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Regionally, the Nigerian Academy of Science is one of eight founding academies of the Network of African Science Academies (NASAC) and has served on its Executive Committee till date. The Academy has played a major role in the development and establishment of academies in Africa. In November 2012 and also 2017, the Nigerian Academy of Science hosted the African academies for the 8^{th} and 13^{th} Annual Meeting of African Science Academies (AMASA), in Lagos and Abuja respectively. The Nigerian Academy has signed agreements with counterparts in many African countries (and beyond) to ensure scientific exchange and partnership.

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The *Proceedings of the Nigerian Academy of Science (PNgAS)* is the peer-review official journal of the Nigerian Academy of Science, one of Africa's leading science Academies and the foremost independent scientific body in Nigeria. The regular edition of the journal is a multidisciplinary publication, with the primary objective of disseminating original research, systematic reviews, and meta-analysis in all Science, Technology, Engineering, and Mathematics (STEM) disciplines, especially those that address national and regional developmental challenges. The journal publishes articles that are based on deep-seated formative research using large and multi-center datasets that leads to a better understanding of the context of science-related developmental challenges and appropriate pathways for accomplishing change in the following scientific disciplines:

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    Chapters in books can be cited as in this guide in the Proceedings of the Nigerian Academy of Science:

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Friday Okonofua, FAS

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Early in 2020, the editorial board of the *Proceedings of the Nigerian Academy of Science* resolved to dedicate themed editions and its regular editions to fielding research results and innovations that address the sustainable development goals (SDGs), especially within the context of the sub-Saharan African region. It is against this background that this special edition of the journal focuses on addressing the Sustainable Development Goal (SDG) 14, with the theme "life under water". The edition features seven original research articles, one review article, and an editorial that elucidate various aspects of this theme, with the idea to galvanize further actions and research for achieving this specific goal in the African region.

Africa is surrounded by vast oceans and portions of water, and has channels, rivers and lakes that dot its internal landscape. Such vast natural water possessions present unique opportunities and economic possibilities, and also some challenges. The specific objective of this themed edition is to provide examples of how scientific research can help in enabling African countries to maximize the opportunities of the available water resources, and also to identify ways to tackle the related challenges. We believe that it is only through empiricism generated through scientific research that African countries can achieve the global goal epitomized in SDG14, of improved water resources and conservation by the year 2030.

The journal is grateful to Professor Olanike Adeyemo, a Fellow of the Nigerian Academy of Science, Professor in the Department of Veterinary Public Health and Veterinary Medicine at the University of Ibadan, and Deputy Vice-Chancellor of the University, for accepting the onerous responsibility of being the Guest Editor of this themed edition of the journal. Her work has included the announcement of the themed edition and call for papers, scrutiny of the submitted papers and coordination of the peer review process, further review and feedbacks to the authors, and final editorial checks leading to the acceptance of the papers. Her in-depth expertise in the discipline, and her association with several international scientists and reviewers in the water science literature has greatly helped to facilitate the publication of this edition.

The journal wishes to take this opportunity to invite themed editions that feature the attainment of the 17 SDGs in the African region. We believe that the elucidation of the issues related to the SDGs consecutively in a themed edition stand the chance of identifying the bottlenecks and challenges that need to be overcome, propelling the rationale use of optimal scientific processes and methods to address them. Only through science and its new and emerging disciplines can the African region achieve the developmental milestones embodied it the SDGs, and meet up with the rest of the world.

GUEST EDITORIAL

SDG 14 - life below water: trend and trajectory in Nigeria

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The United Nations Sustainable Development Goals 14 is focused on the conservation and sustainable use of the oceans, seas and marine resources. Specifically, target 14.1 is aimed at preventing and significantly reducing marine pollution of all kinds in particular from land-based sources including marine debris and nutrient pollution. Aquatic ecosystems in Nigeria are diverse including freshwaters, brackish and coastal waters as well as marine ecosystems. They support a diversity of animal species which serve as food for man and support ecosystem functioning. Furthermore, these ecosystems provide significant services to man including transport, abstraction of water for domestic and industrial use, power generation, reservoir of hydrocarbon deposits which has been the backbone of the Nigerian economy for over five (5) decades (Akinlo et al., 2012), food security, among others. However, the pollution of these ecosystems has been reported by many researchers over the years and remains the subject of studies to date. Aquatic ecosystems such as those in the Niger Delta, Lagos lagoon, Ogun River, River Onne, Calabar River, among others have been reported to have been subjected to various anthropogenic influences from point and diffuse sources with consequent adverse impacts on water quality, aquatic biota, other wildlife, livestock and even humans (Sogbanmu et al., 2016; Ubiogoro and Adeyemo, 2017; Ibor et al., 2017, Adeyemo et al., 2019).

The issue of aquatic pollution and aquatic water quality standards requires a closer look considering the changing environment in Nigeria. Hitherto, the Federal Ministry of Environment and National Environmental Standards and Regulations Enforcement Agency (NESREA) had adopted guidelines by the United States Environmental Protection Agency (USEPA) among other developed countries regulatory guidelines for most water quality parameters; which might not be applicable to Nigeria's situation. There is a need to set contextual standards for water quality parameters, priority pollutants (such as polycyclic aromatic hydrocarbons, heavy metals, BTEX, pesticides) and emerging contaminants (such as pharmaceuticals and personal care products, industrial additives) among others. These pollutants have been and continue to be detected in aquatic ecosystems in Nigeria at levels which are capable of eliciting adverse effects in aquatic life. The paucity of set limits for pollutants exacerbates the potential risk to aquatic biota, animal and human health. Furthermore, as a signatory to the United Nations Sustainable Development Goals (SDGs), Nigeria ought to develop and evaluate strategies to implement the targets of the SDGs including those stated for SDG 14 which is the focus of this themed edition of the Proceedings of the Nigerian Academy of Science.

Consequently, this themed edition contains Seven (7) peer-reviewed research articles, one (1) international perspective/review article. One (1) article presents water quality of an aquatic ecosystem

(River) in Nigeria impacted by abattoir activities, three (3) of the research articles were focused on adverse effects and biomarkers of toxicity in model and wild fish species in Nigerian aquatic ecosystems. Specifically, article 1 describes the adverse effects of ichthyotoxins used in Nigeria on a freshwater fish species, *Clarias gariepinus* (the African sharptooth catfish), article 2 presented information on biomarkers of oxidative stress in *Chrysichthys nigrodigitatus* (Bagrid catfish) sampled from heavy metals-contaminated Lagos lagoon, Nigeria. Article 3 reports human health risk associated with dietary intake of PAHs-contaminated *Orechromis niloticus* (Nile Tilapia) from a tropical creek in Nigeria. Two (2) research articles recommended pollutant valorization and treatment to mitigate the adverse impact from their discharge into aquatic ecosystems. One (1) of the articles described the derivation of renewable energy from sewage sludge while the other reported the role of peroxidase in the efficient bioremediation of crude oil using indigenous *Bacillus* species. The international perspective/review paper provides an extensive exposé on the theme with up-to-date data on biomonitoring approaches, knowledge gaps and future research direction for monitoring, protecting and managing aquatic ecosystems. It provides a parallel on SDG 14 realization in developed countries vis-à-vis a developing country like Nigeria.

This themed edition is envisaged to challenge relevant stakeholders across relevant research institutions, government MDAs, regulatory agencies and policymaking institutions in Nigeria, international, regional and local development partners, non-governmental and civil society organizations to identify areas for collaborations, partnerships and innovation to support the sustainability of life below water in Nigeria.

Authors' contributions: TOS and OKA conceptualized the manuscript. Both authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflict of interest. OKA is the Editor while TOS is the Assistant Editor of the PNgAS Themed edition on SDG 14 – Life Below Water: Status, Current Trends and Future Direction in Nigeria.

References

Adeyemo O K, Adeyemi I G, and Odunsi O O (2019). Physicochemical, heavy metals and microbial pollution of surface and ground water in Bodija Municipal Abattoir and its Environs. *International Journal of Environment, Agriculture and Biotechnology* **4** (6): 1720-1725.

Akinlo A E (2012). How important is oil in Nigeria's economic growth? *Journal of Sustainable Development* **5**: 165-179.

Ibor O R, Adeogun A O, Chukwuka A V, and Arukwe A (2017). Gross pathology, physiological and toxicological responses in relation to metals and persistent organic pollutants (POPs) burden in tilapia spcies from Ogun River, Nigeria. *Marine Environmental Research* **129**: 245-257.

Sogbanmu T O, Nagy E, Phillips D H, Arlt V M, Otitoloju A A, and Bury N R (2016). Lagos lagoon sediment organic extracts and polycyclic aromatic hydrocarbons induce embryotoxic, teratogenic and genotoxic effects in *Danio rerio* (zebrafish) embryos. *Environmental Science and Pollution Research* **23** (14): 14489-501.

Ubigoro O E and Adeyemo O K (2017). Heavy metal pollution of aquatic systems in oil producing communities of Delta state, Nigeria. *Journal of Applied Biosciences* **120**: 11993-11998.

ORIGINAL RESEARCH ARTICLE

Assessment of the health risks associated with human dietary exposure to polycyclic aromatic hydrocarbons in Nile tilapia from Agboyi creek, Southwest Nigeria

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ABSTRACT

Increasing levels of persistent organic pollutants in aquatic ecosystems have been a major challenge in many regions of the world due to their potential adverse effects on ecological receptors and humans via the food chain. This study assessed the risk associated with dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in muscle tissues of Nile tilapia *Oreochromis niloticus* from Agboyi creek in Southwest-Nigeria. The concentrations of PAHs were determined using Gas Chromatography-Mass Spectrometry (GC-MS) following United State Environmental Protection Agency (USEPA) methods. Of the 16 priority PAHs screened, the mean concentration of Acenaphthene (60.51 ± 69.85 µg/kg), the most dominant of all detected PAHs accounted for 19 % of total PAHs while Benzo (a) pyrene with the lowest mean concentration of 0.08 ± 0.17 µg/kg accounted for 0.03 % of total PAHs recorded. Estimated human daily intake (EDI), Hazard Quotient (HQ) and Hazard Index (HI) of PAHs in fish through human consumption were less than the reference dose (RfD) and threshold value. However, obtained toxic equivalent concentration (TEC) for Benzo (b) fluoranthene (35.79 µg/kg) and Dibenz (a, h) anthracene (56.25 µg/kg) as well as the estimated excess cancer risk (ECR) values for 7 most toxic PAHs in fish tissues exceeded the calculated screening value of 0.0027 µg/g and the 'acceptable' range of risk ($> 10^{-6}$) set by the United State Environmental Protection Agency (USEPA) respectively. Dietary exposure to some PAHs recorded in the tissue of Nile tilapia from Agboyi creek may have consequent health implications on the consumers.

Keywords: *Polycyclic aromatic hydrocarbons, Oreochromis niloticus, human health risk*

INTRODUCTION

Increasing levels of potentially toxic substances in aquatic ecosystems and subsequent bioaccumulation in fishery resources raises a major concern due to the potential risks to human consumers, particularly in populations with high consumption rate. Among the complex mixture of toxic substances in the environment, the occurrence and distribution of persistent organic pollutants (POPs) constitutes a major challenge to the health and sustainability of several ecosystems in many regions of the world due their potential adverse effects on organisms and other natural resources. Of particular concern is the levels of poly aromatic hydrocarbons (PAHs) leached daily into rivers, lakes

and oceans from anthropogenic sources such as waste water, industrial effluents and incomplete combustion of fossil fuel and petroleum products (Ekere *et al.,* 2019). According to earlier studies, they have been found at varying concentrations in different environmental matrices (Chen and Liao, 2006; Ezemonye 2006). Nwaichi and Ntorgbo, (2016), also stated in their report that there is an alarmingly high levels of PAH-based pollutants in the aquatic ecosystem due to significant increases in anthropogenic activities along with unavoidable process of biotransformation and biomagnification. And due to their potential carcinogenic, genotoxic and mutagenic effects, the contamination of aquatic ecosystems with PAHs is receiving considerable attention in recent times (Wu *et al.,* 2012; Behera *et al.,* 2018; Ekere *et al.,* 2019; Olayinka *et al.,* 2019). Fish constitutes an important economic resource and a major cultural food in many regions of the world with proven health benefits. A previous report by Xia *et al.* (2010) however noted that dietary intake constitutes a major pathway of PAHs exposure in humans. In addition, increased risks of cancer in humans have been attributed to dietary exposure to elevated concentrations of PAHs (Yoon *et al.,* 2007; Stacewicz-Sapuntzakis *et al.,* 2008). Hence, the risk derived from exposure to chemical pollutants via frequent consumption of fish has been an issue of concern in contrast to the potential health benefits of dietary fish intake (Nwaichi and Ntorgbo, 2016).

In Nigeria, a number of studies have shown a steady increase in the levels of pollutants of priority concerns including PAHs and their bioaccumulation in fishery resources from several ecosystems (Nkpaa *et al.,* 2013; Nwaichia and Ntorgbo, 2016; Tongo *et al.,* 2017; Usese *et al.,* 2017; Igbo *et al.,* 2018; Ekere *et al.,* 2019; Olayinka *et al.,* 2019). The Agboyi creek, a socioeconomically important water body in Southwestern Nigeria and a home to a wide array of fishery resources is also shown to be vulnerable to increasing anthropogenic pressures such as the indiscriminate discharge of untreated domestic and industrial effluents from the surrounding city centers. A previous study revealed contamination of the creek surface water and sediment with organochlorine pesticides (Williams, 2013). However, studies estimating the risk associated with human dietary exposure to reported levels of potentially toxic substances including PAHs in socioeconomically important fish species from Agboyi creek are quite limited. In an effort to aid public health safety, this study was designed to assess the levels and risk associated with human intake of PAHs in muscle tissues of adult tilapia fish, *Oreochromis niloticus* from Agboyi creek.

MATERIALS AND METHODS

Sample Collection and Preservation

Samples of adult *O. niloticus* (n = 40) of average length, 11.3 -15.5cm and corresponding weight of 34.4 - 88.8g were obtained from Agboyi creek during the wet months (May and August, 2019) with the help of a professional fisherman. The fish samples were washed with creek surface water, then wrapped in aluminum foil and immediately transported in polythene bags to the Aquatic Toxicology and Ecophysiology Laboratory located at the Department of Marine Sciences, University of Lagos. In the laboratory, whole fish samples were thoroughly cleaned with distilled water to remove any external dirt and dissected to remove the muscle tissues. They were kept frozen at -4 °C until extraction (Tongo *et al.*, 2018).

Preparation, extraction and clean-up of fish samples

Extraction and pretreatment of samples for PAHs screening followed a step by step procedure for persistent organic pollutants described previously (Tongo *et al.,* 2018; Uyimadu *et al.,* 2018) with slight modification. A homogenous tissue sample was created using a Kenwood commercial-grade food blender (BLP900BK) previously washed with phosphate-free soap and water; then rinsed with hexane between samples to avoid cross contamination. Each sample was grounded to ensure a

homogenous sample. Then 3g of homogenized tissue was weighed into a contaminant-free 150 mL Pyrex Berzelius beaker and mixed with 20g of anhydrous sodium sulfate (sodium sulfate granular, Supelco 2-0296) which had been previously dried by heating to 140ºC overnight. The mixture was stirred frequently, until it was dry and free-flowing, containing no large lumps. The beakers were then numbered with appropriate sample numbers and weighed. Sample extraction was done by a column extraction method using 15 mL of petroleum ether to rinse the column. The sample mixture was then poured into the column, after which 50 mL of acetone/petroleum ether was added to the sample beaker and stirred. The stopcock was closed as the solvent began to elute. At this point, the column was lightly stirred with a glass rod to remove trapped air. Elution was then continued at the rate of 1-2 mL per minute until the solvent level reached the beginning of the sample mixture. Another 50 mL of acetone/petroleum ether was added and elution was continued at the same rate. The stopcocks were rinsed with acetone/petroleum ether to wash any residue lipids or analytes into the concentration tube. The eluent was concentrated to 1 mL by placing the concentrator tubes in a Kuderna-Danish TurboVap concentrator and the extract was then transferred to a 2 mL graduated vial with isooctane. A column chromatography method was used for the clean-up by adding 3 g of activated silica gel which was deactivated with 1 mL distilled water before use. The column was topped with 1 cm of preheated sodium sulfate and then rinsed by eluting twice with 20 ml hexane and discarded. The concentrated extract in iso-octane was transferred to the column and eluted with 50 ml of 20 + 80 DCM / hexane (v/v ratio). The eluent was collected in a 100 ml round bottom flask and then reduced by volume with rotary evaporator to 3 ml. The solvent was then exchanged to iso-octane and the volume further reduced to 1 ml in a stream of nitrogen.

Chromatographic analysis of extract

The final extracts from fish muscle tissues were screened for Naphthalene (NaP), Acenaphthylene (AcPY), Acenaphthene (AcP), Fluorene (Flu), Phenanthrene (Phe), Anthracene (Ant), Pyrene (Pyr), Fluoranthene (FL), Benz[a]anthracene (BaA), Chrysene (Chr), Benzo[b]fluoranthene (BbFL), Benzo[k]fluoranthene (BkFL), Benzo[a]pyrene (BaP), Benzo[g,h,i]perylene (BP), Indeno[1,2,3c,d]pyrene (Ind) and Dibenz[a,h]anthracene (DBA). The analysis of PAHs was carried out on a GC-MS (QP-2010 series, Shimadzu, Japan) following the procedure previously reported by Unyimadu *et al.* (2018). The injector port was set at 300 °C while the oven temperature was held initially at 40 °C and then increased to 120 °C at 25 °C min^{-1}, then to 160 °C at 10°C min^{-1} and finally to 300°C at 5°C min^{-1}. Purified extracts (10 µl) were injected without splitting. The PAHs were separated on a Rxi®-5Sil-MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness of 1,4-bis (dimethyl siloxy) phenylene dimethyl polysiloxane). Ultrapure helium (99.99%) was used as the carrier gas.

Quality Control

Appropriate quality assurance and controls were performed including analysis of procedural blanks to check for purity of reagents, potential laboratory contamination and inferences. Random duplicate samples were analyzed (standard deviation <5) to check the precision of the instrument. PAHs were quantified using the internal calibration method based on five-point calibration curves for individual compounds. Calculated concentrations were reported as less than the limit of detection if the peak area did not exceed the specified threshold (three times the noise). Concentrations below the detection limit (BDL) were assigned zero values for the statistical analysis. The PAHs were denoted by their International Union of Pure and Applied Chemistry numbers. Genuine standards of PAHs (certified reference standard from Accustandard, New Haven, CT, USA) of various concentrations (20 ppm, 40 ppm, 50 ppm and 80 ppm) were used to calibrate the GC-FID before analysis. Recoveries of authentic standards for the individual target PAHs ranged from 75% to 96%.

Human Health Risk Assessment of PAHs levels in Fish

Potential risk to humans associated with PAH intake via fish consumption was assessed through estimation of dietary intake (EDI), Hazard Index, Toxic Equivalent Quotient (TEQ) and Excess Cancer Risk Further toxicological risk from PAHs levels in fish muscles was assessed by comparison with legal limits (Tongo *et al.*, 2017).

Estimated Dietary intake

The daily intake of PAHs from fish was evaluated by multiplying the respective PAH concentration in each fish sample by the fish ingestion rate (IFR) of an average weight adult (70 kg) from Nigeria using (Eqn. 1) as reported by Nasher *et al.* (2016) thus:

Estimated Dietary Intake (µg/kg/day) = (Ci x IFR) / BW (1)

Where: Ci is concentration of individual PAH in fish

IFR is the consumption rate per person per day (0.036 kg/capita/day) (Worldfish, 2018)

BW is the body weight (average adult weight of 70kg in Nigeria)

Estimation of Carcinogenic and Non-Carcinogenic Risk

According to USEPA (1986), values of HQ and HI of contaminants below one (< 1) are considered as safe whereas there may be cause for concern from potential non-carcinogenic health risks when HQ is > 1. The HQ for carcinogenic and non-carcinogenic risks from exposure to PAHs in fish muscle tissues were calculated using equations 2, 3 and 4:

Hazard quotient (HQ non-carcinogenic) = EDI / RfD (2)
Hazard quotient (HQ carcinogenic) = EDI X SF (3)
Hazard index (HI) = (4)

Where; EDI is the estimated daily intake,

RfD is the reference dose and SF is the slope factor in mg/kg/day which was adopted from United State Environmental Protection Agency (USEPA, 2012) following the report of Li *et al.* (2016). Thus, RFD values used in risk estimation were 0.02, 0.02, 0.06, 0.02, 0.04, 0.3 and 0.04 for NaP, AcPY, AcP, Flu, BaP, Phe, Ant, Pyr and FL respectively. On the hand, SF values used in the estimation of risk posed by consumption of BaA, Chr, BbFl, BkFl, BaP, BP, Ind and DBahA in fish tissues were 0.73, 0.007, 0.73, 0.073, 7.3, 0.04, 0.73 and 7.3 respectively.

To estimate the carcinogenic potency from exposure to PAHs in fish, the toxic equivalent quotient was calculated using equation 5 and 6.

$$TEC = TEF \times C_iPAH \quad (5)$$

Where: TEC is the toxic equivalent concentration of individual PAH
TEF is the toxic equivalence factor of individual PAH.

CiPAH is the concentration of individual PAH in fish muscle tissues.

The values of TEF for 7 carcinogenic PAHs (BaA, Chr, BbF, BkF, BaP, InP and DBahA) used in the estimation are 0.1, 0.01, 0.1, 0.01, 1.0, 0.1 and 5.0 respectively (Nisbet and Lagoy 1992).

TEQ = (6)

Where: TEQ is the sum of the toxic equivalent concentration (TEC) of PAH in fish.

The evaluated TEQ value was also compared with a calculated screening value (SV) to assess human health risks posed by PAHs to humans from consumption of fish. According to a previous study, SV is the threshold concentration of total PAHs in fish tissue that is of potential public health concern (Nyarko and Klubi, *et al.,* 2011). The SV was calculated using Eqn. 7 (Tongo *et al.,* 2017).

SV (µg/kg) = [(RL/SF) × BW]/CR (7)

Where:

RL = maximum acceptable risk level (10^{-5} USEPA, 2000)

SF = USEPA oral slope factor (7.30 µg/g day USEPA, 1993)

BW = body weight (70000 g)

CR = fish consumption rate (36 g/day Worldfish, 2018).

The Excess cancer risk was also calculated using the equation below (Tongo *et al.,* 2017; Xia *et al.,* 2010)

ECR =

Where: TEC is the toxic equivalent concentration of individual PAH
IFR is the consumption rate per person per day (0.036 kg/capita/day) (Worldfish, 2018)

BW is the body weight (average adult weight of 70 kg in Nigeria)

Q is the cancer potency of BaP which was assessed as 7.3 µg/g/day by the integrated risk information system of the USEPA (USEPA, 2001).

ED is the exposure duration (70years)

ATn is the average life span (25550 days)

Statistical Analysis

Data obtained from the analysis of duplicate samples were subjected to descriptive statistics and analysis of variance (ANOVA) at 0.05 ($P < 0.05$) level of significance using Microsoft Excel and the Statistical Package for the Social Sciences software (SPSS) version 20.0 for Windows respectively. The results are reported as mean ± standard deviation.

RESULTS

The mean concentrations of total PAHs obtained as 17.74 ± 16.30 µg/kg was observed in the order AcP > BbFL > Bp > BkFL > NaP > Flu > AcPy > Chr > Ant > Ind > Pyr > DBA > Phe > BaA > Bap > Fl in fish muscle tissues. Of all the detected individual PAHs congeners in examined fish muscles, Acenaphthene (AcP) with a mean concentration of 60.51 ± 69.85 µg/kg was the most dominant PAHs (Fig.1). Fluoranthene was below the detection limit (0.002 µg/kg) used in the study. The result also showed relatively low but measurable concentrations of Benzo[a]pyrene (BaP) in examined fish

muscles with a total mean value of 0.084 ± 0.17 µg/kg. Mean concentrations for total carcinogenic PAHs (sum of BaA (1. %), Chr (15%), BkFL (22.2%) BaP (0.1%), BbFL (23.7%), Ind (7.8%), DBA (7.4), BP (22.8) accounted for 47% of the total PAHs in fish.

Generally, the total concentration of higher molecular weight (HMW) PAHs obtained as 162.87 µg/kg was relatively higher than the total lower molecular weight (LMW) PAHs in fish (156.47 µg/kg).

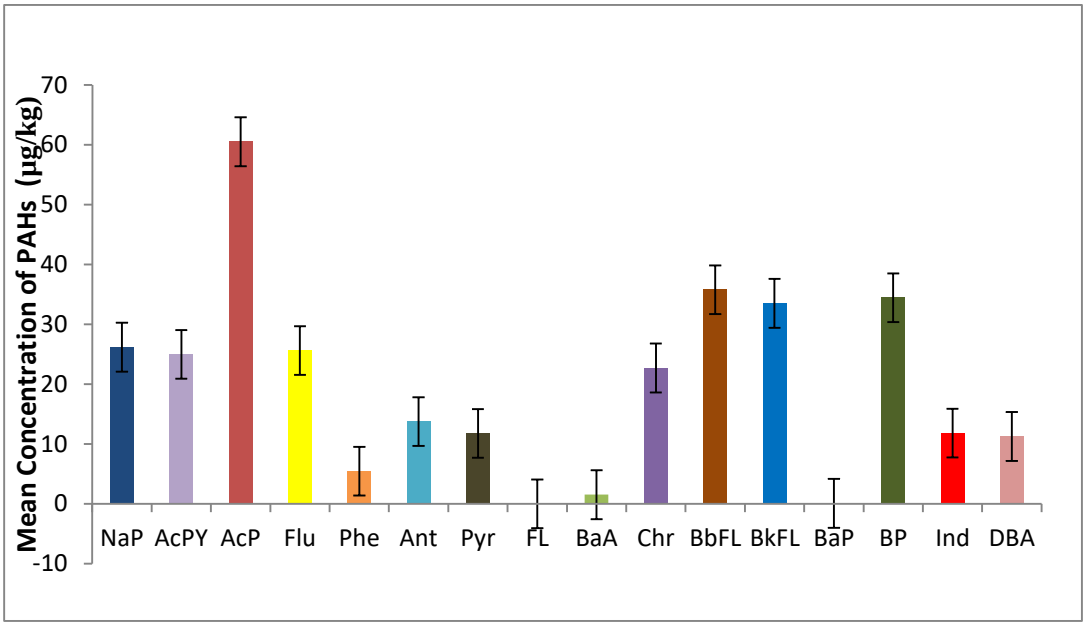

Figure 1: Mean concentration of PAHs in muscle tissues of *Oreochromis niloticus*from Agboyi creek

Human Health Risk

The mean EDI for the seven most toxic PAHs in fish muscle tissues varied from 4.0×10^{-5} to 1.8×10^{-2} µg/kg bw/day. Relatively higher EDI values were recorded for Benzo(b)fluoranthene and Benzo (g, h, i,) perylene, while Benzo (a) pyrene recorded the lowest EDI value of 4.0×10^{-5} µg/kg bw/day (Table I)

Potential Non-Dietary Risk Exposures

The results of non-dietary exposures estimated using HQ for individual PAHs in fish muscle tissue and HI through consumption of fish by adult consumers are presented in Table I. HQ and HI were observed to be less than the threshold value of 1. For carcinogenic risk, the estimated excess cancer risk values for individual toxic PAHs congeners varied from $8.7 \times 10^{-7}$to $5.8. \times 10^{-4}$, all exceeding the USEPA acceptable guideline value of 1×10^{-6} except for Benzo (a) pyrene (Table 1).

Table I: Estimated daily intake, Hazard quotient, Hazard index and Excess cancer risk for 7 most toxic

PAHs in *Oreochromis niloticus* from Agboyi creek.

PAH	Chr	BbFL	BkFL	BaP	BP	Ind	DBA
Estimated dietary intake (µg/kg bw/day)	0.012	0.018	0.017	4.0E-05	0.018	0.006	0.006
Cancer slope factor (mg/kg/day)	0.007	0.73	0.073	7.3	NA	0.73	7.3
Hazard quotient (HQ)	8.2E-08	0.013	0.0013	0.0003	0.129	0.0044	0.042
Excess cancer risk	2.30E-06	3.70E-05	3.4E-05	8.7E-07	3.50E-06	1.2E-05	0.00058
Hazard Index ($\sum_{i=1}^{n} HQ$)	0.19						

An estimated SV value of 0.0027 µg/g was obtained in the present study. Further risk estimation revealed relatively higher toxic equivalent concentration (TEC) values for Benzo (b) fluoranthene (3.58), Benzo (k) fluoranthene (3.35) and Dibenz (a, h) anthracene (56.25) respectively when compared to the SV (Table II).

Table II: Toxic equivalent concentration TEC and Toxic equivalent quotient TEQ for 7 most toxic PAHs in *Oreochromis niloticus*

PAH	Mean (ug/kg)	TEF (Nisbet and Lagoy 1992)	TEC
Chr	22.7 ± 25.19	0.01	0.227
BbFL	35.79 ± 41.24	0.1	3.579
BkFL	33.51 ± 38.57	0.1	3.351
BaP	0.084 ± 0.17	1	0.084
BP	34.45 ±39.33	0.01	0.344
Ind	11.81 ±13.53	0.1	1.181
DBA	11.25 ±13.02	5	56.25
Mean ± SD	21.37 ± 14.0		
TEQ	65.46		

DISCUSSION

Dietary pathways have been identified as a predominant exposure route of contaminants including PAHs in humans (Cheung, *et al.*, 2007; Wu *et al.*, 2012). In the present study, examined fish muscle tissues had relatively low but measurable concentrations of detected USEPA priority PAHs; thus, signifying the potential contamination of Agboyi creek with PAHs. The absence or rather low detection of certain PAHs in fish tissues may also be attributed to their rapid depuration or biotransformation (Dhananjayan, and Muralidharan, 2012). Furthermore, various factors including route and duration of exposure, lipid content of tissues, environmental factors, differences in species,

age, and sex, as well as exposure to other xenobiotics may influence the accumulation and depuration of PAHs in fish (Dhananjayan, and Muralidharan, 2012).

Generally, measured PAHs composition showed a considerable predominance of higher molecular weight PAHs suggesting anthropogenic origin. According to Nwaichia and Ntorgbob (2016), high concentration of heavy molecular weight PAHs indicates a predominant pyrolytic origin for the PAHs pollution. Elsewhere, significantly higher mean percentage concentration of higher molecular weight PAHs, accounting for 88% of the total PAHs in fish has been reported (Tongo *et al.* 2018). Although compositional profile of PAHS in environmental and biological samples are frequently used to identify potential sources of PAHs, Pulster *et al.* (2020) however noted that such comparisons are difficult to assess within organisms due to the complex interactions and species-specific differences between bioaccumulation (e.g., uptake, metabolism and elimination) and the physiochemical parameters affecting chemical exposure and bioavailability in the surrounding environment.

The levels of most of the USEPA priority PAHs detected in this study is lower when compared to the levels observed in muscle tissues of *C. gariepinus* from Ovia River in Southern Nigeria by Tongo *et al.* (2017). In their report, it was observed that the concentration of the lower molecular weight PAHs (LWPAHs) was higher than the higher molecular weight PAHs (HWPAHs) in fish muscle tissues. This was attributed to the omnivorous and detritus feeding habit of *C. gariepinus* as well as the lipophilic nature of PAHs. Relatively higher concentrations of individual PAHs congeners when compared to the results of the present study were also reported in fish muscle tissues from crude oil polluted waters of Ogoniland (Nkpaa, *et al.,* 2013).

In addition, Benzo (a) pyrene (B(a)P) usually used as a marker for the occurrence and effect of carcinogenic PAHs in food (Tongo *et al.,* 2017) were recorded in low but measurable concentrations. The observed BaP levels when compared to levels reported elsewhere (Tongo *et al.,* 2017) was relatively low and did not exceed the existing EU recommended safe limit of 0.002mg/kg for human fish consumption. However, the relatively low levels recorded might still pose unacceptable adverse effects in human consumers and populations with high fish consumption rate. Similarly, higher levels of Flu have been reported in fish tissues by Ohiozebau *et al.* (2017) contrary to the result of the present study. Generally, the obtained levels for individual PAHs congeners in fish muscle tissue varied considerably when compared to previously reported PAHs levels in fish from most contaminated ecosystems in Nigeria (Tongo *et al.,* 2017; 2018; Nkpaa *et al.,* 2013).

The results of estimated human daily intake of PAHs, HQs and HI values for PAHs in examined fish which were lower than the reference dose (RfD) and set threshold value of 1 indicates no potential adverse health effect in consumers. This finding is similar to an earlier report for fish and shellfish from Amariaria Community, downstream of Bonny River, Southern Nigeria (Tongo *et al*., 2018). On the contrary, the toxic equivalent concentration values of Benzo (b) fluoranthene, Benzo (k) fluoranthene and Dibenz (a, h) anthracene which exceeded the calculated screening value, indicates the potential for carcinogenic risk in adult consumers over a life time of exposure. Except for Benzo (a) pyrene, the estimated excess cancer risk (ECR) from lifetime exposure to the 7 most toxic PAHs through fish consumption was also slightly higher than the acceptable risk value ($> 10^{-6}$). This has implications for consumer safety and calls for concern.

CONCLUSION

The present study detected 15 PAHs in muscle tissues of *Oreochromis niloticus* from Agboyi Creek with higher molecular weight PAHs being the most predominance in the fish samples. Estimation of noncarcinogenic health risk indicates no potential negative effects in humans. However, the obtained concentrations of Benzo (a) pyrene (BaP) and the toxic equivalent concentration (TEC) values for

Benzo (b) fluoranthene, Dibenz (a, h) anthracene which exceeded EU recommended safe limit and USEPA screening value in fish respectively, as well as the estimated excess cancer risk (ECR) for 7 most toxic PAHsin adult consumers over a life time exposure may have implications for consumer safety. Hence, there is need for continuous monitoring and stringent environmental regulations in the area.

REFERENCES

Behera B K, Das A, Sarkar D J, Weerathunge P, Parida, P K, Das B K, Thavamani, P, Ramanathan R, & Bansal V (2018). Polycyclic Aromatic Hydrocarbons (PAHs) in inland aquatic ecosystems: Perils and remedies through biosensors and bioremediation. *Environmental pollution*, 241: 212-233

Chen S & Liao C (2006). Health risk assessment on human exposed to environmental polycyclic aromatic hydrocarbons pollution sources. *Science of the Total Environment*, 366, 112–123.

Cheung K C, Leung H M, Kong K Y, & Wong M H (2007). Residual levels of DDTs and PAHs in freshwater and marine fish from Hong Kong markets and their health risk assessment. *Chemosphere,* 66(3): 460–468.

Dhananjayan V & Muralidharan S (2012). Polycyclic aromatic hydrocarbons in various species of fishes from mumbai harbour, India, and their dietary intake concentration to human. *International Journal of Oceanography*, 2012.

Ekere N R, Yakubu N M, Oparanozie T, & Ihedioha J N (2019). Levels and risk assessment of polycyclic aromatic hydrocarbons in water and fish of Rivers Niger and Benue confluence Lokoja, Nigeria. *Journal of Environmental Health Science and Engineering,* 17(1): 383-392.

Ezemonye L (2006). Polycyclic aromatic hydrocarbons (PAH) in aquatic environment of Niger Delta of Nigeria (surface water and sediment). *International Journal of Chemistry*, 6: 135–147.

Igbo J K, Chukwu L O, & Oyewo E O (2018). Assessment of Polychlorinated Biphenyls (PCBs) in water, sediments and biota from e-waste dumpsites in Lagos and Osun States, South-West, Nigeria. *Journal of Applied Sciences and Environmental Management*, 22(4): 459-464

Li J, Dong H, Xu X, Han B, Li X, Zhu C, & Zhang D (2016). Prediction of the bioaccumulation of PAHs in surface sediments of Bohai Sea, China and quantitative assessment of the related toxicity and health risk to humans. *Marine pollution bulletin*, *104*(1-2): 92-100.

Nasher E, Heng L Y, Zakaria Z, & Surif S (2016). Health risk assessment of polycyclic aromatic hydrocarbons through aquaculture fish consumption, Malaysia. *Environmental forensics*, *17*(1): 97-106

Nisbet I C T & LaGoy P K (1992). Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). *Regulatory Toxicology and Pharmacology*, 16(3): 290–300.

Nkpaa K W, Essien K B, & Wegwu M O (2013). Evaluation of Polycyclic Aromatic Hydrocarbon (PAH) concentrations in Crabs and Shrimps from Crude Oil Polluted Waters of Ogoniland in Rivers State, Nigeria. *IOSR J. Environ. Sci. Toxicol. Food Technol*. 4(6): 73- 80.

Nwaichia E O & Ntorgbob S A (2016). Assessment of PAHs levels in some fish and seafood from different coastal waters in the Niger Delta. *Toxicology Reports* 3:167–172.

Nyarko E & Klubi B E (2011). Polycyclic aromatic hydrocarbons (PAHs) levels in two commercially important fish species from the coastal waters of Ghana and their carcinogenic health risks. *West African Journal of Applied Ecology*, 19(1)

Ohiozebau E, Tendler B, Codling G, Kelly E, Giesy J P & Jones P D (2017). Potential health risks posed by polycyclic aromatic hydrocarbons in muscle tissues of fishes from the Athabasca and Slave Rivers, Canada. *Environmental geochemistry and health*, *39*(1): 139-160.

Olayinka O, Adewusi A, Olujimi O & Aladesida A (2019). Polycyclic Aromatic Hydrocarbons in Sediment and Health Risk of Fish, Crab and Shrimp Around Atlas Cove, Nigeria. *Journal of Health and Pollution*, 9(24): 191204

Pulster E L, Gracia A, Armenteros M, Carr B E, Mrowicki J & Murawski S A (2020). Chronic PAH exposures and associated declines in fish health indices observed for ten grouper species in the Gulf of Mexico. *Science of The Total Environment,* 703: 135551

Stacewicz-Sapuntzakis M, Borthakur G, Burns J L & Bowen P E (2008). Correlations of dietary patterns with prostate health. *Molecular Nutrition and Food Research*, 52: 114–130.

Tongo I, Ezemonye L & Akpeh K (2017). Distribution, characterization, and human health risk assessment of polycyclic aromatic hydrocarbons PAHs in Ovia River, Southern Nigeria *Environ Monit Assess*, 189: 247.

Tongo I, Etor E & Ezemonye L (2018). Human health risk assessment of PAHs in fish and shellfish from amariaria community, Bonny River, Nigeria. *Journal of Applied Sciences and Environmental Management,* 22(5): 731-736

Unyimadu J P, Osibanjo O & Babayemi J O (2018). Polychlorinated biphenyls in brackish water fish in the River Niger, Nigeria. *Journal of Health and Pollution*, 8(17): 31-42.
US Environmental Protection Agency (USEPA) (1986). Analysis of Polynuclear aromatic hydrocarbons. Method 8100. US Environmental Protection Agency.

US Environmental Protection Agency (USEPA), (1993). Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons. *Development*, 1-28.

US Environmental Protection Agency (USEPA) (2000). "Risk-Based Concentration Table," United States Environmental Protection Agency, Philadelphia, 2000

US Environmental Protection Agency (USEPA), (2001). Integrated Risk Information System: Benzo[a]pyrene (BaP) (CASRN 50-32-8).

US Environmental Protection Agency (USEPA), (2012). Mid Atlantic Risk Assessment, Regional Screening Level (RSL) Summary Table

Usese A, Chukwu O L, Rahman M, Naidu R, Islam S & Oyewo E O (2017). Concentrations of arsenic in water and fish in a tropical open lagoon, Southwest-Nigeria: Health risk assessment. *Environmental Technology & Innovation*, 8: 164-171

Williams A B (2013). Residue analysis of organochlorine pesticides in water and sediments from Agboyi Creek, Lagos. *African Journal of Environmental Science and Technology*, 7(5): 267-273.

WorldFish, (2018). WorldFish Nigeria Strategy: 2018-2022. Penang, Malaysia: WorldFish. Strategy: 2018-09.

Wu W, Ning Qin N, He W, He Q, Ouyang H & Xu F (2012). Levels, Distribution, and Health Risks of Polycyclic Aromatic Hydrocarbons in Four Freshwater Edible Fish Species from the Beijing Market. *The Sci. World* J., 1–12.

Xia Z, Duan X, Qiu W, Liu D, Wang B, Tao S, Jiang Q, Lu B, Song Y & Hu X (2010). Health risk assessment on dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in Taiyuan, China. *Science of the Total Environment*, 408(22): 5331-5337.

Yoon E, Park K, Lee H, Yang J H & Lee C (2007). Estimation of excess cancer risk on time -weighted lifetime average daily intake of PAHs from food ingestion. *Human Ecological Risk Assess*, 13(3): 669–680.

ORIGINAL RESEARCH ARTICLE

Biomarker profile of a silver catfish *Chrysicthys Nigrodigitatus* in heavy metal polluted areas of the Lagos Lagoon, Nigeria

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Abstract
Aquatic ecosystems are usually exposed to contaminants including heavy metals from anthropogenic activities and high concentration of these metals may alter physiological function in fish muscle. Assessment of biomarker profile will provide the role of antioxidant defence enzymes in response to metal accumulation. The present study aims to find the relationship between biomarker pattern in fish muscle and the contribution of antioxidant defence enzymes to sustain balance from metal toxicity. Adult fish (*Chrysicthys nigrodigitatus*), water and sediment samples were taken from five sampling stations, during the wet and dry season in the Lagos Lagoon. Heavy metals (lead, cadmium, chromium, copper, zinc, iron) levels, antioxidant defence enzyme activity (superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione) and malondialdehyde were analysed in fish muscle using standards methods. Heavy metal levels showed significant difference ($p<0.05$) in fish muscles, water and sediment, with values above recommended standard for aquatic life. PCA showed that elevated activities of antioxidant defence enzymes correlated with increase metal concentrations within the fish muscle, which may indicate possible oxidative stress. Hence, measures should be implemented and sustained in the treatment of effluents before discharging into the aquatic habitat so as to reduce stress and sustained the wild population.

Keywords: *Chrysicthys nigrodigitatus, heavy metals, antioxidant defence enzymes*

Introduction

The brackish water ecosystem is usually exposed to a large volume of contaminants which are attributed to a variety of sources like municipal run-offs and agricultural waste (1). Contaminants including heavy metals have the capacity to affect the quality and uses of this coastal habitat including fisheries, recreation and tourism (2). The concentration of heavy metal within the aquatic environment is subject to seasonal and anthropogenic influence. Seasonal influence such as flooding, erosion, atmospheric deposition and geological weathering while anthropogenic activities such as industries untreated effluent, domestic sewage, surface runoff, and dumpsites leachate that releases toxic metals into the aquatic ecosystems, thereby deteriorating the water quality (3, 4). Heavy metals are metallic elements with relative high density, atomic weight or atomic numbers as compare to that of water (5). Certain heavy metals including lead, cadmium, chromium induces toxicity on aquatic biota even at low level of exposure (6), however some metals (copper, zinc, iron) are essential to life and play critical role in metabolic system of aquatic biota like efficient functioning of specific enzymes sites, but excessive levels can be harmful to the organism (7). The presence of different metals with levels above permissible limits for aquatic life might be highly toxic for metabolic pathways (6, 8, 7) and

are such alter the biochemical and physiological function in fish (7). They can stay in the environment for a lengthy period, which makes them of ecological concern. Even at low levels metals such as lead, cadmium, mercury may induce changes in growth, survival, reproduction and depletion of the wild population of aquatic organisms (8,9). Though metals biological effects differ greatly among biota, and often reflect different patterns of exposure, routes of uptake, metabolism and rates of accumulation (10,11). Fish has been used as the most essential bioindicator of aquatic ecosystem regarding such contamination (11,12). These organisms are found everywhere in almost all aquatic environments with the resident fish usually exposed to contaminants. Fish have a great ecological role in the aquatic environment with their influence in trophic web structure (the organism on top of aquatic food chain), nutrient cycling and energy transfer (2,6). Fish size and organ enhances variety of analytical methods to reflect the effects of water pollution (11). The presence of high metal concentration may affect the nutritional value of the flesh, since fish is an important source of protein for humans resulting in metal related health risks (11,13). Hence, the need to evaluate the extent of heavy metal toxicity.

The large volume of contaminants in the aquatic ecosystem, may induce oxidative stress on the biota including fish, causing temporary or permanent damage in oxidative homeostasis and imbalance of metabolic pathways (12,14). Biomarkers including antioxidant defence enzymes have been used immensely to provide the link between external concentrations of contaminant exposure, internal concentrations of contaminant in tissue and early adverse effects in organism (15,16,17), as such provide critical information on ecosystem structure and function (5). These enzymes in tissues provide the protective shield against the toxicity of reactive oxygen species such as superoxide ion levels on macromolecules due to metal accumulation and this may prevent the alteration in metabolic pathway and redox imbalance (7,18,). Hence, the alteration in the activities of these enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, reduced glutathione (GSH)] and malondialdehyde (MDA) level, might represent a response to pollutant and ideal markers of pollutant induced oxidative damage in fish (7,19,20).

Silver coloured African Catfish *Chrysichthys nigrodigitatus* (Lacepede 1802) is a commercial fish of high value among indigenous African populations (21). It is a benthopelagic fish, whose diet components includes sand particles, phytoplankton, zooplanktons and macrobenthic organisms (22,23). This fish inhabits the lagoon, and its feeding habits predisposes it to metal accumulation, hence there is a possibility that contaminants in the lagoon may exert stress, induce oxidative damage to physiological processes and ultimately affect the wild population. Though no scientific study regarding the seasonal and spatial variation of biomarker profile and heavy metal load in muscle of *Chrysichthys nigrodigitatus* in Lagos Lagoon has been conducted so far. Therefore, the aim of this study was to evaluate the seasonal and spatial biomarker profile in muscle of *Chrysichthys nigrodigitatus* and determine their subsequent response to heavy metal accumulation. It was hypothesized that metal accumulation increases the level of antioxidant defence enzymes in fish muscle. Therefore, this current investigation may provide information on the role of antioxidant enzymes in muscle of fish inhabiting polluted environment.

Materials and Methods

Study area

The Lagos Lagoon is the largest of four lagoon systems in the Gulf of Guinea (24). The distance from Cotonou in the Republic of Benin to the western edge of the Niger delta is about 250km. The lagoon includes forest belt and receives a number of large rivers such as Yewa, Ogun, Ona and Osun rivers, draining more than 103626km^2 of the country and empties into the Atlantic Ocean. The Lagos Harbouris the largest and forms an extensive harbour, which serve as the major outlet of fresh water

from the lagoon system during the rainy season. The central body of the lagoon is located between longitude 003° 23′ 401″and 003° 40′418″E and latitude 06° 22′ 403″and 06° 38′ 420″N. This brackish water is of interest for coastal dynamics and transport of pollutants from the hinterland and the immediate shores of the lagoon (25,26). The lagoon provides home for wide variety of fishery resources, recreational centre, transportation route for ships and container vessels, a dumpsite for residential and industrial discharge and a natural shock absorber to balance forces within the natural ecological system. About 80-85% of the industries in Nigeria are in Lagos State and they all discharge their effluents into the Lagos Lagoon. The effluents discharged are mainly untreated, and very few industries have treatment plants in Nigeria (27). A tropical wet and dry season are the two seasons in Lagos State. Rainy or wet season occurs between April to October while dry season occurs from November to March. Five sampling stations were selected for this study (Apapa, Iddo, Makoko, Ibeshe, Egbin), based on different human activities taking place there and the discharge of different mixture of contaminants into the lagoon (Fig. 1).

Table 1: Description of sampling stations at Lagos Lagoon

S/N	sampling station	GPS of sampling station	Description of sampling station
1.	Apapa	06˚ 27′ 02.3″ N 003˚ 23′ 07.7″ E	transport and shipping activities
2.	Iddo	06˚ 28′ 00.3″ N 003˚ 23′ 01.6″ E	sewage disposal site and fishing activities
3.	Makoko	06˚ 28′ 54.0″ N 003˚ 23′ 06.4″ E	sawmill, wood burning, and fishing activities
4.	Ibeshe	06˚ 34′ 59.9″ N 003˚ 28′ 30.1″ E	textile industry, fishing, sand mining and dredging
5.	Egbin	06˚ 33′ 24.9″ N 003˚ 35′ 51.3″ E	power generation activities

Fig. 1: Lagos Lagoon Displaying Sampled Stations

Sample collection
Fish, sediment and water samples were collected at each sampling station bimonthly for a period of two years, during the wet and dry seasons.

Fish samples
Adult fish samples (n = 546) of *Chrysichthys nigrodigitatus*, were collected with the help of local fishermen from the five selected stations. Total sum of 94,134,112,119 and 87 adult fish samples were collected from Apapa, Iddo, Makoko, Ibeshe and Egbin respectively. Fish samples were identified according to Idodo-Umeh (28). They were placed in aluminium foil lined ice chest at 4℃ to prevent sample deterioration. Samples were labelled and transported to the laboratory for heavy metal and biochemical analysis.

Sediment samples
The sediment samples were collected in triplicates with a 0.25m^2 van Veen grab at each station from an anchored boat and transported to the laboratory. They were labelled for heavy metal analysis. The pH was determined with a water Cyber scan 1000 pH meter and the sediments were acidified to a pH 2 with concentrated hydrochloric acid (HCl) in order to prevent the alteration of organic matter due to microbial activities, air dried and stored in aluminium foil to maintain dryness prior to digestion.

Water samples
Water samples were collected in triplicates at a depth of about 20cm below water surface using 1L reagent bottles with aluminum foil-lined lid that was thoroughly washed and rinsed with acetone, double distilled water and methanol (29). The water samples were acidified to a pH 2 with concentrated hydrochloric acid (HCl) in order to preserve the metals, reduce precipitation and microbial activity.

Estimation of Heavy Metal Level in Fish Muscle
All glasswares and plastic containers were soaked overnight with 10% nitric acid solution and rinsed thoroughly with distilled water before the analysis. One gram of muscle, from fish was dissected for analysis. Dissected samples were transferred to a Teflon beaker and digested in an acid solution to prepare the sample for heavy metal analysis (Kenstar closed vessel microwave digestion) using the microwave digestion program. The samples were digested with 5 ml of nitric acid (65%) and filtered with Whatman filter paper no 41 into a 25ml volumetric flask and distilled water was used to made up to the mark (30). All the digested samples were analyzed in triplicates for metals; Cu, Cd, Pb, Zn, Cr and Fe using Atomic Absorption Spectrophotometer (Perkin-Elmer AAS 700). The instrument was calibrated with standard solutions prepared from commercially available chemicals procured from Merck, Germany (31).

Estimation of Heavy Metal Levels in Sediments
Air dried sediments were stored at 4ºC in an icebox in the laboratory. Dried sediments were homogenized and sieved to remove big particulates. Sieved sediment samples were then digested as follows: 5g of the powdered sediment samples were weighed into a 100 ml beaker. 15ml of freshly prepared mixture of HNO_3 / H_2O_2 ratio 1:1 was added to each sample and covered with a wash glass. It was allowed to stand for 30 minutes during which initial reaction subsided. Digestion was carried out on hot plate whose temperature was allowed to rise gradually until it reached a maximum temperature of 160º C in a fume cupboard. Heating was continued for about 2 hours, reducing the volume in the beaker to about 2 - 5ml. The beaker and its contents were allowed to cool, and the content was transferred with Whatman filter paper no 41 in a 50ml volumetric flask and distilled water

was used made up to mark (30). The digested samples were then analyzed in triplicate for Cu, Cd, Pb, Zn, Cr and Fe using Atomic Absorption Spectrophotometer (Perkin-Elmer AAS 700). The instrument was calibrated with standard solutions prepared from commercially available chemicals procured from Merck, Germany (31).

Estimation of Heavy Metal Levels in Water

From the preserved water samples 25ml were measured into a conical flask, and evaporated to 5ml on a hot plate in fume cupboard at 120ºC. Samples were allowed to cool and filtered in Whatman filter paper no. 41 into 25 ml volumetric flask and distilled water was used to make up the mark (30). All the digested water samples were analysed in triplicates for metals; Cu, Cd, Pb, Zn, Cr and Fe using Atomic Absorption Spectrophotometer (Perkin-Elmer AAS 700). The instrument was calibrated with standard solutions prepared from commercially available chemicals procured from Merck, Germany (31).

Assessment of Antioxidant Enzymes Activities and Lipid peroxidation

Muscle of fish samples were homogenized with 5ml of 0.4M phosphate buffer using Teflon homogenizer. The tissue homogenates were centrifuged at 3000rpm for 15 minutes. The supernatants were analysed for enzyme activities (32), such as the levels of different antioxidants defence enzymes [Superoxide Dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)], non-enzymatic antioxidant [reduced glutathione (GSH)] and biomarker for lipid peroxidation [malondialdehyde (MDA)] following standard protocols. SOD was determined based on the procedure of (32). This involve using a reaction system containing a sodium carbonate buffer of 100mM (pH 10.2), 17.5 µM de EDTA and 1 mM of NBT (nitrotetrazolium blue chloride). The reaction was initiated by the addition of hydroxylamine for a final concentration of 3.7 mM. The increase in absorbance was read at 550nm (33). A unit of SOD was described as the quantity of protein molecules that inhibits the rate of NBT reduction by 50 %. Results were defined as units per milligram protein (U/mg protein). Catalase activity was determined following Sigma protocols that involve the use of ultraviolet (UV) spectrophotometric protocol in a reagent kit (34). After homogenization, 0.2µl of supernatant was place in a cuvette using micropipette, 500µl of 500mM potassium phosphate buffer pH 7.0 and 500µl of 20mM H_2O_2 solution was added and the solution mix by inversion. The reduction in absorbance was determined at the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm ($\varepsilon 240 = 40$ M/cm) (35). Also, a blank solution was prepared using the volume of the same buffer the sample was diluted and absorbance recorded. One unit of catalase activity is defined as the amount of enzyme catalysing the degradation of 1 µmol of H_2O_2 min^{-1} at 37°C and the specific activity corresponding to transformation of substrate (in1mol) (H_2O_2/min/mg protein). The CAT activity is expressed in International Units (U), indicating the quantity of substrate in µmol transformed into product in one minute (µmol min^{-1}) and normalized as a function of the total protein concentration in the homogenate (µmol min^{-1}mg $protein^{-1}$; U mg^{-1}). Measurement of GPx activity according to the method described by (36). The activity of this enzyme was measured by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate. This was measured spectrophotometrically according to (37). The measurement of GSH activity involves homogenizing the tissue in EDTA (0.02 M; pH 6) and subjected to a deproteinisation with sulfosalysilic acid (SSA) at 0.025%. Then maintained for 15 min on ice and centrifuged (3000rpm, 15 min). GSH estimation was measured at 412 nm absorbance. The level of MDA equivalents was analysed by washing the tissue with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were subjected to lipid peroxidation assay immediately. The analysis of the lipid peroxidation was carried out as described (38) with a minor modification. The reaction mixture was prepared by adding 1 mL homogenate into 4 mL reaction solution (15% trichloroacetic acid: 0.375% thiobarbituric acid: 0.25 N NaOH, 1:1:1 w/v) and heated at 100°C for 15 minutes. The mixture was cooled to room temperature, centrifuged (10.000 g for 10

minutes and the absorbance of the supernatant was recorded at 532 nm. MDA results were expressed as nmol mg^{-1} protein in the homogenate. Total protein was estimated using the bicinchoninic acid method (BCA) with the reagent kit made by Sigma-Aldrich with bovine albumin serum as a standard (39).

Statistical analysis

All data were presented as mean±standard error. Antioxidant enzymes and heavy metals data were analysed for fish muscle across sampling stations using Origin 8 software, (Originlab software, USA) and heavy metal concentration in water, sediment and fish muscle during the wet and dry season was calculated using unpaired Student t-test at ($p<0.05$) significant difference. One-way ANOVA was used to calculate significant difference ($p<0.05$) along sampling stations. Principal component analysis from Origin 9 software was used to test the relationship between antioxidants profile and heavy metal concentration.

Results

Heavy metal concentrations

The mean levels of 6 toxicological relevant heavy metals measured in fish muscle, water and sediment during the wet and dry seasons is shown in Tables 2- 4. In fish muscle, at Apapa, the mean concentration followed the pattern Fe > Zn > Cu > Pb > Cd > Cr with values 14.95 µg/g, 14.54 µg/g, 3.47 µg/g, 0.57 µg/g, 0.55 µg/g, 0.29 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Zn > Cu > Pb > Cd > Cr with values 40.14 µg/g, 30.63 µg/g, 5.17 µg/g, 0.82 µg/g, 0.51 µg/g, 0.31 µg/g respectively. At Iddo, the mean concentration followed the pattern Fe > Zn > Cu > Cd > Cr > Pb with values 15.95 µg/g, 7.95 µg/g, 3.88 µg/g, 0.55 µg/g, 0.50 µg/g, 0.17 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Zn > Cu > Cd > Cr > Pb with values 27.66 µg/g, 15.07 µg/g, 9.42 µg/g, 0.68 µg/g, 0.47 µg/g, 0.29 µg/g respectively. At Makoko, the mean concentration followed the pattern Fe > Zn > Cu > Pb > Cr > Cd with values 29.9 µg/g, 12.02 µg/g, 9.50 µg/g, 0.57 µg/g, 0.42 µg/g, 0.18 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Zn > Cu > Pb > Cr > Cd with values 68.27 µg/g, 12.54 µg/g, 9.87 µg/g, 1.09 µg/g, 0.62 µg/g, 0.29 µg/g respectively. At Ibeshe, the mean concentration followed the pattern Fe > Zn > Cu > Cr > Pb > Cd with values 40.49 µg/g, 15.66 µg/g, 7.54 µg/g, 2.05 µg/g, 0.09 µg/g, 0.00 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Zn > Cu > Cr > Pb > Cd with values 57.88 µg/g, 34.01 µg/g, 12.18 µg/g, 3.09 µg/g, 0.27 µg/g, 0.01 µg/g respectively. At Egbin, the mean concentration followed the pattern Fe > Zn > Cr > Cu > Pb > Cd with values 27.83 µg/g, 8.91 µg/g, 1.18 µg/g, 0.20 µg/g, 0.09 µg/g, 0.00 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Zn > Cr > Cu > Pb > Cd with values 69.36 µg/g, 8.62 µg/g, 1.59 µg/g, 0.48 µg/g, 0.27 µg/g, 0.00 µg/g respectively (Table 2). Highest Pb value was recorded in wet season at Makoko with mean values of 1.09±0.42 µg/g, while lowest concentration of Pb was recorded at Ibeshe and Egbin with same mean values 0.09±0.04 µg/g in dry season. Cd concentrations increased in the fish muscle in dry season from Makoko (0.18±0.20 µg/g) to Iddo (0.68±0.51 µg/g) in wet season. Cr concentrations increased in dry season from Apapa (0.29±0.15 µg/g) to Ibeshe (3.09±0.88 µg/g) in wet season. Highest Cu value was recorded in wet season at Ibeshe with mean values of 12.18±4.69 µg/g, while Egbin recorded the lowest concentration of Cu with values 0.20±0.07 µg/gin dry season. Highest Zn value was recorded in wet season at Ibeshe with mean values of 34.01±14.07 µg/g, while Iddo recorded the lowest concentration of Zn with values 7.95±2.69 µg/gin dry season. Fe concentrations increased in the fish muscle in dry season from Apapa (14.95±6.34 µg/g) to Egbin (69.36±27.39 µg/g) in wet season. All the respective metal concentrations showed significant seasonal variation ($p<0.05$). Pb, Cu and Zn showed significant

differences ($p<0.05$) across the five sampling stations. However, all the respective metal concentrations were above permissible limits specified for aquatic life by Food and Agricultural Organization and World Health Organization (40).

In water, at Apapa, the mean concentration followed the pattern Fe > Cu > Zn > Pb > Cr > Cd with values 147.35 mg/L, 0.30 mg/L, 0.12 mg/L, 0.09 mg/L, 0.07 mg/L, 0.06 mg/L respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Pb > Zn > Cr > Cd > Cu with values 2831.77 mg/L, 3.43 mg/L, 0.59 mg/L, 0.35 mg/L, 0.32 mg/L, 0.26 mg/L respectively. At Iddo, the mean concentration followed the pattern Fe > Cu > Cr > Zn > Pb > Cd with values 84.76 mg/L, 0.28 mg/L, 0.22 mg/L, 0.15 mg/L, 0.14 mg/L, 0.07 mg/L respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Pb > Cu > Cd > Zn > Cr with values 10515.79 mg/L, 0.93 mg/L, 0.87 mg/L, 0.52 mg/L, 0.47 mg/L, 0.41 mg/L respectively. At Makoko, the mean concentration followed the pattern Fe > Cu > Zn > Cd > Cr > Pb with values 53.45 mg/L, 0.17 mg/L, 0.13 mg/L, 0.11 mg/L, 0.07 mg/L, 0.04 mg/L respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Cr > Cu > Cd > Pb = Zn with values 833.25 mg/L, 0.36 mg/L, 0.34 mg/L, 0.26 mg/L, 0.25 mg/L, 0.25 mg/L respectively. At Ibeshe, the mean concentration followed the pattern Fe > Cu > Zn > Cr > Cd > Pb with values 76.45 mg/L, 0.53 mg/L, 0.18 mg/L, 0.15 mg/L, 0.11 mg/L, 0.04 mg/L respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Cu > Cr > Zn > Pb > Cd with values 1761.78 mg/L, 1.30 mg/L, 0.55 mg/L, 0.29 mg/L, 0.25 mg/L, 0.21 mg/L respectively. At Egbin, the mean concentration followed the pattern Fe > Cr > Cu > Cd > Zn = Pb with values 65.16 mg/L, 0.15 mg/L, 0.09 mg/L, 0.07 mg/L, 0.04 mg/L, 0.04 mg/L respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Pb > Cr > Zn > Cu > Cd with values 759.70 mg/L, 0.47 mg/L, 0.44 mg/L, 0.43 mg/L, 0.27 mg/L, 0.10 mg/L respectively (Table 3). Highest Pb value was recorded in wet season at Apapa with average values of 3.43±2.01 mg/L, while the remaining stations recorded lower values in dry season. Cd concentrations increased in the lagoon water in dry season from Apapa (0.06±0.04 mg/L) to Iddo (0.52±0.38 mg/L) in wet season. Cr concentrations increased in dry season from Apapa (0.07±0.03 mg/L) to Ibeshe (0.55±0.48 mg/L) in wet season. Highest Cu value was recorded in wet season at Ibeshe with mean values of 1.30±0.77 mg/L, while Egbin recorded the lowest concentration of Cu with values 0.09±0.10 mg/L in dry season. Highest Zn value was recorded in wet season at Apapa with mean values of 0.59±0.27 mg/L, while Egbin recorded the lowest concentration of Zn with values 0.04±0.02 mg/L in dry season. Fe concentrations increased in the lagoon water in dry season from Makoko (53.45±48.98 mg/L) to Iddo (10515.79±8234.23 mg/L) in wet season. Pb, Cd, Cu and Fe showed significant seasonal variation ($p<0.05$) in concentrations. These same metals showed significant differences ($p<0.05$) across the five sampling stations except Cr and Zn. However, all the respective metal concentrations were above permissible limits specified for aquatic life by Food and Agricultural Organization and World Health Organization (40).

In sediment, at Apapa, the mean concentration followed the pattern Fe > Zn > Cd > Pb > Cu > Cr with values 174.85 µg/g, 24.05 µg/g, 14.35 µg/g, 1.67 µg/g, 0.28 µg/g, 0.26 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Zn > Pb > Cr > Cu > Cd with values 3016.88 µg/g, 99.24 µg/g, 61.60 µg/g, 48.96 µg/g, 25.48 µg/g, 16.64 µg/g respectively. At Iddo, the mean concentration followed the pattern Fe > Zn > Cd > Pb > Cr > Cu with values 112.34 µg/g, 42.77 µg/g, 8.45 µg/g, 1.24 µg/g, 0.66 µg/g, 0.36 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Zn > Cr > Pb > Cu > Cd with values 2596.41 µg/g, 77.66 µg/g, 43.32 µg/g, 32.34 µg/g, 21.68 µg/g, 8.30 µg/g respectively. At Makoko, the mean concentration followed the pattern Fe > Zn > Cd > Pb > Cu > Cr with values 106.13 µg/g, 21.28 µg/g, 3.84 µg/g, 1.14 µg/g, 0.36 µg/g, 0.35 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Cr > Cu > Zn > Pb > Cd with values

1697.45 µg/g, 58.45 µg/g, 47.57 µg/g, 38.48 µg/g, 17.50 µg/g, 17.37 µg/g respectively. At Ibeshe, the mean concentration followed the pattern Fe > Zn > Cd > Pb > Cr > Cu with values 64.54 µg/g, 11.27 µg/g, 4.84 µg/g, 0.99 µg/g, 0.51 µg/g, 0.50 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Cr > Cu > Zn > Pb > Cd with values 662.43 µg/g, 90.45 µg/g, 38.99 µg/g, 32.08 µg/g, 20.30 µg/g, 3.40 µg/g respectively. At Egbin, the mean concentration followed the pattern Fe > Zn > Cd > Pb > Cr > Cu with values 90.89 µg/g, 17.53 µg/g, 3.73 µg/g, 0.63 µg/g, 0.27 µg/g, 0.21 µg/g respectively in dry season. While in wet season, the mean concentration followed the pattern Fe > Cr > Cu > Zn > Pb > Cd with values 3264.98 µg/g, 43.67 µg/g, 31.36 µg/g, 16.29 µg/g, 10.70 µg/g, 3.47 µg/g respectively (Table 4). Highest Pb value was recorded in wet season at Apapa with average values of 61.60±32.10 µg/g, while the lowest value was recorded at Egbin 0.63±0.44 µg/g in dry season. Cd concentrations increased in the lagoon sediment in wet season from Ibeshe (3.40±2.93 µg/g) to Makoko (17.37±10.66 µg/g) in the same season. Cr concentrations increased in dry season from Apapa (0.26±0.35 µg/g) to Ibeshe (90.45±57.57 µg/g) in wet season. Highest Cu value was recorded in wet season at Makoko with mean values of 47.57±26.86 µg/g, while Egbin recorded the lowest concentration of Cu with values 0.21±0.10 µg/gin dry season. Highest Zn value was recorded in wet season at Apapa with mean values of 99.24±33.31 µg/g, while Ibeshe recorded the lowest concentration of Zn with values 11.27±9.86 µg/gin dry season. Fe concentrations increased in the lagoon sediment in dry season from Ibeshe (64.54±22.56 µg/g) to Egbin (3264.98±2240.68 µg/g) in wet season. All the respective metal concentrations showed significant seasonal variation ($p<0.05$) and significant differences ($p<0.05$) across the five sampling stations. However, all the respective metal concentrations were above permissible limits specified for aquatic life by Food and Agricultural Organization and World Health Organization (40).

Table 2: Heavy metal concentrations in muscle of *C. nigrodigitatus* from Lagos Lagoon

		Fish muscle		
Heavy metal (µg/g)	**Sampling station**	**Dry**	**Wet**	**FAO/WHO 2016 (µg/g d.w)**
Pb	Apapa	0.57±0.50[b]	0.82±0.48[b]	0.03
	Iddo	0.17±0.08[a]	0.29±0.31[a]	
	Makoko	0.57±0.25b[*]	1.09±0.42[b]	
	Ibeshe	0.09±0.04[a]	0.27±0.35[a]	
	Egbin	0.09±0.04[a]	0.27±0.35[a]	
Cd	Apapa	0.55±0.25[a]	0.51±0.38[a]	0.03
	Iddo	0.55±0.27[a]	0.68±0.51[a]	
	Makoko	0.18±0.20[a]	0.29±0.33[a]	
	Ibeshe	0.00±0.01[a]	0.01±0.02[a]	
	Egbin	0.00±0.01[a]	0.02±0.02[a]	
Cr	Apapa	0.29±0.15[a]	0.31±0.19[a]	0.005
	Iddo	0.50±0.23[a]	0.47±0.27[a]	
	Makoko	0.42±0.21[a]	0.62±0.21[a]	
	Ibeshe	2.05±0.59[a]*	3.09±0.88[a]	
	Egbin	1.18±0.88[a]	1.59±1.06[a]	
Cu	Apapa	3.47±1.86[b]	5.17±1.59[b]	0.05
	Iddo	3.88±0.66[b]*	9.42±6.06[c]	
	Makoko	9.50±3.67[d]	9.87±7.63[d]	
	Ibeshe	7.54±2.27[c]	12.18±4.69[d]	
	Egbin	0.20±0.07[a]	0.48±0.22[a]	
Zn	Apapa	14.54±3.57[ab]	30.63±12.88[b]	0.3-1
	Iddo	7.95±2.69a	15.07±9.64[a]	
	Makoko	12.02±3.37[bc]	12.54±5.20[a]	
	Ibeshe	15.66±8.44[c]*	34.01±14.07[b]	
	Egbin	8.91±1.74[ab]*	8.62±4.44[a]	
Fe	Apapa	14.95±6.34*	40.14±5.89[a]	0.8
	Iddo	15.95±4.11[a]	27.66±15.33[a]	
	Makoko	29.99±13.17[a]*	68.27±25.61[a]	
	Ibeshe	40.49±13.37[a]	57.88±21.81[a]	
	Egbin	27.83±12.07[a]*	69.36±27.39[a]	

Mean±sem (standard error of the mean; SEM). Different letters denote significant difference (p<0.05) across sampling points. Asterisks (*) denote significant difference between seasons.

Table 3: Heavy metal concentrations of water from Lagos Lagoon

Water				
Heavy metal (mg/L)	**Sampling Station**	**Dry**	**Wet**	**FAO/WHO 2016 (mg/L)**
Pb	Apapa	0.09 ± 0.06^{*a}	3.43 ± 2.01^{a}	0.01
	Iddo	0.14 ± 0.06^{b}	0.93 ± 0.48^{b}	
	Makoko	0.04 ± 0.01^{a}	0.25 ± 0.16^{b}	
	Ibeshe	0.04 ± 0.01^{a}	0.25 ± 0.16^{b}	
	Egbin	0.04 ± 0.03^{a}	0.47 ± 0.29^{b}	
Cd	Apapa	0.06 ± 0.04	0.32 ± 0.30^{a}	0.003
	Iddo	0.07 ± 0.03*	0.52 ± 0.38^{b}	
	Makoko	0.11 ± 0.13	0.26 ± 0.06^{a}	
	Ibeshe	0.11 ± 0.12	0.21 ± 0.09^{a}	
	Egbin	0.07 ± 0.06	0.10 ± 0.04^{a}	
Cr	Apapa	0.07±0.03	0.35±0.18	0.05
	Iddo	0.22±0.31	0.41±0.44	
	Makoko	0.07±0.06	0.36±0.39	
	Ibeshe	0.15±0.30	0.55±0.48	
	Egbin	0.15±0.19	0.44 ±0.25	
Cu	Apapa	0.30± 0.35^{ab}	0.26 ± 0.13^{a}	1
	Iddo	0.28±0.14^{*ab}	0.87 ± 0.66^{bc}	
	Makoko	0.17± 0.10a	0.34 ± 0.19^{ab}	
	Ibeshe	0.53 ± 0.41^{*b}	1.30 ± 0.77^{c}	
	Egbin	0.09 ± 0.10^{a}	0.27 ± 0.16^{a}	
Zn	Apapa	0.12 ± 0.07	0.59 ± 0.27	3
	Iddo	0.15 ± 0.05	0.47 ± 0.30	
	Makoko	0.13 ± 0.04	0.25 ± 0.16	
	Ibeshe	0.18 ± 0.25	0.29 ± 0.12	
	Egbin	0.04 ± 0.02	0.43 ± 0.24	
Fe	Apapa	147.35±98.60^{a}	2831.77±1284.51^{a}	0.2
	Iddo	84.76±57.25^{*ab}	10515.79±8234.23^{b}	
	Makoko	53.45±48.98^{a}	833.25 ± 471.38^{a}	
	Ibeshe	76.45± 40.43^{ab}	1761.78±1280.61^{a}	
	Egbin	65.16 ± 17.70^{a}	759.70 ± 138.61^{a}	

Mean±sem (standard error of the mean; SEM). Different letters denote significant difference ($p<0.05$) across sampling points. Asterisks (*) denote significant difference between seasons.

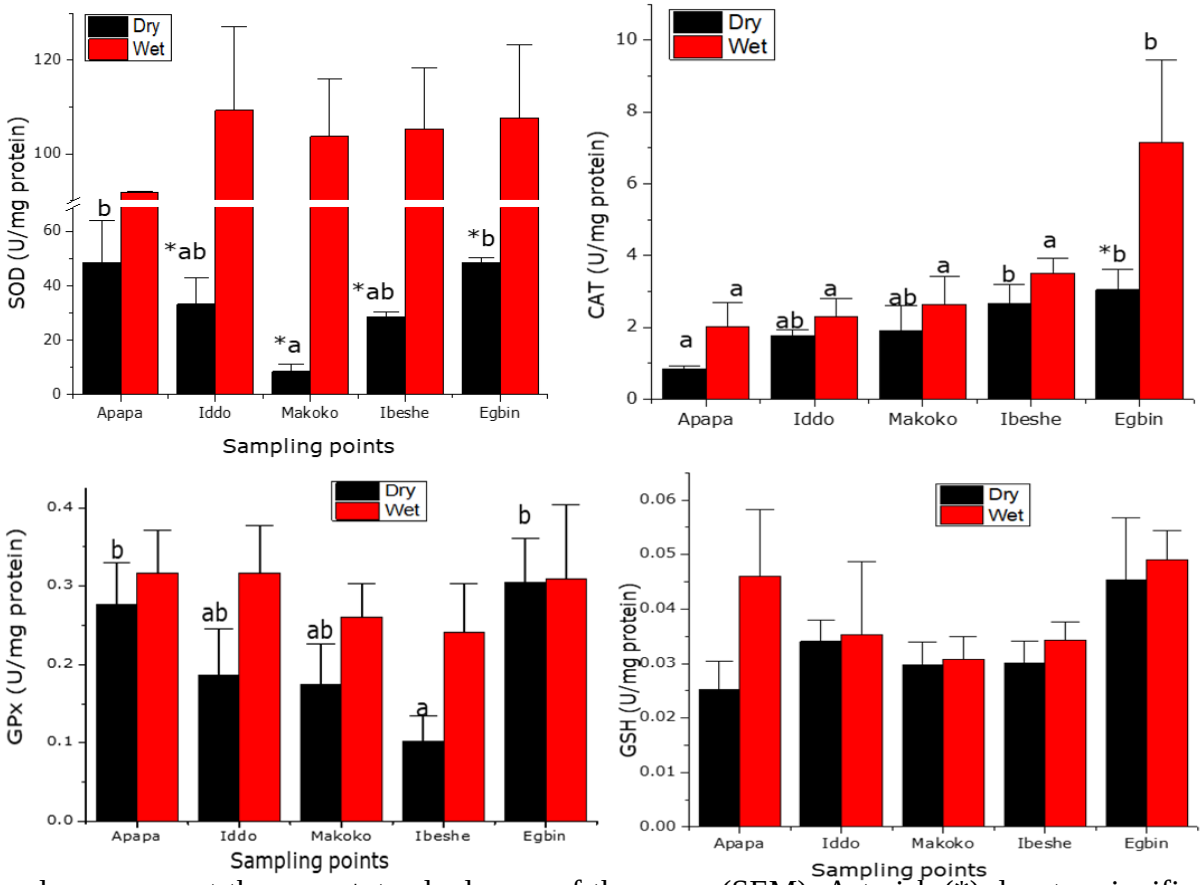
Table 4: Heavy metal concentrations of sediment from Lagos Lagoon

		Sediment		
Heavy metal (µg/g)	**Sampling station**	**Dry**	**Wet**	**FAO/WHO 2016 (µg/g d.w)**
Pb	Apapa	1.67 ± 0.83*	61.60 ± 32.10a	31
	Iddo	1.24 ± 0.88*	32.34 ± 25.99b	
	Makoko	1.14 ± 0.85	17.50 ± 8.52b	
	Ibeshe	0.99 ± 0.90	20.30 ± 15.45b	
	Egbin	0.63 ± 0.44	10.70 ± 11.57b	
Cd	Apapa	14.35 ± 3.24c	16.64 ± 14.41b	0.6
	Iddo	8.45 ± 3.49b	8.30 ± 2.93 ab	
	Makoko	3.84 ± 3.05*a	17.37 ± 10.66b	
	Ibeshe	4.84 ± 3.06a	3.40 ± 2.93a	
	Egbin	3.73 ± 1.97a	3.47 ± 2.66a	
Cr	Apapa	0.26 ± 0.35	48.96 ± 23.89*	26
	Iddo	0.66 ± 0.50	43.32 ± 25.31*	
	Makoko	0.35 ± 0.30*	58.45 ± 43.57	
	Ibeshe	0.51 ± 0.48*	90.45 ± 57.57	
	Egbin	0.27 ± 0.30*	43.67± 29.26	
Cu	Apapa	0.28 ± 0.11*ab	25.48 ± 22.99	16
	Iddo	0.36 ± 0.20ab	21.68 ± 18.29	
	Makoko	0.36 ± 0.26*ab	47.57 ± 26.86	
	Ibeshe	0.50 ± 0.24*b	38.99 ± 14.39	
	Egbin	0.21 ± 0.10*	31.36 ± 16.64	
Zn	Apapa	24.05 ±15.50*a	99.24 ± 33.31bc	120
	Iddo	42.77±24.73*b	77.66 ± 17.75c	
	Makoko	21.28 ± 9.64a	38.48 ± 20.30ab	
	Ibeshe	11.27 ± 9.86a	32.08 ± 11.33a	
	Egbin	17.53 ± 6.85a	16.29 ± 10.69a	
Fe	Apapa	174.85±47.25*a	3016.88±2272.25ab	0.8
	Iddo	112.34±10.82*b	2596.41±2055.07ab	
	Makoko	106.13 ± 56.65b	1697.45±1535.92ab	
	Ibeshe	64.54 ± 22.56b	662.43 ± 470.61a	
	Egbin	90.89 ± 56.57*b	3264.98±2240.68b	

Mean±sem (standard error of the mean; SEM). Different letters denote significant difference (p<0.05) across sampling points. Asterisks (*) denote significant difference between seasons.

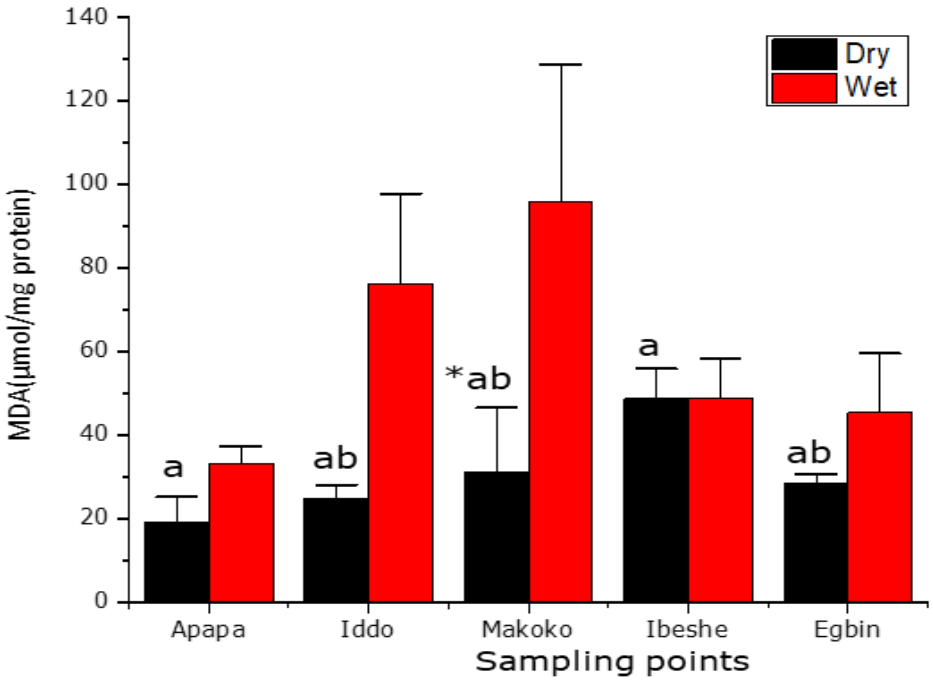
Antioxidant defence enzymes activities

The SOD activity in fish muscle of *C. nigrodigitatus* was significantly higher in wet season than dry season, with the highest activity at Iddo and lowest at Makoko. CAT activity was significantly higher in wet season than dry season, with the highest enzyme activity at Egbin and lowest at Apapa. SOD and CAT showed significant differences across sampling stations. GSH activity in fish muscle did not show any seasonal and spatial differences. GPx activity showed spatial differences with highest activities at Apapa and Iddo in wet season and lowest at Ibeshe in dry season (Fig 2). The level of malonaldehyde in fish muscle was significantly higher in wet season at Makoko and lower at Apapa in dry season (Fig 3).



All values represent the mean±standard error of the mean (SEM). Asterisk (*) denotes significant difference between dry and wet seasons. Different letters denote significant differences between sampling points. The level of significant was set at $p<0.05$

Fig. 2: Activities of antioxidant defence enzymes in muscle of *C. nigrodigitatus*



All values represent the mean±standard error of the mean (SEM). Asterisk (*) denotes significant difference between dry and wet seasons. Different letters denote significant differences between sampling points. The level of significant was set at $p<0.05$.

Fig 3: The levels of malonialdehydein muscle of *C. nigrodigitatus*

Relationship between heavy metal concentration and antioxidant enzyme activities

A PCA was applied to all the data to help discriminate the particular variables that are responsible for the variance of heavy metal concentration and antioxidant defence enzyme activities in *C. nigrodigitatus* (Fig. 4). This was based on the extracted eigenvectors from this analysis (Table 5). Overall, the PCA showed a clear seasonal differentiation between wet and dry seasons, highlighting the different enzyme activities in relation to heavy metals from different anthropogenic inputs. PC1 accounted for 37.7% of variance and showed a clear separation between heavy metal concentration couple with antioxidant enzyme synthesis during the wet season and dry season. There was a negative relationship between dry season and wet season, FMFe, FMZn and FMCu. PC2 explained 19.9% of variance and demonstrate the influence of seasonal trends on contaminants produced from human activities. The PCA confirmed that exposure of *C. nigrodigitatus* to heavy metal may trigger the induction of antioxidant enzymes activities that serve as a protective mechanism against oxidative stress. Correlation analysis showed several statistically significant associations between heavy metal levels and antioxidant enzymes in *C. nigrodigitatus* (Table 5). Wet season was positively correlated with SOD, CAT, GPx, MDA, FMPb, FMCu, FMZn, FMFe, WPb, WCd, WCr, WCu, WZn, WFe, SPb, SCr, SCu, SZn, SFe ($p < 0.05$). On the other hand, dry season showed a negative significant correlation with Makoko and Egbin. More so, sampling stations Apapa, Iddo and Ibeshe show positive correlation between heavy metals in the three media are the predominant stations that generates heavy metals.

Table 5: Extracted Eigenvectors for the relationship between heavy metal concentration and antioxidant enzyme activities in *C. nigrodigitatus*

	PC1(37.7%)	PC2(19.9%)	PC3(11.1%)	PC4(10.6%)
Wet season	0.28009	-0.06441	-0.1153	-0.06542
Dry season	-0.28009	0.06441	0.1153	0.06542
Apapa	0.04012	0.28134	0.13085	-0.1556
Iddo	0.02641	0.11983	0.11667	0.29556
Makoko	-0.02131	0.01186	-0.4469	0.16589
Ibeshe	0.02019	-0.29136	0.26626	0.09767
Egbin	-0.06541	-0.12167	-0.06689	-0.40353
SOD	0.21494	-0.15987	0.07929	0.10282
CAT	0.16326	-0.15702	0.17091	-0.21184
GPx	0.1522	0.16435	0.29926	-0.12868
GSH	0.08975	0.12436	-0.10079	-0.17168
MDA	0.18011	-0.12426	-0.24939	0.22026
FMPb	0.12036	0.17495	-0.34853	0.02642
FMCd	0.07421	0.33439	0.04084	0.22847
FMCr	0.03018	-0.3734	0.17095	-0.06107
FMCu	0.12545	-0.10545	-0.03705	0.40169
FMZn	0.18604	-0.03904	0.23474	-0.00666
FMFe	0.15722	-0.24379	-0.25995	-0.17819
WPb	0.19916	0.22021	0.12678	-0.20864
WCd	0.24995	0.0713	0.0387	0.20223
WCr	0.24755	-0.16348	-0.01418	-0.03939
WCu	0.15458	-0.21448	0.21019	0.26103
WZn	0.26662	0.07769	0.05129	-0.13552
WFe	0.19361	0.06997	0.13105	0.21955
SPb	0.26124	0.14865	0.08971	-0.09318
SCd	0.10869	0.26981	-0.15255	0.05082
SCr	0.25351	-0.15057	-0.06926	-4.64E-04
SCu	0.24191	-0.12901	-0.23081	-0.03144
SZn	0.21773	0.24623	0.1104	0.04575
SFe	0.23878	0.06499	-0.10215	-0.21255

SOD-superoxide dismutase, CAT- catalase, GPx- glutathione peroxidase, GSH-reduced glutathione, MDA-malondialdehyde, FMPb-lead in fish muscle, FMCd- cadmium in fish muscle, FMCr-chromium in fish muscle, FMCu- copper in fish muscle, FMZn- zinc in fish muscle, FMFe- iron in fish muscle, WPb-lead in water, WCd- cadmium in water, WCr- chromium in water, WCu-copper in water, WZn-zinc in water, WFe-iron in water, SPb-lead in sediment, SCd- cadmium in sediment, SCr- chromium in sediment, SCu-copper in sediment, SZn-zinc in sediment, SFe-iron in sediment.

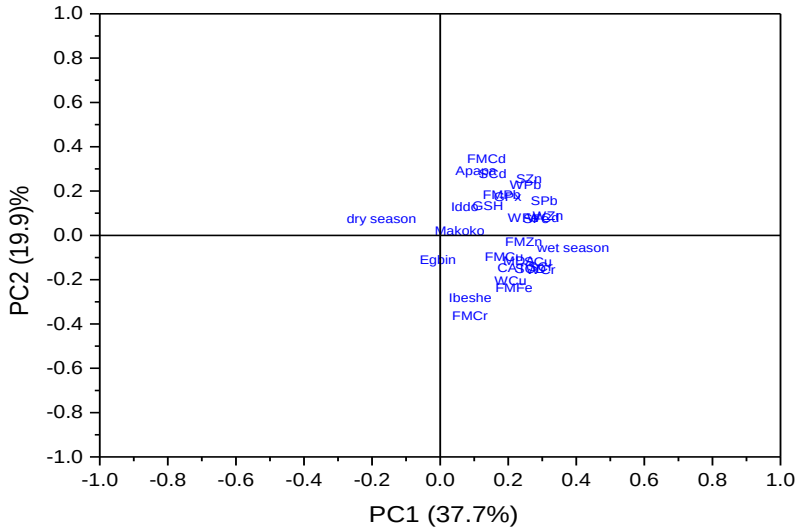


Fig 4: Principal component analysis of antioxidant defence enzymes activities in fish muscle, heavy metal concentration in fish muscle, water and sediment at sampling stations of Lagos Lagoon during the wet and dry season. SOD-superoxide dismutase, CAT- catalase, GPx- glutathione peroxidase, GSH-reduced glutathione, MDA-malondialdehyde, FMPb-lead in fish muscle, FMCd- cadmium in fish muscle, FMCr- chromium in fish muscle, FMCu- copper in fish muscle, FMZn- zinc in fish muscle, FMFe- iron in fish muscle, WPb-lead in water, WCd- cadmium in water, WCr- chromium in water, WCu-copper in water, WZn-zinc in water, WFe-iron in water, SPb-lead in sediment, SCd-cadmium in sediment, SCr- chromium in sediment, SCu-copper in sediment, SZn-zinc in sediment, SFe-iron in sediment.

Discussion

Aquatic habitats are easily destroyed by contaminants from human activities thereby altering the water quality. The results showed the pollution pattern of heavy metals in fish muscle, water and sediment as well as the antioxidant enzymes activities across the sampling stations in Lagos Lagoon during the wet and dry seasons. Heavy metal concentrations results were higher in wet season than dry season. Fish accumulates heavy metals from surrounding water through the gills and ingestion of contaminated food (41). *Chrysichthys nigrodigitatus* is a benthopelagic species that inhabits the bottom sediment and feeds on small fish, shrimps, crabs, polychaete worms and other invertebrates. These organisms inhabit the bottom sediment and as such makes *Chrysichthys nigrodigitatus* susceptible to both water and diet borne metals (41). Fe, Zn and Cu are essential metals that function majorly in metabolic activities and are integrated into the body, while non-essential metals like Cr, Cd and Pb are known to exert toxicity even at trace levels (12). Fe, Zn and Cu are the predominant metals in this fish muscle, which indicates their role in metabolic activities. Fish at Ibeshe has a high concentration of these metals, reflecting the level of anthropogenic perturbations such as textile effluent discharge, sand mining and dredging, and fishing activities. High concentrations of these metals in this fish muscle may have negative consequences on the physiological functioning of this organism. Majority of the metals analysed from water samples in this study, indicates anthropogenic influence. Iddo and Ibeshe are associated with sewage and textile effluent discharges, respectively. The presence of complex mixtures of contaminants in these effluents enhances the concentration of metals within the water column. Iddo station exhibited a high metal content, which may be attributed to domestic sewage inputs into the lagoon (42). Tripathi and Warwade (43) reported a high Fe content

in domestic sewage, which is an indicative feature of its constituent in many black waters (excreta, urine and fecal sludge and grey water). Dredging activities and sand mining at Ibeshe are also possible sources of metals. This agrees with the report made by Ugwu (44) that high metal contaminants in Nigerian waters can be due to increased urbanization and proliferation of industries. Untreated wastes from the hinterlands, leachate from dump sites, sewage discharge may increase urban run-offs when it rains, and these run-offs drain into the aquatic system resulting in increased metal concentrations. This explains the higher metal concentrations in wet season than dry season from this study. Sediments are known as ultimate repository for metals, and as a metal pool that can release metals into the water column via physical and biochemical processes such as bioturbation, resuspension, currents and waves, dredging and shipping activities (45,46). In this study, the sediment samples have a larger concentration of heavy metals especially Fe, Zn, Cd and Pb in wet season. The high Fe concentration exhibited in the sediment is because Nigerian soil has high concentration of Fe (47). Higher Zn levels in sediments during the wet season reflects the formation of colloidal precipitates with organic matter from dissolved salts present in industrial and domestic wastewater which eventually settles out slowly into the sediment (48). The divalent characteristics of Pb and Cd enable them to displace Fe/Mn monosulphides within the sediment, resulting in the formation of large insoluble sulphide precipitates which may not desorb during a resuspension event as such increase the concentration within the sediment (49).

Antioxidant defence enzymes have the capacity of decreasing oxidative stress due to environmental contaminants (9). Heavy metals such as Pb and Cd may enhance the generation of highly reactive molecules known as reactive oxygen species like superoxide anion radical, resulting in oxidative stress (8,9). These species can alter the structure and function of macromolecules such as protein, nucleic acid. The role of SOD is to catalyse the dismutation of superoxide anion radical to water and hydrogen peroxide (9). In the present study, there was a significant increase in SOD activity in wet season than dry season. This indicate that contaminants produced from human activities induces the activity of this enzyme. The role of CAT and GPx is to detoxify the hydrogen peroxide formed by SOD (5,7). CAT activity was observed to be higher in wet season than dry season. Past investigations on antioxidant system on fish, have demonstrated that antioxidant enzymes like SOD and CAT are the first set of enzymes that protect the cell against oxidative imbalance and tissue damage (7).Induction in SOD activity may indicates an adaptive response to heavy metal-triggered superoxide anion radical production while a similar pattern in CAT and GPx enzyme activities was supposed to indicate altered hydrogen peroxide (11, 13).The elevated metal pollution in this lagoon may thrust a larger oxidative stress resulting to significant increase ($p < 0.05$) in SOD and CAT activities in muscle of this fish. Increase activities of these antioxidant enzymes may reflect a preliminary defence system against oxidative damage due to metal exposure (11). Glutathione conjugants (GPx and GSH) represents the secondary line of defence to tackle oxidative damage (11). GSH has the capacity to react with metals within aquatic organisms and a cofactor in GPx activity (55). This enzyme activity did not show any significant difference ($p > 0.05$) in the present study, and this may be due to the increase in glutathione peroxidase (GPx) levels as alternative adaptive response to oxidative stress (7). Decreased activities of these enzymes SOD, CAT, GSH and GPx in dry season reflects the toxic effects of heavy metal which overwhelms enzyme activity, resulting in their inhibition (13). Heavy metal concentrations in fish muscle from this study, were above permissible limits and this may inhibit antioxidant enzymes activity. Scientific evidence has revealed that the concentration of metals above international thresholds for aquatic life may have negative consequences on aquatic organisms (13). The mechanism that indicates cell damage is lipid peroxidation. Malonialdehyde (MDA) is one of the by-products of lipid peroxidation within the cell. This study demonstrates a significant increase in MDA from fish muscle which was higher in wet season. The increase MDA levels may be

attributed to accumulation of metals since this study indicates significant concentrations of heavy metals in fish muscle (11).

The relationship between antioxidant response and heavy metal burden through the PCA confirmed that seasonal trends are the most important driver of heavy exposure thereby inducing antioxidant enzyme activity in *C. nigrodigitatus* (7). The PCA also highlighted the role of human activity from the sampling stations as the main contributors to high metal concentrations in fish muscle, water and sediment.

Conclusion

This study indicated that high concentration of heavy metals in aquatic organism may alter physiological function, thereby inducing oxidative stress. Increase activities of the antioxidant enzymes at elevated metal concentrations in fish muscle were observed and possible severe oxidative damage due to increase malonialdehyde resulting in lipid peroxidation. Also, the increased enzyme activities demonstrated as adaptive mechanisms against oxidative stress was observed. Glutathione conjugants were involved in protecting fish against oxidative stress. Seasonal trends influence the degree of heavy metal concentration, as such may affect the physiological and metabolic functioning of aquatic organism. Future studies are still needed to evaluate the interaction of metals in different fish organs and the relative contributions of enzymatic and low molecular-mass antioxidants in protecting these organs from oxidative damage. This study provides information on antioxidant defence enzyme as one of the mechanism aquatic organisms uses to conserve and sustain their population against oxidative stress from contaminants within the aquatic system.

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REFERENCES

1. Lopez-Galindo C, Vargas-Chacoff L, Nebot E, Casanueva J F, Rubio D, Sole M, & Mancera J M (2010). Biomarker responses in *Solea senegalensis* exposed to sodium hypochlorite used as antifouling. *Chemosphere*. 78: 885.

2. Kroon F, Streten C, & Harries S (2017). A protocol for identifying suitable biomarkers to assess fish health: A systematic review. PLoS ONE 12(4): e0174762.

3. Fakhri M (2012). The state of the Lebanese coastal waters, in: Review and Perspectives of Environmental Studies in Lebanon. INCAM-EU/ CNRS Lebanon, pp. 31–54
4. Rather M, MohdTilwani Y, & Dey A (2019). Assessment of heavy metal contamination in two edible fish species Carassius and Triplophysa kashmirensis of dal Lake, Srinagar, Kashmir, India. *Environ Monit Assess* 2:191–242.

5. Tchounwou P, Yedjou C, Patlolla A K, & Sutton D J (2012). Heavy Metals Toxicity and the Environment. *National Institutes of Health. Public Access.* 101: 133-164.

6. Duffus J H (2002). Heavy metals-a meaningless term? *Pure and Applied Chemistry.* 74(5):793–807.

7. Moniruzzaman M, Midday P, Dhara A, Das D, Ghosal L, Mukherjee D, & Chakraborty S B (2017). Change in redox state and heat shock protein expression in an Indian major carp

Cirrhinus cirrhosis exposed to zinc and lead. *Journal of Toxicological Science.* 42. 6., 731-740.

8. Akporido S O & Onianwa P C (2015). Heavy metals and total petroleum hydrocarbon concentrations in surface water of Esi River, Western Niger Delta. *Research Journal of Environmental Sciences.* 9: 88-100.

9. Das D, Moniruzzaman M, Sarbajna A, & Chakraborty S (2017). Effect of heavy metals on tissue-specific antioxidant response in Indian major carps. *Environmental Science and Pollution Research*. 24: 18010–18024.

10. Adeogun A O & Chukwuka A V (2012). Toxicity of industrial wastewater acting singly or in joint ratios on *Clarias gariepinus*. *American Journal of Environmental Science.* 8: 366-375.

11. Savorelli F, Manfra L, Croppo M, Tornambe A, Palazzi D, Canepa S, Trentini P, Cicero A, & Faggio C (2017). Fitness Evaluation of *Ruditapesmphilippinarum* Exposed to Ni. *Biological Trace Element Research.* 177: 384–393.

12. Akintujoye J F, Anumudu C I, & Awobode H O. Assessment of heavy metal residues in water, fish tissue and human blood from Ubeji, Warri, Delta State, Nigeria. Journal of Applied Science and Environmental Management. 2; 17(2):291-297.

13. Ibanga B, Nkwoji B, Usese I, Onyema C, & Chukwu O (2019). Hydrochemistry and heavy metals concentrations in sediment of Woji creek and Bonny estuary, Niger Delta, Nigeria, *Regional Studies in Marine Science.* 25: 1004-10436.

14. Mukherkee J, Moniruzzaman M, Bhusan S, Lek S, & Ray S (2017). Towards a physiological response of fishes under variable environmental conditions: An approach through neural network. *Ecological indicators.* 78: 381-394.

15. van der Oost R, Beyer J, & Vermeulen N P E (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ ToxicolPharmacol*. 13: 57–149.
16. Kroon F, Streten C, & Harries S (2017). A protocol for identifying suitable biomarkers to assess fish health: A systematic review. PLoS ONE 12(4): e0174762

17. Tchounwou P, Yedjou, C G, Patlolla A K, & Sutton D. J. (2012). Heavy Metals Toxicity and the Environment. *National Institutes of Health. Public Access.* 101: 133-164.

18. Akporido S O & Onianwa P C (2015). Heavy metals and total petroleum hydrocarbon concentrations in surface water of Esi River, Western Niger Delta. *Research Journal of Environmental Sciences.* 9: 88-100.

19. Adeogun A O & Chukwuka A V (2012). Toxicity of industrial wastewater acting singly or in joint ratios on *Clarias gariepinus*. *American Journal of Environmental Science.* 8: 366-375.

20. Savorelli F, Manfra L, Croppo M, Tornambe A, Palazzi D, Canepa S, Trentini P, Cicero A, & Faggio C (2017). Fitness Evaluation of *Ruditapes philippinarum* Exposed to Ni. *Biological Trace Element Research.* 177: 384–393.

21. Akintujoye J F, Anumudu C I, Awobode H O. Assessment of heavy metal residues in water, fish tissue and human blood from Ubeji, Warri, Delta State, Nigeria. *Journal of Applied Science and Environmental Management.* 25; 17(2):291-297.

22. Ibanga B, Nkwoji B, Usese I, Onyema, C, & Chukwu O (2019). Hydrochemistry and heavy metals concentrations in sediment of Woji creek and Bonny estuary, Niger Delta, Nigeria, *Regional Studies in Marine Science.* 25: 1004-10436.

23. Mukherkee J, Moniruzzaman M, Bhusan S, Lek S, & Ray S (2017). Towards a physiological response of fishes under variable environmental conditions: An approach through neural network. *Ecological indicators.* 78: 381-394.

24. Schlenk D. 1999. Necessity of Defining Biomarkers for Use in Ecological Risk Assessments. *Marine Pollution Bulletin*. 39: 48–53.

25. Handy R D, Galloway T S, & Depledge M H. (2003). A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. *Ecotoxicology*. 12: 331–43.

26. Storelli, M (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food and chemical toxicity*. 46: 2782-2788.

27. Moniruzzaman M, Midday P, Dhara A, Das D, Ghosal I, Mukherjee D, & Chakraborty S B (2017). Change in redox state and heat shock protein expression in an Indian major carp *Cirrhinuscirrhosus* exposed to zinc and lead. *Journal of Toxicological Science.* 42: 731-740.

28. Ajani E G (2014). Oxidative stress in fish living in coastal water polluted with sawdust and wood waste along Lagos Lagoon, Nigeria. *Researcher* 6: 1–5.

29. Akinsanya B, Otubanjo O A & Ibidapo C A (2007). Helminth bioload of *Chrysichthys nigrodigitatus* (Lacepede 1802) from Lekki Lagoon, Lagos Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences.* 7:83-87.

30. Omondi R, Yasindi A W, & Magana A M (2011). Spatial and temporal variations of zooplankton in relation to some environmental factors in lake Baringo, Kenya. *Egerton Journal of Science and Technology.* 11:29-50.

31. Esenowo I K, Ugwumba A, Umoh I, & Andem A B (2017). Aspects of the Biology of Silver Catfish (*Chrysichthys nigrodigitatus*) in Nwaniba River, Southeast Nigeria. *Asian Journal of Biology.* 3: 1-9.

32. Webb J E (1958). The ecology of Lagos Lagoon, some physical properties of lagoon deposits. Philosophical Transactions of the Royal Society of London. Series B, *Biological Sciences*. 683: 393-419.

33. Ajao E & Fagade S O (1990). A study of sediments in communities in Lagos Lagoon. *Oil and Chemical*. 785-117.

34. IPEP (2006). The international POPs Elimination Projects (IPEP). Assessment of the Lagos Lagoon for POPs sources, types and impacts. Friend of the Environment (FOTE), Nigeria-Anglophone Africa, pp 123.

35. Oyedepo S O & Fagbenle R L 2011. A study on implementation of preventive maintenance programme in Nigeria power industry- Egbin thermal power plant, case study, *Energy and Power Engineering.* 3:207-220.

36. Idodo-Umeh G (2002). Pollution assessments of Olomoro water bodies using Physical, Chemical and Biological indices: *PhD. Thesis*, University of Benin, Benin City, Nigeria. pp 485.

37. Food and Agriculture Organization of the United Nation (FAO)/SIDA (2003). Manual of methods in aquatic environment research part 9. Analysis of metals and organochlorines in fish. FAO Fish Tech. Pap. (212) pp 32.

38. Kingston H M & Jassie L B (1988). Fundamental relationships in acid decomposition of samples for elemental analysis using microwave energy. *Materials Research Society.* 4:25.
39. Magwere T, Naiks Y S, & Hasler J A (1997). Effect of chloroquine treatment on antioxidant enzymes in rat liver and kidney. *Free Radical Biology and Medicine.* 22: 321-327.

40. McCord J & Fridovich I (1969). Superoxide dismutase, an enzymatic function for erythrocuprein. *Journal of Biological Chemistry.* 244: 6049–6055.

41. Aebi H. (1984) Catalase in vitro. In Methods in enzymology Jan 1 (Vol. 105, 316 pp. 121-126). Academic Press.

42. Lamprecht W & Trautschold I (1963). in *Methods of Enzymatic Analysis*, eds Bergmeyer H U (Academic Press, New York & London), pp 453.

43. Ellman G L & Fiches F T (1959). Quantitative determination of peptides by sulfhydryl groups. *Archives of Biochemistry and Biophysics.* 82: 70-72.

44. Weckbercker G & Cory J G (1988). Ribonucleotide reductase activity and growth of glutathione-depended mouse leukaemia L 1210 cells in vitro. *Cancer Letters.* 40: 257-264.

45. Buege J A & Aust S D (1978). Microsomal lipid peroxidation Methods. *Enzymol*52: 302-310.

46. Smith P K, Krohn R I, Hermanson G T, Mallia A K, Gartner F H, Provenzano M D, Fujimoto E K, Goeke N M, Olson B J, & Klenk D C (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*. 150.1: 76-85.

47. Food and Agriculture Organization of the United Nation (FAO)/WHO (2016): Joint FAO/WHO food standards programme codex committee on contaminants in foods, fifth. Session pp 64-89.

48. Aliko V, Qirjo M, Sula E, Morina V, & Faggio C. (2018). Antioxidant defense system, immune response and erythron profile modulation in goldfish, *Carassius auratus*, after acute manganese treatment. *Fish and Shellfish Immunology.* 76:101–109.

49. Deepali, K K (2010). Metals concentration in textile and tannery, associated soils and ground water. New *York Science Journals*. 3 (4), 82-89.

ORIGINAL RESEARCH ARTICLE

Comparative assessment of the acute and sub-acute effects of some plant based Ichthyotoxins on aquatic organisms: a case study of *Clarias gariepinus*

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Abstract
Plant-based ichthyotoxins are increasingly used for commercial fishing activities in many countries all over the world. This study investigated the acute and sub-acute effects of the aqueous extracts of some plant-based ichthyotoxins, commonly used in Nigeria, on *Clarias gariepinus*. Ichthyotoxins in the bark of *Anarcadium occidentale*, seeds of *Carica papaya*, nuts *of Raphia vinifera* and leaves of *Manihot esculenta* were extracted using referenced methods. Acute effect of the ichthyotoxins to *Clarias gariepinus* was assessed using static bioassays for a 96-h period and sub-acute effect on the biochemical processes in the fish was assessed using static renewal bioassays for 28 days. Acute toxicity studies showed that *A. occidentale* was the most toxic to the fish with a 96h LC_{50} value of 35.159 mg/L compared to the other ichthyotoxins. The 96h LC_{50} values of *C. papaya*, *R. vinifera* and *M. esculenta* were 539.236 mg/L, 828.741 mg/L and 1160.233 mg/L respectively. Sub-acute toxicity studies showed that the ichthyotoxins had minimal effects on the biochemical processes in the fish. Activities of the antioxidant and liver function enzymes investigated did not increase in exposed fish compared to the control. This study showed that some plant-based ichthyotoxins can be acutely toxic to fish at low concentrations and their general application on surface waters expose target and non-target macro invertebrates to the acute effects of the toxins. Hence, there is a need to enlighten indigenous fishermen and regulate the use of plant-based ichthyotoxins which are presumed to be safer than synthetic ichthyotoxins.

Biological: Ecotoxicology. Keywords: *Plant-based Ichthyotoxins, Fish, Toxicity*

Introduction
Aquatic pollution is a global environmental challenge which both the developed and developing countries are steadily trying to curb. Life below water is continually threatened by pollution especially those resulting from anthropogenic activities [1]. Over the last few decades, there has been an increased use of plant based ichthyotoxins (piscicidal plants) for commercial fishing activities in many parts of the world including Nigeria [2, 3]. Plant-based ichthyotoxins are fish poisons derived from plants [4] and active ingredients commonly detected in such plants include alkaloids, tannins, saponins, flavonoids, resin and phenolics [5]. They are perceived to be less harmful to the aquatic environment than synthetic ichthyotoxins [6]. Favourable properties of plant-based ichthyotoxins include availability, rapid biodegradability, less bioaccumulation potential, and reduced toxicity to non-target organisms [7]. Many studies including those of Fafioye et al. [2], Vimal and Das [4],

Odioko et al. [8] and Saha et al. [7], assessing the toxicity of plant-based ichthyotoxins to target and non-target aquatic species have focused mainly on acute toxic effects basically, mortality. The sub-acute biochemical effects of these plants are rarely studied and have been documented by only few reports including Tiwari and Singh [6] and Adamu and Kori-Siakpere [9]. The dearth of knowledge on the sub-acute effects of this category of fish poisons could be attributed to the perceived high degradability (short half-life) of the plant toxins. However, the persistence and degradability of toxins can be altered by prevailing factors including quantity and frequency of use, chemical characteristics of the toxins, as well as biological and physicochemical characteristics of the receiving aquatic ecosystem [10]. Alongside acute toxicity studies, it is vital to investigate the possible sub-acute toxic effects of commonly used plant -based ichthyotoxins to ensure the protection of non-target fish and invertebrate aquatic species inadvertently exposed to these toxins. Fafioye [5] has documented the plant-based ichthyotoxins commonly used for fishing activities in South western Nigeria. This study therefore investigated the acute and sub-acute toxic effects of some of these commonly used plants namely *Carica papaya* (seed), *Raphia vinifera* (nuts), *Anacardium occidentale* (bark) and *Manihot esculenta* (leaves) on *Clarias gariepinus*, a representative freshwater fish species. The status of the selected plants is abundant or common [5] which indicated availability of the plants and facilitated easy collection of plant parts for use in experiments. *Clarias gariepinus* was used as test organism in this study due to its obtainability, it is commonly cultured for commercial purposes in Nigeria, and it is also easy to maintain under laboratory conditions.

Materials and Methods

Collection and preparation of plant samples

Carica papaya (seeds), *Anacardium occidentale* (bark), and *Manihot esculenta* (leaves) were collected from Enugu State, South-eastern Nigeria. While *Raphia vinifera* (nuts) were collected from University of Lagos Nigeria. All plant samples were collected in the early hours of the day between 6:00 and 9:00 am. After collection, the samples were taken to the Herbarium unit of the Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria for proper identification. The plant samples were air dried in the laboratory, at room temperature for two weeks and then oven dried at 32 ˚C for 30 minutes. The seeds of *C. papaya*, the bark of *A. occidentale* and the leaves of *M. esculenta* were pulverised using an electric blender while the nuts of *R. vinifera* was pulverised using an electrical grinding machine. The powder of each plant sample was sieved through a 100 µm sieve to obtain fine powder and transferred into air-tight sterile bottles, labelled, and stored at 4˚C until further analyses.

Aqueous extraction of ichthyotoxins

Ichthyotoxins from the plant samples were extracted by soaking 100 g of the powder from each sample in 1L of distilled water. The solutions were left for 72 h to undergo fermentation and stirred once, morning and evening during this period. After the fermentation period, the solution of each sample was filtered through a Whatman (No.1) filter paper to obtain the aqueous extracts [5]. The extract from each plant sample was then oven dried at 100 ˚C using a Genlab oven. Ten grams of each dried extract was collected and stored at 4 ˚C until use in toxicity studies.

Phytochemical screening of ichthyotoxins

The extracts were screened for presence of active ingredients such as alkaloids, flavonoids, tannins, saponins and phenols using standard methods. The extracts were screened for alkaloids according to methods described by Adegoke et al. [11], for tannins and phenols according to methods described by Boxi et al. [12] and for flavonoids according to methods described by Shahid-Ud-Duaula and Anwarul [13].

Toxicity Studies

Test organism

Clarias gariepinus (African Sharptooth Catfish) was used as test organism in this study. *C. gariepinus* fingerlings (2-3 weeks old) and juveniles (4-6 weeks old) were purchased from a local fish farm in Lagos State, Nigeria and transported in oxygenated polythene bags, to the Research Laboratory, Department of Zoology, University of Lagos, Nigeria. The fingerlings and juveniles were acclimatized separately for seven days in holding tanks, half filled with dechlorinated tap water. They were fed with commercially prepared fish feed (Vital Fish Feed, Nigeria) at 3% body weight during this period and water in the tanks was changed once every other day to avoid pollution by fish metabolic wastes and food remnants. Feeding was discontinued 24 h before the commencement of experiments.

All experimental procedures complied with provisions guiding the use of animal in research by the University of Lagos.

Stock solution of ichthyotoxins

Stock solutions of the extracts were prepared by dissolving 5g of each extract in 1L of distilled water to give a solution of 5 g/L. The stock solutions were serially diluted 1:5 to 1:500 (toxicant: water content) depending on required concentrations, for use in toxicity testing studies.

Acute toxicity studies

The acute toxicity studies were carried out according to standard methods [14]. Briefly, range finding studies were carried out in which *C. gariepinus* fingerlings were exposed to 10, 100, 1000 and 2000 mg/L of each of the plant extracts for 24 h. Graded working concentrations were selected within two concentrations where 10% and 90% mortality was recorded, respectively. After the range finding studies, *C. gariepinus* fingerlings were exposed to graded concentrations of each of the plant extracts. Seven active fingerlings were randomly exposed in triplicates to graded concentrations of each extract and negative controls for 96 h. The fish were exposed to *C. papaya* at 200, 300, 400, 500, 600, 700 mg/L and negative control; *A. occidentale* at 10, 20, 30, 40, 50, 60 mg/L and negative control; *M. esuculenta* at 1000, 1100, 1200, 1300, 1400, 1500 mg/L and negative control and *R. vinifera* at 600, 700, 800, 900, 1000, 1100 mg/L and negative control. Mortality was assessed and recorded once every 24 h during the exposure period.

Sub-acute toxicity studies

Clarias gariepinus juveniles were exposed to sub-acute concentrations (10% 96 h LC_{50}) of the two most toxic ichthyotoxins (*C. papaya* and *A. occidentale*) out of the four tested during acute toxicity studies. The fish were exposed to 53.9 mg/L of *C. papaya* and 3.5 mg/L of *A. occidentale*. Ten active juveniles were exposed to the two ichthyotoxins respectively in two replicates and negative controls. These series of experiment were carried out for a period of 28 days and the semi-static bioassay method was employed to avoid changes in concentration of toxins via evaporation and excessive reduction in dissolved oxygen level. Test media were changed into fresh solution of the same concentration of the ichthyotoxins or control respectively, once every three days, transferring the same exposed test animals into the freshly prepared test media during the 28-day period of the experiment. On the 28^{th} day, two fish per replicate to give four per treatment and untreated control respectively were randomly selected, and the liver was harvested and stored in sterile plain specimen bottles at 4 ˚C until further analysis.

Biochemical studies
Antioxidant stress enzymes, substrates of oxidative stress and liver function enzymes in the liver of the fish exposed to the two ichthyotoxins were analyzed using referenced methods.

Homogenization of liver samples
The liver samples were washed in ice cold 1.15 % potassium chloride (KCL) solution, blotted and weighed. The samples were then homogenized using 0.1M phosphate buffer (pH 7.2) and acid wash sand. The homogenate was centrifuged at 2500 rpm for 15 minutes and the supernatant was decanted into sterile specimen bottles and stored at -20 $^{\circ}$C until analysis.

Determination of antioxidant stress enzyme activity
Superoxide dismutase (SOD) and catalase (CAT) activities in the samples were determined according to methods described by Sun and Zigma [15] and Sinha [16] respectively.

Quantification of substrates of oxidative stress
Glutathione (GSH) content in the samples was determined using the method described by Sedlak and Lindsay [17] while malondialdehyde (MDA) an index of lipid peroxidation, was determined using the method of Buege and Aust [18].

Determination of liver function enzyme activity
Alkaline phosphatase (ALP) activity in the samples was measured using the method described by Wright et al. [19]. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using the method described by Reitman and Frankel [20].

Statistical analysis
The dose-response data obtained from the acute toxicity studies were analyzed using SPSS (Statistical Package for Social Sciences) version 20.0. Indices of measuring acute toxicity (lethal concentration affecting a percentage of exposed organisms) and their 95 % confidence limits were reported.

Data obtained from biochemical studies were analyzed using one-way analysis of variance (ANOVA) and where a significant difference ($p < 0.05$) was obtained, Duncan new multiple range tests was used to detect the source of the difference.

Results

Phytochemical properties of plant extracts
Extracts from each of the plant-based ichthyotoxins contained at least three out of the five active ingredients screened. Extract from the nuts of *R. vinifera* contained the least number (three) out of the five active ingredients screened (Table I).

Table I: Phytochemical composition of the extracts from the plant-based ichthyotoxins

Ichthyotoxins	Alkaloids	Tannins	Saponins	Flavonoids	Phenolics
A. occidentale	+	+	+	-	+
C. papaya	+	+	+	-	+
M. esculenta	-	+	+	+	+
R. vinifera	-	+	+	-	+

+; present, -; absent

Acute toxicity of the plant-based ichthyotoxins against *Clarias gariepinus*
Mortality in fish exposed to the different extracts increased with increasing concentrations of the extracts. Extracts from the bark of *A. occidentale* was the most toxic against the fish species with a

96 h LC_{50} value of 35.159 mg/L while extract from the leaves of *M. esculenta* was the least toxic against the fish species with a 96 h LC_{50} value of 1160.233 mg/L. The 96 h LC_{50} values of the extracts of the seeds of *C. papaya* and the nuts of *R. vinifera* against the fish were 539.236 mg/L and 828.741 mg/L respectively (Table II).

Table II: Acute toxicity parameters of the plant-based ichthyotoxins against *Clarias gariepinus*

Ichthyotoxins	LC_{95} 95% C.L(mg/L)	LC_{50} 95% C.L(mg/L)	LC_{5} 95% C.L(mg/L)	Slope ± S. E	D.F	Probit Line Eqn	T. F
A. occidentale	87.005 (67.263 – 142.623)	35.159 (30.114–40.772)	14.208 (8.454–18.587)	4.180 ± 0.734	4	Y=-6.462+4.180x	1
C. papaya	944.434 (787.183 – 1366.077)	539.236 (490.836 – 601.957)	307.884 (225.211 – 360.467)	6.758 ± 1.254	4	Y=-18.461+6.758x	15.3
M. esculenta	1534.568 (1431.429–1745.139)	1160.233 (1099.232–1210.667)	877.211 (738.984–959.386)	13.544±2.312	4	Y=-41.508+13.544	32.9
R. vinifera	1132.396 (1043.306–1304.318)	828.741 (785.099–872.245)	606.511 (520.483–662.101)	12.132±1.865	4	Y=-35.406+12.132x	23.5

D. F - Degree of Freedom
T. F - Toxicity Factor (ratio of the LC_{50} values to the lowest LC_{50} value in the table)
S. E - Standard Error
L. C - Lethal Concentration
C. L - Confidence limits
Eqn - Equation

Sub-acute effects of the extracts of *Anarcadium occidentale* and *Carica papaya* on the biochemical variables of *Clarias gariepinus*

The activity of SOD antioxidant enzyme reduced significantly ($p < 0.05$) in fish exposed to the extracts of *A. occidentale* and *C. papaya* (1.91 ± 0.27 U/mg protein and 3.32 ± 0.56 U/mg protein respectively) compared to activity recorded in the control (6.04 ± 0.02 U/mg protein). Catalase enzyme activity in fish exposed to extracts of the two plants (*A. occidentale*; 14.41 ± 3.80 U/mg protein and *C. papaya*; 16.25 ± 0.35 U/mg protein) were not significantly different to level of activity recorded in the control (17.58 ± 0.01 U/mg protein). The concentration of GSH in the liver of fish exposed to the extracts of the two plants respectively, were significantly ($p < 0.05$) lower than concentration of the substrate obtained in the control fish (*A. occidentale*; 24.95 ± 1.00 U/mg protein, *C. papaya*; 28.73 ± 1.45 U/mg protein and control; 35.89 ± 0.01 U/mg protein, respectively). The concentration of MDA in fish exposed to the extracts of *C. papaya* (3.44 ± 0.47 U/mg protein) was significantly ($p < 0.05$) higher than concentration obtained in the control (1.51 ± 0.02 U/mg protein), while the concentration of the substrate in fish exposed to the extracts of *A. occidentale* (2.25 ± 0.41 U/mg protein) was not significantly different to concentration in the control (Table III).

The activities of the liver function enzymes screened (AST, ALT and ALP) in fish exposed to the extracts of *A. occidentale* and *C. papaya* respectively, were not significantly different from level of activities of the enzymes recorded in the control fish (Table III).

Table III: Comparative biochemical variables (U/mg protein) of *Clarias gariepinus* exposed to extracts of *Anarcadium occidentale* and *Carica papaya*

Variables (M ± S.D)	Control	Extracts	
		A. occidentale	C. papaya
SOD	6.04 ± 0.02^{b}	1.91 ± 0.27^{a}	3.32 ± 0.56^{a}
CAT	17.58 ± 0.01^{a}	14.41 ± 3.80^{a}	16.25 ± 0.35^{a}
GSH	35.89 ± 0.01^{c}	24.95 ± 1.00^{a}	28.73 ± 1.50^{b}
MDA	1.51 ± 0.02^{a}	2.25 ± 0.41^{a}	3.44 ± 0.47^{b}
AST	88.84 ± 0.08^{a}	75.46 ± 14.82^{a}	72.71 ± 16.31^{a}
ALT	28.71 ± 0.07^{a}	29.88 ± 9.90^{a}	35.13 ± 0.68^{a}
ALP	6.76 ± 0.02^{a}	16.19 ± 6.13^{a}	20.19 ± 14.21^{a}

Means in the same row with different superscripts are significantly different ($p < 0.05$), M – Mean, S.D – Standard Deviation, SOD - Superoxide Dismutase, CAT- Catalase, GSH – Glutathione, MDA – Malondialdehyde, AST- Aspartate Aminotransferase, ALT - Alanine Aminotransferase, ALP - Alkaline phosphatase

Discussion

The plant-based ichthyotoxins evaluated in this study contained phytochemicals commonly detected as active ingredients in plant-based fish poisons [5, 21]. The extracts of all the ichthyotoxins contained tannins, saponins and phenolics. Additionally, alkaloids were detected in extracts of *C. papaya* and *A. occidentale*.

The acute toxicity study results showed that the extract of *A. occidentale* was the most acutely toxic to *C. gariepinus* compared to extracts of *C. papaya*, *R. vinifera*, and *M. esculenta*, respectively. The pattern of acute toxicity of the ichthyotoxins to the fish was *A. occidentale>C. papaya>R. vinifera>M. esculenta*. The higher toxicities of *A. occidentale* and *C. papaya* to the fish may be attributed to the classes of active ingredients detected in the extracts of the plants compared to the extracts of the other plants. They were the only plants that contained alkaloids. Mortality recorded during the acute toxicity studies was dose dependent with an increased mortality at higher concentrations of the ichthyotoxins. This observation conforms with several studies carried out to evaluate acute toxicity of pollutants on fish, including those of Ayotunde and Ofem [22]; Bawa-Allah and Akinnuoye [23].

Exposure to environmental pollutants may induce oxidative stress in organisms and this has been documented by several scholars including Doherty et al. [24], Faramobi et al. [25] and Olagoke [26]. Exogenous substances absorbed by an organism induce an increased production of reactive oxygen species (ROS) which may overwhelm the organism's natural ability to detoxify reactive intermediates and repair damage that may occur in cellular molecules, resulting in oxidative stress [27]. Antioxidant enzymes scavenge ROS and an increase in these enzymes' activities and substrates of oxidative damage are employed as biomarkers of oxidative stress. In this study, the biomarkers of oxidative stress assessed in fish exposed to the extracts of the two most acutely toxic ichthyotoxins (*A. occidentale* and *C. papaya*) to the fish did not increase significantly ($p < 0.05$) compared to the control. The activity of SOD in exposed fish was lower compared to the control while activity of CAT did not differ from the control. The substrates of oxidative damage (GSH and MDA) were also lower in exposed fish compared to the control except for the concentration of MDA in fish exposed to extracts of *C. papaya* which was significantly higher than the concentration in control. The absence of increased oxidative stress enzymes activity in exposed fish compared to the control suggests that the fish were not under oxidative stress.

The increased activity of liver function enzymes such as aminotransferases and alkaline phosphatases are frequently employed as biomarkers indicative of physiological stress in organism exposed to toxic substances [3]. The activities of liver function enzymes; AST, ALT and ALP assessed in fish exposed to the extracts of *A. occidentale* and *C. papaya*, during sub-acute toxicity studies showed that the exposed fish were not under physiological stress. The activities of the enzymes in the exposed fish did not differ significantly ($p < 0.05$) from activities recorded in the control. Dar and Paul [3] has also assessed the sub-acute effects of a plant-based ichthyotoxin (*Anamirta cocculus*) on fish, but they reported a decrease in activities of AST and ALT in tissue of exposed fish compared to the control.

Conclusion

This study has established that acute toxicity of plant-based ichthyotoxins differ greatly depending on plant type and active ingredients present. Some plant-based ichthyotoxins can be acutely toxic to fish at low concentrations. The use of this class of ichthyotoxins in commercial fishing activities usually involve a general application on surface waters facilitating maximum influence on both target and non-target aquatic organism. There is a need for relevant agencies to enlighten indigenous local fishermen on the dangers of plant-based ichthyotoxins to non-target aquatic organisms especially macroinvertebrates that are important links in the aquatic food chain. Although ichthyotoxins may be safer than synthetic toxins with minimal sub-acute effects as observed in this study, unregulated use could result in the collapse of the aquatic food chain if smaller non-target organisms are constantly harmed during fishing activities.

References

Amoatey P & Baawain M S (2019). Effects of pollution on freshwater aquatic organisms. *Water Environment Research*. 91: 1272-1287.

Fafioye O, Adebisi A, & Fagade S O (2004). Toxicity of *Parkiabiglobosa* and *Raphia vinifera* extracts on *Clariasgariepinus* juveniles. *African Journal of Biotechnology*. 3: 627-630.

Dar U Q & Paul V I (2018). Impact of ethanol extract of *Anamirta cocculus* (Linn.) seeds on tissue damage biomarkers of the predatory catfish *Heteropneustes fossilis* (Bloch.). *Journal of Taibah University for Science*. 12: 357-370.

Vimal J B & Das S M (2015). Toxicity of *Euphorbia antiquorum* latex extracts to freshwater fish *Poecilia reticulata. International Journal of Fisheries and Aquatic Studies.* 2: 214-216.

Fafioye O (2005). Plants with piscicidal activities in south western Nigeria. *Turkish Journal of Fisheries and Aquatic Science.* 5: 91-97.

Tiwari S & Singh A (2004). Toxic and sub-lethal effects of oleandrin on biochemical parameters of freshwater air breathing murrel, *Channa punctatus* (Bloch.). *Indian Journal of Experimental Biology*. 42: 413-418.

Saha S, Behera S, Bhakta D, Das A, Mandal A, Kumar S, & Mondal A (2017). Piscicidal effect of *Luffa cylindrical* fruit extract on tilapia fingerlings, *Oreochromis mossambicus* in captive condition. *Journal of Pharmacognosy and Phytochemistry*. 6: 427-430.

Odioko E, Sikoki F D, Vincent-Akpu I F, & Utibe D I (2016). Acute toxicity of sponge plant (*Luffa Cylindrica*) fruit extract on African Catfish (*Clarias Gariepinus,* Buchell 1822) juveniles. *International Journal of Life Sciences Research.* 4: 148-159.

Adamu K M & Kori-Siakpere O (2011). Effects of sublethal concentrations of tobacco (*Nicotiana Tobaccum*) leaf dust on Some biochemical parameters of hybrid catfish (*Clarias gariepinus* and *Heterobranchus Bidorsalis*). *Brazilian Archives of Biology and Technology*. 54: 183-196.

Schafer R B, van den Brink P J, & Liess M (2011). Impacts of pesticides on freshwater ecosystems. *Ecological Impacts of Toxic Chemicals.* 2011: 111-137.

Adegoke A, Iberi P A, Akinpelu D A, Aiyegoro O A, & Mboto C I (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Resources and Natural Products.* 3: 6–12.

Boxi M, Rajesh Y, Raja A, Kumar V, Praveen B, & Mangamma K (2010). Extraction, phytochemical screening, and in-vitro evaluation of antioxidant properties of *Commicarpus chinesis* (aqueous leaf extract). *International Journal Pharmaceuticals and Bioscience.* 1: 537–547.

Shahid-Ud-Duaula A F M & Anwarul B M (2009). Phytochemical screening, plant growth inhibition, and antimicrobial activity studies of *Xylocarpus granatum Malaysian Journal of Pharmaceutical Science.* 7: 9–21.

American Public Health Association (APHA) (1995). *Standard Methods for Examination of Water and Wastewater*. 16th Edition American Public. Health Association, New York. pp. 1193.

Sun M & Zigman S (1978). An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Analytical Biochemistry* 90: 81-89.

Sinha A K (1972). Colorimetric assay of catalase. *Analytical Biochemistry.* 47: 389-394.

Sedlak J & Lindsay R H (1968). Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry.* 25: 1192-1205

Buege J A & Aust S D (1978). Microsomal lipid peroxidation. *Methods in Enzymology.* 52: 302-310.

Wright P J, Leathwood P D, & Plummer D T (1972). Enzymes in rat urine: alkaline phosphatase. *Enzymologia.* 42: 317-327.

Reitman S & Frankel S A (1957). Colorimetric method for the determination of serum oxaloacetic and glutamic pyruvate transaminase. *American Journal of Clinical Pathology.* 28: 56-63.

Cannon J G, Burton R A, Wood S G, & Owen N L (2004). Naturally occurring fish poisons from plants. *Journal of Chemical Education.* 81: 1457-1461

Ayotunde E O & Ofem B O (2008). Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to Nile Tilapia *Oreochromis niloticus* (Linne 1757), Adult. *African Journal of Biotechnology.* 7:2265-2274.

Bawa-Allah K A & Akinnuoye B O (2019). Acute toxicity and adverse effects of aqueous and ethanol extracts of *Parkia biglobosa* pods on biochemical parameters of *Clarias gariepinus. African Journal of Aquatic Sciences*. 44: 89-95.

Doherty V F, Ogunkuade O, & Kanife U C (2010). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in some selected fishes in Lagos, Nigeria. *American-Eurasian Journal of Agriculture & Environmental Science.*7: 359-365.

Farombi E O, Adewole O A, & Ajimoko Y R (2007). Effect of butachlor on antioxidant enzyme status and lipid peroxidation in fresh water African Catfish, (*Clarias gariepinus). International Journal of Environmental Research and Public Health.*5: 423-427.

Olagoke O (2008). Lipid peroxidation and antioxidant defence enzymes in *Clarias gariepinus* as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. *MSc Theses, University of Lagos, Lagos, Nigeria.*70pp.

Almroth B C (2008). Oxidative damage in fish used as biomarkers in field and laboratory studies. *Department of Zoology/Zoophysilogy Goteborg University Sweden.* 74pp.

ORIGINAL RESEARCH ARTICLE

Conversion of sewage sludge from healthcare facility to bio-crude oil using hydrothermal liquefaction technology

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Abstract
Population growth, municipal waste management challenges, fossil fuel depletion and its associated pollution are increasingly becoming public health issues. These necessitate the production of renewable and sustainable biofuels. This study, therefore, employed the use of hydrothermal liquefaction for the conversion of sewage sludge to bio-crude oil.

Samples of the sewage sludge were purposively collected from sewage treatment plant and subjected to laboratory analyses (in triplicates) using standard methods (ASTM and AOAC) to obtain the composition. Sewage sludge (25 kg) from the sewage treatment Plant of University College Hospital, Ibadan, was converted at 300^{o}C to bio-crude oil and biochar using hydrothermal liquefaction. The bio-crude oil was analysed for its physico-chemical properties.

Conversion product yield gave 34% bio-crude oil with cloud point, pour point, viscosity and density of -38.89^{o}C, -36.11^{o}C, 0.86cP and 1.04gml^{-1}respectively. The Higher Heating Value (HHV) of the bio-crude oil was 30.60 MJ/kg. A total of 18 compounds were identified from the analysis of Total Petroleum Hydrocarbon (TPH) in the bio-crude oil with 17 compounds within the diesel range organics of crude oil which showed that bio-crude oil has great potential to serve as alternative to fossil fuels.

Keywords: *Hydrothermal liquefaction, sewage sludge, bio-crude oil.*

Introduction
In the world today, the major challenges facing most countries with crude oil deposit include, depleting fossil fuels, volatile oil prices, mitigation of CO_2 emissions and creation of new job opportunities. Therefore, arresting unprecedented climate change, maintenance of global economic growth and substituting the role played by depleting oil are the major driving forces behind renewable energy development.

In the first quarter of 2015, global atmospheric carbon dioxide levels were reported to have reached a record high of 400 parts per million (ppm), which is a very frightening figure (Dlugokencky and Tans, 2015). In order to mitigate this menace, the global temperature rise has to be restricted to 2 °C and greenhouse gas (GHG) emissions maintained in the range of 445–490 ppm CO_2-equivalent (Edenhofer *et al.*, 2011). Similarly, carbon dioxide emissions have to be reduced to 25-40% and 80-

90% by 2020 and 2050 respectively, as agreed in 2009 'Copenhagen Climate Change Summit'. However, there has been a failure to meet the agreed targets because atmospheric CO_2 has already exceeded the dangerous level, 10 years earlier than had previously been predicted (Stephens *et al*., 2010).

Furthermore, environmental pollution could be traced to a deleterious effect of the exploration and production of fossil fuel (NNPC, 2013). In fact, the exploitation of oil and gas reserves has not always been without some ecological side effects, oil spills, damaged land, accidents and fires, and incidents of air and water pollution have all been recorded at various times and places (Adati, 2012). In fact, virtually every aspect of oil exploration and exploitation has deleterious effects on ecosystem stability and local biodiversity which the peoples' livelihoods depend upon (Zabbey, 2005).

In addition, over the past few decades, first generation biomass or edible biomass (for example grains, corn, oil seeds etc.) used for biofuel production have been in competition with food crops for consumption leading to increasing food prices globally (Putun *et al*., 2006; Karaosmanoglu *et al*., 1999). In fact, farming expansion for fuel and food crops has come at the expense of ecosystem destruction via deforestation and loss of biodiversity (Demirbas *et al*., 2002).

First generation feedstocks, second generation biomass feedstocks (non-edible biomass such us bagasse, agro wastes and residues etc.) are gaining interest as a source of bioenergy, but these are not properly utilized in some places. In most developing countries, large volume of agro wastes and residues, including untreated wastewater are dumped directly into our water resources (Bharathiraja *et al*., 2014), threatening human health, ecosystems, biodiversity, food security and the sustainability of our water resources. This is because a larger part of the population still remains without access to sanitation and wastewater treatment facilities (Adesogan, 2013), and therefore cannot adequately and efficiently manage their agro wastes and sewage (Zandaryaa, 2011). Although, in Nigeria, attention seemed to be focused on solid wastes and hazardous industrial wastes without many realizing that sewage sludge is just as detrimental to human health (Coker *et al.,* 2003).

Some of the water bodies, creeks and tributaries where wastewater or sewage are dumped often find their ways into larger water bodies such oceans, introducing aquatic stress to water body. Sustainable Development goal (SDG) 14 recognises the environmental, economic and social benefits that healthy oceans provide, and that resources and services are being eroded by a range of anthropogenic pressures that are potentially manageable and within the SDG14's scope to improve. As individual activities that damage oceans are often felt far beyond national borders, responsibility for the oceans' health rests with all of us (Sturesso *et al.,* 2018).

The SDG 14.1 (Pollution) recognized that most outflows of nutrients are land sourced. The estimates of the extent and growth of the problem are emerging, with significant negative impact to the health of marine and potentially human food chains. Limited or lack of wastewater treatment plants and a growing inflow of wastewater in coastal systems bordering large urban areas, particularly in developing countries, remains a significant threat (Sturesso *et al.,* 2018).

According to Nagvenkar *et al.* (2009), Hellmer *et al.* (2014) and Betancourt *et al.* (2014), sewage can be a source of contamination and exposure to bacteria (e.g., *E. coli, Salmonella*, etc.), viruses (e.g., hepatitis A, norovirus, etc.), and parasites (e.g., *Giardia, Cryptosporidium*, etc.). Disease-causing germs can be spread from sewage if it is not disposed properly or if people do not practice proper toilet hygiene (cleanliness). If a sewage disposal system is not properly maintained, it will not be able to get rid of the sewage safely. Stefano *et al.* (2001), reported that the diseases commonly caused by these germs include those of bacterial (salmonellosis, shigellosis, diarrhea, trachoma, melioidosis etc.) and viral (gastroenteritis, hepatitis A). Diseases caused by parasites include

giardiasis, dwarf tapeworm infection, threadworm infection, hookworm infection, strongyloidasis etc. These disease-causing germs and parasites can be spread directly by people coming into contact with sewage, indirectly by people coming into contact with animals such as flies and cockroaches which carry the germs and parasites in or on their bodies or through drinking water which has been contaminated by sewage.

Moreover, municipal wastewater production and treatment can be considered as a continuous activity which is most likely not going to end in the future, therefore, it is organizationally, technically, and economically difficult to prevent or strongly reduce the amount of municipal wastewater (Rulkens and Bien, 2004). However, with the recent increase in knowledge, municipal wastewater and sewage sludge can be used to produce energy in the renewable form (Rulkens and Bien, 2004). Therefore, the viable alternative to sludge management and disposal challenge is to utilize the sludge as a source for biofuel production. Hydrothermal liquefaction (HTL) is a favourable thermochemical biomass conversion process for wet biomass feedstocks (Toor *et al*., 2011). The ability to convert high-moisture biomass into liquid fuels without extensive drying makes HTL less energy intensive and therefore more economically viable than other thermochemical conversion techniques (Elliott *et al*., 2015). Moreover, HTL presents a synergistic waste management and bioenergy production opportunity through the utilization of high-moisture waste biomass sources (Minowa *et al.,* 1999).

Studies have shown that biomasses have great potential for the production of renewable energy when subjected to thermochemical conversion (Demirbas *et al.,* 2009; Demirbas, 2011) and since sewage sludge, if not properly managed can predispose the health of the populace as well as the serenity of the environment to challenges of public health. In Nigeria, there is dearth of information on resource recovery from sewage for improved public health status. Therefore, the fabrication of Pyrolizer (using metal scraps) for the utilization of hydrothermal liquefaction renewable energy technology for the conversion of sewage sludge to renewable energy products, will reduce Nigeria's overdependence on fossil fuel, reduce the deleterious effect of oil exploration and production, provide alternative energy, improve the quality of the soil through the use of the biochar, create employment and generate economic returns. It will also eliminate the pollution of sewage or wastewater on water bodies such as rivers, creeks, tributaries and oceans with a view to contributing to the SDG 14.1 goal.

This study, therefore, aims to add to the existing knowledge on the management of sewage sludge, and production of bio-crude oil in Nigeria.

Materials and Methods

Sampling and Sampling Location

Sewage sludge was sampled using purposive sampling method from the sewage treatment plant of the University College Hospital, University of Ibadan, Ibadan, Nigeria and was taken to the laboratory for immediate storage (refrigeration) and analysed in order to avoid compositional changes.

The University College hospital (UCH) is strategically located in Ibadan, which is also the seat of the first University in Nigeria. The University College hospital (UCH) was formally commissioned on the 20 November 1957. The University College Hospital, Ibadan was initially commissioned with 500-bed spaces. Currently, the hospital has 850 bed spaces and 163 examination couches with occupancy rates ranging from 55-60%. The hospital has a staff strength of over 3000 which comprise at least 600 doctors and 1000 nurses (Iyun *et al.*, 2016).

Samples Analysis

Proximate analysis of the sewage sludge sample was conducted to determine the crude protein, fat, dry matter, crude fibre, neutral detergent fibre, acid detergent fibre, lignin and ash content using the official methods described by the Association of Official Analytical Chemist (A.O.A.C., 2005).

Crude protein in the sewage sludge samples was determined by the routine semi-micro kjeldahl technique (Thiex *et al.,* 2002), which consist of four techniques of analysis namely, digestion, distillation, determination and titration. The crude fat was determined by the AOAC official method 988.05 (Thiex, 2008). The dry matter was determined by adapting a modified method used by Gbadamosi *et al*., (2017). Sewage sludge sample (2g) was weighed into a previously weighed crucible. The crucible containing sample taken was then transferred into the oven set at 100^{o}C to dry to a constant weight for 24 hours. At the end of the 24 hours, the crucible containing sample was removed from the oven and transferred to desiccator, cooled for ten minutes and weighed.

The weight of empty crucible was Wo, weight of crucible plus sample was W_1 and weight of crucible containing oven-dried sample was W_3.

% Dry Matter (DM): W_3-W_0 / W_1-W_0 X 100

% Moisture = 100 – % DM.

Ash content, representing the inorganic fraction of the feedstock, was determined by heating the sewage sludge sample at 550 ^{0}C to remove all organic carbon (AOAC 942.05). The Neutral Detergent Fibre (NDF) which is a measure of the insoluble fibre in the sewage sludge feed was determined by adapting the method of analysis used by Van Soest *et al*. (1991), which made use of neutral detergent solution, n-Octanol ($C_8H_{18}O$), Sodium Sulphite anhydrous (Na_2SO_3) and acetone. While the Acidic Detergent Fibre (ADF) was determined using the AOAC 973.18 official method of analysis. The Acid Detergent Lignin (ADL) which is the indigestible non-carbohydrate component of forages was determined using the AOAC 973.18 official method of analysis.

Hydrothermal conversion of sewage sludge to bio-crude oil

Sewage sludge (25 kg) was collected for analysis. Appropriate personal protective equipment was used during collection of the sample. The wet sewage sludge slurry was then fed into a reactor (see Plate 1) with operating temperature set at 300 ^{o}C. At about 1 hour 50 minutes into the hydrothermal liquefaction process, volatile gases began to flow and were condensed (using a condenser attached to cold water source) to the bio-crude oil which was collected in a glassware. The process was left to run for 4 hours after which the bio-crude oil was promptly taken to the laboratory for storage and analyses.

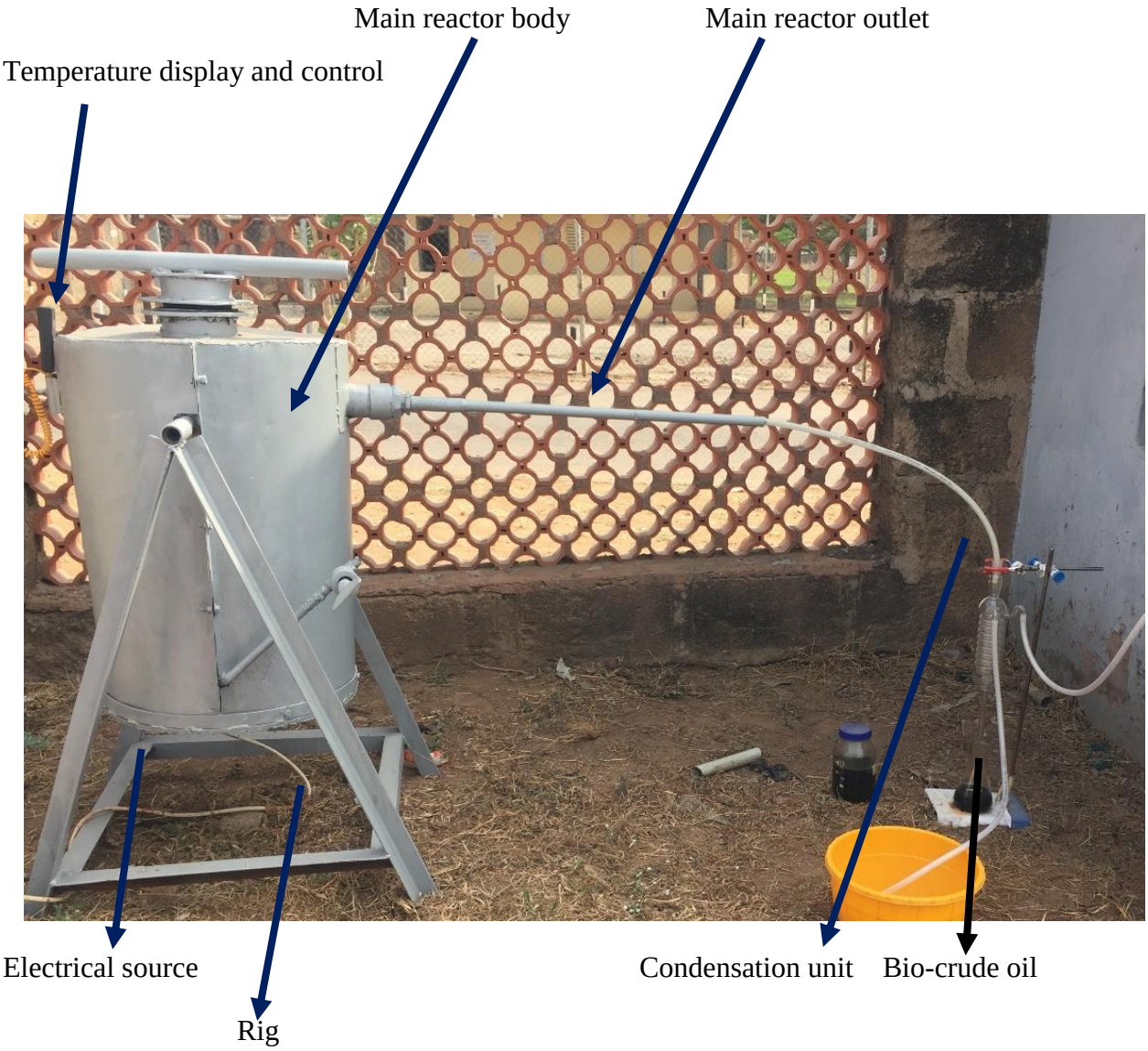

Plate 1: The reactor and condensation unit

Percentage yield of products from hydrothermal liquefaction of sewage sludge

The percentage yield of products was determined using the following formula:

% yield of bio-crude oil = (Mass of bio-crude oil / mass of feedstock) x 100
% yield of bio char = (Mass of biochar / mass of feedstock) x 100
% yield of gas = 100 - (% yield of bio-crude oil + % yield of bio char)

Bio-crude oil characterisation

In this study, the operating temperature of the pyrolyzer was set at 300^0C, such that it will not allow water to be present in the bio-crude oil.

Elemental analysis of bio-crude Oil

The bio-crude oil was analysed for carbon, hydrogen, nitrogen, sulphur and oxygen (CHNSO) to determine some of the elemental composition of the oil. The carbon and hydrogen contained in the bio-crude oil was determined by weighing bio-crude oil samples into a platinum crucible and placing

it in a Leibig-Pregle chamber containing magnesium perchlorate and sodium hydroxide, the sample was then burnt off and the carbon dioxide calculated (Kandpal and Masheshwari, 1995). The nitrogen content of the bio-crude oil was analysed using Kjeldahl method stated earlier.

The sulphur content in the bio-crude oil was analysed by weighing 1g of the sewage sludge into volumetric flask and adding 40 ml of distilled water to make a solution which was then homogenized to ensure the complete dissolution of the sample. The solution was then filtered using a whatman number 1 filter paper. The filtrate (5 ml) was pipetted into a beaker, after which 2 ml of 5% Na_2CO_3 was added, followed by addition of 1 ml of 10% HCl solution. The mixture was then stirred and filtered through a Whatman number 42 filter paper into a 100 ml Beaker, followed by drop wise addition of 10% $BaCl_2$, solution from a Burette with constant stirring and boiling on a water bath for 2 hrs. The hot mixture was carefully filtered through Whatman number 42 filter paper into another 100 ml beaker. $AgNO_3$, (1 ml and 0.1M) was then added to produce a slight opalescence, which confirmed the presence of Barium Sulphate precipitate. The mixture was later filtered into a 250 ml beaker with the precipitate as residue on the filter paper (Raf *et al*, 2006).

% Sulphur = (weight of filter paper plus residue – weight of empty filter paper)/weight of sample X 100

The weight concentration of oxygen was estimated by subtracting from 100 the concentrations of all other elements (C, H, N, S) and of the ash content in the fuel; a method adapted from Karampinis *et al.* (2012).

Determination of higher heating value of the bio-crude oil

The Higher Heating Value (HHV) in MJ/kg was estimated using the Dulong's formula (Brown *et al*., 2010; Zhou *et al*., 2010). The formula makes use of the Carbon, Hydrogen and Oxygen values gotten from the elemental analysis of bio-crude oil as follows:

HHV = 0.3383C + 1.422 (H-(O/8)).

Gas chromatography-mass spectroscopy

The TPH concentrations (C4–C28) for matrices were determined by EPA Method 8015 (modified): GC analysis of Diesel Range Organics (DRO) on SLB®-5ms (20 m x 0.18 mm I.D., 0.18 μm), Fast GC Analysis (Campisano *et al.,* 2017). Gas Chromatography-Mass Spectroscopy GC–MS analysis of the bio-crude oil was performed, using helium as the carrier gas at the Central Research Laboratory, University of Ibadan. One microlitre of the Alkane mix (C_{10} – C_{30} standard) was injected into the gas chromatograph for easy passage into the column to obtain the standard chromatograph, 1 micro litre of the concentrated Dichloromethane and n-hexane extract of the bio-crude oil was then injected into the gas chromatograph for analysis of total petroleum hydrocarbon on full scan mode. The gas chromatography oven was programmed from 50 ^oC to 300 ^oC at a rate of 5^oCmin^{-1} and the hold- time was 5 mins at 50 ^oC and 30mins at 300^oC respectively. The injector temperature was 180 ^0C and a capillary column was used. The identification of the compounds was accomplished using a NIST database and comparing with published mass spectra.

Viscosity

This was done using a viscosity meter. The sample was agitated into viscous slurry and introduced into a capillary tube in order to determine its flow by using Hagen-Poiseuille equation as follows:

$\mathbf{V} = Pr^4\pi t/8l\eta$

Where v= volume of fluid

P= pressure
r= radius
l= length
ɳ= viscosity

Density

The density of the bio-crude oil was determined, using the density bottle method. A 50 cl density bottle with its stopper was weighed while empty. The bottle was then filled with a known volume of bio-crude oil, covered with a stopper and kept in a fixed position. The mass (M) of the bottle and the bio-crude oil in it was measured. The mass of the empty bottle was subtracted from mass (M) of the bottle containing the bio-crude oil to obtain the liquid mass of the known volume of bio-crude oil in the bottle. Density was then calculated by dividing the liquid mass of the bio-crude oil with its volume.

Density = Mass/Volume

Cloud Point

Test jar was filled with bio-crude oil to the mark on the upper part of the test jar and tightened by the cork with the thermometer and placed into a bath containing crushed ice and kept for three second. The jar was immediately removed from the jacket shaking the specimen. At this point, the cloud point was inspected, and the jacket subsequently replaced. Cloud point was recorded on the observation of a cluster or cloud of the liquid specimen at the bottom of the test jar.

Pour Point

Bio-crude oil sample was filled to the level mark in the test jar. The test jar was tightly closed with the cork carrying the test thermometer and placed in a bath containing crushed ice. An interval of at least three minutes was adopted for the inspection of the test jar. This was done by holding the test jar in a horizontal position for a few seconds before returning it to cool. The pour point was recorded at a point when the oil surface stayed in the vertical position for a period of 5 seconds without sagging by inserting a thermometer to cool for 10 seconds and the temperature recorded (lize *et al.*, 2018).

Results and Discussion

Feedstock Composition

The Summary of the Raw Sewage Sludge Composition is presented in Table 1.

Table 1: Proximate Analysis of Raw Sewage Sludge Collected from UCH Sewage Treatment Plant

Sewage Sludge	Value (%)
Crude protein	25.30
Ether extract (fat)	0.85
Moisture	37.47
Ash content	31.05
Neutral Detergent Fibre (NDF)	29.30
Acidic Detergent Fibre (ADF)	19.12
Acidic Detergent Lignin (ADL)	11.22

The proximate analysis of the sewage sludge feedstock showed that the initial sewage sludge composition had a relatively high amount of moisture (37.47%); this may be due to the large amount

of wastewater that finds its way to UCH sewage treatment plant. Moreover, this relatively high moisture content, made the sewage sludge suitable for hydrothermal liquefaction process, which agrees with the Peterson *et al*. (2008), Bosetti *et al.* (2012), Li *et al.* (2010), Toor *et al*. (2011) and Eliott *et al.* (2015) reports. The protein content in the sewage sludge feedstock was 20.30 wt% which is slightly higher than that reported by Derek *et al.* (2011). This may be due to the composition of the sewage that gets to the UCH sewage treatment plant. The sewage sludge also showed compositional fat as 0.85% which is similar to the one gotten by Derek *et al.* (2011). However, the Neutral Detergent Fibre (NDF) and Acidic Detergent Fibre (ADF) (29.30%, 19.12% respectively) were much lower than those reported by Derek *et al.* (2011) studies. This could be traced to disparate sewage composition in the study locations. The Acid Detergent Lignin (ADL), however present a similar percentage composition with the findings of Derek *et al.* (2011). The ash content of 31.05% falls within the range of 20-50 w% recorded by Fonts *et al.* (2012).

Hydrothermal liquefaction Products yield

The hydrothermal liquefaction bio-crude oil yield from this study was 34% of the total product yield (see Figure 1), which is similar to the findings of Eboibi, B.E (2019). This may be attributable to the use of similar operating temperature (300 ^{o}C), which favours the production of bio-crude oil as reported by Huber *et al.* (2006), Minowa *et al*. (1995) and Bosetti *et al*. (2012). The heat and pressure cause the sewage to break down into different fractions — biocrude and an aqueous liquid phase. However, the Bio-char yield (See Plate 2) was 60%, an amount which may be due to the high carbon content and lignin in the sewage sludge feedstock used for the conversion (Karampinis *et al.,* 2012). The remaining yield was attributed to the gas product which is small (6%), because hydrothermal liquefaction carried out at temperatures between 247 and 374^{o}C do not favour gas production (Huber *et al.*, 2006; Bosetti *et al.*, 2012). The gas is of economic value and could be used as energy source.

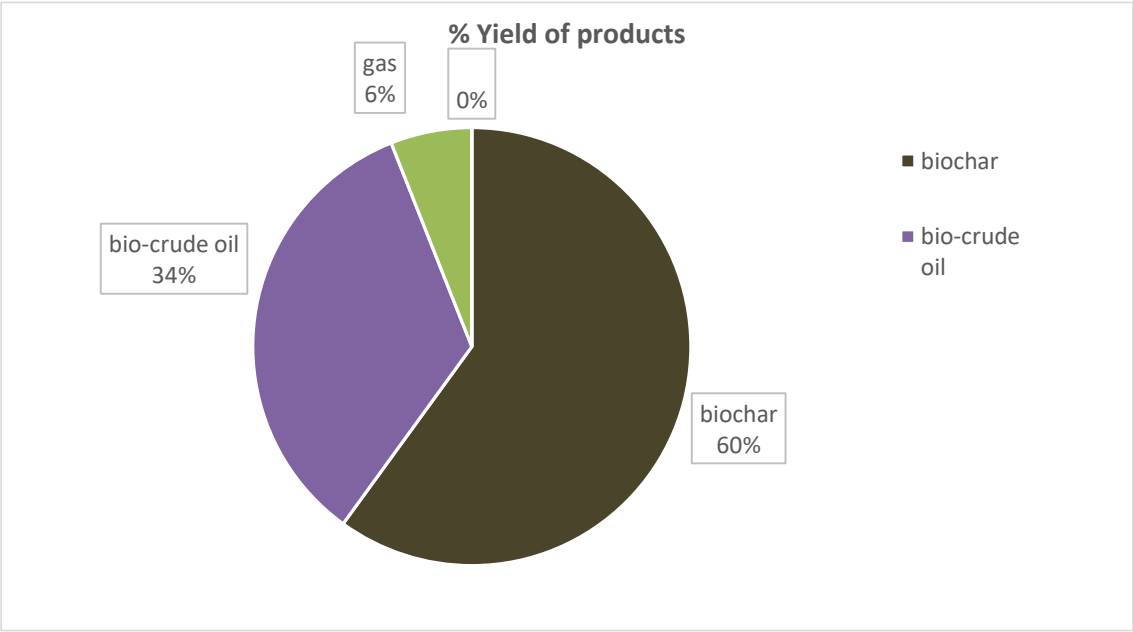

Figure 1: Percentage yields of products from hydrothermal liquefaction of the sewage sludge

Physical characteristics of the bio-crude oil

The bio-crude oil produced was a darkish brown liquid as shown in Plate 2 with a smoke-like smell and a stench that lingers, which agrees with what was reported by Akhtar and Amin (2011) and Jena *et al*. (2011). However, the viscosity of the bio-crude oil produced was significantly lower than that reported by Jarvis *et al.* (2017); this may be due to the use of different operating temperatures and the disparate composition of the initial sewage sludge used. On the other hand, the density of the biocrude oil in this study was very similar to the one reported by Jarvis *et al.* (2017) from the biocrude oil of pine (1.10 g/ml), *Chorella* sp. (0.96 g/ml) and of course sewage sludge (1.00 g/ml). It was also in the range of the density reported for diesel (0.85 g/ml) and biodiesel (0.88 g/ml) (NREL, 2009). In addition, the cloud point and the pour point of the biocrude oil gotten in this study was found to be lower than that reported by Akhil *et al*. (2017) for conventional fuels.

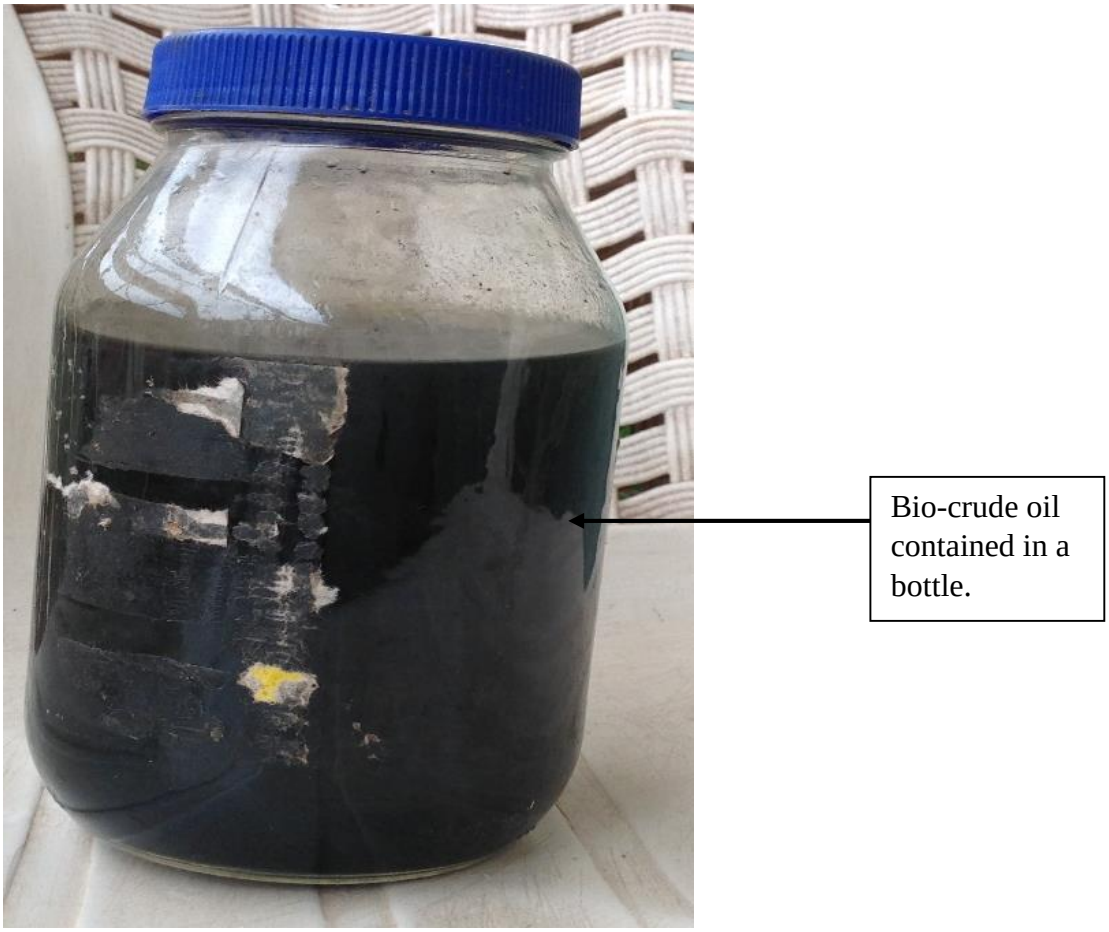

Plate 2: Biocrude oil from the hydrothermal liquefaction of the sewage sludge.

Plate 3: Bio-char from the hydrothermal liquefaction of the sewage sludge.

Elemental analysis and higher heating value of the bio-crude oil produced

The elemental analysis (Table 2) of the bio-crude oil produced in this study showed that the bio-crude oil had a carbon content of 68.05%, which is in consonance with the carbon levels reported by Derek *et al.* (2011). It was, however, lower than the carbon content reported by Jarvis *et al.* (2017), which may be due to the different operating temperatures (200 and 300^0C used). The hydrogen content of the bio-crude oil produced was slightly lower than those reported by Derek *et al.* (2011) and Jarvis *et al.* (2017) from their studies on hydrothermal liquefaction of sewage sludge. The Hydrogen-Carbon mole ratio (H:C) of the bio-crude oil produced (1.40) was also slightly lower than that reported by Jarvis *et al.* (2017), which he reported to be 1.57. This may be traced to the nature and composition of the initial sewage sludge used. The H:C mole ratio of the bio-crude oil in this study was also lower than that of diesel (1.79) and biodiesel (1.87) as reported by NREL (2009).

Table 2: Elemental analysis of bio-crude oil

Bio-crude oil	Value (%)
Carbon	69.02
Hydrogen	8.12
Nitrogen	3.20
Sulphur	0.07
Oxygen	19.56

The nitrogen content (2.94 %) of the bio-crude oil is significant and of great importance. This may be attributable to the nitrogen present in the protein derived from human waste; a finding that is in consonance with Benn and McAuliffe (1986), Li *et al.* (2010) and Toor *et al.* (2013) reports. Similarly, the significant nitrogen content found in the bio-crude oil produced is not different from the one reported by Jarvis *et al*. (2017) at 4.3 % and that reported by Derek *et al.* (2011) at 4.3 %. The sulphur in the bio-crude oil produced is however very low (0.009 %) compared to the sulphur content obtainable in petroleum crude which range from 0.05 to 6 % (Speight, 2006); this may be traced to the fact that biomass always have a relatively low sulphur content (Robbins *et al.*, 2012), which therefore provides a great prospect for its upgrading.

The higher heating value (HHV) of the bio-crude oil produced, which determines the quantitative energy of a fuel is shown to be 30.60 MJ/kg, which falls in the range (25-39 MJ/kg) reported by Suzuki *et al*. (1988), Brown *et al*. (2010), Xiu *et al.* (2010a) and Yin *et al.* (2010) and also from the previous hydrothermal liquefaction of sewage sludge. However, this is lower than the HHV reported for petroleum crude oil (41 – 48 MJ/kg) by Speight (2006) and NREL (2009).

Gas chromatography-mass spectroscopy of the bio-crude oil

The Total Petroleum Hydrocarbon (TPH) constituents in the bio-crude oil produced are represented in Figure 1 and Table ii below. The TPH analysis which defines the measurable number of petroleum-based hydrocarbons in a media, was done to find out the similarity between the TPH in crude oil and that found in the bio-crude oil produced. Crude oil is known to contain hydrocarbons with Carbon range between C_3 and C_{45+}; Gasoline shows ranges of C_6 to C_{10-12} and Diesel fuel with range of C_{8-12} to C_{24-26}. Hence, the total petroleum hydrocarbon analysis result of the bio-crude presented a range of C_{10} to C_{28}, with just one compound (Undecane) in the range of typical crude oil gasoline and the 17 other compounds having range similar to Diesel range organics of crude oil (Manilla and Adeboye, 2009), which further emphasised the potential of bio-crude oil as an alternative to diesel fuel.

Furthermore, the result of the GC-MS for TPH of the bio-crude oil produced had a larger percentage of the compounds identified to be aliphatic hydrocarbons which shows a similarity to typical crude oil hydrocarbons as reported by Potter and Simmons (1998), who stated that typical crude oil contains high concentration of aliphatic hydrocarbons and lower concentration of aromatic hydrocarbons.

Looking at the results obtained from this study, the hydrothermal liquefaction technology seems to be mimicking the geological conditions that nature uses to create crude oil, using high pressure and temperature to achieve in short timesomething that will take mother nature millions of years to do. The *bio-crude oil* can be refined into various fuels and substituting these fuels for fossil fuel can reduce greenhouse gas emissions. The results obtained showed that what we flushed from our toilets that forms part of municipal sewage generated from households and institutions can be converted into bio-crude oil with properties very similar to fossil fuels. Interestingly, plenty of carbon, fats or lipids are associated. The fats or lipids appear to facilitate the conversion of other materials in the sewage, keep the sludge moving through the reactor, and produce quality bio-crude oil that could be refined to yield fuels such as gasoline and biodiesel.

Essentially, the conversion of the sewage to bio-crude oil will help in virtually eliminating the need for sewage residuals processing, transport and disposal for improved public health status and economic benefits. In addition to the bio-crude oil, the liquid phase can be treated to recover compounds or elements such as phosphorus, which can replace phosphorus ore used in fertilizer production. With this technology, sewage can be converted to many useful products.

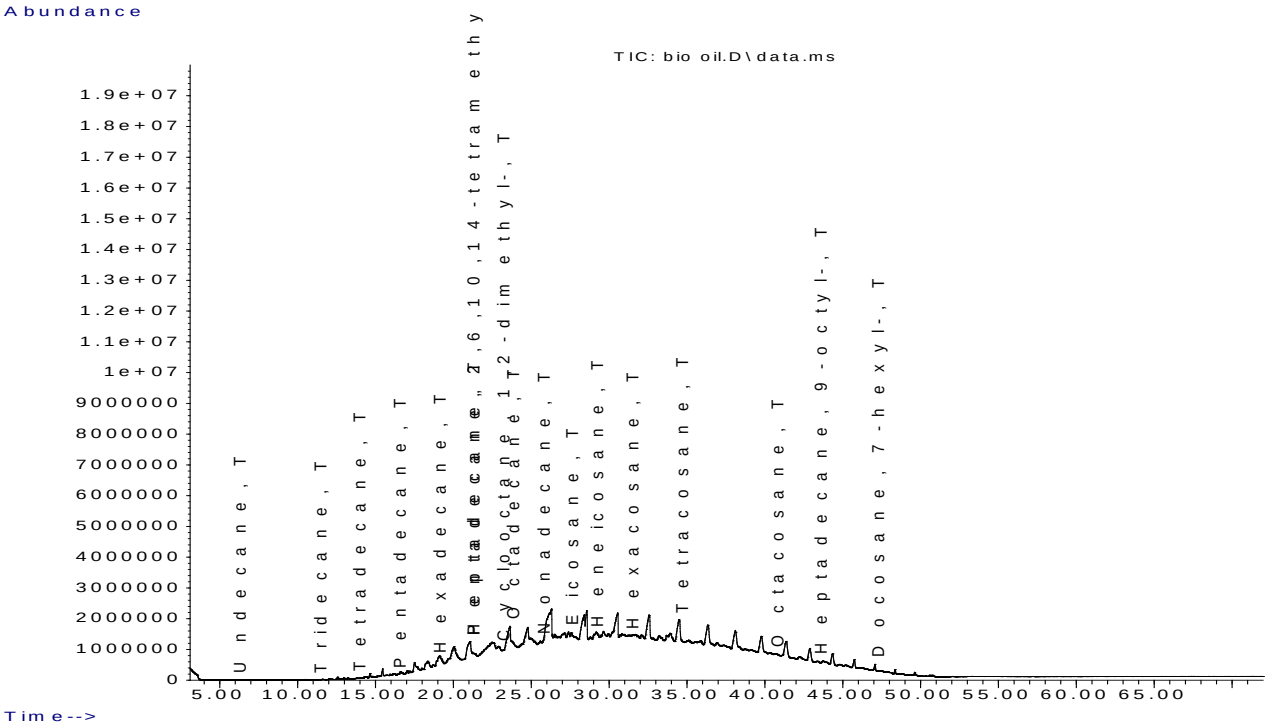

Figure 2: Gas Chromatogram of bio-crude oil from the sewage sludge

Table 3: Total Petroleum Hydrocarbons (TPH) in the bio-crude oil from sewage sludge

#ID	Compound	Formula	Retention time (min)	Concentration (ppm)	Q value
1	Decane	$C_{10}H_{22}$	0.000	0	N.D
2	Undecane	$C_{11}H_{24}$	6.085	0.01	5
3	Tridecane	$C_{13}H_{28}$	11.241	0.23	42
4	Tetradecane	$C_{14}H_{30}$	13.758	0.12	34
5	Pentadecane	$C_{15}H_{32}$	16.356	0.07	46
6	Hexadecane	$C_{16}H_{34}$	18.914	0.12	1
7	Heptadecane	$C_{17}H_{36}$	21.088	46.02	70
8	Pentadecane,2,6,10,14-tetramethyl	$C_{19}H_{40}$	21.880	31.33	58
9	Cyclooctane, 1,2-dimethyl	$C_{10}H_{22}$	23.011	0.96	20
10	Octadecane	$C_{18}H_{38}$	23.635	52.68	92
11	Nonadecane	$C_{19}H_{40}$	25.597	0.06	1
12	Eicosane	$C_{20}H_{42}$	27.411	1.85	51
13	Heneicosane	$C_{21}H_{44}$	29.019	1.49	79
14	Hexacosane	$C_{26}H_{54}$	31.291	2.62	78
15	Tetracosane	$C_{24}H_{50}$	34.472	17.99	97
16	Octacosane	$C_{28}H_{58}$	40.589	0.01	33
17	Heptadecane, 9-octyl-	$C_{25}H_{52}$	43.381	3.08	97
18	Docosane, 7-hexyl-	$C_{28}H_{58}$	47.072	3.08	97

N.D = Not detected

Conclusion

Our study showed that conversion of sewage sludge to bio-crude oil using hydrothermal liquefaction is an effective renewable energy recovery strategy because the TPH analysis of the bio-crude oil showed similarities with petroleum crude oil, which highlights its potentials for production of alternative fuel and value-adding chemicals.

In addition, the world is shifting towards the use of renewable energy technology therefore, the prospect of managing sewage and producing renewable energy, encourages waste to wealth generation schemes, provides alternative energy to fossils fuels and tackles global warming and climate change.

References

Adati K F (2012). Oil Exploration and Spillage in the Niger Delta of Nigeria. *Civil and Environmental Research,* 2(3): 38-51.

Adesogan S. (2013). Sewage Technology in Nigeria: a pragmatic approach. *Science Journal of Environmental Engineering Research.*

Akhil A G, Mohammed P K, Akhilesh S, Muhammad A C A & Khan S (2017). Determination of cloud and pour point of various petroleum products. *International Refereed Journal of Engineering and Science (IRJES),* 6(9): 1-4.

Akhtar J, Aishah N, & Amin S (2011). A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass. *Renewable and Sustainable Energy Reviews,* 15(3): 1615–1624.

AOAC. (1995). Fiber (Acid Detergent) and Lignin in Animal Feed (973.18). Official methods of analysis, 16th edition.

Betancourt W Q, Duarte D C, Vásquez R C, & Gurian P L. (2014). *Cryptosporidium* and *Giardia* in tropical recreational marine waters contaminated with domestic sewage: Estimation of bathing-associated disease risks. *Marine Pollution Bulletin;* 85(1): 268-273. doi: 10.1016/j.marpolbul.2014.05.059.

Bharathiraja B, Yogendran D, Ranjith Kumar R, Chakravarth Y M, & Palani S (2014). Biofuels from sewage sludge- A review. *International Journal of ChemTech Research*: 6(9), 4417-4427.

Bosetti A, Bianchi D, & Franzosi G (2012). Integrated Process for the Production of Bio-Oil from Sludge Coming from a Wastewater Purification Plant. U.S. Patent Application 14/002,321.

Brown T M, Duan P, & Savage P E (2010). Hydrothermal liquefaction and gasification of *Nannochloropsis sp*. *Energy & Fuels,* 24(6): 3639-3646.

Campisano R K, Hall J, Griggs S, Willison S, Reimer H, Mash M, Magnuson L, Boczek, & E. Rhodes (2017). Selected Analytical Methods for Environmental Remediation and Recovery (SAM). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-17/356, 2017.

Coker A O, Sridhar M K C & Martins E A (2003). Management of septic sludge in southwest Nigeria. *In 29th WEDC International Conference, "Towards the Millennium Development Goals*". Abuja, Nigeria.

Demirbas A (2002). Partly chemical analysis of liquid fraction of flash pyrolysis products from biomass in the presence of sodium carbonate. *Energy Conversion and Management.* 43(14): 1801–1809.

Demirbas M, Mustafa B, & Balat H (2009). Potential contribution of biomass to the sustainable energy development. *Energy Conversion and Management*, vol. 50: pp.1746–1760.

Demirbas A (2011). Competitive liquid biofuels from biomass. Applied Energy. 88(1): p. 17-28.

Derek R, Vardon B K, Sharma J S, Guo Y, Zhichao W, Lance S, Yuanhui Z, & Timothy J S (2011): Chemical properties of biocrude oil from the hydrothermal liquefaction of Spirulina algae, swine manure, and digested anaerobic sludge. *Bioresource Technology*, 102: 8295–8303.

Dlugokencky E & Tans P (2015). Trends in Atmospheric Carbon Dioxide: Recent Global CO_2. http://www.esrl.noaa.gov/gmd/ccgg/trends/global.html last accessed on 6 May 2017.

Eboibi B E (2019). Impact of time on yield and properties of biocrude during downstream processing of product mixture derived from hydrothermal liquefaction of microalga. *Biomass Conv. Bioref.* **9,** 379–387.

Edenhofer O, Pichs-Madruga R, Sokona Y, Seyboth K, Kadner S, Zwickel T, & Matschoss P. (Eds.). (2011). *Renewable energy sources and climate change mitigation: Special report of the intergovernmental panel on climate change*. Cambridge University Press.

Elliott D C, Biller P, Ross A B, Schmidt A J, & Jones S B (2015). Hydrothermal liquefaction of biomass: developments from batch to continuous process. *Bioresoure Technology,* 178: 147–156.

Fonts I, Gea G, Azuara M, Ábrego J, & Arauzo J (2012). Sewage sludge pyrolysis for liquid production: a review. *Renewable and Sustainable Energy Review,* 16 (5): 2781–2805.

Gbadamosi T R, Otitoola O C, Akanfe F A, Asimi Tajudeen B F, & Isola O C (2017). Nutritional Analysis of Formulated Complementary Foods from Selected Nigeria Crops. *Technology (IJOSEET),* 2(10): 69-75.

Hellmér M, Paxéus N, Magnius L, Enache L, Arnholm B, Johansson A, & Norder H (2014). Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. *Appl. Environ. Microbiol.*, *80*(21), 6771-6781.

Huber G W, Iborra S, & Corma A (2006). Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical Reviews,*106: 4044–4098.

Iyun A O, Ademola S A, Michael A, Olawoye O, & Oluwatosin O. Perception of healthcare professionals in University College Hospital, Ibadan toward wound care. Nigerian J PlastSurg (2016); 12:47-9.

Jarvis M J, Billing, M J, Hallen T R, & Schmidt J A (2017). Hydrothermal Liquefaction Biocrude Compositions Compared to Petroleum Crude and Shale Oil. *Energy & Fuels,* 31(3): 2896-2906.

Jena U & Das K C (2011). Comparative evaluation of thermochemical liquefaction and pyrolysis for bio-oil production from microalgae. *Energy Fuels* 25: 5472–5482.

Kandpal J B & Masheshwari R C (1995). Combustion of biomass fuels in two cook stoves for their conservation. *Energy conversion and management*, 36(10), 1015-1021.

Karampinis E, Grammelis P, Zethraeus B, Andrijvskaja, J, Kask U, & Hoyne S (2012). In: Proceedings of the 20th European biomass conference and exhibition. *Bioenergy syst. Planners hand b. Bisyplan, Milan.*

Karaosmanoglu F & Tetik E (1999). Fuel properties of pyrolytic oil of the straw and stalk of rape plant. *Renewable Energy,* 16: 1090–1093.

Li H, Yuan X, Zeng G, Huang D, Huang H, Tong J, You Q, Zhang J, & Zhou M (2010). The formation of bio-oil from sludge by deoxy-liquefaction in supercritical ethanol. *Bioresourcee Technology,* 101: 2860–2866.

Lize M S L, Oliveira, Rita C P Nunes, Ygor L L Ribeiro, Dayane M Coutinho, Débora A Azevedo, Júlio C M Dias, & Elizabete F Lucas (2018). Wax Behavior in Crude Oils by Pour Point Analyses, J. Braz. Chem. Soc. vol.29 (10), pp 2158-2168.

Manilla & Adeboye (2009). An analysis of hydrocarbon present in sediment of some flood plain lakes in Bayelsa State, Niger-Delta, Nigeria. *Asian journal of Microbiology, Biotechnology and Environmental Sciences.* 11(3): 495-499.

Minowa T, Yokoyama S, & Kishimoto M (1995). Oil production from algal cells of *Dunaliellatertiolecta* by direct thermochemical liquefaction. *Fuel* 74:1735–1738.

Nagvenkar G S & Ramaiah N (2008). Abundance of sewage-pollution indicator and human pathogenic bacteria in a tropical estuarine complex. Environmental Monitoring and Assessment;155(1-4):245-256. doi:10.1007/s10661-008-0432-1. 2.

National Renewable Energy Laboratory. (2009). *Biodiesel Handling and Use Guide*, 4th ed.; Golden, CO, USA.

NNPC Annual Statistical Bulletin (2013).
Retrieved from:
www.nnpcgroup.com/PublicRelations/OilandGasStatistics/AnnualStatisticsBulletin.aspx
nosdra.gov.ng (2012). Information on the Vision and Mission statement and the background of the establishment of National Oil Spill Detection and Response Agency.

Peterson A A, Vogel F, Lachance R P, Fröling M, Antal M J & Tester J W (2008). Thermochemical biofuel production in hydrothermal media: a review of sub- and supercritical water technologies. *Energy and Environmental Science,* 1:32–65.

Potter T L & Simmons K E (1998). *Composition of petroleum mixtures*: Amherst., Mass Amherst Scientific publishers, Total Petroleum Hydrocarbon criteria working group series, Vol.2, pg. 102.

Putun E, Uzun B B, & Putun A E (2006). Fixed-bed catalytic pyrolysis of cotton-seed cake: Effects of pyrolysis temperature, natural zeolite content and sweeping gas flow rate. *Bioresoure Technology,*97: 701–710.

Raf D, Jan B, Fernand R, & Marleen P (2006). The Analysis of the Total Sulphur Content of Wastewater Treatment Sludge by ICP-OES. Environmental Engineering Science, vol.23(6), pp904-907.

Robbins M P, Evans G, Valentine J, Donnison I S & Allison G G (2012). New opportunities for the exploitation of energy crops by thermochemical conversion in northern Europe and the UK.

Progress of Energy and Combust Sciences, 38:138–55. http://dx.doi.org/ 10.1016/j.pecs.2011.08.001.

Rulkens W H & Bien J D (2004). Recovery of energy from sludge: Comparison of the various options. *Water Science and Technol*ogy, 50(9): 213– 221.

Speight J G (2006). *The Chemistry and Technology of Petroleum*, 4th ed.; Chemical Industries; CRC Press.

Stefano D, Antonio S, Suzanne K, & Karek K (2001). The Importance of Pathogenic Organisms in Sewage and Sewage Sludge, *Journal of the Air & Waste Management Association*, 51:6, 848-860, DOI: 10.1080/10473289.2001.10464313

Stephens E, Ross I L, Mussugnug J H, Wagner L D, Borowitzka M A, Posten C, Kruse O, & Hankamer B (2010). Future prospects of microalgal biofuel production systems. *Trendsin Plant Sciences,* **15:** 554-564

Sturesson A, Weitz N, & Persson Å (2018). SDG 14: Life Below Water. A Review of Research Needs. Technical annex to the Formas report Forskningför Agenda 2030: Översikt av forskningsbehov och vägar framåt. Stockholm Environment Institute, Stockholm.

Suzuki A, Nakamura T, Yokoyama S, Ogi T, & Koguchi K (1988). Conversion of sewage sludge to heavy oil by direct thermochemical liquefaction. *Journal of Chemical Engineering of Japan*, 21(3), pp. 288-293.

Thiex N, (2008). Evaluation of analytical methods for the determination of moisture, crude protein, crude fat, and crude fiber in distillers dried grains with solubles. *Journal of AOAC international*, 92(1), 61-73.

Thiex N J, Manson H, Anderson S, & Persson J A (2002). Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. *Journal of AOAC International*, 85(2), 309-317.

Toor S S, Reddy H, Deng S, Hoffmann J, Spangsmark D, Madsen L B, Holm-Nielsen J B, & Rosendahl L A (2013). Hydrothermal liquefaction of *Spirulina* and *Nannochloropsis salina* under subcritical and supercritical water conditions. *Bioresource Technology,*131: 413–419.

Toor S S, Rosendahl L, and Rudolf A (2011). Hydrothermal liquefaction of biomass: A review of subcritical water technologies. *Energy,* **36**(5): p. 2328-2342.

Van Soest P V, Robertson J B, and Lewis B A (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of dairy science*, 74(10), 3583-3597.

Vardon D R, Sharma B K, Blazina G V, Rajagopalan K, Strathmann T J (2012). Bioresource technology thermochemical conversion of raw and defatted algal biomass via hydrothermal liquefaction and slow pyrolysis. *Bioresoure Technology,* 109:178–187. http://dx.doi.org/10.1016/j.biortech.2012.01.008.

Xiu S, Shahbazi A, Shirley V, & Cheng D (2010). Hydrothermal pyrolysis of swine manure to bio-oil: Effects of operating parameters on products yield and characterization of bio-oil. *Journal of Analytical and Applied Pyrolysis, 88*(1), pp. 73-79.

Yin S, Dolan R, Harris M, & Tan Z (2010). Subcritical hydrothermal liquefaction of cattle manure to bio-oil: Effects of conversion parameters on bio-oil yield and characterization of bio-oil. *Bioresource Technology,* 101(10), pp.3657-3664.

Zabbey N (2005). Impacts of extractive industries on the biodiversity of the Niger Delta region, Nigeria‖. Paper delivered at National Workshop on coastal and marine biodiversity management, 7 – 9 Sept 2005, Pyramid Hotel, Calabar, Cross River State, Nigeria.

Zandaryaa S (2011). *Global challenge of wastewater* – example from different continents," presented at the 2011 World Water Week in Stockholm. Pg. 21-27.

Zhou S, Wu Y, Yang M, & Tong J (2010). Bio-oil production from sub- and supercritical water liquefaction of microalgae *Dunaliellatertiolecta* and related properties. *Energy and Environmental Science*, 3:1073–1078.

ORIGINAL RESEARCH ARTICLE

Effects of abattoir activities on the water quality of Oko-Oba river in Agege, Lagos, Nigeria: a seasonal variation assessment

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Abstract
Surface water from the Oko-Oba River in Agege, Lagos Nigeria which runs beside the Oko-Oba Abattoir was assessed for its physicochemical quality in relation to temporary seasonal variation. The samples were collected in May and September 2019 (wet and dry season) and analysed for physicochemical (including some potentially toxic metals (PTMs)) and microbiological parameters using standard protocols of American Public Health Organization (APHA) and American Society for Testing and Materials (ASTM). Many physicochemical and microbiological parameters analysed for the samples did not meet regulatory standards for surface water. Some significant correlations were observed between the seasons, sampling points and parameters. The results of this study showed that seasonal variations affected some the water quality parameters of the Oko-Oba River. Further studies are recommended to provide information on the effect of the Abattoir activities on aquatic life in other to ensure sustainable river bodies.

Key Words: *Abattoir, seasonal variation, physicochemical parameters, microbiological analysis, correlation study*

Introduction
Abattoirs provide meat and other materials required for domestic and export purposes (Nwanta et al., 2008). They also generate employment opportunities for large groups of people directly and indirectly while providing revenue for government (Alhaji and Baiwa, 2015). However, abattoir activities such as, slaughtering of animals, washing of meat, evisceration (removal of content of stomach), removal of bones, storage of blood and fat, hair removal and burning of waste among others lead to the generation of large amounts of liquid and solid wastes. Abatoir liquid wastes include the blood and washwater from the processing of meat and meat parts while abatoir solid wastes include the excrements removed from the intestines and stomach of the animals, wasted meat and fats (Akanni et al., 2019) among others. These wastes adversely impact on the surrounding environments. Pathogens present in animal carcasses or animal wastes may be washed into waste water discharged into surrounding water body(s) leading to occurences of pathogenic organisms in water bodies (Abattoirs are usually situated near water bodies such as rivers, stream or canals in Lagos**)** (Augustyn**,** et al, 2016**).** Thus, causing the degradation of surounding land and lowering of both surface and ground water qualities (Ekpetere et al., 2019). Abattoir activities if not managed may lead to the pollution of the soil and water resources.

Ojekunle and Lateef (2017) assessed the impact of abattoir wastewater discharge on the water qualities of some surface and ground water bodies in Abeokuta Ogun state Nigeria. Out of all the parameters accessed in their study, the values for hardness, lead (Pb), Copper (Cu), Zinc (Zn), Cadmium (Cd), Iron (Fe) and faecal coliform for most their samples exceeded the stipulated permissible values by FEPA for surface water. The impact of abattoir waste water on river Illo Ota in Ogun state, surface and ground water bodies in Oshogbo Osun all in Nigeria, were studied by Omole and Longe (2008) and Akanni et al., (2019) respectievly. They all found that at least one or more water quality parameters did not meet the standard values for surface water. Oluseyi et al, (2019) compared the effects of abatoir waste water from two Abattiors in Lagos Nigeria (Oko-Oba and Itire Abatoir) on their surrounding surface and ground water. They also found that some water quality parameters did not comply with the limits. Barakat et al., (2016) and Pejman et al. (2009) studied seasonal variation on surface water quality of various water bodies but not around Abattoirs. Their findings were different and unique to the water bodies studied however, water quality varied with climatic seasons.

Previous studies on the Oko-Oba River water quality by Oluseyi et al., (2019) and Ojo (2014) in 2011 and 2014 respectively were only base on a season. They did not consider the seasonal climatic variation and its relationship with water quality of the River. The aim of this study is to access the effect of the abattoir activities near the Oko-Oba River in Agege Local Government Area of Lagos, Nigeria on the River with a view of understanding how seasonal variation impacts on the River water quality.

Material and method

Study area

Lagos located in South Western Nigeria, is the commercial nerve centre of the country and is home to over 18 million people which is about 10 % of the country's population (Ojeh, 2016). It is estimated that about 8000 ruminant livestock (valued at 1.6 billion Naira) are eaten daily in Lagos (Agbeze, 2017). To meet the demand, animals (especially cattle) are killed in Abattoirs and distributed across the state.

The study area was Oko-Oba river beside Oko–Oba Abattoir of Agege Local Government Area, Lagos, Nigeria (Figure 1). Oko-Oba abattoir is the largest of the authorized abattoirs in Lagos state, Nigeria. It accounts for over 30 % of the meat processed for consumption in Lagos (Agbeze, 2017). The river was sampled at upstream, midstream and downstream with respect to the abattoir (Figure 1). Three samples each were taken from upstream, midstream and downstream of the river. The sampling points were geo-referenced with the aid of a handheld Global Positioning System (GPS) and recorded as coordinates of the locations (Figure 1).

There are two seasons in Nigeria. They are the dry and wet season. The wet season spans from April to July and September to October while the dry season spans from November to March. Dry season characteristics are found in the month of August (the break between double maxima rainfall) extensions into September is now being observed due to climate change (Adedeji et al., 2018; Salau, 2016; Odekunle, 2004).

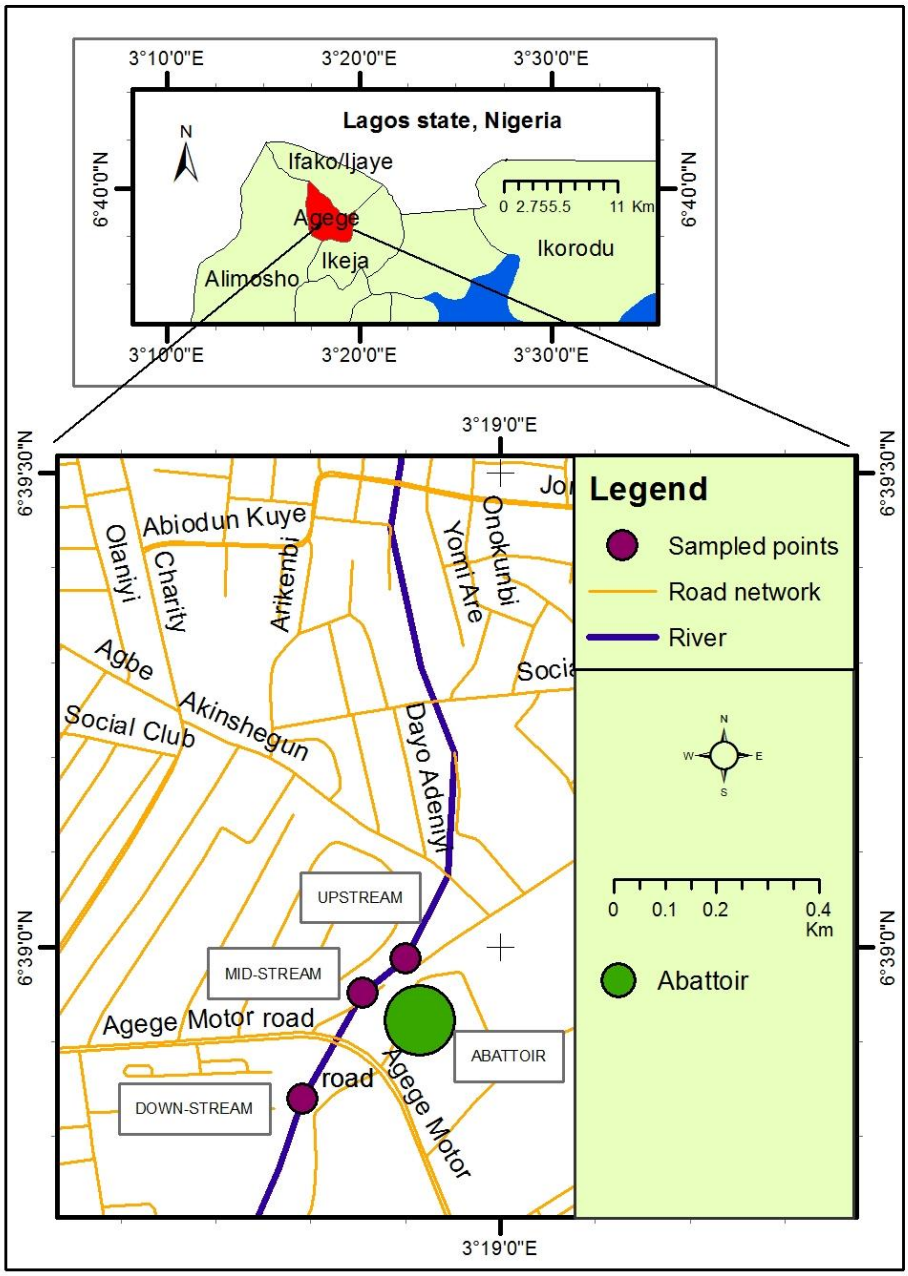

Figure 1: Map of the study area showing sampling points on the Oko-Oba River by the Oko-Oba Abattoir, Agege, Lagos Nigeria (Author, 2020).

Sampling

Surface water samples were collected on the 17th of July 2019 (wet season) and the second batch was collected on the 2nd September 2019 (dry season) (2nd of September was still part of the August break which is a dry season in Nigeria because of observed climate change). Samplings were done in the mornings around 10am to 11am when abattoir activities were at its peak. The water samples were collected and stored in pre-washed plastic bottles that had been soaked in 1% nitric acid solution. Sterile bottles (250 ml capacity) were used to collect samples for the microbial analyses at 20 cm to 30 cm depth from the river. Samples were appropriately labeled and preserved in a cooler with ice packs before being transported to the laboratory for analysis. The samples from the three

sampling points at each location (upstream, midstream and downstream) were mixed to generate a composite sample prior to analyses (Oluseyi et al., 2019).

Physico-chemical analyses of Surface water

Sample analyses began immediately on arrival of the samples in the laboratory. Physico-chemical parameters determined for the samples include pH, total suspended solids (TSS), total solids (TS), total dissolved solid (TDS), chloride, (TS), chemical oxygen demand (COD), biological oxygen demand (BOD) and potentially toxic metals (PTMs), such as lead and cadmium (Odour and colour were also observed). Standard protocols of American Public Health Organization (APHA, 2005) and American Society for Testing and Materials (ASTM) were employed for analysis of surface water physicochemical parameters. pH of samples was determined electrometrically using calibrated pH meter (Mettler Toledo pH Meter; FP20). A calibrated Methrohm conductivity meter was used to measure the conductivity of samples.

TSS, TS and TDS were determined by gravimetric methods. BOD was determined using the modified Winkler's Method in 350ml bottles. COD was determined by back titration after refluxing sample mixture with excess oxidising agent (potassium dichromate solution). Acidity, alkalinity and chloride of the samples were determined by titration method using sodium hydroxide (NaOH), hydrochloride (HCl) and silver nitrate ($AgNO_3$), respectively. In the titration for acidity, alkalinity and chloride phenolphthalein, methyl orange and potassium dichromate indicators were employed (Rahmanian et al., 2015).

Potentially Toxic Metals (PTMs) analyses

Water samples were digested with nitric acid and concentrations of PTMs commonly called heavy metals were determined using a calibrated Buck Scientific Model 210 VGP, Serial Number 1619 Flame Atomic Absorption Spectrophotometer (AAS) as in EPA (2016). Specifically, only lead and cadmium were determined because their concentrations have been reported to be higher than other PTMs in animal tissues and blood (Nwude et al., 2010).

Microbial analyses

Microbial analyses of surface water samples were according to the standard methods for water examination as stipulated in APHA (1991; 2005). Pour plate technique was employed for microbial analysis of water collected from the Oko-Oba river. Specifically, total bacteria count (TBC), total coliform count (TCC), total faecal coliform count (TFCC), total fungi count (TFC) were analysed. The water samples were serially diluted, aliquots of diluted samples were inoculated into sterile petri dishes in duplicates and sterile molten prepared culture media were poured into the inoculated plates. The plates were swirled to ensure even distribution of the inoculums. The plates were then allowed to solidify and incubated at 37 ^{O}C for 48 h (TBC and TCC), 44 ^{O}C for 48 h (TFCC) and at 28 ^{O}C for 2-5 days (TFC). The developed colonies were counted in duplicates using colony counter. The culture plates with discrete colonies were taken and recorded. The mean colonies counted were then multiplied by the dilution factor to give the total number of bacteria for population in CFU/ml of the water analysed (Fawole and Oso, 2007).

Quality control and calibration

The samples from field were kept at 4 ^{0}C till analyses. All laboratory glass wares were washed and soaked in 1% nitric acid solution overnight before being used for sampling and analyses. The stipulated sample holding times for water quality parameters analyses as recommended in APAH (2005) were not exceeded. pH meter was calibrated, with three buffer solutions of pH 4.0, 7.0, and

10.0, before measurements were taken. The conductivity meter was calibrated using standard solutions of known conductivity. Each pH and conductivity measurement were taken after submerging and holding the pH probe for a couple of minutes to achieve a stabilized reading. Each pH and conductivity readings, the probes were submerged in 50 ml of sample and allowed to stabilize for some minutes (when the stability indicator disappears) before the value was recorded A four-point calibration on the AAS with a range of 0.00 to 3.00 mg/l for lead was 0.00 to 1.00 mg/l for cadmium for used for quantification. Cross contamination was prevented by rinsing with distilled water and blotting the probes (the pH and conductivity meter) while for the aspirator of the AAS deionised water was used to rinse the tip and aspirate through before the next measurement. Analyses of the various parameters were conducted within the stipulated holding times allowed for each parameter as recommended in APAH (2005). Each water sample parameter was analysed in triplicates. Blanks were carried out for every parameter using distilled water and blank values were subtracted from values obtained for analyses of field samples.

Statistical analysis

Physicochemical parameters of sample were expressed as mean± standard deviation. One-way Analysis of Variance (ANOVA) was used to compare water quality for upstream, midstream and downstream samples from the Oko-Oba River in both the wet and dry seasons. Pearson correlation test was used to compare all water quality parameters for wet season as well as dry season. T- test was carried out to compare the dry and wet season values for each parameter. All the statistical analyses are conducted using Microsoft Excel version 2007.

Results and Discussion

Physicochemical characteristics of surface water upstream, midstream and downstream of the Oko-Oba River Agege, Lagoon

Physical examination of upstream, midstream and downstream samples from the river all appeared brownish, but the upstream samples had the lightest colour in both rainy and dry season. However rainy season samples were generally not as dark as dry season samples. Colour variation in water is an indicator of impurity. Dark colours are due to anthropogenic activities (Daramola et al., 2019) which in this case major activity was abattoir activities beside the river which produces both liquid and solid waste usually deposited directly and indirectly as runoff into the river.

The mid and downstream samples had offensive odours in both dry and rainy season however the odour from dry season samples were more offensive. The offensive smell may have been as a result putrefaction of liquid and solid waste deposited directly and indirectly by run-offs and waste streams of effluents into the river from the Abattoir. Putrefaction of organic waste like blood, animal fat, faeces and waste meat produce hydrogen sulphide. Hydrogen sulphides are known to have offensive smell (Daramola et al, 2019). Offensive odours were observed by Ezeoha and Ugwuishwu, (2011), Akanni et al., (2019) in their studies of rivers by/ around Abattoirs in Nigeria. They attributed the offensive odour to the poor waste management practices of the Abattoirs near the rivers.

Results from the analyses of physicochemical parameters are shown in Table 1 (Table 1S, Table 2S, Table 3S and Table 4S in supplementary data). The salinity of Oko-Oba River ranged between 0.429±0.09 and 1.01±0.15 g/l indicating that it was an oligohaline Estuary. Fresh water has a salinity of less than 0.5g/l. Estuaries are divided into oligohaline (0.5 - <5.0 g/l), mesohaline (5.0 – 18.0 g/l), polyhaline (18.0 – <30.0 g/l), euhaline/ near oceans (seas) have salinity above 30 g/l like oceans (EPA, 2006). Oligohaline habitats are valuable habitats because they act as nursery areas (Feyer et al., 2015; Rozas and Hackney, 1983). Hence the river should be preserved.

Results from analyses of water samples showed that values for upstream samples (for TDS, conductivity, TSS, TS, acidity, alkalinity, lead and cadmium) were lower than for midstream and downstream except for salinity, chloride, BOD, and COD. BOD and COD values were highest in midstream samples followed by upstream samples and downstream samples gave the lowest values which may be attributed to the direct deposition of effluents and waste into the river at Midstream. Lower TDS, conductivity, TSS, TS, acidity, alkalinity, lead, cadmium, TBC and TFCC indicates better water quality (Duressa et al., 2019).

The trend in TDS, conductivity, TSS, TS, acidity, alkalinity, lead and cadmium values may have been due to the direction of River water flow and the impact of Abattoir activities on the River. The River flows from upstream to midstream and then to downstream (as shown in Figure 1) and the values from analyses of physicochemical parameters also increased in like manner. Upstream water samples were taken 400 m before the Abattoir, midstream samples were taken by the Abattoir where all the activities producing waste occurred and the wastes were dumped in the river. Downstream samples were taken about 250 m from the midstream sampling point. Thus, upstream is not impacted directly by the Abattoir activities while the midstream and downstream are impacted by the activities of the Abattoir.

Lower chloride content and salinity experienced at midstream may have been a direct effect of dilution from the waste being directly deposited. Chloride, salinity, conductivity, TS, TDS, BOD and COD values were lower in rainy season than in dry season. This must have been as a result of dilution of the river water by rains which are regular in the rainy season. In dry season, the water is more concentrated, slightly acidic, has more dissolved solids and ions which gave rise to higher conductivity and salinity. Similar trends were observed Sogbamu et al., (2020) and Nwoji et al., (2010) in their seasonal variation study of surface water.

Nwude et al., (2010) reported higher levels of lead and cadmium compared to other potentially toxic metals are found in animal tissues and blood. Blood containing lead and cadmium released into the effluents and subsequently may contaminate the rivers. Oko-Oba River was analysed for lead and cadmium concentration. The concentration of lead ranged between 0.00 ± 0.00 and 0.17 ± 0.06 mg/l in the –Oba River with the water samples from midstream giving the highest values (of 0.17 ± 0.06 mg/ in wet season and 0.13 ± 0.15 mg/l in dry season) and the upstream water samples having the lowest values (Table 1). Cadmium was also detected and quantified in the river water samples and the values obtained were between 0.003 ± 0.006 and 0.017 ± 0.015 mg/l. The upstream samples gave lower values for cadmium while the downstream samples gave highest value for cadmium especially in the dry season (Table 1). All the water samples exceeded their regulatory limits for lead and cadmium except upstream water samples in dry season which complied with the limit for lead. Lead and cadmium can bio-accumulate and bio- magnify along the food chain. Hence the concentration found present potential risk to both aquatic life and humans who will eventually feed on them. Lead and cadmium are carcinogens, (ATSDR., 2011) while lead impacts the central nervous system of the exposed individual. Lead could also cause delayed mental and physical growth in children while affecting the attention span and learning abilities of children (ATSDR., 2011).

The physicochemical parameters (Table 1) of upstream and midstream samples showed 53.85 % compliance (that is only 8 out of 13 physicochemical parameters analysed) with the National Environmental Standards and Regulations Enforcement Agency (NESREA) Nigeria and the Federal Ministry of Environment in Nigeria (formerly known as Federal protection agency (FEPA)) (NESREA 2011; FEPA 2001 and 1988) limits for surface water. TSS, Alkalinity, BOD, COD, lead

and cadmium for the two seasons (dry and wet season) did not comply with their regulatory limits. Similarly analyses of downstream samples gave values for physiochemical parameters that showed 46.15 % compliance with the set limits for surface water (that is only 7 out of 13 physicochemical parameters analysed). TS, TSS, Alkalinity, BOD, COD, lead and cadmium values from downstream samples did not comply. The high values of BOD and COD can be attributed to the (especially the very high dry season values) to the presence of excess bacteria in the water, organic and inorganic pollutants in the river water samples. Table 2 showed the presence of a high number of microorganisms. Bacteria consume the available oxygen from the water column cause high alkalinity such as observed in study to disposal of domestic and municipal waste into the river by Haque et al., (2019). At the Oko-Oba river, liquid waste was found to be deposited directly into the river and solid waste were washed into the river by run-offs.

Ojo, (2014) and Oluseyi et al., (2019) also studied the same sampling site, in 2014 and 2011 respectively and a comparison of their results is as shown in Table 2. The pH values for the River was different from Oluseyi et al., (2019) but similar to the 2014 study. The BOD, TDS and TSS values varied from the various studies however, BOD values were close, though there was a gradual increase from 2011 to 2014 and 2019. TDS increased from 2011 to 2014 and TDS decreased in 2019. TSS reduced was found to initially reduce in 2014 but had increased in this study.

Using the classification as described in Table 3 according to Tekenah et al., (2014), indicates the river is acceptable base on pH but base on the TSS value, the river is slightly polluted however, based on the BOD and COD values the river is highly polluted.

Table 1: Physicochemical characteristics of surface water of the Oko-oba River Agege, Lagoon in dry and wet season 2019

	Wet season			**Near Dry Season**			
Parameter	Downstream	Midstream	Upstream	Downstream	Midstream	Upstream	**FEPA 1991 LIMIT**
pH	7.22 ± 0.09	7.55 ± 0.02	7.30 ± 0.08	6.52 ± 0.09	6.88 ± 0.07	6.60 ± 0.21	6-9a
TDS (mg/l)	898.93 ±17.83	609.77 ± 4.31	329.90 ± 9.24	1169.67 ±15.37	726.60 ± 11.96	653.20 ± 2.76	2000a
Conductivity (µS/cm)	2031.1 ± 97.73	1398.1 ± 41.11	760.13 ± 18.97	2932.0 ± 37.47	1813.7 ± 28.50	1644.7 ± 32.87	4000 [b]
TSS (mg/l)	**323.33 ± 113.72**	**170.00 ± 81.85**	**140.00 ± 36.06**	**283.33 ± 189.30**	**166.67 ± 125.83**	**216.67 ± 57.74**	**30a**
TS (mg/l)	**1222.3 ± 114.93**	779.77 ± 78.34	469.90 ± 26.97	1453.0 ± 200.13	893.27 ± 116.56	869.87 ± 55.87	1000a
Acidity (mg/l)	203.3 ± 41.63	110.00 ± 30.00	103.33 ± 23.09	326.67 ±70.24	96.67 ± 50.33	86.67 ± 5.77	NS
Alkalinity (mg/l)	**1050.0 ± 229.13**	**550.00 ± 427.20**	**433.33 ± 104.08**	**966.0 ± 76.38**	**566.00 ± 57.74**	**566.67 ± 256.58**	**250a**
Chloride (mg/l)	307.67 ± 114.12	260.33 ± 54.23	485.17 ± 40.99	363.17 ± 92.65	307.83 ± 51.68	611.83 ± 92.54	600c
Salinity (g/l NaCl)	0.506 ± 0.19	0.429 ± 0.09	0.800 ± 0.07	0.598 ± 0.15	0.507 ± 0.09	1.010 ± 0.15	2.0a
BOD (mg/l)	**81.00 ± 34.64**	**115.00 ± 11.27**	**86.67 ± 22.28**	**110.67 ± 47.61**	**194.33 ± 28.99**	**436.00 ± 163.24**	**40a**
COD (mg/l)	**177.27 ± 65.98**	**244.87 ± 29.67**	**178.27 ± 51.26**	**243.97 ± 109.84**	**387.47 ± 84.83**	**948.80 ± 386.99**	**60c**
Lead (mg/l)	**0.13 ± 0.23**	**0.17 ± 0.06**	**0.03 ± 0.06**	**0.03 ± 0.06**	**0.13 ± 0.15**	0.00 ± 0.00	**0.015a**
Cadmium (mg/l)	**0.007 ± 0.006**	**0.007 ± 0.06**	**0.003 ± 0.006**	**0.017 ± 0.015**	**0.007 ± 0.006**	**0.013 ± 0.006**	**0.002a**

TDS-Total dissolved solids, TSS- Total Suspended Solids, TS-Total Solids, BOD- Biological Oxygen Demand, COD-Chemical Oxygen demand, b-FEPA 1988, a-FEPA 1991 C- NESREA 2010. **Note:** Values are Mean ± SD (n=3); NESREA, 2010; NS- Not specified. The bolden data in the table shows that the parameter did not comply with its limit.

Table 2: Physicochemical characteristics of surface water of the Oko-Oba River Agege, Lagos in 2011, 2014 and 2019

Parameter	**2011 Study by Oluseyi et al., (2019)**	**2014 by Ojo, (2014)**	**This study**
pH	3.8 to 4.6	6.50 ± 0.30 to 7.00 ± 0.40	6.52± 0.09 to 7.55±0.02
BOD (mg/l)	85.2 to120.36	66.10 ± 0.80 to 151.00 ± 6.60	81.00±34.64 to 436.00 ±163.24
TDS (mg/l)	226.05 to 618.82	2450 ± 40.0 to 6000.00 ± 87.50	329.90 ±9.24 to 1169.67±15.37
TSS (mg/l)	10305.04 to 1100.48	5.00 ± 0.50 to 32.00 ± 40.00	140.00 ±36.06 to 323.33±113.72

Table 3: Classification of surface water according to Tekennah et al., (2014.)

Parameter	Class 1	Class 2	Class 3	Class 4	Class 5
pH	6.5 – 8.0	6.0 – 8.4	5.0 – 9.0	3.9 – 10.1	<3.9 – >10.1
BOD (mg/l)	1.5	3.0	6.0	12.1	>12.1
COD (mg/l)	10	20	40	80	>80
TSS (mg/l)	20	40	100	278	>278

Value of classes; Class 1= excellent, Class 2 = acceptable, Class 3 = slightly polluted, Class 4 = polluted, Class 5=heavily polluted

Microbiological characteristics of surface water upstream, midstream and downstream of the Oko-Oba River Agege, Lagoon

Pathogenic microorganisms in rivers may cause health risk to humans and animals. The TBC, TCC, TFCC, and TFC of the Oko-Oba River were determined for both seasons and the results are shown in Table 4. TBC, TCC, TFC, and TFC values obtained from water samples analyses were high. These high levels are indications of contamination from its environment and could pose a health risk to the consumers of this water resource. The midstream had the highest TBC, TFCC, and TCC in both seasons. This trend was similar to the trend observed with BOD, and COD. This may be due to the proximity of the Abattoir to the midstream. The Abattoir was by the river at the midstream point of sampling and waste steams were discharged at the mid-stream.

Higher TBC and TFCC levels were found in the wet season than in the dry season. A different trend was observed for TFC and TF where higher levels were recorded in the dry season. Higher numbers of pathogenic microorganisms are expected in dry season than in wet seasons due to less rainfall, less surface runoffs and less dilutions of the rivers (Edokpayi et al 2015). However, Edokpayi et al., (2018) reported higher TBC and TFCC in the wet season compared to the dry season. Their findings were similar to this study. They attributed the trend to the discharge of ill-treated waste water from activities on the riverbank and the slightly higher temperature experienced in the wet season of the region in South Africa because, higher temperature favours incubation of bacteria. In this study location, abattoir liquid waste containing some solids was discharged directly into the river. Also, higher temperature is associated with Lagos in June than September when the samples were collected. Ojeh, et al, (2016) who studied urban-rural temperature differences in Lagos between 2014 and 2015 stated that observed mean daily temperature for June was higher (27.1 ^{0}C) than September (26.3 ^{0}C).

Oluseyi et al., (2019) also studied Oko-Oba River water in 2011 found that the river water had, total bacteria count and total fungi count values in the range of 1.80 X 10^{05} to 3.10 X 10^{05}cfu/ml and 3.0 X 10^{03} to 8.0 X10^{03}cfu/ml respectively. In this study TBC and TFC values had a range of 1.29 X 10^{07}± 3.87 X 10^{06} to 4.40X10^{10} ±7.45 X 10^{10} cfu/ml and 1.37 X 19^{02}±1.05 X 10^{02} to 7.30X10^{03}±1.87 X 10^{03} cfu/ml respectively (Table 3). Higher TBC and similar TFC values were obtained in this study compared with the 2011 study. The difference in these studies may be attributed to the difference in time. The river has continually received waste since 2011 till date. The results obtained from this study, indicate that water from Oko-Oba River poses as a potential health risk and is not fit for any domestic use.

Table 4: Microbiological characteristics of surface water of the Oko-oba River Agege, Lagoon in dry and wet season 2019 (cfu/ml)

	Wet season			Near Dry Season		
Parameter	**Downstream**	**Midstream**	**Upstream**	**Downstream**	**Midstream**	**Upstream**
TBC	$5.50\ X10^{08} \pm 7.78\ X10^{08}$	$4.40X10^{10} \pm 7.45X10^{10}$	$3.13X\ 10^{08} \pm 3.01X10^{08}$	$1.36\ X10^{07} \pm 6.01X10^{06}$	$1.29X10^{07} \pm 3.87X10^{06}$	$5.93X10^{06} \pm 1.91X10^{06}$
TCC	$1.32\ X10^{04} \pm 1.35\ X10^{04}$	$3.13X10^{04} \pm 2.01X10^{04}$	$3.57X\ 10^{04} \pm 1.65X10^{04}$	$1.10X10^{05} \pm 7.87X10^{04}$	$7.70X10^{04} \pm 3.30X10^{04}$	$2.60X10^{04} \pm 9.85X10^{03}$
TFCC	$6.33X\ 10^{03} \pm 5.13\ X10^{03}$	$7.33X10^{03} \pm 6.43X10^{03}$	$1.83X\ 10^{03} \pm 2.75X10^{03}$	$2.17X10^{03} \pm 1.26\ X110^{03}$	$7.33X10^{03} \pm 1.01X10^{04}$	$2.17X10^{03} \pm 1.04X10^{03}$
TFC	$4.40X10^{02} \pm 1.04\ X10^{02}$	$1.90\ X10^{02} \pm 9.54X10^{01}$	$1.37X19^{02} \pm 1.05X10^{02}$	$7.50\ X10^{03} \pm 1.21X10^{03}$	$4.33X10^{03} \pm 9.87X10^{02}$	$7.30X10^{03} \pm 1.87X10^{03}$

TBC-Total bacteria count, TCC-Total coliform count, TFCC –total faecal coliform count, TFC-total fungi count. Note: Values are Mean ± standard deviation (SD) (n=3)

Relationship between physicochemical and bacteriological parameters of Oko- Oba Surface water in dry and rainy season

One-way ANOVA was used to compare the physicochemical and microbiological properties for upstream, midstream and downstream of the Oko-Oba River in both seasons using Excel 2007and the results are shown in Table 5 and Table 6. p values of 0.382594 and 0.867863 for wet and dry season respectively were obtained which are more than the traditional p value of 0.05. The *p*-values obtained were associated with F value of 0.98 and 0.142169. This result shows that there is no significant difference between the values of the Oko-Oba River parameters at the upstream, midstream and downstream statistically in both seasons. This is quite surprising however, looking at Table 1 and Table 2, shows that the river is generally polluted, and many parameters did not comply their limits. The results from one-way ANOVA carried out to compare the upstream, midstream and downstream of the Oko-Oba River in both seasons suggests that though the river is being polluted by the Abattoir other factors/ sources of pollution maybe occurring along the river which may require further investigation.

Matrix correlation study of all the parameters were carried out for wet and dry season and the results are shown in Table 7 and Table 8 respectively. The r-values displayed in Table 7 and Table 8 reveals several relationships between parameters of the water samples. There were some similarities and differences in the r values for dry and wet seasons. Correlation between the physicochemical and microbiological parameters showed that some parameters were strongly associated while some were not. Important relationships ($r \geq 0.600$) were observed between TDS and conductivity, TSS, TS, acidity, alkalinity, lead, cadmium, TFCC in wet season. Similarly, important relationships ($r \geq 0.600$) observed between TDS and conductivity, TSS, TS, acidity, alkalinity, lead, cadmium, TFCC in wet season was also observed with dry season but in addition TDS also correlated positively with TBC, TCC. Solute in water is TDS when it dissolves in water but in suspended state it measured as TS. Solutes are responsible for increasing the conductivity of water. The type of solute impacts on the acidity and alkalinity of a water sample. Lead and cadmium in water are solute which dissolves in water to form ions. Eliku and Leta., (2018) also carried out matrix correlation on many parameters for Awash River in Ethiopia. They found many strong relationships especially with parameters that were related with solutes such as nitrates, phosphate and conductivity among others in dry and wet seasons. Particularly of note in wet season, there was a strong positive correlation between BOD and TBC, COD and TBC, COD and TFCC some positive but not strong correlation (0.35 to 5.90) was observed between BOD and TCC, BOD and TFCC, COD and TCC. However, in the dry season, negative strong correlation was observed between TBC and BOD, TCC and BOD, TBC and COD, TCC and COD. Islam et al., 2019 in their review stated that a positive association was found between COD and the bacterial diversity in combination with other factors such as dissolved organic carbon by Llirós et al., (2014), while in contrast another study by Kagalou et al., (2009) found negative correlation between COD and bacterial count in contaminated water bodies.

Correlation and statistical mean difference between each physicochemical and microbiological parameters of Oko-Oba River in wet and dry season were analysed. The results are shown in Table 9 (Tables 6S to Table 20S in supplementary data). Only pH, TDS, TS, conductivity and TF showed positive correlation between their wet and dry season values at a probability less than 0.05. Thus, seasonal variation was only obvious with these parameters statistically.

A probability value of less than 0.05 indicates that the relationship was not due to chance but was significant. Edokpayi et al, (2018) also carried out a correlation study of dry and wet season parameters of Nzhelele rivers of South Africa. They found significant strong relationship between the turbidity, chloride, nitrates, of dry and wet season of their samples.

Table 5: Comparison of the upstream, midstream and downstream physicochemical and microbiological properties of the Oko-Oba river in wet season using one-way ANOVA

Groups	Count	Sum	Average	Variance
DSSA	17	5.5E+08	32358016	1.78E+16
MSSA	17	4.4E+10	2.59E+09	1.14E+20
USSA	17	3.13E+08	18436704	5.78E+15

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	7.43E+19	2	3.71E+19	0.980272	0.382594	3.190727
Within Groups	1.82E+21	48	3.79E+19			
Total	**1.89E+21**	**50**				

DSSA- mean value for downstream in wet season, MSSA- mean value for midstream in wet season, USSA- mean value for upstream in wet season

Table 6: Comparison of the upstream, midstream and downstream physicochemical and microbiological properties of the Oko-Oba river in dry season using one-way ANOVA

Groups	Count	Sum	Average	Variance
DSSB	17	13686356	855397.3	1.15E+13
MSSB	17	13022827	813926.7	1.04E+13
USSB	17	5967542	372971.4	2.2E+12

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	2.29E+12	2	1.14E+12	0.142169	0.867863	3.204317
Within Groups	3.62E+14	45	8.04E+12			
Total	3.64E+14	47				

DSSB- mean value for downstream in dry season, MSSA- mean value for midstream in dry season, USSA- mean value for upstream in dry season

Table 7: Correlation coefficient (r) among physicochemical and microbial parameters of Oko- oba river in wet Season 2019

	pH	TDS	Conductivity	TSS	TS	Acidity	Alkalinity	Salinity	Chloride	BOD	COD	Lead	Cadmium	TBC	TCC	TFCC	TF
pH	1.000																
TDS	-0.262	1.00															
Conductivity	-0.251	**1.000**	1.000														
TSS	-0.586	**0.936**	**0.931**	1.000													
TS	-0.350	**0.996**	**0.995**	**0.964**	1.000												
Acidity	**-0.659**	**0.898**	**0.893**	**0.996**	**0.935**	1.000											
Alkalinity	-0.565	**0.944**	**0.940**	**1.000**	**0.971**	**0.993**	1.000										
Salinity	-0.452	**-0.742**	**-0.750**	-0.458	**-0.678**	-0.373	-0.481	1.000									
Chloride	-0.452	**-0.742**	**-0.750**	-0.458	**-0.678**	-0.373	-0.481	1.000	1.000								
BOD	**0.995**	-0.165	-0.153	-0.503	-0.255	-0.581	-0.480	-0.538	-0.538	1.000							
COD	**0.971**	-0.022	-0.011	-0.374	-0.114	-0.459	-0.350	**-0.653**	**-0.653**	**0.990**	1.000						
Lead	0.488	**0.714**	0.722	0.421	**0.647**	0.334	0.444	**-0.999**	**-0.999**	0.573	**0.684**	1.000					
Cadmium	0.264	**0.861**	0.867	**0.626**	**0.811**	0.551	**0.646**	**-0.980**	**-0.980**	0.359	0.489	**0.971**	1.000				
TBC	0.966	-0.005	0.007	-0.358	-0.097	-0.443	-0.333	**-0.666**	**-0.666**	**0.987**	**1.000**	**0.697**	0.504	1.000			
TCC	0.562	**-0.946**	-0.942	**-1.000**	**-0.971**	**-0.992**	**-1.000**	0.484	0.484	0.477	0.346	-0.447	-0.649	0.330	1.000		
TFCC	0.425	**0.762**	0.769	0.484	**0.699**	0.400	0.506	**-1.000**	**-1.000**	0.513	**0.630**	**0.997**	**0.985**	**0.644**	**-0.510**	1.000	
TF	-0.576	**0.940**	0.936	**1.000**	**0.967**	**0.994**	**1.000**	-0.469	-0.469	-0.492	-0.363	0.432	**0.636**	-0.346	**-1.000**	0.495	1.000

TDS-Total dissolved solids, TSS- Total Suspended Solids, TS-Total Solids, BOD- Biological Oxygen Demand, COD-Chemical Oxygen demand, TBC-Total bacteria count, TCC-Total coliform count, TFCC –total faecal coli form count, TFC-total fungi count, Note: Bold values indicate the correlation is significant at the 0.05 level

Table 8: Correlation of Physicochemical and Microbial Parameter in dry season 2019

	pH	TDS	Conductivity	TSS	TS	Acidity	Alkalinity	Salinity	Chloride	BOD	COD	Lead	Cadmium	TBC	TCC	TFCC	TF
pH	1.000																
TDS	-0.556	1.000															
Conductivity	-0.565	**1.000**	1.000														
TSS	**-0.918**	**0.840**	**0.846**	1.000													
TS	**-0.634**	**0.995**	**0.996**	**0.888**	1.000												
Acidity	**-0.632**	**0.996**	**0.996**	**0.888**	1.000	1.000											
Alkalinity	**-0.661**	**0.991**	**0.993**	**0.904**	**0.999**	**0.999**	1.000										
Salinity	-0.477	-0.465	-0.456	0.089	-0.378	-0.379	-0.345	1.000									
Chloride	-0.477	-0.465	-0.456	0.089	-0.378	-0.379	-0.345	**1.000**	1.000								
BOD	-0.075	**-0.787**	**-0.780**	-0.326	**-0.724**	**-0.725**	**-0.699**	**0.912**	**0.912**	1.000							
COD	-0.131	**-0.751**	**-0.744**	-0.273	**-0.684**	**-0.685**	**-0.657**	**0.934**	**0.934**	**0.998**	1.000						
Lead	**0.905**	-0.149	-0.159	**-0.661**	-0.243	-0.242	-0.277	**-0.806**	**-0.806**	-0.493	-0.542	1.000					
Cadmium	**-0.991**	**0.663**	**0.671**	**0.963**	**0.732**	**0.731**	**0.756**	0.354	0.354	-0.060	-0.004	**-0.839**	1.000				
TBC	0.248	**0.667**	**0.659**	0.156	0.592	0.593	0.563	**-0.970**	**-0.970**	**-0.985**	**-0.993**	**0.638**	-0.115	1.000			
TCC	-0.072	**0.869**	**0.864**	0.462	**0.817**	**0.818**	**0.796**	**-0.842**	**-0.842**	**-0.989**	**-0.979**	0.360	0.206	**0.948**	1.000		
TFCC	**0.980**	-0.382	-0.392	**-0.822**	-0.469	-0.468	-0.500	**-0.641**	**-0.641**	-0.270	-0.324	**0.971**	**-0.945**	0.434	0.126	1.000	
TF	**-0.990**	0.433	**0.443**	**0.853**	0.518	0.517	0.548	0.596	0.596	0.215	0.270	**-0.956**	**0.962**	-0.382	-0.069	**-0.998**	1.000

TDS-Total dissolved solids, TSS- Total Suspended Solids, TS-Total Solids, BOD- Biological Oxygen Demand, COD-Chemical Oxygen demand, TBC-Total bacteria count, TCC-Total coliform count, TFCC –total faecal coli form count, TFC-total fungi count, Note: Bold values indicate the correlation is significant at the 0.05 level

Table 9: Results from correlation of between physicochemical and microbiological parameters of Oko-Oba River in wet and dry season

Parameters	Correlation value in Oko-Oba River	p-value (two tailed) in Oko Oba river	Comment with respect to pat 0.05 level
pH (wet and dry)	**0.854886**	**0.001545**	Significant
TDS (wet and dry)	**0.622805**	**0.000966**	Significant
Conductivity (wet and dry)	**0.367268**	**0.003046**	Significant
TSS (wet and dry)	0.990969	0.468631	not – significant
TS (wet and dry)	**0.972696**	**0.049396**	Significant
Acidity (wet and dry)	-0.93472	0.192016	not – significant
Alkalinity (wet and dry)	0.142857	0.595774	not - significant
Salinity (wet and dry)	0.697817	0.365348	not - significant
Chloride (wet and dry)	0.697817	0.365348	not - significant
BOD (wet and dry)	-0.83075	0.581189	not - significant
COD (wet and dry)	-0.81738	0.562956	not - significant
Lead (wet and dry)	1	0.42265	not - significant
Cadmium (wet and dry)	0.944911	0.225403	not - significant
TB (wet and dry)	0.4381	0.41162	not - significant
TC (wet and dry)	-0.89309	0.286349	not - significant
TFC (wet and dry)	0.640464	0.470529	not - significant
TF (wet and dry)	**0.402823**	**0.025161**	significant

Similar comments / conclusion was obtained when conducted at 0.01level of confidence

Conclusion

Our results show that there were significant and non-significant seasonal variations in the water quality parameters analysed for the Oko-Oba River samples and the water is unfit for consumption since it did not comply with the regulatory limit for many parameters. Results from this study, indicates the river is acceptable base on pH but base on the TSS value, the river is slightly polluted however, based on the BOD and COD values the river is highly polluted. Physicochemical and microbiological analyses of samples showed that upstream samples had lower values except for chloride, salinity, COD, BOD, TFCC, TBC and TCC. Chloride, salinity, had lower concentration at the midstream due to dilution while COD, BOD, TFC, TFCC, TBC and TCC had higher concentrations at the mid-stream due to direct discharge of effluent into the river. However, statistical analyses revealed that there was no significant difference between the upstream, midstream and downstream of the Oko-Oba river in both dry and wet season. This suggests that though the Abattoir is contributing to the pollution of the river some other factors may also be contributing to the pollution of the river. We therefore recommend that these factors be investigated. We also recommend further studies such as animal studies on assessment of the risk associated with the abattoir activities to aquatics organisms and man to aid the understanding of the nature of impact to the fishes and organisms dwelling in the River in line with the UN SDG goal of sustainable life under water (SDG goal 14). Furthermore, the Abattoir should ensure the proper and adequate treatment of its effluents before being discharge into the Oko-Oba River.

Note Supplementary Data available

Reference

Adedeji T, Utah E U & Sombo T E (2018). Monthly variation and annual trends of rainfall across major climatic zones in Nigeria. IOSR Journal of Applied Physics. 10 (4): 15-28.

Agbeze C (2017). Oko-Oba Agege Abattoir where unethical practices, negligence rule. Bussiness Day. 20thAugust, 2017, https://businessday.ng/opinion/article/oko-oba-agege-abattoir-unethical-practices-negligence-rule/ (accessed 6thApril 2020).

Al-Bayatti K, Al-Arajy K H, & Al-Nuaemy S H (2012). Bacteriological and physicochemical studies on Tigris River near the water purification stations within Baghdad Province. Journal of Environmental and Public Health. 2012; 1-8 doi:10.1155/2012/695253.

Augustyn L, Babula A, Joniec J, Stanek-Tarkowska J, Hajduk E, & Kaniuczak J (2016). Microbiological indicators of the quality of river water, used for drinking water supply. Polish Journal of Environmental Study. 25 (2): 511–519.

Akanni A, Ogbiye A, & Onakunle O (2019). The impact assessment of abattoir waste facility discharge on water in Osogbo, Nigeria. Cogent Engineering. 6 (1); 1-9.

Alhaji N B & Baiwa M (2015). Factors affecting workers' delivery of good hygienic and sanitary operations in slaughterhouses in North-Central Nigeria. Sokoto Journal of Veterinary Sciences. 13 (1): 29-37.

APHA (1999). American Public Health Association, Standard methods for examination of water and wastewater, American Public Health Association (APHA) Washington.

APHA (2005). American Public Health Association, standard methods for examination of water and waste waters, 21st edition American Public Health Association (APHA) Washington.

ATSDR (2011). Environmental Health and Medicine Education; Case Studies in Environmental Medicine (CSEM) - Cadmium Toxicity by Agency for Toxic Substance and Disease Registry (ATSDR). Page 1-63. https://www.atsdr.cdc.gov/csem/cadmium/docs/cadmium.pdf.

ATSDR (2019). Environmental health and medicine education; Case Studies in Environmental Medicine (CSEM) -leadtoxicity By Agency for Toxic Substance and Disease Registry (ATSDR). Page 1-185. https://www.atsdr.cdc.gov/csem/lead/docs/CSEM-Lead_toxicity_508.pdf.

Barakat A, El-Baghdadi M, Rais J, Aghezzaf B, & Slassi M (2016). Assessment of spatial and seasonal water quality variation of Oum Er Rbia River (Morocco) using multivariate statistical techniques**.** International Soil and Water Conservation Research**.** 4 (4): 284-292, ISSN 2095-6339, https://doi.org/10.1016/j.iswcr.2016.11.002.

Daramola J, Ekhwan T M, Adepehin E J, Mokhtar J, Lam K C, & Er A C (2019). Seasonal quality variation and environmental risks associated with the consumption of surface water: implication from the Landzun Stream, Bida Nigeria.Heliyon 5 (2019) e02121: 1-11.

Edokpayi J N, Odiyo J O, Msagati T A, & Potgieter N (2015). Temporal variations in physico-chemical and microbiological characteristics of Mvudi River, South Africa. International Journal of Environmental Research and Public Health. 12 (4): 4128-4140. doi:10.3390/ijerph120404128.

Edokpayi J N, Odiyo J O, Popoola E O, & Msagat T A M (2018). Evaluation of microbiological and physicochemical parameters of alternative source of drinking water: A case study of Nzhelele River, South Africa. Open Microbiological Journal. 12 (1): 18–27.

Ekpetere O K, Ekeh O F, & Ofodum N M (2019). Impact of abattoir wastes on groundwater quality in the FCT Abuja-Nigeria: A Case Study of Gwagwalada Satellite Town. Journal of Environment and Earth Science. 9 (4): 90-104.

Eliku T, & Leta S (2018). Spatial and seasonal variation in physicochemical parameters and heavy metals in Awash River, Ethiopia. Applied Water Science**.** 8, (177). .https://doi.org/10.1007/s13201-018-0803-x.

EPA (2006). Volunteer Estuary Monitoring Manual, Chapter 14; Salinity, Second Edition, USA Environmental Protection Agency. EPA-842-B-06-003.

Fawole M O & Oso B A (2007). Laboratory manual of microbiology, Spectrum books limited, p. 1 – 33.

FEPA (2001). Federal Environmental Protection Agency (FEPA), Guidelines and Standards for Environmental Pollution Control in Nigeria, Ministry of Environment, Lagos, Nigeria, 2001.

FEPA (1988). Federal Environmental Protection Agency (FEPA), Guidelines and Standards for Environmental Pollution Control in Nigeria, Ministry of Environment, Lagos, Nigeria, 1988.

Feyrer F, Cloern J E, Brown L R, Fish M A, Hieb K A, & Baxter R D (2015). Estuarine fish communities respond to climate variability over both river and ocean basins. Global Change Biology. 21 (10): 3608-3619. DOI: 10.1111/gcb.12969.

Haque M A, Jewel M A S, & Sultana M P (2019). Assessment of physicochemical and bacteriological parameters in surface water of Padma River, Bangladesh. Applied Water Science. 9, (10). https://doi.org/10.1007/s13201-018-0885-5.

Islam, M, Shafi S, Bandh S A, & Shameem N (2019). Chapter 3 - Impact of environmental changes and human activities on bacterial diversity of lakes, Editor(s): Suhaib A. Bandh, Sana Shafi, Nowsheen Shameem in Freshwater Microbiology; Perspectives of Bacterial Dynamics in Lake Ecosystems, Academic Press, 2019,Pages 105-136, ISBN 9780128174951 https://doi.org/10.1016/B978-0-12-817495-1.00003-7

Kagalou I, Tsimarakis G, & Bezirtzoglou E (2009). Inter-relationships between Bacteriological and Chemical Variations in Lake Pamvotis – Greece. Microbial Ecology in Health and Disease. 14(1):37-41. DOI: 10.1080/08910600276000274 8.

Llirós M, Inceoğlu Ö, García-Armisen T, Anzil A, Leporcq B, Pigneur L M, Viroux L, Darchambeau, F, Descy J, & Servais P (2014). Bacterial Community Composition in Three Freshwater Reservoirs of Different Alkalinity and Trophic Status. PLoS ONE 9(12): e116145. https://doi.org/10.1371/journal.pone.0116145.

NESREA (2011). National Environmental (surface and ground water quality control) Regulations, 2011. National Environmental Standards and Regulations Enforcement Agency (NESREA) of the Federal Ministry of Environment in Nigeria. 1-37.

Nkwoji J A, Yakub A, Ajani G E, Balogun K J, Renner K O, Igbo J K, Ariyo A, & Bello B O (2010). Seasonal variations in the water chemistry and benthic macro invertebrates of a South Western Lagoon, Lagos, Nigeria Journal of American Science. 6 (3): 85-92.

Nwanta J A, Onunkwo J I, Ezenduka V E, Phil-Eze P O, & Egege S C (2008). Abattoir operations and waste management in Nigeria: A review of challenges and prospects. Sokoto Journal of Veterinary Sciences. **7** (2): 61-67.

Nwude D O, Okoye P A C, & Babayemi J O (2010). Blood heavy metal levels in cows at Slaughter at Awka Abattoir, Nigeria. International Journal of Dairy Science. 5: 264-270. DOI:10.3923/ijds.2010.264.270

Odekunle O T (2004). Rainfall and the length of the growing season in Nigeria. International Journal of Climatology. 24: 467-479.

Ojekunle O Z & Lateef S T (2017). Environmental impact of abattoir waste discharge on the quality of surface water and ground water in Abeokuta. Journals of Environmental and Analytical Chemistry. 7(5): 1-10.

Ojeh V N, Balogun A, & Okhimamhe A (2016). Urban-Rural temperature differences in Lagos. Climate. 4 (2) https://doi.org/10.3390/cli4020029.

Ojo J O (2014). Environmental Impact Assessment of Effluents from Oko-Oba Municipal Abattoir at Agege, Lagos State, Nigeria. Global Advanced Research Journal of Agricultural Science. 3 (10): 317-320.

Oluseyi T O, Folarin T, & Adaramaja B T (2019). Physico-chemical and microbial characterisation of abattoir wastewater: Environmental Impact and Health Concern. 8 (1):38-49.

Omole D O, & Longe E O (2008). An assessment of the impact of Abattoir effluents on River Illo, Ota, Nigeria. Journal of Environmental Science and Technology. 1 (2): 56-64.

Rahmanian N, Ali S H B, Homayoonfard M, Ali N J, Rehan M, Sadef Y, & Nizami A S (2015). Analysis of physiochemical parameters to evaluate the drinking water quality in the State of Perak. Malaysia. Journal of Chemistry 2015: 1-10. | https://doi.org/10.1155/2015/716125.

Rodrigue C & Cunha C (2017). Assessment of microbiological quality of recreational waters: indicators and methods. Euro-Mediterranean Journal for Environmental Intergration. 2 (25): 1-18.

Rozas L P, & Hackney C T (1983). The importance of oligohaline estuarine wetland habitats to fisheries resources. Wetlands**.** 3: 77–89. https://doi.org/10.1007/BF03160732.

Salau O R (2016). The changes in temperature and relative humidity in Lagos State, Nigeria. World Scientific News. 49(2): 295-306.

Sogbanmu T O, Fatunsin O T, Echebiri F O, Otitoloju A & Olayinka K O (2020). Sawmill activities near the Lagos Lagoon, Nigeria: polycyclic aromatic hydrocarbons and embryotoxic evaluations of sediment extracts using clariasgariepinus. Bulletin of Environmental Contamination and Toxicology. 104: 809 - 819. doi.org/10.1007/s00128-020-02845-6.

Tekenah W E, Agi, P I & Babatunde B (2014). Analysis of surface water pollution from abattoirs and the interrelationship between physico-chemical properties (A case study of the New Calabar River). IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT). 8 (5): 10-18

ORIGINAL RESEARCH ARTICLE

Efficiency of crude oil degradation and peroxidase production by indigenous bacteria isolated from Ogoni land, River State, Nigeria

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Abstract

Detrimental impacts of crude oil spills on life below water require urgent intervention. With the emergence of microbial remediation technology as a viable strategy for clean-up of oil spill, low degradation efficiency by many bacteria remains a major challenge. Exploring new bacterial isolates with improved crude oil degradation efficiency is therefore crucial. In this study, bacterial isolates from crude oil contaminated site in Ogoniland, Rivers State, Nigeria were screened for ability to grow on crude oil and glucose (control) as sole carbon sources. Three isolates exhibited higher growth on crude oil based medium (COBM) than on glucose based medium, and were identified using 16S rRNA sequencing as *Bacillus cereus and Paenibacillus alvei* strains 1 and 2. They were further investigated for their growth kinetics, degradation efficiency and total peroxidase production on varying concentrations of crude oil (30, 50 and 75 g/L) at 30°C and 180 rpm for 288 h. Results revealed exponential decline in residual crude oil during the logarithmic growth phase of the three bacteria. Total peroxidase activity increased as crude oil degradation progressed. Highest enzyme yields of 1.79 U/mg, 1.39 U/mg and 1.69 U/mg were recorded from *B. cereus*, *P. alvei* strain 1 and *P. alvei* strain 2, respectively at 240 h of cultivation in 50 g/L COBM with degradation efficiency of 87.52%, 90.90% and 84.95%. Remarkably, these bacteria showed ≥ 80% crude oil degradation efficiency at the peak of peroxidase production which suggests that the enzyme played significant role in crude oil degradation by the bacterial isolates.

Keywords: *Bacteria; Bioremediation; Crude oil*

Introduction

Oil spillage is a challenging problem globally which requires urgent intervention. Despite the advances made to prevent oil spill accidents, many countries still suffer from aquatic and terrestrial spills due to the unpredictable manner of oil spill occurrence and the inability to totally prevent spillage [1]. Oil spills on surface and groundwater endanger aquatic lives and may inhibit survival of the ecosystem since crude oil polycyclic aromatic hydrocarbon components are neurotoxic and carcinogenic [2].

It has been extensively reported that crude oil spills have detrimental impacts on life below water and this has been an issue of great concern for decades as in the case with the Deepwater Horizon oil spill in Gulf of Mexico in 2010 [3, 4]. In Nigeria, public attention has been drawn to an environmentally challenged community, Ogoniland located in Rivers State, in the Niger Delta region of the country where repeated oil spills have polluted and adversely affected lands, rivers, creeks, ground waters as well as aquatic lives for decades. Hence, crude oil pollution is a global menace which has consistently attracted research in bioremediation [5, 6].

Microbial remediation technology is currently emerging as a viable strategy for clean-up of up oil spills due to its cost-effectiveness and environmental friendliness [7 – 9]. Degradation of crude oil hydrocarbons by indigenous microorganisms forms part of the major mechanism of remediating polluted environment [10]. It has been reported that indigenous microorganisms gain adaptation to the environment which enhances their metabolic ability to degrade crude oil hydrocarbons [11 – 13]. Recent reports indicate that degradation of crude oil hydrocarbons by bacteria is more effective than degradation by algae, yeasts, and fungi, [14]. In spite of the tremendous progress made with bioremediation of crude oil and other pollutants, low efficiency of microbes in degrading high concentrations of crude oil hydrocarbons still remains an overwhelming challenge [9]. Therefore, continuous search for discovery of microbes capable of overcoming this challenge is pivotal to the success of bioremediation technology.

Microbial degradation of crude oil depends on integral enzyme activity in microorganisms which utilize crude oil hydrocarbons to meet their carbon and energy needs [11, 12, 15 – 17]. Biodegradation of crude oil hydrocarbons is done by the activity of extracellular and intracellular enzymes [18 – 20]. Microorganisms initiate degradation of polycyclic aromatic and aliphatic hydrocarbons by co-metabolism using enzymes which include peroxidases, laccases, and oxygenases [21-23]. Peroxidases are redox enzymes which catalyse a variety of reactions and play diverse roles in all forms of life either in aquatic or terrestrial habitat. They have been reported as useful biocatalysts in the degradation of organic pollutants [24, 25]. They are able to catalyze the oxidative cleavage of carbon–carbon bonds in various organic compounds [26].

Nigeria is one of the largest producers of crude oil in the world [27 – 29]. As a result of persistent and long-term oil exploration, repeated oil spills have occurred in this region for decades which have polluted and adversely affected lands, rivers, creeks and ground waters. In particular, Ogoniland in Rivers State, situated in the Niger Delta region of Nigeria has extensively been reported to be affected by crude oil spills [29] which prompted the choice of soil samples from this area.

This study therefore investigated the crude oil degradation efficiency of some isolated bacteria from a crude oil contaminated site in Ogoniland, Rivers State. The role of peroxidase and its production were also determined during the biodegradation process.

Materials and Methods

Materials

Media components were products of Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade and also obtained from Sigma-Aldrich. Brent crude oil with approximately 0.37% sulfur was obtained from the Department of Petroleum Resources (DPR), Nigerian National Petroleum Corporation (NNPC), Port Harcourt, NE, Nigeria and used for this study.

Soil collection

Soil samples were collected at 0-20 cm depth from ground surface at different areas of oil spill site in Ogoniland, Rivers State, Southern Nigeria during rainfall season using sterile trowel after clearing debris from the soil surface. The samples (50g) were collected into sterile plastic containers with appropriate labels and transported to the laboratory for microbial isolation and identification.

Bacterial isolation and identification

Bacterial isolation and identification were carried out at the Biotechnology Unit, Federal Institute of Industrial Research Oshodi, Lagos, Nigeria. Bacteria were isolated by spread plate technique following serial dilution of soil samples on *Luria*-Bertani (LB) agar plates. Pure colonies were subcultured onto fresh LB plates and were identified based on cultural, morphological and biochemical characteristics. The observed morphology of the bacteria was compared with bacterial morphology provided by Bergey's Manual of Systematic Bacteriology [30] for initial identification of isolates.

Preparation of seed culture

Seed culture of each bacterial isolate was prepared by growing a loopful of slant culture in sterile nutrient broth composed of peptone (5 g/L), NaCl (5 g/L), beef extract (1.5 g/L) and yeast extract (1.5 g/L), pH 7.4. The culture was incubated at 30°C for 24 h at 180 rpm in a shaking incubator (Stuart, UK).

Screening of bacterial isolates capable of degrading crude oil

Ten bacterial isolates were screened for crude oil degradation by inoculating 5% (v/v) seed culture of each bacterial isolates into crude oil based medium consisting crude oil (10 g/L) as the only carbon source, KH_2PO_4 (0.2 g/L), NH_4NO_3 (2.0 g/L), NaCl (0.8 g/L), KCl (0.8 g/L), $KH_2PO_4 \cdot 12H_2O$ (0.2 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), $FeSO_4 \cdot 7H_2O$ (0.002 g/L) and $MgSO_4$ (0.2 g/L), pH 7.4. The control contained 10 g/L glucose as sole carbon source. These were incubated in shaking incubator at 180 rpm and 30^{o}C for 144 h. Bacterial growth was monitored through optical density at 620 nm [31]. Three isolates which exhibited higher growth on crude oil based medium than in glucose based medium (control) were selected for further studies and their molecular identification was done using 16S rRNA sequencing.

Molecular characterization and identification of selected bacteria

Identification of bacteria under study was done using 16S rRNA sequencing. Genotypic characterization of bacterial strains was analyzed by 16S rRNA sequencing according to the method described by Paju *et al*. [32]. DNA extraction was carried out on isolates using Zymo Bacterial DNA extraction kit according to manufacturer's instructions. The 16S rRNA gene of the bacteria was amplified through polymerase chain reaction (PCR) using the primer pair 27F- 5′-AGAGTTTGATCCTGGCT CAG -3', and 1492R 5'-GGTTACCTTGTTACGACTT -3' (BIOMERS, Germany). Solis Biodyne 5x HOT FIREPol Blend Master mix was used for the PCR reaction which was performed in 25 µl reaction mixture. The reaction concentration was brought down from 5x concentration to 1x concentration containing Solis Biodyne 1x Blend Master mix buffer, 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphates, (dNTP) (Solis Biodyne), 25 pMol of each primer, 2 U of Hot FIREPol DNA polymerase, proofreading enzyme, 5 µl of the extracted DNA, and sterile distilled water. Thermal cycling was done in EppendorfVapo protect thermal cycler (Nexus Series) for an initial denaturation at 95°C for 15 min followed by 35 amplification cycles of 30 s at 95°C; 1 min at 61°C, 1 min 30 s at 72°C and a final extension step

of 10 min at 72°C. The amplified product was separated on 1.5% agarose gel electrophoresis. DNA bands were visualized by ethidium bromide staining. The DNA molecular weight marker used was 100bp DNA ladder (Solis Biodyne). Amplified PCR products were sent to GATC Biotech, Germany for product purification and sequencing. The sequencing was done according to the Sanger sequencing method [33]. Resulting 16S rRNA sequences were compared with sequences present in the GenBank database and the National Center for Biotechnology Information (NCBI) database using the Standard Nucleotide BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Identified bacterial strains were maintained on fresh nutrient agar slants and stored at 4°C.

Biodegradation of varying crude oil concentrations

The identified bacteria, *Bacillus cereus*, *Paenibacillus alvei* strain 1, and *Paenibacillus alvei* strain 2 which demonstrated highest growth on crude oil-based medium (COBM) were selected for further biodegradation studies. Effects of varying crude oil concentrations (30, 50 and 75 g/L) on degradation efficiency of the microbes were investigated. Seed culture of each isolate was used as inoculum (5%, v/v) for the varied concentration of COBM. This was incubated with mixing in a shaking incubator (180 rpm) at 30^{o}C for 288 h. The COBM contained crude oil (30, 50 and 75 g/L) as sole carbon source, KH_2PO_4 (0.2 g/L), NH_4NO_3 (2.0 g/L), NaCl (0.8 g/L), KCl (0.8 g/L), $KH_2PO_4 \cdot 12H_2O$ (0.2 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), $FeSO_4 \cdot 7H_2O$ (0.002 g/L) and $MgSO_4$ (0.2 g/L), pH 7.4. Microbial growth was measured and monitored through optical density at 620 nm during the biodegradation period [31]. Residual crude oil concentration and degradation efficiency of selected isolates were determined at 48 h intervals over the biodegradation period. Aliquots of cultures were taken at 48 h intervals and centrifuged at 10,000 rpm for 20 min at 4°C. Clear supernatants were collected and used as source of total peroxidase.

Measurement of residual crude oil and evaluation of degradation efficiency

The residual crude oil in the biodegradation medium was determined at 420 nm [34] at 48 h intervals over 288 h biodegradation period. Samples (5 mL) from each biodegradation medium were mixed vigorously with equal volume of toluene using a vortex mixer to extract crude oil hydrocarbons from samples. A prepared standard curve of crude oil in toluene from 0 to 1000 mg/L (Figure 1) was used to estimate the amount of crude oil in samples. Residual crude oil was estimated as the difference between the initial and final concentrations of total crude oil. Degradation efficiency was estimated in percentage.

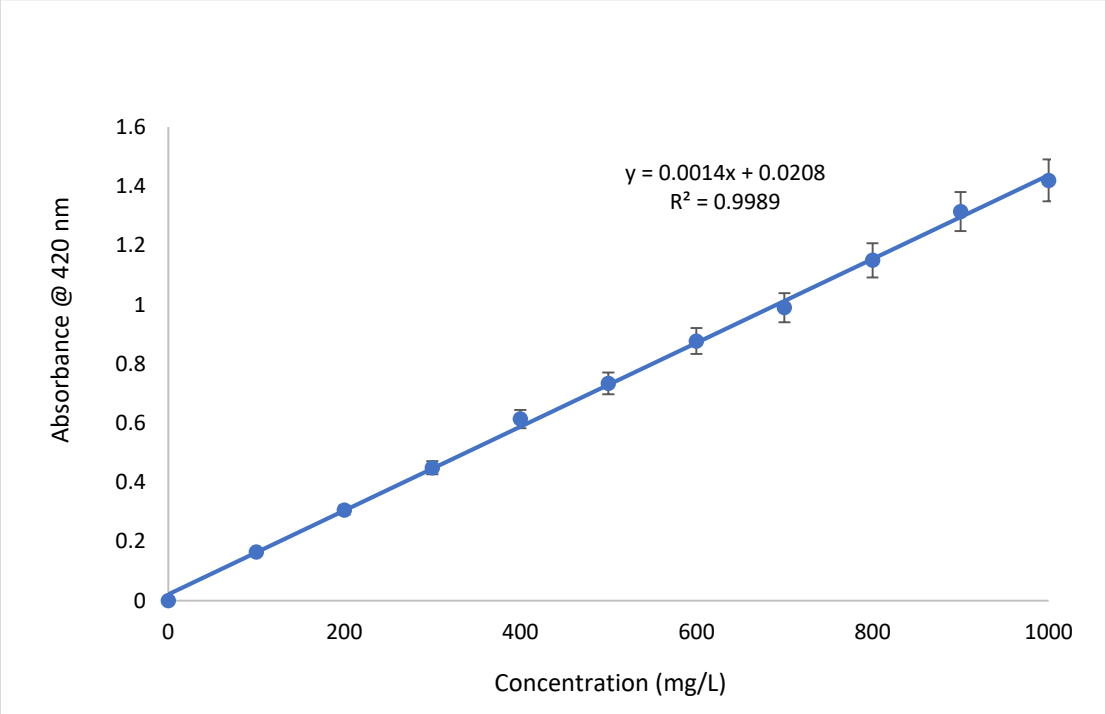

Figure 1. Standard curve of crude oil in toluene at 420 nm

Peroxidase production during degradation

Production dynamics of total peroxidase by bacteria under study during crude oil biodegradation process was determined by evaluating the enzyme activity over the study period. The method of Hunter *et al*. [35] with slight modifications was used to determine total peroxidase activity over the biodegradation period via oxidation of 0.24 mM 2,2'-azino-di-[3~ethyl benzothiazoline-6-sulphonic acid] (ABTS) buffered with 50 mM sodium acetate buffer, pH 5.0 in the presence of 5 mM H_2O_2 at 414 nm for 5 min. The reaction mixture (3 mL) contained 1 mL ABTS, 1 mL culture supernatant and 1 mL H_2O_2. One unit (U) of peroxidase activity was defined as the amount of enzyme that oxidized 1 micromole of ABTS per minute at pH 5.0 and 30^{o}C. Protein concentration was routinely determined by Bradford method [36] using bovine serum albumin as standard and the specific activity of peroxidase was expressed as U/mg protein.

Statistical Analysis

All studies were done in triplicates. Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Graphpad Prism 7 (GraphPad Software, La Jolla, CA, USA). Differences between means at 5% ($P < 0.05$) level were considered significant.

Results

Bacterial isolation and identification

In this study, bacteria were isolated from soil samples collected from a selected oil spill site in Ogoniland, Rivers State, Nigeria. The soil samples haboured *Bacillus* sp., *Clostridium* sp., *Corynebacterium* sp. And *Paenibacillus* sp. (Table 1). *Bacillus* sp. dominated the crude oil spill site

Table1: Bacterial isolation and identification

Isolate code	Colour	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Methyl red test	Voges-Prokauer test	Citrate utilization	Urease activity	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	Spore test	NO_3 reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Raffinose	Saucin	Sorbitol	Lactose	Microbial identity
BNS1	Cream	+	Rods	+	–	–	–	+	–	–	–	+	+	–	–	–	+	–	–	+	–	–	–	–	–	–	+	*Corynebacterium* sp. 1
BNS2	Cream	+	Rods	+	–	–	–	–	–	–	+	+	–	–	–	+	+	+	–	+	–	–	–	+	+	–	–	*Corynebacterium* sp. 2
BNS3	Cream	+	Rods	–	–	–	+	–	–	–	–	–	–	+	+	+	+	+	–	+	+	–	–	–	–	–	+	*Clostridium* sp.
BNS4	Cream	+	Rods	+	+	–	+	–	+	+	–	+	+	+	+	+	+	+	–	+	–	–	+	–	–	–	+	*Bacillus* sp. 1
BNS5	Cream	+	Rods	+	+	–	+	–	+	–	–	–	+	–	+	+	+	–	–	–	+	+	–	–	–	–	–	*Bacillus* sp.2
BNS6	Cream	-	Rods	+	–	–	–	–	–	+	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	*Paenibacillus*sp.
BNS7	Cream	+	Rods	+	+	–	+	–	–	+	–	+	+	+	+	–	+	+	+	–	+	+	–	–	–	–	–	*Bacillus* sp. 3
BNS8	Cream	+	Rods	+	+	–	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Bacillus* sp. 4
BNS9	Cream	-	Rods	+	+	+	+	–	–	–	+		–	–	–	+	+	–	–	–	–	–	–	–	–	–	+	*Bacillus* sp. 5
BNS10	Cream	+	Rods	+	+	–	+	–	+	–	–	+	–	+	+	+	+	+	+	+	–	+	–	+	–	–	+	*Bacillus* sp. 6

Screening of bacterial isolates for growth on media and molecular characterization

Growth of ten bacterial isolates (Table 1) cultivated in 10 g/L crude oil based medium and 10 g/L glucose-based medium was monitored for 144 h (Figures 2a - j). The bacterial isolates had varying degree of growth in COBM. *Corynebacterium* sp. 2 (Figure 2b), *Clostridium* sp. (Figure 2c), *Bacillus* sp. 2 (Figure 2e) and *Bacillus* sp. 3 (Figure 2g) effectively utilized crude oil by exhibiting growth which was similar to their respective control (in glucose based medium). It was observed that all the isolates grew well in glucose based medium with utilization of glucose within 48h compared with a prolonged lag phase exhibited by isolates in crude oil-based medium. However, after the lag phase, some of the bacterial isolates exhibited higher growth rate in COBM than in control. The growth of *Corynebacterium* sp. 1, *Bacillus* sp. 4and *Bacillus* sp. 6 was lower in COBM than in control**.** *Bacillus* sp. 1 (Figure 2d), *Paenibacillus*sp. (Figure 2f), and *Bacillus* sp. 5 (Figure 2i) exhibited higher growth in COBM than in glucose-based medium which served as control. Molecular characterization of these isolates confirmed their identities as *Bacillus cereus* and *Paenibacillusalvei* (strains 1 and 2) (Figures 3a-c).

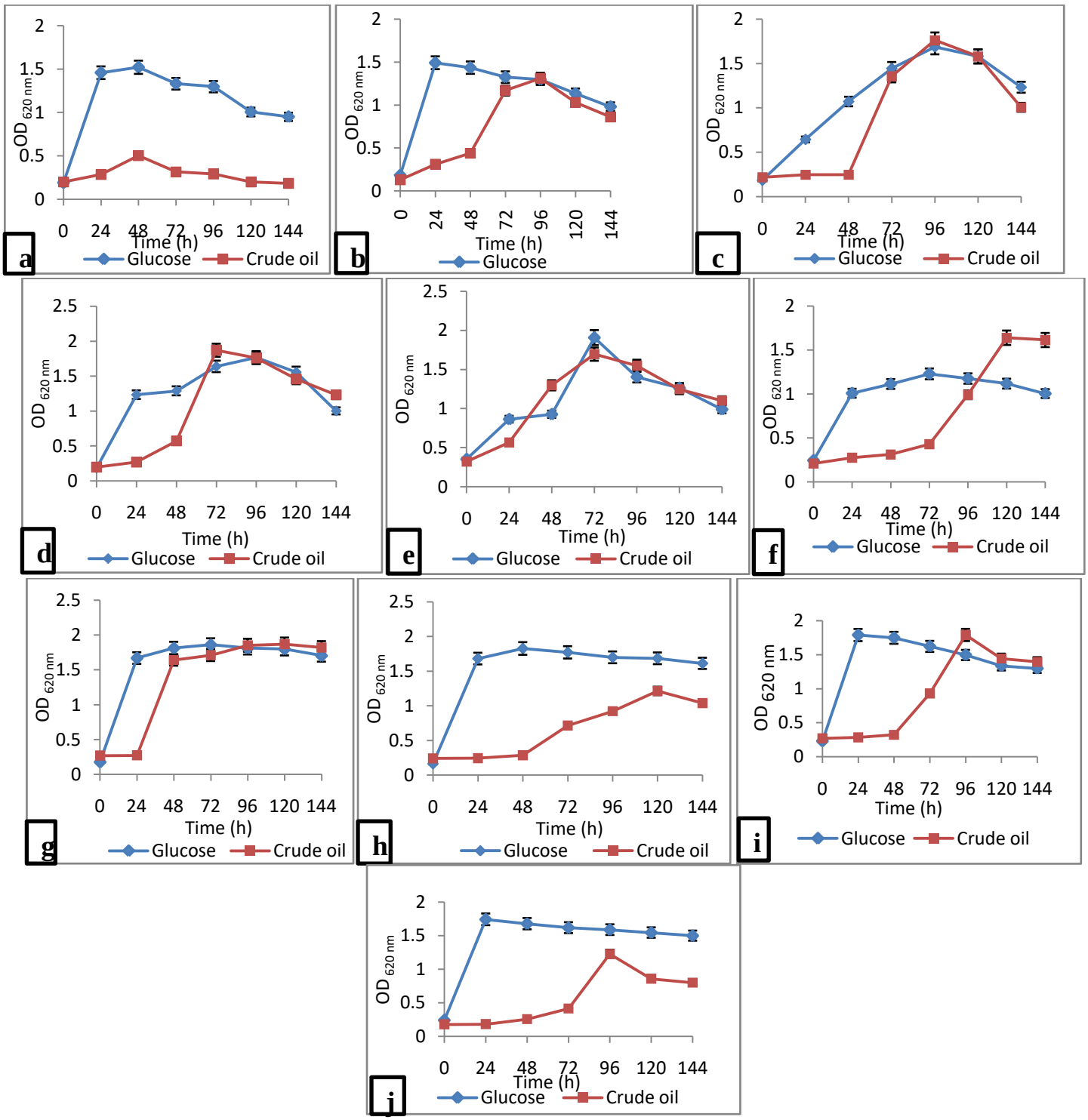

Figure 2. Growth profile of bacterial isolates in crude oil and glucose (control) based media over 144 h cultivation period [(a) *Corynebacterium* sp. 1 (b) *Corynebacterium* sp. 2 (c) *Clostridium* sp. (d) *Bacillus* sp. 1 (e) *Bacillus* sp. 2 (f) *Paenibacillus* sp. (g) *Bacillus* sp. 3 (h) *Bacillus* sp. 4 (i) *Bacillus* sp. 5 (j) *Bacillus* sp. 6] (Error bars represent Mean ± Standard Deviation)

TTAAAAGCCGCAGAGTATAATGCTGGTCTCAGACGATGGATTAATAGCTTGCTCTTA
TGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGG
ATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGA
AATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGT
TGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATC
GGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGA
ATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCT
TTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGC
ACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTATGTGGCATGCGTTATCCGGAATTATTGGGGCGTAGAGCGCGCGCAGGT
GGTTTCTTAGGTCTGATGTGAAACCCCGCGGCTCAACCGTGGAGGGCCGTTGTGAAA
TTGGGAGACTCCCCTGCGGAACAGGAGAGGCACTTTCGTATGTAGCGGCTGAAATCT
GTGCACAAATTGATGTACCACCAGTTTGGCAACGCTTATTTTCCGTCTGGGCCGCGA
ACGTGGGCCCGTGATCTGGAGAAACGTCCCCAGGAGATATCCTGCTCGCCCCAGCA
GTGCAGGTCTGTTGTATGTCGTGAGGGGGTTTCTCCTCCTGTA

a. 16S ribosomal RNA gene partial sequence of *Bacillus cereus*

CATGTACGGTATCGATTATGAGAGTATGATGCTGGTCGAGGGGACTTGATGGAGTGC
TTGCACTCCTGATGGTTAGCGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCAT
AAGACTGGGATAACCCACGGAAACGTGAGCTAATACCAGATAGGCATTTTCCTCGC
ATGAGGGAAATGAGAAAGGCGGAGCAATCTGTCACTTATGGATGGACCTGCGGCGC
ATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGA
GAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTG
ATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCCTAGGAGAGTA
ACTGCTCTTAGGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGGGGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGCGC
GCGCAGGCGGCAATGTAAGTTGGGTGTTTAAACCTAGGGCTCAACCTTGGGTCGCAT
CCAAAACTGCATAGCTTGAGTACAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGT
GAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTGTA
ACTGACGCTGAGGCGCGAAAGCGTGGGGGAGCAAACAGGATTAAATACCCTGGTAG
TCCACGGCGTAACGATGAATGCTAGGTGTTAGGAGTTTCGATCCCTTGGTGCGAAGT
TAACACCATTATGCATTGCCGCCCGGGCGATGGCGGCGTCGAGACTGCAACGTCAG
AGAAATGACGCGGACGCCCCACAAGCTAGTGCAGTATGTGGTGTAATTCGAGCACG
CGCACGAAAACTTTACC

b. 16S ribosomal RNA gene partial sequence of *Paenibacillus alvei* (strain 1)

CATGTACGGTATCGATTATGAGAGTATGATGCTGGTCGAGGGGACTTGATGGAGTGC
TTGCACTCCTGATGGTTAGCGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCAT
AAGACTGGGATAACCCACGGAAACGTGAGCTAATACCAGATAGGCATTTTCCTCGC
ATGAGGGAAATGAGAAAGGCGGAGCAATCTGTCACTTATGGATGGACCTGCGGCGC
ATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGA
GAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTG
ATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCCTAGGAGAGTA
ACTGCTCTTAGGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGC
AGCCGCGGTAATACGTAGGGGGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGCGC
GCGCAGGCGGCAATGTAAGTTGGGTGTTTAAACCTAGGGCTCAACCTTGGGTCGCAT
CCAAAACTGCATAGCTTGAGTACAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGT
GAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTGTA
ACTGACGCTGAGGCGCGAAAGCGTGGGGGAGCAAACAGGATTAAATACCCTGGTAG
TCCACGGCGTAACGATGAATGCTAGGTGTTAGGAGTTTCGATCCCTTGGTGCGAAGT
TAACACCATTATGCATTGCCGCCCGGGCGATGGCGGCGTCGAGACTGCAACGTCAG
AGAAATGACGCGGACGCCCCACAAGCTAGTGCAGTATGTGGTGTAATTCGAGCACG
CGCACGAAAACTTTACC

c. 16S ribosomal RNA gene partial sequence of *Paenibacillus alvei* (strain 2)

Figure 3. 16S ribosomal RNA gene partial sequence of *Bacillus cereus* (a); 16S ribosomal RNA gene partial sequence of *Paenibacillus alvei* (strain 1) (b); 16S ribosomal RNA gene partial sequence of *Paenibacillus alvei* (strain 2) (c)

Bacterial growth kinetics and residual crude oil.

This study showed that the selected bacteria (*B. cereus, P. alvei* strain 1 and *P. alvei* strain 2) degraded crude oil with increase in cell density in the presence of the varying concentrations of crude oil. *B. cereus* (Figure 4a) and *P. alvei* strain 1 (Figure 5a) exhibited high growth during the first 192 h of study with 3.49 g/L and 5.80 g/L residual crude oil, respectively while *P. alvei* strain 2 (Figure 6a) showed exponential growth in the first 96 h with 9.9 g/L residual crude oil reaching 4.95 g/L at 192 h. *B. cereus* (Figure 4b) and *P. alvei* strain 1 (Figure 5b) exhibited quick logarithmic growth and prolonged stationary phase on 50 g/L COBM while growth rate of *P. alvei* strain 2 was low (Figure 6b). Crude oil concentration decreased to 17.90 g/L, 28.80 g/L and 40.80 g/L for *B. cereus, P. alvei* strain 1 and *P. alvei* strain 2, respectively at 96 h before the onset of stationary phase. Different crude oil degradation capacity was observed in 75 g/L COBM for *B. cereus* (Figure 4c), *P. alvei* strain 1 (Figure 5c) and *P. alvei* strain 2 (Figure 6c) with residual crude oil as low as 18.70 g/L, 12.10 g/L and 13.70 g/L, respectively at 192 h.

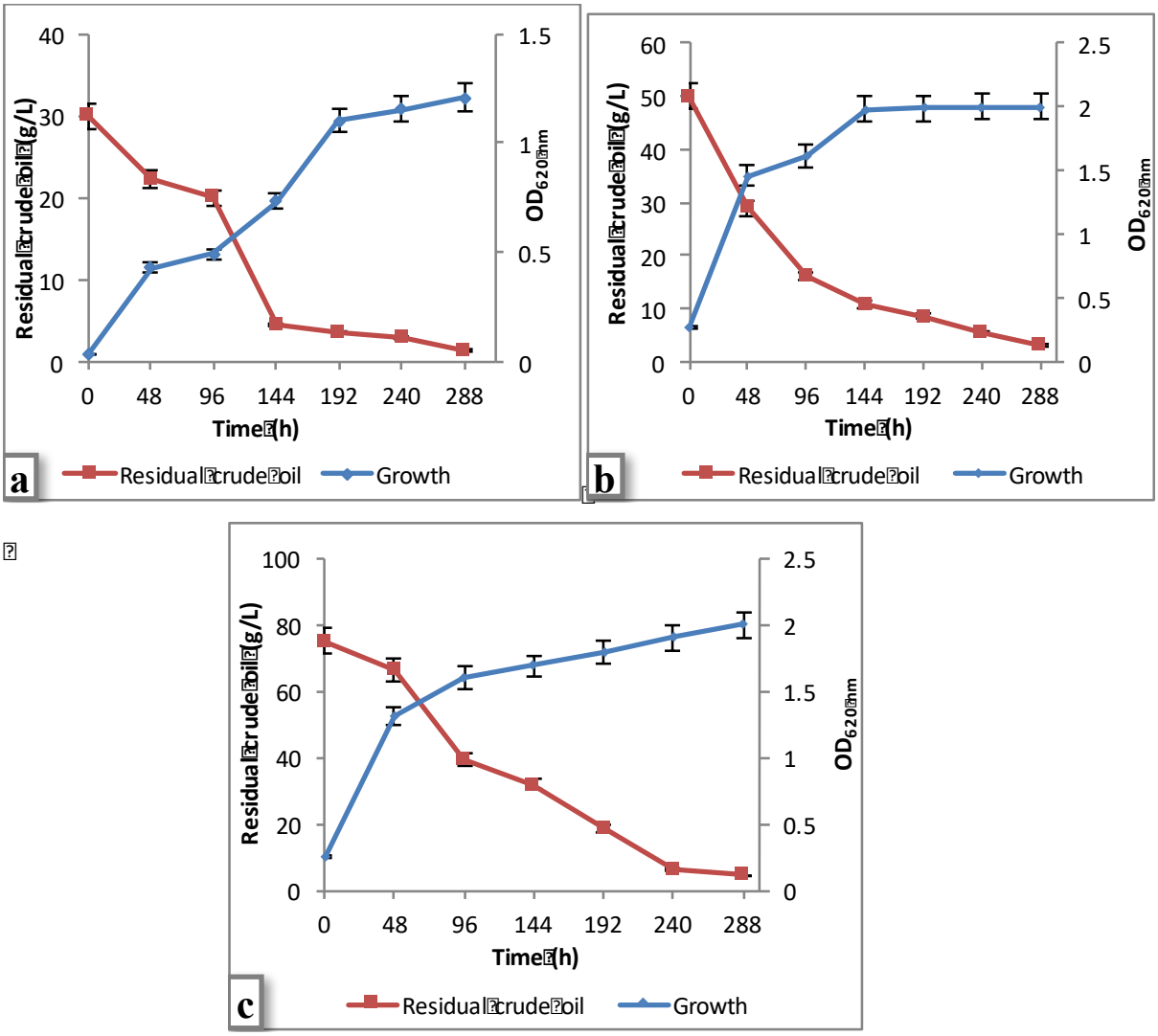

Figure 4. Growth profile and residual crude oil by *Bacillus cereus* in (a) 30 g/L (b) 50 g/L (c) 75 g/L crude oil based medium (Error bars represent Mean ± Standard Deviation)

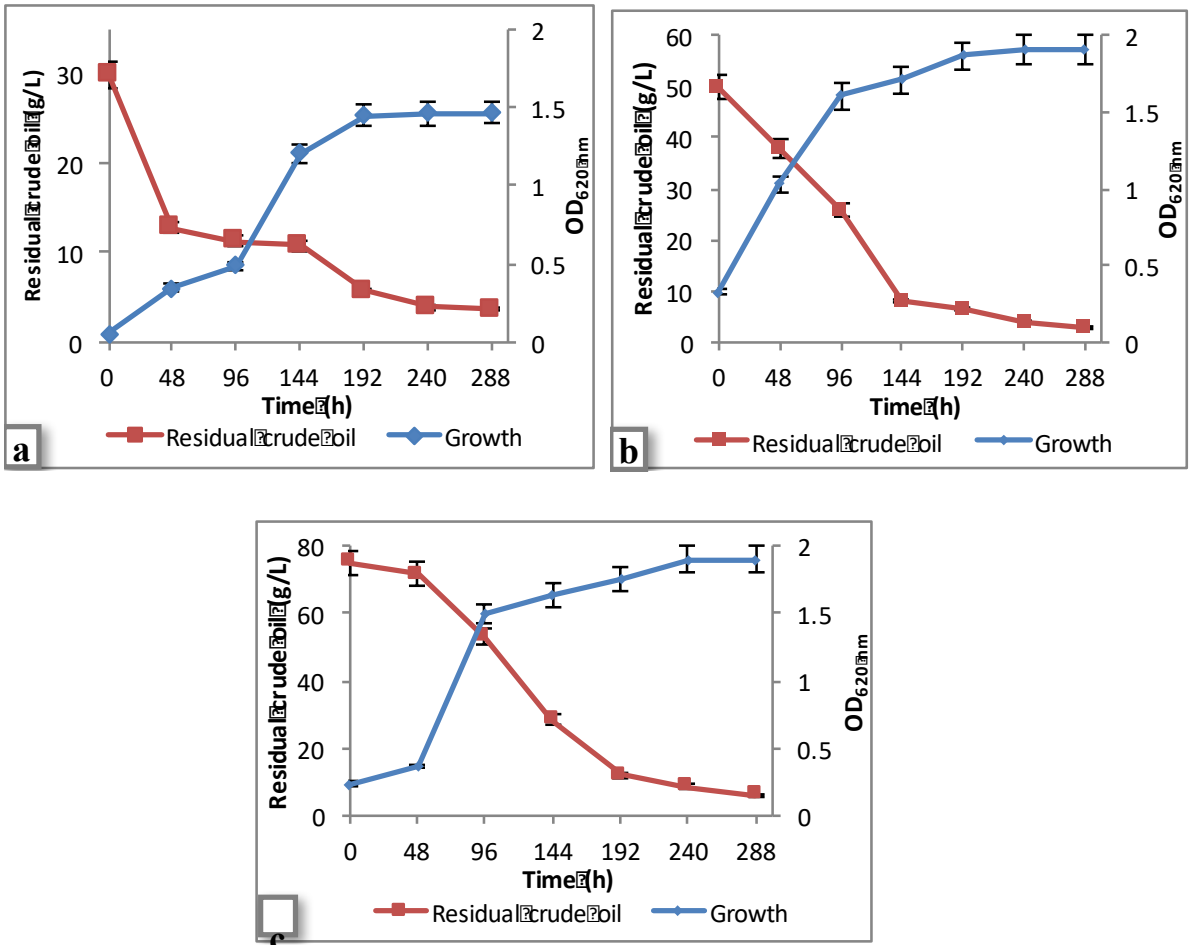

Figure 5. Growth profile and residual crude oil by *Paenibacillus alvei* strain 1 in (a) 30 g/L (b) 50 g/L (c) 75 g/L crude oil based medium (Error bars represent Mean ± Standard Deviation)

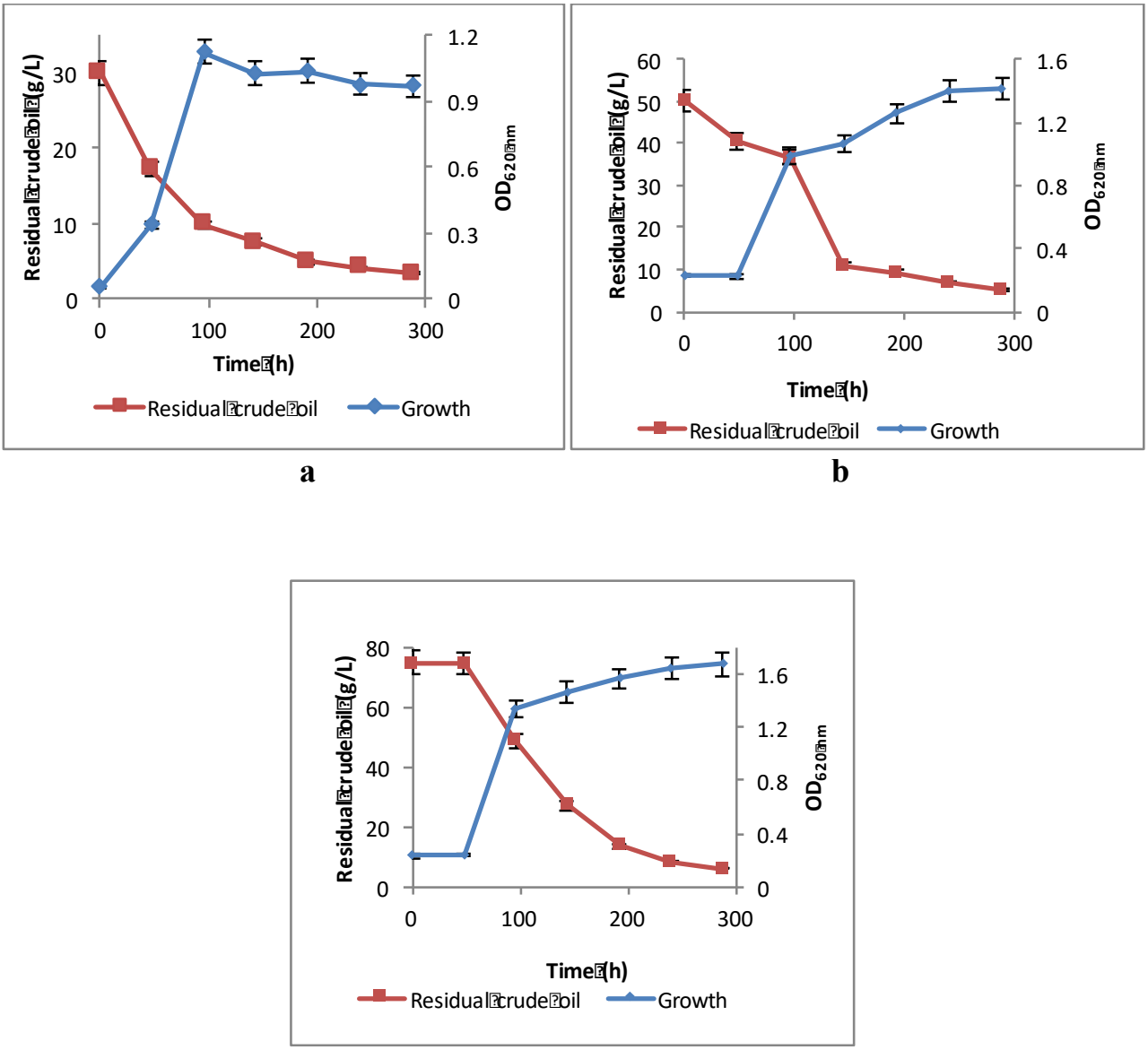

Figure 6. Growth profile and residual crude oil of *Paenibacillus alvei* strain 2 in (a) 30 g/L (b) 50 g/L (c) 75 g/L crude oil based medium (Error bars represent Mean ± Standard Deviation)

Degradation efficiency and peroxidase activity

Maximum peroxidase activity of 1.85 U/mg was obtained when *B. cereus* was cultivated in 30 g/L COBM at 144 h with 85.16% degradation efficiency. Peroxidase production decreased thereafter with increase in cultivation period. A marked increase in peroxidase production which resulted in increase in degradation efficiency was observed between 96 h and 144 h with minimal increase in degradation efficiency thereafter as peroxidase activity decreased. Degradation efficiency of 26% was observed at 48 h when there was no peroxidase activity (Figure 7a). In 50 g/L COBM, highest peroxidase activity of 1.79 U/mg was observed at 240 h with degradation efficiency of 87.52% (Figure 7b). Same trend was observed at 75 g/L crude oil concentration with peroxidase activity and degradation efficiency of 1.74 U/mg and 91.67%, respectively at 240 h (Figure 7c).

Production of peroxidase by *P. alvei* strain 1 was highest at 192 h (2.96 U/mg) with degradation efficiency of 80.5% in 30 g/L COBM (Figure 8a) while maximum peroxidase production in 50 and 75 g/L COBM was 1.39 U/mg and 1.18 U/mg with degradation efficiency of 90.9% and 88.3%, respectively at 240 h (Figures 8b and c). Minimal increase in degradation efficiency was observed as enzyme production decreased. Production of peroxidase by *P. alvei* strain 2 in 30 g/L COBM was 1.23 U/mg at 192 h with degradation efficiency of 83.5% (Figure 9a). Highest peroxidase production of 1.69 U/mg and 1.30 U/mg was observed at 240 h with degradation efficiency of 84.95% and 88.62%, respectively at 50 g/L and 75 g/L crude oil concentration (Figures 9b and c) after which peroxidase activity decreased.

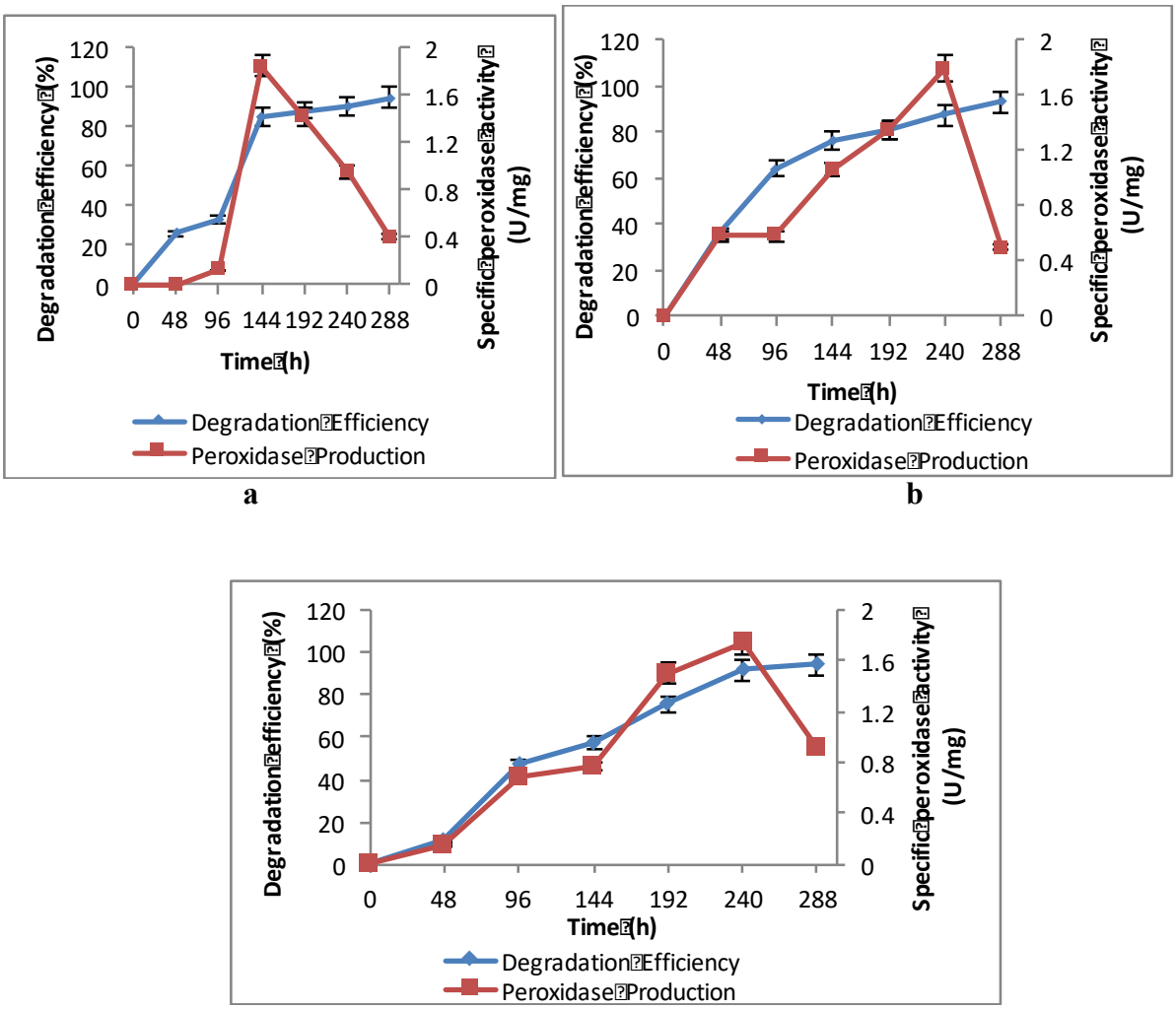

Figure 7. Degradation efficiency and peroxidase production by *Bacillus cereus* in (a) 30 g/L (b) 50 g/L (c) 75 g/L crude oil based medium (Error bars represent Mean ± Standard Deviation)

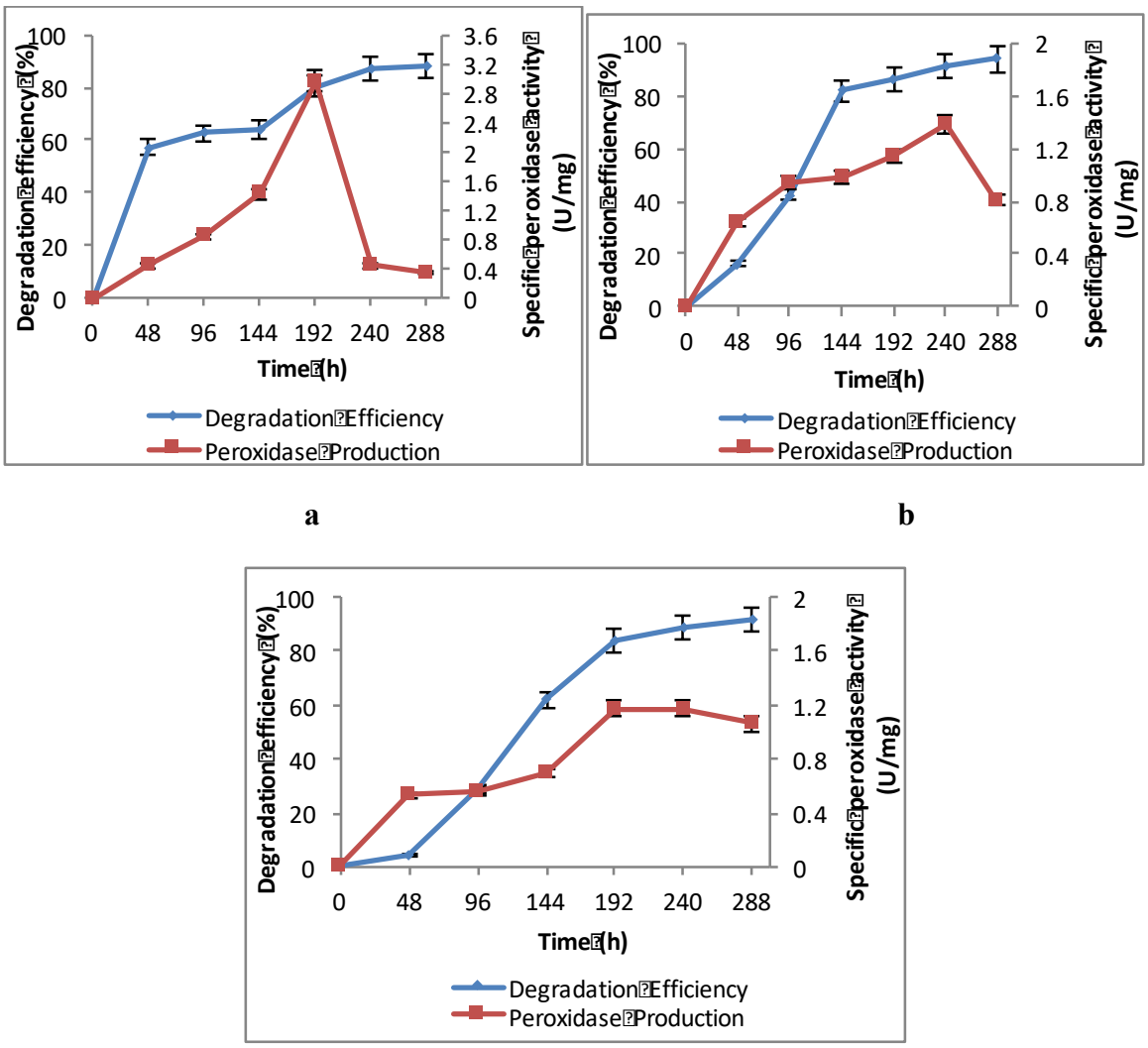

Figure 8. Degradation efficiency and peroxidase production by *Paenibacillus alvei* strain 1 in (a) 30 g/L (b) 50 g/L (c) 75 g/L crude oil based medium (Error bars represent Mean ± Standard Deviation)

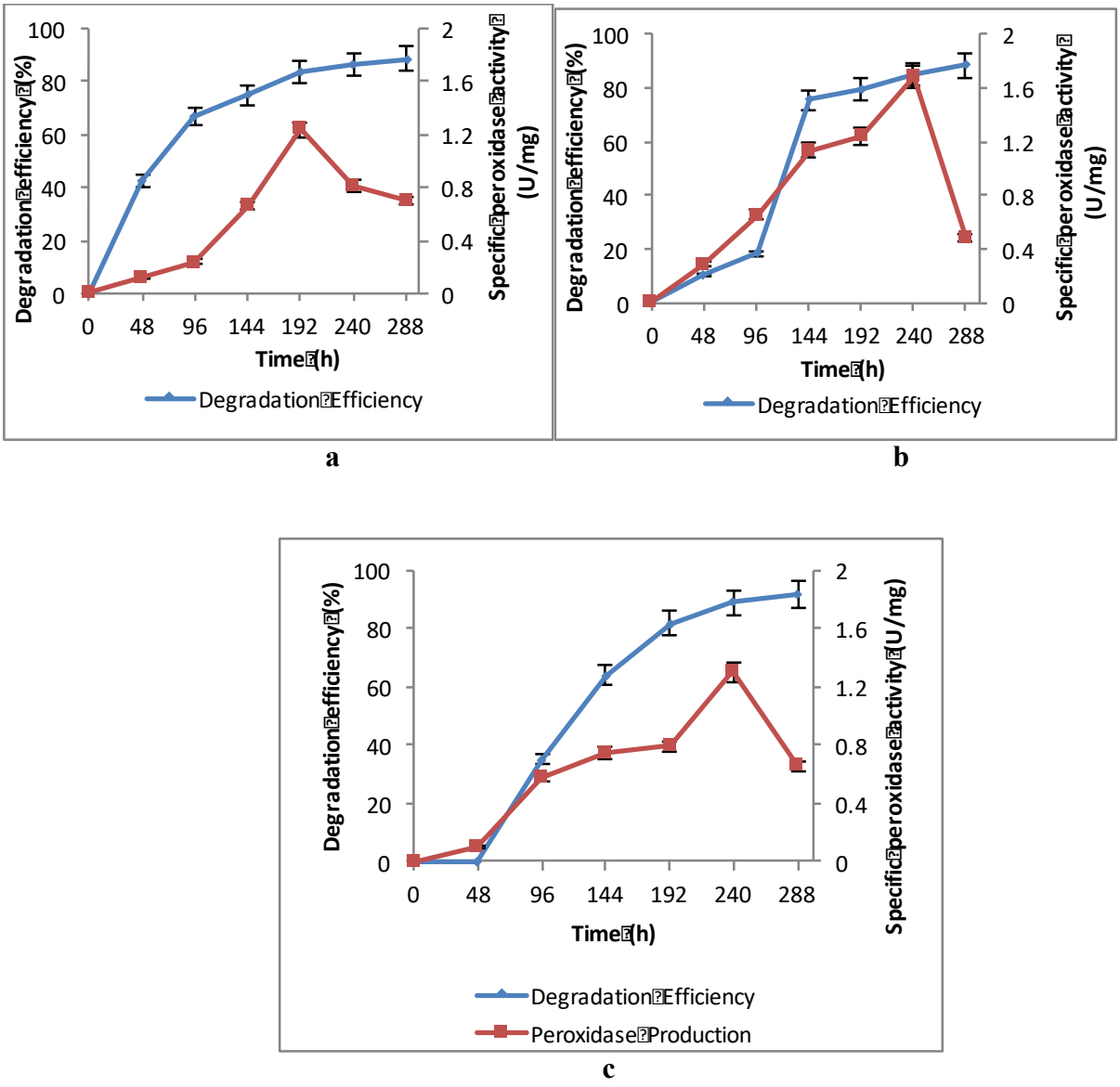

Figure 9. Degradation efficiency and peroxidase production by *Paenibacillus alvei* strain 2 in (a) 30 g/L (b) 50 g/L, (c) 75 g/L crude oil based medium (Error bars represent Mean ± Standard Deviation)

Discussion

Bacillus sp. dominated the crude oil spill site possibly due to the ability of the organisms to produce spores, which are resistant to toxic effects of hydrocarbons [37]. The ability of members of the genus to survive periods of unfavourable conditions by producing endospores which reproduce during favourable conditions has been reported [38].

The high growth profile of some isolates in COBM indicates that the isolates had enzymatic machinery to degrade and utilize hydrocarbons present in crude oil for growth. Previous and recent studies have demonstrated that bacterial growth kinetics on crude oil is a significant measure of the ability of a bacterium to utilize hydrocarbons as carbon source [11, 39 – 41]. However, the growth of *Corynebacterium* sp. 1, *Bacillus* sp. 4and *Bacillus* sp. 6 was lower in COBM than in the glucose based medium (control). This could probably be due to the inability of the bacteria to

secrete specific enzymes required for crude oil degradation [42]. On the other hand, reduced bacterial growth on crude oil could be due to the inability of the bacteria to tolerate the toxicity of the crude oil hydrocarbons and limiting oxygen supply [41, 43, 44]. All the bacterial isolates preferentially metabolized glucose within 48 h due to the simpler structure of glucose compared with the mixture of simple and complex hydrocarbons in the crude oil-based medium [45].

Degradation of crude oil by the selected bacteria (*Bacillus cereus, Paenibacillus alvei* strain 1, and *Paenibacillus alvei* strain 2) with increase in cell density in the presence of the varying concentrations suggests that the bacterial isolates effectively utilized crude oil hydrocarbons for growth. Similar results were reported by Christova *et al*. [39] on cryogel immobilized *B. cereus* cells which degraded 93% of aliphatic fraction in 10 g/L crude oil at the end of 48 h. Shibulal *et al*. [40] also reported *P. ehimensis* BS1 which transformed heavy crude oil to light aliphatic and aromatic compounds and achieved 67.1% biodegradation of 10 g/L crude oil at 216 h. There have been reports that *Paenibacillus* species are found in oil reservoirs [46]and are associated with hydrocarbon degradation including heavy weight polycyclic aromatic hydrocarbons [47, 48].

The highest rate of crude oil degradation was observed within the log phase of bacterial growth as revealed by the degradation efficiency of about 80% recorded at 192 h on 50 g/L COBM which resulted in remarkable decrease in crude oil concentration in the media. This was followed by a reduced rate of degradation at the stationary phase until the end of the degradation period. Similar trend in microbial growth and crude oil biodegradation was reported by Xu [49]. Growth at different crude oil concentrations indicates high tolerance of *B. cereus, P. alvei* strain 1 and *P. alvei* strain 2to crude oil and their ability to degrade crude oil hydrocarbons. The result clearly shows that degradation and microbial growth increased with the cultivation period [50]. Prolonged stationary phase was observed in the three bacteria at the end of the log phase which resulted in decline in crude oil removal rate. This might be due to accumulation of metabolic wastes or depletion of oxygen and available nutrients. The result from this study is remarkable with 87.52%,90.90%, and 84.95% degradation of 50 g/L crude oil achieved by *B. cereus, P. alvei* strain 1, and strain 2 at 240 h, respectively when compared with some previous studies [51 - 54]. Lower crude oil degradation efficiency of 66% and 76% by bacterial isolates was reported in the studies by Adebusoye *et al*. [51] and Vinothini *et al*. [52], respectively. Omotayo *et al*. [53] reported crude oil degradation efficiency of 93% by *Micrococcus varians* at the end of 30 d cultivation period while Okoh [54] reported that 4.90% and 29.6% crude oil were degraded in his study at the end of 15 d cultivation of *Stenotrophomonas, maltipholia,* and *Burkholderiacepacia,* respectively. Still in the search for improved biodegradation of crude oil, Deng *et al.* [43] in a very recent study reported that it took a consortium of bacteria to degrade 97.5% of 50 g/L crude oil at the end of 180 d.

Interestingly, crude oil biodegradation efficiency increased with cultivation period of bacteria and increased peroxidase activity. However, a decline of peroxidase activity was observed towards the end of the cultivation period suggesting that these bacteria possessed alternative mechanism for crude oil degradation which was evident at the stationary phase of growth. It further implied that degradation of hydrocarbon still continued in the presence of oxygen and water from the environment using some other enzymatic processes. It has been reported that accumulated hydrogen peroxide could be split by catalase, an intracellular enzyme found in all aerobic bacteria [55]. Catalase is another enzyme that is inductively expressed in the presence of crude oil to split

accumulated hydrogen peroxide into molecular oxygen and water thereby preventing cells from damage by reactive oxygen species [56].

The decrease in peroxidase activity during the stationary growth phase of bacteria could be due to increase in concentration of inhibitory metabolites to peroxidase synthesis caused by degradation of crude oil hydrocarbons [37]. Remarkably, *B. cereus, P. alvei* strain 1, and *P. alvei* strain 2recorded ≥ 80% degradation efficiency at the peak of peroxidase production suggesting that peroxidase played major role in biodegradation of crude oil. This is similar to the report by Shekoohiyan *et al*. [57] that peroxidase-mediated process is a promising method to achieve effective biodegradation of total petroleum hydrocarbons. Mohsenzadeh *et al*. [58] also reported increase inperoxidase activity with crude oil degradation by four fungal strains, *Acromonium* sp., *Alternaria* sp., *Aspergillus terreus* and *Penicillium* sp. Though Pi *et al.* [59] reported that they did not find correlation between oil biodegradation efficiency and peroxidase activity by bacterial strains in their study, hydrocarbon degradation efficiency of bacteria has been reported to be primarily dependent on the expression and activity of their hydrocarbon degrading enzymes [60, 61]. Biodegradation of crude oil over varying concentrations in this study is particularly striking since high levels of hydrocarbons has been reported to strongly inhibit bacterial growth which results in poor biodegradation efficiency [45].

Conclusion

Crude oil degradation efficiency exhibited by *B. cereus, P. alvei* strain 1, and *P. alvei* strain 2, demonstrates the tolerance of these bacteria to varying concentrations of crude oil with potential for application as environmental remediation agents for removal of crude oil hydrocarbons from oil spill site. The relationship between bacterial growth and peroxidase activity serves as an important measure of biodegradation efficiency. The increased production of peroxidase during the biodegradation process gives insight into the significant role played by the enzyme in crude oil biodegradation.

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Conflict of interest

All authors declare that they have no conflict of interests.

Ethical Approval

This article does not contain any studies with animals performed by any of the authors

References

Acevedo F, Pizzul L, Castillo M D, González M E, Cea M, Gianfreda L, & Diez M C (2010). Degradation of polycyclic aromatic hydrocarbons by free and nanoclay immobilized manganese peroxidase from *Anthracophyllum discolor*. *Chemosphere*.80: 271-278.

Adebusoye S A, Ilori M O, Amund O, Teniola O D, & Olatope S O (2007). Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World Journal of Microbiology and Biotechnology*. 23: 1149–1159.

Agrawal N & Shahi S K (2017). Degradation of polycyclic aromatic hydrocarbon (pyrene) using novel fungal strain *Coriolopsisbyrsina* strain APC5. *International Biodeterioration and Biodegradation*. 122: 69–81.

Al-Hawash A B, Dragh M A, Li S, Alhujaily A, Abbood H A, Zhang X, & Ma F (2018). Principles of microbial degradation of petroleum hydrocarbons in the environment. *Egyptian Journal of Aquatic Research*. 44: 71–76.

Al-Hawash A B, Zhang X, & Ma F (2019). Removal and biodegradation of different petroleum hydrocarbons using the filamentous fungus *Aspergillus* sp. RFC-1. *Microbiology Open*. 8: 619.

Al-Saleh E, & Obuekwe C (2014). Crude oil biodegradation activity in potable water. *International Biodeterioration& Biodegradation*. 93: 18–24.

Bradford M (1976). A rapid method of the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248-254.

Carpenter A (2019). Oil pollution in the North Sea: the impact of governance measures on oil pollution over several decades. *Hydrobiologia*. 845: 109–127.

Christova N, Kabaivanova L, Nacheva L, Petrov P, & Stoineva I (2019). Biodegradation of crude oil hydrocarbons by a newly isolated biosurfactant producing strain. *Biotechnology and Biotechnological Equipment*. 33: 863-872.

Coconi-Linares N, Ortiz-Vázquez E, Fernández F, Loske A M, & Gómez-Lim M A (2015). Recombinant expression of four oxidoreductases in *Phanerochaetechrysosporium* improves degradation of phenolic and non-phenolic substrates. *Journal of Biotechnology*. 209: 76–84.

De Vos P, Garrity G M, Jones D, Krieg N R, Ludwig W, Rainey F A, Schleifer K, & Whitman W B (Eds). (2009). The Firmicutes in *Bergey's manual of systematic bacteriology* (Springer, New York) Vol 3.

Deng Z, Jiang Y, Chen K, Gao F, & Liu X (2020). Petroleum depletion property and microbial community shift after bioremediation using *Bacillus halotolerans* T-04 and *Bacillus cereus* 1-1. *Frontiers in Microbiology.* 11:353.

Deora A, Giri R, Suneja S, Goyal S, & Kukreja K (2012). Isolation and characterization of pyrene degrading bacteria. *Pollution Research*. 31: 25–32.

Dombrowski N, Donaho J A, Gutierrez T, Seitz K W, Teske A P, & Baker B J (2016). Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill. *Nature Microbiology*. 1: 16057.

Dvorák P, Nikel P I, Damborský J, & de Lorenzo V (2017). Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnology Advances*. 35: 845–866.

Elum Z A, Mopipi K, & Henri-Ukoha A (2014). Oil exploitation and its socioeconomic effects on the Niger Delta region of Nigeria. *Environmental Science Pollution Research*. 23: 12880–12889.

Fabien B, Machado B, Cros C, Incardona J P, Scholz N L, & Block B A (2014). Crude oil impairs cardiac excitation-contraction coupling in fish. *Science*. 343: 772-776.

Heipieper H J, & Martínez P M (2010) in Handbook of Hydrocarbon and Lipid Microbiology, eds Timmis K N (Springer, Heidelberg), pp 1563 – 1573.

Hunter C L, Maurus R, Mauk M R, Lee H, Raven E L, Tong, H, Nguyen N, Smith M, Brayer G D, & Mauk A G (2003). Introduction and characterization of a functionally linked metal ion binding site at the exposed heme edge of myoglobin. *Proceedings of the National Academy of Sciences of the United States of America* 100: 3647–3652.

Ivshina I B, Kuyukina M S, Krivoruchko A V, Elkin A, Makarov S O, Cunningham C J, Peshkur T A, Atlas R M, & Philp J C (2015). Oil spill problems and sustainable response strategies through new technologies. *Environmental Science Processes and Impact*. 17: 1201-1219.

Kadri T, Rouissi T, Brar S K, Cledon M, Sarma S, & Verma M (2017). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: a review. *Journal of Environmental Science*.51: 52–74.

Kleindienst S, Paul J H, & Joye S B (2015). Using dispersants after oil spills impacts on the composition and activity of microbial communities. *Nature Reviews Microbiology*. 13: 388–396.

Li J, Zhang Y, Huang Q, Shi H, Yang Y, Gao S, Mao L, & Yang Xe (2017). Degradation of organic pollutants mediated by extracellular peroxidase in simulated sunlit humic waters: A case study with 17β-estradiol. *Journal of Hazardous Materials* 331: 123-131.

Li X, Li H, & Qu C (2019). A Review of the mechanism of microbial degradation of petroleum pollution. *IOP Conference Series: Material Science and Engineering*. 484: 012060.

Lindén O, & Pálsson J (2013). Oil Contamination in Ogoniland, Niger Delta. *Ambio*. 42: 685–701.

Ma Y L, Lu W, Wan L, & Luo N (2015). Elucidation of fluoranthene degradative characteristics in a newly isolated *Achromobacter xylosoxidans* DN002. *Applied Biochemistry and Biotechnology*. 175: 1294–1305.

McGenity T J, Folwell B D, McKew B A, & Sanni G O (2012). Marine crude-oil biodegradation: a central role for interspecies interactions. *Aquatic Biosystems*. 8: 10.

Meng L, Liu H, Bao M, & Sun P (2016). Microbial community structure shifts are associated with temperature, dispersants and nutrients in crude oil-contaminated seawaters. *Marine Pollution Bulletin*. 111(1–2): 203–212.

Mohamed M E, Aldousary M, Hamzah R Y, & Fuchs G (2006). Isolation and characterization of indigenous thermophilic bacteria active in natural attenuation of bio-hazardous petroleum pollutants. *International Biodeterioration and Biodegradation*. 58: 213-223.

Mohsenzadeh F, Rad A C, & Akbari M (2012). Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum-polluted soils. *Journal of Environmental Health Science and Engineering.* 9: 26.

Mukherjee A K, Bhagowati P, Biswa B, Chanda A, & Kalita B (2017). A comparative intracellular proteomic profiling of *Pseudomonas aeruginosa* strain ASP-53 grown on pyrene or glucose as sole source of carbon and identification of some key enzymes of pyrene biodegradation pathway. *Journal of Proteomics.* 167: 25–35.

Muthuswamy S, Arthur R B, Sang-Ho B, & Sei-Eok Y (2008). Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. *Clean*. 36: 92–96.

Najafi A R, Rahimpoura M R, & Jahanmiri A H (2011). Interactive optimization of biosurfactant production by *Paenibacillus alvei* ARN63 isolated from an Iranian oil well. *Colloids and Surfaces B: Biointerfaces*. 82: 33–39.

Odu C T I, Esuruoso O F, Nwoboshi L C, & Ogunwale J A (1985). Environmental study of the Nigeria Agip Oil Company, operational area soil and freshwater vegetation, (Union Graft Publications, Milan), pp 22 – 25.

Okoh A I (2003). Biodegradation of Bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. *African Journal of Biotechnology*. 2: 104-108.

Olajuyigbe F M, & Ehiosun K I (2016). Assessment of crude oil degradation efficiency of newly isolated actinobacteria reveals untapped bioremediation potentials. *Bioremediation Journal*. 20: 133–143.

Olajuyigbe F M, Ehiosun K I, & Jaiyesimi K F (2015). Preliminary study towards enhanced crude oil biodegradation reveals congeneric total peroxidases with striking distinctions. *Advances in Enzyme Research*.3: 66-74.

Omotayo A E, Ojo O Y, & Amund O (2012). Crude oil degradation by microorganisms in soil composts. *Research Journal of Microbiology*. 7: 209-218.

Osuagwu E S, & Olaifa E (2018). Effects of oil spills on fish production in the Niger Delta. *PLoS ONE*. 13: e0205114.

Overholt W A, Marks K P, Romero I C, Hollander D J, Snell T W, & Kostka J E (2016). Hydrocarbon-degrading bacteria exhibit a species-specific response to dispersed oil while moderating ecotoxicity. *Applied and Environmental Microbiology*. 82: 518-527.

Paju S, Bernstein J M, Haase E M, & Scannapieco F A (2003). Molecular analysis of bacterial flora associated with chronically inflamed maxillary sinuses. *Journal of Medical Microbiology*. 52: 591–597.

Palanisamy N, Ramya J, Kumar S, Vasanthi N S, Chandran P, & Khan S (2014). Diesel biodegradation capacities of indigenous bacterial species isolated from diesel contaminated soil. *Journal of Environmental Health Science and Engineering*. 12:142.

Pi Y, Bao M T, Li Y, Li G, Lu J, & Sun P (2015). Characterization of crude oil degrading microbial cultures isolated in Qingdao China. *RSC Advances*. 5: 97665-97674.

Pozdnyakova N, Makarova O, Chernyshova M, Turkovskaya O, & Jarosz-Wilkolazka A (2013). Versatile peroxidase of *Bjerkanderafumosa*: substrate and inhibitor specificity, *Enzyme and Microbial Technology*. 52: 44–53.

Raed S A, & Shimaa R H (2014). Bacterial biodegradation of crude oil using local isolates. *International Journal of Bacteriology*. 2014: 863272.

Rahman K S M, Rahman T J, Kourkoutas Y, Petsas I, Marchant R, & Banat I M (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. *Bioresource Technology*. 90: 159–168.

Sanger F, Nicklen S, & Coulson A R (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*. 74: 5463–5467.

Shekoohiyan S, Moussavi G, & Naddafi K (2016). The peroxidase-mediated biodegradation of petroleum hydrocarbons in a H_2O_2-induced SBR using in-situ production of peroxidase: Biodegradation experiments and bacterial identification. *Journal of Hazardous Materials.* 313:170-178.

Shibulal B, Al-Bahry S N, Al-Wahaibi Y M, Elshafie A E, Al-Bemani A S, & Joshi S J (2017). The potential of indigenous *Paenibacillusehimensis* BS1 for recovering heavy crude oil by biotransformation to light fractions. *PLoS ONE* 12: e0171432.

Shiyin L, Lixiao N, Panying P, Cheng S, & Liansheng W (2004). Effects of pesticides and their hydrolysates on catalase activity in soil. *Bulletin of Environmental Contamination and Toxicology.* 72: 600–606.

Song M, Yang Y, Jiang L, Hong Q, Zhang D, Shen Z, Yin H, & Luo C (2017). Characterization of the phenanthrene degradation-related genes and degrading ability of a newly isolated copper-tolerant bacterium. *Environmental Pollution*. 220: 1059–1067.

Ulrici W (2000) in *Biotechnology: Environmental Process II: Contaminated soil areas, different countries and contaminants, monitoring of contaminants*, eds Rehm H J Reed G (Wiley-VCH, Weinheim), pp 5–41.

Vinothini C, Sudhakar S, & Ravikumar R (2015). Biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus*. *International Journal of Current Microbiology and Applied Sciences*. 4: 318-329.

Wolińska A, Kuźniar A, Szafranek-Nakonieczna A, Jastrzębska N, Roguska E, & Stępniewska Z (2016). Biological activity of autochthonic bacterial community in oil-contaminated soil. *Water, Air & Soil Pollution.* 227**:** 130.

Xenia M E, & Refugio R V (2016). Microorganisms metabolisms during bioremediation of oil contaminated soils. *Journal of Bioremediation and Biodegradation.* 7: 2.

Xu J (2012) in Introduction to enhanced oil recovery (EOR) processes and bioremediation of oil-contaminated sites, eds Romero-Zerón L (InTechOpen, London), pp 207-244.

Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, Gao X, Li F, Li H, & Yu H (2018). Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. *Frontiers in Microbiology*.9: 2885.

Zhang S, Ning Y, Zhang X, Zhao Y, Yang X, Wu K, Yang S, La G, Sun X, & Li X (2015). Contrasting characteristics of anthracene and pyrene degradation by wood rot fungus *Pycnoporussanguineus* H1. *International Biodeterioration and Biodegradation*. 105: 228–232.

ORIGINAL RESEARCH ARTICLE

Green synthesis of TiO_2particles using lime juice/lime Peel extract: photo degradation of dye in water

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Abstract
In this work, titanium dioxide (TiO_2) particles were synthesized from bulk Titania using lime juice or lime peel as capping agents. The synthesized TiO_2 nanoparticles were characterized using powder X-Ray Diffraction (p-XRD), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and Ultra-Violet Spectroscopy (UV-VIS). The photocatalytic property of the titania nanoparticles was tested by applying these nanoparticles to degrade methylene blue dye solution. The p-XRD pattern revealed an anatase structure. The SEM results showed that the nanoparticles capped with the lime peel extract had better shape and size distribution than those obtained from lime juice. This is quite interesting as the peel which would have been ordinarily considered a waste was found to be useful. The degradation of methylene blue showed that the titania nanoparticles could be applied to degradation of dye contaminated water. This process is simple, non-toxic, inexpensive and can be used for the remediation of effluents from dye and textile industries before they are discharged into the ocean. This in turn will keep the ocean clean and preserve the aquatic life.

Keywords: *Titanium dioxide nanocubes, Green synthesis, Dye Degradation*

Introduction
Bulk titania is known in paint and pigment industries but with the advent of nanoscience, efforts have been intensified to prepare nano titania with interesting properties which has led to its numerous applications including in sunscreen cream, photocatalysis, photovoltaics, environmental remediation, antibacterial and so on (Chen and Mao 2007).

In recent years, biological methods for the synthesis of titanium dioxide (TiO_2) nanoparticles have been developed andadopted. The use of bacteria, yeasts, fungi and plant extract have been recently reported as well as eco-friendly chemical procedures involving low toxicity reagents and mild conditions of temperature and pressure (Chatterjee *et al*., 2016, Abdul Jalill *et al*., 2016).

This has allowed the development of nanoparticles displaying novel properties in their composition, size and biocompatibility (Nicolas *et al*., 2014). Phytonanotechnology has become an upcoming new area of research for biological synthesis of metal/oxide nanoparticles. Plant extracts offer more advantages with the presenceof functional groups in them which can be used as both reducing agent and capping agent. This is a better method as it allows for controlling the particle size growth thus providing stabilization during synthesis (Prasad, 2014). Various parts of

the plants can be used for synthesis of nanoparticles including leaves, stems, roots, bark and peel (Baker, *et al*., 2013).

A study by Sundrarajan *et al* (2011) reported the development of a facile and eco-friendly method for the synthesis of titanium dioxide nanoparticles from titanium isopropoxide solution using *nyctanthes* leaves extract. The shape and morphology were studied by Scanning Electron Microscopy (SEM) which showed the nanoparticles' sizes to be between 100 and 150 nm.

In 2017, Hariharan *et al* the studied the antibacterial effect of TiO_2 nanoparticles on *E. coli* strain. In their work a comparative study on the anticancer activity between commercially available TiO_2 and the green synthesised TiO_2 was carried out. It was found that the green synthesised TiO_2 had a good anticancer activity. When compared with the bulk, nanostructures have wide range of valuable properties including high surface area, stability, availability, and possibility for surface modification making them as a suitable carrier for attachment of various drugs (Jafari *et al*., 2020).

According to Dobrucka in 2017, the biosynthesis of titanium dioxide nanoparticles using an aqueous solution of *Echinacea purpurea* herba extract as a bioreductant is a novel and interesting method. The size of TiO_2 nanoparticles was found to be in the range of 120 nm. Moreover, the alkaline reaction of the solution (pH = 8) resulted in the increase in absorbance at 280 nm.

Another research group reported the synthesis of TiO_2 nanoparticles from leave extract of Aloe Vera plant using Titanium chloride as the precursor (Rao *et al*., 2015).

In a study by Strambeanu *et al* (2015), the biosynthesis of TiO_2 nanoparticles was done using an isolate of *Bacillus mycoides* with titanyl hydroxide and these produced colonies with novel morphologies at 37 °C. Biosynthesized nanoparticles had anatase polymorphic structure, spherical morphology, polydisperse size (40–60 nm). An advantage of biosynthesized titania over chemically produced ones is that they do not display phototoxicity. In order to design less expensive and greener solar cells, biosynthesized nanoparticles were evaluated in Quantum Dot Sensitized Solar Cells (QDSSCs) and then compared with chemically produced TiO_2 nanoparticles. Solar cell parameters such as short circuit current density (ISC) and open circuit voltage (VOC) revealed that biosynthesized TiO_2 nanoparticles have the same efficiency as the chemically produced TiO_2.

In these syntheses, the use of toxic titanium precursor (chloride, hydroxide or isopropoxide) which leads to the production of toxic titania nanoparticles is still a major concern. It therefore becomes necessary to find alternative less toxic titanium precursor.

One of the sources of water pollution is the effluents from the textile and dye industries. Most dye industry waste is composed of toxic and non- biodegradable components such as rhodamine, methyl orange and other polyaromatic dyes. When these dyes are released into the water bodies, they are harmful to plants, animals and humans (Konstantinou and Albanis 2004). In addition, they cause eutrophication, and some are carcinogenic. Although numerous methods have been adopted for the treatment of polluted water/industrial waste, there is still the need for cheaper and ecofriendly, efficient alternative. It is in view of this that titania nanoparticles with good photocatalytic property be employed.

In the present work, readily available and non-toxic bulk titania was to produce the precursor, while lime juice and lime peel extract were used as capping agents. The titanium dioxide

nanoparticles were characterised with powder- X-ray diffraction, Scanning electron microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS). The nanoparticles were then tested as a photocatalytic degrader by reacting it with methylene blue and monitored with the UV spectroscopy.

Experimental

Preparation of Lime Juice

Fresh limes were purchased from Oyingbo Market in Lagos state, Nigeria. The limes were cut, and the juice was squeezed out over a white cloth into a beaker. The sieved solution was then centrifuged at 3000 rpm for 10 mins. After which it was then filtered, using Whatman No 1 filter paper and then refrigerated until needed. The experiments were carried out in the Nanoscience laboratory, Chemistry Department, University of Lagos.

Extraction from Lime Peel

Lime peel was collected from left over of the fruit used and then cut into small pieces. 150 g of the peel was directly taken into the beaker and extracted with 450 mL of water and left for 2 hrs at 90 °C. The extract was filtered using Whatman No 1 filter paper. The filtrate was refrigerated until it was needed for the synthesis of titania nanoparticles.

Preparation of Titanium Dioxide Nanoparticles

The procedure was adapted from literature but with modifications (Abdul Jalill *et al*., 2016). Bulk titania was purchased locally from Turraco Industrial Chemical Company in Lagos. Up to 1.0 g of bulk titanium dioxide was dissolved in 10 mL of nitric acid and the mixture was heated in order to get a homogeneous solution of titanate salt which now serves as the precursor. 50 mL of the juice/peel extract was mixed with 5 mL of the precursor in a conical flask and the resulting solution was stirred at 50 °C and 1000 rpm for 7hrs on a hot plate. A colour change was observed from milk to tea colour. The mixture was allowed to cool to room temperature after which it was centrifuged at 3000 rpm for 10 min. This was repeated three times. The obtained precipitate (titanium dioxide nanoparticles) was dried at room temperature for 24 hrs.

Figure 1: (a) Lime peel extract only, (b) lime peel extract and bulk titanium dioxide solution, (c) lime peel extract and titanium dioxide solution after 7 hrs of stirring, (d) Titanium dioxide nanoparticles.

Photodegradation Study on Methylene Blue Using Titanium Dioxide Nanoparticles

The solutions were prepared following procedures in literature with some modifications. (Barkul et al., 2017). A total of 0.05 g of methylene blue was weighed into a 1000 mL (1.5×10^{-4} mol.dm^{-3}) volumetric flask and made to the mark with water and this was labelled as the stock. Then 0.01

g of titania nanoparticles was weighed and dissolved in 45 mL of water (1.0 $x10^{-4}$ $mol.dm^{-3}$). The solution was stirred for 10 mins.

5 mL of methylene blue solution was taken from the stock and added to the beaker containing 45 ml of the stirred TiO_2 solution to give a final dye concentration of 0.15 $x10^{-4}$ $mol.dm^{-3}$. The mixture was subjected to irradiation from the sun and the reaction was carried out at different times: 0 min, 30 min and finally at 60 min. The resulting solutions were run on the UV spectrophotometer by scanning from 400 to 800 nm.

Another batch of methylene blue and TiO_2 solution was prepared, kept in the dark and also monitored at 0 min, 30 min and 60 min. These were also run on the UV spectrophotometer.

Characterization of titanium dioxide (TiO_2) nanoparticles

TiO_2 nanoparticles were characterized by powder X-Ray Diffraction (XRD), Energy-dispersive X-Ray Spectroscopy (EDS), Scanning Electron Microscopy (SEM) and Ultra-Violet Spectroscopy (UV-VIS). The p-XRD, SEM and EDX instruments were located in the Geology Department, University of Ibadan. The p-XRD analyses were run on Rigaku D/Max-lllC X-ray diffractometer developed by the Rigaku Int. Corp. Tokyo, Japan and set to produce diffractions at scanning rate of 2 $^{\circ}$/min from 15 to 70° at room temperature with a CuKa radiation set at 40 kV and 20 mA. JEOL JSM-7600F scanning electron microscope +EDAX was used to determine the morphology and chemical composition of the samples. UV-Vis spectroscopy analysis was carried out by scanning solutions obtained from different samples in the range 400 – 700 nm on Thermo Scientific UV-Vis spectrophotometer located in the central research laboratory, University of Lagos.

Results and Discussion

Powder X-ray Diffraction (p-XRD)

The p-XRD analysis confirmed the formation of TiO_2 nanoparticles (Figure 2). Six distinct diffraction peaks were observed at 25.6°, 35.8°, 47.5°, 53.1°, 61.6°, 66.8° which were indexed to planes: 101, 004, 200, 105, 211, 204 and 116 respectively. The diffraction pattern was matched with the standard JCPDS card No.21-1272 which suggests an anatasephase crystalline structure. Similar results were obtained from TiO_2 nanoparticles synthesized by Sundrarajan *et al*., 2011. Our results showed no traces of impurities in the XRD pattern and this is comparable with results obtained from using less environmentally chemicals and methods (Natarajan et al., 2018).

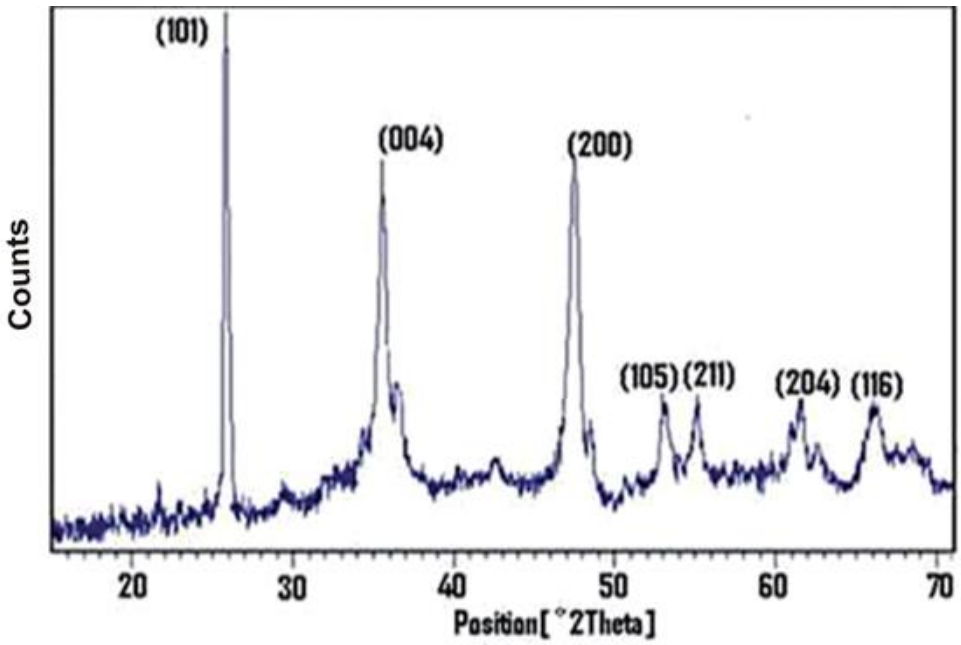


Figure 2: The p-XRD pattern for titanium dioxide nanoparticles

Energy Dispersive X-ray Spectroscopy (EDS)

The EDS spectra obtained for titanium dioxide particles synthesized from both lime juice and lime peel extract are shown in Figures 3 and 4. In both cases, Ti and O were confirmed thereby corroborating the results from p-XRD that TiO_2 were formed.

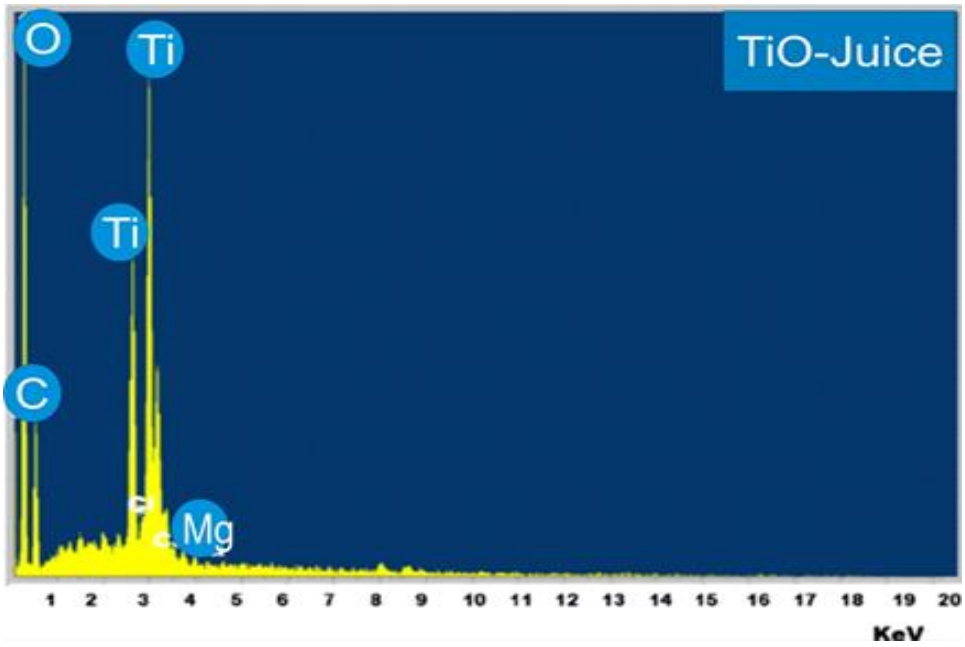


Figure 3 shows EDS spectrum of TiO_2 nanoparticles capped with lime juice

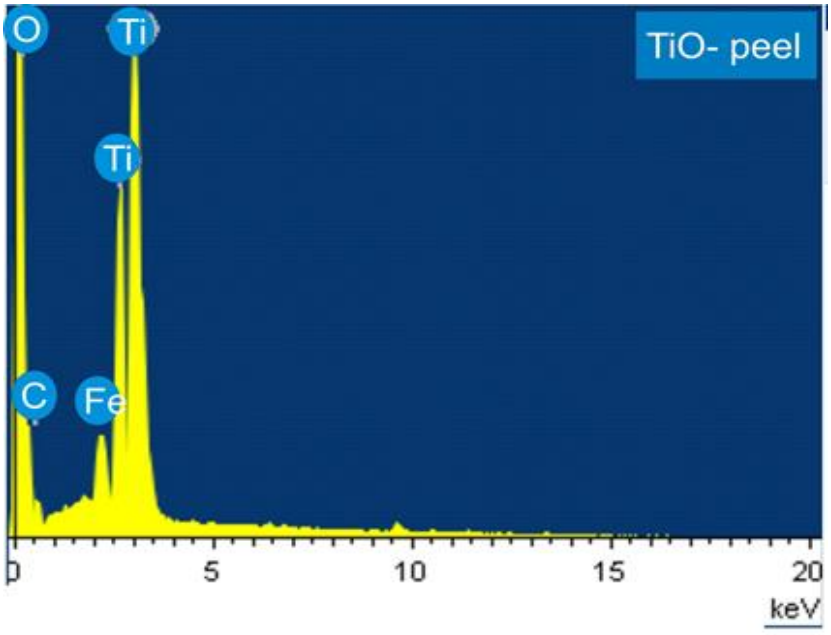


Figure 4 shows EDS spectrum of TiO_2 capped with lime peel extracts

Scanning Electron Microscopy (SEM)

Figures 5 and 6 show the SEM images obtained for titanium dioxide capped with lime juice and lime peel extract respectively. The images revealed that the titanium dioxide particles capped with lime peel extracts have uniform shape (cubes) and size, with an average size of about 10 µm (Figure 6) while nanoclusters were obtained with titanium oxide capped with lime juice and shows agglomeration (Figure 5). This shows that the TiO_2 cubes obtained from the lime peel extract are monodisperse implying that the peel possesses a better capping ability than the juice.

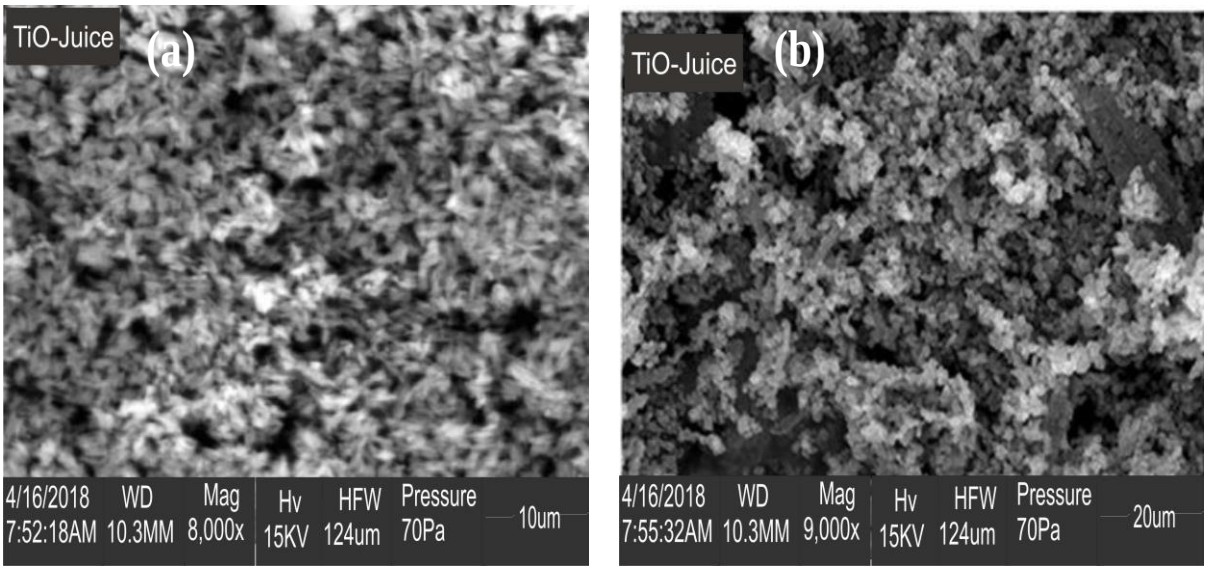


Figure 5 - SEM images of TiO_2nanoparticles capped with lime juice at different magnifications.

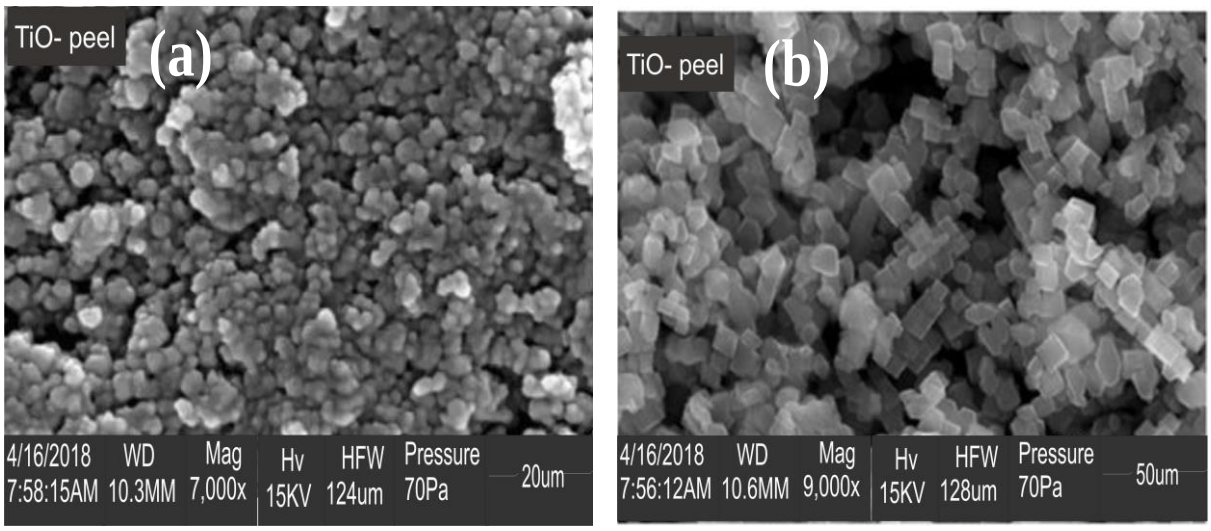


Figure 6 - SEM images of TiO_2nanoparticles capped with lime peel extract at different magnifications.

UV Analysis for The Degradation of Methylene Blue

On exposure to solar radiation, it was observed that the absorbance of the methylene blue (Figure 7) solution decreases considerably as reaction time increases.

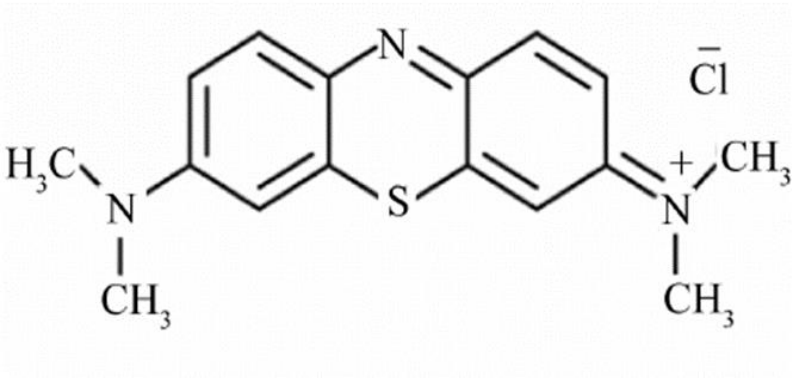
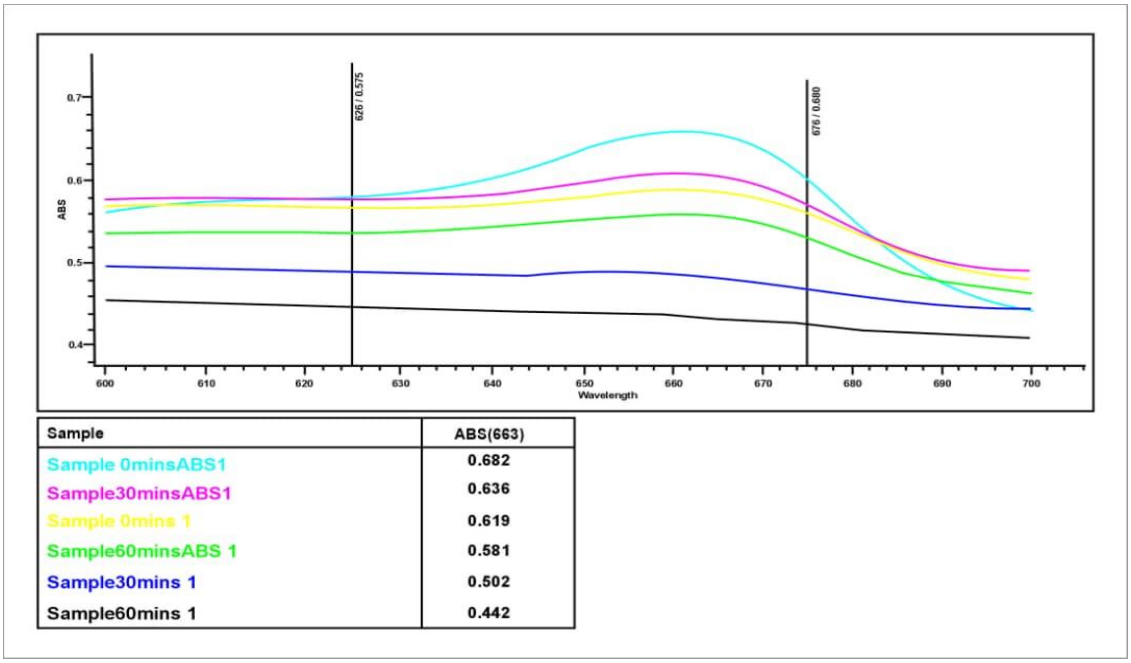


Figure 7 shows the structure of methylene blue

At wavelength of maximum absorbance 663 nm, absorbance at 0 min, 30 min and 60 min are 0.619, 0.502 and 0.442 respectively (Figure 8). On the other hand, in the absence of solar radiation (ABS), the dye solution showed only a little decrease in the absorbance of the dye solution. The absorbance values of 0.682, 0.636 and 0.581 at 0 min, 30 min and 60 min respectively. These results show that the degradation process is very rapid in the presence of light and very slow in the dark.



Sample	ABS(663)
Sample 0minsABS1	0.682
Sample30minsABS1	0.636
Sample 0mins 1	0.619
Sample60minsABS 1	0.581
Sample30mins 1	0.502
Sample60mins 1	0.442

Figure 8 showing the UV-VIS spectra of the degradation of methylene blue as a function of time. (ABS are absorbance values obtained in the dark).

The physical degradation of methylene dye solution by the nanoparticles over time was shown in Figure 9. It was observed that the colour faded rapidly over time which implies that the longer the time of exposure, the better the photocatalytic degradation of methylene blue. Compared with other works previously reported where smaller sized doped- titania nanoparticles were used in the degradation of dyes and textile effluents, our titania cubes which are larger in size and undoped were able to achieve the degradation of dye within a short period of time of comparable efficiency (Barkul *et al*., 2017, Natarajan *et al*., 2018, Raliya *et al*., 2017). This shows the feasibility of this simple eco-friendly process.

Previous studies have shown that dyes can photo-decompose under visible light irradiation in aqueous TiO_2 suspensions. The mechanism is that the dyes, rather than titania particles, are excited by the irradiating source to appropriate singlet or triplet states, this is then followed by electron injection into the conduction band (or a surface state) of the titania while the dyes are converted to cationic dye radicals. The injected electron can reduce surface chemisorbed oxidants, typically oxygen, to also yield the oxidizing species or radicals that can bring about degradation. Thus, titania played an important role in electron-transfer mediation but itself is not excited (Zhao *et al*., 1998).

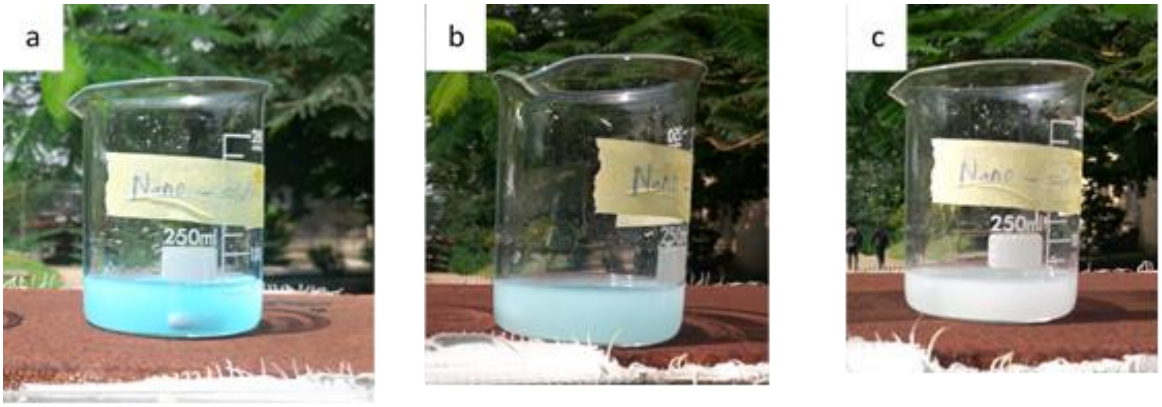


Figure 9 shows degradation of methylene blue dye solution as a function of time. (a) at 0 min (b) at 30 mins (c) at 60 mins.

Conclusion

Titanium dioxide (TiO_2) particles have been successfully synthesized by green synthetic pathway. Lime juice and lime peel extract were utilized as natural capping agents in the synthesis. The identification of the titanium dioxide nanoparticles was done by characterization techniques such as XRD, EDX and SEM. Lime peel extract proved to be a better capping agent as the particles obtained were monodisperse than those obtained from the lime juice as seen in the SEM images. The synthesized titanium dioxide particles using the peel extract when tested for its photocatalytic property proved they possessed excellent photocatalytic activity as the methylene blue solution was degraded rapidly on exposure to solar radiation. This indicates that the green synthesized titania particles could also be applied to degradation of dye contaminated water or effluents from textile industries.

References

Abdul Jalill R D H., Nuaman R S, & Abd A N (2016). Biological synthesis of Titanium Dioxide nanoparticles by *Curcuma longa* plant extract and study its biological properties. *World Scientific News.* 49: 204-22

Baker S, Rakshith D, Kavitha K S, Santosh P, Kavitha H U, Rao Y, & Satish S (2013). Plants: Emerging as nanofactories towards facile route in synthesis of nanoparticles. *BioImpacts*. 3: 111-117

Barkul R P, Patil M.K, Patil S M, Shevale V B, & Delekar S D (2017) Sunlight-assisted photocatalytic degradation of textile effluent and Rhodamine B by using iodine doped TiO2 nanoparticles. *Journal of Photochemistry and Photobiology A: Chemistry*. 349: 138-147.

Chatterjee A, Nishanthini D, Sandhiya N, & Jayanthi A (2016). Biosynthesis of Titanium Dioxide Nanoparticles Using *Vigna Radiata. Asian journal of pharmaceutical and clinical research.*9: 85-88.

Chen X, & Mao S (2007). Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications, and Applications. *Chemical Review.* 107: 2891-2959.

Dobrucka R (2017). Synthesis of Titanium Dioxide Nanoparticles Using *Echinacea purpurea* Herba *Iranian Journal of Pharmaceutical Research.*16*:*756-762.

Hariharan D, Srinivasan K, & Nehru L C (2017). Synthesis of TiO_2 nanoparticles using *Cynodon Dactylon* leaf extract for Antibacterial and Anticancer (A549 Cell Lines) Activity. *Journal of Nanomedicine Research*. 5: 1-5.

Jafari S, Mahyad B, Hashemzadeh H, Janfaza S, Gholikhani T, & Tayebi L (2020). Biomedical Applications of TiO_2 Nanostructures: Recent Advances. *International Journal of Nanomedicine*. 15: 3447-3470.

Konstantinou I K & Albanis T A (2004). TiO_2-assisted photocatalytic degradation of azo dyes in aqueous solution: kinetic and mechanistic investigations: a review. *Applied Catalysis B: Environmental*. 49: 1–14.

Natarajan K, Bajaj H C, & Tayade R J (2018) Effective removal of organic pollutants using GeO_2/TiO_2 nanoparticle composites under direct sunlight. *Materials Chemistry Frontiers*, 2: 741-751.

Nicolás A O, Luis A S, Vicente MD, Denisse M B, & José MP (2014). Use of titanium dioxide nanoparticles biosynthesized by *Bacillus mycoides* in quantum dot sensitized solar cells. *Microbial Cell Factories.* 13: 1-10.

Prasad R (2014). Synthesis of Silver Nanoparticles in Photosynthetic Plants. *Journal of Nanoparticles.* 2014: 1-9.

Raliya R, Avery C, Chakrabarti S, & Biswas P (2017). Photocatalytic degradation of methyl orange dye by pristine titanium dioxide, zinc oxide, and graphene oxide nanostructures and their composites under visible light irradiation. *Applied Nanoscience.* 7: 253-259.

Rao K G, Ashok C H, Rao K V, Chakra C H S, & Rajendar V (2015). Synthesis of TiO_2 Nanoparticles from Orange Fruit Waste. *Synthesis.* 2: 82-90.

Strambeanu N, Demetrovici L, & Dragos D (2015) in *Natural Sources of Nanoparticles*. (Springer International Publishing Switzerland), pp9-19.

Sundrarajan M, & Gowri S (2011) Green Synthesis of Titanium Dioxide Nanoparticles by *Nyctanthes Arbortristis* Leaves Extract. *Chalcogenide Letters*. 8:447-451.

Zhao J, Wu T, Wu K, Oikawa K, Hidaka H, & Serpone, N (1998). Photoassisted Degradation of Dye Pollutants. 3. Degradation of the Cationic Dye Rhodamine B in Aqueous Anionic Surfactant/TiO2 Dispersions under Visible Light Irradiation: Evidence for the Need of Substrate Adsorption on TiO_2 Particles. *Environmental Science Technology*. 32:2394-2400.

REVIEW ARTICLE

Biomonitoring of aquatic pollution: status and trends from genomics to populations

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Abstract

Biomonitoring offers an appealing tool for the assessment of pollution in aquatic ecosystem. Biological processes, species, or communities of bioindicators are used to assess the quality of the environment and how it changes over time. Bioindicators include algae, macrophytes, zooplanktons, insects, bivalves, molluscs, gastropods, fish, amphibians, and others. Changes in aquatic ecosystems are often attributed to anthropogenic disturbances, including pollution. Major contributors to aquatic pollution include wastewater, metals and metalloids, industrial effluents, contaminated sediments, nutrients, polycyclic aromatic hydrocarbons, flame retardants, persistent organic pollutants, pharmaceuticals and illicit drugs, emerging contaminants (such as microplastics and engineered nanoparticles), pesticides, herbicides, and endocrine disruptors. In this review, we discuss categories of aquatic pollutants, status and trends of aquatic biomonitoring and approaches, from genomics to populations. We conclude by offering recommendations for research and regulatory testing.

Keywords: *Bioindicators, Pollution, Biomonitoring, Ecotoxicological tools*

Introduction

According to Adeyemo (2003), Nigeria's vast freshwater resources are among those most affected by environmental stress imposed by human population growth, urbanization, and industrialization. Fish and marine resources in the country face total collapse or extinction, due to over-fishing and destruction of marine life and natural habitats by pollution of water bodies. Unregulated and excessive use of pesticides for agriculture and the deliberate disposal and dumping of toxic and hazardous wastes into water bodies are significant causes of massive fish kills and loss of aquatic life and habitats in the country. Biomonitoring has been defined as the act of observing and assessing the state and ongoing changes in ecosystems, components of biodiversity and landscape, including the types of natural habitats, populations, and species. These has been said to lie at the core of environmental conservation, management, and restoration, albeit primarily in the developed world. In human studies, "exposomics" is an emerging area that falls between environmental monitoring and public health surveillance (Smith et al, 2015). The "exposome" is

defined as the summation of environmental exposures for individuals, beginning *in utero*. Samples of human biospecimens such as blood, saliva, or urine are analyzed for chemical contaminants using advanced methodologies and looking for contaminants such as lead, phthalates, dioxins, or mercury, among others.

In aquatic ecosystems, biomonitoring studies are used to measure exposure, response, and recovery of aquatic communities to disturbances and provide an understanding of the relationship between physical, chemical, and biological components (Gurtz, 1994). Biomonitoring studies are important in assessing aquatic ecosystem health because organisms' function as sensors of the quality of their environment more than can be achieved with water quality measurements. Aquatic biomonitoring has become an essential task in most of the developed world because of strong anthropogenic pressures affecting the health of lakes, rivers, oceans, and groundwater. A typical assessment of the environmental quality status, such as is required by Europe, North America and other legislation, relies on matching the composition of assemblages of organisms identified using morphological criteria present in aquatic ecosystems to those expected in the absence of anthropogenic pressures. In developing countries like Nigeria, this assessment method is being utilized as well to evaluate the health status of aquatic ecosystems (Olaniran et al., 2019; Nwabueze et al., 2020). In this review/perspective paper, we provide an overview of aquatic biomonitoring, categories of aquatic pollutants, aquatic biomonitoring status and trends, aquatic biomonitoring approaches from genomics to populations and end with recommendations for research and regulatory testing.

Aquatic biomonitoring: would usually involve fish, macroinvertebrate, and algae. Rosenberg and Resh (1996) provided a comprehensive review of current biomonitoring approaches. These approaches span a range of scales:

1. Biochemical and physiological measurements, including metabolic studies and measures of enzyme activities.

2. Individual attributes such as morphological, behavioural, and life-history parameters, or the use of "sentinel organisms" that bioaccumulates toxic materials.

3. Population and assemblage level responses, such as using occurrence or abundance of indicator species as a measure of sensitivity to a pollutant.

4. Community-level approaches which synthesize many types of data into summary responses to a pollutant.

5. Ecosystem-level scales that assess effects of stressors on processes and function.

Categories of Aquatic Pollutants

Persistent Organic Pollutants: Persistent organic pollutants (POPs) are chemicals of global concern due to their potential for long-range transport, persistence in the environment, ability to bio-magnify and bio-accumulate in ecosystems, as well as their significant negative effects on human health and the environment. POPs have been the object of biomonitoring studies for more than 60 years. The most commonly encountered POPs are organochlorine pesticides, including, dichlorodiphenyltrichloroethane (DDT) and its metabolites and degradates; hexachlorocyclohexanes, including lindane; dieldrin; mirex; trans-nonachlor; polychlorinated biphenyls (PCBs); and several other POPs now listed in the Stockholm Convention. The same physical and chemical characteristics that lead to persistence in the environment lead to persistence

in biological systems, with limited metabolism, slow elimination, and resulting bioaccumulation. Many of these pollutants have multiple effects on health. Some are potent endocrine disruptors, affecting development, reproduction and behaviour. Others are potent immune disruptors, lowering the natural defenses against disease. For example, p, p-DDE is both an anti-androgen and a weak estrogen and also affects immune parameters in fish exposed to this pesticide (Martyniuk et al, 2016).

Heavy metals: The term 'heavy metal' is used synonymously with 'trace metal' and includes both essential and non-essential trace metals. All of these have the potential to be toxic to living organisms if present or bioavailable above a threshold. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). Three measures of heavy metals level in aquatic habitats are usually carried out in pollution studies--namely concentrations in water, sediments, and biota. For example, A study (Adeyemo, 2007a) on lead levels in rivers, sediments and fishponds in the Ibadan metropolitan area, South-West Nigeria revealed that lead levels in surface waters ranged between 0.5–2.35 mg l^{-1} (mean: 0.76 mg l^{-1}) and 1.15–2.20 mg l^{-1} (mean: 1.34 mg l^{-1}) during the dry and rainy seasons, respectively. Lead levels in river sediments ranged between 0.9–4 mg kg^{-1} (mean 1.86 mg kg^{-1}) and 1.15–2.2 mg kg^{-1} (mean 1.49 mg kg^{-1}) during the dry and rainy seasons, respectively. Lead levels in fishponds were even higher, at 1.09–2.9 mg l^{-1} (mean 1.88 mg l^{-1}). The need for a detailed assessment of sources of lead in Nigerian aquatic ecosystems and further research into the distribution of lead in different biota (such as aquatic plants, invertebrates, and fish) in relation to the environment was recommended. Heavy metals are accumulated by many aquatic organisms to remarkably high tissue and hence body concentrations. These accumulated concentrations are easily measured, not liable to contamination, and provide a time-integrated measure of metal supply over weeks, months, or even years, according to the species analyzed. Many of these heavy metals cause kidney dysfunction and an assortment of other health issues such as gastrointestinal disorders, ataxia, paralysis, and death. Some heavy metals are carcinogens. Chronic exposure to these chemicals may be costly for the people involved (Omenka and Adeyi, 2016).

Pharmaceuticals, and Personal Care Products (PPCPs): The extensive use and misuse of antimicrobials for treatment and prophylaxis in livestock production generally and aquaculture is of great concern to environmental and public health. Other categories of PPCPs pollutants aside from antibiotics, including hormones, antimicrobial agents, synthetic musks, among others, have raised significant concerns about their persistent input and potential threat to the ecosystem and human health. As an important group of organic pollutants with intensive studies in recent years, PPCPs have been found to be ubiquitous in the aquatic environment throughout the world. They constitute a large and diverse group of organic compounds such as soaps, lotions, toothpaste, fragrances, and sunscreens, which are widely used in high quantities throughout the world, together with their metabolites and transformation products (Kummerer, 2010). In Nigeria, regulation and monitoring of aquaculture and other livestock production activities at best is lax. For example, in a study by Saka et al, (2017), all the isolates (100%) from fish muscle of live-fish obtained from a market in Nigeria had multiple antibiotic resistance (MAR) indices >0.2 to a wide range of antibiotics (ampicillin, cefixime, augmentin, ciprofloxacin, cefuroxime, nitrofurantoin, ofloxacin, ceftazidime and gentamycin), which portends potential source of resistant bacteria for humans. In a similar study carried out on Lafenwa effluents and its receiving water in Abeokuta, Ogun state, 31% Enterobacteriaceae and 66% Pseudomonas isolates were resistant to five antibiotics (Ceftazidime, Cefpodoxime, Cefotaxime, Ertapenem and Amoxicillin-clavulanate) and 77% of the isolates had MAR indices >0.2 to the same set of antibiotics (Akpan et al., 2020).

Endocrine-Disrupting Chemicals (EDCs): The occurrence of endocrine-disrupting chemicals (EDCs), including natural and synthetic estrogens, is well documented in the aquatic environment (Folmar et al, 1996, Yoon et al. 2010; Vidal-Dorsch et al. 2012, González et al, 2020). The understanding of EDCs and the effects that they have on aquatic biota has improved over the past two decades with increased precision of analytical methods and innovative assays designed to detect and quantify estrogenic responses. Baldigo et al, (2015) determined the levels of plasma vitellogenin (Vtg) and Vtg messenger ribonucleic acid (mRNA) in male fathead minnows (*Pimephales promelas*) exposed to wastewater effluents and dilutions of 17α-ethinylestradiol (EE2), estrogen activity, and fish assemblages in 10 receiving streams and reported that EE2, plasma Vtg concentration, and Vtg gene expression in fathead minnows, and 17β-estradiol equivalents (E2 Eq values) were highly related to each other ($R^2 = 0.98–1.00$). In Nigeria, several chemicals used in agriculture as pesticides and herbicides rank among these pollutants and have been proven to have deleterious effects in animals that are exposed to them in varying quantities and also pollution of air, soil, ground and surface water. For example, in the Ogun River, Nigeria, 15 PCB congeners, lindane, dieldrin, 4-isononylphenol and 4-tert-octylphenol analysed in fish muscle and sediments samples have been associated with 24% prevalence of intersex. This correlated with gonadal histopathological changes, significantly higher plasma estradiol-17β, luteinizing hormone, follicle stimulating hormones as well as hepatic transcript levels of Vtg, zona radiata and aromatase in male fish (Ibor et al., 2016).

Our study on carbendazim revealed significant decreases in testosterone and 11-ketotestosterone levels in exposed African Sharptooth Catfish (*Clarias gariepinus)*. Testicular histological and ultra-structural studies also revealed germinal and Sertoli cell degeneration and necrosis, displacement of Sertoli cysts, capillary endothelial wall necrosis and basement membrane disruption (Aina et al, 2019). In similar studies, catfish exposed to formalin had generalized massive vacuolations of the skin, multifocal necrosis of hepatocytes, massive lymphoid depleted spleen and seminiferous tubules (Adeyemo et al., 2011a). Malachite green exposed catfish (Adeyemo et al., 2011b) had disrupted and depleted seminiferous tubules, focal localized vacuolation of skin and generalized fatty degeneration of liver while copper sulphate exposure (Alarape et al., 2013) resulted in necrotic ovaries, matted lamellae of the gills and multifocal severe degeneration of the seminiferous tubules. All three (formalin, malachite green and copper sulphate) had significantly detrimental effects on egg, milt quality and hatchability.

Oil and Oil-Dispersing Agents: Oil pollution from exploration and production processes, natural seeps, atmospheric contribution, freight accidents, industrial discharge, and urban run-off is a significant hazard for the marine environment (Wilson and LeBlanc, 2000). Oil dispersants (chemical agents such as surfactants, solvents, and other compounds) are used to reduce the effect of oil spills by changing the chemical and physical properties of the oil. However, polycyclic aromatic hydrocarbons (PAHs) and other components of oil and the dispersant used in the clean-up process may persist in the marine environment for a long time thereby creating pathways for lingering biological exposure and associated adverse effects (Sogbanmu and Otitoloju, 2014). Our study (Adeyemo et al, 2015) on the effects of the exposure of inland silversides (*Menidia beryllina)* embryos at 30–48 h post-fertilization to water accommodated fractions of oil (WAF, 200 ppm, v/v), dispersants (20 ppm, v/v, Corexit 9500 or 9527), and mixtures of oil and each of the dispersants to produce chemically enhanced water accommodated fractions (CEWAFs) over a 72-h period revealed that that significantly more treated embryos were in a state of deterioration, with significantly more embryos presenting arrested tissue differentiation compared with controls (Figure 1), amongst other effects.

Figure 1: Some abnormalities observed in hatchlings of Menidia embryos exposed to dispersants and CEWAFs were (a) head malformation, (b) pericardial edema, and (c) Spinal deformities (Adeyemo et al, 2015).

Aquatic biomonitoring: Status and Trends

Environmental pollution due to anthropogenic activities is a major public health concern in Nigeria. Some studies have been conducted to determine the status of aquatic pollution in Nigeria. For example, seasonal variations in ecological parameters have been found to exert profound effect on the distribution and population density of both animal and plant species. Adeyemo (2008 a,b,c,d) conducted a field study to determine spatiotemporal pollution status of rivers in Ibadan, Nigeria. The metrics used were physicochemical parameters and nutrient load of rivers and their sediment. Habitat assessment was also conducted by evaluating the structure of the surrounding physical habitat that influences the quality of the water resource and the condition of the resident aquatic community. The results revealed that colour, total suspended solid, total solids and total nitrogen were generally higher during the dry season, which suggests that the run-offs have only a diluting effect on these parameters. The overall sensitivity of the watershed to physicochemical environmental pollution revealed that during dry season, of the 22 sample points, 3 (13.6%) were unpolluted; 6 (27.3%) were slightly polluted; 10 (45.4%) were moderately polluted; 2 (9.1%) were seriously polluted and 1 (4.5%) was exceptionally polluted.

In a similar field study on water quality parameters of one of the major rivers traversing an industrial estate in Ibadan to determine its suitability for aquatic life. It was revealed that the physico-chemical properties of the river were not within the World Health Organization standard and thus rendered unsuitable for aquatic life (Kupoluyi et al., 2018). Furthermore, the pollution status of other aquatic ecosystems in Nigeria such as the coastal waters of the Niger Delta, the Lagos lagoon, Lekki lagoon, among others have demonstrated the impact of anthropogenic pressures due to infrastructure development, exploitation of aquatic resources, shipping and other forms of water transportation, oil prospecting and extraction, sand mining and so on (Nwaichi and

Ntorgbo, 2016; Sogbanmu et al., 2016). The trend in aquatic biomonitoring in Nigeria is being driven towards hazard evaluation, integrated approaches and development of ecological risk indices targeting various priority and emerging pollutants and ecosystems (Benson et al., 2018; Edegbene et al., 2019; Sogbanmu et al., 2019).

Aquatic Biomonitoring approaches: from Genomics to Population

Genomic Markers: Since 1999, when the term "*toxicogenomics*" was coined to describe the application of genomics to toxicology, different genomic approaches have been available through a combination of advanced biological, instrumental, and bioinformatic techniques which can yield a previously unparalleled amount of data concerning the molecular and biochemical status of organisms. Ankley et al (2006) stated that "*among other applications, genomic information can be used to design microarrays or "gene chips" for some or all of the genes in an organism. These chips can be used to determine which genes are up- or down-regulated (as transcribed messenger RNA [mRNA]) in a cell, tissue, organ, or organism under specific physiological conditions or in response to an environmental perturbation, such as exposure to a toxic chemical".*

All responses to external stressors, including toxicants, have been linked to changes in normal patterns of gene expression (Ellwood and Foster, 2004, Grifiitt et al, 2012, Thomas and Meyer, 2012,). Different mechanisms of toxicity can generate specific patterns of gene expression reflective of mechanism or mode of action (MOA). The application of transcriptomics to toxicity research has become increasingly important because of the availability of many genes that can be queried with microarrays or RNAseq, which runs fifteen thousand to whole transcriptomes for some animal models (Hoffmann and Sgro, 2011, Marx, 2013, Qian et al, 2014).Proteomics also provides additional critical insights into biological pathways since not all mRNA sequences are transcribed, and many proteins are modified, for example, by phosphorylation, posttranslational cleavage) before becoming physiologically active (Dole-Olivier et al, 2009, Martyniuk et al, 2009, Unterseher et al, 2011). Consequently, alterations in protein profiles can be used, in conjunction with transcriptomics, to understand responses of an organism to toxicants (Wooley et al, 2010, Carter et al, 2012, Martyniuk and Denslow, 2012, Garcia et al, 2013), providing broad characterizations of the proteins expressed within cells, organs, or, in some instances, whole organisms. Methods typically include protein isolation and separation steps with techniques such as 2D gel electrophoresis or high-pressure liquid chromatography, followed by tandem mass spectrometry (MS/MS) analyses to identify peptide profiles or amino acid sequences as a basis for determining specific proteins (Whittaker, 1960, Yates et al, 2009, Wang et al, 2012, Wang et al, 2013a).

While transcriptomics and proteomics are excellent tools, metabolomics captures a more integrated assessment of the physiological state of an organism. Different high-resolution MS and nuclear magnetic resonance (NMR) techniques are the primary methods for generating metabolomic data. Transcriptomics, proteomics, and metabolomics measure responses at different biological levels of organization and thus provide different insights into the biochemical and molecular status of an organism. However, all three approaches have an excellent potential for defining toxicity pathways, particularly if used together (Carrigg et al, 2007, Gonzalez et al, 2011, Garcia et al, 2013, Mizrahi-Man et al, 2013, Davis et al, 2017). Adeyemo et al (2015), in their study which assessed developmental abnormalities and differential expression of genes induced in oil and dispersant exposed *Menidia beryllina* embryos quantified abundances of transcripts of target genes for sexual differentiation and sex determination (StAR, dmrt-1, amh, cyp19b, vtg and chg-L,), growth regulation (ghr)and stress response (cyp1a and Hsp90) concluded that molecular endpoints were most sensitive, especially the expression of star, cyp19b, cyp1a, hsp90 and

recommended that these biomarkers could be used as early indicators of long term effects of Corexit 9500 and 9527 usage on *M. beryllina* in oil spill management and oil pollution events.

High-Throughput Cell Assays and Metagenomics

The application of high-throughput sequencing protocols in metagenomics (Thomas and Meyer, 2012) offers the opportunity for a cost-effective and comprehensive means of assessing biotic diversity and ecological relationships for many complex animal, plant and microbial ecosystems. However, addressing complex biological questions with metagenomics requires the interaction of researchers who bring different skill sets to problem solving. It is now possible to get one (1) litre of water from a location, extract the environmental DNA (eDNA), sequence this with high throughput sequencers and identify all the organisms present at a site (Deiner et al, 2017).

High-throughput cell assays that detect and integrate the response of multiple chemicals acting via a common mode of action enhances environmental monitoring practices (Wang et al, 2013b). In the United States, the Environmental Protection Agency has launched a large project using over 800 different in vitro assays to characterized contaminants into bins of similar modes of action. Results from these assays can be found at the CompTox Chemical Dashboard (https://comptox.epa.gov/dashboard) where, to date, they have tested over 875,000.

In Figure 2 below, we illustrate an example with p,p'-DDT. Plotted here is the AC50 of various assays as a function of the magnitude of the reaction as a scaled function (Scaled Top) to be able to represent all of the assays in one chart. Over 246 different assays were performed with this pesticide but only the 29 that are plotted here were active at AC50 concentrations below the cytotoxicity levels in the cells. Among the assays that were positive in this region, we find assays relating to cell cycle arrest, channels, cytochrome P450's, malformation, nuclear receptors, phosphatase, transferase and transporter activities. The most potent of all of the assays was a phosphatase assay with an AC50 of 0.16 uM. Among the nuclear receptors that are active, estrogen receptor 1 (ESR1) is the most active with an AC50 of 3.26. Other nuclear receptors include ESR2, PGR and NR112. These assays suggest that DDT is highly active with multiple endpoints.

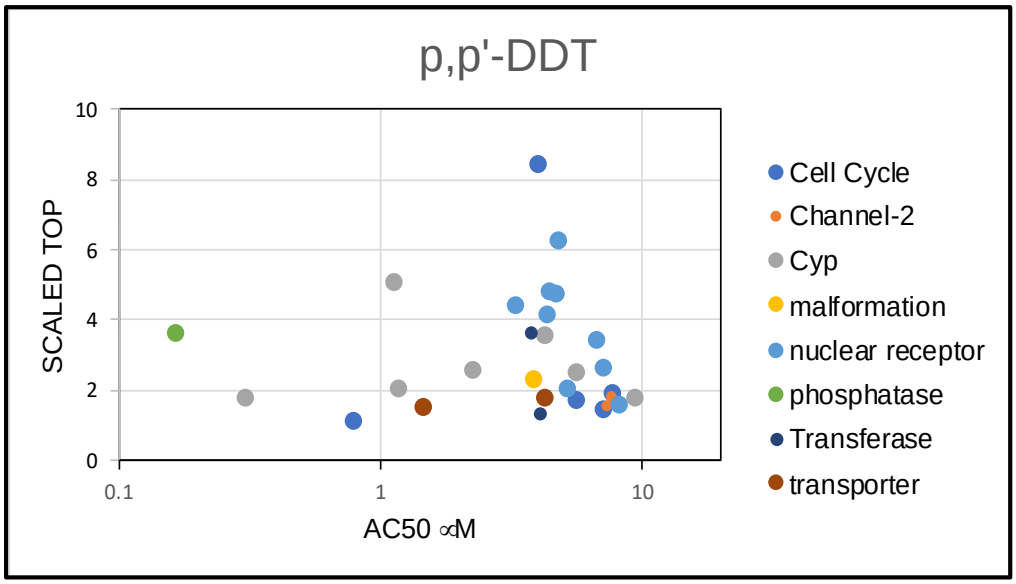

Figure 2: ToxCast bioanalytical plot for p,p'-DDT. The y-axis (scaled top) represents the highest response of each assay divided by the activity cutoff of the assay to be able to represent all assays in the same graph. The x-axis is the AC50 (uM) for each assay

These technologies are providing useful information to characterize environmental hazards. Additionally, it is important to complement high-throughput screening data with reliable in vivo effects data. Ultimately, this connection allows high-throughput screening data to be used to prioritize and predict effect thresholds for risk assessment. For example, Mehinto et al, (2018) reported that a linkage between in vitro and in vivo responses is key to demonstrating that in vitro cell assays can be predictive of ecologically relevant outcomes. Their study investigated the potency of 17β-estradiol (E2), estrone (E1), nonylphenol (NP), and treated wastewater effluent using the GeneBLAzer estrogen receptor transactivation assay and two life stages of the inland silverside (*Menidia beryllina*); and it was concluded that in vitro cell assays were more sensitive than live fish models, making it possible to develop in vitro effect thresholds which could be used to protect aquatic organisms.

Tissue responses: In chemical monitoring, the presence of pollutants in tissues are evaluated by chemical analysis, but biomonitoring methods evaluate not only the presence, but also the response of the organisms to these pollutants by the assessment of various physiological, cellular, biochemical and histopathological biomarkers. Detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. For example, studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological and/or biochemical parameters (Adeyemo, 2007b; Nwabueze et al., 2020). Histopathology provides direct and reliable evidence by identifying cellular alterations in various organs because of environmental pollution. Sublethal levels of pollutants have been reported to cause biochemical or physiological effects at the subcellular level in an organism (Sogbanmu et al., 2018; Olaniran et al. 2019). A variety of pollutants and xenobiotics cause various gill lesions such as lamellar necrosis, epithelial lifting and hyperplasia (Sogbanmu et al., 2018), in response to a wide range of contaminants. Other tissues that could be used as biomarker of pollution include the liver, which is the site of metabolism and plays a key role in biochemical transformations of pollutants under detoxification processes; toxicant exposure, accumulation lesions and other histopathological alternations are therefore common in the liver.

Whole-organism responses: contaminants in aquatic systems usually remain either in soluble or suspension form and finally tend to settle down to the bottom or are taken up by the organisms. The progressive and irreversible accumulation of heavy metals in various organs of marine biota ultimately leads to metal-related diseases in the long run because of their toxicity. Badejo et al (2010) reported that fish and shrimp from Lagos Lagoon, Nigeria, bioaccumulated Zinc, Iron and Manganese in their tissues and linked this to the prevailing improper discharge of wastes from various human activities in the study area especially from industrial, oil sectors and municipal discharges. The result also showed that fish and shrimp significantly bioconcentrated Zinc, Iron and Manganese in comparison to their levels in the surface water. Similarly, developmental abnormalities, reduced hatching success, decreased heartbeat, embryo death have been observed in *Danio rerio* (zebrafish) embryos exposed to sediments from the Lagos lagoon (Sogbanmu et al., 2016; 2020) as well as environmentally relevant concentration of PAHs mixtures (Sogbanmu et al., 2016).

Population-level Responses: aquatic pollution has been established to have affected the normal function of aquatic ecosystems including reproduction, feeding, and have also affected the habitats of aquatic flora and fauna (Schmeller et al., 2018). Ola-Davies et al (2015) reported diazinon-induced clastogenity and pathological changes in ovaries and testes of *Clarias gariepinus*. Chemicals such as diazinon, an organophosphate pesticide, originating from agricultural activity

enter the aquatic environment through atmospheric deposition, surface run-off or leaching. Pollutants enter the food chain through accumulation in soft bottom sediment and aquatic organisms.

The concept of Adverse Outcome Pathway has become important in both environmental and human toxicology. First proposed by Ankley for fish (Ankley et al, 2010), it is now used in human health as well. The idea behind this concept is that there is an initiating event which is called the molecular initiating event (MIE) where a toxicant binds to a protein or receptor to initiate a cascade of events that ultimately lead to adversity. These events start at the molecular level but increase in complexity as they alter cellular homeostasis, organ function and ultimately the organism and population to cause morbidity or mortality, considered adverse effects. The concept offers a way to organize effects from contaminants, with the idea that the effects must result in adversity if the contaminant is to be regulated. A lot of effort has gone into establishing these pathways for environmental organisms and humans and it has been used to develop a knowledge base that can be accessed through the AOP wiki https://aopwiki.org.

Recommendations for Research and Regulatory Testing

The protection of water quality and aquatic ecosystem as a vulnerable resource, essential to sustain life, development and environment is of utmost importance to prevent further pollution and degradation of Nigeria's aquatic resources A long-term research need involves relating the molecular and biochemical responses measured by toxicogenomic methods to specific toxicity pathways and to (adverse) alterations in survival, growth, and reproduction. Significant parallels exist between toxicogenomic data and the endpoints considered as potentially useful biomarkers (such as changes in CYP enzymes, metallothionein, heat shock proteins, vitellogenin). A database of pollutants or contaminant detection and levels in various aquatic ecosystems in Nigeria as well as biological effects elicited in aquatic organisms at environmental concentrations is imperative for future risk evaluations and guidelines development particularly for emerging environmental contaminants. These efforts in tandem with consistent monitoring and evaluation by relevant environmental regulators in Nigeria will support Nigeria' strides towards sustaining life below water (UNSDG 14).

References

Adeogun, A O, Ibor O R, Chukwuka A V, Regoli F, and Arukwe A 2019. The intersex phenomenon in *Sarotherodon melanotheron* from Lagos lagoon (Nigeria): occurrence and severity in relation to contaminants burden in sediment. Environmental Pollution, 244, 747-756.

Adeyemo O K 2003. Consequences of pollution and degradation of Nigerian aquatic environment on fisheries resources. Environmentalist, 23(4), pp.297-306.

Adeyemo O K 2007a. Lead levels in rivers, sediments and fishponds in the Ibadan metropolitan area, south-west Nigeria. African Journal of Aquatic Science, 32(2), pp.153-157.

Adeyemo O K 2007b. Haematological profile of *Clariasgariepinus* (Burchell, 1822) exposed to lead. Turkish Journal of Fisheries and Aquatic Sciences, 7(2), pp.163-169.

Adeyemo O K, Akano O F, Emikpe B O 2011a. Effect of formalin on spawning success and organ histology in *Clarias gariepinus*, Research Journal of Environmental Toxicology 5(7):1-9.

Adeyemo O K, Alarape S A and Emikpe B O, 2011b. Reprotoxic effect of malachite green on African Catfish *Clarias gariepinus* (Burchell 1822), Journal of Fisheries and Aquatic Science, 6 (5): 1-8.

Adeyemo O K, Kroll K J and Denslow N D 2015. Developmental abnormalities and differential expression of genes induced in oil and dispersant exposed *Menidiaberyllina* embryos. Aquatic Toxicology, 168, pp.60-71.

Aina O O, Ozegbe P C, and Adeyemo O K 2019. Age related Histology and Immunohistochemistry of some intermediate filaments in the Testis of the African Catfish (*Clarias gariepinus*). Nigerian Journal of Physiological Sciences: Official Publication of the Physiological Society of Nigeria, 34(2), pp.121-124.

Akpan S O, Odeniyi O A, Adebowale O O, Alarape S A, and Adeyemo O K 2020. Antibiotic resistance profile of gram-negative bacteria isolated from Lafenwa abattoir effluent and its receiving water (River Ogun) in Abeokuta, Ogun State. Onderstepoort Journal of Veterinary Research (*in press*.)

Alarape S A, Ajani F, Adeyemo O K and Shobiye J O 2013. Effect of copper sulphate on spawning success in African Catfish (*Clarias gariepinus*, Burchell 1822), Journal of Fisheries and Aquatic Science, 8 (6), 714-720.

Amaeze N H, Egonmwan R I, Jolaoso A F, and Otitoloju A A 2012. Coastal environmental pollution and fish species diversity in Lagos lagoon, Nigeria. International Journal of Environmental Protection, 2(11), 8-16.

Ankley G T, Daston G P, Degitz S J, Denslow N D, Hoke R A, Kennedy S W, Miracle A L, Perkins E J, Snape J, Tillitt D E, and Tyler C R, 2006. Toxicogenomics in regulatory ecotoxicology. Environmental Science and Technology, 40(13), 4055-4065.

Ankley G T, Bennett R S, Erickson R J, Hoff D J, Hornung M W, Johnson R D, Mount D R, Nichols J W, Russom C L, Schmieder P K, and Serrrano J A, 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry: An International Journal, 29(3), pp.730-741.

Badejo A, Adeyemo O K, and Ojo S O 2010. Seasonal levels of essential metals in fresh and fried marine shrimp and fishes from Lagos lagoon, Nigeria. International Journal of Environmental Sciences, 1(4), pp.454-461.

Baldigo B P, George S D, Phillips P J, Hemming J D, Denslow N D, and Kroll K J, 2015. Potential estrogenic effects of wastewaters on gene expression in *Pimephales promelas* and fish assemblages in streams of southeastern New York. Environmental toxicology and chemistry, 34(12), pp.2803-2815.

Bawa-Allah K A, Saliu J K, and Otitoloju A A 2018. Heavy metal pollution monitoring in vulnerable ecosystems. A case study of the Lagos lagoon, Nigeria. Bulletin of Environmental Contamination and Toxicology, 100(5), 609-613.

Benson N U, Adedapo A E, Fred-Ahmadu O H, Williams A B, Udosen E D, Ayejuyo O O, and Olajire A A 2018. New ecological risk indices for evaluating heavy metals contamination in

aquatic sediment: a case study of the Gulf of Guinea. Regional Studies in Marine Science, 18, 44-56.

Carrigg C, Rice O, Kavanagh S, Collins G, and O'Flaherty V, 2007. DNA extraction method affects microbial community profiles from soils and sediment. Applied Microbiology and Biotechnology, 77(4), pp.955-964.

Carter M Q, Xue K, Brandl M T, Liu F, Wu L, Louie J W, Mandrell R E, and Zhou J, 2012. Functional metagenomics of *Escherichia coli* O157: H7 interactions with spinach indigenous microorganisms during biofilm formation. PloS One, 7(9), p.e44186.

Davis J M, Ekman D R, Skelton D M, LaLone C A, Ankley G T, Cavallin J E, Villeneuve D L, and Collette T W, 2017. Metabolomics for informing adverse outcome pathways: androgen receptor activation and the pharmaceutical spironolactone. Aquatic Toxicology, 184, pp.103-115.

Deiner K, Bik H M, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge D M, De Vere N, and Pfrender M E, 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Molecular ecology, 26(21), pp.5872-5895.

Denslow N D, Griffitt R J, Martyniuk C J. 2012. Advancing the Omics in aquatic toxicology: SETAC North America 31st Annual Meeting. Ecotoxicology and Environmental Safety, 76(2), 1-2.

Dole-Olivier M J, Castellarini F, Coineau N, Galassi D M P, Martin P, Mori N, Valdecasas A, and Gibert J, 2009. Towards an optimal sampling strategy to assess groundwater biodiversity: comparison across six European regions. Freshwater Biology, 54(4), pp.777-796.

Edegbene A O, Elakhame L A, Arimoro F O, Osimen E C and Odume O N 2019. Development of macroinvertebrate multimetric index for ecological evaluation of a river in North Central Nigeria. Environmental Monitoring and Assessment, 191(5), 274.

Ellwood M D and Foster W A, 2004. Doubling the estimate of invertebrate biomass in a rainforest canopy. Nature, 429(6991), pp.549-551.

Fatta-Kassinos D K, Kümmerer, Pharmaceuticals in the environment: sources, fate, effects and risks. 2010. Environmental Science and Pollution Research, 17, 519–521.

Folmar L C, Denslow N D, Rao V, Chow M, Crain D A, Enblom J, Marcino J, and Guillette Jr L J, 1996. Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant. Environmental Health Perspectives, 104(10), pp.1096-1101.

Garcia G D, Gregoracci G B, Santos E D O, Meirelles P M, Silva G G, Edwards R, Sawabe T, Gotoh K, Nakamura S, Iida T, and de Moura R L, 2013. Metagenomic analysis of healthy and white plague affected *Mussismiliabraziliensis* corals. Microbial Ecology, 65(4), pp.1076-1086.

González A, Clemente J C, Shade A, Metcalf J L, Song S, Prithiviraj B, Palmer B E, and Knight R 2011. Our microbial selves: what ecology can teach us. EMBO Reports, 12(8), pp.775-784.

González A, Kroll K J, Silva-Sanchez C, Carriquiriborde P, Fernandino J I, Denslow N D, and Somoza G M, 2020. Steroid hormones and estrogenic activity in the wastewater outfall and receiving waters of the Chascomús chained shallow lakes system (Argentina). Science of The Total Environment, p.140401.

Griffitt R J, Denslow N D, and Martyniuk C J, 2012. Advancing the Omics in Aquatic Toxicology: SETAC North America 31st Annual Meeting. Ecotoxicology and Environmental Safety, 76(2), 1-2.

Gurtz M E 1994. Design of biological components of the National Water-Quality Assessment (NAWQA) program, chap. 15 of Loeb, SL, and Spacie, Anne, eds., Biological monitoring of aquatic systems: Boca Raton, Fla.

Hoffmann A A and Sgro C M 2011. Climate change and evolutionary adaptation. Nature, 470(7335), pp.479-485.

Ibor O R, Adeogun A O, Fagbohun O A, and Arukwe A 2016. Gonado-histopathological changes, intersex and endocrine disruptor responses in relation to contaminant burden in Tilapia species from Ogun River, Nigeria. Chemosphere, 164, 248-262.

Kupoluyi A Y, Alarape S A, and Adeyemo O K 2018. Impact of industrial effluents on Alaro River in Oluyole Industrial Estate, Ibadan and its suitability for aquatic life. Sokoto Journal of Veterinary Sciences, 16(1): 38-44.

Leopold M A and Dreier D A 2019. Ecotoxicological risk assessment of endocrine disruptors: current and future approaches in Elsevier Reference Collection in Earth Systems and Environmental Sciences

Liu J L and Wong M H 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environment international, 59, pp.208-224.

Martyniuk C J, Alvarez S, McClung S, Villeneuve D L, Ankley G T, and Denslow N D 2009. Quantitative proteomic profiles of androgen receptor signaling in the liver of fathead minnows (*Pimephales promelas*). Journal of Proteome Research, 8(5), pp.2186-2200.

Martyniuk C J and Denslow N D 2012. Exploring androgen-regulated pathways in teleost fish using transcriptomics and proteomics. Integrative and Comparative Biology, 52(5):695-704.

Martyniuk C J, Doperalski N J, Prucha M S, Zhang J L, Kroll K J, Conrow R, Barber D S, and Denslow N D 2016. High contaminant loads in Lake Apopka's riparian wetland disrupt gene networks involved in reproduction and immune function in largemouth bass. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 19, pp.140-150.

Marx C J 2013. Can you sequence ecology? Metagenomics of adaptive diversification. PLoS Biol, 11(2), p.e1001487.

Mehinto A C, Kroll K J, Jayasinghe B S, Lavelle C M, Van Dervort D, Adeyemo O K, Bay S M, Maruya K A, and Denslow N D 2018. Linking in vitro estrogenicity to adverse effects in the inland silverside (*Menidia beryllina*). Environmental Toxicology and Chemistry, 37(3), pp.884-892.

Mizrahi-Man O, Davenport E R and Gilad Y 2013. Taxonomic classification of bacterial 16S rRNA genes using short sequencing reads: evaluation of effective study designs. PloS One, 8(1), p.e 53608.

Nwabueze C J, Sogbanmu T O, and Ugwumba A A A 2020. Physicochemical characteristics, animal species diversity and oxidative stress responses in dominant fish from an impacted site on the Lagos Lagoon, Nigeria. Ife Journal of Science, 22(2), 81-93.

Nwaichi E O and Ntorgbo S A 2016. Assessment of PAHs levels in some fish and seafood from different coastal waters in the Niger Delta. Toxicology Reports 3, 167-172.

Ola-Davies O E, Fagbohun A F, Emikpe B O, and Adeyemo O K 2015. Diazinon-induced clastogenity and pathological changes in ovaries and testes of *Clarias gariepinus*. Agricultural Sciences, 6(01), p.146.

Olaniran E I, Sogbanmu T O, and Saliu J K 2019. Biomonitoring, physico-chemical and biomarker evaluations of abattoir effluent discharges into the Ogun River from Kara Market, Ogun State, Nigeria using *Clarias gariepinus*. Environmental Monitoring and Assessment, 191, 44.

Omenka S S and Adeyi A A 2016. Heavy metal content of selected personal care products (PCPs) available in Ibadan, Nigeria and their toxic effects. Toxicology Reports, 3, pp.628-635.

Qian X, Ba Y, Zhuang Q, and Zhong G 2014. RNA-Seq technology and its application in fish transcriptomics. Omics: A Journal of Integrative Biology, 18(2), pp.98-110.

Rainbow P S 1995. Biomonitoring of heavy metal availability in the marine environment. Marine Pollution Bulletin, 31(4-12), pp.183-192.

Reddy P B and Rawat S S 2013. Assessment of aquatic pollution using histopathology in fish as a protocol. International Research Journal of Environment Sciences, 2(8), pp.79-82.

Saka B A, Adeyemo O K, and Odeseye A O 2017. Multiple antibiotic resistance indices of *Aeromonas hydrophila* isolates of muscle of catfish (*Clarias gariepinus*, Burchell 1822) from selected markets in Ibadan, Nigeria. African Journal of Clinical and Experimental Microbiology, 18(2), pp.73-78.

Schmeller D S, Loyau A, Bao K, Brack W, Chatzinotas A, De Vleeschouwer F, Friesen J, Gandois L, Hansson S V, Haver M, and Le Roux G 2018. People, pollution and pathogens–Global change impacts in mountain freshwater ecosystems. Science of the Total Environment, 622, pp.756-763.

Smith M T, de la Rosa R, and Daniels S I 2015. Using exposomics to assess cumulative risks and promote health. Environmental and Molecular Mutagenesis, 56(9), pp.715-723.

Sogbanmu T O and Otitoloju A A 2014. Joint action toxicity and biochemical effects of binary mixtures of Forcados light crude oil and three dispersants against *Clarias gariepinus*. International Journal of Environmental Research, 8(2), 395-402.

Sogbanmu T O, Nagy E, Phillips D H, Arlt V M, Otitoloju A A, and Bury N R 2016. Lagos lagoon sediment organic extracts and polycyclic aromatic hydrocarbons induce embryotoxic, teratogenic

and genotoxic effects in *Danio rerio* (zebrafish) embryos. Environmental Science and Pollution Research, 23(14), 14489-501.

Sogbanmu T O, Osibona A O, Oguntunde A O, and Otitoloju A A 2018. Biomarkers of toxicity in *Clarias gariepinus* exposed to sub lethal concentrations of polycyclic aromatic hydrocarbons. African Journal of Aquatic Science, 43(3), 281-292.

Sogbanmu T O, Osibona A O, and Otitoloju A A 2019. Specific polycyclic aromatic hydrocarbons identified as ecological risk factors in the Lagos lagoon, Nigeria. Environmental Pollution 255, 113295.

Sogbanmu T O, Fatunsin O T, Echebiri F O, Otitoloju A A, and Olayinka K O 2020. Sawmill activities near the Lagos lagoon, Nigeria: Polycyclic aromatic hydrocarbons analysis and embryotoxic evaluations of sediment extracts using *Clarias gariepinus*. Bulletin of Environmental Contamination and Toxicology 104(6), 809-819.

Thomas T, Gilbert J, and Meyer F 2012. Metagenomics-a guide from sampling to data analysis. Microbial informatics and experimentation, 2(1), p.3.

Unterseher M, Jumpponen A R I, Oepik M, Tedersoo L, Moora M, Dormann C F, and Schnittler, M 2011. Species abundance distributions and richness estimations in fungal metagenomics–lessons learned from community ecology. Molecular Ecology, 20(2), pp.275-285.

Vidal-Dorsch D E, Bay S M, Maruya K, Snyder S A, Trenholm R A, and Vanderford B J 2012. Contaminants of emerging concern in municipal wastewater effluents and marine receiving water. Environ Toxicol Chem 31:2674–2682

Wang J F, Stein A, Gao B B, and Ge Y 2012. A review of spatial sampling. Spatial Statistics, 2, pp.1-14.

Wang J F, Jiang C S, Hu M G, Cao Z D, Guo Y S, Li L F, Liu T J, and Meng B 2013a. Design-based spatial sampling: Theory and implementation. Environmental Modelling and Software, 40, pp.280-288.

Wang J, McLenachan P A, Biggs P J, Winder L H, Schoenfeld B I, Narayan V V, Phiri B J, and Lockhart P J, 2013b. Environmental biomonitoring with high-throughput sequencing. Briefings in Bioinformatics, 14(5), pp.575-588.

Whittaker R H 1960. Vegetation of the Siskiyou mountains, Oregon and California. Ecological Monographs, 30(3), pp.279-338.

Wooley J C, Godzik A, and Friedberg I 2010. A primer on metagenomics. PLoS Computational Biology, 6(2), p. e1000667.

Yates J R, Ruse C I, and Nakorchevsky A 2009. Proteomics by mass spectrometry: approaches, advances, and applications. Annual Review of Biomedical Engineering, 11, pp.49-79.

Yoon Y, Ryu J, Oh J, Choi B G, Snyder S A 2010. Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). Science of the Total Environment 408, 636-643.

Zolfaghari-Baghbaderani A, Emtyazjoo M, Poursafa P, Mehrabian S, Bijani S, Farkhani D, and Mirmoghtadaee P 2012. Effects of three types of oil dispersants on biodegradation of dispersed crude oil in water surrounding two Persian Gulf provinces. Journal of Environmental and Public Health Article ID 981365, 8 pages.

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