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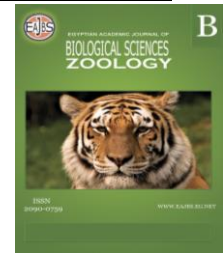


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A comparative Study on Bioaccumulation of BTEX and Oxidative Stress Biomarker Response in Parasitised and Non -Parasitised *Parachanna obscura* (GUNTHER, 1861) from Lekki Lagoon, Lagos, Nigeria

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ABSTRACT

Background: The study was aimed at investigating the bioaccumulation of BTEX in *Parachanna obscura*, its enteric parasite, *Procamallanus sp*, water and sediment collected from Lekki lagoon in Lagos, Nigeria. Parasite intensity, antioxidant enzymes, and lipid peroxidation activities were also investigated in the fish. Ninety-two (92) samples of *P. obscura* were investigated for BTEX and parasitic infection for a duration of six months. Physicochemical parameters were measured in-situ using a handheld multiparameter probe (Horiba Water Checker Model U50). BTEX was analyzed using Agilent 7890B gas chromatography system with FID and 7683 series injectors. Histopathological analysis of the fish intestine was conducted following standard histopathological measures.

Results: BTEX was not detected in the sediment but m+p- Xylene and O-Xylene 6.938 µg/g and 5.854 µg/g were detected in the water respectively. *P. obscura* showed high accumulation of total BTEX in the intestine than in the liver especially Benzene even though benzene was not detected in the water. The concentration of BTEX was similar in ranges for the parasitized and unparasitized tissues. The parasite recovered from the fish was *Procamallanus spp*. The parasite showed little or no depurative capacities for BTEX. The decrease in superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in conjunction with an increase in Malondialdehyde (MDA) characterized relatively higher susceptibility in the fish. Lipid peroxidation damage is one of the foremost damages to cell components that could be inflicted by BTEX.

Conclusions: It is therefore pertinent that regular monitoring of BTEX in the water body as well as in the biota should be undertaken and regulatory agencies need to ensure that all the oil depots and tank farms situated along the Lagos lagoon treat all their storm waters and effluents in order to reduce its hydrocarbon level before discharge into the lagoon to safeguard human health and integrity of the aquatic habitat.

INTRODUCTION

Lagos being a metropolitan city and the center for economic development in Nigeria with over 21 million inhabitants makes it highly vulnerable to environmental pollution. It is located in the low-lying coastal zone of Nigeria rivers, creeks, lagoons, and estuaries are dominant in this coastal landscape. Twenty-five percent of the land mass of the State is water. Epe, Lekki, Lagos and Ologe lagoons are the four major lagoons in the state. Wastes from industries located in the state are sadly often disposed of in these water bodies. In Nigeria, imported petroleum products are stored in tank farms, many of which are in Lagos, and these products are distributed to other parts of the country through pipelines connected all over Nigeria. Nigeria has a wide pipeline network and depots for distributing refined petroleum products (Renner *et al.*, 2008). There is about 5000km of pipelines and about 20 oil depots altogether in Nigeria (Adewuyi and Olowu, 2012). Frequent petroleum spillages, impacting the terrestrial ecosystem, occur due to the vandalization of the pipelines. In Nigeria, a total number of 2,097 oil spill incidents were recorded between 1997 and 2001 (Renner *et al.*, 2008). In 2005, 117 cases of fire outbreaks were recorded as a result of pipeline vandalization and rupture of Nigerian National Petroleum Corporation (NNPC) pipelines (Renner *et al.*, 2008). According to Shell Petroleum Development Company of Nigeria Limited, in the Niger Delta region alone, 1301 oil spill incidents were recorded around SPDC facilities between 2007 and March 2013 resulting from equipment failure, corrosion, or human error (Doherty, 2014).

Surface water contamination caused by anthropogenic activities from point and non-point sources has become one of the main environmental issues and a challenge to the world population. Water contaminations have kept on creating upsetting ramifications for well-being and economic development in Nigeria (Esrey *et al.*, 1991). Contamination of aquatic ecosystems by petrogenic chemicals such as BTEX has been detected in sediment, water, and aquatic flora and fauna worldwide (Smith and Guentzel, 2010), and also in Nigerian water bodies (Enuneku and Ilegomah, 2015), such as Lekki lagoon (Doherty and Otitolaju, 2016). The largest petroleum product depots in Nigeria with a large network of pipelines distribute petroleum products all over the country. Consequently, Lekki lagoon has been subjected to incremental oil spillages due to numerous tank farms which facilitate the importation, storage, and distribution of petroleum products all over Nigeria (Adeyemi *et al.*, 2009; Akinsanya *et al.*, 2015).

Surface run-off and erosion are the sources of diverse pollutants discharged into the rivers. Direct human discharges of wastes, industrialization and agricultural activities at different segments of the rivers no doubt accentuate pollution stress on the aquatic environments and endanger the lives of plants and fisheries resources (Pius and Happiness, 2012). These unhealthy activities have accentuated the levels of toxicants in water bodies. Among the toxicants, BTEX from petrochemical industries has been proven to impact the quality of water with deleterious effects on human health. When the physical and chemical conditions of the ecosystem are changed from their normal ranges, similar changes are expected to occur in the individual organism, populations and the communities of the ecosystem (Adakole, 2000). High levels of the monocyclic aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) have been reported in different areas of the environment by researchers (Akpoborie *et al.*, 2008; De Oliveira *et al.*, 2007; Guimaraes *et al.*, 2010; Osu and Asuoha 2010; Osuji and Achugasim 2010).

The acronym BTEX refers to a specific family of chemicals which are volatile organic compounds. Such compounds include benzene, toluene, ethylbenzene, and

xylene. Natural sources of BTEX are forest fires and volcanoes. These compounds are constituents of crude oil, hence their occurrence in sea water is associated with natural gas and petroleum-related anthropogenic activities. BTEX compounds are among the most produced chemicals in the world. They are associated with the production of petroleum products, paints, lacquers, pharmaceutical products, thinners, rubber products, adhesives, ink, and cosmetic products (Akinsanya, Goh, Lim, & Tinga, 2015).

BTEX compounds have a higher solubility in water than the aliphatics, which are the other organic compounds known to be present in gasoline (Farhadian *et al.*, 2008). A large percentage of petroleum products is made up of BTEX, which is about 18% in standard gasoline essentially because they are added to unleaded petroleum products to increase fuel performance (Budavari, 1996). The main concern over BTEX is their volatile nature, and ability to migrate away from the original areas of pollution. BTEX compounds are well-known toxicants to a wide range of terrestrial biota as well as aquatic organisms (Au, 2004). BTEX, like other hydrocarbons, have a high degree of lipophilicity which enables them to penetrate biological cell membranes, leading to their concentration in fatty deposits (Doherty, 2014).

Benzene is recognized as a carcinogenic compound, while the others (toluene, ethylbenzene, and xylene) are regarded as non-carcinogenic. Several studies have demonstrated the effects of exposure to BTEX on fish. Exposure of fish to other hydrocarbons has also been widely reported (Akinsanya, Goh, Lim, and Tinga, 2015; Sogbanmu *et al.*, 2016).

BTEX (benzene; toluene; ethylbenzene; and o-, m-, and p-xylenes) are classified as priority pollutants regulated by many environmental organizations around the world. These monocyclic aromatics are highly water-soluble and volatile compared to their aliphatic counterparts (Yang *et al.*, 2017). Small aromatics frequently enter the air, soil, sediments, and groundwater due to accidental oil spills, leakage of gasoline and other petroleum fuels from underground storage tanks and pipelines, and improper oil-related waste disposal. Owing to their potential acute toxicity and health hazard to humans and aquatic life, BTEX is often required to be analyzed as one group whenever a hydrocarbon fuel is suspected to have been spilled (especially in relatively confined areas), (Yang *et al.*, 2017).

Benzene, toluene, ethylbenzene, and xylenes are aromatic compounds of similar chemical structure, properties, and behavior in the environment. These compounds are important and widely used solvents and industrial chemicals. As constituents of crude oil, they are present in refined petroleum products in the context of gasoline and other fuels. BTEX compounds are used as indicators for evaluating the size, age, and toxicity of petroleum fuel spills and plumes (Popek *et al.*, 2018). Studies have reported that BTEX compounds are neuro-and hepato-toxins that have the ability to cause significant cognitive and behavioral effects in humans when they are exposed to it (Santiago *et al.*, 2014). Health implications that may arise from chronic exposure to BTEX compounds can range from mild to severe implications including cancer, liver cirrhosis, leukemia and changes to the nervous system (Zhang *et al.*, 2012). BTEX compounds are therefore unsafe at all concentrations to all life forms (Irwin *et al.*, 1997). The persistence of BTEX bioaccumulation tendency and global contamination resulted in the ban and restriction in many countries. Despite the restriction, BTEX is still detected in the environment and in tissue samples. The African snakehead, *Parachanna obscura*, is an emerging aquaculture candidate in Nigeria and the sub-Saharan region as a whole. The species is preferred for its palatable fillet and strong flesh integrity, and because that species is high in protein and fat contents thereby making it is a good healing agent for post-operation patients. Snakeheads are also sold as live, fried, or

smoked fish foods in ethnic markets, beaches and restaurants in India, South-Eastern Asia, Japan, and in Epe Lagoon, Lagos states of Nigeria (Adegbehingbe *et al.*, 2018).

Snakeheads have medicinal uses which have been reported in Malaysia and Indonesia; extracted oils from the species *Channa striata* are used to reduce scarring following surgery. Considering these huge benefits offered by *Parachanna obscura*, it is necessary to exploit the fish for commercial purposes in order to derive its enormous economic gains (Adegbehingbe *et al.*, 2018).

In areas where there are frequent incidences of petroleum product spillage, BTEX is reported as a major compound causing environmental pollution. The consistent pollution of the environment of Lagos with monocyclic aromatic hydrocarbons, represented by BTEX, necessitates constant monitoring and assessment of their impacts. The need to therefore detect and assess the impact of pollution, particularly low concentrations of contaminants, on environmental quality has led to the development of biological markers. Biochemical biomarkers are increasingly used in ecological risk assessment of the ecosystem to identify the incidence and effects of xenobiotics. This is because of their potential to act as an early warning signal against potentially damaging effects caused by stressors (Doherty *et al.*, 2014). Ideally, biochemical biomarkers will identify effects at a subcellular level before they are apparent at higher levels of biological organization (Olsen *et al.*, 2001). Hydrocarbons can cause oxidative stress through the generation of reactive oxygen species (ROS) (Ziech *et al.*, 2010). This will have harmful effects on the cells through deoxyribonucleic acid (DNA) damage, protein oxidation, and lipid peroxidation (Ziech *et al.*, 2010). These harmful effects can be prevented when antioxidant systems are produced to eliminate ROS; the organism in a contaminated environment is, therefore, able to overcome the oxidative stress when this occurs (Ahmad *et al.*, 2004).

The fish species is highly sensitive to petrogenic stressors such as BTEX as it readily bio-accumulates the xenobiotics in its lipid-rich tissues. These chemicals may elicit oxidative stress (Akinsanya *et al.*, 2014; Isibor, 2020) and histopathological injuries (Akinsanya *et al.*, 2015; Akinsanya *et al.*, 2018) which may serve as indices in estimating the exposure of the fish to the toxicants and serve as a guideline in the protection of the entire aquatic habitat. Its piscivorous and insectivorous feeding habit puts it in the vulnerable ecotoxicological position in the food web as the ultimate receptor of the BTEX residue in the various linked food chains. Being linked to multi-bioaccumulation channels (Kalfakakour and Akrida-Demertzi, 2000), the fish may have accumulated an impressive amount of BTEX that may be unhealthy for the consumers. Furthermore, environmental contaminants reduce immunocompetence in many species (Luebke *et al.*, 1997). As a result of exposure to BTEX in the environment, this fish may undergo immunosuppression which may foster susceptibility to parasites. Parasite intensity in fish may also serve as indices in estimating the extent of pollution in an aquatic environment. Accumulation of contaminants by gastrointestinal parasites from their host fish may also ameliorate the concentration in the host (Bosch *et al.*, 2015). Hence, the parasite and microbial load on the fish may serve as a reliable supportive tool with bioaccumulation indices in the determination of the ecotoxicological implications of BTEX in the lagoon. Despite these debilitating perturbations on the water body, an appreciable percentage of Lagos populace still depends on the lagoon for potable and recreational water. Lekki lagoon, Epe also serves as a source of fish for animal protein needs.

BTEX has a variety of health implications. Benzene and Toluene exposure, for example, can result in neurological and cardiovascular symptoms, as well as kidney and liver damage than death. Ethylbenzene has the potential to harm the developing and

nervous systems. Xylene exposure can result in developmental, hepatic, neurological, and renal problems. As a result of the foregoing submissions, it is now necessary to analyze BTEX concentrations in the aquatic environment and compare them to regulatory criteria.

The study was aimed at assessing the bioaccumulation, histopathological alterations, parasitological investigations, immunological and oxidative stress responses of BTEX in *Parachanna obscura* in Lekki Lagoon, Epe, Nigeria.

MATERIALS AND METHODS

Study Area:

The Epe lagoon, Lekki situated in Lagos State, South-Western, Nigeria, lies between longitudes 4 00' and 4 15' E and latitudes 6 25' and 6 37' N (Fig. 1), with a surface area of approximately 247 km and a maximum depth of 6.4 meters, although certain points of the lagoon are shallow measuring less than 3.0 meters deep (Akinsanya *et al.*, 2007). The Lekki lagoon is part of an intricate system of waterways that includes lagoons and creeks that stretches 200 kilometers along the coast of South-Western Nigeria, from the Dahomey border to the Niger Delta. It is nourished by the River Oni, which flows to the north-east, and the Rivers Oshun and Saga, which flow to the north-west. The lagoon opens into the Gulf of Guinea via the Lagos harbor.

Stilt-rooted trees with dense undergrowth of shrubs and herbs such as *Elaeis guineensis*, *Raphia sudanica* and *Cocos nucifera* (Edokpayi *et al.*, 2008) characterize the vegetation of the lagoon. The lagoon which experiences both dry and rainy seasons typical of the southern part of Nigeria supports a major fishery in Nigeria. The rich fish fauna of the lagoon includes *Gymnarchus niloticus*, *Heterotis niloticus*, *Malapterurus electricus*, *Chrysichthys nigrodigitatus*, and *Clarias gariepinus*, *Synodontis Clarias*, *Parachanna obscura*, *Sarotherodon melanotheron* and *Tilapia zilli*.

For the purpose of this study, three stations were randomly selected and sampled from August to January.

Collection of Samples:

A total of 92 *Parachanna obscura* species were collected at the sample stations. These were purchased at Oluwo market from the local fish mongers over 5 trips between the months of August 2020 - January 2021 excluding the month of October due to the nationwide protest. The blood of each fish was extracted using syringes and put in an EDTA (Ethylenediaminetetraacetic acid) sample bottle containing anticoagulant to prevent clotting and transported in a cooler containing ice.

At each sampling site, surface sediments were collected from three positions. Each surface sediment subsample (5 cm in thickness) was collected with a stainless-steel grab of the Van Veen type from an anchored boat. Each sediment sample was wrapped in aluminum foil and placed in a Ziploc bag to prevent contamination and kept in an ice chest during its transportation to the laboratory for analysis.

Water samples were also collected in plastic bottles and transferred to the laboratory for analysis.

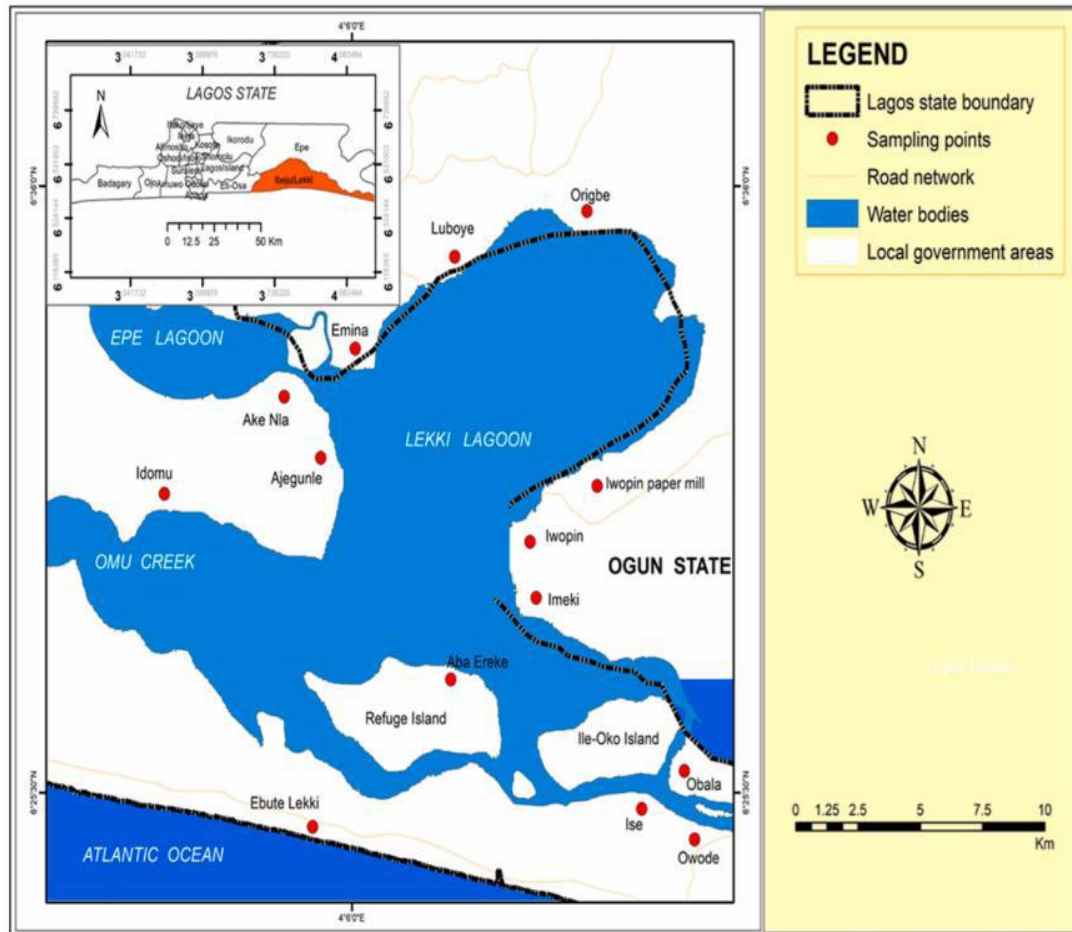


Fig. 1: Map of the Epe Lagoon, Lekki.

Source: Akinsanya *et al.*, 2021

Determination of Fish Morphological Parameters:

The weights, standard lengths, and total lengths of each fish species were recorded. The total length (TL) of the fish was measured from the tip of the snout to the tip of the lobe of the caudal fin while the standard length (SL) of the fish was measured from the tip of the snout to the caudal peduncle. A standard weighing balance (SF-400) was used to measure the weight to the nearest 0.01 gram (g) while a tape rule recorded in centimeters (cm) was used to measure the lengths.

The sex and maturity of the collected specimen were determined by gross examination of the gonads i.e. the presence or absence of testis or ovaries.

The condition factor of the fish describes the state of well-being of the fish. This could be translated to how fatty or lean the fish is. It is represented mathematically as the ratio of the weight of the whole fish to the cube of its standard length. Generally, specimens of fish exhibiting higher weight are considered to be in a better condition and vice versa.

$$K = \frac{100W}{L^3}$$

Where K= condition factor

L= Standard length in centimeters

W= weight of fish in grams

Determination Of Physicochemical Parameters:

Physicochemical parameters such as Dissolved oxygen (DO), Electrical Conductivity, PH, Salinity, Temperature, Total Dissolved Solids (TDS) and Turbidity

were measured using a handheld multiparameter probe (Horiba Water Checker Model U-10) with the aid of a motorized canoe.

Determination of BTEX in Water, Soil and Biological Media:

BTEX was determined in all media with the aid of a gas chromatography-mass spectrometer (GC-MS).

Determination of oxidative stress biomarkers:

The post mitochondria fraction of the organs of the animal was prepared for the analysis by weighing the liver and intestine of the fish and then homogenized with 0.1 phosphate buffer (PH 7.2) by putting the organs each into a mortar which was blended with a pestle till a consistent mixture is observed. The resulting homogenate was centrifuged at 2500 rpm for 15 min. The supernatant was decanted and stored at -20 °C.

Triglycerides:

Triglycerides were analyzed in the 65 fish samples using the enzymatic method described by Tietz (1990).

Glucose:

The glucose concentrations in the liver and intestine of the 16 uninfected and 49 infected fish were determined within 30 min of collection using the method of Wedermeyer and Yasutake (1977).

Catalase (CAT):

Catalase (CAT) was assayed calorimetrically at 620nm and expressed as moles of hydrogen peroxide (H₂O₂) consumed /min/ mg protein as described by Quinlan *et al.* (1994). The reaction mixture (1.5ml) contained 1.0ml of 0.01M pH7.0 phosphate buffer, 0.1ml of Plasma and 0.4ml of 2M H₂O₂. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). The specific activity of catalase was expressed as moles of reduced per minute per mg protein.

Superoxide Dismutase (SOD):

Superoxide Dismutase activity in liver homogenates was determined using the procedure described by Marklund and Marklund (1974). The method is based on the ability of SOD to inhibit the autoxidation of pyrogallol. In 970µL of buffer (100 mM Tris - HCl, 1mM EDTA, pH 8.2), 10µL of homogenates and 20µL pyrogallol 13mM were mixed. An assay was performed in thermostated cuvettes at 25°C and changes in absorption were recorded by a spectrophotometer (Spectronic 20D, Philips model PU 9100, manufactured in England, 2018) at 480nm. One unit of SOD activity was defined as the amount of enzyme that can inhibit the auto-oxidation of 50% of the total pyrogallol in the reaction.

Glutathione (GSH):

Reduced glutathione (GSH) was determined by the method of Ellman (1959). To the liver homogenate 10% TCA was added and centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH8.0). The absorbance was read at 412nm.

Glutathione Peroxidase (GPx):

Glutathione peroxidase catalyses the reduction of hydrogen peroxide and lipid peroxide into water and lipid alcohol through the oxidation of reduced glutathione (GSH) into glutathione disulphide (GSSG) (Arthur, 2000). Samples were incubated using hydrogen peroxide in the presence of glutathione for a particular time period. The amount of utilized hydrogen peroxide is then determined by directly 5, 5'- estimating GSH content using Ellman's reagent.

Malonaldehyde (MDA):

Malondialdehyde (MDA) an index of lipid peroxidation was determined by adding 1.0 ml of the supernatant was added to 2 ml of (1:1:1) TCA-TBA HCL reagent (thioarbituric acid 0.37%, 0.24 n HCL and 15% TCA) tricarboxylic acid-thioarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 mins, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 mins. The supernatant was removed and the absorbance read at 532 against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of $1.5 \times 10^5 \text{ M/cm}$.

Histopathological Analysis:

The liver and intestine tissues were preserved in Bouin's fluid for 6 h and then decanted. Afterwards, 10% of phosphate-buffered formalin was added to preserve the tissue. Random selection was made from the preserved tissues for analysis. The selected tissue was routinely dehydrated in an ascending series of alcohol of 1% at 30 min intervals. The liver and intestine tissues were then embedded in molten paraffin wax and allowed to solidify. The blocked tissues were sectioned at 4-5 microns, processed and stained with haematoxylin and eosin (H&E) stains. The stained tissues were washed off in tap water. The tissues were then mounted using DPX mountant dried and examined under the binocular dissecting microscope (American Optical Corporation, Model 570) at the pathology laboratory of the department of veterinary pathology, University of Ibadan, Nigeria where the samples were taken for analysis and recording.

Quality Assurance/ Quality Control:

All standards replicate, and blanks were prepared at the same time and used immediately to prevent contamination or compromise of quality. The standards were calibrated, and the calibration curves were verified with ICV standards. The ICV standard was prepared from an independent (second source) material at or near the mid-range of the calibration curve. The acceptance criteria for the ICV standard were $\pm 20\%$ of its true value. The analysis data for the ICV was kept on file with the sample analysis data. The calibration curve was verified at the end of each analysis batch and after every 20 samples using continuing calibration verification (CCV) standard and a continuing calibration blank.

A certified Standard Reference Material (SRM) was prepared with each analytical batch of samples using the same preparation method as that employed for the samples with the frequency of 1 in 20 samples per matrix. The SRM results for each analyte were validated to be within the specifications supplied by the vendor or within 75 - 125% of the true value. Samples that exceeded the linear calibration were diluted and reanalyzed to a sensitive line for which quality control data was already established. All reagents used were analar grade which was permissible and standard reagents for laboratory analysis as obtained from the vendor with their certificate of analysis. Gases purchased from the gas vendor were of high purity as shown in the certificate of analysis. Standard reagents, of high purity with certificate of analysis, were obtained from certified manufacturers. Storage and handling of all reagents and standards are strictly in compliance with the safety precautions necessary as indicated in the MSDS of each respective reagent/standard.

All glassware was treated with chromic acid before being washed with detergent. They then cleaned all glassware by detergent washing with hot water, and rinse with tap water, distilled water and acetone and oven-dried at 150 to 200 °C for 30 min. The volumetric flask was rinsed with dichloromethane only. After drying and cooling, they were sealed and stored in a clean environment to prevent post-cleaning contamination.

1. Ethical Permission:

Ethical approval was obtained from the University of Lagos College of Medicine's health research ethics committee with reference number CMUL/HREC/05/20/724.

Statistical Analysis:

The data obtained from bioaccumulation analysis, oxidative stress analysis, morphometric values and physicochemical parameters were evaluated using Analysis of variance (ANOVA), Statistical Package for the Social Sciences (SPSS), IBM 20.0 version. The condition factor of the fish species of the Lagoon ecosystem was calculated. The condition factor (K) of the fish was also calculated using the formula;

$$K = \frac{100W}{SL^3}$$

Where W = weight in gram

L = standard length (cm)

K = condition factor

Parasitic mean intensity was calculated using the formula according to Ezewanji *et al.*, (2005).

$$\text{Percentage Prevalence} = \frac{\text{Number of infected fish}}{\text{Number of fish examined}} \times 100$$

(Akinsanya *et al.*, 2014)

$$\text{Parasite Abundance} = \frac{\text{Number of collected parasites}}{\text{Number of fish examined}} \quad (\text{Akinsanya } et al., 2014)$$

$$\text{Mean intensity} = \frac{\text{Number of collected parasites}}{\text{Number of infected fish}} \quad (\text{Akinsanya } et al., 2014)$$

Immunology analysis data were evaluated using Microsoft Office Excel.

Pearson Chi-Square, Correlation and Regression analysis were also used. To analyze the prevalence, mean and median intensity of parasite infestation Quantitative parasitology software (QP, version 3.0) was used.

RESULTS

The mean concentration of BTEX in the infected liver of *Parachanna obscura* as shown in Table 1 were benzene with a mean of (77.4848), toluene with a mean of (9.8822), ethylbenzene with a mean (6.68685), m+p- Xylene (7.1226) and o- Xylene (6.9120) (µg/g). Total BTEX detected was (1.0809E2) (µg/g). Chlorobenzene was not detected.

The mean concentration of BTEX in the uninfected liver of *Parachanna obscura* detected were benzene with a mean of (1.2513E2), toluene with a mean of (9.6060), ethylbenzene with the mean (6.6722), m+p- Xylene (7.2468) and o- Xylene (6.9946) (µg/g). Total BTEX detected was (1.5565E2) (µg/g). Chlorobenzene was also not detected (Table 2).

In the intestine of the fish, the mean concentration of BTEX was higher in the infected intestine (1.9457E2) (µg/g) than in the uninfected intestine (1.6045E2) (µg/g) as shown in Table 3. Benzene had the highest concentration in both the infected (1.6080E2) and uninfected intestine (1.2700E2) (µg/g) followed by Toluene (10.8944) (µg/g) in the infected intestine and (10.1126) (µg/g) while Ethylbenzene, m+p-Xylene, and O-Xylene were in a similar range of values of (7.3562), (7.3850) and (7.2630) (µg/g) respectively in the infected intestine and (7.1618), (7.3034), and (7.1798) (µg/g) respectively in the uninfected intestine. Chlorobenzene on the other hand showed the lowest concentration in both infected (0.8764) (µg/g) and uninfected intestine (1.6916) (µg/g), however, its

concentration in the uninfected intestine was a tad bit higher than in the infected intestine.

Table 1: Mean Concentration of BTEX ($\mu\text{g/g}$) in the Infected Liver of *Parachanna obscura*

Parameters	N	Mean	Std. Deviation	Std. Error Mean	p-value
Benzene	5	77.4848	62.273	27.848	.050
Toluene	5	9.8822	0.299	0.134	.000
Chlorobenzene	5	.0000	0.00	0.00	NA
Ethylbenzene	5	6.6868	0.21502	0.096	.000
m+p-Xylene	5	7.1226	0.09249	0.041	.000
O-Xylene	5	6.9120	0.21316	0.095	.000
Total BTEX	5	1.0809E2	61.88418	27.675	.017

Table 2: Mean Concentration of BTEX ($\mu\text{g/g}$) in the uninfected Liver of *Parachanna obscura*

Parameters	N	Mean	Std. Deviation	Std. Error Mean	p-value
Benzene	5	1.2513E2	117.49731	52.54639	.076
Toluene	5	9.6060	.82486	.36889	.000
Chlorobenzene	5	.0000	.00000 ^a	.00000	.000
Ethylbenzene	5	6.6722	.26416	.11814	.000
m+p-Xylene	5	7.2468	.15468	.06917	.000
O-Xylene	5	6.9946	.63544	.28418	.042
Total BTEX	5	1.5565E2	117.79290	52.67859	NA

Table 3: Mean Concentration of BTEX ($\mu\text{g/g}$) in the infected intestine of *Parachanna obscura*

Parameters	Mean	Std. Deviation	Std. Error Mean	p-value
Benzene	1.6080E2	147.70182	66.05426	.072
Toluene	10.8944	0.79252	0.35443	.000
Chlorobenzene	0.8764	1.95969	0.87640	.374
Ethylbenzene	7.3562	0.39457	0.17646	.000
m+p-Xylene	7.3850	0.18547	0.08294	.000
O-Xylene	7.2630	0.37846	0.16925	.000
Total BTEX	1.9457E2	145.38231	65.01694	.040

Table 4: Mean Concentration of BTEX ($\mu\text{g/g}$) in the Uninfected Intestine of *Parachanna obscura*

Parameters	Mean	Std. Deviation	Std. Error Mean	p-value
Benzene	1.2700E2	136.78394	61.17164	.106
Toluene	10.1126	0.64640	.28908	.000
Chlorobenzene	1.6916	2.31729	1.03632	.178
Ethylbenzene	7.1618	0.55750	.24932	.000
m+p-Xylene	7.3034	0.18034	.08065	.000
O-Xylene	7.1798	0.48155	.21535	.000
Total BTEX	1.6045E2	137.69129	61.57741	.060

There was no adsorption of BTEX on the bottom sediment from the overlying water of Epe Lagoon (Table 5). BTEX was not detected in the sediment of the Epe

lagoon but it was detected in the water. The total BTEX detected in the water is 12.792 µg/l. m+p-Xylene and O-Xylene were the only components found in the water with values of 6.938 µg/l and 5.854 µg/l respectively. Benzene, toluene chlorobenzene, and ethylbenzene were not detected in the water.

Table 5: Mean Concentration of BTEX in water (µg/l) and sediment (µg/g)

Components	Soil	Water	Adsorption
Benzene	0.000	0.000	0
Toluene	0.000	0.000	0
Chlorobenzene	0.000	0.000	0
Ethylbenzene	0.000	0.000	0
m+p-Xylene	0.000	6.938	0
O-Xylene	0.000	5.854	0
Total BTEX	0.000	12.792	0

A total of Ninety-two specimens collected were subjected to parasitological analysis. The helminth parasite *Procamallanus spp (Spirocamallanus)*, phylum Nematoda was recovered. A total of 37 fish samples were infected by the *Procamallanus spp (Spirocamallanus)* as shown in (Table 6). These parasites were recovered from the gastrointestinal tract of *Parachanna obscura*. The prevalence of intestinal helminth infections was 40.2% for the combined sexes (Table 7).

The prevalence of *Procamallanus spp* in relation to the size of P. Obscura is represented in Table 8. The length groups 10-15 cm had a prevalence of 40.0%, 16-20 cm recorded 0% of infection, the highest length groups 21-25 cm recorded 57.9% of infection, 26-30 cm recorded a prevalence of 47.6%, while 31-35 cm recorded a prevalence of 44.4%. There was however significant relationship between sex and the number of helminth parasites found in both the population samples. The results of gastrointestinal helminth infections in *Parachanna obscura* show that the female specimens recorded a prevalence of 57.6% which suggests that they are more susceptible to parasitic infections than the male specimens with a prevalence of 33.3%.

Table 6: Overall prevalence of parasitic infection recorded in *Parachanna obscura*

Total number examined	92
Total number infected	37
Percentage of infection	40.2

Table 7: Prevalence of intestinal helminth infections in relation to sex in *Parachanna obscura*.

	Male	Female	Total
Number examined	66	26	92
Number infected	22	15	37
Percentage of infection (%)	33.3	57.6	40.2

Table 8: Prevalence of parasitic infection in relation to the length of *Parachanna obscura*.

Length cohorts (cm)	10-15	16-20	21-25	26-30	31-35	36-40
Total Number examined	5	10	19	48	9	1
Number infected	2	-	11	20	4	-
Percentage of Infection	40.0	-	57.9	47.6	44.4	

The order of BTEX components in the fish parasites was chlorobenzene > m+p-Xylene > ethylbenzene > o-xylene > benzene > toluene (Table 9). The highest component of BTEX detected in the parasite was chlorobenzene which had a range of 4.316- 54.984 $\mu\text{g/g}$ and a mean of 21.289 $\mu\text{g/g}$. This was followed by m+p-Xylene with a range of 7.068 to 12.357 $\mu\text{g/g}$ and a mean of 8.813 $\mu\text{g/g}$. The next in the decreasing order was ethylbenzene which ranged from 6.280- 10.422 $\mu\text{g/g}$ and had a mean of 7.633 $\mu\text{g/g}$. O-xylene ranged from 6.072 to 8.575 $\mu\text{g/g}$, with a mean of 6.823 $\mu\text{g/g}$. The concentration of benzene in the parasite ranged from 0.00 to 8.885 $\mu\text{g/g}$, while the mean was 2.962 $\mu\text{g/g}$. Toluene was not detected in the parasite of the fish.

Table 9: Concentration of BTEX ($\mu\text{g/g}$) in parasite (*Procamallanus spp.*)

Components	MIN	MAX	MEAN
Benzene	0.000	8.885	2.962
Toluene	0.000	0.000	0
Chlorobenzene	4.316	54.984	21.289
Ethylbenzene	6.280	10.422	7.633
m+p-Xylene	7.068	12.357	8.813
O-Xylene	6.072	8.575	6.823
Total BTEX	23.74	95.22	47.52

Histopathological Alterations in The Fish Tissues:

The results of the histopathological alterations in the fish tissues are shown in plate 1. The fish host shown different pathological consequences such as mild stunting of villi, thickening of the muscularis mucosa and loss of villous structure. Consequently, these pathological alterations will affect absorption in the fish host and affect the marketability of the fish host.

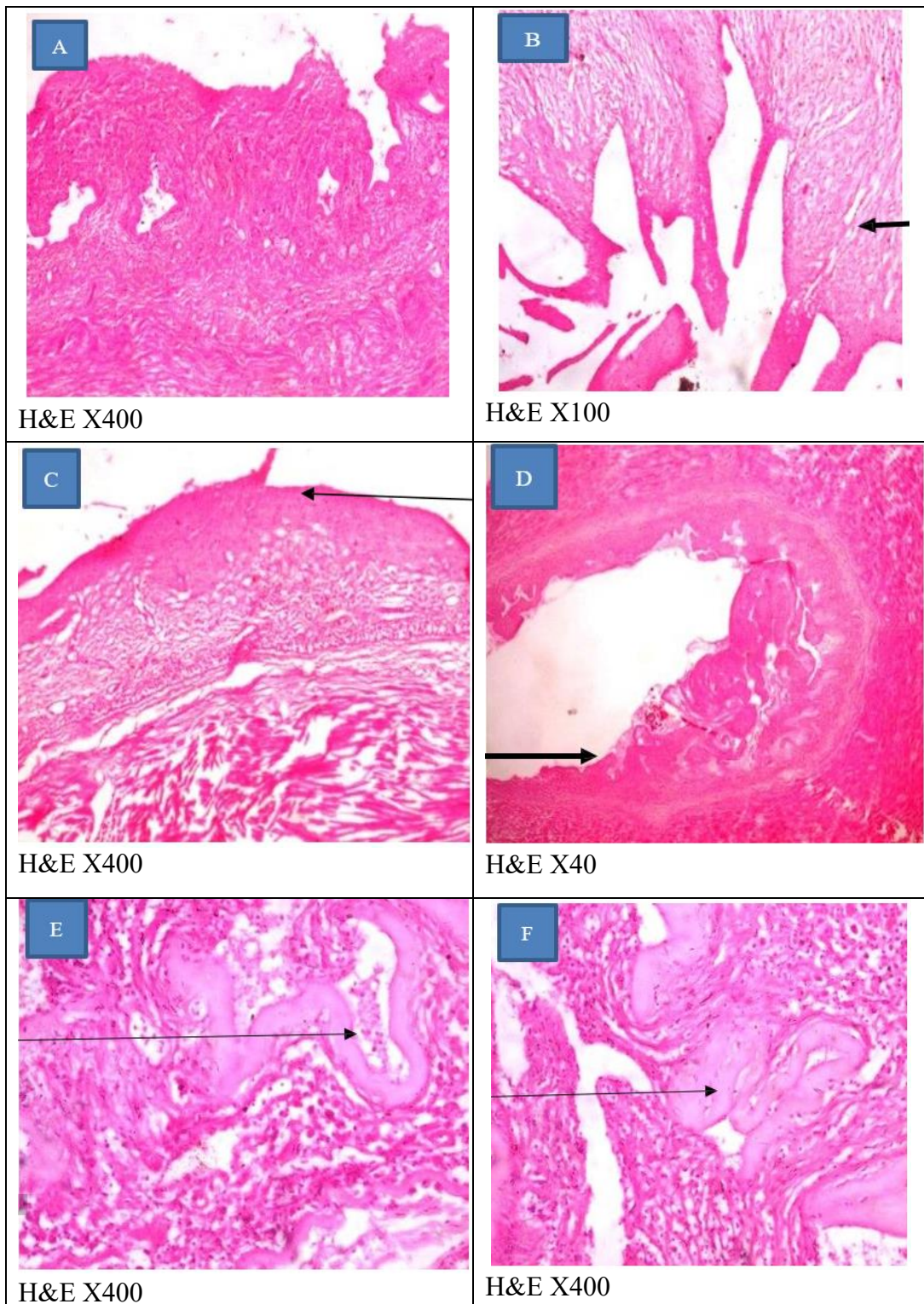


Plate 1: Histopathological alterations in the fish host:

A: Photomicrographs of intestinal tissue show normal villi structure, normal mucosa, submucosa and muscularis with well-preserved crypt-villous architecture, **B:** Photomicrographs of intestinal tissue show a mild increase in the connective tissue of the submucosa (black Arrow), **C:** Photomicrographs of intestinal tissue show focal area of loss of villous structure (slender Arrow), **D:** Photomicrographs of intestinal tissue show mild stunting of villi and loss of intestinal glands (Thick black arrow), **E:** Photomicrographs of intestinal tissue show mild presence of detritus within the lumen, **F:** Photomicrographs of intestinal tissue show mild thickening of the muscularis mucosa (slender arrow).

DISCUSSION

A comparison of the total BTEX content in water and sediment shows that BTEX was absent in the sediment but present in water. The isomers of xylene m+p-Xylene and o-xylene were detected in the water at concentrations of 6.938 and 5.854 respectively. However, other components such as Benzene, Toluene, chlorobenzene, and ethylene were not detected. A probable cause for this is that BTEX easily gets into the water before reaching the sediment and even if they do get into the sediment they can also be released into the water after binding. This observation correlates with the findings of Akinsanya *et al.*, (2020) where BTEX and PAH accumulation in fish water and sediment was studied in Epe Lagoon. Although BTEX was detected in the sediment in this study, its concentration, however, was lower in comparison to the concentration in water. However, this report contrasted with investigations by Isibor *et al.*, (2021) which showed higher concentrations of BTEX and PAH congeners in the sediment medium than in the water which may be attributable to the repository nature of the bottom sediment in conjunction with the hydrophobic nature of the contaminants. Also worthy of note is that effluents from industries release BTEX into water bodies, as they are being used as solvents especially those that are involved in oil and gas. Domestic hazardous wastes have also been implicated as sources of BTEX. Robinson *et al.*, (2005) reported the presence of BTEX in human care products, pesticides, pharmaceuticals, and some detergents. All this increases the concentration of these compounds in municipal waters, hence may explain the reason for the higher BTEX content in water over sediment. Doherty and Otitolaju (2016) also reported higher total BTEX content in water in comparison with sediment from the Lagos lagoon.

Fishes are able to concentrate or store certain substances in the tissues of their body in amounts that are higher than those in their surrounding environment and are described as accumulators (Beeby 2001). They are able to provide important information on the number of chemicals in their habitats and by extension provide knowledge about the biological availability of target substances. In this study, BTEX was found in the intestine and liver of *Parachanna Obscura* in high concentrations above the prescribed limits and standards however, this concentration was more in the intestine than in the liver. This could be attributable to the detoxifying function of the liver by either making the chemical more water-soluble or by bio-transforming it to less harmful substances (Akinsanya *et al.*, 2020). Akinsanya *et al.*, (2019) also reported a lower average total concentration of BTEX in the liver of the fish *Chrysichthys nigrodigitatus* compared to the intestine.

Benzene was not detected in water or sediment but had the highest concentration in the fish followed by toluene. This would mean that there are other solid substances in water that the fish feed upon that harbor these chemicals. Furthermore, some other aquatic animals may have accumulated these chemicals in their bodies from other habitats, and have over time migrated to the water body sampled in this study.

Higher concentrations of BTEX components in the intestine and liver of the fish than permissible limits indicate a serious prognostic of health and environmental challenges worthy of specified research to ascertain. Though, the intestine of the fish accumulated higher concentrations of the BTEX than the liver, concentrations in both tissues are alarmingly higher than set standards; particularly the concentration of benzene which may elicit harmful effects on consumers of the fish. Health effects of accumulated benzene include disruption of the bone marrow, thereby causing decreased red blood cells, hence anemia (Akinsanya *et al.*, 2019). A comparative assessment of BTEX and heavy metals Bioaccumulation in *Chrysichthys nigrodigitatus* in Epe Lagoon

also showed that BTEX accumulated more in the intestine of the fish than in the liver (Akinsanya *et al.*, 2019). The concentration of BTEX in the infected and uninfected intestines of the fish showed no significant difference. This also holds true for the infected and uninfected liver of the fish. This explains that the parasite was not able to reduce the toxicant load of BTEX in the fish to a significant level.

Parasites are ubiquitous and are capable of infecting every living organism. In aquatic organisms, the presence of parasites in the host is in equilibrium with their elusive lifestyle on the planet (Mansour *et al.*, 2002). Parasites are known to attack their hosts and parasitic diseases can be transmitted from one host to another with grave consequences. So, the presence of parasitic infections in fishes is an indicator of environmental stress (Schludermann *et al.*, 2003). Scientists are however beginning to prove that parasites do not only affect the health of the host organism but can also interact with pollutants in the aquatic environment. Studies have shown that unpredictable or contradictory results emerge if infected animals are used in ecotoxicological research without considering the possible effects of parasites on biomarker responses (Sures *et al.*, 2017). Sures, (2003) also justified the use of parasites for metal monitoring with their excellent accumulation capacity.

The total BTEX concentration recorded in the parasite was 47.52. Benzene which was significantly high in the intestine and liver of fish was the lowest in the parasite while toluene was not detected. Overall, the rate at which the parasite accumulated BTEX was quite low. This probably explains the reason for similar ranges of BTEX concentration in both parasitized and unparasitized intestines and liver. This implies that the parasite, *Procamallanus* spp is a poor accumulator of BTEX. Interestingly, Akinsanya *et al.*, (2019) in a similar study reported that the parasite *Wenyonia acuminata*, infecting *Synodontis clarias* in the same study area did not bioaccumulate BTEX from fish. In another study by Isibor *et al.*, 2020 the concentration of BTEX in the intestine of the *Malapterurus electricus* fish indicated that its enteric parasite *Electrotaenia malopteruri* significantly sequestered the toxicant's burden from the fish. Although the parasites were generally of low intensity in the fish, they, however, exhibited some ability to sequester chlorobenzene, ethylbenzene, o-xylene, m+p-xylene, and 1,4-dichlorobenzene from the intestine of the fish.

Activities of CAT and SOD are two indicators of oxidative stress. There is usually a rise in ROS and reactive metabolites as a result of the interactions between different enzyme systems, including detoxifying enzymes (Machala *et al.*, 1997). In this study, the liver and intestines of *P. obscura* showed an increased level of MDA. Increased malondialdehyde (MDA) levels are commonly considered an indicator of lipid peroxidation derived from oxidative stress anomalies promoted by exposure of fish to pollutants (Garcia *et al.*, 2020). This may be attributed to the elevated oxidation of molecular oxygen (O₂) to produce superoxide radicals, an indication of the important roles of the fish tissues, particularly the liver in the detoxification process (Sreejai *et al.*, 2010). Increased MDA levels could also be a result of impairment in antioxidant enzymes due to enhanced ROS formation. This could lead to alterations in the cell membrane and cellular dysfunction (Kaur *et al.*, 2017). Lipid peroxidation damage is one of the foremost damages to cell components that could be inflicted by BTEX. The antioxidant defense system includes enzymes such as SOD, CAT, glutathione peroxidase (GPx), glutathione S-transferase (GST), and other low molecular weight scavengers such as GSH (Storey 1996). The activities of antioxidant enzymes such as GPX were also increased especially in the intestine (84.72) and liver (42.41). The GPx detoxifies H₂O₂ or organic hydroperoxides that are produced in lipid peroxidation. Total protein was 10.48 in the intestine and 16.66 in the liver. Excess protein can also be a sign of

bone marrow disorder. BTEX has been implicated as a cause of bone marrow disorder in humans.

Histopathological biomarkers are useful tools in the determination of aquatic ecotoxicological impacts (Oliveira Ribeiro *et al.*, 2006). The present study revealed significant alterations in the intestinal tissues of *P. obscura* with increasing length. This is attributable to an increase in appetite (characterized by increased feeding rate) in fish with greater lengths. Histopathological alterations observed in the intestinal tissues are attributable to outcomes of the interactions between the xenobiotics and the oxidative defense system of the fish. Lipid peroxidation damage is one of the foremost damages to cell components that could be inflicted by BTEX.

The immune system is part of an organism's biological defense against infections or foreign substances. There are some review papers on fish immunotoxicology although limited. Dunier (1996) discussed the immunosuppressive effects of pollutants from industry (effluents, heavy metals) and agriculture (pesticides) on freshwater fish as a sentinel model for the aquatic environment studies on fish.

In this study, immunoglobulin G levels were assessed in *Parachanna Obscura* using the fish immunoglobulin G Elisa kit. IgG has the function of promoting phagocytosis by phagocytic cells and promoting the degradation of extracellular microorganisms and toxins. Fish P7 and P2 showed the highest concentrations of igG in their blood 43.683 and 42.017 respectively. This could mean either of two things; there is some form of exposure to a contaminant or parasitic infection or to both. This could mean that there is a high level of infection and the immune system of the fish is producing more immunoglobulin G to counteract this effect. Interestingly, when the immune system is compromised, the system will produce less immunoglobulin G even though there is an infection or exposure to a contaminant. Although the number of scientific studies is small, there have been reports that strongly suggest an increase in the risk of infection caused by a decrease in immune function due to exposure to chemical substances (Rodsather *et al.*, 1977).

Conclusion

This study provided information on surface water quality characteristics, levels of BTEX in sediment, surface water, biota and parasite samples from the Epe Lagoon. The current study is a reflection of the negative impact of man on the environment as observed in the sensitivity to changes in the tissue histology, chemical composition of fish and parasites and the biochemical response from the fish host. Physicochemical parameters were within the standard range according to FEPA 2003. However, this does not give an accurate representation of the quality of the water. The use of biological organisms can provide early warning signals of pollution in the environment. A high concentration of BTEX in *P. obscura* of Lekki lagoon compromised the immunity of the fish, thereby increasing the levels of *Procamallanus* infection. Findings ascertained that the water had adequate conditions of BTEX but *P. obscura* had accumulated dangerous levels of contaminants over time. The parasite *Procamallanus* accumulated low concentrations of BTEX considering the high concentration in the fish. This implies that the parasite had low accumulation potential and therefore could not reduce the burden of BTEX on the fish. This explains the high concentration of MDA and GPX in the fish indicative of oxidative stress.

It is therefore pertinent that regular monitoring of BTEX in the water body as well as in the biota should be undertaken in order to mitigate the environmental pollution and forestall the ensuing debilitating challenges. It may also become important for researchers to begin to consider the use of parasites as an early warning signal which requires caution so as to prevent escalation of the pollution status of the water body.

There is therefore an urgent need for the regulatory agencies to ensure that all the oil depots and tank farms situated along the Lagos lagoon treat all their storm waters and effluents in order to reduce their hydrocarbon level before discharge into the lagoon to safeguard human health and integrity of the aquatic habitat.

List of Abbreviations

SOD: Superoxide dismutase

MDA: Malondialdehyde

CAT: Catalase

GPx: Glutathione peroxidase

BTEX: Benzene, toluene, ethylene and xylene

Ethics approval/ consent: Ethical approval was obtained from the University of Lagos College of Medicine health research ethics committee with reference number CMUL/HREC/05/20/724.

Availability of Data and Materials: The authors declared that all the data obtained for this research are available

Competing of interests: The authors declare that there are no competing interests in this research.

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