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# Histological based Biomonitoring: A Baseline Ecotoxicological Evaluation of Ekerekana and Okochiri Creeks using Mudskipper

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**Abstract:** The effect of toxic chemicals on aquatic lives has been a problem in many communities and populations around the world today. Ekerekana and Okochiri communities in Okrika local government area of Rivers state Nigeria, whose river channel is a receptacle to Nigeria National Petroleum Corporation Port Harcourt Refinery's effluent is not an exemption. It is perceived that this effluent has a negative ecological and human health impact on the affected communities. This study involved the use of Histology as a biomarker to tackle these environmental problems. The ecotoxicological study was carried out in Ekerekana (EKE station) and Okochiri (OKO station), while an aqua-culture centre – African Regional Aquaculture Centre (ARAC) at Buguma, Rivers state was used as the reference or control site. This followed a gross anatomical study of harvested fishes by applying a condition Factor (CF) equation and a fish health assessment index (HAI) protocol. Histological assessment protocol in a qualitative and semi-qualitative scenario was also done. The Environmental Water Quality Index (EWQI) result for the experimental sites were poor (10.5) and marginal (57.5) for EKE and OKO respectively. Sediment quality show elevated Pd. A comparative fish study shows: no significant difference for CF in ARAC (4.62) and OKO (6.65); HAI was better in ARAC (16.0) than OKO (55.5). This study was ecologically relevant; it revealed that Ekerekena and Okochiri creeks are contaminated with a moderate level of pollution.
**Keywords:** Histology; Biomarker; Ecotoxicology; Fish; Bio-Monitoring

## INTRODUCTION

The Port Harcourt Refining Company (PHRC) located at Alesa Eleme, in Eleme Local Government Area of Port Harcourt, Rivers State, is a subsidiary of the Nigerian National Petroleum Corporation (NNPC). PHRC is a government owned oil and Gas Company primarily specialized in refining crude oil into petroluem products. The company operates two oil refineries including an old plant commissioned in 1965 that can process 60,000 barrels of oil per stream day and the new plant commissioned in 1989, which has a capacity of 150, 000 bpsd. Both oil refineries possess a combined capacity of 210,000 barrels per stream day making PHRC the "biggest oil refining company in Nigeria" [1]. The PHRC refining process requires large volumes of process water which is need to be recycled to meet up the demand. PHRC has constructed paved waste water drainage running from their facility to a receptacle which is a creek at Ekerekana community. The creek connects with other adjoining rivers of Okochiri Community. It is evident that both the waste water and process water are treated in a water treatment plant, but the level of treatment can only be ascertained through the analysis of the discharged effluent at different points of their exit into the adjoining environment [2].

Over the years Ekerikana creek in Okrika Local Government Nigeria has served as the recipient water body (environment) where the refinery effluents / run off are discharged. The level of impact of these discharges on the adjoining Ekerekana /Okochiri river channels have always been a cause of concern to the affected communities. The waste water bears a pungent smell, and as such the locals refer to the primary affected creek as the "smelling river". Fishing activities have thus been stopped around the immediate effluent receiving creeks in Ekerekana community, making the adjacent community (Okochiri) river channels as the nearest available alternative for subsistence fishers to earn livelihood. Nevertheless, the creek serves as home to many aquatic foods such as fish, crab, prawn, crayfish, etc [3]. It is therefore the main objective of this research to provide some valuable information on the status of the treated waste water and also the level of impact on the creek.


**Fig-1: Picture of PHRC effluent paved drainage through Ekerekana community *(A)* discharging into a receptacle *(B)* – a creel in Ekerekana community, which directly flows into Okochiri River.**

This study is meant to determine the ecological integrity and pollution status of an aquatic ecosystem. The terms "Pollution" and "contamination" have often been used erroneously as synonymous entities by environmental analysts. In environmental health, contamination is the presence of foreign substance beyond the prestine or allowable level for that environment while pollution is the presence of foreign substance beyond the prestine or allowable level for that environment with evidence of biological effect. Contamination and pollution both mean the substance of foreign material in the ambient environment. The mere presence of a foreign substance above the natural level for that environment alone can trigger contamination; however, it requires a much high concentration to pollute the same environment. Organisms also adapt or tolerate some level of contaminants after chronic exposure. This is why the concentration of contaminant to cause pollution changes from organism to organism and from one environment to the other. Contamination can sometimes affect the process, whereas pollution is usually the products of the process being interactive with the environment and hence causing problems. Pollution is after the end process, and contamination is during the process. Contamination happens to the substance in use, whereas pollution happens to nature, soil, water, air, light, etc.

After an aquatic contamination, monitoring and impact assessment are commonly based on measurement of chemical contaminants in the physical compartments (water and sediment) of the environment. In some cases these chemicals are analysed as a factor of their accumulation in biota - bioaccumulation assessment. However, those measurements fail to give information on the bioactivity of the compounds, making the scientifical bases of determining pollution to be incomplete. It is thus necessary to monitor contamination impact for determination of pollution by carrying out studies that take into cognizance the pharmacodynamics or biological activities of anthropogenic chemicals in the organism's natural environment. So ecotoxicological methods, which consider the measure of the effect of contaminants, are needed as a complement to chemical analyses of the physical environment. The incorporation of an effective suite of bioassays and biomarkers of exposure and effect can provide insights into the etiology of any higher-level adverse effects that may be observed.

This study used a multivariant approach to determine the pollution status and the ecological integrity of Ekerekana and Okochiri river channels. Many studies have also used a combination of endpoints to link exposures with effects e.g.at lower levels of biological organisation studies using subcellular biomarkers with metal bioaccumulation [4, 5]and subcellular biomarkers and condition indices with metal and organic bioaccumulation [6, 7]. Linking of higher tier organisation with pollutant exposure has been achieved using histopathology with metals and organic pollutants [8, 9], fish health with water quality changes [10], biological traits and water quality [11] and fish communities and ecological traits associated with water quality changes [12, 13].

Many studies to determine impacts of aquatic contaminants in waters have clearly demonstrated the potential and usefulness of applying biological-effects techniques in impact assessments, particularly concerning sublethal and long-term impacts at higher levels of biological organization in selected organisms [14-36].

The use of fish and the attributes of fishes have long been applied to assess the integrity or ecological state of and/or in identify impacts affecting aquatic ecosystems [37]. Initially fish community attributes were used to assess ecological integrity of stressed ecosystems [38]. The notion of using fish end points at different levels of biological organisation ranging from subcellular to ecosystem is now firmly entrenched in ecological risk assessment [39, 40].

The aim of this study is to determine pollution status of the Ekerekana/Okochiri river channels. This would provide scientific evidence whether the study river channels are contaminated and/or polluted. The ultimate purpose of ecotoxicological assessment and monitoring is to protect ecosystems from anthropogenic degradation. Using suborganismal assays, the modes of action of substances can be detected and, if basal cytotoxicity or key functions are affected, they can give valuable information on possible consequences for populations and communities.

## MATERIALS AND METHOD

### Study Area

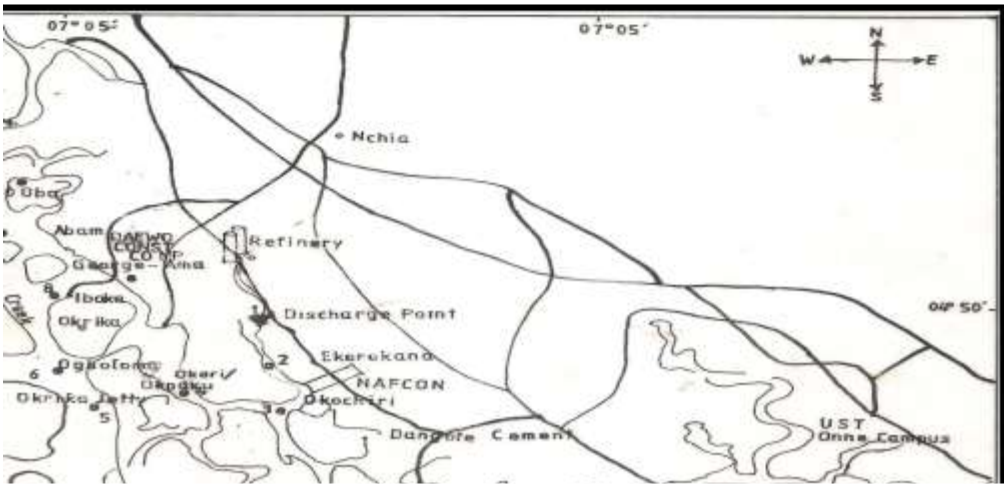


**Fig-2: Map showing Ekerekana (2), Okochiri (3), the PHRC discharge point (1) and the connectivity of rivers flowing down stream into the larger Okirika River which connects to the Atlantic Ocean. Source: (www.josrjournal.com)**

### Experimental Area

Two (2) main sites were identified for the purpose of the study. Ekerekana (Station 1 or EKE) and Okochiri (Station 2 or OKO) communities are both in Okrika Local Government Area (LGA) of Rivers State, Nigeria. The Okrika LGA lies between latitude 40351 and 40451N and longitude 7000N and 70151E. It is situated in the tropical rainforest belt dominated by secondary forest and bush fallow and the soil type is of coastal plain terrace and sedimentary in origin. EKE and OKO communities are intersected by creeks, rivulets and rivers forming an inter-connected river channels or system. EKE community creeks form the upstream part of the river system and a receptacle to PHRC waste water effluence via a pave and open gutter - point-source drainage system. Their river system are salt water with the Okochiri part of the river system connecting at its upstream to the tributaries of Bony River which discharge into the Atlantic Ocean about 56km from the North Bank of the Bight of Benin. Indigenous people of both communities are predominantly subsistent fishers, though increasing population and development is creating some semblance of urbanization with increase in commercial activities. Nevertheless, the people still engage in traditional fishing practices and even the working class among them still engage in part-time fishing to augment resources.

### Reference Area

African Regional Aqua-Culture Centre (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 of Science and Technology (RSUST) for the award of Master of Science (M. Sc) and Post graduate Diploma (PGD) in Aquaculture. Hands-on training programmes for farmers across the aquaculture value chain is a regular feature in the ARAC curriculum. ARAC has two centre, 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 Akuko-toru Local Government Area of Rivers State, which is responsible with the culture of marine fishes. The reference specie was harvested from the Buguma centre and used as control for this study.

**Study Specie**

**Fig-3: Mudskipper specie (*Periophthalmuspapillo*) used for the study**

Mudskipper fish (*Periophthalmus papillo*) was used as a biological indicator specie for this study. Mudskippers are member of the subfamily Oxudercinae (*Periophthalmin*) [41], within the family Gobidae (gobies). They are completely amphibious fish that can use their pectoral fin to walk on land [42]. They are found in tropical, subtropical and temperate zone. Mudskipper are very sensitive to ambient environment, they easily accumulate heavy metals in their tissue as compare to other fishes. The fish is a good monitor of aquatic pollution of PAH, as studies have shown that mudskipper species have high plasma enzyme level causing a change in its protein metabolism that enables bioavailability of PAH [43, 44]. Due to their natural abundance, and considerable resistance to their highly polluted habitat and benthic habitat they can be used as viable bio-indicator [45]. Its feeding habit is carnivorous because it feed on small prey such as crabs and other anthropods. It is an indigenous fish as is been consumed by member of the study communities (i.e. EKE and OKO). It is called *ishila* by the locals.

**Sampling Technique**

Sampling was done for water, sediment and fish. A total number of four (4) samples at two weeks interval was taken for water and sediment from EKE (i.e. the point of discharge of the PHRC effluence into the Ekerekana creek) and OKO (i.e. a creek in Okochiri community, about 2.5Km away from the effluent discharge point, where fishing activities take place). A total of twenty fishes (20) were sampled for the experiment study from the Okochiri community location (OKO) only (i.e excluding EKE were fishing activities has been stopped by community authorities). While, ten (10) fishes were sampled from ARAC, the Buguma centre, for the control study.

The water samples for heavy metal and PAH were collected using polypropylene bottles and glass bottles respectively. Samples were iced and sent to the laboratory for further analysis.

Sediment samples were collected using a stainless steel at EKE and an erkman grab sampler at OKO. Samples were put in an aluminum foil, iced and sent to the laboratory for heavy metals and PAH analysis.

Fish were caught using gills net. Fish were sacrifice immediately after inspected for gross anatomical pathological. Target organs were then collected and fixed in 10% formalin solution put in labelled vials and sent to the laboratory for further histological preparation.

**Laboratory Analysis**

**Heavy metals**

Atomic Absorption Spectrometre (AAS) was used to analyse water and sediment samples for heavy metals**.** Samples were first prepared for AAS by the Digestion Procedure A [46]. For water sample, the procedure involves adding of 5cm3 of concentrated HNO3 to a well-mixed 100cm3 of the water sample in a beaker. The solution was evaporated to near dryness on hot plate, making sure that the sample does not boil (using low to medium heat). The beaker and content was allowed to cool to room temperature. Another 5cm3 conc. HNO3 was added to the beaker and it was immediately covered with a watch glass. The beaker was returned to the hot plate and a set a gently reflux action of action of the solution by increasing the temperature of the hot plate (Medium to high heat). There was a continous heating with an addition of conc. HNO3 as necessary until light-coloured residue is obtained (which indicated that digestion is completed). For sediment samples, 5g air-dried and sieved sediment sample was put a 100ml of distilled water. 2ml of HNO3 and 6ml HCL in the ratio 1:3 is added to the sample and heated to digest the sample. Digested samples are introduced to the pre-calibrated AAS for analysis.

**PAH Analysis**

Water and sediment samples for PAH were analysed using a Gas Chromatography. Extraction of

PAH from water samples was done by measuring out 250ml of sample into a separation funnel and into a container rinsed with Dichloromethane. The organic extract was passed through a receiving container containing columns cotton, silica-gel and anhydrous sodium sulphate. The silica-gel aids the clean-up of the extract by disallowing the passage of debris from the extract while the anhydrous sodium sulphate acts as a dehydrating agent to rid the extract of every form of moisture/water. The collected organic extract was washed and injected into the Gas Chromatography. For sediment, extraction was done collecting 1gm of samples into 10ml of extraction solvent (Dcm), mixed thoroughly and allowed to settle. The mixture was carefully filtered into a clean paper fitted into butcher funnels. The extract was concentrated to 1ml and then transferred for clean-up/separation. Afterwards the recovered concentrated organic extract is analysed via the Gas Chromatographic method.

**Gross Anatomical Assessment (Fish Necropsy)**

Harvested fish from both reference and experimental sites were grossly studied with respect to their external and internal anatomy. Their external anatomy were studied immediately fish were harvest (before being sacrificed) to observe their weight and length relation - Condition Factor (CF), ectopatasite numbers and body lesions. After sacrificing (before resection of target organs for histological studies), internal organs are inspected for any gross organ alterations - colour change, hemorrhage, atrophy and other anormalities). The observed internal and external lesion are recorded and scored in terms of the severity of the lesion using a Health Assessment Index (HAI) protocol by Adams *et al*., [47].

**Histological Analysis**

Selected target organs (liver, kidney and gills) were resected from fishes harvest from both experiment and reference sites. These organs were collected in vial filled with preservative (10% neutrally-buffered formalin solution) and transported to the laboratory. Tissues from the organs are subjected to further treatment by dehydrating in agraded series of ethanol bathes (*30%, *50%, *70%, *80%, *90%, *96% and *2x100%) for 1 hour and embedded in paraffin. The embedded tissues were sectioned at 4-5µm thickness on a wax microtome. The tissue sections were mounted onto a glass microscope slides and stretched using an albumin solution [48]. The slides were then dried on a hot plate and kept in the oven overnight. Once dried, the slides were stained with routine standard histological stains, Haematoxylin and Eosin (H&E) [36].

**Evaluation and Assessment**

**Environmental Water Quality (EWQ) Index**

Analytical values of physical parameters (dissolved oxygen concentration DO, temperature, total disposed solids TDS and pH) and chemical parameters (heavy metals and PAH) were used to estimated EWQ Index in accordance with CCME [49] protocol for Water Quality Index. Estimated EWQ index is ranked by relating it to the following categories: EXCELLENT (95-100)-Water quality is protected with a virtual absence of threat or impairment, conditions very close to natural or pristine level; GOOD (88-94)- Water quality is protected with only a minor degree of threat or impairment. Conditions rarely depart from natural or desirable levels; FAIR (65-89) - Water quality is usually protected but occasionally threatened or impaired. Conditions sometimes depart from natural or desirable levels: MARGINAL (45-64) - Water quality is frequently threatened or impaired. Conditions often depart from natural or desirable levels; POOR: (0-44) - Water quality is almost always threatened or impaired [49].

**Gross Anatomical Assessment**

These involve physical assessment of the weight and length of the fish, external and internal assessment, looking out for leisure on the skin, eyes as well as on the target organs which include gills, liver and kidney. This phase is divided into two procedures, Condition factor and health assessment index.

**Condition Factor (CF)**

This is a measure of the length and weight of the fish using a meter rule and a sensitive weighing balance. The length weight relationship of a fish is an important fishery management tool. Its importance is pronounced in estimating the average weight at a given length group [50] and is assessing the relative well-being of a fish population [51]. It is advantageous to use two measurable and convertible sizes of fish for estimating the condition factors. Fulton [52], proposed mathematical formula was used in quantifing the condition of fish:

$$K = 10^N W/L^3$$

Where,

K = the condition factor or coefficient of condition often simply referred to as the k factor

W = the weight of the fish in grams (g)

L = the length of the fish in millimeters (mm)

N =Five, having weighed and measured thousands of fishes

The value of K is influenced by age of the fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development. In some fish species, the gonads may weigh up to 15% or more of the total body weight. With females, the K value will decrease rapidly when the eggs are shed. The K value can be used to assist in determining the stocking rate of fishes in particular water. If the K value reaches an unacceptably low level in water which is totally or partly dependent on stocking, the stocking rate can be reduced accordingly until the K value improves and reaches an acceptable level. On the basis of comparison of the K value with general appearance, fat content etc., the following standards were adopted: 

- Excellent condition, trophy class fish (1.60) 
- A good, well proportional fish (1.40) 
- A fair fish, acceptable to many anglers (1.20) 
- A poor fish, long and thin (1.00) 
- Extremely poor fish (0.80)

### Health Assessment Index (HAI)

The health assessment index was used to assess to the health of fish population in the field using Adams *et al*., [47] protocol. According to Adams *et al*., [47], it is important because it help to account for the differences in severity of damage or level of effect of the fish. Below shows some variable of the HAI which are assigned values of 10, 20, 30, depending on the extent of abnormality or observed damage. To calculate an HAI for each fish within a sample, numerical values for all variables are summed. The HAI for a sample population is then calculated by summing all the individual fish HAI values and dividing by the total number of fish examined for that sample. A standard deviation for each sample is calculated as

$$SD=\sqrt{\sum_{i=1}^{n}\frac{(Vi-X)^2}{n-1}}$$

Where,

n= number of fish per site
X= average index for each fish
Vi= index value for fish i

The coefficient of variation (CV) is calculated as

$$CV=\frac{SD}{X}\times 100$$

### Histological Assessments

Qualitative and semi-quantitative histological assessments were conducted.

### Qualitative Analysis

Using a Light microscopy (Olympus BH2) a qualitative assessment (histological description) were made on all mounted histological slides at X100, X400 and X1000 magnification. The percentage prevalence of tissue histopathology was noted. Histological findings in slides from experimental fishes were later compared with slides of reference (control) fishes. Micrographs of assessed slides were taken using Image Manager Software (Pixel IT).

### Semi-quantitative analysis

Scores where apportioned to observed histological alterations based on the severity or potential to cause loss of function of the organs. The sum of the calculated organ index values gives an overall fish index value (Ifish) which is indicative of combined histological responses of sampled organs per fish specimen [53]. Furthermore, a modified classification system by Van Dyk *et al*., [9, 54] based on a proposed scoring scheme by Zimmerli *et al*., [55] was used to evaluate the degree of histological changes. This classification system is based on the calculated mean organ index values:

- Class 1 (index value <10): Slight histological alterations.
- Class 2 (index value 10-25): Moderate histological alterations.
- Class 3 (index value 26-35): Pronounced alterations of organ tissue.
- Class 4 (index value >35): Severe alterations of organ tissue.

### Statistical Analysis

t-square statistical distribution for independent variables was used to analyse CF data, while Mann-Whitney U statistical distribution was used to analyse HAI data. Both statistical analysis where done at a significant level of 0.05.

## RESULTS

### Environmental Water Quality Index (Ewqi)

*Physico-Chemical water quality parameters*

**Table-1: Physico-Chemical parameters used in evaluation of EWQI for EKE and OKO**

| Parameters | EKE Mean | OKO Mean | CCME Guideline | Remark |
|---|---|---|---|---|
| Temperature (C | 32.3 | 28.2 | Ambient (31.0) * | Non-applicable |
| Conductivity (µs/cm). | 669.7 | 399.9 | 150-500 ** | Non-applicable |
| Salinity ppm | 889.8 | 2560.5 | - | Non-applicable |
| TDS | 69.75 | 392,2 | - | Non-applicable |
| DO (mg/L) | 2.89 | 2.22 | 5.5-9.5 | Applicable |
| PH | 6.5 | 6.0 | 6.0-9.5 | Applicable |
| Pb (mg/L) | 0.001 | 0.173 | 1-7 | Applicable |
| Cd (mg/L) | 0.001 | 0.035 | 0.017-0.10 | Applicable |
| Cr (mg/L) | 0.001 | 0.14 | 1 | Applicable |
| Hg (mg/L) | 0.001 | <0.001 | 0.026 | Applicable |
| Cu (mg/L) | 0.001 | 0.04 | 2-4 | Applicable |
| PAH (mg/L) | 0.001 | 0.003 | - | Non-application |

*Key: * = SON (2007)* [56] *Guideline and ** = USEPA (2012) Guideline*

Using CCME (2001) [49] and SON guideline for mathematical evaluation of EWQI, **EKE** EWQI was estimated to be **10.5** and **OKO** EWQI was estimated to be **57.5.**

**Sediment Quality Analysis**

The result of heavy metals (mg/g) and PAH (mg/g) present in the sediment from the two stations is represented in the graph below.

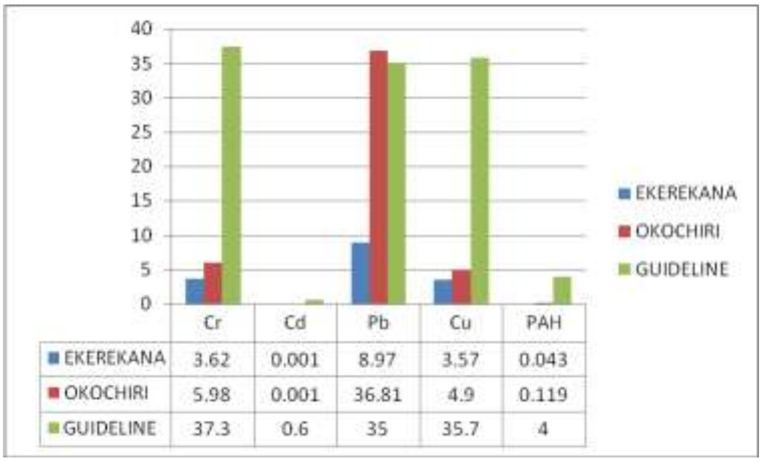


**Fig-4: Graoh showing heavy metal and PAH concentration in sediment for Ekerekana and Okochiri stations**

**Gross Anatomical Assessment – Fish Necropsy**
**Condition factor**

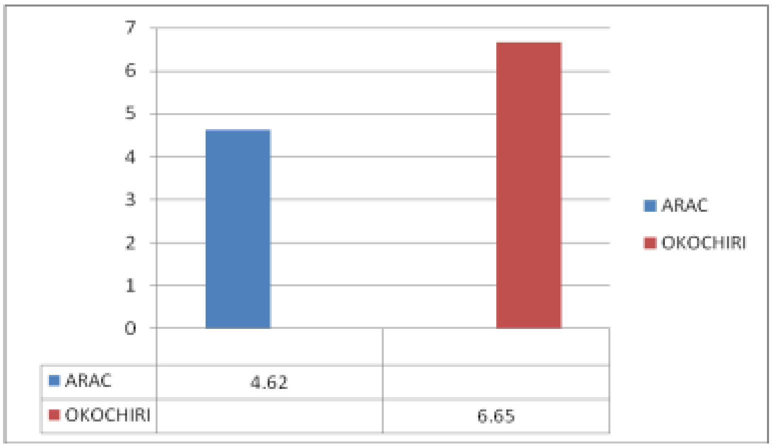


**Fig-5: Graph comparing mean condition factor of fishes harvested for ARAC and OKOCHIRI**

**Health assessment index (HAI)**

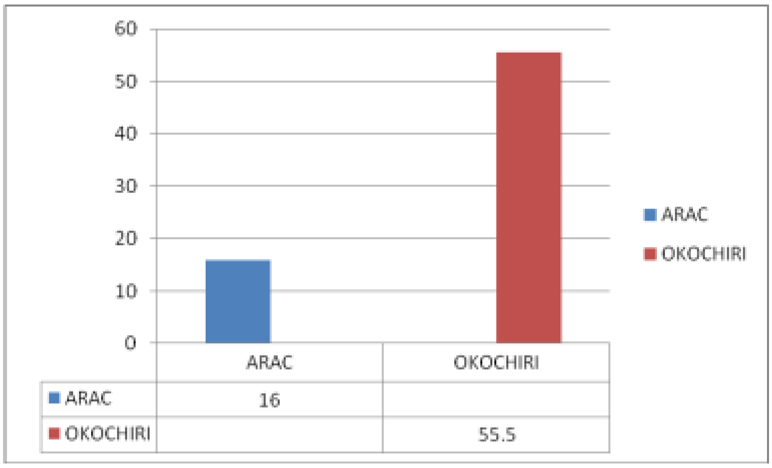


**Fig-6: Graph comparison of mean HAI values of fishes from OKO and ARAC**

## Histological Assessment

### A. Qualitative Assessment

### Liver

Bellow (Fig-7) is the observation of the micrographs of Liver. **(A)**: the histological organization is a characteristic tubulosinusoidal and lacks the lobular configuration observed in mammals; the hepatic parenchyma is very homogenous and the hepatocytes polygonal in shape, often weakly basophilic when compared to mammals with a single, clear, dark and spherically located nucleus; hepatocyte were arranged acinar form, while the hepatic cord forming hepatocytes are in columns separating irregularly dispersed sinusoids; there is the presence of Hepatopancreas tissue with a characteristic brownish stain (which physiologically consists of pancreatic exocrine and endocrine glands) with each accompanying a biliary duct. **(B):** there is distortion of a major portion of the liver parenchyma with multifocal to diffuse lesions involving vacuolated cells; there is multifocal necrosis with putative manifestation on adjacent tissues resulting in breakdown of cellular membrane. **(C)**: Shows a variable distorted liver architecture with diffuse hypertrophy of hepatocytes; degeneration of hepatocytes with a breakdown of sinusoidal walls, presence of degenerated tissues in central vein with evidence of Frank's Necrosis. (**D**): Diffuse intercellular haemorrage with evidence of coagulative Necrosis; Diffuse Hepatocyte vacuolation.

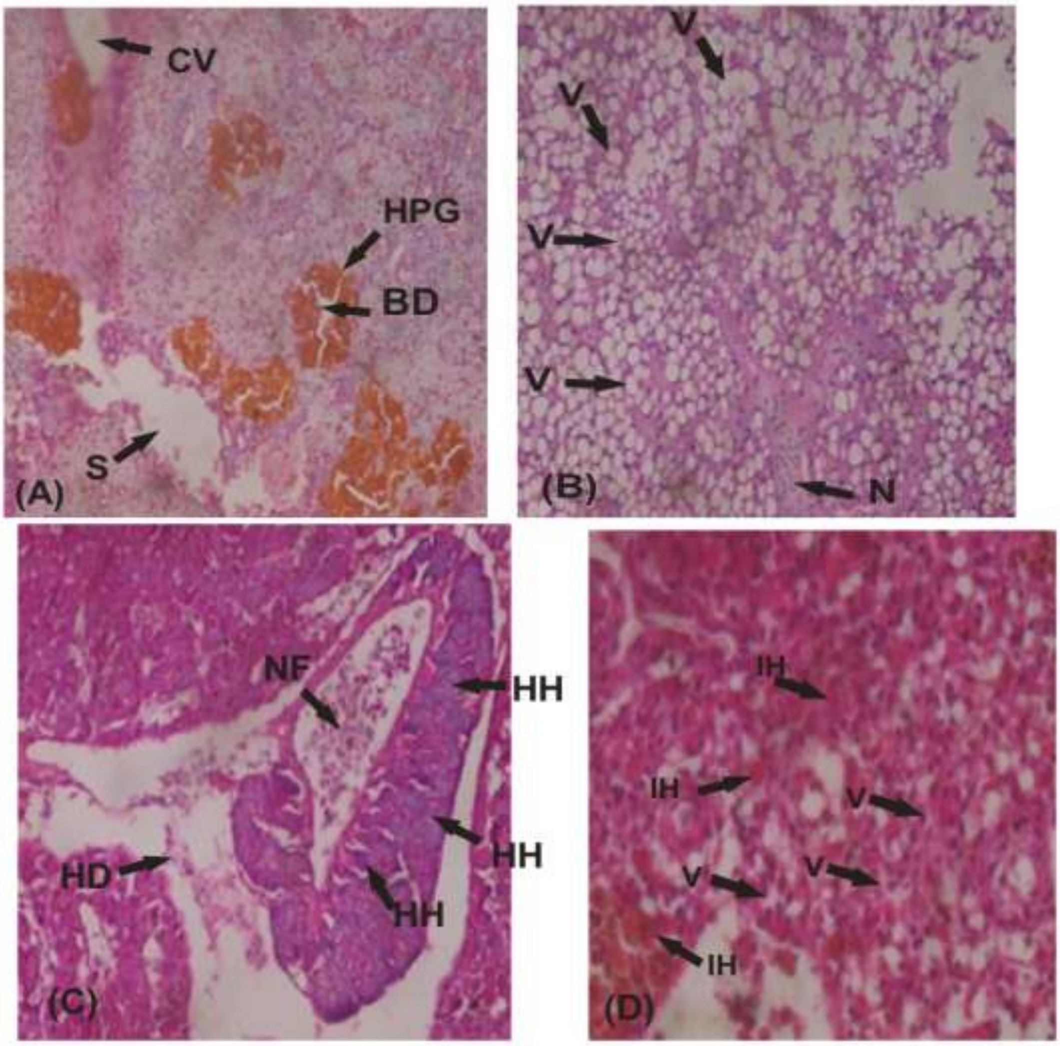


**Fig-7: Showing normal and histopathologic tissue micrograph of the liver. A.) Normal tissue at x 400 magnification, showing Sinusoid (S), Hepatopancreatic gland (HPG), Bill ducts (BD) and Central Vein (CV). B) Histopathologic tissues at x 400 magnification showing, Multifocal to diffuse Vacuolation (V) and tissue Necrosis (N). C) Histopathologic tissue at 400 X magnification showing Hepatocyte Hypertrophy (HH), and Hepatocyte Degeneration (HD) and Necrotic Foci (NF)(Frank's Necrosis). D) Histopathologic tissue showing at x 1000 magnification showing diffuse intercellular haemorrhage (IH) and diffuse cellular vacuolation (V)**

**Table-2: The percentage prevalence of liver histopathology of fishes harvested from OKO and ARAC**

| Alteration | Prevalence (%) | |
|---|---|---|
| | OKO (n=20) | ARAC (n=10) |
| **Circulatory Disturbance (CD)** | | |
| Intercellular haemorrhage | 20 | 10 |
| **Regressive Change (RC)** | | |
| Intracellular deposits | 30 | 10 |
| Frank necrosis | 50 | 10 |
| Fatty change | 60 | 20 |
| Vacuolation other than steatosis | 80 | 30 |
| Melano-macrophage centres | 20 | 0 |
| **Foci of Cellular Alteration (FCA)** | | |
| Vacuolated foci | 10 | 0 |
| Necrotic foci | 40 | 0 |
| **AVERAGE % PREVALENCE** | 62 | 16 |

**Kidney**

Bellow (Fig-8) is the observation of the micrographs of Kidney. **(A)**: the normal histological architecture of the kidney showing the renal corpuscles (i.e. bowman's capsule and glomerulus) and some tubules. **(B):** there is diffuse inflammation of the renal corpuscles (involving almost all renal corpuscle in the micrograph) with an evidence of Melanomacrophage centre (MMC) resulting in severe distortion of the corpuscular architecture; there is concomitant intracellular haemorrhage **(C)**: Presence of MMC with mild distortion of Kidney architecture **(D)**: Presence of MMC with severe distortion of Kidney architecture; Eodematic tubular cells resulting in tubular distortion.

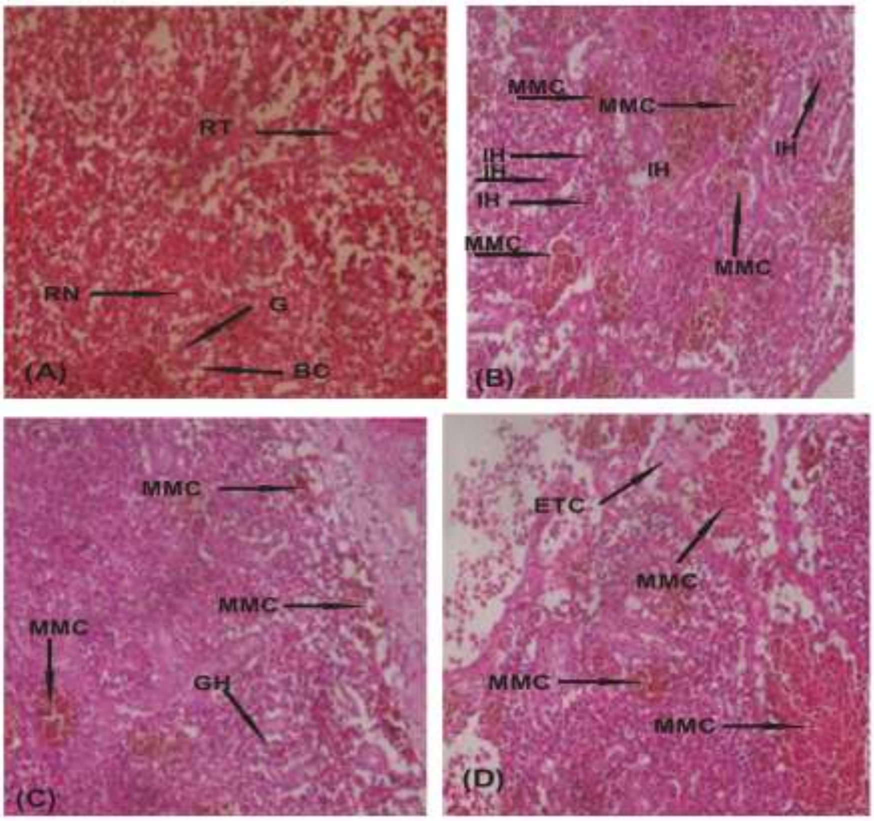


**Fig-8: Showing normal and histopathologic tissue micrograph of the kidney A) Normal kidney tissue at 400 X magnification showing the Renal Corpuscles (RC) (with Bowman's space (BS) and Glomerulus (G)) and renal tubules (RT). B) Histopathologic tissue at 400 X magnification showing MMC with renal corpuscle distortion and Intracellular haemorrhage (IH). C) Histopathologic tissue at 400 X magnification showing inflammation (MMC) and glomerular haemorrhage (GH) with mild distortion of kidney architecture. D.) histopathologic tissue at 400 X magnification showing inflammation (MMC) and eodematic tubular cell (ETC)**

**Table-3: showing the percentage prevalence of kidney histopathology of fishes harvested from OKO and ARAC**

| Alteration | Prevalence (%) | |
|---|---|---|
| | OKO (n=20) | ARAC (n=10) |
| **Circulatory Disturbance (CD)** | | |
| Intercellular haemorrhage | 30 | 10 |
| Interstitial oedema | 10 | 0 |
| **Progressive changes (PC)** | | |
| Hyperplasia | 30 | 10 |
| **Regressive Change (RC)** | | |
| Architectural &Structural alterations | 40 | 0 |
| Necrosis | 80 | 20 |
| Melano-macrophage centres | 10 | 0 |
| **AVERAGE % PREVALENCE** | 33.3 | 13.0 |

**Gills:** Bellow (Fig. 9) is the observation of the micrographs of Gills. **(A)**: shows normal histological with a core of fibromuscle and cartilage surrounded by columnar epithelium forming papillary projections (primary and secondary lamellae) on their surface with rich vascularization. **(B):** shows hyperplasia and lifting of epithelial cells. **(C)**: shows engorged capillaries with talengiectasia and epithelial fusion. (**D**): Telengiectasia and partial fusion of secondary lamella

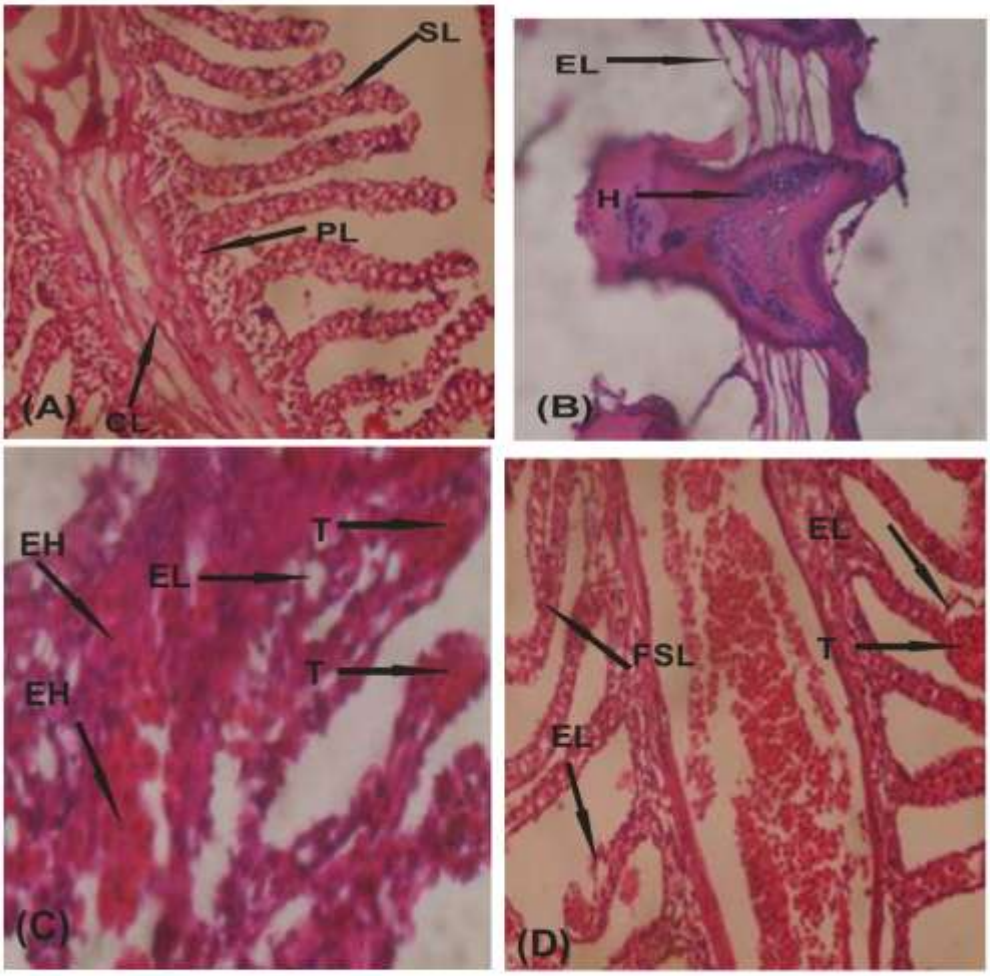


**Fig-9: Showing normal and histopathologic tissue micrograph of the Gills. A) Normal Gills at 400 X magnification showing Capillary lumen (CL), Primary lamella (PL) and secondary lamella (SL). B) histopathologic tissue at 100 X magnification showing Hyperplasia (H) and Epithelial lifting (EL). C) histopathologic tissue at 400 X magnification showing Extracellular Haemorrhage (EH) with Talengiectasia (T) and Epithelial lifting (EL). D) histopathologic tissue at 400 X magnification showing Talengiectasia, epithelial lifting (EL) and partial fusion of secondary lamella (FSL).**

**Table-4: Showing the percentage prevalence of Gills histopathology of fishes harvested from OKO and ARAC**

| Alteration | Prevalence (%) | |
|---|---|---|
| | OKO (n=20) | ARAC (n=10) |
| **Circulatory Disturbance (CD)** | | |
| Hyperaemia | 55 | 20 |
| Epithelial lifting | 20 | 0 |
| **Progressive Change (PC)** | | |
| Hyperplasia | 30 | 0 |
| **AVERAGE % PREVALENCE** | 35 | 20 |

**B. Semi-Quantitative Histological Assessment**

The qualitative histological assessment data was further quantified according to the Protocol by Bernet *et al*., [53], modified by Van Dyk *et al*., [9]. OKO had the highest mean Gills, liver and kidney index values while the reference site, ARAC, had the lowest mean Gills, liver and kidney index values. The quantified results showed that OKO and ARAC had fish index values of 20.2 and 9.3 respectively. Following Man Whitney Test statistical analysis, significant differences ($p<0.05$) were noted between OKO and ARAC.

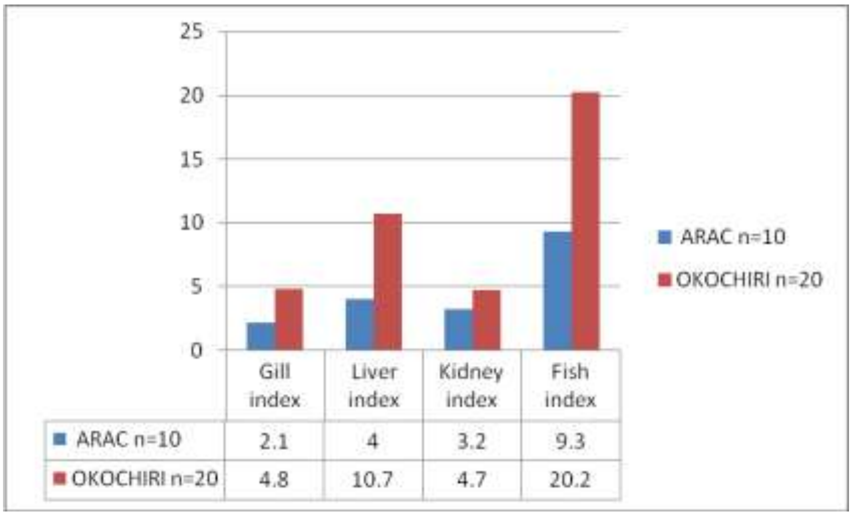


**Fig-10: Showing comparison of semi-quantitative histological assessment**

## DISCUSSION

### Water Quality

The physico-chemical water parameters were measured and used in evaluation of EWQI. Some parameters like temperature, conductivity and sanity were not applied in the evaluation of EWQI and there effects are analysed separately:

- *Temperature*: The temperature of surface (environmental) water recorded at EKE was 32.3 $^{O}$C which was above the normal allowable temperature for aquatic life [56], equivalent to ambient temperature (31.0 $^{O}$C). This would affect aquatic life and result in reduce aquatic organism, especially fishes, activities in creeks around the study site. This is consistent with the locals complain that immediately after the construction of the PHRC effluent discharge drains into the Ekerekana creek they experienced dwindling fishing yield. The experience pungent smell from the creek might not be unconnected with the temperature rise beyond ambient temperature. This can be explained by the principles of "Ideal Gas Law" which relates temperature to kinetic energy of gas molecules. In OKO, the recorded temperature was 28.17 $^{O}$C which is below the ambient temperature (30.5$^{O}$C). This might just be one of the reasons why some fish communities are still found in Okochiri axis of the river channels.
- *Conductivity:* Conductivity was 669.7 and 399.9 µs/cm for EKE and OKO respectively. Studies of inland fresh waters indicate that streams supporting good mixed fisheries have a range between 150 and 500 µhos/cm. Conductivity outside this range could indicate that the water is not suitable for certain species of fish or macroinvertebrates. Industrial waters can range as high as 10,000 µmhos/cm [57]. Conductivity in water is affected by the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions (ions that carry a negative charge) or sodium, magnesium, calcium, iron, and aluminum

cations (ions that carry a positive charge) [57]. Hence EKE is not properly conditioned for fish habitation.

- *Salinity:* Salinity is the measure of the concentration of total dissolved salts in soil or water, mainly sodium chloride. Ekerekana and Okochiri river channels are natural marine aquatic ecosystem with relatively high Salinity because of its nearness to Bony River estuary which opens up to the Atlantic ocean. Salinity for this study was 889.8 and 2560.5 ppm for EKE and OKO respectively. Primary salinity occurs naturally and is the result of rainfall interacting with geographical features over thousands of years. Secondary salinity is the result of human land use and either produces more salt or causes primary salinity to rise to the surface of the land [58]. Guidelines for Salinity (in ppm) are: Fresh water = < 1,000, Slight Salinity = 1,000 - 3,000, Moderate Salinity = 3,000 - 10,000 and High Salinity = 10,000 - 35,000 [59]. From the foregoing Ekerekana and Okochiri river channels salinity are at the levels of freshwater and slight salinity respectively. From the foregoing, EKE and OKO salinity score would be freshwater and moderate salinity levels respectively. Certainly, this would distort its pristine estuarine salinity level aquatic system and thus affect the natural aquatic biodiversity of these river channels.

**EWQI**: EWQI values were estimated as 10.5 and 57.5 EKE and OKO reflectively. Based on CCME, 2001 score ranking, water quality was rated as poor at EKE and marginal at OKO. EKE EWQI result was in accordance with the perception of the community leaders who feel that the Ekerekana creeks are contaminated and thus might not be good for any fishing activities, for which reason fishing activities were stopped. OKO EWQI score was marginal. Relatively, EWQI of OKO is better than EKE, but fishe would still be stressed under this condition and heavy metals might bio-accumulate in fish tissues. This can posed a public health risk to those who eat such fishes due to bio-concentration of such hazardous chemicals in their body.

**Sediment Quality**

Sediment's trace elements of Cd, Cr, Cu and PAH measured for both stations were found to be less than CCME (2001) guideline levels for aquatic life protection, except for Pb in Okochiri station which was found to be higher (Pb = 36.8mg/g) than the CCME guideline value (Pb = 35.7mg/g). The concentration of chemicals adsorbed to sediments—generally affects the quality of habitat for sediment-dwelling organisms, which live in contact with the sediments and may ingest sediment particles. Chemicals adsorbed to sediments can also re-enter the water column depending on environmental conditions such as dissolved oxygen concentrations, pH, and temperature. The chemical characteristics of sediment depend on the natural geology of the basin and erosional processes that transport minerals into the waterbody, as well as human activities that cause pollution to enter the river system.

**Gross Anatomical Assessment**

**Condition factor (CF)**

CF value for fishes from OKO was 7.8 x $10^{-4}$ while those from ARAC were 8.4 x $10^{-4}$. Condition factor compares the wellbeing of a fish and is based on the hypothesis that heavier fish of a given length are in better condition [60]. Condition factors decreases with increase in length [61, 62] and also influences the reproductive cycle in fish [63]. Hence, fish from ARAC are in better condition than those from the experimental site (OKO).

**Health Assessment Index (HAI)**

From this study, it was discovered that the mean HAI score for fishes from OKO (55.5) is greater than that from ARAC (16.5). HAI is a simple inexpensive necrosy-based means of rapidly assessing the general health status of fish in field situations. HAI is not a biomarker, but rather a protocol for documenting lesions or changes that have advanced to the point of being grossly visible. It is not intended to be diagnostic but serves to highlight possible problems that should be investigated with more diagnostic specific tests [64]. HAI variables are numeral values based on the degree of severity or damage incurred by an organ tissue from environmental stressors (internally or externally). A low index value indicates a good health status while a high value translates to poor health condition. Hence, from this study fishes from OKO have a poorer gross anatomical health outcomes than ARAC.

**Histological Assessment**

The identifaction of bio-marker responses to environmental toxicants determines the pollution status of that environment. For this reason, environmental bio-indicators are studied at higher levels of biological organisation - histopathological alterations.

**Qualitative Analysis**

The results found in this study were indicative of moderate to severe histological alterations:

- *Liver histopathology:* Alteration found in the liver organ include, intracellular deposit, frank necrosis, vacuolation other than steatosis, melanomacrophage centre, granulomatosis, infiltration, vacuolated foci and necrotic foci. There was an average % prevalence of 62 and 16 in the liver histopathology of fishes from OKO and ARAC respectively.

- *Kidney histopathology:* Alteration found in the kidney organ include, intracellular haemorrhage, interstitial edema, architectural and cellular alteration, necrosis, hyaline droplet, eosinophilic cytoplasm, melanomacrophage centre. Most of the changes observed in the kidney are regressive changes. There was an average % prevalence of 33.3 and 13 in the kidney histopathology of fishes from OKO and ARAC respectively.
- *Gills histopathology:* alteration found in the gill include, telegiectasia, epithelial lifting and hyperplasia. There was an average % prevalence of 35 and 20 in the Gills histopathology of fishes from OKO and ARAC respectively.

General, the qualitative histological findings of this ecotoxicological study were consistent with toxicological studies of effect, simulated in a laboratory scenario with similar trace elements to the target chemicals as shown below:

- *Lead (Pb):* Olojo *et al*., [65], showed that Clarias gariepinus exposed to sublethal Pb had hepatic cirrhosis; detached bile connective tissue; parenchyma degeneration; increase of fibro-connective tissue; blood sinusoid congestion and necrosis.
- *Cadmium (Cd):* A similar studies with similar histopathological features showed that Cd was the most toxic among other trace elements [66]. Exposure of O. niloticus to cadmium for 7 days showed the following histological alterations: In the liver, there was severe fatty vacuolations, generalised necrosis of hepatocytes, fatty change, congestion of liver sinusoids and central veins; Kidneys showed severe glumerular shrinkage and necrosis, lymphocytic infiltration in the distal renal convoluted tubules [67].
- *Chromium (Cr):* In a study Oncorynchus tshawytscha (Chinook salmon) exposed to Cr6+ showed lipid droplets in the liver; increase in gill epithelium; apoptosis of chloride cells; hypereosinophillic chloride cell cytoplasm; pyknosis; karyorrhexis; necrosis of kidney tubules and gross alteration to kidney and spleen [68]. In another study of the lethal effects of chromium on the histological alterations of Cyprinus carpio showed: gills - clubbing of the secondary lamella in the ends, fusion of adjacent secondary lamella, epithelial lifting, necrosis and curling of secondary lamella; liver - Hepatic cirrhosis, fatty changes, degeneration of parenchyma cells results in atrophy, tissue ischemic and extensive necrosis; kidney - Hypertrophy of epithelial cells, contraction of glomerulus, increase of space inside the grouping of tubules, distortion of architecture, glomerular edema, Bowman's capsule atrophy and dispersed interrenal cells with pyknosis of some nuclei [69]
- *Copper (Cu):* Study has shown that exposure Nile tilapia (Oreochromis niloticus) to sublethal level of Cu cause histopathological alteration in: gills - were edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis; the liver [70]. Rainbow trout (Oncorynchus mykiss) exposed to Cu sulphate for 28 days showed histological alterations in the liver (non-homogenous regions; congestion of the central veins, dark-stained hepatocytes; increase number of kupffer cells; vascular degeneration and sinusoidal degeneration) [71].

**Semi-Quantitative analysis**

The qualitative histological assessment data was further quantified according to the protocol by Bernet *et al*., [53], modified by Van Dyk *et al*., [9]. OKO had the highest mean Gills, liver and kidney index values while the reference site, ARAC, had the lowest mean Gills, liver and kidney index values. The quantified results showed that OKO and ARAC had fish index values of 20.2 and 9.3 respectively. Following Man Whitney Test statistical analysis, significant differences ($p<0.05$) were noted between OKO and ARAC. Based on the Zimmerli [55] Histological scoring scheme, OKO, which is the experimental site has fish that are moderately pathologic while the ARAC, the control site, has fish that are mildly pathologic. Although, majority of the organs of the fishes from the experimental site were moderately dysfunctional; some of the organs (especially in the kidney and gills) were in terminal stages of necrosis with Franks necrosis very conspicuous. This is an indication that the aquatic contaminants of OKO creek are impacting on the ecological ecosystem. The indicator fish species is known to inhabit polluted environments because of its resilience to toxicants. Much cannot be said about other environmentally sensitive and fragile species which form part of that ecosystem. There is no scientific doubt that the ecological ecosystem of the study creek is under threat. There should be a concerted effort to reverse or neutralize the stressor or otherwise many aquatic species would be driven to extinction. Most importantly human resources would be lost forever, and the consequence can be ramifying in both economic and health terms,

**CONCLUSION**

This study was ecologically relevant. It was able to demonstrate that Ekerekena and Okochiri creeks are contamination with a moderate level of pollution. The study has once more given credence to the use of histology as a biomarker to assess sublethal level of environmental stressors. Our study also highlighted the fact that no single investigative approach is able to measure the adverse effects of environmental contaminants. Histological based ecotoxicological studies using both chemical and biological methods are essential.

## RECOMMENDATIONS

- Because Ekerekana and Okochiri river channels are recipient of a point source industrial waste disposal, there should be regular bio-monitoring to forestall potential ecological impact.
- An epidemiological survey with Integrated Health Risk Assessment (IHRA) should be conducted in order to determine the general health impact on the affected communities.
- Furthermore, a forensic analysis should be conducted on established contaminants in order to identify polluting environmental system for effect management of such waste.


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## CONFLICT OF INTEREST

We write to state that there is no conflict of interest

## AUTHORS' CONTRIBUTIONS

We write to state that below is the author's contribution for the Manuscript titled: HISTOLOGICAL BASED BIOMONITORING: A BASELINE ECOTOXICOLOGICAL EVALUATION OF EKEREKANA AND OKOCHIRI CREEKS USING MUDSKIPPER. 'Author A' (Allison, Theodore Athanasius) designed the study the design, protocol, the write-up and intellectual content and 'Authors B' (Paul, Chikwuogwo Wokpeogu) reviewed the design, protocol, the write-up and managed the literature searches, managed the analyses of the study, wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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