

Histological Based Environmental Monitoring Of The Lower Nun River: Using The Histo-Morphometry of Kidney, Gills, Liver, And Testes of *Coptodon zillii*

ABSTRACT:

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 and feral fish from two stations along the LNR, Peremabiri (PER) and Igbomatoru (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 analyzed 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.*

Comment [ak1]: meaningless

Comment [ak2]: should be in italics and write its common name redbelly tilapia

INTRODUCTION

Niger Delta region is an ecologically threatened geographical region, in the South-South geopolitical 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 (WWF, 2020). 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 (NDES, 1995).

Beyond the legitimate crude oil activities by Multi-National Oil Companies (MNOOC), 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 (Reuters, 2017). Crude oil related crimes have continued to rise in the Niger Delta despite efforts by the nation's security agencies, to curb the menace (Vanguard, 2014). Makeshift refineries, usually hidden in oil-soaked clearings, support tens of thousands of people locally (Reuters, 2017). 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 (Punch, 2019).

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 midwived 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 (NDES, 1995):

- Northern Niger Delta border is located at about (5°33'49"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 southern basin 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-eastern basin 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 Oporoza 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 ecotoxicologically 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 (Vanguard, 2014).

Peremabiri (PER) and Igbomatoru (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 transverse 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 (Vanguard, 2014). Agip was once forced to suspend her crude oil production activity in Bayelsa State, due to the activities of vandals and oil theft (Vanguard, 2014).

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 (IEOD, 1995; Abowei and Hart, 2008). 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 (Abowei and Hart, 2008).

Most studies of the LNR were based on assessing contamination of the ecosystem; which includes sediment quality (Aghoghovwia et al., 2018; Alagoa et al., 2023), water quality (Ifelebuegu, et al., 2017; Aigberua and Tarawou, 2018; Williams et al., 2023), hydrodynamics of pollutants (Okuyade et al., 2016) bio-diversity (Ifelebuegu, et al., 2017), biological contaminants (Ezekwe et al., 2013; Kigigha et al., 2018) and bioaccumulation of xenobiotics in sentinel aquatic organisms (Wala et al., 2016; Aigberua and Tarawou, 2017; Alagoa, J., Yabefa, J.A., Yowei, E.J. (2021),). There is a study gap on the bioavailability and bioactivity or effect of observed contaminants on biological organism (Bernet et al., 1999; Allison and Paul, 2014), 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 (Allison and Paul, 2014). 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 (e.g. Stott et al., 1983; Berthou et al., 1987; Sole´ et al., 2003; Davies and Topping, 1997; Lyons et al., 1997; Edwards and Sime, 1998; Harvey et al., 1999; Fernley et al., 2000; Jewett et al., 2002; Peterson et al., 2003; Auffret et al., 2004; Bocquene´ et al., 2004; Budzinski et al., 2004; Gerhardt et al., 2004; Laubiart et al., 2004; Lee and Anderson, 2005; Beiras and Saco-Alvarez, 2006; Cajaraville et al., 2006; Marigo mez et al., 2006; Ordas et al., 2007; Martınez-G3mez et al., 2010; Allison and Paul, 2014).

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 (Chapman, 2007). 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 (Chapman, 2007). Pollution is contamination that can result in adverse biological effect to resident communities (Chapman, 2007; Allison and Paul, 2014). 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.

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 (Chapman, 2007), 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. 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. 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.

Target organs such as liver, testes, kidney and gills, have a tendency to accumulate high levels of heavy metals (Yilmaz, 2003). 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 (Roganovic-Zafirova *et al.*, 2003, Allison and Paul, 2014).

MATERIALS AND METHOD

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 (Britannica, 2014). 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 (Britannica 2014). 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 (Vanguard, 2014). IGB is an area notorious for illegal oil bunkering and refining activities. (Vanguard, 2014)

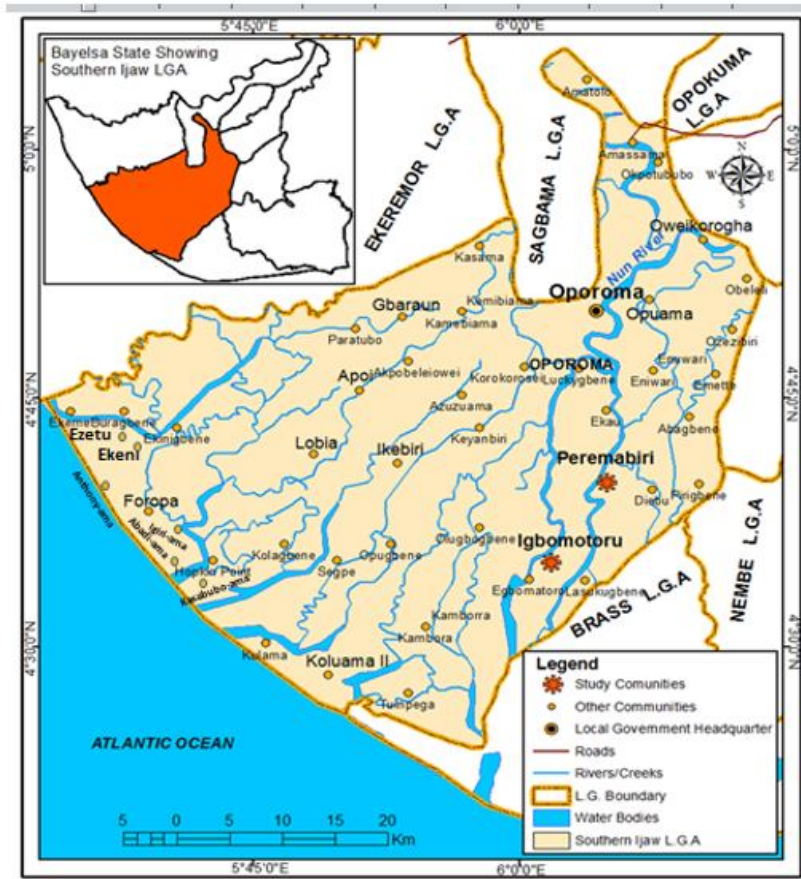


Figure 1: Map of Southern Ijaw Local Government Area Showing the lower Nun River with an indication of Peremabiri and Igbomatoru

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 aquaculture 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.

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 (GISD, 2019). 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 (Fish Base, 2008; GISD, 2019).



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

SAMPLE SELECTION

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). (Fatoba et al., 2015)
- 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. (Osuji et al., 2006)
- IV. Pb and Cr is associated with crude oil piping system. (Iyebor et al., 2020):
- V. The sediments of the LNR system revealed a high mobility and bioavailability for Pb and Cr. (Aigberua and Tarawou, 2018):
- VI. Trace metal levels were moderately elevated with Ni recording the highest percentage in clams harvest from LNR. (Wala et al. 2016)
- 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. (Aigberua and Tarawou, 2017).

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 (USEPA, 2016a), the following chemicals were chosen for this study: Pb, Ni, Cr, Cu and Cd

Fish Selection

EROCIPS (Emergency Response to coastal Oil, Chemical and Inert Pollution from Shipping), (2006), "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 (Harvey et al., 1999; Pietrapiana et al., 2002; Budzinski et al.,2004; EROCIIPS, 2006; Joly-Turquin et al.,2009).

In a survey of fish diversity of the LNR in Southern Ijaw LGA, Oguntade et al.,2014, Allison and Okadi2009, and Sikoki et al.,1998 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 (Oguntade *et al.*, 2014).

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 (Agbabiaka, 2012; Basiao and Taniguchi, 1984; Bruton and Gophen, 1992; Williams and Bonner, 2008).

SAMPLING AND EVALUATION

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 (2013) 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)

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 Ekman 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 (SON, 2007; USSG,2018)

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 (2015) 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 evaluated 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) (Fulton, 1902).
- **Health Assessment Index (HAI):** This is a field assessment tool for quantitative evaluation of fish health (Adams et al., 1993). 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 Adams et al. (1993).

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. (1999) protocol modified by Van Dyk et al. (2009), 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., (2009a, b) based on a proposed scoring scheme by Zimmerli et al., (2007) 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)

RESULTS AND DISCUSSION

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 (Fresh Water)	Remark
	1 st	2 nd	3 th	4 th	Mean		
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 (Fresh Water)	Remark
	1 st	2 nd	3 th	4 th	Mean		
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 degradation concerns of LNR basin (Vanguard, 2014; Reuters, 2017; Punch, 2019).

SEDIMENT QUALITY

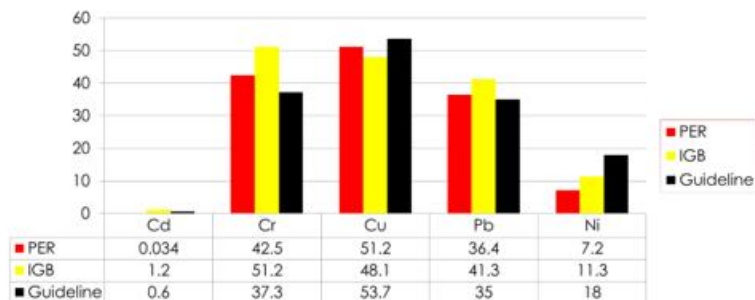


Figure 3: Graph showing TC concentration in sediment for PER and IGB stations compared to the Guideline MATC standard (CCME, 2001) for fresh water sediment

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 (Kosari and Vanae, 2007)

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



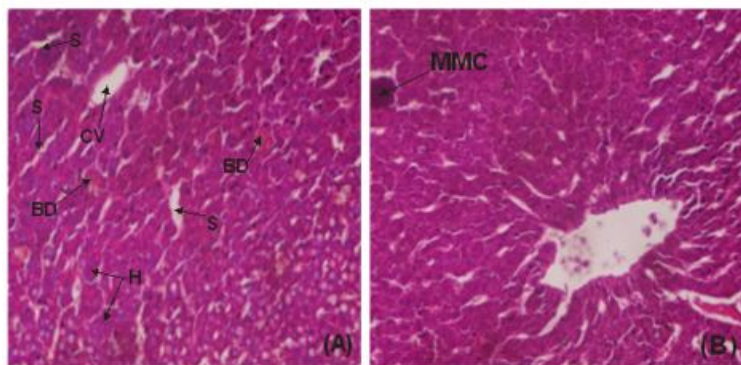
Findings: HAI = IGB > PER > ARAC - (**P<0.05**).

Remarks: Result is consistent with 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



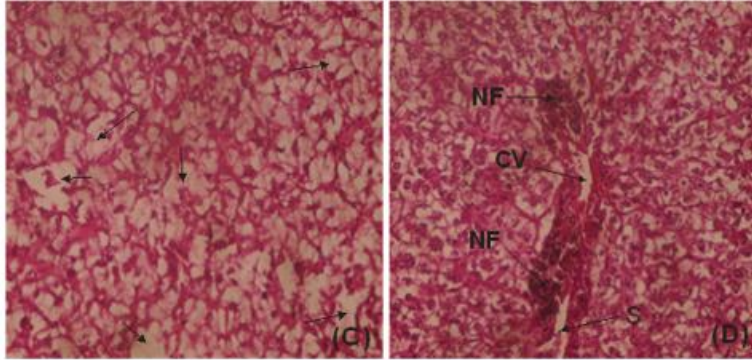


Figure 4: Photomicrographs (H&E; 400X magnification) of Liver of fishes harvested from PER, IGB and ARAC. A: The histology of a normal Liver of fishes showed Hepatocytes (H), Sinusoids (S), Central Vein (CV) and Bile Duct (BD). B: A normal architecture, but with a Melano-Macrophage Centre (MMC). C: Micro-vesicular steatosis (Arrows). D: Showing Diffuse Micro-vesicular steatosis with 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

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Kidney

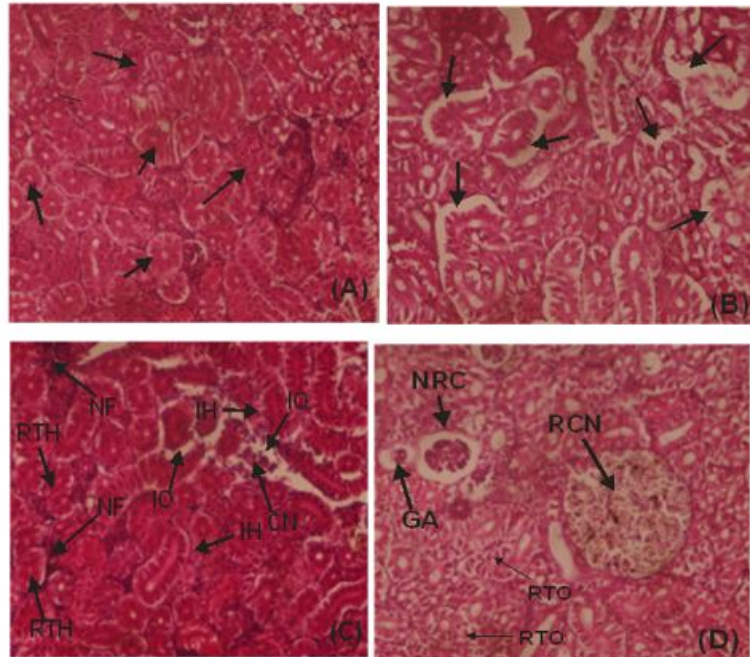


Figure 5: Photomicrographs (H&E: 400 x magnification) of Kidney of fishes harvested from PER, IGB and ARAC. A: is a normal histological structure of the kidney specimens sampled in this study, consisted of Renal Tubules (Arrows) with normal lumen and interstitium B: Diffuse Interstitial Oedem with dilated 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



Gills

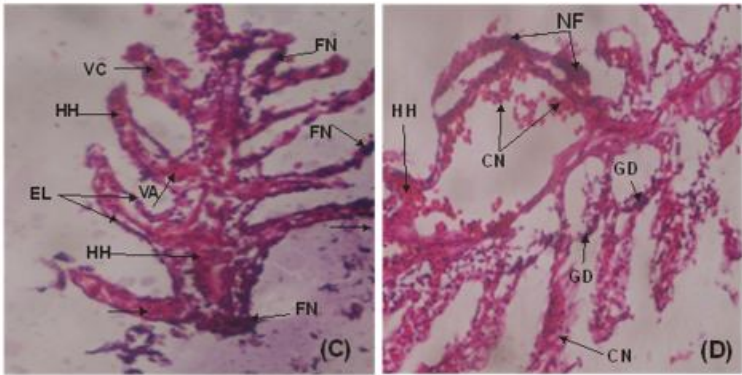
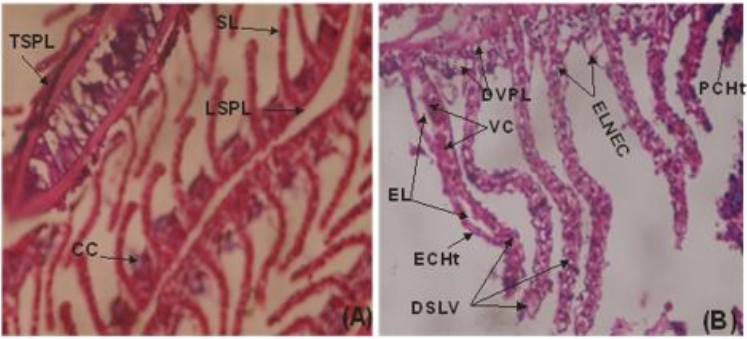
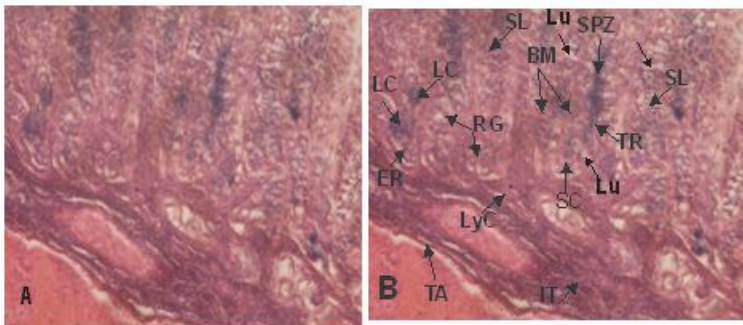


Figure 6: Photomicrographs (H&E; 400 x magnification) of Gills of fishes harvested from PER, IGB and ARAC. A: The histology of a normal Gill architecture showing Longitudinal Section of Primary Lamella (LSPL), Secondary Lamella (SL), Chloride Cells (CC) and Transverse Section of Primary Lamella (TSPL). 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 (DSLVL). 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



Testes



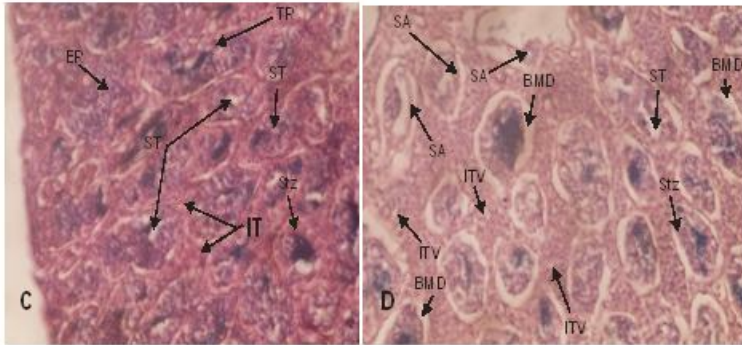


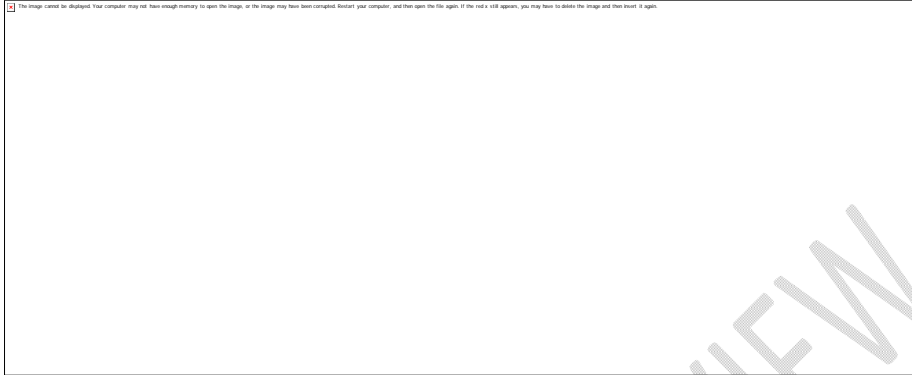
Figure 7: Photomicrographs (H&E; 400 x magnification) of Testes of fishes harvested from PER, IGB and ARAC. **A:** Seminiferous Lobules (SL) in different stages of spermatogenesis (RG-Regressive Gonade, ER – Early Recrudesce, TR - Terminal Recrudesce). **B:** The histology of a normal Testes architecture 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)	ARAC(n=10)
1. CIRCULATORY DISTURBANCE (CD)			
Interstitial Oedema	10	30	0
2. REGRESSIVE CHANGE (PC)			
Disorganization of Lobule	5	35	0
Detachment 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.



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



Using the I_{org} pollution classification (Zimmerli et al. 2007)

- 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

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

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.

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 to provide 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

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UNDER PEER REVIEW