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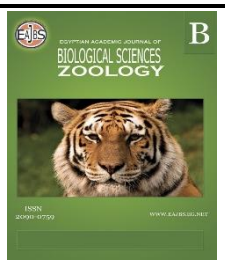

Bioaccumulation of Polybrominated Diphenyl Ethers (Pbdes), Oxidative Stress Biomarker Response and Histopathological Alterations in *Malapterurus electricus* (Gmelin, 1789) From Lekki Lagoon, Lagos, Nigeria

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ABSTRACT

Poly-brominated Di-phenyl Ethers (PBDEs) are important chemical flame retardants, added to many consumer products to reduce the spread of fire. This study was aimed at assessing the bio-accumulation of poly-brominated diphenyl ethers (PBDEs), oxidative stress, and histopathological alterations in *Malapterurus electricus* from Epe Lagoon, Lagos, Nigeria. Water samples were collected from three different stations of the lagoon and the physiochemical parameters were analyzed using Horiba. Also, fish specimens of *Malapterurus electricus* were randomly selected from vendors at the Epe Lagoon, Lagos Nigeria. These specimens were then dissected to remove intestine, liver and parasite for examination. The excised liver, intestine and parasite (*Electrotaenia malopteruri*) were then taken to the laboratory to analyze for bioaccumulation of PBDEs, oxidative stress, and histopathological assessment. Results showed that the physico-chemical parameters of water body had their mean values either below or within established permissible limits. BDE-183 had the highest concentration accumulated in the liver meanwhile BDE-209 congener was not detected at all across the intestine, liver and parasite. The oxidative stress response parameters, GPx (glutathione peroxidase) was highest for both intestine and liver, GPx, and SOD (superoxide dismutase) were higher in the uninfected fish than the infected one. Histopathological findings in the uninfected fish showed normal villi structure, normal mucosa, sub-mucosa, and muscularis while the infected fish showed a mild increase in the connective tissue of the sub-mucosa and a focal area of loss of villous structure. The results reflect the biological effects of PBDEs pollution on the aquatic organisms and in combination with oxidative stress markers and histopathology of the guts; the endangered state of the Epe lagoon was unveiled.

INTRODUCTION

In accordance with established scientific literature, Polybrominated diphenyl ethers (PBDEs) represent a class of halogenated organic compounds renowned for their fire-

retardant properties, as detailed by Madgett *et al.* (2022). These substances are integral components in the fabrication of various commercial and consumer goods, including but not limited to textiles, paint, building materials, automobiles, polyurethane foams used in furniture, mattresses, carpets, car seats, plastics, and electrical appliances. They are extensively employed as non-reactive additives in textiles, polyurethane foams, thermoplastics, and electronic products, as elucidated by La Guardia *et al.* (2012). The composition of PBDE congener groups, such as TriBDEs, HexaBDEs, HeptaBDE, and NonaBDE, varies in their respective proportions, as discussed by La Guardia *et al.* (2012) and Winid (2015).

PBDEs have emerged as a significant environmental pollutant due to their recalcitrant nature and capacity for bioaccumulation, resulting in adverse toxic effects. Elevated concentrations of PBDEs have been detected in air, water, sediment, and soil within densely populated regions, particularly in proximity to manufacturing, recycling, and waste disposal facilities, which constitute point sources of PBDEs in non-living media, as demonstrated by Osuala *et al.* (2020). PBDEs are characterized by their persistence, lipophilicity, and accumulation in the adipose tissues of animals. Human exposure to PBDEs may occur through the consumption of contaminated foods, particularly those with high-fat content, such as fatty fish and crabs. The presence of PBDEs in the environment is regarded as a significant concern due to their potential carcinogenic and endocrine-disrupting properties, as noted by Vonderheide et al. (2008). Consequently, these factors prompted the European Union (EU) to enact a ban on the production and use of PBDEs in August 2004, in accordance with Harrad and Porter (2007).

As indicated in prior research findings, Nigeria has been identified as one of the African nations functioning as a recipient of electronic waste, thus serving as a significant source of Polybrominated Diphenyl Ethers (PBDEs) in the environment, a fact established by Leung *et al.* (2010). Comprehensive investigations have unveiled the presence of PBDEs in virtually all environmental compartments, encompassing both indoor and outdoor environments, aquatic ecosystems, particles, and sediments within water bodies, terrestrial mammals, avian species, marine mammals, fish, and even in human populations, all exhibiting PBDE levels deemed to be of concern, a pattern observed in studies conducted by Chang *et al.* (2020), Covaci *et al.* (2005), Adewuyi and Adeleye (2013), Wang et al. (2015), and Kalantzi *et al.* (2011).

The persistence and toxicity of PBDEs in the environment have raised mounting apprehensions, particularly considering that their presence and adverse effects have been reported across various continents, indicating a widespread global issue, in accordance with findings by Fayiga and Ipinmoroti (2017) and Zhang *et al.* (2016).

Polybrominated Diphenyl Ethers (PBDEs) are recognized as crucial chemical flame retardants that find application in an array of commercial and household products, including but not limited to electronic components, circuit boards, plastics, furniture, carpets, toys, paints, foams, and rubber, with the primary purpose of retarding the spread of fires. However, it is essential to acknowledge that when introduced into the environment, PBDEs assume the status of environmental contaminants. The release of PBDEs into the environment is chiefly attributed to the production, utilization, and disposal of products and materials treated with PBDEs, a phenomenon well-documented in the research conducted by Shaw and Kanaan (2009) and Lee and Kim (2015).

Tetra, Penta, Octa, and Decca BDEs are the most common PBDEs presently used. Their log Kow value is around 6.15, 7.32, 8.65 and 12.61 respectively. PBDEs exhibit very low water solubility and high binding affinity to lipid tissues. Low water solubility and high adsorption affinity to substrates increase their persistence in the environment. Due to their remarkable persistence, Polybrominated Diphenyl Ethers (PBDEs) are ubiquitously distributed in the atmosphere, water bodies, and sediment matrices. Within aquatic

ecosystems, these compounds exert their influence on aquatic organisms through a dual mechanism. First, they are encountered directly through partitioning from the surrounding water. Second, PBDEs enter the food web, where their concentrations are subject to amplification, a phenomenon known as biomagnification. Owing to their hydrophobic properties and tenacious persistence, PBDEs tend to accumulate within biological tissues, giving rise to potential disruptions of the endocrine system and the induction of neurotoxic effects.

Among the various aquatic organisms, fish hold a position of paramount importance, given their crucial role as a primary protein source for human consumption. In this context, the electric catfish, scientifically known as Malapterurus electricus, is a species that inhabits the western and central regions of tropical Africa, including the Nile River. This species thrives in a wide array of major freshwater systems across the African continent and holds particular culinary appeal, particularly when prepared in a smoked form, making it a favored choice among consumers.

The contamination of freshwater with Polybrominated Diphenyl Ethers (PBDEs) presents a potential hazard to the well-being of aquatic organisms, including fish, and this, in turn, carries indirect implications for human health. Even though numerous PBDE congeners have been phased out or prohibited, the existing reservoir of these compounds in the environment retains its capacity to inflict harm, specifically in terms of endocrine disruption and neurotoxic effects, due to the enduring nature of these chemicals. Therefore, it is imperative to maintain vigilant monitoring of the presence of these substances in the environment, even after regulatory measures have been enforced, as emphasized by Gaion *et al.* (2021).

While the occurrence of PBDEs in freshwater fish species has drawn some attention, the available data remain somewhat limited. Recent times have witnessed mounting concerns surrounding PBDEs and other brominated flame retardants, owing to a substantial upsurge in PBDE concentrations over the past three decades, as highlighted in the research by Akinsanya *et al.* (2022). Consequently, there is an imperative need for further research endeavors focused on PBDEs to furnish valuable insights into strategies for mitigating and controlling their potential adverse effects.

The Epe Lagoon has faced increased anthropogenic activities such as domestic waste deposition, fishing, indiscriminate sand mining and inland water transportation. This was due to the rapid population growth, and agricultural and urban development experienced at her bordering wetland over the past decade (Akagha *et al.*, 2020). Consumer products such as foam cushions, clothing, bedding, computers, electronics, carpets lightings and other textiles containing PBDEs as flame retardants; dumped into the lagoon as disposables constitute great harm to the aquatic life therein, as they alter the hydrochemistry of the lagoon. Studies have shown that PBDEs of the lagoon are capable of causing endocrine disruption in fishes and that because of their low water solubility and high binding affinity are very persistent in the environment, resulting in their high bioaccumulation and bio-magnification potential (Akinsanya *et al.*, 2022).

The demand for fish protein has exponentially increased due to the rapid increase in human population. *Malapterurus electricus* are highly desired as food among consumers, especially when they are smoked. It is desirable to know the health status of the fish population as these fish are being consumed by man. Consequently, what would affect fish would indirectly affect man. Thus, this study was conducted to examine the effect of the accumulation of the contaminant; Poly-brominated Diphenyl Ethers (PBDEs) on the liver, intestine and parasite inherent in the electric catfish, *Malapterurus electricus*. Information gathered from the study will be useful in developing strategies for protecting fish health, conservation, management, and sustainability of fish in aquatic bodies which in turn would protect human health.

The enteric parasites of both terrestrial and aquatic organisms are useful bioindicators because they are affected by anthropogenic and natural environmental factors (Vidal-Martínez *et al.,* 2015). Their communities are far less speciose than those of free-living organisms, especially in benthic marine environments. Their taxonomy and life cycles are relatively well-known. From a parasite point of view, each host is an island (or habitat), and statistically, each host becomes a 'sampling unit' with its own set of parasite species. Parasites of top predators are also considered top trophic consumers, and consequently good bioindicators of food web accumulation (Tellez and Merchant, 2015).

Due to their specific biology and physiology, aquatic parasites show a high affinity to accumulate metals, whereas the uptake of some lipophilic substances (e.g. PCBs, PAHs) was found to be rather low until recent times (Le *et al.,* 2014). Lipophilic chemicals mainly accumulate in fat, whereas hydrophilic substances are distributed more evenly among tissues. Most of the parasites are not able to produce their fatty acids and therefore rely on taking them up from the host. As a consequence, they have a low percentage of fat and are not able to bio-concentrate lipophilic substances above the levels of the host tissues (Le *et al.,* 2014).

Although most PBDEs have been banned, the ones that already exist are still capable of causing harm due to their persistent nature (Gaion *et al.,* 2021). It is therefore important to monitor closely the presence of these chemicals in the aquatic environment. Besides, fish forms an important part of the human diet and therefore the aquatic environment should be monitored for pollutants possibly present. Furthermore, although some attention has been received as regards the occurrence of PBDEs in freshwater species, the data available is still limited. There is a need for more research in this line to provide sufficient data that can be acted upon for control on mitigation.

The aim of this study was to assess the bioaccumulation of polybrominated diphenyl ethers (PBDEs), oxidative stress and histopathological alterations in *Malapterurus electricus* from Epe Lagoon, Lagos, Nigeria.

MATERIALS AND METHODS

1. The Study Area:

The Epe Lagoon is found in Lagos State, South-West, Nigeria. It lies between latitudes N 06^0 31.893' E 003^0 31.912' and longitudes N 06^0 33.710' E 004^0 03.710'. It has a surface area that is above 243 km^2 and a maximum depth of about 2.8 meters (Uwadiae, 2010, Edokpayi *et al.,* 2008). The lagoon is fresh, lotic and non-tidal in characteristics. It is sandwiched between two lagoons, the Lekki Lagoon (freshwater) in the east and the Lagos Lagoon (brackish water) in the west (Akagha *et al.,* 2020, Uwadiae, 2010). It connects to the sea through the Lagos Harbour. The vegetation around the lagoon is characterized by stilt-rooted trees with dense under-growths of shrubs and herbs; Oil palm; *Elaeis guineensis*, Coconut palm; *Cocos nucifera*, and Raffia palm; *Raphia sudanica*, (Edokpayi *et al.,* 2008). Fauna present in this lagoon includes Electric catfish; *Malapterurus electricus*, African sharp-tooth catfish; *Clarias gariepinus*, Bagrid catfish; *Chrysichthys nigrodigitatus* Aba Knife fish; *Gynmnarchus niloticus*, red-tailed synodontis; *Synodontis clarias*, and African arowana; *Heterotis niloticus*.

2.Determination of Water Physicochemical Parameters:

The dissolved oxygen (DO), electrical conductivity, pH, salinity, temperature, total dissolved solids (TDS), and turbidity were assessed using a handheld multiparameter probe (Horiba Water Checker Model U-10). A motorized canoe was used to collect data from the lagoon.

3. Determination of PBDEs in Environmental Media:

Environmental media such as *M. electricus* samples, surface water samples, bottom

sediment samples, and fish enteric parasites. One hundred and twenty (120) samples of lifeless but fresh *M. electricus* fish were procured from the fishermen. Surface water samples were collected using sampling bottles, and bottom sediment using Van Veen grab sampler. Both samples (n=120) were randomly collected at scattered points in the lagoon.

3.1.Determination of PBDEs in Water Samples:

The EPA Method 3510C was adopted in the extraction of organic fractions from the water samples (Lee *et al.*, 1982) and was employed.

100 mL of water sample was measured into a 250 mL separatory funnel, and then extracted three times with 20 mL of methylene chloride, giving ~60mL of final extracting solvent. Cover and shake the separatory funnel vigorously for 1 – 2 mins, periodically venting the funnel to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of 10 min and then the organic layer into a clean beaker/round-bottom flask. Sample extracts were concentrated (organic layer) to about 1 – 2 mL using a rotary evaporator prior to fractionation for PBDEs. Then add about 1 – 3 spatula, full of activated Sodium sulphate to the concentrated extract, in order to eliminate water/aqueous portions (Barlas and Akbulut, 2006).

3.2.Determination of PBDEs in Sediment Samples:

The Microwave-assisted method (Barlas and Akbulut, 2006), was used in the extraction of organic fractions from the sediment samples. Into a 250 mL of Teflon bottle, about 10g ± 0.05g of the homogenized sample was weighed and then about 1 – 3 spatula, full of activated sodium sulphate were added to the samples in the Teflon bottles so as to eliminate any water/aqueous portions. 20mL of 1:1 acetone: hexane was employed for the extraction procedure thrice, resulting in ~60mL of final extracting solvent. Then, the covered Teflon bottles were sonicated in an ultrasonic bath for 30 minutes at a temperature of 70 °C. The organic layer was decanted into a clean round-bottom flask/beaker, and further dried with sodium sulfate. A clean-up procedure using a silica gel column was carried out and then the sample extract was concentrated to ~2 mL using a rotary evaporator prior to analysis using a gas chromatography-mass spectrometer (GC-MS).

3.3.Determination of PBDEs in Biological Tissues:

In this analysis, KOH Refluxing/Vortex Extraction (Vassilaros *et al.*, 1982) – EPA Method 3611C cleanup method was employed. 15 g wet weight of excised liver and intestine samples of the fish specimens was weighed into a crucible and then macerated and homogenized. From the homogenized tissue, 10 g was taken and placed in a 50 mL centrifuge tube, and 15 mL of 6N KOH was added to it, the tubes were now sealed and incubated for 18 h in a 35 ºC water bath, shaking vigorously for 30 seconds for every ½ hour for the first 4 h and was allowed to cool later on.

15 mL of methylene chloride or ethyl ether was then added to the centrifuge tube, vortex for 1 min and centrifuged at 2000 rpm for 5 min to ease phase separation. Using a Pasteur pipette, the upper/aliquot layer was removed into a 250 mL round-bottom flask. Solvent centrifugation was repeated twice and all aliquots fractions were combined in the round-bottom flask. Prior to fractionation cleanup, sample extracts concentration to about 5 – 10 mL was carried out by rotary evaporator using alumina gel column and GC-MS analysis.

4. Parasite Collection, Preservation and Identification:

In compliance with ethical standard practice lifeless but fresh samples (n= 210) of *P. obscura* were used as specimens for the study. For easy identification, the specimens were arranged on a work table. With the aid of a dissecting set, the body cavity of each fish was dissected from the throat to the vent. The gastrointestinal was detached and cut into sections. The liver and intestine were immediately placed in clean Petri dishes containing normal saline solution to remove dirt and clear. The parasites recovered were counted and recorded after which they were fixed in 70% alcohol (Opara and Fagbemi, 2008) in a sample bottle.

All parasites found were preserved and transferred to vials after being thoroughly sealed and labelled. Parasites were identified using the standard taxonomic keys and photomicrographs. The total parasite collected was 210 enteric parasites using Akinsanya et al. (2007).

5. Biochemical Analysis:

5.1.Homogenizing Sample:

The dissected liver and intestine were removed and weighed. The organs were homogenized with 0.1phosphate buffer (PH 7.2) putting the organ into the mortar and blended with a pestle together. The resulting homogenate was centrifuged at 2500 rpm for 15 min. The supernatant was decanted and stored at -20°C.

5.2.Determination of Catalase Activity (CAT):

Catalase activity will be determined according to the method of Sinha (1971). The reaction mixture contains 5% Potassium heptaoxodichromate, 0.2 M Hydrogen Peroxide, Dichromate/acetic acid solution and 0.01 M Phosphate buffer pH 7.0. 0.1 ml of the sample will be mixed with 4.9 ml of distilled water to give a 1 in 5 dilution of the sample. The assay mixture will contain 4 ml of 0.2 M Hydrogen Peroxide and 5 ml of 0.01 M Phosphate buffer in a 10 ml flat bottom flask. 1 ml of properly diluted enzyme preparation (test sample) will be rapidly mixed with the reaction mixture by gentle swirling motion. The reaction will run at room temperature. 1 ml portion of the reaction mixture will be withdrawn and blown into a test tube containing 2 ml of dichromate/acetic acid reagent at 60-second intervals for 3 mins. An extinction coefficient for H_2O_2 at 240 nm of 40.0 M-1cm-1 would be used for the calculation.

5.3.Determination of Reduced Glutathione (GSH):

The total sulphydryl groups, protein-bound sulphydryl groups and free sulphydryl groups like GSH in biological samples can be determined using Ellman's reagent, 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) as described by Sedlak and Lindsay (1968) and Jollow *et al,* 1974. This method is based on the development of a relatively stable yellow complex formed as a result of the reaction between Ellman's reagent and free sulphydryl groups. 0.2 ml of sample will be mixed with 1.8 ml of distilled water to give 1 in 10 dilution. About 3 ml of precipitating reagent (4% sulphosalicyclic acid) will be added to the diluted sample and then allowed to stand for 10 minutes for precipitate to occur. 0.5 ml of supernatant will be withdrawn and added to 4 ml of phosphate buffer followed by 0.5 ml of Ellman's reagent. The blank will be prepared with 4 ml of 0.1 M phosphate buffer pH 7.4, 1 ml of diluted precipitating solution and 0.5 ml of Ellman's reagent. The absorbance will be read within 20 minutes of color development at 412nm against blank using a spectrophotometer.

The chromic acetate produced will be measured with a spectrophotometer at 570 – 610 nm. It should be noted that dichromate has no absorbance at this wavelength and hence its presence in the assay mixture will not interfere with the determination of chromic acetate. Catalase preparation (in samples) will be allowed to split H_2O_2 for different periods of time. The reaction will be stopped and the mixture will be heated. The remaining hydrogen peroxide will be determined by measuring chromic acetate.

5.4.Determination of Glutathione Peroxidase (GPx):

0.2ml of 0.4M phosphate buffer pH 7.0, 0.1ml of 10mM sodium azide, 0.2ml of plasma, 0.2ml of glutathione salt (GSH), and 0.1ml of 0.2mM Hz02 were added to evaluate glutathione peroxidase (GPx) activity. The mixture was incubated at 37°C for 10 minutes. The reaction was arrested by 0.4ml of 10% TCA and centrifuged. Ellman's reagent was used to measure the glutathione content of the supernatant.

5.5.Malondialdehyde (MDA):

This will be assayed by measuring the Thiobarbituric acid (TBA) reactive products present in the test sample using the procedure of Vashney and Kale (1970) and expressed

as micromolar of malondialdehyde (MDA)/g tissue. The assay will be based on the reaction of chromogenic reagent (2 – TBA) with MDA (end product of lipid peroxidation) under acidic conditions to yield a stable pink chromophore with maximum absorbance at 532 nm. The reaction mixture will contain 30% Trichloroacetic acid (TCA) solution, 0.75% Thiobarbituric acid (TBA) solution in 0.1 M HCl and 0.15 M Tris KCl buffer (pH 7.4). An aliquot of 0.4 ml of the test sample will be mixed with 1.6 ml of Tris KCl buffer (which will be placed into the test tube before the test sample). Then, 0.5 ml of 30% TCA will be added followed by 0.5 ml of 0.75% TBA and the mixture will be placed in a water bath for 1hr between 90 – 95ºC. This will then be cooled in ice and centrifuge at 3000r.p.m. for 15 mins. The clear pink supernatant will be collected and absorbance will be measured against a reference blank of distilled water at 532nm in a spectrophotometer. Malonaldehyde (MDA) which is an index of LPO will be calculated with an extinction coefficient of 1.5 x 105 M-1cm-1.

5.6.Determination of Superoxide Dismutase (SOD) Activity:

The level of SOD activity will be determined by the method of Misra and Fridowich (1972). The mixture contains 0.05 M carbonate buffer (pH 10.2) and 0.3 mM epinephrine. The sample (0.1 ml) will be diluted in 0.9 ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2 ml of the diluted enzyme preparation will be added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction will start by adding 0.3 ml of freshly prepared 0.3 mM epinephrine to the mixture which will be quickly mixed by inversion. The reference cuvette will contain 2.5 ml of 0.05 M carbonate buffer, 0.3 ml of epinephrine (substrate) and 0.2 ml of distilled water. An increase in absorbance at 480nm will be monitored every 30secs for 150 seconds. An extinction coefficient for epinephrine at 480 nm of 4020 M-1cm-1 was used in calculating activity.

6.Determination of PBDEs in Tissues:

KOH Refluxing/Vortex Extraction– EPA Method 3611C cleanup method was employed in the analysis. We weighed 15 g wet weight of excised liver, and intestine, and pooled parasite samples of the fish specimens into a crucible then macerated and homogenized it. From the homogenized tissue, 10 g was placed in a 50 mL centrifuge tube, 15 mL of 6N KOH was added, and the tubes were sealed and incubated for 18 h in a 35 °C water bath, shaking vigorously for 30 seconds for every ½ hour for the first 4 h and allowed to cool afterwards. We then added 15 mL of methylene chloride or ethyl ether to the centrifuge tube, vortex for 1 min and centrifuge at 2000 rpm for 5 min to facilitate phase separation. The upper/aliquot layer was then removed using a Pasteur pipette into a 250 mL round-bottom flask. Repeat solvent centrifugation twice and combine all aliquot fractions in the round-bottom flask.

Sample extract concentration to about 5 – 10 mL was carried out by rotary evaporator prior to fractionation cleanup using alumina gel column and GC-MS analysis.

7. Histopathological Assessment of Infected Tissues:

Fish liver and intestine were fixed in Bouin's fluid for six hours and transferred to 10% phosphate-buffered formalin to preserve the tissue. The tissue samples were cut and placed inside well-labelled tissue embedding cassettes. They were then processed using an automatic tissue processor for 17 - 19 hours. The dehydration of the tissues took place in increasing concentrations of alcohol (70%, 95%, and then twice in absolute alcohol at 30 minutes duration). Tissue samples were impregnated in molten paraffin three times and later embedded in molten paraffin wax and allowed to solidify. Each of the blocks was placed on the rotary microtome and sectioned at 4- 5 microns to expose their surfaces. The sections were gently placed on each well-labeled slide. With the aid of curved floating forceps, the sections were floated out on a hot water bath already maintained at 45°C each.

The labelled slides are then used to pick the sections that are free of creases, whilst ensuring that each of the sections adheres to the centre of the slide. Slides are subsequently dried on

a hot air oven already maintained at 60°C to ensure proper attachment, (Bancroft *et al*., 2014). The slide sections were dewaxed in xylene dehydrated using descending grades of alcohol (absolute alcohol to 95%, 80%, 70%, and water). The sections were stained using Haematoxylin and Eosin stains. Hematoxylin colours the nucleus while Eosin colours the cytoplasm of the cell.

The stained tissues were washed off in tap water and the overstrained ones were destined in 1% acid alcohol. The tissues were finally mounted using Di-N-butyl phthalate in xylene (D.P.X) and dried. Thereafter, they were examined under a microscope. The photomicrograph and the interpretation of the pathological lesions were done at the Pathology Laboratory of the Department of Veterinary Pathology, University of Ibadan, Nigeria.

8. Quality Control/ Quality Assurance:

Prior to the analysis of samples, the instrument was calibrated for analysis. This was carried out by injecting a series of Calibration standards. The volume introduced was 1 µL. The calibration standard that was commercially obtained was used in the preparation of a five-point calibration curve. The calibration curve was inspected to ensure that, the R2 value is ≥0.995. Using the area response and the amount of standard material, the response factor (RF) was calculated for each analyte/component in the calibration standard. The relative standard deviation percentage (%RSD) of the Rf was also calculated for each analyte over the calibration curve. The value ascertained not to have exceeded 15% for the curve to be deemed valid. For the weight ranges, the average response factor was calculated and used for sample quantification. In the case, where any component alkane exceeded 15%, then the weight range response factors were evaluated and used having met that standard. Using the initial calibration standards, continuous calibration standard (5µg/ml or 10µg/ml), standard reference material (SRM), and instrument blank, sample analysis and quality control were discovered.

9. Statistical Analysis:

The descriptive statistics of the PBDE concentrations in the tissues, bioaccumulation analysis, the parasite abundance, oxidative stress analysis, and physicochemical parameters were evaluated using Microsoft Excel 2010, and Analysis of variance (ANOVA) in Statistical Package for the Social Sciences (SPSS, IBM 20.0 version) to establish the relationship between each variable**.**

RESULTS

1. Pysico-chemical Parameters of Epe Lagoon:

The results of statistical analysis of some physical and chemical parameters of the water samples obtained from three different stations on the Epe Lagoon (Table 1). Results showed that the lagoon has a mean pH of 7.1 with a mean temperature of 24.474^{o}C, Salinity of 0.386 ppt, turbidity of 6.929 NTU, dissolved oxygen of 5.596 mg/l, electrical conductivity of 0.448 µs/cm, and total dissolved solids of 0.297 g/l.

However, the temperature was shown to be minimum at 27.42 ^{o}c and maximum at 30.97 ^{o}c, pH was minimum at 6.610 and maximum at 7.800, Turbidity was minimum at 0.600 and maximum at 11.90, while dissolved oxygen was minimum at 2.770 and maximum at 11.58. In addition, Total Dissolved solids were observed to be minimum at 0.100 and maximum at 0.430, Conductivity was minimum at 0.150 and maximum at 0.670 while salinity was minimum at 0.100 and maximum at 1.500.

2.Soil Adsorption Coefficient (Kd) Of Poly-Brominated Diphenyl Ethers From Water And Sediment:

The soil adsorption coefficient (Kd) of the PBDE congeners on the bottom soil of the Epe lagoon from the overlying water column (Table 2). The order of the adsorption

coefficient decreased from BDE-183 (5.25) > BDE- 100 (4.00) > BDE-47 (2.00) > BDE-28 (1.33). The congeners BDE-99, BDE-154, BDE-153, and BDE-209 had their Kd values reading zero as they were not detected in the water samples.

Table 1: The physicochemical parameters of the water sample obtained from the three different stations (40 sub-stations; n= 120 samples) at the Epe Lagoon.

Parameters	Min	Max	Mean SD
Temperature (oc)	27.41	30.97	29.474 1.368
pH	6.610	7.800	7.111 0.494
Conductivity (µs/cm)	0.150	0.670	0.448 0.190
Turbidity (NTU)	0.600	11.90	6.929 4.502
DO (mg/L)	2.770	11.58	5.596 2.971
TDS (mg/L)	0.100	0.430	0.297 0.122
Salinity (ppt)	0.100	1.500	0.386 0.498

Table 2: Soil adsorption coefficient (Kd) of PBDEs from water

Components	**Sediment**	**Water**	**Kd**
BDE-28	0.08	0.06	**1.33**
BDE-47	0.02	0.01	**2.00**
BDE-100	0.04	0.01	**4.00**
BDE-99	0.01	0.00	*
BDE-154	0.02	0.00	*
BDE-153	0.05	0.00	*
BDE-183	0.21	0.04	**5.25**
BDE-209	0.28	0.00	*

Emboldened figures indicate significant adsorption coefficients. BDE-28 (2,4,4'-Tribromodiphenyl ether), BDE-47 (2,2',4,4'-Tetrabromodiphenyl ether), BDE-100 (2,2',4,4',6-Pentabromodiphenyl ether), BDE-99 (2,2',4,4',5,-Penabromodiphenyl ether), BDE-154 (2,2',4,4',5,6'-Hexabromodiphenyl ether), BDE-153 (2,2',4,4',5,5'-Hexabromodiphenyl ether), BDE-183 (2,2',3,4,4',5',6-Heptabromodiphenyl ether), BDE-209= 2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether, n=120

3.Bioaccumulation of PBDEs in the Intestine of *M. electricus*:

Table 3 shows the various levels of accumulation of each PBDE congener across the infected intestine, uninfected intestine and parasite of the electric fish, *Malapterurus electricus*.

Results showed that for all the congeners (BDE-28, BDE-47, BDE-100, BDE-99, BDE-154, BDE-153, and BDE-183) with the exception of BDE-209, there was no significant difference between their accumulated levels across the infected intestine, uninfected intestine and parasite. Only, BDE-209 was shown to be absent across all three; the infected intestine, uninfected intestine and parasite of the fish.

4.Bioaccumulation of PBDEs in the Liver of *M. electricus*:

Table 4, shows the various levels of accumulation of each PBDE congener across the infected liver, uninfected liver and parasite of the electric fish, *Malapterurus electricus*. Results showed that with the exception of BDE 209, the accumulated levels of each congener (BDE-28, BDE-47, BDE-100, BDE-99, BDE-154, BDE-153, and BDE-183), across the infected liver, uninfected liver and parasite were not significantly different from each other while for BDE-209, nothing was found in the infected liver, uninfected liver and parasite. Furthermore, BDE-183 had the highest concentration in the liver.

Table 3: Bioaccumulation of PBDEs in the intestine of *M. electricus***.**

Components	Infected		Uninfected		Parasite		Sig. Value
	Mean	SD	Mean	SD	Mean	SD	
BDE-28 (2,4,4'-Tribromodiphenyl ether)	0.002	0.004	0.000	0.000	0.000	0.000	0.600
BDE-47 (2,2',4,4'-Tetrabromodiphenyl ether)	0.011	0.013	0.015	0.007	0.000	0.000	0.203
BDE-100 (2,2',4,4',6-Pentabromodiphenyl ether)	0.014	0.019	0.013	0.006	0.000	0.000	0.367
BDE-99 (2,2',4,4',5,-Penabromodiphenyl ether)	0.017	0.027	0.021	0.020	0.000	0.000	0.439
BDE-154(2,2',4,4',5,6'-Hexabromodiphenyl ether)	0.0002	0.0004	0.000	0.000	0.000	0.000	0.600
BDE-153(2,2',4,4',5,5'-Hexabromodiphenyl ether)	0.0007	0.001	0.000	0.000	0.000	0.000	0.600
BDE-183(2,2',3,4,4',5',6-Heptabromodiphenyl ether)	0.028	0.064	0.004	0.009	0.000	0.000	0.600
BDE-209 (2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether)	0.000	0.000	0.000	0.000	0.000	0.000	-

Mean concentrations of PBDEs (n=120) in the infected intestine, uninfected intestine and parasite with significant value greater than 0.05 are not significantly different from each other while components with significant value lesser than 0.05 are significantly different from each other.

Table 4: Bioaccumulation of PBDEs in the liver of *M. electricus*.

Components	Infected		Uninfected		Parasite		Sig Value
	Mean	SD	Mean	SD	Mean	SD	
BDE-28 (2,4,4'-Tribromodiphenyl ether)	0.002	0.004	0.000	0.000	0.000	0.000	0.600
BDE-47 (2,2',4,4'-Tetrabromodiphenyl ether)	0.000	0.000	0.003	0.007	0.000	0.000	0.354
BDE-100 (2,2',4,4',6-Pentabromodiphenyl ether)	0.005	0.007	0.000	0.000	0.000	0.000	0.304
BDE-99 (2,2',4,4',5,-Penabromodiphenyl ether)	0.000	0.000	0.000	0.000	0.000	0.000	0.600
BDE-154 (2,2',4,4',5,6'-Hexabromodiphenyl ether)	0.0002	0.0004	0.000	0.000	0.000	0.000	0.600
BDE-153 (2,2',4,4',5,5'-Hexabromodiphenyl ether)	0.0007	0.0016	0.000	0.000	0.000	0.000	0.600
BDE-183 (2,2',3,4,4',5',6-Heptabromodiphenyl ether)	0.0282	0.0642	0.004	0.009	0.000	0.000	0.615
BDE-209 (2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether)	0.000	0.000	0.000	0.000	0.000	0.000	-

Mean concentrations of PBDEs (n=120) in the infected liver, uninfected liver and parasite with significant value greater than 0.05 are not significantly different from each other while components with significant value lesser than 0.05 are significantly different from each other.

5. Oxidative Stress in the Intestine of *M. electricus*:

Figure 1 shows the results of the different levels of biochemical markers indicative of oxidative stress. Results showed that the level of glutathione peroxidase (GPx) was higher in the uninfected fish than the infected fish while the level of hepatic protein (PRO) was the same for both infected and non-infected fish. In addition, the level of superoxide dismutase (SOD) was slightly higher in the uninfected fish than the infected fish while the levels of Catalase (CAT), Malondialdehyde (MDA) and reduced glutathione (GSH) were shown to be negligible (close to zero).

6.Oxidative stress in the Liver of *M. electricus:*

Figure 2 shows the results of the different levels of biochemical markers indicative of oxidative stress. Results showed that the level of glutathione peroxidase (GPx) was slightly higher in the uninfected fish than the infected fish while the level of hepatic protein (PRO) was the same for both infected and non-infected fish. Moreover, the level of superoxide dismutase (SOD) was higher in the uninfected fish than in the infected while the levels of catalase (CAT), malondialdehyde (MDA) and reduced glutathione (GSH) were shown to be negligible (close to zero).

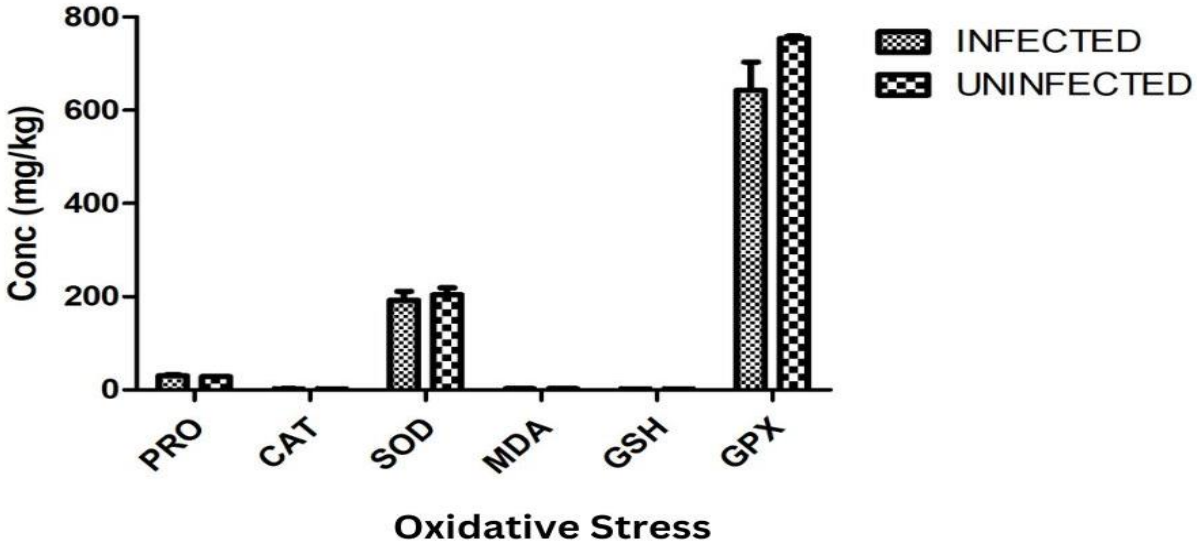

Fig. 1: Oxidative stress in the intestine of *Malapterurus electricus.*

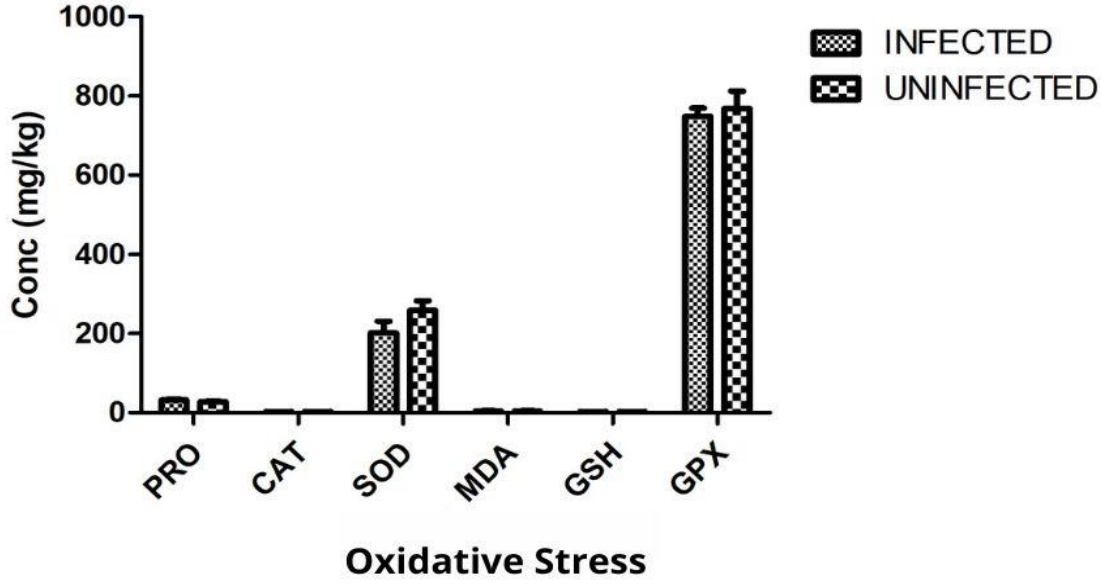

Fig. 2: Oxidative stress in the liver of *Malapterurus electricus.*

7.Lipid Profile of PBDEs in *Malapterurus electricus*:

Figure 3 shows the results of the lipid profile of PBDEs in *Malapterurus electricus*. Results showed the level of cholesterol (CHOL) to be the highest with a mean value (1.3007), while triglycerides (TRIG) were the lowest with a mean value (0.445). Meanwhile, High-Density Lipids-HDL (0.6508) was present in levels higher than Low-Density Lipids-LDL (0.4477). The lipid profile in decreasing order was CHOL>HDL>LDL>TRIG

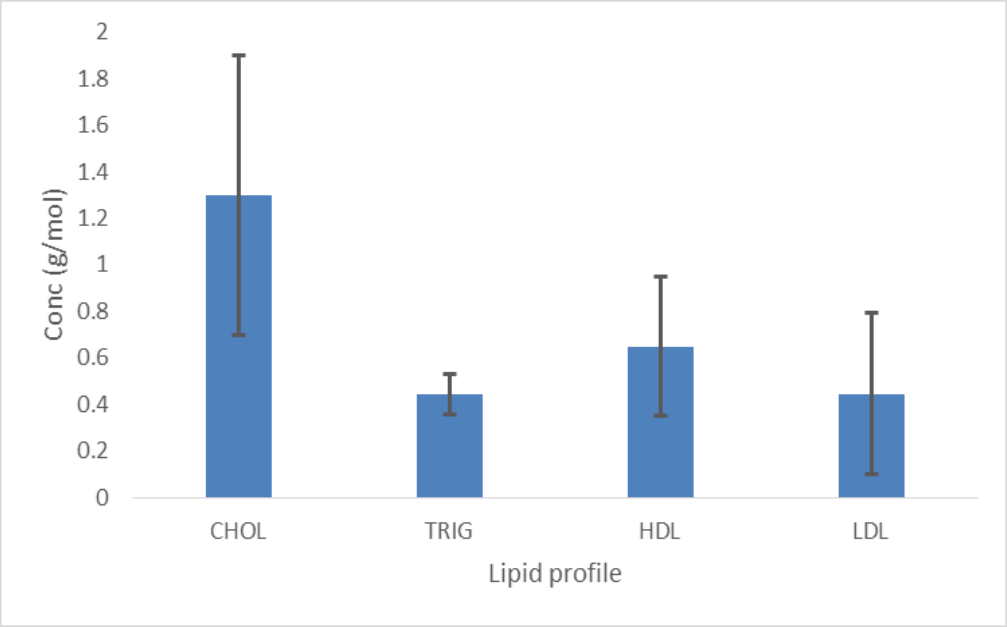

Fig. 3: Lipid Profile of PBDEs in *M. electricus* CHOL- cholesterol, HDL- high-density lipids, TRIG- triglycerides, LDL- low-density lipids

8. Histopathological Assessment of *M. electricus* intestine:

The photomicrographs of the histopathological assessment of the uninfected intestines of *Malapterurus electricus* in the lagoon (Fig. 4) showed normal villi structure (black arrow), normal mucosa (red arrow), sub-mucosa (blue arrow), and muscularis (green arrow). The normal villous architectural structures were well preserved.

Meanwhile, the histopathological assessment of the infected intestines (Fig. 5), showed a mild increase in the connective tissue of the submucosa (black arrows) and a focal area of loss of villous structure (slender arrows).

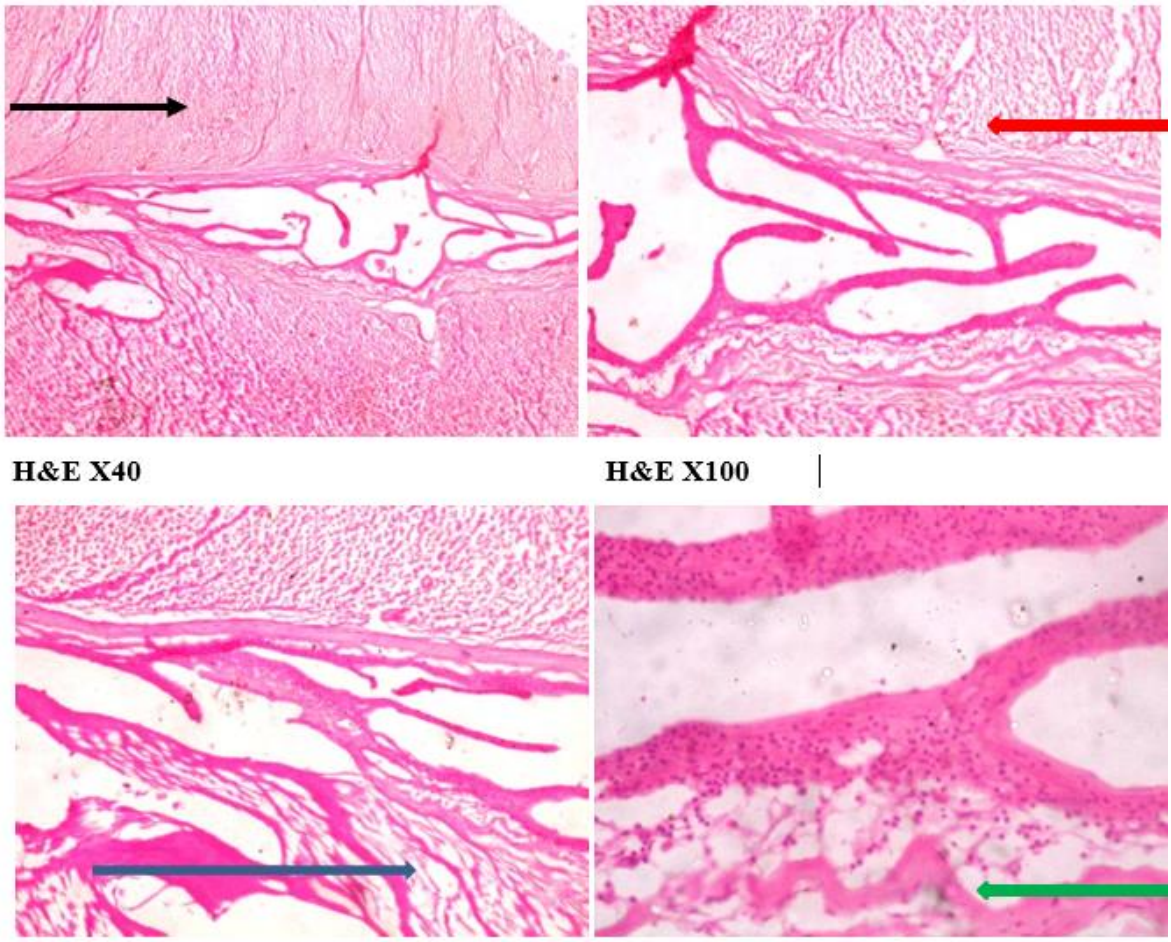

Fig 4: Photomicrographs of uninfected intestinal tissue on H and E stain at magnification of X 40, X 100, and X 400 showing normal villi structure (black arrow), normal mucosa (red arrow), sub-mucosa (blue arrow) and muscularis (green arrow) with no obvious distortion of histo-architectural integrity. The normal crypt-villous architecture is well preserved.

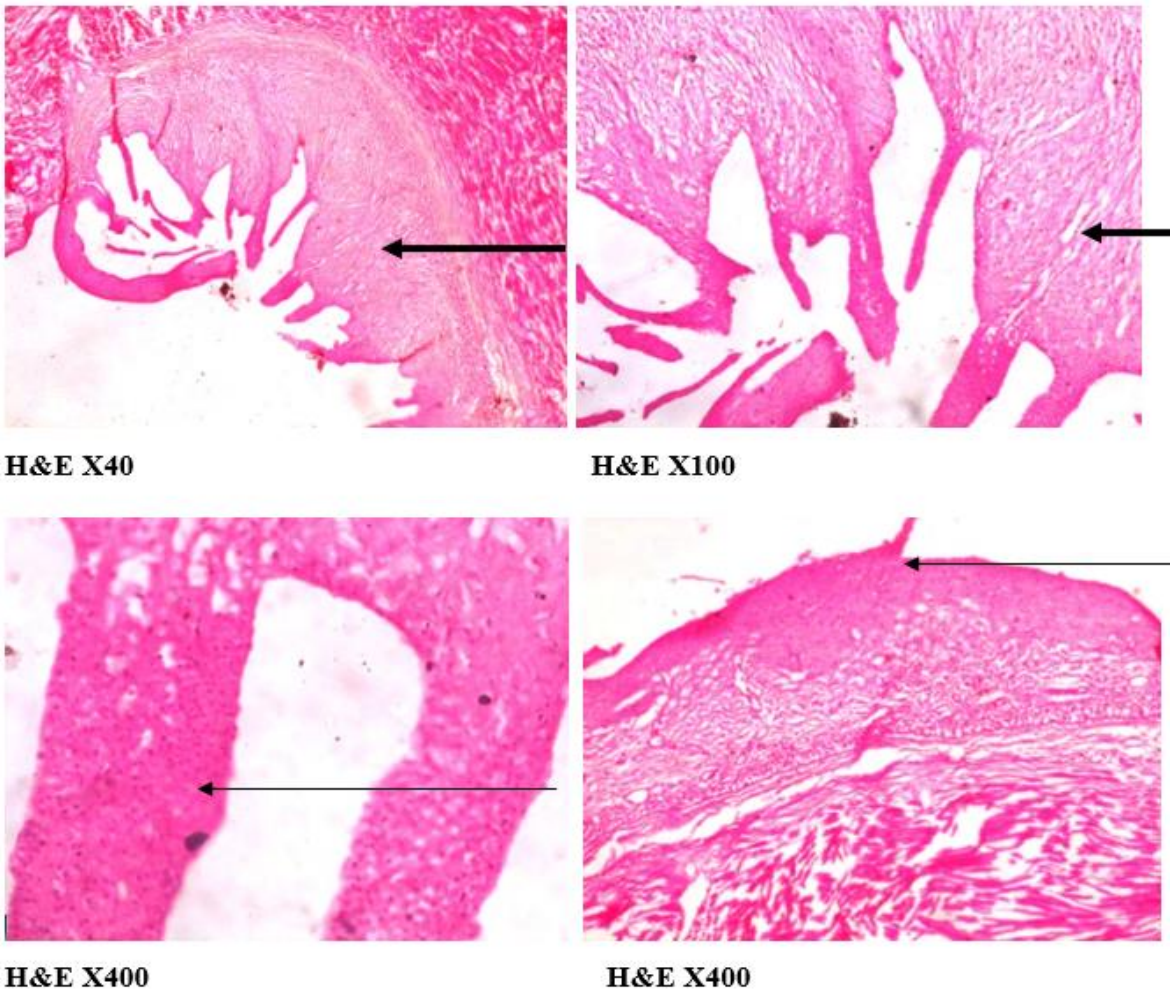

Fig. 5: Photomicrographs of infected intestinal tissue on H and E stain at a magnification of X 40 and X 100 showing a mild increase in the connective tissue of the submucosa (thick black arrows). Higher magnifications of X 400 show a focal area of loss of villous structure (slender arrows).

DISCUSSION

The findings from this study showed the state of health of the electric catfish, *Malapterurus electricus* alongside the status of the Epe Lagoon with respect to pollution by Polybrominated Diphenyl Ether compounds (PBDEs). The results of the assessment of the physico-chemical parameters of the lagoon showed that the mean value of studied parameters was below or within the established permissible limits for Surface Water. This observation suggests that the lagoon may be a productive water system.

The temperature of the water was below the FMEnv permissible limit of 35^0c. This indicates that water was safe enough for fish and all other aquatic organisms to thrive, as temperature plays a great role in the growth, reproduction and survival of fish (Volkoff and Rønnestad, 2020, Islam *et al.*, 2019). Similarly, for pH of water, the value was within FMEnv permissible limit of 7.0-8.5. This also indicates that the water is healthy enough for the survival of fish and any other aquatic organisms. The conductivity value was below FMEnv permissible limit of 10,000 µs/cm. This can be due to low nutrient concentration in the water and could also indicate pristine or background conditions of water (Cormier *et al.*, 2018). Turbidity of the water was below permissible limits of 10 NTU, (Rawat *et al.*, 2017). Low turbidity indicates high water clarity and quality. It also shows that the water is low in

suspended organic and nonorganic substances such as mud and fine sands (Azis *et al.,* 2015). The dissolved oxygen content of water was below FMEnv permissible limits of 10 mg/l. The value observed was lower than the 10mg/l maximum limit (Ukamaka *et al.,* 2022). Thus water is capable of supporting aquatic life such as fish. It is also worthy of note that dissolved oxygen concentrations in water are directly dependent on oxygen-generation processes such as photosynthesis and consumption by living organisms like bacteria (Aniyikaiye *et al.,* 2019). Total Dissolved Solids of water were also below the FMEnv permissible limits of 500 ppm. A low level of total dissolved solids indicates high water quality. This is also in line with Moran, 2018 that stated that total dissolved solids are usually low for freshwater bodies at less than 500 ppm. Besides, the salt content of water was low. The salinity level was below established permissible limits. Low salinity levels show that the water can be useful for other purposes like watering crops while still serving as a good habitat for fish and other aquatic organisms.

The soil adsorption coefficient (kd) of polybrominated diphenyl ethers from waters showed that humans who depend on the lagoon for her services may be exposed to elevated concentrations of polybrominated diphenyl ethers through the primary activities in that area. Results from the adsorption coefficient of the investigated PBDE congeners were in this order: BDE-183 (5.25) > BDE- 100 (4.00) > BDE-47 (2.00) > BDE- 28 (1.33). This shows that from the abilities of PBDE congeners to be adsorbed from the overlying water column to the bottom sediment, BDE-183 was ranked highest. However, because PBDEs are naturally hydrophobic and lipophilic due to their high octanol-water partition coefficients; these adsorbed congeners can be very persistent in the environment and thus, are indicative of ecological concern (Mizukawa *et al.,* 2009). In addition, results also showed that PBDE congeners BDE-99, BDE-154, BDE-153, and BDE-209 were absent. This could be due to the complete precipitation of the PBDE congeners from the overlying water, and thus can't be found.

In this study, among the fishes that were investigated, some were found to be infested by parasites (*Electrotaenia malopteruri*), especially in the intestine and liver. A comparison of the levels of each of the different PBDE congeners across the infected intestine, uninfected intestine and parasite of *Malapterurus electricus* showed that there was no significant difference between accumulated levels except for BDE-209 which was not detected. The non-detection of the congener, BDE-209 could be attributed to the relatively labile nature of BDE-209, decomposing under environmental conditions to yield a large range of lower Brominated PBDEs (Wu *et al.,* 2018, Odunsanya, 2008). Results of the bioaccumulation of Polybrominated Diphenyl Ethers in the intestine also showed that though most of the congeners were present in the infected intestine and uninfected intestine, none was found in the parasite. This can be attributed to some environmental factors as they have a profound effect on several fish-parasite interactions (Khan, 2012). Moreover, taking a general view of the levels of all the congeners in both the infected and uninfected intestine, it can be deduced that the PBDE congeners were higher in the infected intestine than in the uninfected intestine. This can be attributed to the weak hormonal system of the infected fish to fight against contaminants meanwhile for the uninfected because their hormonal system is still active and competent can fight against contaminants in fish.

Similarly, the comparison of the levels of each of the different PBDE congeners across the infected liver, uninfected liver and parasite of *Malapterurus electricus* showed that there was no significant difference in accumulated levels of each congener across the infected liver, uninfected liver and parasite. Only BDE-209 was not detected across all three tissues. None of the congeners were also found in the parasite meanwhile BDE-183 was observed to be the highest accumulated in the liver. This implies that the liver of the electric catfish, *Malapterurus electricus* had a better adaptation sequestration of BDE-183. It also supports the adsorption coefficient of BDE-183 ranking highest. This may pose a threat to

the consumers of the fish if the exposure of the fish is not mitigated (Akinsanya *et al.*, 2022). It was also observed that the two most abundant congeners amongst PBDEs; 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) were absent in the infected liver while for the uninfected liver, they were just negligible (Yang *et al.* 2017). On the other hand, when compared with that of the intestine it was observed that the two congeners BDE-47 and BDE-99 were present both in the infected and uninfected intestine. In addition, taking a general view of the results, it was observed that PBDE congeners were present in higher concentrations in the intestine than in the liver. This difference can be attributed to the functional difference of the two studied organs (Akinsanya *et al.*, 2022, Lee *et al.*, 2015). While the liver is the main organ for detoxification in vertebrates and hence center for the breakdown of metabolic products, the intestine is the organ for the digestion of food. Besides, in vertebrates, the intestine comes before the liver and thus it can be said that contaminants would get to the intestine before getting to the liver, leading to higher concentrations in the intestine than in the liver.

Oxidative stress is an imbalance between the production of ROS and the ability of the antioxidant systems to readily detoxify these reactive intermediates. This imbalance is induced by many chemical pollutants at sub-lethal concentrations. To protect against it, cells possess specific antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), Glutathione S-transferases (GST), reduced glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD). Of these enzymes, glutathione peroxidase (GPx), is capable of decomposing superoxide anion radical (SOD) and hydrogen peroxide (Akinsanya *et al.*, 2020).

The results of the oxidative stress in the intestine of *Malapterurus electricus* showed that the level of glutathione peroxidase (GPx) was higher in the uninfected fish than in the infected fish. Meanwhile, the level of superoxide dismutase (SOD) was slightly higher in the uninfected fish than in the infected fish. Similarly, for the liver, results of oxidative stress showed the level of glutathione peroxidase (GPx) to be higher in the uninfected fish than the infected fish. However, this time around it was a slight increase. The difference wasn't much when compared to that of the intestine. Besides, taking a look at all the biomarkers indicative of oxidative stress for both the intestine and liver, the level of GPx was highest while Catalase (CAT), Malondi-aldehyde (MDA) and reduced glutathione (GSH) were negligible. Increased GPx levels may promote reductive stress which is characterized by a lack of essential ROS required for cellular signaling processes whereas reduced GPx levels can promote the susceptibility to oxidative stress (Lubos *et al.*, 2011).

Furthermore, the level of glutathione peroxidase (GPx), and superoxide dismutase (SOD) being higher in the uninfected fish for both intestine and liver can be attributed to the absence of parasites in the fish. Meanwhile, the lesser level observed in the infected fish for both the intestine and liver can be attributed to the presence of the parasite, *Electrotaenia malopteruri* inherent in the fish. The parasite present in the infected fish might absorb some of the PBDEs, thereby helping to share the toxicant burden and thus reducing stress. The rationale behind this is that as the toxicant burden is being shared by the parasite in the infected fish the response to stress by producing antioxidant enzymes will reduce that's why we had less amount of antioxidants being produced in the infected fish. On the other hand, the uninfected fish had a higher amount because there was no parasite present in it that could help share the toxicant burden. Although the uninfected fish can be said to be healthy, due to the absence of a parasite that would have helped share the burden, the uninfected fish would end up producing enough antioxidant enzymes just to mitigate the stress imposed by the toxicant and that was why the amount of antioxidant enzymes in the uninfected fish was higher. This study is consistent with that of the observations of Akinsanya *et al.*, 2020, who discovered bioaccumulation of Pyrethroid in Parasite *Wenyonia acuminata* (Cestoda: Caryophyllaeidae) and Host fish *Synodontis clarias* from Lekki Lagoon, Lagos Nigeria.

The lipid profile of PBDEs in *Malapterurus electricus* was in this order; CHOL>HDL>LDL>TRIG. The results showed that Cholesterol was highest with a mean value of 1.3007 while triglycerides (TRIG) were lowest with a mean value of 0.445. A major component of the compound, polybrominated diphenyl ethers being cholestesterol suggests that this compound can pose a health threat to fish and then to man as they feed on them.

The histopathological comparisons made between the uninfected and infected fish emphasises the usefulness of histology in the assessment of toxic substances in aquatic organisms from the contaminated ecosystem. Histology provides the opportunity to detect the effect of pollutants in various organs and systems of organisms. The results of the histopathological assessments of the intestine of the electric catfish, Malapterurus electricus showed that the intestine of the infected fish might have been impacted by the toxicant as well as the parasite, marked by a mild increase in the connective tissue of the submucosa and a focal area of loss of villous structure meanwhile the uninfected intestine showed normal villi structure, normal mucosa, sub-mucosa, and muscularis.

4.1 Recommendations and Conclusion

Considering the increased anthropogenic activities in the Epe Lagoon, regulators should ensure strict adherence and implementation of environmental best practices in the treatment and disposal of waste discharge into the environment by local companies. The lagoon should be closely monitored on a regular basis. Environmental education should be organized and carried out for both residents and all staff of the local companies around the lagoon. This may not need to be too lengthened or strenuous; it just needs to cover the key points, and specifically in relation to waste disposal. So that all the residents and all staff of the local companies around the lagoon are aware of its implications.

This study clearly showed that assessment of an aquatic body just based on the physico-chemical parameters may not adequately reflect the true biological state of the aquatic ecosystem. Initially, the Epe lagoon appeared to be in a productive state as the mean value of studied parameters was either below or within the established permissible limits for Surface Water. However, with further health assessment of the *Malapterurus electricus* in the Epe lagoon, severe biological effects were revealed. BDE-209 was not detected at all in this study. From the soil adsorption coefficient (kd) to the bioaccumulation of PBDE congeners across infected fish, uninfected fish and parasites for both intestine and liver, there was no trace of content. The non-detection of the congener, BDE-209 could be attributed to the relatively labile nature of BDE-209, decomposing under environmental conditions to yield a large range of lower Brominated PBDEs (Wu *et al*.., 2018, Odunsanya, 2008). On the other hand, BDE-183 was not only detected but was ranked highest for soil adsorption coefficient (kd) and also for bioaccumulation in the liver. This implies that BDE-183 has good adsorption ability and that the liver of the electric catfish, *Malapterurus electricus* had a better adaptation sequestration of BDE-183.

Because PBDEs are naturally hydrophobic and lipophilic due to their high octanol-water partition coefficients; BDE-183 can be very persistent in the environment and thus, are indicative of ecological concern (Mizukawa *et al.,* 2009).

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