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Histological Based Biomonitoring: A Baseline Ecotoxicological Evaluation of Ekerekana and Okochiri Creeks Using *Sarotherodon Melanotheron*

Allison, Theodore Athanasius, Paul, Chikwuogwo wokpeogu*
Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Science, University of Port Harcourt Nigeria

***Corresponding author**
Paul, Chikwuogwo wokpeogu

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Abstract: The aim of this study was to ascertain the pollution status of Ekerekana (EKE) and Okochiri rivers channels in Okirika Local Government Area of Rivers State, Nigeria. These creeks and river channels are receptacle to waste water effluent of Port Harcourt Refining Company situated at Eleme in Rivers State. Chemical and bio-monitoring was done. This involved the assessment of Condition Factor, Health Assessment Index and Histological assessment of target organs in qualitative and semi-quantitative (SQHA) scenario. Fish harvested from the experimental site (OKO) were compared with Fish harvested from a control site – African Regional Aqua-Culture Centre (ARAC). Results showed that the Environmental Water Quality Index for EKE (9.0) and OKO (35.9) were both poor with abnormal levels of temperature, conductivity, pH, Dissolved Oxygen (DO) at EKE and conductivity, salinity and Cd at OKO. Gross Anatomical Assessment showed that CF for OKO (1.9) was greater than ARAC (1,8), HAI for OKO (83.6) was greater than ARAC (18). QHA showed higher % prevalence in histological alteration in Gills, liver and Kidney of fish from OKO than ARAC. Histological alterations were consistent with toxicological study of exposure to similar substances. SQHA showed that OKO (30.3) had a higher fish index value than ARAC (7.7). Indicating that fish from OKO had pronounced alterations of organ tissue (class 4 = index value 26-35), while ARAC had slight histological alterations (class 1 = index value <10). It was concluded that EKE and OKO creeks and river channels were polluted and aquatic lives were seriously under threat.
Keywords: Condition Factor, Health Assessment Index, Histological Assessment, Qualitative and Semi- Quantitative.

INTRODUCTION

The creeks and river channels of Ekerekana (EKE) and Okochiri (OKO) communities of Okirika Local Government Area of Rivers State in Nigeria are receptacle to waste water effluence of Port Harcourt Refining Company (PHRC). PHRC is located at Alesa Eleme, in Eleme Local Government Area of Rivers State, Nigeria. PHRC is a government owned oil and Gas Company primarily specialized in refining crude oil into petroleum products and a subsidiary of the Nigerian National Petroleum Corporation (NNPC). The company operates two oil refineries plants with a combined capacity of 210,000 barrels per stream day making PHRC the biggest oil refining company in Nigeria.

There is a palpable fear by the indigenes of EKE and OKO communities that the PHRC effluent might be polluting their creeks. This is heightened by the fact that the company's effluence bears a pungent smell, disrupting social and economic activities along the path of their concrete drainage and at the immediate receiving waterbody in EKE community. This has warranted the local populace calling the effluent receiving creek in EKE community "The Smelling River". For which reason all fishing activities have been stopped in EKE by the community authority. Thus, fishing activities are only carried out in the adjoining community, OKO, where commercial and subsistent fishers of EKE and OKO strive to make a living from the fishing business.

Water is essential to all forms of life and makes up 50-97% of the weight of all plants and animals and about 70% of human body [1]. Water is also a vital resource for agriculture, manufacturing, transportation and many other human activities. Despite its importance, water is the most poorly managed resource in the world [2].

The availability and quality of water always have played an important role in determining the quality of life. Water quality is closely linked to water use and to the state of economic development [3]. Most of the water bodies in the areas of the developing world are the end points of effluents discharged from industries.

This research is an ecotoxicological study with the aim of using histology to determine the pollution status of Okrika river basin with sampling points at Ekerekana (EKE) and Okochiri (OKO). Traditionally, histology has been used to identify morphologic changes in the context of nonclinical safety assessment, clinical diagnosis, and evaluation response to therapy. There is a strong correlation between specific histology findings, clinical outcomes, and some clinical chemistry parameters. Because of this history, histology is currently used in biomarker qualification as a reference standard to evaluate the sensitivity and specificity of potential biomarkers and reversibility of morphologic changes. Because of the variation in the practice of histology, this guidance is offered to facilitate quality, consistency, and scientific rigor in biomarker qualification studies where histology is used as a reference standard [4].

MATERIALS AND METHODS

Study area

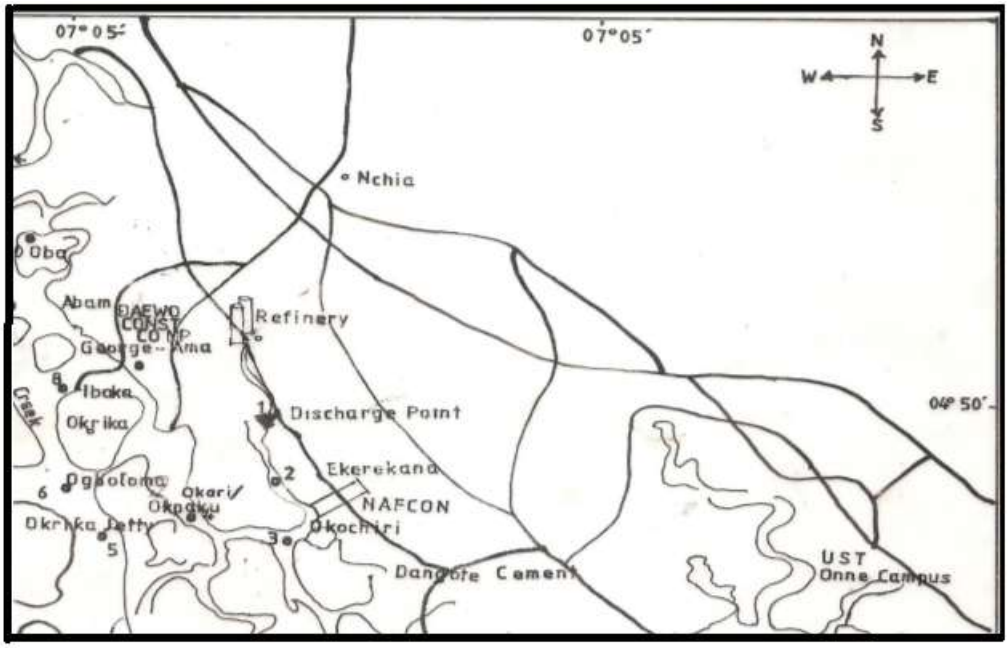


Fig-1: Map showing Ekerekana (2), Okochiri (3), the PHRC discharge point (1) and the connectivity of rivers flowing down stream into the larger Okirika River which connects to the Atlantic Ocean. Source: (www.josrjournal.com)

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. Ekerekana and Okochiri creeks, rivulets and rivers are inter-connected and consist of brackish water under the influence of tidal cycles. The vegetation in the river basin is basically mangrove, with pockets of barrier islands typical of forest species, especially at the southern fringe. Seven species of mangrove distributed in five families, known to inhabit the West African coastal waters are all represented within the study area. These include: *Rizophora racemosa*, *Rizophora harrisonii*, *Rizophora mangle*, *Avicennia Africana* (white mangrove), *Laguncularia racemosa* (Black mangrove). 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 engages 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 are 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

Sarotherodon melanotheron (*S. Melanotheron*), the blackchin tilapia, is a pale (variable light blue, orange, golden yellow) cichlid whose common name refers to the dark pigmentation usually but not always concentrated on the underside of the head (the chin) in adult animals. *S. melanotheron* is a demersal (bottom-associated) species inhabiting fresh to brackish water where it occurs. It switches from a more carnivorous habit as juveniles to an adult diet that focuses mainly on detritus, algae, periphyton and the organisms and material inhabiting or fouling submerged hard surfaces [14, 15]. The species is common in quiet muddy brackish water habitats where aquatic vegetation is abundant [13].

Selection of analytes

A suite of chemicals were selected by reviewing historical fish tissue chemistry data within the okirika river and by reviewing water quality data collected by the Standard Organisation of Nigeria (SON) survey on a wide range of common water contaminants. A total of 6 chemicals of potential concern (COPC) were identified for this study, which are; Cadmium (Cd), Cromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), and Polyaromatic Hydrocarbon (PAH).

Sampling

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 OKC (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 [16]. 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 continues 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.*, [5].

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 [6]. 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) [6].

EVALUATION AND ASSESSMENT

Environmental Water Quality (EWQ) Index

Analytical values of physical parameters (dissolved oxygen concentration DO, conductivity, temperature, total disposed solids TDS, salinity and pH) and chemical parameters (heavy metals and PAH) were checked against standard guidelines water quality values for the protection of aquatic lives. The result was used to estimate EWQ Index in accordance with CCME [7] protocol for Water Quality Index:

Formula: EWQI = 100, [7]…….Eqn 1

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 [7].

Gross Anatomical Assessment

These involve physical assessment on 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 [8] and is assessing the relative well-being of a fish population [9]. It is advantageous to use two measurable and convertible sizes of fish for estimating the condition factors. Fulton [10], proposed mathematical formula was used in quantifing the condition of fish:

$$K = 100W/L^3$$

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 (cm)

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*., [5] protocol. According to Adams *et al*., [5], 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

Histological Assessments

Qualitative and semi-quantitative histoogical assessments were conducted.

Qualitative Histological Analysis (QHA)

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 Histological Analysis (SQHA)

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 [11]. Furthermore, a modified classification system by Van Dyk *et al.*, [12] based on a proposed scoring scheme by Zimmerli *et al.* [11] 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)

Physical and Chemical water quality parameters results (Tables) were use in the estimation of EWQI for the experimental areas (EKE and OKO). A mathematical analysis applying the CCME [7] equation showed that:

EKC EWQI = 9.0

OKC EWQI = 35.9

Sediment quality analysis

The result of heavy metals in the sediment from the two experimental stations is represented in fig-2.

Table-1: Physical water quality parameters and their standard guidelines for surface water

Parameters	Day 1		Day 2		Day 3		Day 4		Mean		STD
	EKE	OKO	EKE	OKO	EKE	OKO	EKE	OKO	EKE	OKO	
Temperature (°C)	30.0	28.9	30.3	28.0	32.2	28.8	36.5	27.0	32.3	28.2	Ambience (31)
Conductivity (µs/cm)	399.9	310.0	399.9	140.7	399.9	40.4	399.9	135.8	399.9	156.7	0-100*
Salinity(ppm)	70	98	70	104	70	87	68	108	69.5	116.6	13515 –35040**
TDS (mg/L)	399.9	70	390.0	71	388.8	74	390.0	68	392.2	70.8	<1000**
DO (mg/L)	3.09	2.20	2.04	2.21	2.35	2.19	4.09	2.30	2.9	2.2	>5.5**
pH	5.7	6.8	5.6	6.4	5.9	6.4	5.8	6.5	5.8	6.5	6.5-9.0*

*– CCME [7] surface water quality objectives for the protection of aquatic life

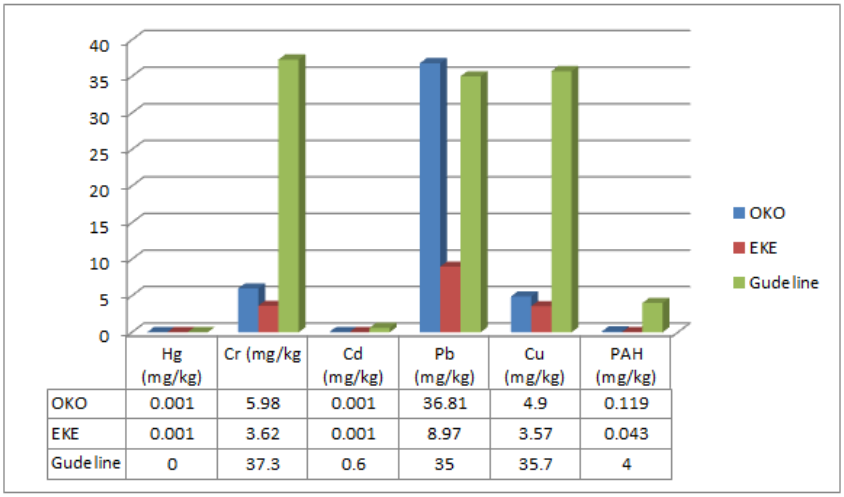
** – USEPA [17], National Recommended Ware Quality Criteria

Table-2: Chemical water quality parameters and their standard guidelines for surface water

Parameters	Day 1		Day 2		Day 3		Day 4		Mean		STD
	EKE	OKO	EKE	OKO	EKE	OKO	EKE	OKO	EKE	OKO	
Cd	0.051	0.001	0.053	0.051	0.032	0.001	0.020	0.001	0.04	0.014	Max. level
Cr	0.125	0.001	0.185	0.001	0.124	0.001	0.127	0.001	0.14	0.001	0.009**
Cu	0.036	0.001	0.041	0.001	0.052	0.001	0.033	0.001	0.034	0.001	0.05**
Hg	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	<0.001	<0.001	0.003**
Pb	0.218	0.001	0.193	0.001	0.179	0.001	0.100	0.001	0.173	0.001	0.001**
PAH	0.005	0.001	0.004	0.001	0.002	0.001	0.010	0.001	0.005	0.001	0.008**

*– CCME [7] surface water quality objectives for the protection of aquatic life

** – USEPA [17], National Recommended Ware Quality Criteria



	Hg (mg/kg)	Cr (mg/kg	Cd (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	PAH (mg/kg)
OKO	0.001	5.98	0.001	36.81	4.9	0.119
EKE	0.001	3.62	0.001	8.97	3.57	0.043
Gude line	0	37.3	0.6	35	35.7	4

Fig-2: Graph showing heavy metal profile from the experimental stations using NOAA (2009) [18]. PAH –BCWQ [19] Guidelines

Gross anatomical assessment – fish necropsy

Condition factor

The condition factor for fishes from OKO ranged from 1.5 to 2.2 with a mean value of 1.85, while that of ARAC ranged from 1.6 to 2.0 with a mean value of 1.81. There was no significant changes or differences between both sampled site when employing and using the T (independent) sample test ($p<0.05$).

Table-3: Condition factor estimation of fishes harvested from Okochiri

EXPERIMENTAL FISH (OKO)				CONTROL FISH (ARAC)			
ish Tag	ENGTH (cm)	EIGHT (g)	F	h Tag	LENGTH (cm)	WEIGHT (g)	F
1	4.1	3.9	.9	**1**	7.1	1.4	.8
2	5.1	6.4	.6	**2**	7.1	8	.0
3	5.0	3.4	.6	**3**	7.1	6	.9
4	5.0	7.8	.7	**4**	6.1	1.7	.0
5	5.0	9.8	.5	**5**	7.1	7.8	.8
6	4.0	7.8	.7	**6**	8.1	00.6	.7
7	5.0	5.5	.9	**7**	6.1	1.2	.7
8	4.0	6.7	.7	**8**	4.1	1.4	.8
9	6.1	3	.8	**9**	5.1	2.9	.8
10	5.1	0.7	.8	**10**	8.1	3.4	.6
11	4.0	4.8	.0	**2**	6.6	3.4	.8
12	4.1	0.3	.8				
13	5.0	1.7	.8				
14	4.1	5.8	.0				
15	4.0	8.4	.8				
16	4.1	8.5	.1				
17	4.1	1.2	.9				
18	3.4	8.2	.2				
19	3.1	8.8	.2				
20	3.0	3.2	.0				
21	4.4	4.3	.9				

M1 = Mean of Experimental Fish, M2 = Mean of Control Fish

Table-4: Standard Mann-Whitney U Test for Health Assessment Index

Parameters	M1 Exp.	M2 Con.	T1	T2	U Score	U Critical	Inference (U score >55 = non-Significance)
Length	14.4	16.6	223.5	239.5	171	55	No significant diff. in HAI btw experiment & control groups ($p>0.05$)
Weight	54.3	83.4	224	241	169	55	No significant diff. in HAI btw experiment & control groups ($p>0.05$)
CF	1.9	1.8	321.5	143	88.5	55	No significant diff. in HAI btw experiment & control groups ($p>0.05$)

M1 = Mean of experimental, M2 = Mean of control, T1 = Rank sum of experiment; T2 = Rank sum of Control, U Score = estimated U value, U critical = Table U value at 0.05 significance

Health assessment index (HAI)

Gross anatomical assessment showed that:

- OKO fishes had: Black spots on skin (80%), ectoparasites on 10% of fishes, and 70% fin lesions were noted following external examination. On internal examination, liver exhibited fatty change and focal discolouration (40%) and gills showed frayed and pale to very light colour (50%),
- ARAC fishes had: Black spots on skin (80%), moderate ectoparasites infestation on 0% of fishes. and 10% fin lesions were noted following external examination. No abnormality was noticed on internal examination.

Fish from OKO showed to have the higher HAI when compared fish from ARAC. Man Whitney U test (Table) showed significance differences ($p<0.05$) on HAI between OKO and ARAC on Fins, Eyes, Gills and Kidney, while the Skin, Parasite Index and Liver showed no significant difference.

Table-5: HAI results for Okochiri

S/N	SKIN		FINS		EYES		GILLS		PARASITES		LIVER		KIDNEY		Sum of Variables
T1	3	30	3	30	B	30	M	30	0	0	C	30	M	30	180
T2	3	30	3	30	B	30	M	30	1	10	OT	30	M	30	191
T3	1	10	1	10	B	30	OT	30	0	0	D	30	M	30	140
T4	2	20	1	10	N	0	M	30	0	0	D	30	N	0	90
T5	1	10	2	20	N	0	N	0	0	0	A	0	M	30	60
T6	0	0	1	10	N	0	M	30	0	0	A	0	M	30	70
T7	2	20	1	10	N	0	F	30	0	0	A	0	M	30	90
T8	0	0	1	10	N	0	F	30	0	0	A	0	M	30	70
T9	1	10	1	10	N	0	N	0	0	0	A	0	M	30	50
T10	0	0	1	10	N	0	N	0	0	0	A	0	N	0	10
T11	1	10	1	10	E	30	N	0	0	0	OT	30	M	30	110
T12	3	30	1	10	E	30	M	30	0	0	OT	30	M	30	160
T13	1	10	1	10	E	30	N	0	0		OT	30	N	0	80
T14	1	10	2	20	N	0	N	0	0	0	D	30	N	0	60
T15	1	10	0	0	N	0	M	30	0	0	A	0	N	0	40
T16	1	10	0	0	N	0	N	0	0	0	A	0	N	0	10
T17	1	10	1	10	N	0	N	0	0	0	OT	30	N	0	50
T18	2	20	2	20	N	0	M	30	0	0	OT	30	M	30	130
T19	1	10	2	20	N	0	M	30	0	0	A	0	N	0	60
T20	1	20	0	0	N	0	N	0	0	0	A	0	N	0	20
M1		13.5		12.5		9		16.5		0.5		15		16.5	83.6
HAI	**83.6**														

KEY:

Skin

0 = Normal, no skin aberrations
1 = Mild skin aberrations
2 = Moderate skin aberrations
3 = Severe skin aberrations

Fins

0 = No active erosion
1= Light skin erosion
2 = Moderate active erosion with some hemorrhaging
3 = Severe active erosion with hemorrhaging

Eyes

N = No aberration, good clear eyes
B = Generally, an opaque eye (one or both).
E = Swollen, protruding eye (one or both)

Gills

N = Normal, no apparent aberration
F = Frayed, erosion of tips of gills lamellae.
M = Marginate, gills with light, discoloured margins along tips of the lamellae
OT = Other, deviation in liver not fitting other categories.

Liver

A = Normal, solid red or light red colour.
C = Fatty liver, coffee with cream colour.
D = Nodules in the liver, cysts or nodules.
OT = Other, deviations in liver not fitting other categories

Kidney

N = Normal, firm dark red colour, lying relatively flat along the length of the vertebral column.
M = Mottled, gray discolouration

Parasites

0 = No observed parasites
1 = Few observed parasites

Table-6: HAI results for ARAC

S/N	SKIN		FINS		EYES		GILLS		PARASITES		LIVER		KIDNEY		Sum of Variables
T1	1	10	0	0	N	0	M	30	0	0	D	30	N	0	70
T2	1	10	1	10	N	0	N	0	0	0	A	0	N	0	10
T3	3	30	0	0	N	0	N	0	0	0	A	0	N	0	30
T4	2	20	0	0	*N*	0	N	0	0	0	A	0	N	0	20
T5	0	0	0	0	N	0	N	0	0	0	OT	0	N	0	0
T6	1	10	0	0	N	0	N	0	0	0	OT	0	N	0	10
T7	1	10	0	0	N	0	N	0	0	0	A	0	N	0	10
T8	2	20	0	0	N	0	N	0	0	0	A	0	N	0	20
T9	1	10	0	0	N	0	N	0	0	0	A	0	N	0	10
T10	0	0	0	0	N	0	N	0	0	0	A	0	N	0	0
M2		12		1		0		3		0		3		0	18
HAI	**18**														

Table-7: Standard Mann-Whitney U Test for Health Assessment Index

Parameters	M1 Exp.	M2 Con.	T1	T2	U Score	U Critical	Inference (U score >55 = non-Significance)
Skin	13.5	12	318.5	126	91.5	55	No significant diff. in HAI btw experiment & control groups (p>0.05)
Fins	12.5	1	388	77	22	55	Significant diff. in HAI btw experiment & control groups (p>0.05)
Eyes	9	0	340	125	70	55	No significant diff. in HAI btw experiment & control groups (p>0.05)
Gills	16.5	3	355	110	55	55	Significant diff. in HAI btw experiment & control groups (p>0.05)
Parasite	0.5	0	315	150	95	55	No significant diff. in HAI btw experiment & control groups (p>0.05)
Liver	15	3	350	130	65	55	No significant diff. in HAI btw experiment & control groups (p>0.05)
Kidney	16.5	0	365	100	45	55	Significant diff. in HAI btw experiment & control groups (p>0.05)
HAI	83.6	18	388	77	22	55	Significant diff. in HAI btw experiment & control groups (p>0.05)

M1 Exp.= Mean of experimental, M2 Com.= Mean of control, T1 = Rank sum of experiment; T2 = Rank sum of Control, U Score = estimated U value, U critical = Table U value at 0.05 significance

HISTOLOGICAL ASSESSMENT

A. Qualitative Histological Analysis (QHA) Results

Gills Assessment

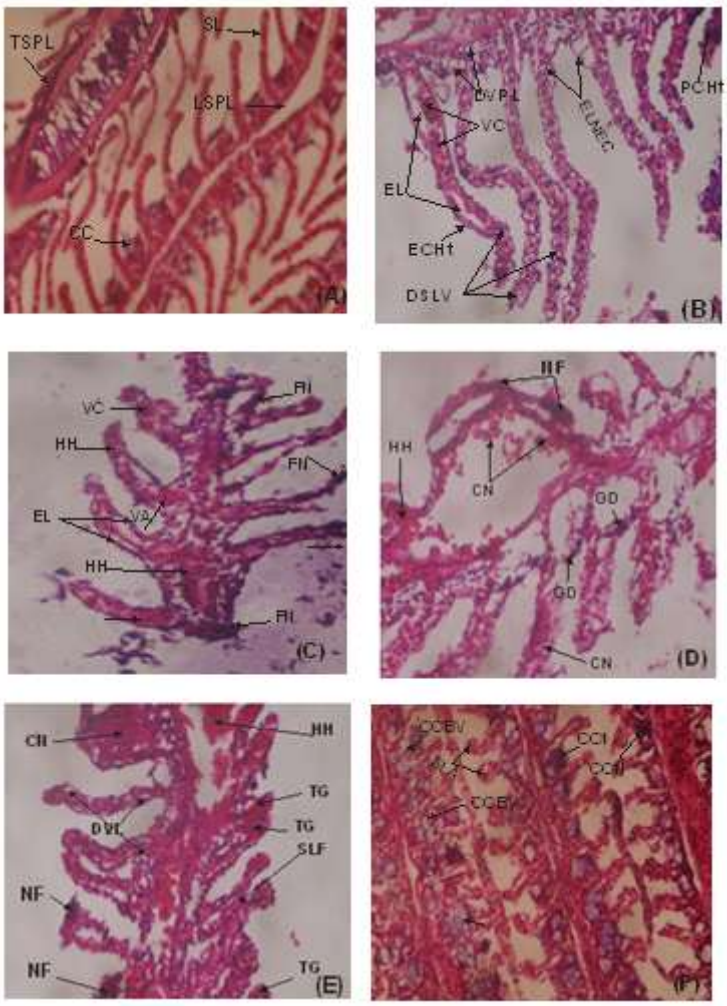


Fig-3: **Micrographs (400 x magnification) of Gills of fishes harvested from OKC and ARAC**

- A: The histology of a normal Gill from ARAC 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).
- E. Early stages of vascular rupture, showing: Showing Talemgiectasia (TG), Haemmorrhage (HH), focal areas of Coagulative Necrosis (CNF), Necrotic Foci (NF), Secondary Lamella Fusion (SLF) and Diffuse Vacuolization (DVL)
- F. Early stages of structural degeneration and cellular death showing; Chloride Cell Inflamation (CCI), Chloride Cell Swelling, Blebbing and Vacuolization (CCBV), Chloride Cell Necrosis (CCN)

Table-8: Percentage prevalence of gill alterations of specimen from OKO and ARAC

Alteration	**% Prevalence**	
	OKC	**ARAC**
Circulatory Disturbance		
Haemorrhage/Hyperaemia/Aneurysm	65	20
Intercellular Oedema	0	0
Regressive Changes		
Epithelium		
Architectural and Structural Alteration	55	10
Plasma Alterations*	10	0
Deposits	0	0
Nuclear Alterations	30	0
Atrophy	20	
Necrosis	60	10
Rupture of the Pillar Cells	45	0
Supporting Structures		
Architecture and Structural Alteration	75	20
Plasma Alteration*	40	0
Deposits	30	0
Nuclear Alteration	55	0
Atrophy	35	0
Necrosis	40	10
Progressive Changes		
Epithelium		
Hypertrophy	10	0
Hyperplasia	5	0
Supporting Tissues		
Hypertrophy	25	10
Hyperplasia	10	0
Inflammation		
Exudates	5	
Infiltration	10	0
Tumour		
Benign Tumour	0	0
Malignant Tumour	0	0
MEAN	32.9	10.0

*Plasma Alterations: (plasma cell Aggregation or Granular Degeneration/fatty Vacuolization or Glycogen Degeneration/thickening of Connective Tissue Fibres or Hyaline Degeneration

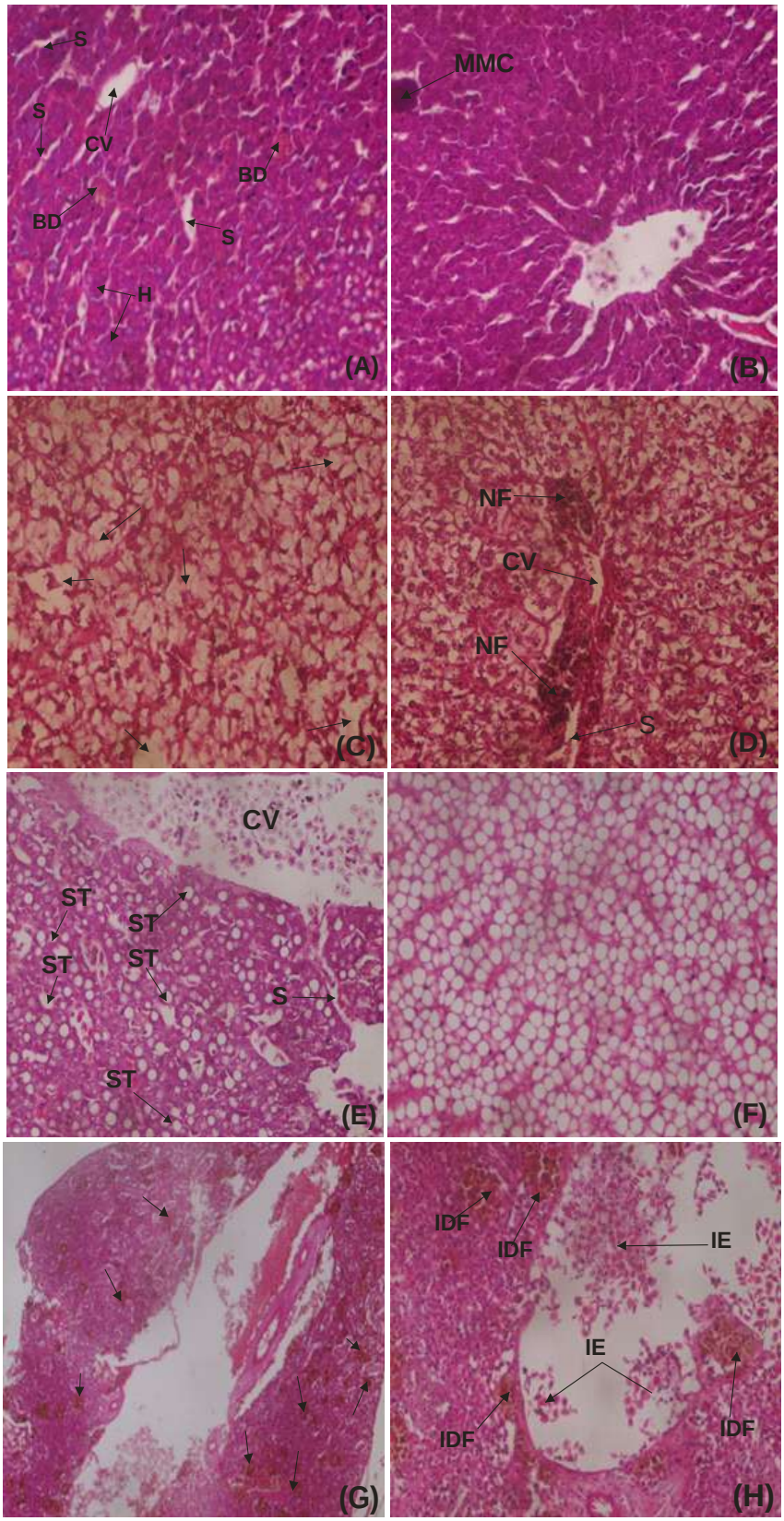


Fig-4: Micrographs (400 x magnification) of Liver of fishes harvested from OKC and ARAC

- **A**: The histology of a normal Liver from control (ARAC) fishes showing Hepatocytes (H), Sinusoids (S), Central Vein (CV) and Bile Duct (BD).

- B: A normal architecture, but with a Melano-Macrophage Centre (MMC)
- C: Showing Diffuse Glycogen Degeneration (Arrows)
- D: Showing Diffuse Glycogen Degeneration with Necroti Foci (NF) in the walls of the Central Vein (CV) and Sinusoids (S)
- E: Showing Diffuse Steatosis (ST) with Inflammatory Exudates in the Central Vein (CV)
- F: Showing Fatty Degeneration with total loss of Liver Parenchyma
- G: Diffuse Intra-cellular Deposits (Arrows)
- H: Focal Areas of Intra-cellular Deposits (IDF) and Inflammatory Exudates (IE) in Central Vein

Table-9: Percentage prevalence of liver alterations of fish from OKO and ARAC

Alteration	**% Prevalence**	
	OKC	**ARAC**
Circulatory Disturbance		
Haemorrhage/Hyperaemia/Aneurysm	45	20
Intercellular Oedema	25	10
Regressive Changes		
Liver Tissue		
Architectural and Structural Alteration	65	10
Plasma Alterations	35	0
Deposits	10	0
Nuclear Alterations	25	0
Atrophy	10	0
Necrosis	40	10
Vacuolar Degeneration	25	0
Interstitial Tissues		
Architecture and Structural Alteration	55	10
Plasma Alteration	15	0
Deposits	10	0
Nuclear Alteration	15	0
Atrophy	10	0
Necrosis	30	0
Bile Duct		
Architectural and Structural Alteration	5	0
Plasma Alterations	0	0
Deposits	0	0
Nuclear Alterations	0	0
Atrophy	0	0
Necrosis	15	0
Progressive Changes		
Liver Tissue		
Hypertrophy	10	0
Hyperplasia	0	0
Interstitial Tissues		
Hypertrophy	5	0
Hyperplasia	0	0
Bile Duct		
Hypertrophy		
Hyperplasia		
Wall proliferation of Bile Ducts or Ductules		
Inflammation		
Exudates	30	0
Infiltration	30	0
Tumour		
Benign Tumour	0	0
Malignant Tumour	0	0
MEAN	24.2	12.0

*Plasma Alterations: (plasma cell Aggregation or Granular Degeneration/fatty Vacuolization or Glycogen Degeneration/thickening of Connective Tissue Fibres or Hyaline Degeneration

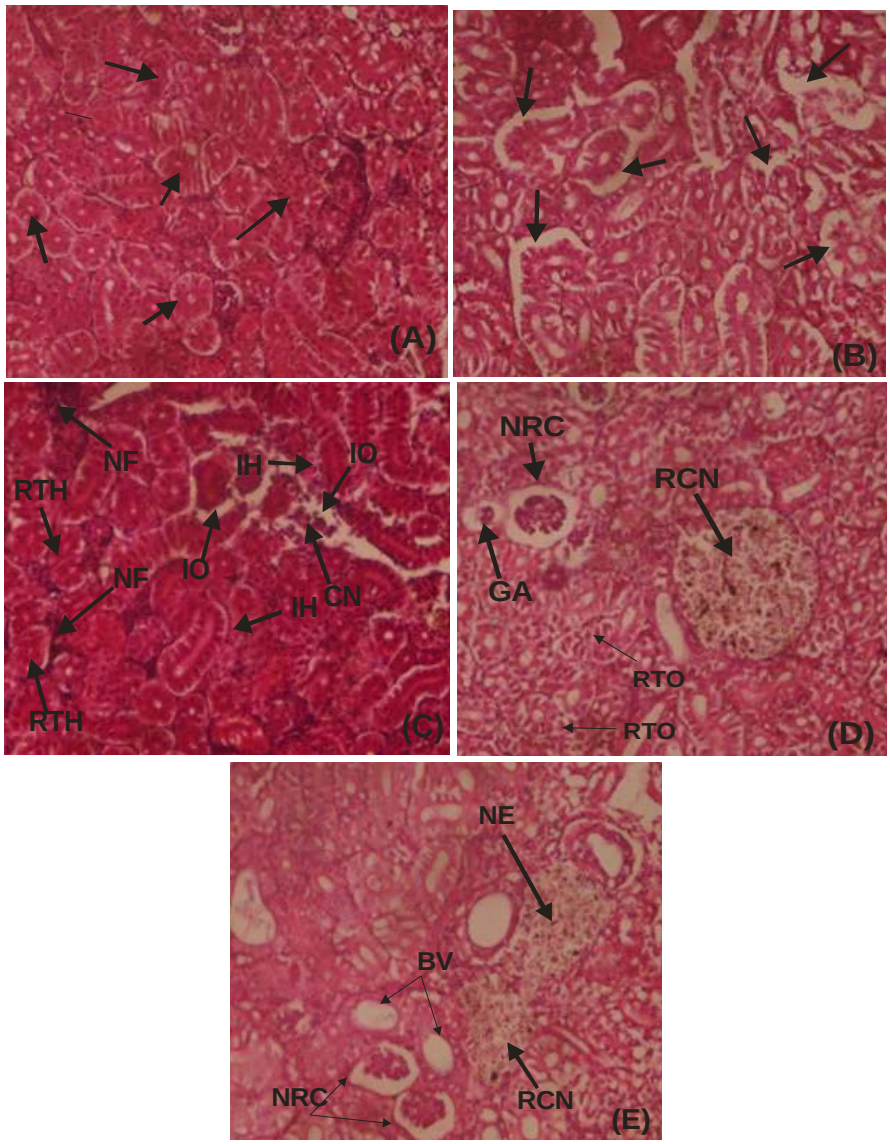


Fig-5: Micrographs (400 x magnification) of Kidney of fishes harvested from OKO and ARAC.

- **A**: The histology of a normal trunk Kidney from control (ARAC) fishes showing Renal Tubules (Arrows),
- **B**: Diffuse Interstitial Oedema
- **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), Bloov Vessls, Renal Corpuscle Necrosis and Necrotic Exudate (NE)

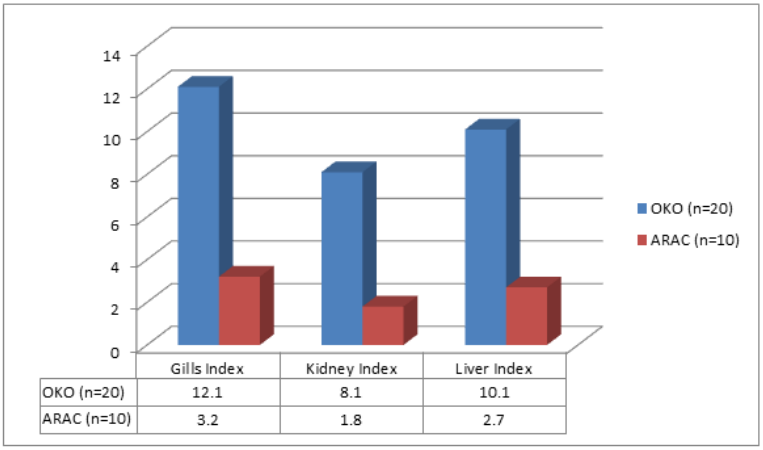
Table-10: Percentage prevalence of kidney alterations of specimens from OKC and ARAC

Alteration	% Prevalence	
	KC	ARAC
Circulatory Disturbance		
Haemorrhage/Hyperaemia/Aneurysm	0	0
Intercellular Oedema	5	0
Regressive Changes		
Tubules		
Architectural and Structural Alteration	0	0
Plasma Alterations	0	0
Deposits	0	0
Nuclear Alterations	5	0
Atrophy	0	0
Necrosis	5	0
Glomerulus		
Architecture and Structural Alteration	5	0
Plasma Alteration	0	0
Deposits	0	0
Nuclear Alteration	0	0
Atrophy	0	0
Necrosis	5	0
Interstitial Tissues		
Architecture and Structural Alteration	0	0
Plasma Alteration	0	0
Deposits	5	0
Nuclear Alteration	0	0
Atrophy	5	0
Necrosis	0	0
Progressive Changes		
Tubules		
Hypertrophy	0	0
Hyperplasia	5	0
Glomerulus		
Hypertrophy	5	0
Hyperplasia	0	0
Interstitial Tissue		
Hypertrophy	0	0
Hyperplasia	0	0
Inflammation		
Exudates	5	0
Infiltration	0	0
Tumour		
Benign Tumour	0	0
Malignant Tumour	0	0
MEAN	2.9	2.7

*Plasma Alterations: (plasma cell Aggregation or Granular Degeneration/fatty Vacuolization or Glycogen Degeneration/thickening of Connective Tissue Fibres or Hyaline Degeneration

B. Semi-Quantitative Histological Analysis (SQHA)

The semi-quantitative histological assessment showed that 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 30.3 and 7.7 respectively. Following Man Whitney U Test analysis at $P<0.05$, there was significant differences between fish from OKO and ARAC.



	Gills Index	Kidney Index	Liver Index
OKO (n=20)	12.1	8.1	10.1
ARAC (n=10)	3.2	1.8	2.7

Fig-6: Graph showing the comparison of fish index with the organs index

DISCUSSIONS

Physical Water Quality

Temperature

Aquatic organisms often have narrow temperature tolerances. Thus, although water bodies have the ability to buffer against atmospheric temperature extremes, even moderate changes in water temperatures can have serious impacts on aquatic life, including bacteria, algae, invertebrates and fish [13]. The temperature of the EKE (32.3^{O}C) was above the ambient temperature while OKO (28.2^{O}C) was bellow ambient temperature (31^{O}C). Since fish harvested for the study were primarily from OKO, it can be concluded that the temperature of the river did not affect the health of the aquatic lives habiting the river. Nevertheless, fish migration to the EKE river channels and would be adversely affected. Biodiversity on the EKE river channels would adversely affect aquatic plant and animal colonies because of their variability in adapting to sustained temperature rise. High temperature would also cause low levels Dissolved Oxygen (DO) concentration. This can cause environmental stress on fishes, further compounding the energy flow in the aquatic ecosystem of EKE.

Conductivity

The conductance of Eke (399.9µS/cm) and OKO (156.7µS/cm) rivers were above the normal surface water conductivity ranges (0-100µS/cm). Municipal, agricultural and industrial discharge can contribute ions to receiving waters or can contain substances that are poor conductors (organic compounds) changing the conductivity of receiving waters. Thus conductivity can also be used to detect pollution source [20, 21] readings can help locate potential pollution source because polluted water usually has a higher conductivity than unpolluted waters [21]. In unpolluted rivers and streams, geology and groundwater are the primary influence on conductivity level [21]. Based on the fact the conductivity of EKE is higher than OKO, it can thus be inferred that the polluting source is the EKE axis of the creeks and river channels.

Salinity

The level of salinity in aquatic systems is important to aquatic plants and animals as species can survive only within certain salinity ranges [22]. Although some species are well-adapted to surviving in saline environments, growth and reproduction of many species can be hindered by increases in salinity [23]. The salinity of EKE (69.5ppm) and OKO (116.6ppm) are below the normal range for estuarine marine aquatic value (13515 –35040ppm). The abnormal reduction of salinity level below the pristine estuarine levels can be due to high quantum of effluence discharge and storm water recharge. Overtime, this will no doubt affect the biodiversity of aquatic organisms living in these creeks and rivers.

Total Dissolved Substance (TDS)

TDS or turbidity are suspended solids in streams or river water, and are often the result of sediments carried by the water. The sources of these sediments includes natural and anthropogenic (human) activities in watershed, such as natural or excessive soil erosion from agriculture, forestry or construction, urban runoff, industrial effluents, or excess phytoplankton growth [17]. Higher TDS increases water temperature because suspended particles absorb more heat. This in turn, reduces DO concentrations because warm water holds less DO than cold water. TDS for EKE (392.2mg/l) was higher than the normal range (<1000mg/l) for marine aquatic environment, while OKO (70.8mg/l) was below the normal range. It can thus be said that TDS was not a contributing factor to the harvested fish pathology.

pH: The pH of an aquatic ecosystem is important because it is closely linked to biological productivity. pH outside the normal range can acutely cause physiological and in chronic exposure cause limitations in growth and reproduction. Low pH can also affect the toxicity of aquatic compounds such as ammonia and certain metals by making them more readily available for bio-uptake by plants and animals. This can produce conditions that are toxic to aquatic

life. Although the tolerance of individual species varies, pH values between 6.5 and 8.5 usually indicate good water quality and this range is typical of most major drainage basins of the world [23]. The pH of EKE was slightly higher than the normal pH range (6.5 – 9.0) while OKO was within the normal range. Since fish harvested for the study were primarily from OKO, it can be concluded that pH of the rivers did not pose any risk to the lives of the aquatic habitat.

Dissolved Oxygen (DO)

Oxygen that is dissolved in the water column is one of the most important components of aquatic systems. Oxygen is required for the metabolism of aerobic organisms, and it influences inorganic chemical reactions. Oxygen is often used as an indicator of water quality, such that high concentrations of oxygen usually indicate good water quality [23]. DO levels for both stations appeared to be lower than the standard Oxygen concentration for surface water (5mg/l) for protection of warm water fish. This could be as a result of accumulation of heavy metals, especially lead which has been known to reduce the oxygen level of water. The oxygen level of these rivers can pose risk of hypoxia on the aquatic live. The highest measures were in (EKE) 4.09mg/l, while the lowest was in (OKO) 2.04mg/l.

Chemical Water Quality

The chemical parameter considered for the estimation of EWQI were heavy metals (i.e. Pb, Cr, Hg and Cd) and PAH.

Cd: Cd level in EKE (0.04mg/l) and OKO (0.014mg/l) were higher than the standard value (0.009mg/l). Water in EKE and OKO are contaminated with Cd, which can pose serious risk to the aquatic ecosystem of EKE and OKO. Cd contamination can be one of the causes of pathologies found in the fishes harvested from OKO. Bioavailability refers to the availability of a metal to enter and affect a biological system. The most bioavailable and therefore most toxic form of cadmium is the divalent ion (Cd+2). Exposure to this form induces the synthesis of a low molecular weight protein called metallothionen, which can then bind with cadmium and decrease its toxicity. This normally takes place in the liver of fish and humans. But if the cadmium concentration is high, the metallothionen detoxification system can become overwhelmed and the excess cadmium will be available to produce toxic effects [24-26]. Cadmium effects on aquatic organisms are analogous to those in humans, and include skeletal deformities and impaired functioning of kidneys in fish. The effects of cadmium on aquatic organisms can be directly or indirectly lethal and can impact populations and ecosystems as well as individuals. Cadmium impairs aquatic plant growth. This affects the entire ecosystem because green plants are at the base of all food chains. When aquatic plants that are exposed to cadmium do not grow normally, there will be less food available for aquatic animals [24-26]. Reduced long-term survival and growth were observed in marine isopods (a group of marine invertebrates) when sublethal cadmium exposure occurred during embryonic and larval development. Some individual animals were more sensitive to the toxic effects of cadmium than others. Differential survival of cadmium-exposed isopods can result in long-term changes in population structure [24-26].

Cr: Cr level in EKE (0.14mg/l) was higher than the standard value (0.05mg/l), while OKO (0.001mg/l) was lower than the standard value. Water in EKE is contaminated with Cr, while fishes harvested from OKO are not of any direct risk due to Cr poisoning. Nevertheless, because of fish migratory pattern in search food spawning areas, fishes from OKO can still be affected by EKE Cr contaminants. Chromium is an essential trace nutrient that is required in small amounts for carbohydrate metabolism, but becomes toxic at higher concentrations. Cr and Cd interact synergistically; the combined toxicities of these two metals is greater than the sum of their individual toxicities. Low concentrations of hexavalent chromium cause sublethal toxic effects in aquatic plants and animals. For example, 62 ppb inhibits growth in algae and 16 ppb inhibits growth in chinook salmon [27]. This is consistent with the overall finding that aquatic animals are more sensitive to metals than are aquatic plants [26]. Although reducing the growth of a plant or animal is not directly lethal, the smaller size increases the vulnerability of the organism to predators. What begins as a sublethal effect of a metal may end up as a lethal effect. As is the case with other metals, chromium toxicity to aquatic organisms increases as water temperature increases and as pH and salinity decrease. Additionally, chromium is more toxic in soft water than in hard water and there are species differences in sensitivity. The concentration of chromium that caused death in 50% of the exposed population was 3 ppm in soft water and 72 ppm in hard water for fathead minnows and 18 ppm in soft water and 133 ppm in hard water for goldfish [28, 27].

Cu: Cu level in EKE (0.034mg/l) was higher than the standard value (0.003mg/l), while OKO (0.001mg/l) was lower than the standard value. Water in EKE is contaminated with Cu, while fishes harvested from OKO are not of any direct risk due to Cu poisoning. Nevertheless, because of fish migratory pattern, fishes from OKO can still be affected by EKE Cu contaminants. Cu is an essential trace nutrient that is required in small amounts by humans, other mammals, fish and shellfish for carbohydrate metabolism and the functioning of more than 30 enzymes. It is also needed for the formation of haemoglobin and haemocyanin, the oxygen-transporting pigments in the blood of vertebrates and shellfish respectively. However, Fish and crustaceans are 10 to 100 times more sensitive to the toxic effects of copper than are mammals. Algae,

especially blue-green algae species, are 1,000 times more sensitive to the toxic effects of copper than are mammals [29, 30, 26]. This is an exception to the general principle that aquatic animals are more sensitive than aquatic plants to the toxic effects of metals. The effects of Cu on aquatic organisms can be directly or indirectly lethal. Gills become frayed and lose their ability to regulate transport of salts such as sodium chloride and potassium chloride into and out of fish. These salts are important for the normal functioning of the cardiovascular and nervous systems. Cu also adversely affects olfaction (sense of smell) in fish. Cu can affect olfaction by competing with natural odorants for binding sites [31]. Fish rely on their sense of smell to find food, avoid predators and migrate. Fish normally migrate back to where they were born; spawning then occurs in the "home" river or stream. Successful homing depends on olfaction [32].

Hg: Hg level in EKE (<0.001mg/l) and OKO (<0.001mg/l) were lesser than the standard value (0.001mg/l). Water in EKE and OKO are not contaminated with Hg. But it is worthy to note that, exposure of aquatic ecosystem to Hg can cause bioconcentration and biomagnification of Hg, which varies by age, size, and species of exposed fish. Hence, more biomagnification takes place in the tissues organism higher up the food chain. Fish can tolerate ten times as much methylmercury as humans and are more tolerant than their wildlife predators. High enough levels of Hg cause decreased hatching rate of fish, waterfowl, and marine bird eggs and reduced growth and development of the fish fry and baby birds that have hatched from the eggs. These impacts can have severe repercussions at the population and ecosystem levels because food chains will be impacted and there will be a shift in the species composition of the ecosystem [26].

Pb: Pb level in EKE (0.173mg/l) was higher than the standard value (0.008mg/l), while OKO (0.001mg/l) was lower than the standard value. Water in EKE is contaminated with Pb, while fishes harvested from OKO are not of any direct risk due to Pb poisoning. Nevertheless, because of the migratory pattern of fishes in search food spawning areas, fishes from OKO can still be affected by EKE contaminants. Lead bioconcentrates in the skin, bones, kidneys, and liver of fish rather than in muscle and does not biomagnify up the food chain. This makes lead less problematic via this route of exposure. However, people who eat whole fish and wildlife, who, of course, eat the whole fish, can potentially be exposed to high concentrations of lead [26]. When lead inhibits enzymes needed for photosynthesis, it reduces growth of plants, specially phytoplankton. This means less food for animals; this has repercussions for the entire ecosystem. Embryos and fry are more sensitive to the toxic effects of lead than are adults [27, 26]. High level of lead is also known to cause a decrease level of oxygen in water, and cause health risks on the aquatic lives such as; hypoxia, damage to the central nervous system and inability to synthesize red blood cells.

PAH: PAH level in EKE (0.005mg/l) and OKO (0.001mg/l) were lower than the standard value (0.007mg/l). Water in EKE and OKO are not contaminated with Hg

Environmental Water Quality Index (EWQI)

The CCME (2001) ranking, EWQI for EKE (9.0) and OKO (35.9) were both classified as Poor. The implication of this is that, the water quality of EKE and OKO is not good enough for the protection of aquatic life and can also impact on human health through water use and food chain.

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

CF: CF estimation for OKO (1.9) was greater ARAC (1, 8). This indicates that fish from OKO are in nutritive state than ARAC. Nevertheless, statistical analysis (Table-4) showed that there was no a significant change or difference between fish harvested from OKO and ARAC. CF uses biometric data to assess the nutritional wellbeing of a fish and is based on the hypothesis that heavier fish of a given length are in better condition [8, 6]. CF is not a diagnostic tool and is used in assessment of some physical symptoms of an underlying pathology. Fish therefore may appear normal but might have an insidious pathology at a sublethal level.

HAI: HAI estimation for OKO (83.6) was greater ARAC (18). This indicates that fish from OKO have worse gross or macroscopic pathology of the external and internal organs than ARAC. Statistical analysis (Table-7) showed that there was significant change or difference between fish harvested from OKO and ARAC. HAI is a simple necrosy-based tool for the rapid assessment of the general health status of fish in field situations [6]. HAI is not a biomarker, but rather a

protocol for documenting lesions or changes that have advanced to the point of being grossly visible [6].

Histological Assessment

QHA: The qualitative assessment of specimen slides showed that:

- **Gills**: Had histological findings (Fig-3) with highest percentage prevalence (Table-8) in the following alterations: OKO – Supportive structures architectural alterations (75%), Haemorrhage (65%) and epithelial necrosis (65%). ARAC – Haemorrhage (20%), supportive structures architectural alterations (20%) and epithelial necrosis (10%).
- **Liver**: Had histological findings (Fig-4) with highest percentage prevalence (Table 9) in the following alterations: OKO – Liver tissue architectural alterations (65%), Haemorrhage (65%) and plasma alterations (35%). ARAC – Haemorrhage (20%), interstitial oedema (10%) and liver architectural alterations (10%).
- **Kidney:** Had histological findings (Fig-5) with highest percentage prevalence (Table 10) in the following alterations: OKO –Haemorrhage (70%), tubular architectural alterations (60%) and interstitial tissue architectural alterations (40%). ARAC – Haemorrhage (20%), glomerular architectural alterations (20%) and interstitial oedema (10%).

General, the qualitative histological findings of this ecotoxicological study were consistent with other toxicological studies, which were laboratory simulation of the effects of fish exposure to some hazardous substances:

- **Pb**: Olojo *et al*., [33], 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.
- **Cd**: 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 [34].
- **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 [35]. In another study of the lethal effects of Cr 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 [36].
- **Cu**: Study has shown that exposure of *Oreochromis niloticus* to sublethal level of Cu caused histopathological alteration in: gills - were edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis [37]. Rainbow trout (*Oncorynchus mykiss*) exposed to Cu sulphate for 28 days showed histological alterations in the liver: congestion of the central veins, dark- stained hepatocytes; increase number of kupffer cells; vascular degeneration and sinusoidal degeneration [38].

SQHA: SQHA showed that OKO (30.3) had a higher fish index value than ARAC (7.7). This indicates that fish harvested from OKO had worse hispathological outcome when compared to fish harvested from ARAC. Based on the Zimmerli [11] classification of the degree of histological alteration, OKO had pronounced alterations of organ tissue (class 4 = index value 26-35), while ARAC had slight histological alterations (class 1 = index value <10). Following Man Whitney U Test analysis at $P<0.05$, there was significant differences between fish from OKO and ARAC.

CONCLUSION

EKE EWQI was poor with abnormal levels of temperature, conductivity, pH, DO, salinity, Cd Cr, Cu and Pb. OKO EWQI was also poor with abnormal levels of conductivity, salinity and Cd. Notable finding in sediment analysis was the elevated levels of Pb in OKO. Conductivity results showed that the contaminating source was from EKE to OKO axis of the creek and river channel. Gross anatomical analysis of harvested fish did not show any significant difference among study sites in terms of CF, but HAI showed notable anomalies with worse outcomes in OKO. Histological analysis further authenticated gross pathologies with fishes from OKO showing pronounced histological alterations of organ tissues. Thus:

- EKE and OKO creeks and river channels are POLLUTED and aquatic lives are seriously under threat
- The level of POLLUTION has capability of imposing serious health and economic cost human living in EKE and OKO communities
- Following the observed contamination pattern and the kind of pollutants involved, the likely polluting source is the PHRC effluents.

RECOMMENDATIONS

- A detail forensic analysis should be done to ascertain or confirm the polluting agent(s)
- Environment protection agencies should check the PHRC effluent and ascertain their level of compliance in the treatment of their waste.
- Epidemiological assessment should be carried out to assess the level of human health impact of the elevated substances.
- Fish consumption study should be done to ascertain the level of bioaccumulation of target chemicals in indigenous fish and their edibility status
- Human and Health Ecological Risk Assessment study should be done to determine the impact of fish consumption on local human populace.

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