HISTOLOGICAL BASED BIOMONITORING: A BASELINE ECOTOXICOLOGICAL EVALUATION OF NEW-CALABAR RIVER USING CHRYSICHTHYS NIGRODIGITATUS.

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ABSTRACT. *The aim of this study was to evaluate the impact of environmental contaminants on gross anatomy (external and internal) and histopathologic biomarkers in liver, kidney and gills of African Silver Catfish (Chrysichthys Nigrodigitatus) collected from New Calabar River (NCR) in comparison to a reference site (African Aquaculture Centre, ARAC). NCR was chosen for this study because of its reported elevated levels of toxicants. For this study, chemical monitoring and bio-monitoring assessments were carried out in three sampling stations (Choba, Ogbogoro and Iwofe) of NCR. The chemical assessment involved EWQI assessment, sediment quality analysis, and heavy metals and PAH assessment. The histological bio-monitoring assessment involves the sampling of twenty table-sized fish harvested from the wild - the Choba station of NCR, which were compared with ten table-sized fishes of the same specie harvested from an aqua-cultured centre, ARAC, which has a controlled water quality system. EWQI was marginal, Sediment analysis showed slightly elevated level of PAH, Fish health Assessment Index (HAI) and fish biometric (CF) were worse in fishes harvested from NCR. Histopathological assessment was done in a qualitative and semi-quantitative scenario and fishes from NCR shows worse results as compared to those from ARAC. The qualitative assessment of target organs show alterations of target, which includes: Neurotic foci, vacuolation, melano-macrophageal centre and fatty changes in liver; intercellular haemorrage, intestinal oedema and melano-macrophageal centre in kidney and; telangiecstasia, epithelial lifting and hyperplasia in gills. Semi-quantitative histological assessment showed that NCR and ARAC had fish index values of 31.1 and 3.6 respectively. Following Man Whitney Test statistical analysis, significant differences ($p<0.05$) were noted between NCR and ARAC.*

KEYWORD: Histology, Ecotoxicology, biomonitoring, biomarker,

INTRODUCTION

The degradation of aquatic ecosystem restricts its uses and can render a whole water body useless. The consequence is not only on aquatic life, but also on water resource base itself. Pollution may intensify water scarcity and inflict a huge cost on other uses. Water quality deterioration has a serious economic impact because the value of the resources is grossly affected and frequently needs significant restoring and management input to bring it back to a useable state (Meybeck et al., 1996, Codd, 2000, Schlacher et al., 2007). Water resources are frequently under inspection as pollution increases. Therefore an urgent need has arisen for sensitive bio-monitoring tools in toxicant impact assessment to indicate the effect of pollution on fish health in polluted aquatic ecosystems. Histopathological assessment of fish tissue allows for early warning signs of disease and detection of long term injury in cells, tissues or organs. Various biochemical and biological studies of fish have been used to assess the consequences of environmental toxicants on fish, but histology is able to enhance and add quality to the research carried out by identifying cellular alterations and quantifying the results.

Ecotoxicology and the effect of Heavy Metals and PAH on Fish species

Aquatic organisms can acquire trace elements from food, suspended particles or directly from the water (Carvalho and Fowler, 1993). Many of these pollutants are non- biodegradable compounds and dangerous due to their innate ability to constantly remain within the ecosystem (Hernandez-Hernandez et al., 1990). Fish are often highly exposed to these aquatic contaminants especially in areas where the dilution rate of wastewater is low. This has adverse effects particularly when contaminants: are not or only slightly decomposable; exhibit a high biological effectiveness; pose a high potential for accumulation; and influence each other in an additive way, in case of multiple contaminants.

Heavy metal pollution is ubiquitous in our environment (Don-Pedro et al.,2004) and result from diverse activities such as industrial affluence, foundry waste, wearing of metals parts and equipment, paints, auto-mobiles, mining, and rock weathering. These are subsequently deposited on soil surface and may be leached through municipal drainages to near by ponds, streams, and rivers which are common amphibian habitats and hiding places. The major concern of heavy metals lie with their acute toxicity and ability to bioaccumulate in biological systems (Otitoloju and Don-Pedro, 2002), resulting in a number of deleterious effects such as immunosupression (Carey and Bryant, 1995). According to Vuuren et al. (1999) metal pollutants are currently considered to be some of the most toxic contaminants present world-wide.

One of the major contributors of PAH to environment in Niger Delta, Nigeria is crude oil spill. There are no consistent figures of the quantity of crude oil spilled in the Niger delta, but it is widely believed that an estimated 13 million barrels (1.5 million tons) of crude oil have been spilled since 1958 from over 7000 oil spill incidents; a yearly average of about 240,000 barrels (UNDP, 2011). Oil spills affected at least 1500 communities in the eight crude oil-producing states in Nigeria, and were mainly from the 5284 oil wells that were drilled (as at 2006) and the 7000 km of crude oil pipeline that crisscrosses the Niger delta region (UNDP, 2006). The oil spills often resulted in contamination of surface water with hydrocarbons and trace metals (Ordinioha et al., 2009). Oil pollution, one of the environmental consequences of crude oil exploration and exploitation activities, produces aqua-toxicological effects, which are deleterious to aquatic life (Kori-Siakpere, 2000; Agbogidi et al., 2005).

One of the core missions of ecotoxicology is to understand the mechanisms by which contaminants perturb normal biological performance (their mode of action), in order to develop appropriate measures to prevent adverse outcomes resulting from environmental contaminants. Histological biomarkers can thus be effectively used as a sensitive ecotoxicological tool for assessing sublethal

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level of toxicity and chronic pollutant exposure in an ecosystem. Toxicants causes physiological and biochemical changes in tissues of living organism. These changes, if no reversible, ultimately result tissue and cellular alteration which are better expressed via histological studies.

Fish is becoming a more widely used and far reaching sentinels organism for Aquatic ecosystem monitoring. Fish in their natural environments are typically exposed to numerous stressors including unfavorable or fluctuating temperatures, high water velocities and sediment loads, low dissolved oxygen concentrations, limited food availability, and among other types of natural episodic variables (Adams and Goede, 1993; Wedemeyer, 1984). Previous and recent studies have shown that fish found in polluted aquatic systems, or exposed to heavy metals and other bio-accumulative chemicals, displayed histological alterations of target organs. Alterations were identified in gonads (Barnhoorn et al., 2004; Pieterse et al., 2010) gills (Van Dyk et al., 2009a), liver (Van Dyk et al., 2007; Marchand et al., 2009) and kidney (Vinodhini and Narayanan, 2009a) after exposure to pollutants. However no notable research has been carried out on the health status of fish in the locations selected for this study using histology as a biomonitoring tool, hence this study would be the first time a prognosis would be conferred on the environmental health status of the NCR.

MATERIALS AND METHOD

Study Area

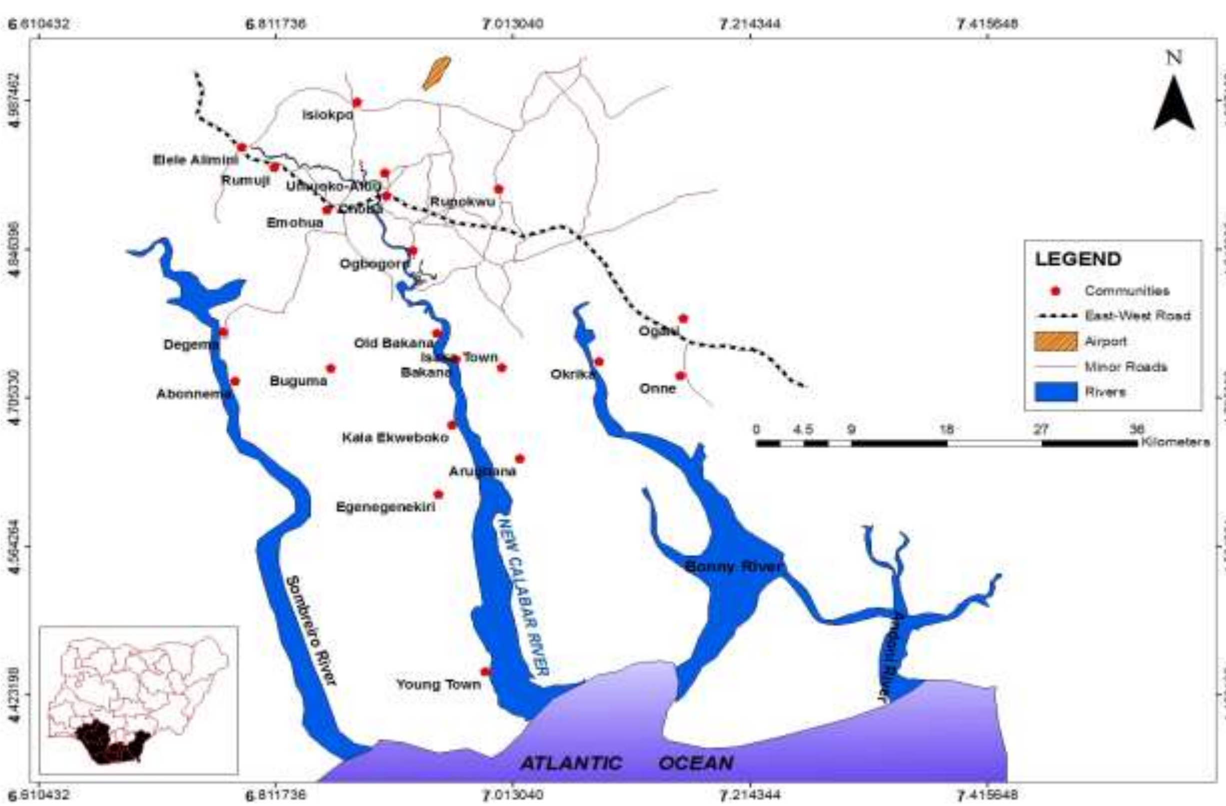


Figure 1. Map of lower Niger Delta, adopted from Francis and Elewuo (2012), showing the New Calabar River drainage system and study area with the sampling stations (Choba, Ogbogoro and Iwofe). Inset is map of Nigeria showing position of the lower Niger Delta.

Experimental Area: The experimental study was carried out in New Calabar River (NCR). Sampling for water, sediment and fish where done in three different stations (Choba, Ogbogoro and Iwofe) of NCR. NCR and its tributaries are located in Rivers States, one of the oil bearing states, in the Niger Delta Region of Nigeria. It is a low lying deltaic rivers which rises at approximately latitude 5˚10'N and longitudes 6˚50'E near Elele-Alimini and flows Southward for roughly 150km before its discharge into the Atlantic Ocean at about latitude 4˚20'N and longitudes 7˚00'E. It occupies a low relief region, ranging from 0-50m above sea level at the low zone, to 50-100 above sea level at its source. The soil of the river basin consist of clays, silt and sand, with high organic matter, classed as either 'lithosols' (mangrove soil type) or 'hydrmorphic' soil type (alluvial deposits). The bedrock of the basin consist of post-cretaceous, recent sedentary formation. The river is unidirectional in the upper reach and tidal in the lower reach. Its upstream reach is fresh water with tropical lowlands rain (dense) forest through secondary forest/farmland vegetation. The downstream reach is however brackish and consist of Mangrove swamp forest. The climate of the area is characterized by wet and dry seasons. The wet season period stretches from April to October each year with monthly average rainfall of 254mm (Department of Meteorological Services Report, Rivers State, 2000), and however with occasional precipitation in the dry season month of November-March. NCR river basin is sub-urban in structure with about 40% of the rural populace predominantly artisanal fishermen. However, the life style of the indigenes has been greatly influenced by the presence of the University of Port Harcourt and the several industries along its river bank. Population growth results in conversion of open land into non-permeable surfaces which increase the rate of run-off and can negatively impact water quality (Jiang et al., 2001). Human activities including defecation, washing, recreation (swimming/bathing and fishing) and waste disposal are prevalent especially where human settlements exist along the river banks. Similarly, several industries are located along the river banks. The social and health impacts are numerous since most communities living on the river banks use the shores as dumpsites for their wastes, which are washed into the ecosystems during rains.

Reference Area: Fishes from African Regional Aquaculture Centre (ARAC) were used for the control study. ARAC was chosen for this study because it is an internationally recognized aquaculture centre with a good control of their fish tank water quality. ARC 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 involved in fisheries and aquaculture research, development and training. 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.

Study Species
Chrysichthys nigrodigitatus belongs to the family Claroteidae. C. Nigrodigitatus has several characteristics required in a sentinel species: it is widely studied; wide geographic distribution; great abundance; and it is frequently subjected to long term water contamination with a high survival rate. C. nigrodigitatus is highly commercial, marketed fresh, smoked or dried, and apart from being a cheap source of highly nutritive protein, it also contains other essential nutrients required by the body (Abowei and Hart, 2008). The males when fully grown usually have a broader head which they use to dig out their breeding nests in their native habitat. They are Omnivorous; feed on seeds, insects, bivalves and detritus. Feeding becomes specialized with age and size; larger fish may feed on decapods and fish. The Fish can be used for aquaculture gamefish and in public aquariums. It occurs in shallow waters of lakes (less than 4 m), over mud and fine sand bottom, in rivers and in swamps.

Selection of Target Analytes
Target Analytes for this study was done base on: 1. Published evidence of environmental systems or sources of contaminants & contamination of NCR (Chinda, 1998; Woke and Aleleye 2007; Wegwu and Akaninwor 2006; Akaninwor and Egwin 2006; Sikoki et al., 2013). 2. Analytes recommended for WQI: Acceptable & Health parameters (CCME, 2001). 3. Target analytes prioritized based on water and sediment sampling results, land use within the watershed, geographic characteristics and analytical costs. (USEPA, 2000).

Sampling
Water and sediment sampling were carried out in all three delineated study stations (Choba, Ogbogoro and Iwofe) of NCR. ARAC was chosen as the reference site for fish samples based on the consideration that the centre has a highly controlled fish culture tanks that can hardly be contaminated. Four different water and sediment samples were collected, at one monthinterval, spanning the dry and rainy season (between February and September).Water sampling was done in accordance with USEPA (2013)and sediment sampling was done in accordance with USEPA(2001).

Fish sampling was carried out in Choba station only, and sampling were done during the rainy season, between August and September 2014. During this period the river receives the most organic and industrial loads carried out by runoff. Gill nets were used at site to attain the required sample size of twenty fish for the specie (Marchand, 2009; DWA, 2012).

Physico-chemical and Environmental Water Quality Index (EWQI)
The physico-chemical water quality parameters were recorded at three sites in the NCR. Site 1: Inflow to NCR (Choba); Site 2: (Ogbogoro); Site 3: Most southern part of the NCR (Iwofe)]. Water temperature (C), pH, Dissolved oxygen and conductivity (µS) were analysed in situ with a HORIBA, U-50 series Multi-parameter water quality checker. Water samples for heavy metal analysis were taken from the river using polypropylene bottles; 2ml of sulphuric acid was added and frozen for further analysis. Atomic absorption Spectrophotometry (Buck Scientific Model210) using element specific hollow cathode lamps in default condition by flame absorption mode was used to approximate the metal concentration within samples. The empirical screening values obtained from the physico-chemical EWQ parameters (Table 1.1) were further analysed

mathematical to give a baseline EWQ screening score to NCR. Essentially, the EWQI model consists of three measures of variance from selected water quality objectives (Scope; Frequency; Amplitude). The "Scope (F1)" represents the extent of water quality guideline non-compliance over the time period of interest. The "Frequency (F2)" represents the percentage of individual tests that do not meet objectives. The "Amplitude (F3)" represents the amount by which failed tests do not meet their objectives. These three factors combine to produce a value between 0 and 100 that represents the overall water quality (CCME 2001).

Sediment Quality Analysis

An aliquot of the sediment sample was filtered and made up to 50ml with distilled water. The filtrate was stored in plastic bottles for heavy metals and PAH analysis.Samples will be determined using GBC Avanta atomic absorption spectrophotometer (AAS).

Gross anatomical Assessment: Fish Necropsy

Fish captured were externally examined for ectoparasites, lesions deformities and identification of sex. Fishes were weighed and standard length recorded for evaluation Condition Factor (CF) using the formula Weigh/cube of length x 100000 (Carlander, 1977). Fishes were then sacrificed by cutting through the spinal cord anterior to the dorsal fin. Once sacrificed a thorough fish necropsy was carried out to note for any gross anatomical abnormalty of the external features and internal abnormalities of organs within the visceral cavity and gills.

A Fish Health Assessment Index (HAI) (Adams et al., 1993), was applied to compare the health of fishes from NCR and ARAC .

Histological Assessment

The selected target organs; liver, kidney and gills were resected for histological preparation during fish necropsy . Liver, kidney and gill samples were fixed in 10% neutrally-buffered formalin (10% NBF) for 48 hours. Samples were finally transported to the Histology Laboratory at the University of Port Harcourt for further processing. The samples fixed in formaldehyde solution were dehydrated in agraded series of ethanol bathes (*30%, *50%, *70%, *80%, *90%, *96% and *2x100%) for 1 hour and embedded in paraffin. The embedded organs 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 (Humason, 1979). 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) (Van Dyk and Pieterse, 2008). Qualitative and semi-qualitative histoogical assessments were conducted. Using a Light microscopy (Olympus BH2) a qualitative assessment (histological description) were made on all mounted slides and the percentage prevalence was noted. Assessed slides were later compared to slides of the reference site, ARAC. Micrographs of assessed slides were taken using Image Manager Software (Pixel IT). The semi-qualitative analysis gave scores to observed 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 (Bernet et al, 1997)

Statistics

Mean and standard deviations were calculated for data obtained for physico-chemicalproperties of water and for the presence of heavy metals and PAH in water, sediment and fish tissuessamples. A One-way ANOVA and Man Whitney test at significant levels of p-values less than 0.05 was used to compare HAI, CF and Semi-quantitative assessment results fromNCR and reference site (ARAC)

RESULT

Physico-Chemical and EWQI

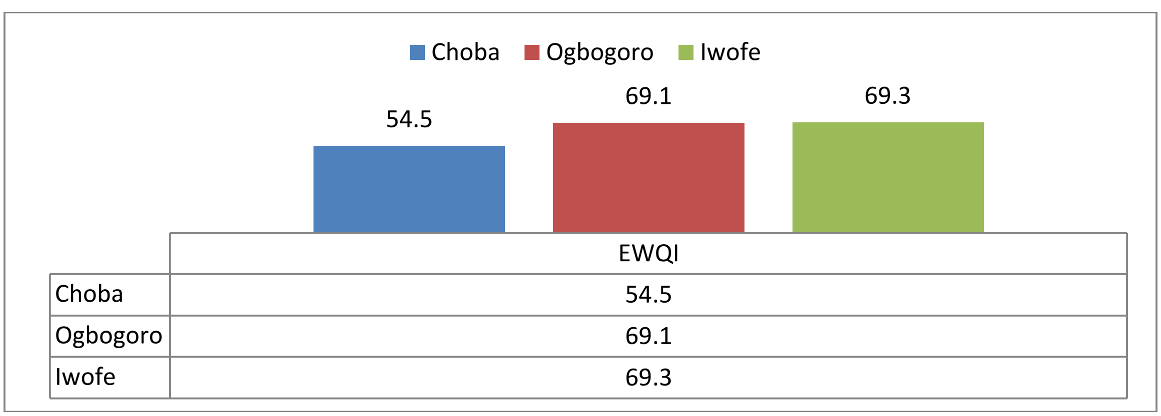
Physical watervariables, such as temperature, pH, Conductivity, and dissolved oxygen (DO), and chemical water variables, such as Cd, Cr, Cu, Pb and Zn were done and recorded (Table 1.1).

Table. 1. Mean values of Physico-Chemical parameters of water from NCR

PARAMETERS	CHOBA	OGBOGORO	IWOFE	GUIDELINES (SON, 2007)
Temperature (oC)	24.3	22.2	24.3	Ambient
pH	5.1	6.7	6.7	5.5-9.0
DO (mg/L)	3.7	4.2	4.5	>5mg/L
Conductivity	1152.5	1343.5	1186.0	1000 (uS/cm)
Cd (mg/L)	<0.001	<0.001	<0.001	0.003 mg/L
Cr(mg/L)	<0.001	<0.001	<0.001	0.05 mg/L
Cu (mg/L)	<0.001	<0.001	<0.001	1 mg/L
Pb (mg/L)	<0.001	<0.001	<0.001	0.01 mg/L
Zn (mg/L)	<0.001	<0.001	<0.001	3mg/LL

SON: Standard Organization of Nigeria

Physico-Chemicalwater parametersvalues were used to mathematically the EWQ index for each station and presented in Fig. 1.1.



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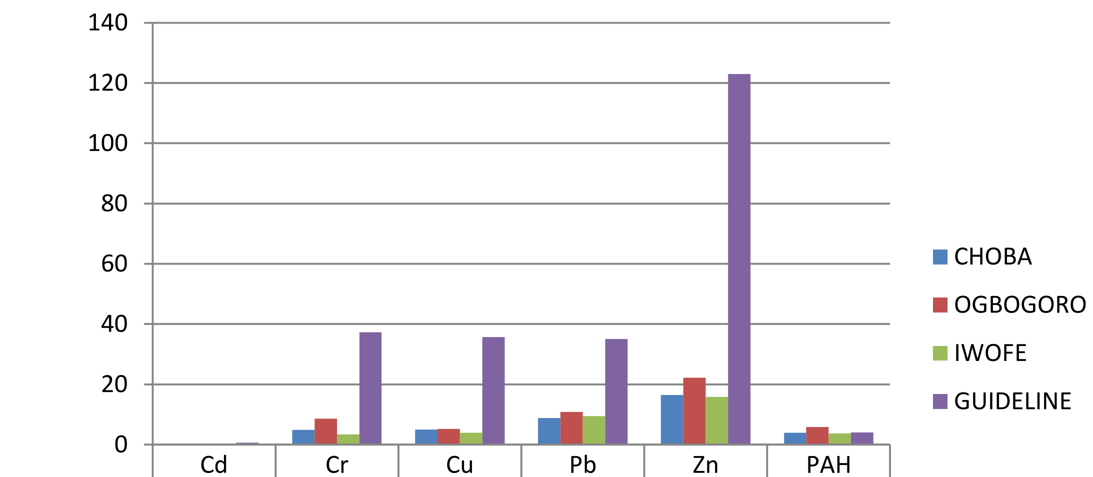
Canadian water quality (CCME, 2001) guidelines for the protection of aquatic life = 0-44(poor), 45-64 (marginal), 65-79 (fair), 80-88, (good) and 89-94 (very good)

Fig. 1: Graph showing the comparison of EWQ Indexes between stations (Choba, Ogbogoro and Iwofe) using CCME (2001) as a guidelines.

Sediment Quality Analysis

The result of Chemicals of Potential Concern (COPC) present in the sediment from the three stations are represented in fig. 2..



	Cd	Cr	Cu	Pb	Zn	PAH
CHOBA	0.04	4.84	5.03	8.82	16.44	3.9
OGBOGORO	0.001	8.62	5.2	10.85	22.22	5.84
IWOFE	0.001	3.45	3.98	9.47	15.84	3.76
GUIDELINE	0.6	37.3	35.7	35	123	4

***Cd, Cr, Cu, Pb& Zn Guideline** = (NOAA, 2009)*
***PAH Guideline** = effects range low based on NSTPA (2006)British Columbia Water Quality Guidelines.*

Fig. 2: Graph showing the comparison of COPC in sediment samples from Choba, Ogbogoro and Iwofe stations and against sediment quality guidelines of NOAA (2009) and NSTPA (2006).

Gross Anatomical Fish Assessment

Fish necropsy

Collected fish specimens were examined for any external macroscopic abnormalities in terms of skin, fins, scales, opercula and eyes. The fish necropsy was performed on fishes harvested from

NCR compared with those acquired from the reference site (ARAC) to determine any internal abnormalities within the visceral cavity. All abnormalities were recorded and Fish HAI score was given on all pathological variables. NCR fishes: Black spots on skin (20%), ectoparasites on 60% of fishes, and 30% fin lesions were noted following external examination. On internal examination, liver exhibited fatty change and focal discolouration (30%) and gills showed frayed and pale to very light colour (30%). ARAC fishes: Black spots on skin (15%), moderate ectoparasites infestation on 15% of fishes, and 10% fin lesions were noted following external examination. No abnormality was noticed on internal examination. Fish from NCR showed to have the higher HAI when compared fish from ARAC (Fig. 3) . One-way ANOVA and Man Whitney tests showed significance differences ($p<0.05$) between experimental sites (NCR) and the reference site (ARAC)

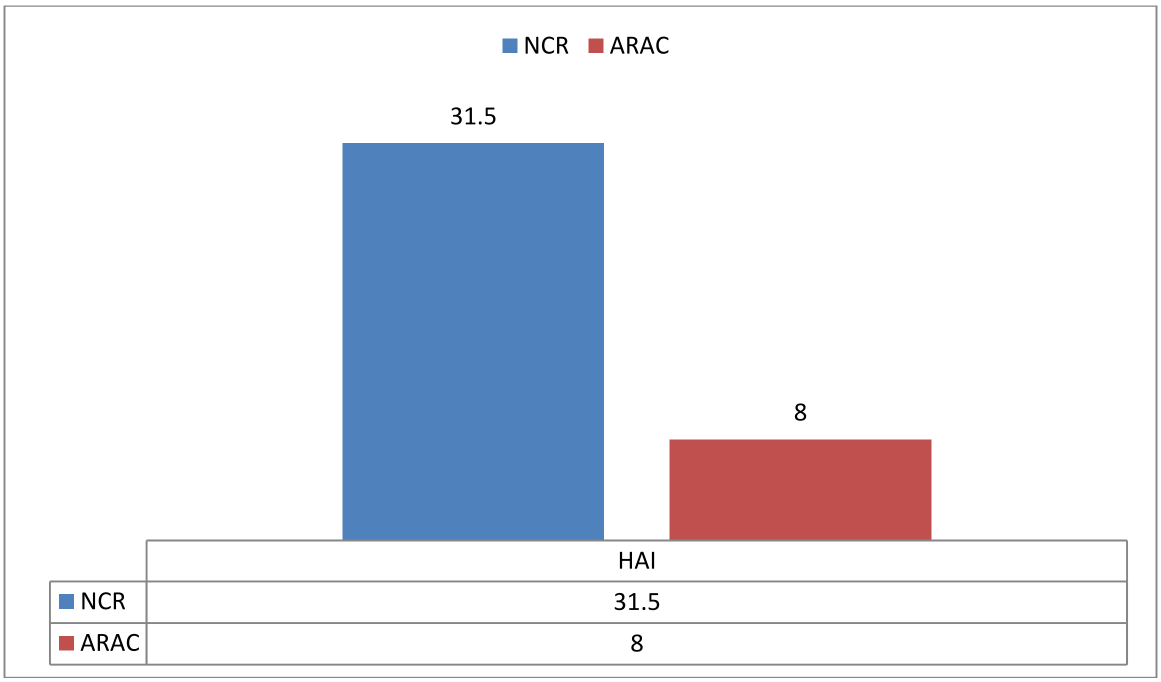
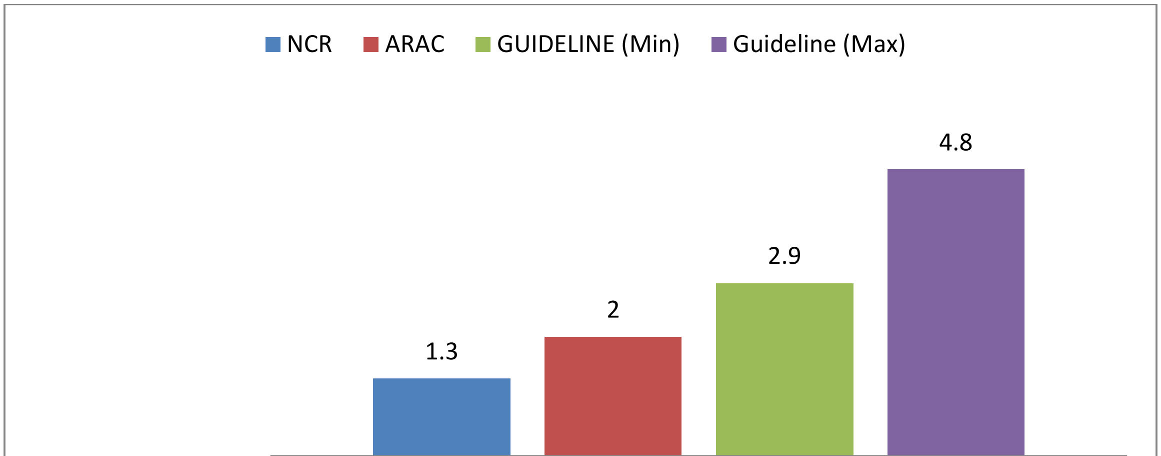


Fig. 3: Graph showing a comparison of the HAI values for C. Nigrodigitatus from NCR and reference site ARAC

Biometrics (Condition Factor, CF)

The CF indices for C. Nigrodititatus from NCR and ARAC were calculated using the total length and weight recorded and the results are represented in fig. 4. The condition factor for fishes from NCR ranged from 0.2 to 2.0 with a mean value of 1.3, while that of ARAC ranged from 0.7 to 2.2 with a mean value of 2.0. There were significant changes or differences between both sampled site when employing or using the one-way ANOVA and Post Hoc tests ($p<0.05$).



	CF
NCR	1.3
ARAC	2
GUIDELINE (Min)	2.9
Guideline (Max)	4.8

Guideline for good Condition Factor for mature fresh water fish (Bagenal, 1978)

Fig. 4: Graph showing a comparison of CF for C. Nigrodititatus from NCR and reference site (ARAC)

Histological Assessment

Qualitative histological assessment

Liver histology and histopathology

A variety of histological alterations were identified in the liver tissue (plate. 4.1A-B). These alterations included: a) MMCs (fig. 5A), inflammatory response, b) fatty change. c) Intracellular deposits were identified as small brown deposits visible within the cytoplasm of the affected hepatocytes. b) Glycogen vacuoles were identified as very small rounded open spaces within the cytoplasm of hepatocytes and were visibly smaller than the other vacuoles. c) Vacuolation was identified as round, empty spaces within the cell cytoplasm and the contents of the cells were most probably dissolved as a result of the H&E staining technique. d) Necrosis of hepatocytes (fig. 5B) was identified as having a darkly stained eosinophilic cytoplasm. e) Oedema was identified as groups of deposits with dark pigmentation within the C. Nigrodigitatus liver tissue. The liver tissue exhibited the most histological alterations than any other selected target organs. All the identified histological alterations and the percentage prevalence are represented in Table 2.

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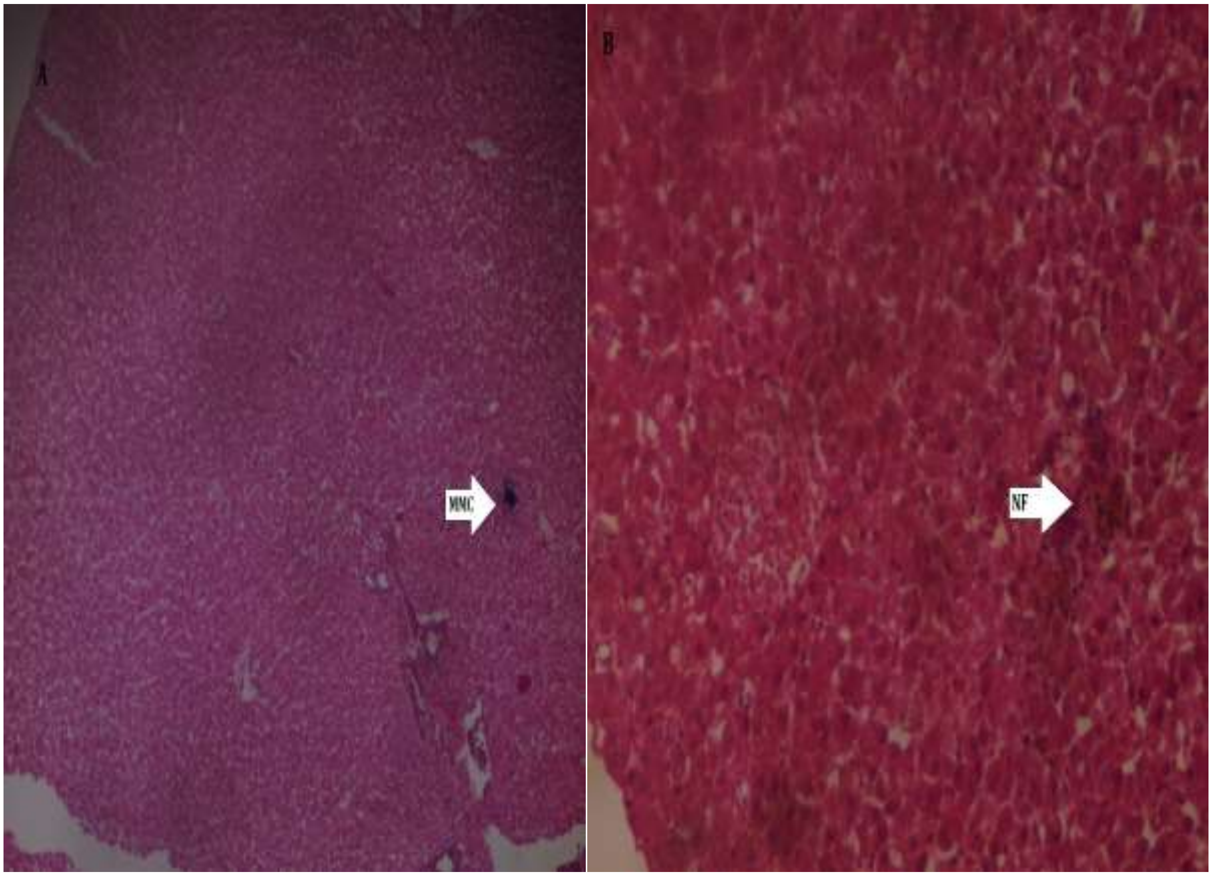


Fig. 5.:photomicrograph of Liver histopathology A (40X) and B (100X) ; a.) MMC. Intercellular deposits b.); NF.Necrotic foci.

Table 2: Percentage prevalence of liver alterations of fish from NCR and ARAC

Alterations	**Prevalence (%)**	
	NCR (n=20)	**ARAC (n=10)**
Circulatory Disturbance (CD)		
Intercellular haemorrhage	15	**0**
Regressive Change (RC)		
Intracellular deposits	60	20
Frank necrosis	50	10
Fatty change	30	40
Vacuolation other than steatosis	55	15
Melano-macrophage centres	30	0
Inflammatory (I)		
Infiltration	20	0
Granulomatosis	30	0
Foci of Cellular Alteration (FCA)		
Vacuolated foci	10	0
Necrotic foci	20	0

Kidney histology and histopathology

Histological alterations noted in the kidney tissue were circulatory disturbance (CD) and regressive changes (RC). These include: a) Necrotic foci (fig.6C) – tubular and interstitious were spotted; b) Necrosis of hepatocytes (fig. 6A) was identified as having a darkly stained eosinophilic cytoplasm. c) Structural alterations were identified (fig. 6A) d). RC found in specimen from ARAC were Interstitialoedema only (see Table 4.2)

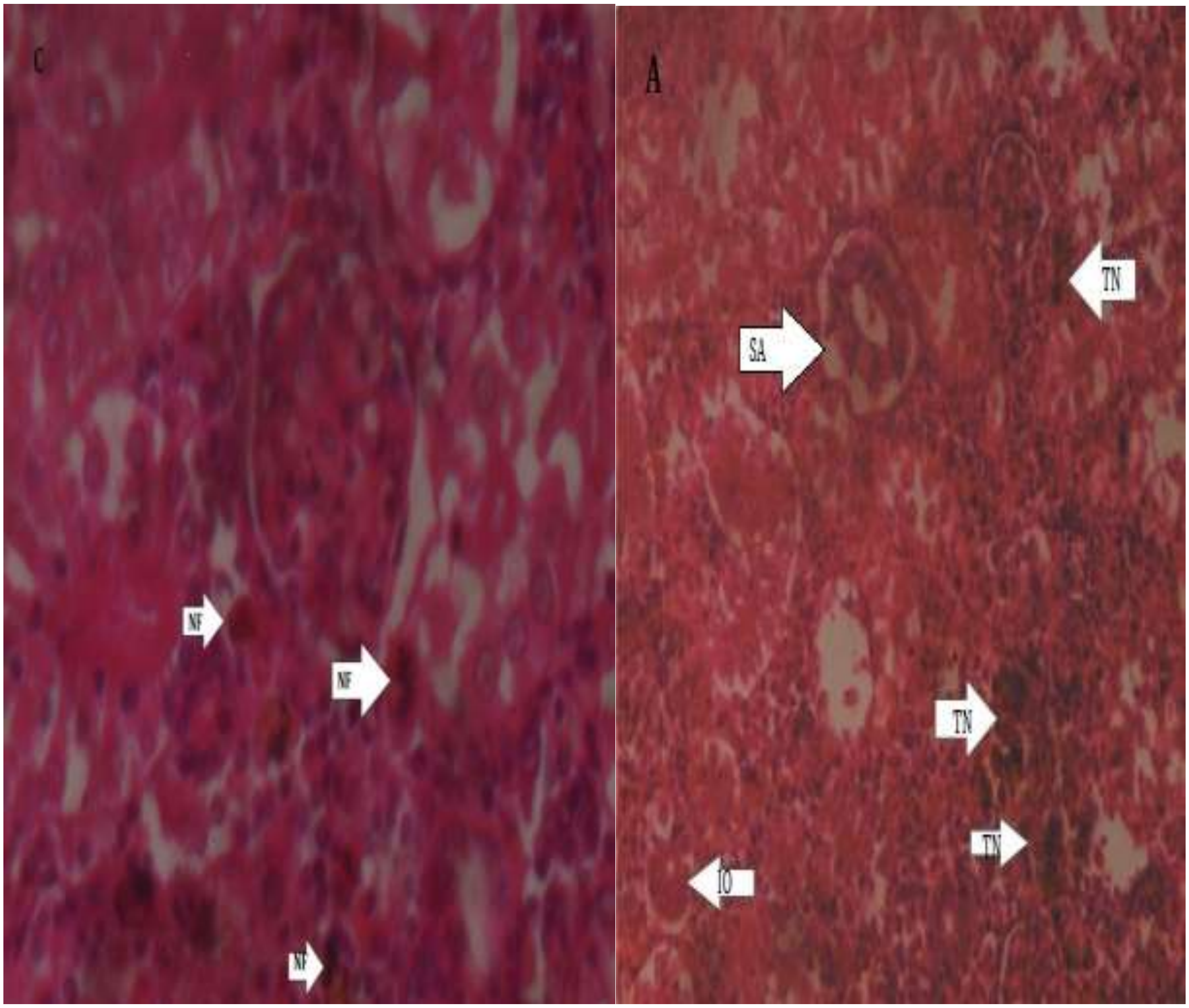


Fig. 6:photomicrograph ofkidney histopathology C (400X) and A (100X): C: showing NF (necrotic foci) – hepatocyte, identified as having a darkly stained eosinophilic cytoplasm. A: showing TN (Tubular necrosis), IO. (Interstitial oedema)and SA. (Structural alteration).

Percentage prevalence of kidney alterations of specimens from NCR and ARAC

Alteration	**Prevalence (%)**	
	NCR	**ARAC**
Circulatory Disturbance (CD)		
Intercellular haemorrhage	30	10
Interstitial oedema		
Regressive Change (RC)		
Architectural &Structural alterations	40	0
Necrosis		
Hyaline droplet degeneration	20	0
Eosinophilic cytoplasm	30	0
Melano-macrophage centres	20	10

Gill histopathology

Qualitative histological assessment results showed that CD and PC were identified in gill tissue (Table 4). CD changes include: Telangiectasia and Epithelial lifting. PC changes spotted were Hyperplasia. The only changes seen in ARAC specimen were epithelial lifting (fig. 7).

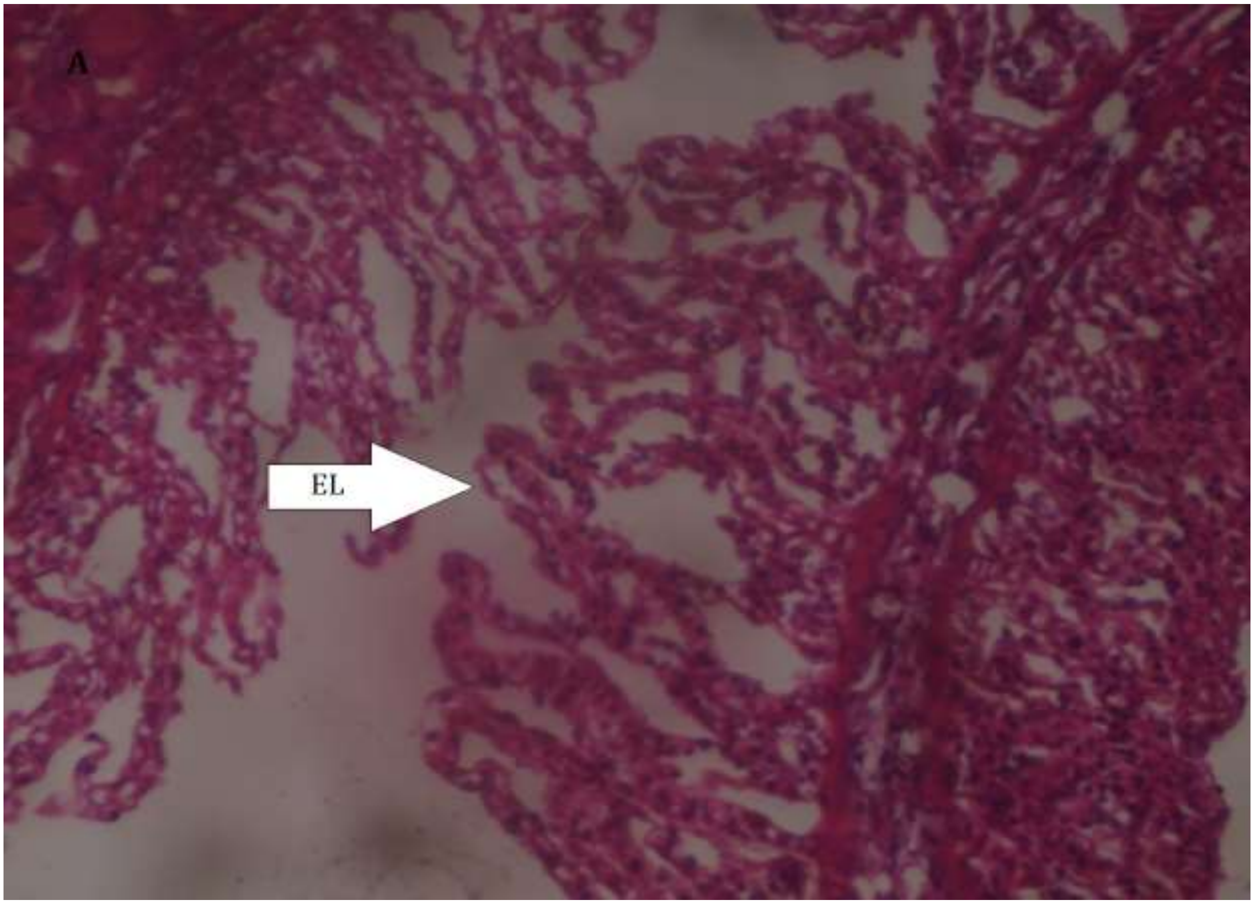


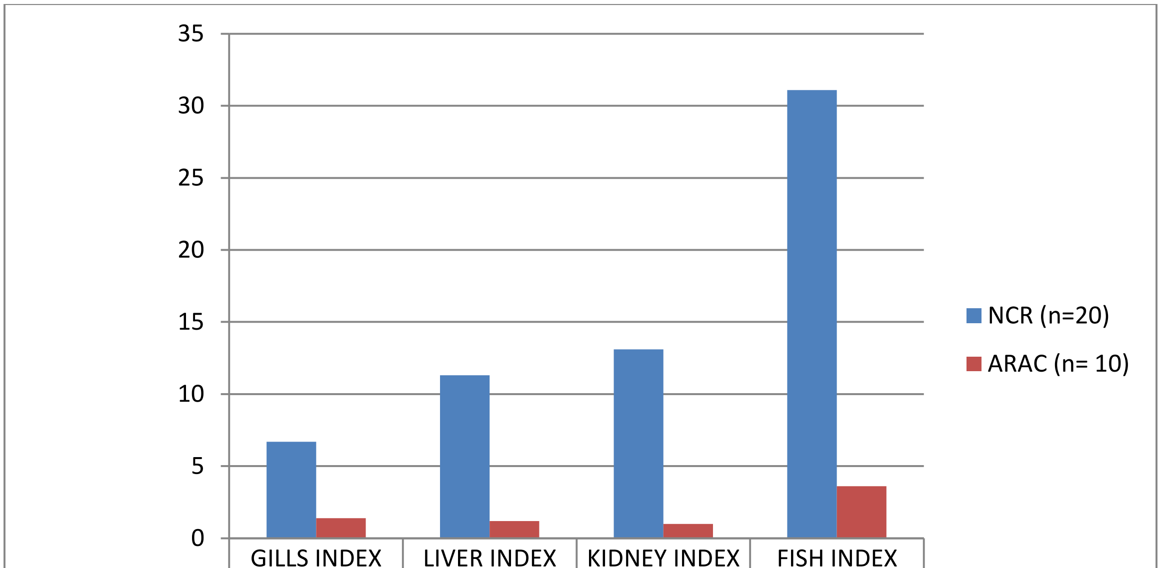
Fig. 7: A Gills histopathology micrograph (400X) showing epithelial lifting of the gills of C. nigrodigitatus.

Table 4: Percentage prevalence of gill alterations of specimen from NCR and ARAC

Alteration	**Prevalence %**	
	NCR	**ARAC**
Circulatory Disturbance (CD)		
Telangiectasia	20	0
Epithelial lifting	40	10
Progressive Change (PC)		
Hyperplasia	20	0

Semi-quantitative Histological Assessment

The qualitative histological assessment data was further quantified according to the protocol by Bernet et al. (1989, modified by Van Dyk et al. (2009a). NCR 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 total organindex or fish results showed that NCR and ARAC had values of 31.1and 3.6 respectively (fig. 8). Following Man Whitney Test statistical analysis, significant differences ($p<0.05$) were noted between NCR and ARAC.



	GILLS INDEX	LIVER INDEX	KIDNEY INDEX	FISH INDEX
NCR (n=20)	6.7	11.3	13.1	31.1
ARAC (n= 10)	1.4	1.2	1	3.6

Fig. 8: Graph showing the comparison ofsemi-quantitative histological assessment of C. Nigrodigitatusfrom NCR and ARAC.

DISCUSSION

The physico-parameters for each of the three sampling stations of NCR was measured and used in determining an environmental water quality index EWQI for the respective stations. The EWQI values for theChoba, Ogbogoro and Iwofe were 54.5, 69.1 and 69.3 respectively. The values showed that the water can be ranked as marginal at Choba station, and fair at the Ogbogoro and Iwofe stations. The EWQI result shows that the water quality gets better as the river flows down stream in the direction of Choba, Ogbogoro and Iwofe. This order of increase in EWQI between station might be due to dilution and flow factors because of the downstream tidal pattern (i.e. the upper reaches of NCR are non-tidal while the lower reaches are tidal) and proximity to the estuary which is a short distance to the Atlantic ocean. This order is also a reflection of the cosmopolitan pattern of the host communities of the station: Choba is more remote to the estuary and more populated with more built-up areas (including the University of Port Harcourt Campus) and a relatively higher soci-economic (a popular Choba market and) and industrial (Ndomiefood factory) along its river bank, while Ogbogoro and Iwofe follows in the same order with lesser population and industrial activities, but more important is their closeness to the estuary leading up to the ocean. The overall EWQI of NCR is an indication of the various anthropogenic activities at those stations. Contaminants such as discharge of untreated or partially treated effluent by various industries at the bank of river, domestic sewage, abattoir waste, runoff water from agricultural lands near the banks of the river may be responsible for the marginal state of EWQI.

Sediment's heavy metals (Cd, Cr, Cu, Pb and Zn) concentration profile values for the three sampling stations of Choba, Ogbogoro and iwofe, were within the normal NOAA (2009) screening guideline range for the protection of aquatic life. Nevertheless, Ogbogoro station recorded the highest values in Cr, Cu, Pb and Zn, while Cd value was highest in Choba. Iwofe recorded the lowest values in Cd, Cr, Cu and Zn, while the lowest values of Pb was recorded in Choba. Of all three stations, Ogbogoro recorded a relatively marginal outcome in heavy metal concentration profile, which calls for concern. PAH concentrationin sediment was highest in Ogbogoro (5.84mg/kg) , which was higher than NOAA (2009) sediment screening guideline for fresh water river (4.0mg/kg). The rest station recorded PAH values less than the NOAA guideline range as follows: Iwofe (3.76mg/kg) and Choba (3.76). The highscreening level ofPAH in Ogbogoro might be a reflection of the high level of oil servicing companies activities in the Ogbogoro station compared to the other stations of the NCR. Although the results of sediment analysis did not show any alarming concentrations of other substances tested for, except for PAH, nevertheless, this expose fishes to mixture of known pollutants. Fishes are known to bioaccumulate chemicals, especially heavy metals and PAH, to a considerably higher concentrations than the levels found in the water or sediment. This is mainly due to the lipid solubility and resistance of these compounds to several degenerative processes in animal tissue (Miranda et al.,2008; Vaclavik et al., 2006). It has also been reported that accumulation of toxic metals can enhence (and particular cases, even directly cause) pathology in fish (Moiseenko et al., 2008) and also interfere with molecular and cellular events, inducing some negative or harmful effects in the energy reserves, resulting in reduced growth and/ or reproduction (Moiseenko et al., 2008) .

The fish necropsy performed enabled the assessment of fish macrmorphormetry and HAI.Macromorphometric details involved the assessment of the fish weight and length relationship otherwise calledconditions factor,CF.InthecurrentstudythemeanCFforC.Nigrodigitatuswas1.3forNCRand2.0forARAC.This meansthatfishesfromARACareinacomparativelybetterhealthyconditionthanthosefromNCR,indicator than ARAC aquatic conditions favours better growth and health conditions.This result comparedfavourablywithotherreportsfromsimilarstudieswithsimilarfishspeciecarriedoutbyAboweiandEzekiel(2013)inAmasomaRiverPlains.Severalstudieshaveshownthataquaticpollutioninducesbiochemicalandphysiologicalchangesinfish.AdecreaseinhealthconditionwouldaffectthenormalphysiologyoffishandinduceorresultsinchangesinbiometricindicessuchasCFandHSI(Kleinkaufetal.,2004).Fish HAIrecorded ascore of 31.5 for NCR and 8 for ARAC. This indicates thatfishharvestedfrom NCR had more observable microscopic lesion and parasite infestation as compared to Fishes ARAC.
ThehighNCRHAIscorewasattributedtoseveralfactorssuchasliverwithfattychange,discoloration andthe presence of ectopara site on the skin and gills. The health assessment index (HAI) adapted from Adams et al. (1993)is a usefully tool that allows statistical comparisons of fish health among

data sets (Adams et al., 1993). Comparisons can be made among sample sites, between different aquatic systems, concurrently or over time to establish temporal patterns in fish population health. The HAI is a simple inexpensive means of rapidly assessing the general health status of fish in field situations. C. Nigrodigitatus from NCR exhibited a higher HAI score as compared to the reference sites (ARAC). HAI is a non-diagnostic tool and thus points to areas of scientific concern that warrant further investigation.

The Histological Assessment done in this study was analysed in qualitative and semi-quantitative scenario. The qualitative analysis involve the description of histological alterations in target organs, hence their discussions are as follows:

Gill histopathology: The gills are sensitive indicators of environmental stress, including exposure to harmful compounds present in aquatic ecosystems as a result of anthropogenic activities (Hinton et al., 1992). The gills in fish are vulnerable to toxicants and irritants because they are in direct contact with the surrounding water and have a rich blood supply to pick up oxygen for respiration from the water (Roberts, 2001). Therefore, functional impairment of gills caused by pollutants can jeopardize the health status of the fish. In the current study, histological alterations in varying degrees were identified in gills. These were mostly focal circulatory disturbances and progressive changes. Circulatory disturbances are related to pathological conditions of blood and tissue fluid flow. Epithelial lifting in focal areas was noted in both NCR and ARAC. Epithelial lifting is characterized by detachment of epithelial cells due to the outflow of serous fluids into the interstices of gill tissue (Van Dyk et al., 2009a). This alteration has been observed in various other studies (Erkmen, 2000; Cengiz and Unlu, 2002, 2003; Bernet et al., 2004 and Vinodhini and Narayanan, 2009a). Epithelial lifting may be a defence mechanism of fish in response to toxicants. The lifting up of epithelium increases the distance through which toxicant has to travel to reach the blood stream (Morgan and Tovell, 1973; Cengiz, 2006). Telangiectasis of the secondary lamellae was noted in fishes from NCR. The appearance of the secondary lamellae results from a collapse of the pillar cell system and breakdown of vascular integrity with a release of large quantities of blood that pushes the lamellae epithelium outward (Alazemi, et al., 1996). Telangiectasia has been reported in mosquito fish, catfish, common carp and Nile tilapia in polluted system or under exposed conditions (Jiraungkoorskul et al., 2002; Cengiz and Unlu, 2002,2003; Cengiz, 2006; Van Dyk, 2009a and Botha, 2010). Progressive changes identified in the study is hyperplasia of mucous and epithelial cells found only in fish from NCR. Cengiz (2006) stated that gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and increase in the toxicant-blood diffusion distance. This defence mechanism takes place at the expense of the respiratory efficiency of the gills and eventually, the respiratory impairment must outweigh any protective effect against pollution uptake. Van Dyk et al. (2009a) identified mucous hyperplasia in fish from a polluted stream while hyperplasia of epithelium has been reported by several authors (Leino et al., 1987; Neskovic et al, 1996; Cengiz, 2006; Chezhian, et al., 2009 and Wani et al., 2011). Generally, on the basis of literature these alterations could be related to exposure to various chemicals and poor water quality. However, these alterations could also be understood as a form of defensive mechanism against exposure to pollutants rather than as irreversible toxic effects.

Liver histopathology: The liver is a detoxification organ and is essential for both the metabolism and the excretion of toxic substances in the body (Hinton and Lauren, 1990). Hence, as a result the liver was the most affected target organ on the basis of macroscopic and histological observationsin the current study. Observed histological alterations include; regressive changes, inflammatory changes and focal cellular alterations. The histological responses in the liver were mostly associated with regressive changes. Regressive changes included vacoulation, fatty change, pleumorphism, necrosis, increase in MMCs and intracellular deposits. Vacuolated hepatocytes are associated with the inhibition of protein synthesis, energy, depletion, dissagregation in microtubule or shift in substrate utilization (Hinton and Lauren, 1990). This alteration along with fatty degeneration was observed in both fish species. Vacoulation as well as fatty change have been reported in previous studies (Jiraungskoorskul et al., 2002; Van Dyk et al., 2007; Sarma et al., 2011). Inflammatory responses were noted in fish specimen from NCR. It is primary a protective mechanism although occasionally may initiate severe disease in certain sites. Necrosis as stated by Roberts (1989) is where cellular damage is not immediately lethal and the changes are often reversible when source of damage is removed. Neurotic foci were Necrotic changes have been related with exposures to algae and phenol (Roberts, 2001; Barse et al., 2006; Abdel-Hameid, 2007). The changes with a high prevalence in NCR were increased in MMCs and intracellular deposits. MMCs are cells that belong to the unspecific immune system (Marigomez et al., 2006). The occurrence of MMCs has been previously described as a normal feature of normal fish liver histology (Brusle and Gonzalez, 1996). However an increase in MMCs has been related to a stress response or a detoxification process to exposure to toxicants. Furthermore, Marigomez et al. (2006) proposed that high MMC prevalence might be the direct consequences of enhanced parasitic infestation. Focal cellular alteration was also identified in NCR. Marigomez et al. (2006) and Carrola et al. (2009) have previously reported the occurrence of this alteration. Generally, C. Nigrodogitatus specimen from NCR showed a higher liver alteration occurrence than the reference site, ARAC. Macroscopic observations support these findings as several liver abnormalities were observed in NCR.

Kidney: the kidney receives the largest proportion of post branchial blood and therefore renal alterations might be good indicators of environmental pollution (Oritz et al., 2003; Cengiz, 2006). In the current study histological alterations in the kidney were identified. These include circulatory disturbance and regressive changes. The circulatory disturbance was intercellular hemorrhage found on both NCR and ARAC fish. Regressive changes included eosinophilic cytoplasm, necrosis, hyaline droplets degeneration as well as increase in MMCs. MMCs were the only regressive change noted in ARAC fishes. Necrotic changes have been observed in fish kidney exposed to various chemicals by various authors (Srivastava et al., 1990; Dass and Mukherjee, 2000; Oritz, et al., 2003 and Silva and Martinez, 2007). Alterations such as necrosis may lead to functional problems ultimately leading to the death of fish. According to Takashima and Hibiya (1995), hyaline droplet degeneration of the epithelial cells is one of the typical changes that occur in the renal tubules. Coarse eosinophilic granules appear in the cytoplasm. The size of the granules often varies from large to small granules. The accumulation of these granules can lead to necrosis (Hinton and Lauren, 1990; Takashima and Hibiya, 1995). Eosinophilic granules of irregular size appear in the cytoplasm in hyaline droplets degeneration and the accumulation of these granules can lead to necrosis (Hinton and Lauren, 1990; Takashima and Hibiya, 1995). Oulmi et al. (1995) found the frequent deposition of brightly stained hyaline droplets degeneration within the cells of the tubules of kidney following exposure to a herbicide. According to Wester and Canton, (1986); Chaudhuri et al. (1999) the presence of hyaline droplets degeneration has been suggested to be indicator of renal toxicity for a variety of chemical including pesticides. However hyaline droplet degeneration was also noted in a reference group in a study by Bernet et al. (2004). In the current study organic chemical such as PAH was significantly present in NCR and could have an effect on the kidney of the fish species studied. Botha (2010) also obtained similar results to that of the current study in C. gariepinus from a polluted site.

Semi-quantitative histological assessment involved the application of a protocol by Bernet et al. (1999). Furthermore, the quantified results were classed based on the classification system according to a scoring scheme by Zimmerli, (2007) to evaluate the degree of histological changes in target organs of fish samples. Results showed that mean liver, kidney and gill indices for C. Nigrodigitatus from ARAC were within class 1 which implies structure with slight histological alterations. The mean gills index for NCR was also within class 1. However the mean liver and kidney indices values for NCR fell in class 2 which is interpreted as the structure with moderate alterations. Although alterations were identified these findings could suggest that the organs are still in a functional state.

CONCLUSION

The EWQI has shown that NCR is of a marginal quality. The PAH concentration in sediment in Ogbogoro station is above the recommended guideline of 4.0 mg/kg. Thedifferenttypesofpathologyfoundinthetestspeciesresultfromcomprehensiveimpactofnumeroustoxicsubstances,foundintheNCRwaterandsediment,onthefish.Histologicalanalysisoffishorgansandtissuesidentifiedextensivealterationstoallorgansexamined,theliver,gillandkidney;manyofthesealterationsareirreversible.Theresultsofthisstudyshowthatwaterandsedimentqualityandlivingconditionsforaquaticspecies,particularlyfish,intheNCRaresubstandard.Theoccurrenceofsignsofintoxicationbased on the findings of his topathological alterations draw a conclusion that the ecological health conditions of NCR are unsatisfactory and give a clear indication that the river is polluted and the need to decrease pollution.

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