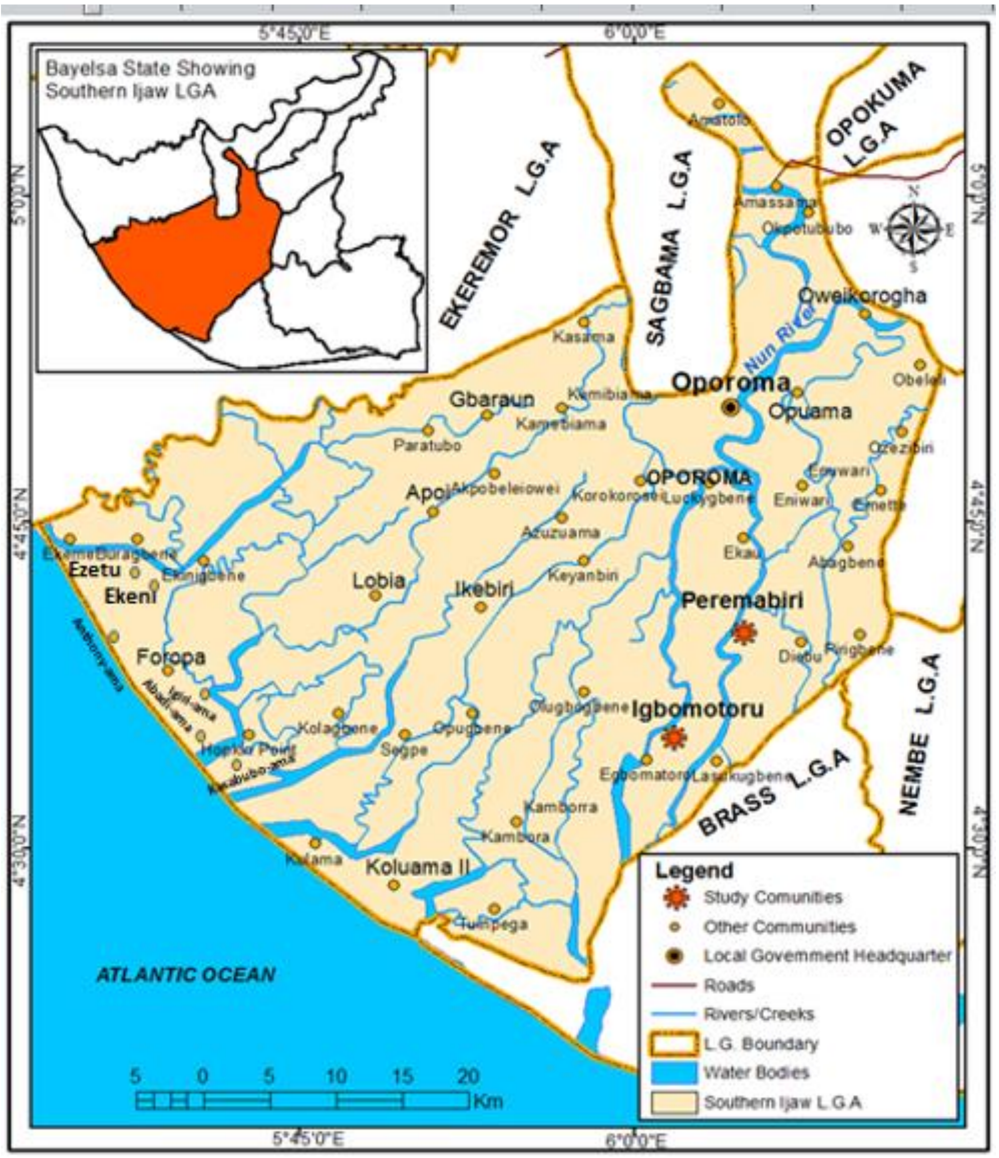

Fig. 1. Map of Southern Ijaw Local Government Area Showing the lower Nun River with an indication of Peremabiri and Igbamatoru

Reference Area: Control fishes for this study were acquired from African Regional Aqua-Culture Centre (ARAC) located at Aluu in Ikwerre Local Government Area of Rivers State. ARAC is involved in fisheries and aquaculture research, development and training. ARAC was established in 1979 as an African sub-region aquaculture development centre by FAO/UNDP and handed over to Nigerian Government in 1987, operated by the Nigerian Institute for Oceanography and Marine Research (ARAC/ NIOMR). ARAC is affiliated to the Rivers State University (RSU) for the award of Master of Science (M.Sc) and Post graduate Diploma (PGD) in Aquaculture. Hands on training acquaculture programme for farmers are the value chain featured in the ARAC curriculum. ARAC has two centres, one located at Aluu, in Ikwerre Local Government Area of Rivers State, which is responsible with the culture of brackish water fish, while the other is located at Buguma in Asari-toru Local Government Area of Rivers State; which is responsible with the culture of marine fishes.

2.2 Study Specie

The chosen bio-indicator fish specie for this study is *Tilapia zillii*, now known as *Coptodonzillii. Coptodonzillii* is a resident fish of the Nun River estuary. It has a maximum length of 40cm (SL) and a maximum published weight of 300 grams with a total of 13 to 16 dorsal spines [43]. The non-breeding colouration of *C. zillii* is dark olive on top and light olive to yellow-brown on the sides, often with an iridescent blue sheen. Lips are bright green and the chest is pinkish. Six to seven dark vertical bars cross two horizontal stripes on the body and caudal peduncle. Fins are olivaceous, covered in yellow spots with the dorsal and anal fins displaying an outline of a thin orange band. Caudal fin often grey with pale interstices with dots covering the entire fin. Adults display a black spot outlined in yellow. *C. zillii* from 2 to 14cm (SL) have an entirely yellow to grey caudal fin with no dots, developing a greyish caudal fin with dots with increasing size. Spawning colouration is shiny dark green on top and sides, red and black on the throat and belly, and obvious vertical bands on the sides. Heads turn dark blue to black with blue-green spots. Eggs are green to olive green, sticky, 1-2 mm in diameter; relatively smaller than eggs of other cichlids [43,44].

Fig. 2. Picture of *C. zillii*, resident fish harvested from the Lower Nun River

2.3 Sample Selection

2.3.1 Target chemical selection

Selection of target Chemical (TC) were done based on findings from literature review of chemical analysis studies done on crude oil spill and ecological samples from LNR part of Southern Ijaw Local Government Area of Bayelsa State. The findings are that:

I. Several heavy metals are associated with crude oil include lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn), nickel (Ni), vanadium (V), chromium (Cr) [45].
II. Heavy metals most frequently detected in oil spill are in the order; Pb>Ni>V>Zn>Cd. (Mustafa et al., 2015);
III. Ni and V are major heavy metal contaminants in crude oil [46].
IV. Pb and Cr is associated with crude oil piping system. [47]:
V. The sediments of the LNR system revealed a high mobility and bioavailability for Pb and Cr. [12]:
VI. Trace metal levels were moderately elevated with Ni recording the highest percentage in clams harvest from LNR. [17]
VII. Heavy Metals (Pb, Ni, Cu, Zn, Mn and Fe,) concentrations in Muscle of Tilapia zillii from some LNR estuaries in Southern Ijaw LGA of Bayelsa were found to be above the limits recommended by various agencies including Food and Agricultural Organization (FAC) and World Health. [12].

With respect to the foregoing findings of the prevalence of some particular heavy metals in LNR, and the US EPA Heavy metals weight-of-Evidence (W-O-E) classification for carcinogen [48], the following chemicals were chosen for this study: Pb, Ni, Cr, Cu and CD.

2.3.2 Fish selection

EROCIPS (Emergency Response to coastal Oil, Chemical and Inert Pollution from Shipping), [49], “Protocol for Selection of Sentinel Species” was used to select the appropriate sentinel specie for this study. It is widely accepted that sentinel organism selected for monitoring of biological-effect techniques should be a resident organism with well-documented features [26,32,49-51].

In a survey of fish diversity of the LNR in Southern Ijaw LGA, Oguntade et al.,[52], Allison and Okadi [53], and Sikoki et al.,[5] have found *Coptodon Zilli* to be among 53 resident fish species in 18 families of the LNR. Highest biodiversity were found in the Sciaenidae and Cichlidae. The dominant fish of the ONE were Pseudotholythus and Tilapia species [52].

Based on the foregoing, Tilapia fish species, *Coptodonzillii* was selected for the study. Outside the fact the *Coptodon zilli* is a dominant fish species in the study area, it is also a widely studied and well documented fish species [6, 54-56].

3. SAMPLING AND EVALUATION

3.1 Surface Water

Surface water was sampled from designated stations of the experimental sites (PER and IGB) for water quality study. Water sampling method was done in accordance with CCME, (2001), UNEP (2008) and USEPA [57] surface water sample guidelines. Four samples of surface water from each station, at two weeks interval, were collected in vials and transported to the laboratory via iced tanks for further analysis, using Atomic Absorption Spectrometer (AAS). Analytical results were used to evaluate Environmental Water Quality Index (EWQI) for PER and IGB, using CCME 2001 water quality Index equation. EWQI was then ranked as: EXCELLENT:(EWQI = 95-100); GOOD: (EWQI = 88-94); Fair (EWQI = 65-89); MARGINAL: (EWQI = 45-64); POOR: (EWQI = 0-44) (CCME, 2001).

3.2 Sediment

Sediment from the designated field stations (PER and IGB) were sampled for sediment quality. This was done in accordance with SOEPA (2001) sediment sampling guidelines. An Erkman Grab Sampler was used to collect sediment from the river bed for further laboratory analysis using AAS. Analytical results were used to evaluate Sediment quality by comparing the results with guideline standards of Maximum Allowable Toxicant Concentration (MATC) for aquatic life in fresh water body [58, 59].

3.3 Fish Study

Angling method was used to harvest Ten (10) fishes from each designated station (PER and IGB) of experimental site and reference site (ARAC) for gross anatomical and histopathological studies. This was done in accordance with USEPA [60] fish sampling guidelines.

A. Gross Anatomy Analysis (Fish Necropsy)

- **Condition Factor (CF)**: Sampled fishes external anatomy was evaluated with respect to their weight and length relation using the mathematical formula $K = 10^N W/L^3$; where the constant K, which represents the CF, were evaluted for both experimental and reference sites and compared. Observed CF was ranked as Excellent (1.60); Good (1.40); Fair (1.20); Poor (1.00); Extremely Poor (0.80) [61].

- **Health Assessment Index (HAI):** This is a field assessment tool for quantitative evaluation of fish health. Immediately after harvesting the fish from both experimental and reference sites, each fish was quickly assessed for external and internal body lesions and parasites infestation using a standard check list. The observed internal and external lesion were recorded and scored in terms of the severity of the lesion using a Health Assessment Index (HAI) protocol by

B. Histological Evaluation

- **Qualitative Histological Assessment:** Harvested fish organs were preserved in 10% buffered formalin solution and taken to the laboratory for histological tissue preparation (Drury and Wallington, 1980). Light microscopy (Olympus BH2) was used to identify and interpret tissue slides and micrograph specimens at 40X, X100 and X400 magnification. The percentage prevalence of tissue histopathology from various target organs of the different sites were observed and compared with the control.

- **Semi-Quantitative Histological Assessment:** A standard semi-quantitative histological assessment, in accordance Bernet et al. [16] protocol modified by Van Dyk et al. [62], was done to quantify the histopathological alterations observed in the liver, kidney, gills and testes histology slides. Furthermore, a modified classification system by Van Dyk et al. [62,63] based on a proposed scoring scheme by Zimmerli et al., [64] was used to evaluate the degree of histological changes, which was further used to certify and stratify pollution. Its stratification system is based on the classes of the calculated mean organ index values: Class 1 (index value <10): Class 2 (index value 10-25): Class 3 (index value 26-35): Class 4 (index value >35).

4. RESEARCH FINDINGS

Field Study

- EWQ at LNR was not suitable to sustain aquatic life (poor)
- SQ revealed high concentration of potent carcinogenic heavy metals.
- HAI showed that fishes in LNR are in poorer health conditions, as compared to the reference site.
- EPS showed that LNR was slightly polluted at IGB axis

WATER QUALITY

Table 1. Result showing physical and Target Chemical (TC) parameters used in evaluation of EWQI for PEREMABIRI Station (PER)

Parameters	PER					CCME MATC	Remark
	1st	2nd	3th	4th	Mean	(Fresh Water)	
Temperature (C^{O})	28.5	30.0	32.3	29.1	30.0	Ambient (<31.0) *	N/A
TDS (mg/L).	386.3	**510.1**	**669.7**	317.3	470.9	<500	N/A
Salinity ppm	798.4	667.2	789.8	878.4	783.5	<1000**	N/A
DO (mg/L)	**4.3**	**3.2**	**4.8**	5.8	**4.5**	5.5-9.5	AP
PH	7.3	6.6	6.5	7.2	6.9	6.0-9.5	AP
Pb (mg/L)	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	AP
Cd (mg/L)	<0.001	<0.001	<0.001	<0.001	<0.001	0.0001	AP
Cr (mg/L)	**0.014**	**0.004**	**0.002**	**0.003**	**0.006**	0.001	AP
Cu (mg/L)	**0.012**	**0.025**	**0.013**	**0.023**	**0.018**	0.002	AP
Ni (mg/L)	<0.001	<0.001	<0.001	<0.001	<0.001	0.025	AP

*Key: TDS = Total Dissolved Solid; DO = Dissolved Oxygen; PH = Hydrogen Ion Concentration: CCME MATC = CCME (2001) MATC standard for Fresh Water; * = SON (2007) Guideline; ** = USGS (2015); Bold (Red) = failed tests; N/A = Not applicable EWQI estimation; and AP = Applicable for EWQI estimation*

Findings: Using CCME (2001) guideline for mathematical evaluation of EWQI, PER EWQI was estimated to be 41.9 **(POOR)**

Table 2. Result showing physico-chemical and Chemical parameters used in evaluation of EWQI for IGBOMOTORU station (IGB)

Parameters	IGB					CCME MATC	Remark
	1st	2nd	3th	4th	Mean	(Fresh Water)	
Temperature (C^{O})	25.6	30.3	26.4	28.8	27.8	Ambient (<31.0) *	N/A
TDS (mg/L).	**587.7**	480.4	**669.7**	486.3	**556.0**	<500	N/A
Salinity ppm	598.4	601.2	689.8	578.4	617.0	<500**	N/A
DO (mg/L)	**3.4**	**3.8**	**3.1**	5.8	**4.0**	5.5-9.5	AP
PH	6.3	6.9	7.5	6.2	6.7	6.0-9.5	AP
Pb (mg/L)	<0.001	**0.017**	**0.024**	<0.001	**0,012**	0.001	AP
Cd (mg/L)	**0.004**	**0.002**	**0.011**	**0.031**	**0.012**	0.0001	AP
Cr (mg/L)	**0.023**	**0.013**	**0.031**	**0.021**	**0.022**	0.001	AP
Cu (mg/L)	**0.034**	**0.024**	**0.041**	**0.012**	**0.028**	0.002	AP
Ni (mg/L)	<0.001	<0.001	<0.001	<0.001	0,001	0.025	AP

*Key: TDS = Total Dissolved Solid; DO = Dissolved Oxygen; PH = Hydrogen Ion Concentration: CCME MATC = CCME (2001) MATC standard for Fresh Water; * = SON (2007) Guideline; ** = USGS (2015); Bold (Red) = failed tests; N/A = Not applicable EWQI estimation; and AP = Applicable for EWQI estimation*

Findings: Using CCME (2001) guideline for mathematical evaluation of EWQI, IGB EWQI was estimated to be 44.9.(**Marginally Poor**)

Remarks: EWQI of this study has scientifically proven that the LNR is CONTAMINATED, which has validated news media reports on the environmental degradationconcerns of LNR basin [3,4].

SEDIMENT QUALITY

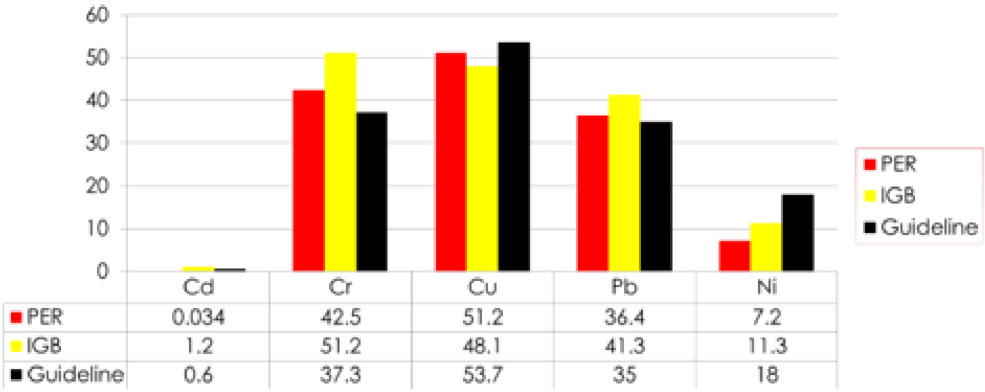

Fig. 3. Graph showing TC concentration in sediment for PER and IGB stations compared to the Guideline MATC standard (CCME, 2001) for fresh water sediment

Findings: Summary of TC above MATC: PER = **Cr** and **Pb,** IGB = **Cd, Cr** and **Pb**

Remarks: Long term contamination activities in the study area basin might have resulted to precipitation of TC and ultimate settlement in LNR sediment resulting in the POOR SQ [65]

FISH: GROSS ANATOMY

Condition Factor

Table 3. Comparing the mean fish CF for PER, IGB and ARAC

	Mean Weight	Mean length	Mean CF	Paired Sample Correlation	No of Sample (N)	Correlation	Sig.
ARAC	83.8	63.1	1.91	ARAC & PER	10	0.175	0.628
PER	54.3	140.3	1.98	ARAC & IGB	10	-0.183	0.612
IGB	54.3	132.5	1.99	PER & IGB	10	0.042	0.861

Findings: There was NO SIGNIFICANT DIFFERENCE btw PER, IGB and ARACS (P>0.05.

Remarks: There was inconsistency of PER and IGB CF with their corresponding EWQI & SQ. This might be due to fish migratory potential and LNR hydrology at the different experimental sites.

HEALTH ASSESSMENT INDEX (HAI)

Table 4. Comparing the mean Health Assessment Index (HAI) for PER, IGB and ARAC

	Mean HAI	Paired Sample Correlation	No of Sample (N)	Correlation	Sig.
ARAC	18.0	ARAC & PER	10	-287	**0.001**
PER	49.0	ARAC & IGB	10	0.754	**0.00**
IGB	60.5	PER & IGB	10	-400	**0.002**

Findings: HAI = IGB > PER > ARAC - **(P<0.05**).

Remarks: Result is consistentwith the SQ findings, but inconsistent with EWQI. Indicating that LNR sediment quality is a potential cause of observed fish pathology

FISH: HISTOLOGICAL ASSESSMENT

QUALITATIVE ASSESSMENT

LIVER

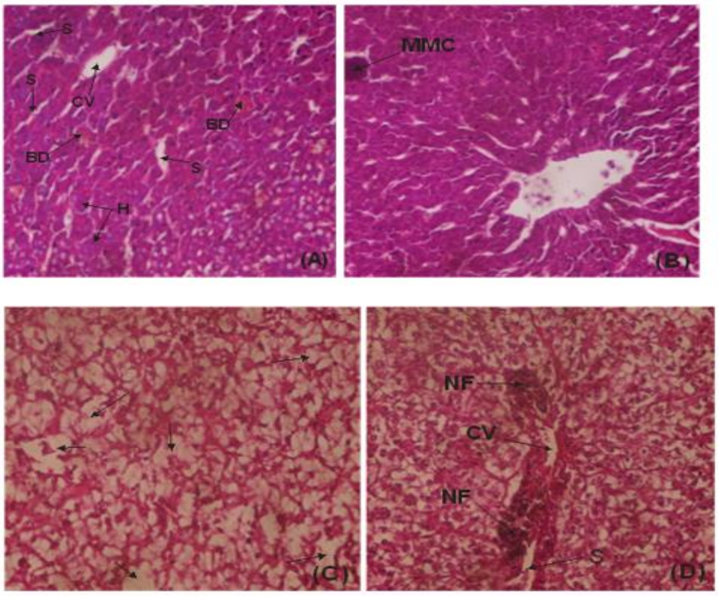

Fig. 4. Photomicrographs (H&E: 400X magnification) of Liver of fishes harvested from PER, IGB and ARAC

A: The histology of a normal Liver of fishes showed Hepatocytes (H), Sinusoids (S), Central Vein (CV) and Bile Duct (BD). B: A normal architecture, but with a Melano-Macrophage Centre (MMC). C: Micro-vesicular steatosis (Arrows). D: Showing Diffuse Micro-vesicular steatosiswith Necrotic Foci (NF) around the Central Vein (CV) and Sinusoids (S)

Table 5. Showing the percentage prevalence of liver histopathology of fishes harvested from PER, IGB and ARAC

Alterations	**% Prevalence**		
	PER $(\mathbf{n = 10})$	**IGB** $(\mathbf{n = 10})$	**ARAC9n=10)**
1. CIRCULATORY DISTURBANCE (CD)			
Intercellular Haemorrhage	10	10	0
2. REGRESSIVE CHANGE (RC)			
Intracellular Deposit	20	35	10
Frank Necrosis	0	10	0
Fatty Change	20	40	10
Vacuolation other than Steatosis	0	10	0
Melano-Macrophage Cetres (MMC)	10	35	0
Foci of Cellular Alteration (FCA)	5	20	0
Vacuolated Foci			0
Necrotic Foci	10	20	2.5
Average % Prevalence	**9.4**	**22.5**	

KIDNEY:

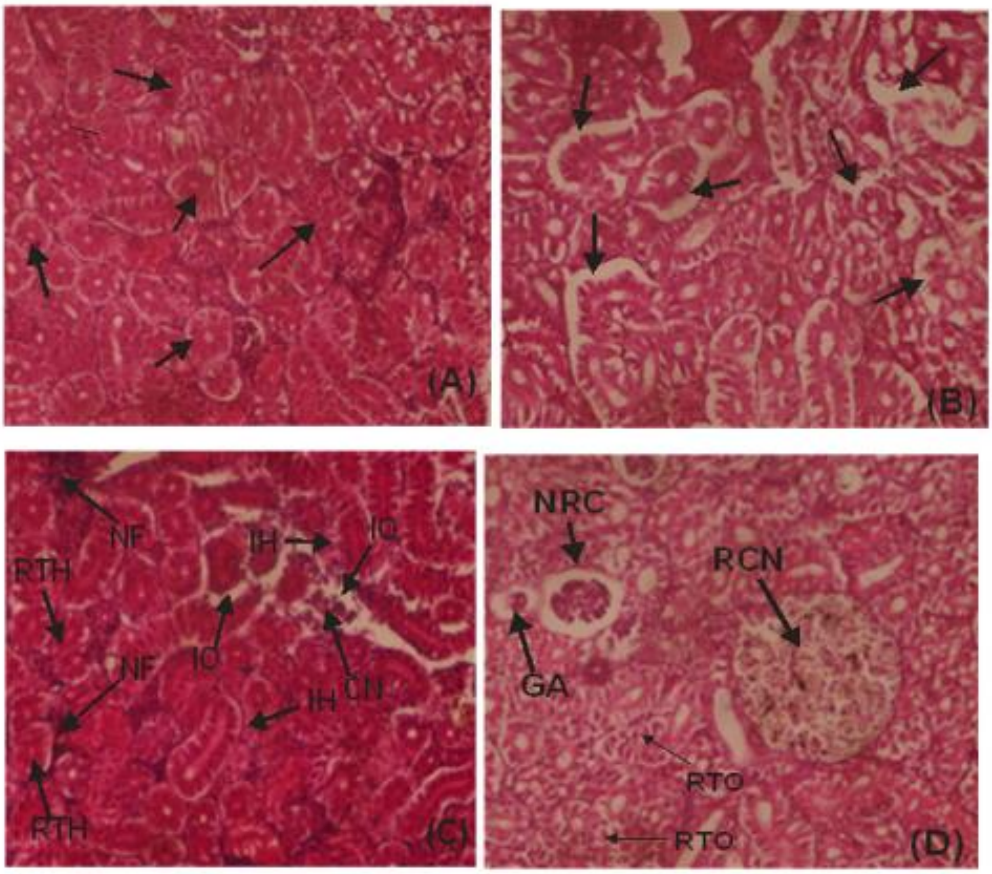

Fig. 5. Photomicrographs (H&E: 400 x magnification) of Kidney of fishes harvested from PER, IGB and ARAC

A: is a normal histological structure of the kidney specimens sampled in this study, consisted of Renal Tubules (Arrows) with normal lumen and interstitium B: Diffuse Interstitial Oedem with dilated renal lumen C: There is generalized vascular rupture with Interstitial Haemorrhage (IH) showing; Diffuse Coagulative Necrosis (CN), Areas of Interstitial Oedema (IO), Renal Tubular Hypertrophy (RTH) and Necrotic Foci (NF). D: Showing Normal Renal Corpuscle (NRC), Renal Corpuscle Necrosis and Glomerular Atrophy. There is also renal tubule oedema. E: Showing Normal Renal Corpuscle (NRC), Blood Vessels (BV), Renal Corpuscle Necrosis and Necrotic Exudate (NE)

Table 6. Showing the percentage prevalence of kidney histopathology of fishes harvested from PER, IGB and ARAC

Alterations	% Prevalence		
	Per $(\mathbf{N}=\mathbf{10})$	**IGB(** $\mathbf{N}=\mathbf{10})$	**ARAC 9N=10)**
1. Circulatory Disturbance (CD)			
Intercellular Haemorrhage	0	20	0
Interstitial Oedema	20	35	10
2. Progressive Change (PC)			
Hyperplasia	0	0	0
3. Regressive Change (RC)			
Architectural & Structural Alteration	15	40	0
Necrosis	10	30	0
Melano-Macrophage Centers (MMC)	10	25	10
Average % Prevalence	**11.0**	**30.0**	4.0

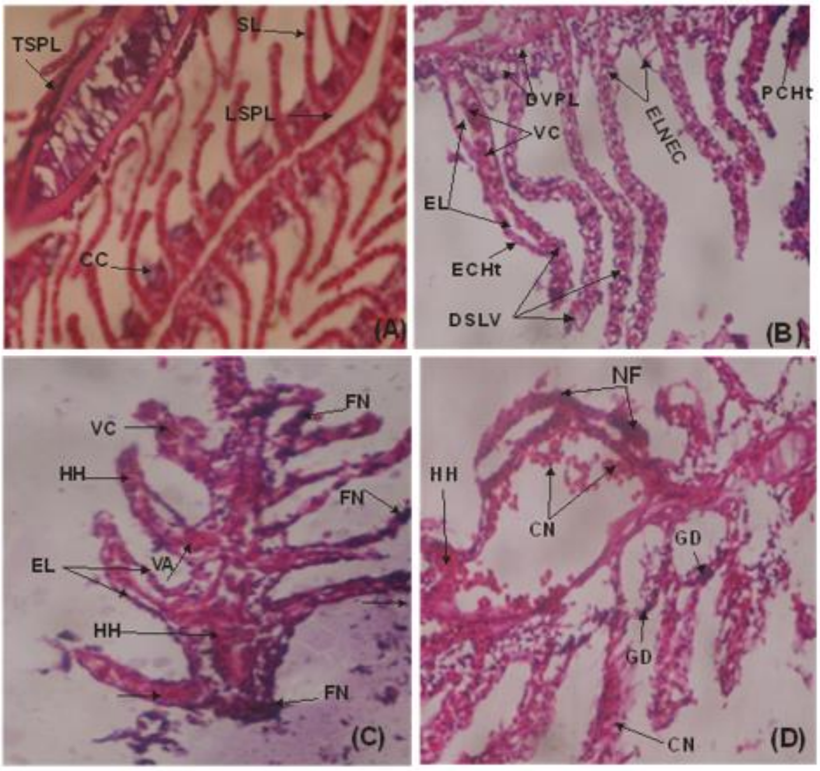

Fig. 6. Photomicrographs (H&E; 400 x magnification) of Gills of fishes harvested from PER, IGB and ARAC

A: The histology of a normal Gill architecture showing Longitudinal Section of Primary Lamella (LSPL), Secondary Lamella (SL), Chloride Cells (CC) and Transverse Section of Primary Lamella (TSPL). B: Early stage of degeneration with diffuse vacuolization showing; Epithelial Lifting (EL) with Normal Epithelial Cells (ELNEC), Diffuse Vacuolization of Primary Lamella (DVPL), Pillar Cell Hypertrophy (PCHt), Vascular Congestion (VC), secondary lamella Epithelial Cell Hypertrophy (ECHt) and Diffuse Secondary Lamella Vacuolization (DSLV). C: There is generalized necrosis of vascular walls with diffuse coagulative necrosis leading to disruption of the Gill's architecture, showing; Epithelial Lifting (EL), Vascular Congestion (VC), Haemorrhage (HH), Vascular Aneurism (VA) and Frank Necrosis (FN). D: Late stages of Gills Degeneration with disruption of Gill's architecture, showing; Diffuse Vacuolization (DVL), rupture of vascular wall with Haemorrhage (HH) and Diffuse Coagulative Necrosis (CN), diffuse necrosis with Necrotic Foci (NF) leading to the Degenerative Granulation (DG)

Table 7. Showing the percentage prevalence of Gills histopathology of fishes harvested from PER, IGB and ARAC

Alteration	**% Prevalence**		
	Per $(n = 10)$	**IGB$(n = 10)$**	**ARAC9n=10)**
1. CIRCULATORY DISTURBANCE (CD)			
Hyperaemia	30	50	10
Epithelial Lifting	20	45	0
Telangiectasia	5	10	0
2. PROGRESSIVE CHANGE (PC)			
Hyperplasia	0	10	0
3. REGRESSIVE CHANGE (RC)			
Structural Alteration	30	35	10
Vacuolation	5	15	0
Atrophy			
Necrosis	5	15	0
Average % Prevalence	**13.6**	**25.7**	2.9

TESTES

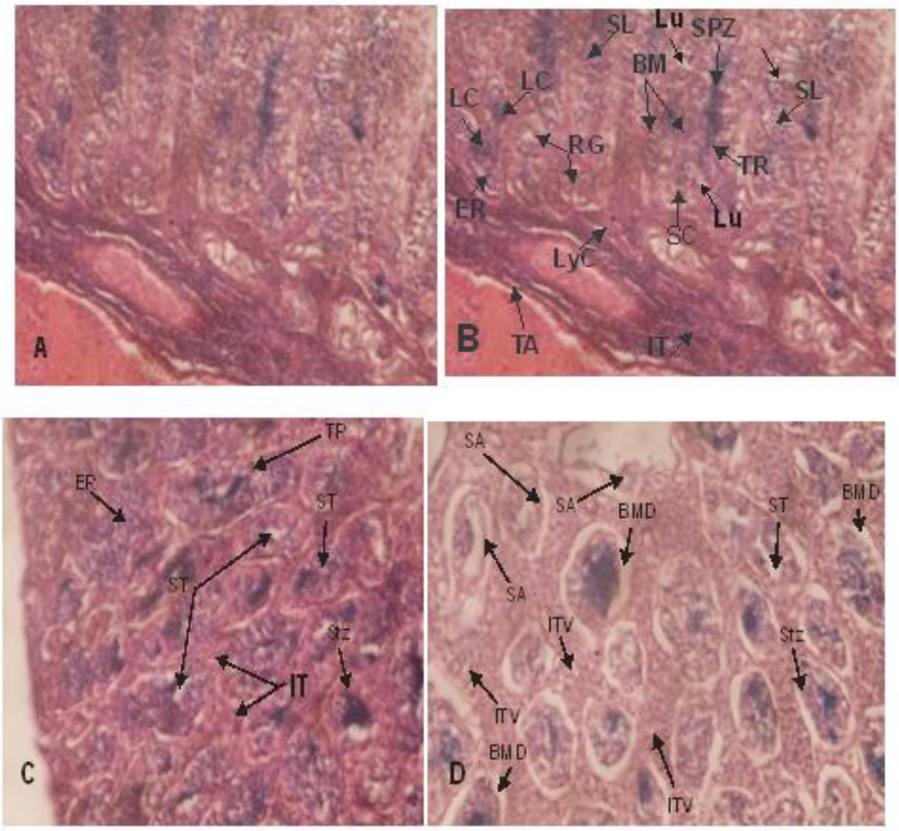

Fig. 7. Photomicrographs (H&E; 400 x magnification) of Testes of fishes harvested from PER, IGB and ARAC

__A__: Seminiferous Lobules (SL) in different stages of spermatogenesis (RG-Regressive Gonade, ER – Early Recrudesce, TR - Terminal Recrudesce). __B:__ The histology of a normal Testes architecture showing the Seminiferous Lobules (SL) and their Basement Membranes (BM). The SL contains the germinal epithelium represented by Sertoli cells (SC) enveloping daughter cells of spermatogonia, lobule (spermatocytes) cysts (LC), Seminiferous lobule lumen (Lu) and Spermatozoa (SPz). The interstitial Tissue (IT) contains Leydig cells being limited by an external wall, the Tunica Albuginia (TA). __C:__ showed sections of seminiferous Tubule (ST) consisting of low cuboidal epithelial cells with many of its lumen engorged with spermatozoa (SPz). __D:__ Showed notable histopathology of the study showing lobular structure alteration (SA), with marked and diffuse basement detachment. There is also decrease in spermatids and spermatozoa in some of the seminiferous lobules (SL), Interstitial Vacuolation (ITV)

Table 8. Showing the percentage prevalence of Testes histopathology of fishes harvested from PER, IGB and ARAC

Alteration	**% Prevalence**		
	Per $(n = 10)$	**IGB**$(n = 10)$	**ARAC9** $n = 10$ **)**
1. CIRCULATORY DISTURBANCE (CD)			
Interstitial Oedema	10	30	0
2. REGRESSIVE CHANGE (PC)			
Disorganization of Lobule	5	35	0
Defachment of Basement Membrane	10	15	0
Inhibition of Spermatogenesis	5	10	0
INTERSTITIAL TISSUE			
Structural Alteration	5	10	0
Vacuolation	5	15	0
Average % Prevalence	6.7	19.2	0

SEMI-QUANTITATIVE HISTOPATHOLOGICAL ASSESSMENT:

Table 9. Comparing the mean organ indices of per and igb to the reference site, ARAC

		Paired Difference					T	df	Sig. (2 Tailed)
ORGAN	SITES	Mean Organ Index (Iorg)	Std. Deviation	Std. Error Mean	95% confidence Interval of the difference				
					lower	Upper			
LIVER	ARAC	3.4	4.8	1.5	- 7.4	-0.4	-2.6	9	**0.03**
	PER	7.5	9.5	3.0	-17.3	-3.7	-3.5	9	**0.01**
	IGB	12.7	8.1	1.8	9.1	1.6	3.0	19	**0.01**
KIDNEY	ARAC	2.6	3.8	1.2	-3.1	2.3	-0.3	9	0.74
	PER	4.5	7.6	2.4	-11.3	-0.4	-2.4	9	**0.03**
	IGB	9.2	4.7	1.0	-6.9	-2.6	-4.6	19	**0.00**
GILLS	ARAC	6.9	9.6	3.0	-11.6	2.2	-1.5	9	0.15
	PER	9.2	15.1	4.8	-21.5	0.1	-2.2	9	0.05
	IGB	13.5	8.1	1.8	-8.2	-0.6	-2.4	19	**0.02**
TESTES	ARAC	0.7	1.1	0.3	-1.0	0.6	-0.6	9	0.59
	PER	1.2	1.7	0.5	-2.0	0.3	-1.7	9	0.12
	IGB	4.9.	2.1	1.2	-1.0	0.1	-2.0	19	**0.02**

Finding: I_{org}: IGB > PER > ARAC.

Remark: I_{org} order is consistent with the SQ, but inconsistent with EWQI. SQ is a potential cause of fish histopathology. EWQI inconsistencies might be due to surface water Hydrology (water flow and recharge variability) at PER & IGB.

ENVIRONMENTAL POLLUTION STATUS

Table 10. Pollution stratification using the mean Organ Indices of ARAC, PER and IGB

	Mean Organ Index (Iorg)				Ranking
	Liver	Kidney	Gills	Testes	
ARAC	3.4	4.6	6.9	0.7	**3.7**
PER	7.3	4.5	9.1	1.2	**5.5**
IGB	12.7	9.5	13.5	4.9	**10.2**

Using the Iorg pollution classification [64]
I. Class 1 (index value <10): Not Polluted
II. Class 2 (index value 10-25): Slightly Polluted
III. Class 3 (index value 26-35): Moderately Polluted
IV. Class 4 (index value >35): Severe Pollution

Finding: PER is Not Polluted; IGB is slightly polluted

Remarks: EPS is consistent with SQ, but inconsistent with EWQI, hence HEAVY METALS (other than physical parameters) are the likely cause of POLLUTION

FISH INDEX (IFISH):

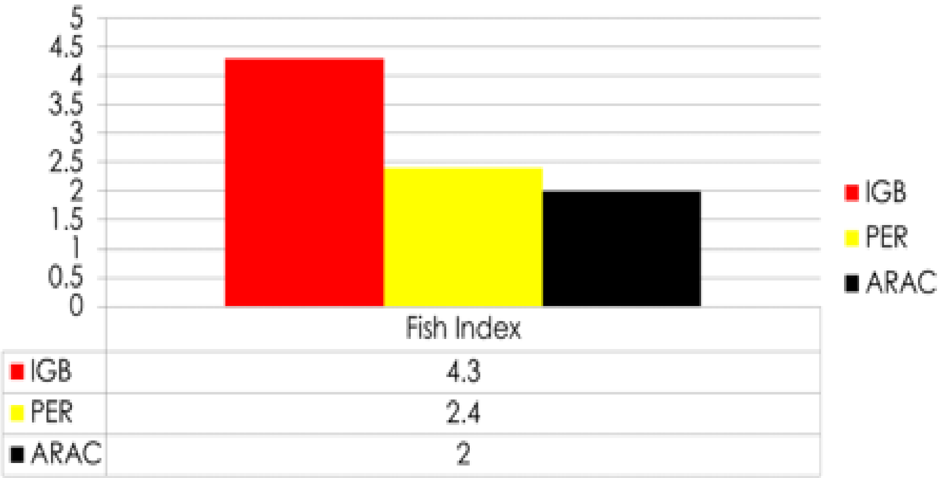

Fig. 8. Graph showing comparison of Fish Index assessment values)

Finding: Ifish= IGB>PER>ARAC

Remarks: Finding is consistent with SQ, inconsistent with EWQI and corroborates HEAVY METALS as the cause of Fish pathology

5. RESULTS AND DISCUSSION

Environmental Water Quality Index (EWQI) values were ranked as poor at PER and marginally poor at IGB respectively (CCME, 2001). The Peremabiri (PER) and Igbomotoru (IGB) EWQI results were consistent with the physical perception of observers and users of the lower reaches of the Nun River (Vanguard, 2014). It can therefore be inferred that the poor water quality parameters of this study are one of the likely causes of the fish lesion observed in this study. Sediment's trace elements of Cadmium Cd, Chromium Cr, Copper Cu, and lead Pb measured at IGB station were found to be above the Maximum allowable toxicant concentration (MATC) guideline levels for freshwater aquatic life protection, except for Nickel. While in PER station, Cr, Cu and Pb was found to be higher than the MATC guideline value, except for Cd and Nickel. For the fact that these heavy metals (Cr, Cu, Cd and Pb) are known to be associated crude oil exploration and processing Fatoba et al. it can therefore be concluded that the alleged longtime illegal oil refining around the vicinity of the said communities might be the potential cause of the high sediment levels of the target chemicals. Based on Carlander classification of mean CF, all sites, ARAC, PER and IGB, fell under excellent condition (trophy class fish) with CF >1.60. Some fishes might be grossly anatomically observed to be in good condition but still have lesion that are causing serious organ dysfunction (Bernet et al. and Marchand et al. HAI showed that fish caught from the experimental sites had poor health conditions as compared to the reference sites. This might be attributed to the poor EWQI of the experimental sites, which was consistent with findings in previous studies by Marchand et al., and Allison and Paul.The qualitative histological study showed that IGB had the highest prevalence of histopathological lesions in the liver, kidney, gills and testes followed by PER, with ARAC having the least. This was consistent with EWQI findings from this study, implying that surface water contaminants are potential cause of lesions. Nevertheless the finding was also inconsistent with the sediment quality result which can be adduced to surface water hydrology affect suspended particles in sediment and weather.The histopathological Organ Index (Iorg) of the target organs (liver, kidney, gills and Testes) showed that fishes harvested from IGB had worse lesions, followed by PER and ARAC having the least. This implies that fishes from the LNR axis at IGB and PER are affected by observed surface water and sediments contaminants of the LNR. The hierarchy in severity of the histopathological findings between both experiments sites of PER and IGB correlates with the EWQ indices for the two sites, with IGB having a comparatively worse EWQI. It can be inferred therefore, that the water quality parameter that were above the MATC levels are the potential causes of the organ histopathologies recorded. Findings were consistent with reports in previous studies by Van Dyk et al. and Marchand et al. The aquatic environmental pollution status (EPS), using organ index histological alterations Zimmerli et al. showed that ARAC and PER were ranked class 1 (not polluted), while IGB was ranked within the range of Class 2 (mildly polluted). The PER findings were inconsistent with EWQI and sediment results of the sediment quality results. This inconsistency might be due to fish migratory patterns in avoidance of hazardous environment.

6. CONCLUSION

This study was ecologically relevant. It was able to demonstrate that the lower Nun River, around Peremabiri and Igbomotoru water bodies, are contamination with a slight level of pollution at Igbomotoru axis. The study has once more given credence to the use of histology as a biomarker to assess sublethal level of environmental stressors, and in determination of the pollution status of an ecosystem. This study has also highlighted the fact that no single investigative approach is able to measure the adverse effects of environmental contaminants, Nevertheless, histological based ecotoxicological studies using both chemical and biological methods are essential in ecological risk and impact assessment.

7. RECOMMENDATIONS

- Forensic Environmental Audit (FEA) to ascertain the environmental agent that is source to the heavy metals found in the LNR
- Regular bio-monitoring by environmental agencies toprovide baseline data for the environmental protection and forestall further environmental degradation.
- Bioaccumulation study of fisheries by food agencies for food edibility advisory
- An epidemiological survey with Integrated Health Risk Assessment (IHRA) should be conducted in order to determine the human health impact on the study communities.
- Artisanal training of local on petroleum refining and licensing of local modular refineries so as to forestall illegal refining and bunkering
- Increase local pipeline security contracts as a means for community security and local empowerment
- A concomitant use of conventional pipeline security to protect and prevent pipeline from vandalism and sabotage.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abowei JF, Hart AI. Artisanal fisheries characteristics of the fresh water reaches of lower Nun River, Niger Delta, Nigeria. Journal of Applied Sciences and Environmental Management. 2008;12(1). Adams SM, Brown AM, Goede RW. A quantitative health assessment index for raid evaluation of fish condition in the field. American Fish Society. 1993;122:63-73.
2. Aghoghovwia OA, Izah SC, Miri FA. Environmental risk assessment of heavy metals in sediment of Nun River around Gbarantoru and Tombia Towns, Bayelsa State, Nigeria. Biological evidence. 2018;8.
3. Alagoa KJ, Lelei KE, Charles EE. heavy metal concentration in soft sediments of river-nun at amassoma axis. EPRA International Journal of Multidisciplinary Research (IJMR). 2023;9(4):307-11.
4. Ifelebuegu AO, Ukpebor JE, Ahukannah AU, Nnadi EO, Theophilus SC. Environmental effects of crude oil spill on

the physicochemical and hydrobiological characteristics of the Nun River, Niger Delta. Environmental monitoring and assessment. 2017;189:1-2.

5. Olarinmoye OM, ABL. Fish gills as morphological indicators of exposure to four environmentally relevant pharmaceuticals. Asian Journal of Fisheries and Aquatic Research. 2013; 22(2):1–6. Available:https://doi.org/10.9734/ajfar/2023/v22i2565
6. Ramos AR, Campelo DA, da Silva Carneiro CL, Zuanon JA, da Matta SL, Furuya WM, Salaro AL. Optimal dietary L-glutamine level improves growth performance and intestinal histomorphometry of juvenile giant trahira (*Hoplias lacerdae*), a Neotropical carnivorous fish species. Aquaculture. 2022;547:737469.
7. Aigberua A, Tarawou T. Assessment of heavy metals in muscle of tilapia zilli from some Nun River Estuaries in the Niger Delta Region of Nigeria. Academic Journal of Chemistry. 2017;2(9):96-101.
8. Ebuete AW, Puanoni NI, Ebuete YI, Ebuete E. Water quality index (NSFWQI) of the river Nun, Bayelsa State, Nigeria. American Journal of Environment and Climate. 2023;2(2):15-22.
9. Okuyade W, Abbey T. Pollutants spread in a bifurcating river: The River Nun, Bayelsa, Nigeria. Journal of Scientific Research and Reports. 2016;12(6):1-9.
10. Ezekwe IC, Ezekwe AS, Endoro PO. Biological contaminants in the River Nun and environmental ethics of riverside communities in the Niger Delta: The case of Amassoma, Bayelsa, Nigeria. Estudos de Biologia. 2013 Nov 27;35(84).
11. Kigigha LT, Seiyaboh EI, Obua VJ, Izah SC. Contamination of River Nun at Amassoma, Bayelsa State, Nigeria due to microbial diversity in sediments. Environmental Toxicology Studies Journal. 2018;2(1):2.
12. Wala C, Hart AI, Babatunde BB, Zabbey N. Assessment of human health risk from heavy metal loads in freshwater clam, Ergeria radiata, from the Nun River, Niger Delta, Nigeria. Journal of Environment and Earth Science. 2016;6(9):10-20.
13. Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T. Histopathology in fish: proposal for a protocol to assess aquatic pollution. Journal of fish diseases. 1999;22(1):25-34.
14. Allison TA, Paul CW. Histological based biomonitoring: a baseline ecotoxicological evaluation of New-Calabar River using Chrysichthys nigrodigitatus. Int J Environ Poll Res. 2014;2(3):17-41.
15. Stott GG, Haensly WE, Neff JM, Sharp JR. Histopathologic survey of ovaries of plaice, Pleuronectes platessa L., from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the Amoco Cadiz crude oil spill. Journal of Fish diseases. 1983;6(5):429-37.
16. Berthou F, Balouet G, Bodennec G, Marchand M. The occurrence of hydrocarbons and histopathological abnormalities in oysters for seven years following the wreck of the Amoco Cadiz in Brittany (France). Marine Environmental Research. 1987;23(2):103-33.
17. Solé M, Raldua D, Piferrer F, Barceló D, Porte C. Feminization of wild carp, Cyprinus carpio, in a polluted environment: Plasma steroid hormones, gonadal morphology and xenobiotic metabolizing system. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2003;136(2):145-56.
18. Davies JM, Topping G. The impact of an oil spill in turbulent waters: The Braer.
19. Lyons BP, Harvey JS, Parry JM. An initial assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in the intertidal teleost Lipophrys pholis. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 1997;390(3): 263-8.
20. Harvey JS, Lyons BP, Page TS, Stewart C, Parry JM. An assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in selected invertebrate and vertebrate species. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 1999;441(1):103-14.
21. Fernley PW, Moore MN, Lowe DM, Donkin P, Evans S. Impact of the Sea Empress oil spill on lysosomal stability in mussel blood cells. Marine Environmental Research. 2000;50(1-5):451-5.
22. Jewett SC, Dean TA, Woodin BR, Hoberg MK, Stegeman JJ. Exposure to hydrocarbons 10 years after the Exxon Valdez oil spill: evidence from cytochrome P4501A expression and biliary FACs in

nearshore demersal fishes. Marine Environmental Research. 2002;54(1):21-48.

23. Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB. Long-term ecosystem response to the Exxon Valdez oil spill. Science. 2003;302(5653): 2082-6.
24. Auffret M, Duchemin M, Rousseau S, Boutet I, Tanguy A, Moraga D, Marhic A. Monitoring of immunotoxic responses in oysters reared in areas contaminated by the Erika oil spill. Aquatic Living Resources. 2004;17(3):297-302.
25. Bocquené G, Chantereau S, Clérendeau C, Beausir E, Ménard D, Raffin B, Minier C, Burgeot T, Leszkowicz AP, Narbonne JF. Biological effects of the Erika oil spill on the common mussel (*Mytilus edulis*). Aquatic Living Resources. 2004;17(3):309-16.
26. Budzinski H, Mazéas O, Tronczynski J, Désaunay Y, Bocquené G, Claireaux G. Link between exposure of fish (*Solea solea*) to PAHs and metabolites: Application to the Erika oil spill. Aquatic Living Resources. 2004;17(3):329-34.
27. Laubier L, Le Moigne M, Flammarion P, Thybaud E, Cossa D. The monitoring programme of the ecological and ecotoxicological consequences of the Erika oil spill. Aquatic Living Resources. 2004;17(3):239-41.
28. Lee RF, Anderson JW. Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. Marine Pollution Bulletin. 2005;50 (7):705-23.
29. Beiras R, Saco-Alvarez L. Toxicity of seawater and sand affected by the Prestige fuel-oil spill using bivalve and sea urchin embryogenesis bioassays. Water, Air, and Soil Pollution. 2006;177(1):457-66.
30. Cajaraville MP, Garmendia L, Orbea A, Werding R, Gómez-Mendikute A, Izagirre U, Soto M, Marigómez I. Signs of recovery of mussels health two years after the Prestige oil spill. Marine Environmental Research. 2006;62:S337-41.
31. Marigomez I, Soto M, Cancio I, Orbea A, Garmendia L, Cajaraville MP. Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill (Monitoring Campaign 2003). Marine Pollution Bulletin. 2006;53(5-7):287-304.
32. Ordás MC, Albaigés J, Bayona JM, Ordas A, Figueras A. Assessment of *in vivo* effects of the prestige fuel oil spill on the mediterranean mussel immune system. Archives of environmental Contamination and Toxicology. 2007;52:200-6.
33. Chapman PM. Determining when contamination is pollution—weight of evidence determinations for sediments and effluents. Environment international. 2007;33(4):492-501.
34. Yilmaz AB. Levels of heavy metals (Fe, Cu, Ni, Cr, Pb, and Zn) in tissue of Mugil cephalus and Trachurus mediterraneus from Iskenderun bay, Turkey. Environmental Research. 2003;92(3):277-81.
35. Roganovic-Zafirova DR, Jordanova M, Panov S, Velkova-Jordanovska L. Hepatic capillariasis in the Mediterranean barbel (Barbus meridionalis petenyi Heck.) from Lake Ohrid.
36. Fatoba PO, Ogunkunle CO, Ihaza CO. Assessment of metal pollution of soil and Diagnostic species associated with oil spills in the Niger Delta, Nigeria. Environmental Research, Engineering and Management. 2015;71(3):13-22.
37. Osuji LC, Adesiyan SO. Extractable hydrocarbons, nickel and vanadium contents of Ogbodo-Isiokpo oil spill polluted soils in Niger Delta, Nigeria. Environmental Monitoring and Assessment. 2005;110:129-39.
38. Iyebor EW, Amauche NS, Ubong U, Obed N. Assessment of hydrocarbons in soil along crude oil pipeline route in Rivers State, Nigeria. International Journal of Innovative Research and Development. 2020;9(7).
39. Pietrapiana D, Modena M, Guidetti P, Falugi C, Vacchi M. Evaluating the genotoxic damage and hepatic tissue alterations in demersal fish species: a case study in the Ligurian Sea (NW-Mediterranean). Marine Pollution Bulletin. 2002;44(3):238-43.
40. Joly-Turquin G, Dubois P, Coteur G, Danis B, Leyzour S, Le Menach K, Budzinski H, Guillou M. Effects of the Erika oil spill on the common starfish Asterias rubens, evaluated by field and laboratory studies. Archives of Environmental Contamination and Toxicology. 2009;56:209-20.

41. Oguntade OR, Oketoki OT, Ukenye EA, Mojekwu TO, Usman BA, Adeleke MT. Survey of the present and fast disappearing fish species along two rivers in the Niger Delta.
42. Allison ME, Okadi D. Species distribution and abundance in the lower Nun River, Niger delta, Nigeria. Journal of Fisheries International. 2009;4(1):13-8.
43. Sikoki FD, Hart AI, Abowei JF. Gill net selectivity and fish abundance in the lower Nun river, Nigeria. J Applied Science and Environmental Management. 1998;1:13-9.
44. Agbabiaka L. Food and feeding habits of Tilapia zilli (pisces: cichlidae) in river Otamiri south-eastern Nigeria. Algae. 2012;71(8.82).
45. Basiao ZU, Taniguchi N. An investigation of enzyme and other protein polymorphisms in Japanese stocks of the tilapias Oreochromis niloticus and *Tilapia zillii*. Aquaculture. 1984;38(4):335-45.
46. Bruton MN, Gophen M. The effect of environmental factors on the nesting and courtship behaviour of Tilapia zillii in Lake Kinneret (Israel). Hydrobiologia. 1992;239: 171-8.
47. Williams C, Bonner TH. Texas Freshwater fishes: Tilapia zillii redbelly tilapia. Texas State University-San Marcos, Biology Department, Aquatic Station; 2008.
48. United States Environmental Protection Agency, (USEPA). surface water sampling: environmental investigations standard operating procedures and quality assurance manual. Region 4 Science and Ecosystem Support Division (SESD), Athens; 2013.
49. Fulton TW. The rate of growth of fishes. 2oth Annual Report of the Fishery Board of Scotland. 1902;(3):326-446.
50. Van Dyk JC, Marchand MJ, Smit NJ, Pieterse GM. A histology-based fish health assessment of four commercially and ecologically important species from the Okavango Delta panhandle, Botswana. African Journal of Aquatic Science. 2009; 34(3):273-82.
51. Van Dyk JC, Marchand MJ, Pieterse GM, Barnhoorn IE, Bornman MS. Histological changes in the gills of Clarias gariepinus (Teleostei: Clariidae) from a polluted South African urban aquatic system. African Journal of Aquatic Science. 2009;34 (3):283-91.
52. Zimmerli S, Bernet D, Burkhardt-Holm P, Schmidt-Posthaus H, Vonlanthen P, Wahli T, Segner H. Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. Aquatic Sciences. 2007;11-25.
53. Kosari S, Vanae M. Survey of the source and amount of pollutants concentration of heavy metal in Tembi River and Bohlol Lake. InProceedings of the 1st Conference on Geological and Environmental and Medical; 2007.
54. Tabasian H, Abdoli A, Valikhani H, Khosravi M. An investigation into socio-economic impacts of invasive redbelly tilapia Coptodon zillii (Gervais, 1848): A case study from the Shadegan Wetland, Iran. Scientific Reports in Life Sciences. 2021 Sep 30;2(3):25-38.
55. Erarto F, Getahun A. Impacts of introductions of alien species with emphasis on fishes. Int. J. Fish. Aquat. Stud. 2020;8:207-16.
56. Ajagbe SO, Odulate DO, Akegbejo-Samsons Y, Kehinde AS, Ajagbe RO, Ojubolamo MT. Species composition, abundance and diversity of Ichthyofauna of Ikere-Gorge, Oyo State, Nigeria. Acta Ecologica Sinica. 2021 Dec 1;41(6):575-83.
57. Popoola MO, Schedel FD, Hebert PD, Schliewen UK. First DNA barcode library for the ichthyofauna of the Jos Plateau (Nigeria) with comments on potential undescribed fish species. PeerJ. 2022 Apr 13;10:e13049.
58. Villanueva JD, Le Coustumer P, Huneau F, Motelica-Heino M, Perez TR, Materum R, Espaldon MV, Stoll S. Assessment of trace metals during episodic events using DGT passive sampler: a proposal for water management enhancement. Water resources management. 2013 Sep;27 :4163-81.
59. Backwash CC, Flotation DD, East E, Zone EE, Assessment EE, East-Northeast EN, East-Southeast ES, Blooms HH, Bank HH, Area MM, North N. ANZECC Australian and New Zealand Environment and Conservation Council BCF Bioconcentration Factor CCC Criteria Continuous Concentration CCME Canadian Council of Ministers of the Environment CD Chart Datum.
60. Smith WC. A histology-based health assessment of selected fish species from two rivers in the Kruger National Park.

University of Johannesburg (South Africa); 2012.
61. Rakhi SF, Reza AH, Hossen MS, Hossain Z. Alterations in histopathological features and brain acetylcholinesterase activity in stinging catfish Heteropneustes fossilis exposed to polluted river water. International aquatic research. 2013 Dec;5:1-8.
62. Kaptaner B, Kankaya E, Doğan A, Çelik İ. Histopathology and oxidative stress in the liver of Chalcalburnus tarichi living in lake Van, Turkey. Life Science Journal. 2014;11(8):66-77.
63. Okito GM, Agbohessi P, Matunguru J, Micha JC, Ntakimazi G, Nshombo V, Mbalassa M, Jauniaux T, Mandiki R, Kestemont P. Histopathological disturbances in two fish species Oreochromis niloticus (Linnaeus 1758) and Labeo barbus pellegrini (Bertin et Estève 1948) from downstream mining sites in the upper Ulindi and Elila river basins, Eastern DR Congo. Biolife International Quarterly Journal of Biology & Life Sciences. 2023 Mar 25;11.
64. Georgieva E, Yancheva V, Stoyanova S, Velcheva I, Iliev I, Vasileva T, Bivolarski V, Petkova E, László B, Nyeste K, Antal L. Which is more toxic? Evaluation of the short-term toxic effects of chlorpyrifos and cypermethrin on selected biomarkers in common carp (Cyprinus carpio, Linnaeus 1758). Toxics. 2021 May 31;9(6):125.
65. Todorova K, Velcheva I, Yancheva V, Stoyanova S, Dimitrova P, Tomov S, Georgieva E. Interactions of lead with other heavy metals (cadmium, nickel and zinc) in toxic effects on the histological structure of gills of the common carp Cyprinus carpio Linnaeus, 1758. Acta Zool Bulgar. 2019 Mar 1;71(1):95-102.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/117884