IJMAAS International Journal of Microbiology and Applied Sciences

Volume 1 Issue 1 April, 2023

Research Article

Microbiological and Polycyclic Aromatic Hydrocarbon Profile of *Pomadasys commersonnii* Harvested from Crude Oil Impacted Creeks in Borikiri, Port Harcourt, Nigeria

Benibo, Nkoba*, Obire, Omokaro, Douglas, S. Ibietela and Nrior, R. Renner

Department of Microbiology, Faculty of Science,
Rivers State University, Nkpolu-Oroworukwo,
Port Harcourt, Nigeria.
*** Corresponding Author:** *E-mail: nkobabenibo1972@yahoo.com

ABSTRACT

This study evaluated the microbiology and level of PAH contamination of *Pomadasys commersonnii* and surface water along Ikpukulu Creek in Borikiri. Standard microbiological procedures were used to assess and identify total heterotrophic bacteria (THB), total fungi and hydrocarbon utilizing bacteria (HUB) and fungi (HUF). PAHs were analyzed using Gas chromatographic–Flame Ionization Defector. Results revealed that, THB counts of *Pomadasys commersonnii* ranged from 2.4 x 10^6 to 1.2 x 10^7 cfu/g, HUB; ranged from 2.0 x 10^3 to 1.3 x 10^4cfu/g, Fungi; ranged from 1.9 x 10^3 to 2.3 x 10^5 cfu/g and HUF ranged from 2.2 x 10^2 to 2.5 x 10^3cfu/g. Genera of HUB and HUF identified from fish and water samples were; *Pseudomonas*, *Bacillus*, *Aeromonas*, *Proteus*, *Serratia*, *Citrobacter*, *Staphylococcus*, *Escherichia*, *Corynebacterium*, *Acinetobacter*, *Flavobacterium*, *Micrococcus*, *Enterobacter*, *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium*, *Cladosporium*, *Metshnikowia*, *Candida*, *Cryptococcus*, *Leucosporidium*, *Rhodotorula*, *Aspergillus*, *Alternaria*, *Geotrichium*, *Saccharomyces*, and *Trichoderma*. PAHs level in fish samples ranged from 0.025mgkg^{-1} – 0.035mgkg^{-1} with a mean of 0.031mgkg^{-1}, while PAHs in water ranged from 0.0035 – 0.0045mgL^{-1} with a mean of 0.004 mgL^{-1}. Highest PAH recorded was benzo(b)fluoranthene (60%). Results showed that the fish biominified pyrenes, benzo(a)anthracene and benzo(b)fluoranthene. Contamination factor (c^1f) was 0.12 ($c^1f < 1$), which indicates, Cancer Risk Index using indeno1,2,3,(cd)pyrene as bioindicator showed no cancer risk in consumption of *Pomadasys commersonnii* harvested from Ikpukulu Creek. There is significant difference at $p<0.05$ in PAH level between surface water and *Pomadasys commersonnii* indicating the fish has the tendency of biomagnifying some PAHs. Therefore, the inhabitants should be enlightened of the dangers of these pollutants (PAHs) in food chains. Some of the hydrocarbon utilizing microbes in this study could be useful in bioremediation of hydrocarbon and PAH contaminated environments.

Keywords: Crude oil, PAH, Creek, *Pomadasys commersonnii*, biomagnify, carcinogen

Introduction

Contamination of water bodies with hydrocarbons from partial refined crude oil discharged into the ecosystem is a common occurrence in contemporary time in Rivers State (Ekweozor, 1996). Apart from interfering with the chemistry of both water and sediments, the survival of both micro and macro fauna are threatened by the components of crude oil in the ecosystem (FME, 2006). Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrocarbons, with fused aromatic (benzene) rings between 2 – 6. PAHs occur in the environment naturally or as a result

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

of man's activities. PAHs arising from fossil fuel are called pyrogenic while those from crude oil are called petrogenic. About thirty parent PAHs are found in crude oil of which sixteen are regarded as important pollutants due to their high carcinogenic, mutagenic and teratogenic activity (Douglas *et al*., 2020). The 16 listed PAHs as priority pollutants are; naphthalene, acenaphthylene, acenaphthene, flourene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo (a) anthracene, benzo (b) fluoanthene, benzo (k) flouranthene, benzo (a) pyrene, benzo (g,h,i) pyrene, Indeno (1, 2, 3 – cd) pyrene, and dibenz (a,h) anthracene (Douglas *et al*., 2020; Ariyo and Obire, 2022). PAHs are the first substance to be associated with carcinogenesis (Eisler, 1987). Among them benzo (a) pyrene is the known human carcinogen and is as an indicator for PAH exposure. PAHs are fractions of the crude oil that enter into the food chain and are bioaccumulated and biomagnified through feeding (Edori and Edori, 2021). Polycyclic aromatic hydrocarbons (PAHs) are a group of hydrocarbons of carbon between 2 - 6 are fractions of the crude oil that enter into the food chain and are bioaccumulated and biomagnified through feeding.

In order to maintain osmotic pressure, fishes always drink water, in this manner, PAHs gain entrance into the fish and alter some vital enzymatic reactions thus leading to cell necrosis (Temitayo *et al*., 2019). Due to the specific heat of water, water current, wave actions and convection, the marine ecosystem and estuaries are able to stabilize the physicochemistry of the water. By photo-oxidation some PAH component are rid of the ecosystem within a short time, some are biodegraded by hydrocarbonclastic microbes (Vinithin *et al*., 2015). However, some gets adsorbed onto surfaces in the ecosystem and become persistent (Jih *et al*., 2018). Research works have been ongoing in various ecosystems due to continuous discharge of petroleum and refined products from artisanal refineries along the coasts of Niger Delta. Some studies have established that there are bacterial strains that have adapted to these discharges and hence have the capacity to degrade the pollutants even at very high concentrations (Obire and Nwaubeta, 2002). A study carried out on Bonny River on hydrocarbon degraders-Drilling fluid utilizing bacteria genera isolated were: *Pseudomonas*, *Bacillus*, *Micrococcus* and *Enterobacter*, with *Pseudomonas* having the highest frequency of 35.7%, followed by *Bacillus* with the frequency of 30.7%, *Micrococcus* had 15.4% and *Enterobacter* 15.4%.Fungi genera isolated include *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* (Nrior *et al*., 2017).

Some PAHs e.g. Benz(a) pyrene are well reported carcinogens by the IARC (International Agency for Research on Cancer). Standards are therefore set by EU NO 835/2011 establishing permissible levels of PAH markers in seafoods. Such include 0.006mgkg^{-1} of Benzo(a) pyrene or 0.01mgkg^{-1} of the total level of Benzo(a) pyrene, chrysene, Benzo(a) anthracene and Benzo(b) fluranthene (PAHs 4) or 0.005mgkg^{-1} or indeno 1,2,3, (cd) pyrene. PAHs are lipophilic and easily bioaccumulate in the tissues of fish. The consumption of seafood forms part of the culture of the people of Rivers State. Fish contains the heart friendly omega-3-protein without cholesterol (EUEPA, 2009).

Pomadasys commersonnii (commonly known as spotted grunter) is found in coastal water and estuaries. It can tolerate fresh water. If feeds mostly on crustaceans, worms and bivalves. It is ray finned and a native of brackish and marine waters of West Indian Ocean (Ibim and Bongili, 2018). It has a maximum length of 80cm, found within a depth of 0 – 49m in the water body. It belongs to the class Actinoptcroii, order pereformes (Ibim and Bongili, 2018). It is also known as *Pomadasys perculare*, *Pristopoma operculare*, *Labrus commersonnii*, *Pristipoma opercularis* (Ibim and Bongili, 2018). It is one of the choice fish though expensive in Rivers State. Apart from tilapia, mullet and sardine, *Pomadasys commersonnii* is one of the abundant fishes harvested along the Ikpukulu Creek (Vincent-Akpu and Okosiemiema, 2019).

Materials and Methods

Study Location

The study area are creeks in Borikiri Wetlands connecting the Bie-Ama and Pereama, Borikiri Sandfill area of the Port Harcourt, Rivers State Nigeria. It lies approximately between Longitude 7^0 1.0" E to 7^0 $2^1$27.33"E and Latitude 4^0 $43^1$32.2" N to 4^0 45^1 0"N (Vincent-Akpu and Okosiemiema, 2019).

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

These are marketing points of kerosene, gasoline, lubricating oils and diesel from artisanal refineries conveyed from "kpo fire" camps along the coastal areas of Rivers State.

This study area is an Estuary with brackish water salinity ranging from 10% - 30%. The climate is tropical wet with lengthy rainy season and very short dry season. The vegetation is mangrove with subsoil characterized by typical fibrous clayey mud that shows a large value of compressibility (Ideriah *et al*., 2012). Several anthropogenic activities take place along the creek, such as disposal of refuse and excreta, frequent blasting of crude oil conveying boats as well as recreation.

Sampling

Fish (*Pomadasys commersonnii*) were harvested by net throwing in the intertidal zones and open sea. Twenty fishes were randomly collected from each of the four sampling sites (Ikpukulu jetty, MTN Mast, Island and Okilo Polo) every month from November 2019 – October 2020. Samples were washed thoroughly with sea water and placed in foils and labeled and taken to the Institute of Pollution Studies (IPS) Rivers State University Nkpolu-Oruworukwu, Port Harcourt in an ice-chest as described by Isaac *et al*. (2018)

Sampling for surface water was done from November, 2019 to October, 2020. A total of five water samples were collected each month from each of the four sample stations; making 20 water samples per month. Sample bottles were carefully opened under the water within a depth of about 10cm from the surface. Bottles were rinsed with surface water samples about 3 times before sample collection.

The five composites of each sample stations were mixed together and homogenized to obtain a bulk composite (APHA, 1995). Water samples for microbiological analysis were properly labeled and put in ice box. Water samples were filtered and preserved on site with hydrochloric acid for PAH analysis and transported in an ice chest to the laboratory after proper labeling (APHA, 1995).

Samples of collected *Pomadasys commersonnii* were allowed to thaw and cool to room temperature. Fish samples were homogenized using clean sterile ceramic mortar and pistil. One gram (1g) of each part of sample was added to 9ml of normal saline for the serial dilution.

Microbiological Analysis

Total Heterotrophic bacterial Counts (THBC)

Total heterotrophic bacteria from the samples were enumerated using the spread plate technique as described by Prescott *et al.* (2005). An aliquot (0.1 ml) of the dilutions 10^{-4} to 10^{-6} were aseptically transferred unto properly dried nutrient agar plates in duplicate, spread evenly using a bent glass rod and incubated at 37ºC for 24 hours. After incubation, the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh nutrient agar plates using the streak plate technique.

Discrete colonies on the plates were aseptically transferred into 10% (v/v) glycerol suspension, well label and stored as stock cultures for preservation and identification (APHA, 1995). Total Heterotrophic Bacterial Counts (THBC) were calculated from the mean value of colonies counted from the duplicate plates using the below formula: THBC (CFU/g) = Number of Colonies/ (Dilution (10^{-7}) x Volume plated (0.1 ml)).

Total Heterotrophic Fungal Counts

The total Heterotrophic fungi were enumerated using the spread plate method as described by Prescott *et al*. (2005). An aliquot (0.1 ml) of the dilutions 10^{-2} to 10^{-3} was aseptically transferred unto properly dried Sabouraud Dextrose Agar plates containing antibiotic (tetracycline and penicillin) to inhibit bacterial growth in duplicate (Cheesebrough, 2006).

Plates were spread evenly using a bent glass rod and incubated at 35ºC for 3 to 5 days. After taking counts, the fungal colonies were sub-cultured onto Sabouraud Dextrose Agar slant in bijou bottles for preservation (APHA, 1995).

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

Isolation and Enumeration of Hydrocarbon Utilizing Bacteria (HUB)

Collected water samples from the ice box were cooled to room temperature and diluted in tenfold serial dilution with sterile physiological saline to give an initial 1:10 dilution. 0.1 ml of prepared dilutions were pipetted out and placed on mineral salts medium supplemental with 50 $ugml^{-1}$fungizol miconazole nitrates to prevent fungal contamination.

Isolation and enumeration were done using spread plate techniques (Okpokwasili and Amanchukwu, 1988; Chikere *et al*., 2009) using vapour phase transfer technique on mineral salts agar for HUB. The plates were incubated at 30ºC for 7 days for HUB, while for THB, incubation was at 28ºC for 24hours. Enumeration of isolates was done and expressed as CFU/ml for surface water and CFU/g for fish gills and tissues.

Hydrocarbon Utilizing Fungal Counts

The population of hydrocarbon utilizing fungi was determined by inoculating 0.1 ml aliquot of the serially diluted samples onto mineral salt agar media using vapour phase transfer method according to Nrior *et al*. (2017). The mineral salt medium used was amended with 250mg of tetracycline to inhibit the growth of hydrocarbon utilizing bacteria.

The plates were inverted and incubated at 28ºC for 5 days. Fungal counts were expressed and recorded as Colony Forming unit per gram (CFU/g) for fish gills and tissues and CFU/ml for surface water.

Identification of Bacterial and Fungal Isolates

Bacterial isolates were identified based on cultural, morphological and biochemical characteristics using Holt *et al*. (1994).

On the other hand, the fungal spores /isolates that utilized carbohydrate as their sole carbon energy source were viewed macroscopically and microscopically using Lactophenol Cotton Blue Stain and the slide culture technique. This was carried out using the steps as described in Cheesbrough (2006).

Polycyclic Aromatic Hydrocarbon (PAH) Analysis

PAH Analysis of Surface Water

Two hundred and fifty milliliters (250ml) of water samples were measured into a separating funnel rinsed with dichloromethane. To the 250ml water sample 25ml dichloromethane was added. The mixture was shaken vigorously to extract all organic materials. The organic extract was passed through a column containing cotton wool, silica gel and anhydrous suphate for cleaning and dehydration. The organic extract obtained was injected into gas chromatographic column. A µl of the concentrated sample was injected by means of hypodermal syringe through a rubber septum into the column, the vapour fraction of the PAHs were automatically defected as it emerges from the column by the flame ionization Detector (FID). The results were expressed in mgl^{-1}.

PAHs Analysis of *Pomadasys commersonnii*

Samples of collected *Pomadasys commersonnii* were allowed to thaw and cool to room temperature. Fish samples were homogenized using clean sterile ceramic mortar and pistil. Two grams (2g) of sample were weighed into a clean extractor container. 20ml of extraction solvent (hexane) was added into sample and mixed thoroughly and allowed to settle. The mixture was carefully filtered into Buchner funnels. The extracts were concentrated to 2ml and transferred for clean-up. For clean-up, 1cm of moderately packaged glass wool was placed at the bottom of 10mm internal diameter x 250mm long chromatographic column. Slurry of 2g activated silica in 10ml dichloromethane was prepared and placed into the chromatographic column. To the top of the column, 0.5cm of sodium sulphate was added.

The column was rinsed with additional 10ml of dichloromethane. The column was pre-eluted with 20ml of hexane. This was allowed through the column at the rate of about 2 minutes until the liquid in the column was just above the sodium sulphate layer. Immediately, 1ml of the extracted sample was transferred into the column. The extraction bottle was rinsed with 1ml of hexane and the dissolved extract was added to the column as well. The stop cork of the column was opened and the eluent was collected in a

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

10ml graduated cylinder. Hexane was added to the column in 1-2ml increment just before the exposure of the sodium sulphate layer to air. Accurately measured volume of 8-10ml of the effluent was collected and labeled-polyaromatic. The concentrated polyaromatic fractions were transferred into labeled glass vials with Tefion and rubber crimp caps for gas chromatographic analysis.

One microliter (1µl) of the concentrated sample was injected into the column by means of hypodermic syringe through a rubber septum into the column. Separation occurs as the vapour constituents' partition between the gas and liquid phases. The sample was automatically detected as it emerged from the column by the Flame Indicator Detector (FID) (RPI / NNPC, 1985a; RPI / NNPC, 1985b).

Results

Results of the total heterotrophic bacterial and total fungal counts in the surface water and in *Pomadasys commersonnii* from November 2019 to October, 2020 are as shown in Figure 1 and Figure 2 respectively. The THB counts ranged from 2.4 x 10^6 to 1.2 x 10^7cfu/g while total fungi ranged from 1.9 x 10^3 to 2.3 x 10^5 cfu/g.

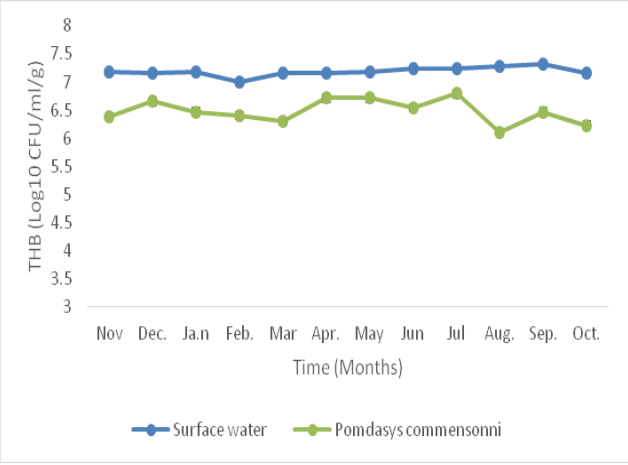

Fig. 1: Total Heterotrophic Bacterial Counts in Surface Water and *Pomadasys commersonni* (Nov. 2019- Oct. 2020)

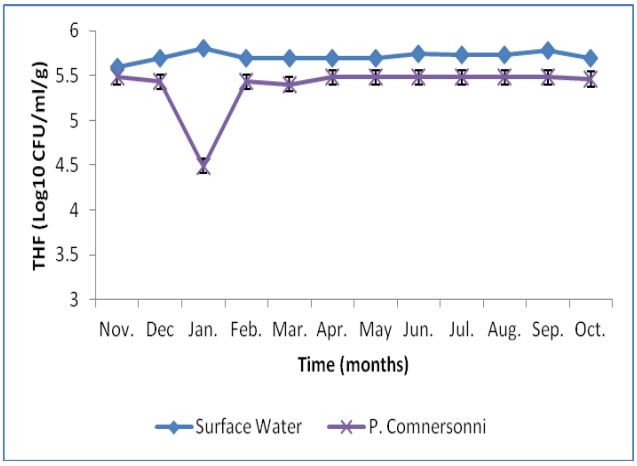

Fig. 2: Total Heterotrophic Fungi (THF) Count in Surface Water and *Pomadasys commersonni* (Nov. 2019- Oct. 2020)

The results of the counts of hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) are as shown in Figures 3 and 4 respectively. The counts of hydrocarbon utilizing bacteria ranged from 2.0 x 10^3 to 1.3 x 10^4cfu/g, while counts of Hydrocarbon utilizing fungi ranged from 2.2 x 10^2 to 2.5 x 10^3cfu/g.

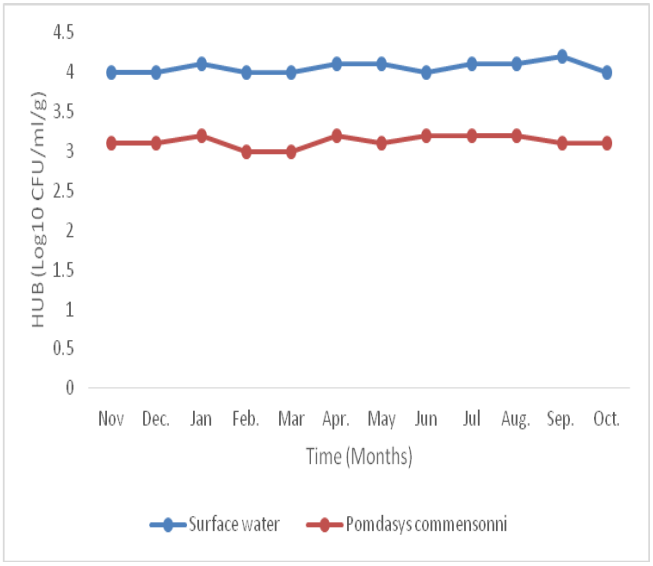

Fig. 3: Hydrocarbon Utilizing Bacteria (HUB) Count in Surface Water and *Pomadasys commersonni* (Nov. 2019- Oct. 2020)

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

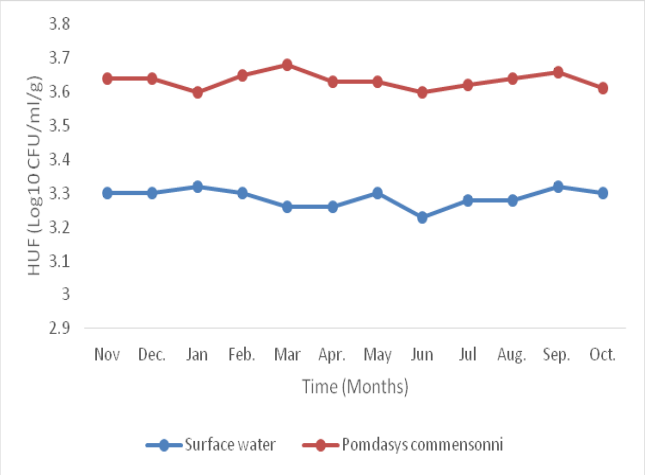

Fig. 4 Hydrocarbon Utilizing Fungi (HUF) Count in Surface Water and *Pomadasys commersonni* (Nov. 2019- Oct. 2020)

The occurrences of identified Hydrocarbon utilizing Bacteria (HUB) and the Hydrocarbon Utilizing Fungi (HUF), and their percentage (%) occurrences in the surface water samples and in the fish *Pomdasys commersonni* samples during this study are as shown in Table 1 below.

Table 1: Occurrence (%) of Hydrocarbon Utilizing Bacterial (HUB) and Hydrocarbon Utilizing Fungal (HUF) isolates from Surface water and Fish – *Pomdasys commersonni*

Hydrocarbon Utilizing Bacterial (HUB)			**Hydrocarbon Utilizing Fungal (HUF)**		
HUB isolate	**Surface Water Occurrence (%)**	***Pomdasys commersonni* Occurrence (%)**	**HUF isolate**	**Surface Water Occurrence (%)**	***Pomdasys commersonni* Occurrence (%)**
Proteus sp.	3(4.69)	1(3.03)	*Mucor* sp.	2(4.76)	4(11.43)
Pseudomonas spp	7(10.94)	3(9.09)	*Penicillium* sp.	19(45.24)	5(14.29)
Aeromonas sp.	3(4.69)	2(6.06)	*Rhizopus* sp.	1(2.38)	2(5.71)
Escherichia coli	4(6.25)	1(3.03)	*Fusarium* sp.	0(0.00)	1(2.86)
Bacillus sp.	6(9.38)	7(21.21)	*Cladosporium* sp.	5(11.90)	4(11.43)
Serratia sp	4(6.25)	4(12.12)	*Metshnikowia* sp.	0(0.00)	3(8.57)
Klebsiella sp.	4(6.25)	0(0.00)	*Candida* sp.	2(4.76)	2(5.71)
Vibrio sp.	3(4.69)	0(0.00)	*Cryptococcus* sp.	2(4.76)	1(2.86)
Enterococcus sp.	3(4.69)	0(0.00)	*Leucosporidium* sp.	1(2.38)	2(5.71)
Citrobacter sp.	1(1.56)	1(3.03)	*Rhodotorula* sp.	1(2.38)	1(2.86)
Staphylococcus sp	4(6.25)	4(12.12)	*Aspergillus* sp.	4(9.52)	3(8.57)
Streptococcus sp	2(3.13)	0(0.00)	*Alternaria* sp.	0(0.00)	2(5.71)
Entrobacter sp	3(4.69)	1(3.03)	*Geotrichium* sp.	0(0.00)	2(5.71)
Shiegella sp	4(6.25)	3(9.09)	*Saccharomyces* sp.	3(7.14)	2(5.71)
Corynebacterium sp	2(3.13)	2(6.06)	*Trichoderma* sp.	2(4.76)	1(2.86)
Acinetobacter sp	2(3.13)	1(3.03)			
Chromobacterium sp	2(3.13)	0(0.00)			
Flavobacterium sp	1(1.56)	2(6.06)			
Micrococcus sp	2(3.13)	1(3.03)			
Alcaligenes sp	3(4.69)	0(0.00)			
Norcadia sp	1(1.56)	0(0.00)			
Total	**64 (100%)**	**33 (100%)**	**Total**	**42 (100%)**	**35 (100%)**

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

The mean values or levels of PAHs in surface water and *Pomadasys commerconnii* as analyzed are shown Figure 5 and 6 respectively. The PAH levels in surface water ranged from 0.003mgl^{-1} to 0.005mgl^{-1} with a mean value of 0.004mgl^{-1}. The PAH level of *Pomadasys commerconnii* ranged from 0.02mkg^{-1} – 0.04mgkg^{-1} with a mean value of 0.031mgkg^{-1}.

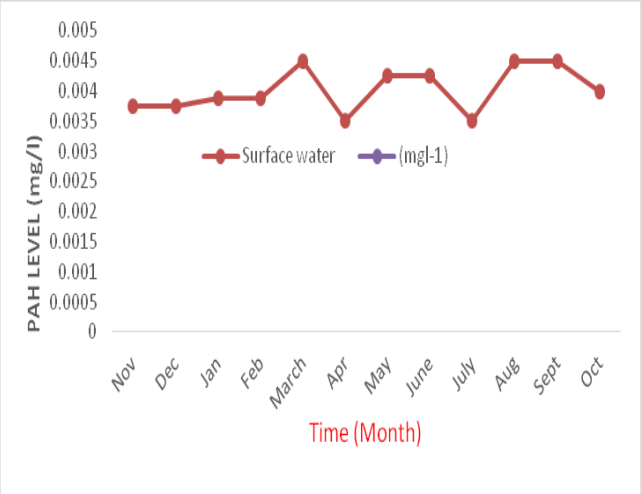

Fig. 5: Changes in PAH Levels in Surface Water (mg/l) in Crude Oil Polluted Stations in Borikiri Wetlands

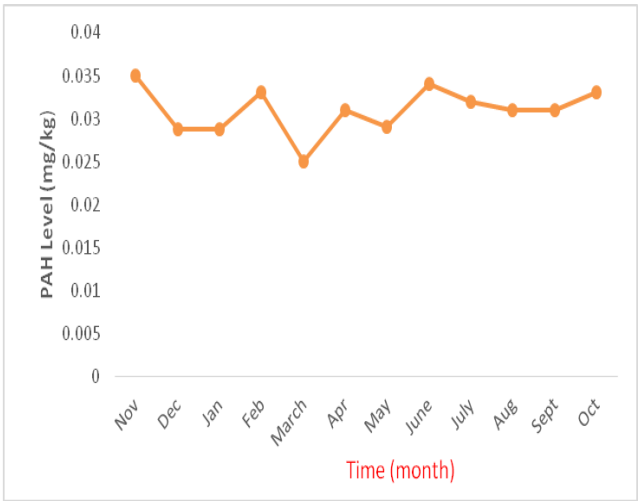

Fig. 6: Changes in PAH Level in *Pomadasys commersonni* (mg/kg) in the sample Stations in Borikiri Wetlands

The PAHs components are shown in Figures 7 and 8 and in Table 2 below. Specifically, Table 2 shows that out of the 14 PAHs components analyzed; pyrene, Benzo (a) anthracene and Benzo (b) fluoranthene are not biomagnified by *Pomadasys commerconnii*.

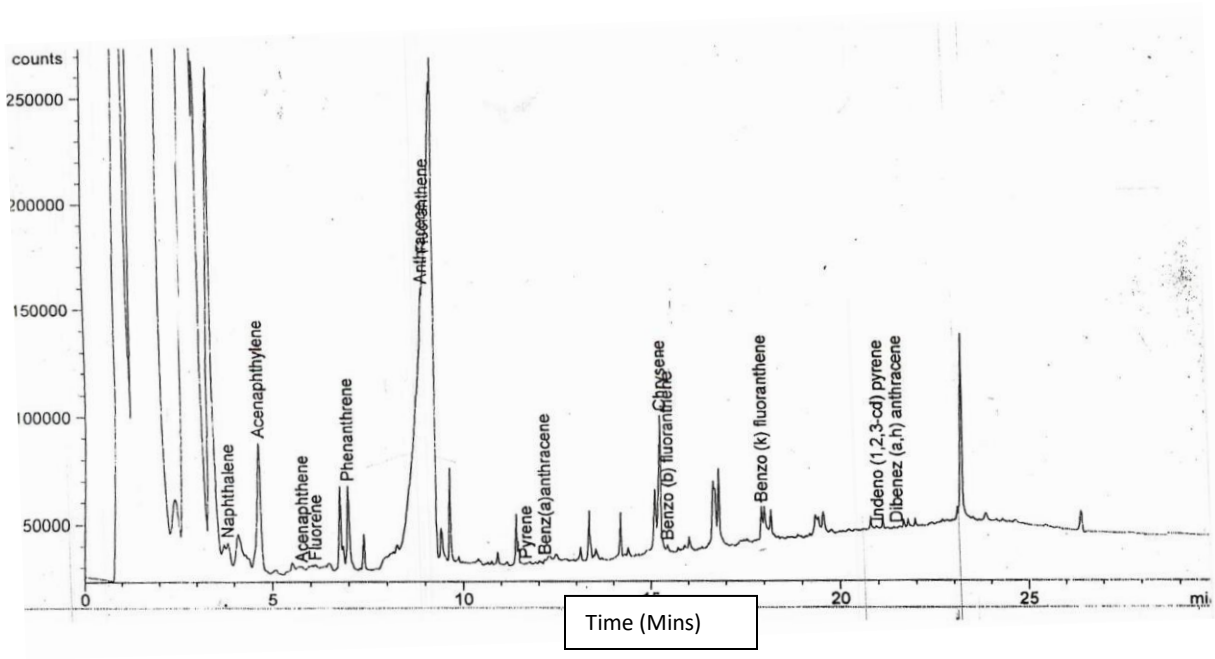

Fig. 7: Chromatogram of PAHs in *Pomadasys commersonni* from Borikiri Wetlands

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

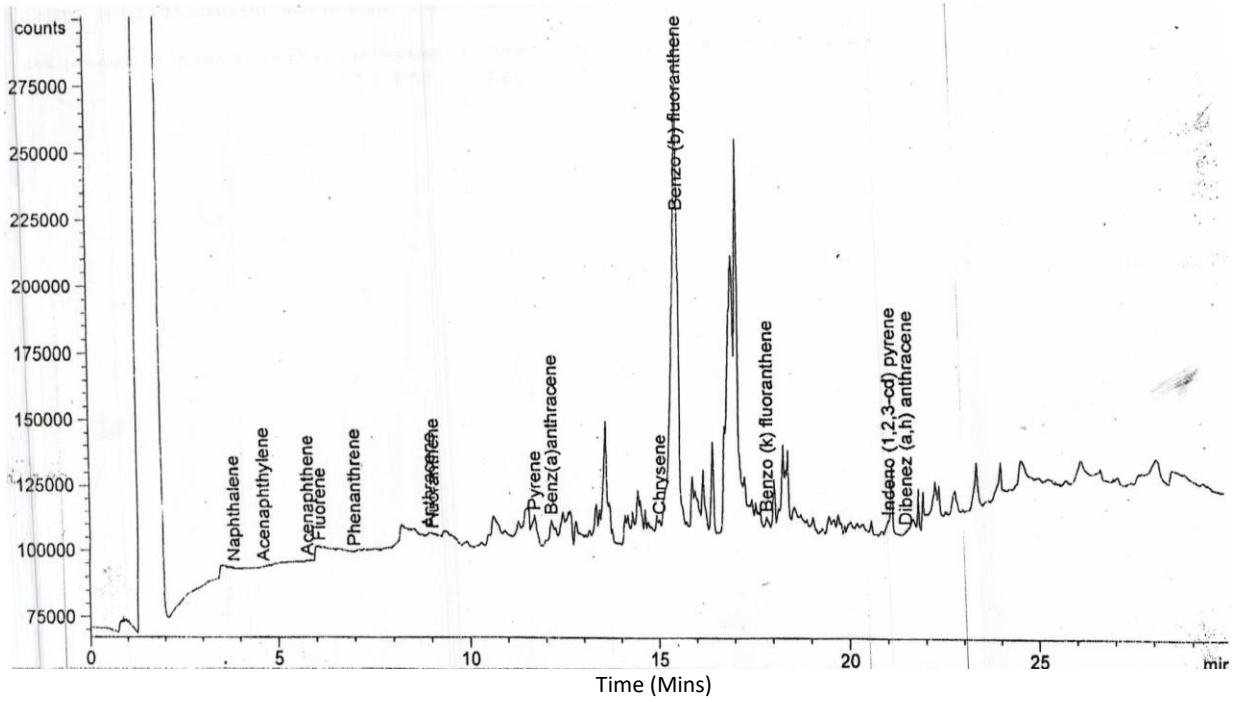

Fig. 8: Chromatogram of Polycyclic Aromatic Hydrocarbons in Surface Water from Borikiri Wetlands

Table 2: Mean values of PAHs Components in Surface Water and *P. commersonnii* in Ikpukulu Creek (Nov. 2019 – Oct. 2020)

PAH Components	Surface Water (mgl^{-1})	*Pomadasys commersonni*	Inference
Naphthalene	0.000016	0.00045	Biomagnified
Acenaphthylene	0.00006	0.0034	Biomagnified
Acenaphthene	0.000075	0.00014	Biomagnified
Fluorene	0.00009	0.0002	Biomagnified
Phenanthrene	0.00003	0.0017	Biomagnified
Anthracene	0.00006	0.014	Biomagnified
Fluoranthene	0.00006	0.0066	Biomagnified
Pyrene	0.0003	0.000026	Biominified
Benzo (a) anthracene	0.00019	0.000068	Biominified
Chrysene	0.000099	0.0025	Biomagnified
Benzo (b) fluoranthene	0.0024	0.00048	Biominified
Benzo (k) Fluorathene	0.00015	0.00063	Biomagnified
Indeno (1,2,3) (d) pyrene	0.0002	0.0006	Biomagnified
Dibenes (a, k) anthracene	0.00024	0.00064	Biomagnified
Total	**0.004**	**0.031**	

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

Estimated Daily intake of food EDI using indeno 1,2,3, (cd) pyrene

$$= \frac{0.0006\text{mgkg}^{-1} \; x \; 0.1\text{kg}}{70kg} \quad \text{.....Equation 1}$$

= 0.00000086mg/kg/day

Reference Estimated Daily intake of food:

$$\text{EDI} = \frac{\text{Mass of reference contaminant } (mgkg^{-1}) \text{ x } 0.1\text{kg}}{70kg}$$

.....Equation 2

0.1 kg = 100g = mass of seafood ingested (USEPA, 2011)

70kg = mass of an average man

Reference concentration = 0.005mg/kg

∴ Reference EDI of *Pomadasys commerconnii*

$$= \frac{0.005\text{mgkg}^{-1} \; x \; 0.1\text{kg}}{70kg} = 0.000007\text{mg/kg/day}$$

Cancer Risk Index = $\frac{0.00000086\text{mg/kg/day}}{0.000007\text{mg/kg/day}}$

= 0.12

The Contamination factor (c^1f):

$$C^1f = \frac{Mass\ of\ contaminant\ (mgkg^{-1})}{Mass\ of\ Standard\ (mgkg^{-1})} =$$

$$\frac{0.0006mgkg^{-1}\ indeno\ 1,2,3cd\ pyrene}{0.005mgkg^{-1}} \quad \text{.....equation 3}$$

Contamination factor of PAH (indeno1,2,3,(cd) pyrene) in *Pomadasys commerconnii* = 0.12

Discussion

This study has revealed the microbiological and polycyclic aromatic hydrocarbon (PAH) profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks and of the surface water in Borikiri, Port Harcourt, Nigeria. The results of Total heterotrophic bacterial count (THBC) from these crude oils impacted surface water falls within 1.2 x 10^5 $Cfuml^{-1}$ - 5.0 x10^7 $Cfuml^{-1}$ (Fig 1) with non-exceeding A x 10^7 $Cfuml^{-1}$ where $1 \geq A \leq 9$. Unimke *et al.* (2014) recorded bacterial densities of 1.2 x 10^5 - 2.2 x 10^6 $Cfuml^{-1}$ for THBC and 1.2 - 1.3 x 10^5 $Cfuml^{-1}$ hydrocarbon utilizing bacteria (HUB) for surface water samples in crude oil impacted Imo River estuary and 2.6 x 10^7 - 2.8 x 10^9 THBC. Total heterotrophic fungi densities of surface water 5.3 x 10^5 $cfuml^{-1}$ (Fig. 2); by implication, it simply means that the density of fungi available to metabolize the crude oil in the surface water. These results showed the presence of high active indigenous microbial flora in the fish and surrounding environment.

Results from this research work shows that, the mean bacterial density in surface water from the research sites, 1.6x10^7 $Cfuml^{-1}$, 1.2x10^4 $Cfuml^{-1}$(0.075%) are hydrocarbon utilizers (Fig 4). According to Zobell (1971), hydrocarbon utilizing bacteria take advantage of the presence of crude oil in the ecosystem and use them as energy and carbon source since carbon in the marine environment is a limiting factor being in the concentration of 20mg/l. According to this research, one bacterial cell can decompose 5.0 x 10^{-12} mg crude/hr in a litre of water. Going by this, a bacterial density of 1.2 x 10^4 $cfuml^{-1}$ which is equivalent to 1.2 x10^7 $Cful^{-1}$ of the HUB recorded in this research, is a sure way of carrying out a natural attenuation method of bioremediation in the surface water.

It has been reported that, fungi biodegrade PAHs to carbon (IV) oxide and water. A fungal density of 9 x 10^4 reported in this present studywhich is included in the range of fungal density is recorded by Zobel (1971), as enough density for microbial crude oil degradation.

The following hydrocarbon utilizing bacteria were identified from the fish and water samples: *Pseudomonas* spp, *Bacillus* spp, *Aeromonas* sp, *Proteus* sp, *Serratia* sp, *Citrobacter* sp, *Staphylococcus* sp, *Escherichia coli*, *Corynebacterium* sp, *Acinetobacter* sp, *Flavobacterium* sp, *Micrococcus* sp, *Enterobacter* sp. While, the following fungal isolates were also identified; *Penicillum* sp, *Mucor* sp, *Rhizopus* sp, *Fusarium* sp, *Cladosporium* sp,

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

Metshnikowia sp, *Candida* sp, *Cryptococcus* sp, *Leucosporidium* sp., *Rhodotorula* sp., *Aspergillus* sp., *Alternaria* sp., *Geotrichium* sp., *Saccharomyces* sp., *Trichoderma* sp. The bacterial isolates from this research samples like *Micrococcus* sp., *Pseudomonas aeruginosa* and *Bacillus* sp. are reported to possess catabolic genes catechol 2, 3 dioxygenase (nah H), alkane mono-oxygenase (alk B) and catechol 1, 2 dioxygenase (cat A) genes which are PAHs degrading (Oboh, 2006). *Micrococcus*, *Bacillus* and *Citrococcus* are reported by Atlas and Bartha (1992) as alkaliphilic oil utilizing bacteria and are responsible for biodegradation and eventual clean-up of spills in our environment. According to Adekunle and Adebambo (2007), microbial communities exposed to hydrocarbons become adapted, exhibiting selective enrichment and genetic changes. This adaptation makes them extremophiles. These microorganisms are able to withstand this environmental stress due to their ability to release stress mitigating compounds e. g. alkaloids, peptides, and exopolysaccharides which enable them use hydrocarbons as carbon and energy sources. Bacterial densities recorded from other crude oil impacted ecosystems are similar to that reported by Obiukwu and Otokunefor (2014) in these research site viz: Ekerekana creek, Okari Creek, Ogoloma, Edemebikri, Elechi Creek 1.4×10^5 - 5.0×10^7 $Cfuml^{-1}$ for surface water; 2.5×10^4 - 2.8×10^9 $Cfug^{-1}$ for sediment. The bacterial isolates were *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp., *Klebsiella* sp., *Vibrio* sp., *E. coli*, *Flavobacterium* sp., *Micrococcus* sp., *Streptococcus* sp. (Obiukwu and Otokunefor, 2014). *Chromobacterium* sp. and *Corynebacterium* sp. were not recorded in these creeks although crude oil impacted. It could thus be said that, some bacteria may be indigenous to some ecosystems just like some macrofauna. Otokunefor and Obiokwu (2014) also recorded a bacterial density of 1.2×10^4 - 1.6×10^9 $Cfuml^{-1}$ from refinery effluents. It shows that bacterial densities from refinery effluents are in close proximity with crude oil impacted ecosystems. The bacterial isolates have representatives which can biodegrade all the fractions of total petroleum hydrocarbons (TPH) presented in this research; *Pseudomonas* sp., *Bacillus* sp., *Enterobacter* sp., *Staphylococcus* sp., *Citrobacter* sp., *Micrococcus* can biodegrade n – alkanes $C_{14} – C_{30}$ as well as resins and asphaltenes being equipped with catabolic genes (nah H, cat A and Alk B).

The Hydrocarbon utilizing Bacteria (HUB) and their percentage (%) occurrences' in the surface water samples and in the fish *Pomdasys commersonni* samples during this study were: *Proteus* sp. 3.03%, *Pseudomonas* spp 9.09%, *Aeromonas* sp. 6.06%, *Escherichia coli* 3.03%, *Bacillus* sp. 21.21%, *Serratia* sp12.12%, *Citrobacter* sp. 3.03%, *Staphylococcus* sp12.12, *Entrobacter* sp 3.03, *Shiegella* sp 9.09%, *Corynebacterium* sp 6.06%, *Acinetobacter* sp 3.03%, *Flavobacterium* sp 6.06%, and *Micrococcus* sp 3.03% (Table 1).

The fungi isolated from this research sites and their percentage (%) occurrences were: *Mucor* sp. 11.43, *Penicillium* sp. 14.29, *Rhizopus* sp. 5.71, *Fusarium* sp. 2.86, *Cladosporium* sp.11.43, *Metshnikowia* sp. 8.57, *Candida* sp. 5.71, *Cryptococcus* sp. 2.86, *Leucosporidium* sp. 5.71, *Rhodotorula* sp. 2.86, *Aspergillus* sp. 8.57, *Alternaria* sp. 5.71, *Geotrichium* sp. 5.71, *Saccharomyces* sp. 5.71, *Trichoderma* sp. 2.86 (Table 1).

Candida sp produces the biosurfactants, sophorolipids and lipomannan that can biodegrade alkanes of carbon chains $C_{10} – C_{16}$ the nephtalene, gasoline and kerosene fractions. *Rhodotorula* sp is known as a nuisance in the aviation industry as they contaminate aviation fuel and metabolize it causing adverse effect on the machine. *Rhodotorula* sp is however a potent petroleum degrader and is good news in crude oil impacted ecosystem (Obiukwu and Otokunefor, 2014).

Total heterotrophic fungi densities of surface water 5.3×10^5 $cfuml^{-1}$; by implication, it simply means that the density of fungi available to metabolize the crude oil in the surface water. Separating the yeast densities from fungi, the yeast density of surface water has a mean of 4.7×10^5 $Cfuml^{-1}$ of which 09.4% (4.4×10^3 $Cfuml^{-1}$) is hydrocarbon utilizing Yeast (HUY).The HUY metabolize the petroleum products, they are reduced to the forms other fungi can metabolize. The biota also showed a yeast density ranging from 1.4×10^3 - 4.5×10^3 $Cfug^{-1}$ of hydrocarbon utilizers. The yeast density of 4.6×10^4 $Cfug^{-1}$ from *Pomadasys commersoni* (gills), 3.5×10^3 $cfug^{-1}$ from *Pomadasys commersoni* (tissue). Unimke *et al*. (2014) records fungi densities of surface water 1.2×10^5 $Cfuml^{-1}$; Hydrocarbon utilizing fungi of surface water 7.2 - 8.9

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

x 10^4 $Cfuml^{-1}$ from Imo River, a crude oil impacted estuary.

The fungi isolated from Ekerekana Creek, Okari, Ogoloma, Edemebikri and Elechi Creek were *Penicillium* sp, *Aspergillus* sp, *Fusarium* sp, *Candida* sp, *Mucor* sp, and *Saccharomyces* sp. The research of Otokunefor and Obiokwu (2014) did not isolate *Metchikowi* sp, *Leucosporidium* sp, *Alternaria* sp, and *Geotrichium* sp. Fungal isolates identified by Douglas and Longjohn (2022) include: *Aspergillus flavus*, *Rhizopus arrhizus*, *Aspergillus niger*, *Rhizopus* sp, *Mucor* sp, *Microsporum* sp, *Cunninghamella* sp and *Candida* sp. from Bonny River.
This research however, similarly isolated *Penicillium* sp as the most dominant fungal isolate.

The PAH level ranged from 0.003 - 0.005 m/l in all the stations with a mean of 0.004 mg/l. There is no significant difference in the PAH levels in the various stations in the different month with $P \leq 0.05$ probability. Daka *et al*. (2020) recorded 0.009 - 0.0122 mg/l in surface water in Olugbobiri, Bayelsa State. The PAH level in the research site shows a contamination factor (c^1f) of 0.571 which implies low contamination since $c^1f < 1$

Edlund and Jansson (2006) reported *Pseudomonas* and *Flavobacterium* spp as the most dominant species of bacteria in an environment with very high concentrations PAH. *Pseudomonas* spp are reported to reduce PAH components of high molecular weights fractions (Chrysene, Benzo (a) pyrene, incleno 1, 2, 3, (cd) pyrene and benzo (g,h,l) phrylene) (Temitayo *et al*., 2019). In a consortium of five strains of bacteria, Temitayo *et al*. (2019) reported complete removal of indeno (1,2,3 cd) pyrene, Benzo (k) flouranthere, Benzo (b) fluoranthene and dibenzo (a,h) anthracene as well as significant reduction of all PAHs. Their presence in this research could be the reason for such small quantity of PAHs.

The levels of PAHs in surface water and *Pomadasys commerconnii* are shown in Figures 5 and 6 respectively. The PAH levels in surface water ranged from $0.003mgl^{-1}$ to $0.005mgl^{-1}$ with a mean value of $0.004mgl^{-1}$. The PAH level of *Pomadasys commerconnii* ranged from $0.02mkg^{-1}$ – $0.04mgkg^{-1}$ with a mean value of $0.031mgkg^{-1}$. There is no significant difference in the monthly PAH levels of *Pomadasys commerconnii* with $P < 1$ at 0.05 probability level.

The PAHs components are shown in Table 4 and Figs. 7- 8. Specifically, Table 2 shows that out of the 14 PAHs components analyzed; pyrene, Benzo(a) anthracene and Benzo(b)fluoranthene were not biomagnified by *Pomadasys commerconnii*. This implies that, it biominifies Benzo(a)anthracene, pyrene and Benzo(b) fluoranthene.

It thus has a mechanism of utilizing these PAHs as energy sources or it has a device of eliminating these PAHs from its system. Also, anthracene is its highest contaminant ($0.14mgkg^{-1}$ i.e. 45.15%) while its least contaminant is pyrene ($0.000026mgkg^{-1}$ i.e. 0.0084%). PAH is lipophilic and easily accumulate in fatty tissue of fish.

According to EU No 835/2011, benzopyrene is the PAH marker using dietary administration. Initially, PAH 4 (Benzo(a)pyrene, chrysene, benzo(a) anthracene, and benzo(b) fluranthene) were used as a PAH marker. The EU standard stipulates a $0.006mg^{-1}$ limit of Benzo(a) pyrene in seafood.

Currently, EU has indicated a $5\mu gkg^{-1}$ ($0.005mgkg^{-1}$) limit of indeno 1,2,3, (cd) pyrene as the most suitable indicator of PAH contamination of sea food. Going by this stipulation, the level of indeno (1,2,3-cd) pyrene in *Pomadasys commerconnii* is $0.0006mgkg^{-1}$ which is lower than the EU standard and giving a c^1f of 0.12 ($0.0006mgkg^{-1}$/ $0.005mgkg^{-1}$) giving a $c^1f < 1$ implying low contamination.

Research has shown that raw fish from unpolluted water do not contain detectable concentrations of PAHs. PAH concentrations in fish are usually low due to rapid metabolization, while higher molecular weight PAHs, do not seem to accumulate in fish (Eisler, 1987). The tendency of fish to metabolize PAHs extensively and rapidly may explain why benzo(a)pyrene, for example, is frequently undetected, or only detected in low concentrations in livers of fish from environments heavily contaminated with Polycyclic aromatic hydrocarbons (PAHs).

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

Conclusion

In spite of the ongoing crude oil marketing in the coastal area of Ikpukulu Creek, and the continuous discharge of crude oil into the surface water, *Pomadasys commerconnii* is not affected enough for its consumption to be of health risk by Polycyclic aromatic hydrocarbon contamination. . Hence, due to the recalcitrant and carcinogenic nature of PAHs in the environment, it is important to create awareness of the danger of this substance in the environment and how to reduce activities that may lead to increase in its concentration in the environment to the lowest minimum. Therefore, there is need for proper enlightenment campaign to educate the people of the areas on the dangers of the increase in these pollutants in the environment, which may lead to biomagnifications of PAHs in fish and biomagnify in food chains. Results from this study indicate high microbial populations of active hydrocarbon utilizing bacteria and fungi, some of which have been implicated in bioremediation of hydrocarbons and PAHs.

References

Adekunle, A. A., Adebambo, O.A. (2007). Petroleum hydrocarbon utilization by fungi isolated from *Detariumsenegalense* (J.F Gmelin) seeds. *J. Am. Sci. 3(1)*: 69-76.

American Public Health Association, APHA. (1995). *Standard Methods of Analysis of Water and Waste Water*. 19th ed. APHA-AWWA-WEF, Washington, DC, USA.

Ariyo, A. B., and Obire, O. (2022). Polycyclic aromatic hydrocarbons (PAHs) and Heavy metal content of abattoir Wastewaters in Bayelsa and Rivers State. *International Journal of Scientific and Management Research*. *5 (5)* 25 – 33.

Atlas, R.M. and Bartha, R. (1992). Hydrocarbon biodegradation and oil spill bioremediation. Adv. *Microbiol. Ecol*. *12*: 287 – 338.

Cheesebrough, M. (2006). *District laboratory practices in tropical countries (Second Edition)*. Cambridge: Cambridge University Press.

Chikere, C.B., Okpokwasili, G.C, lchiakor, O. (2009). Characterisation of hydrocarbon utilizing bacteria intropical marine sediments. *African Journal of Biotechnology*. *8(11)*: 2541-2544.

Daka, E.R., Atalawei, E.S., and Gobo, A.E. (2020). Comparative assessment of surface water quality in Olugbobiri and Ogboinbiri creeks, Bayelsa state, Nigeria. *IIARD International Journal of Geography and Environmental Management*. 6.

Douglas, S. I. and Longjohn, I. (2022). Evaluation of the Microbiology and Some Physicochemical Properties of Bonny River, Rivers State, Nigeria. *Acta ScientificMicrobiology*. *5 (3)*: 33-44.

Douglas, S.I., Ugboma, C.J., and Onwukwe, O.J. (2020). Effect of Three Organic Amendments on Polycyclic Aromatic Hydrocarbon Degradation from Crude oil Polluted Artisanal Refining Site. *International Journal of Current & Applied Sciences*. 9(12): 488 – 502.

Edlund, A., and Jansson, J.K. Changes in active bacterial communities before and after dredging of a highly polluted Baltic Sea sediments. *Applied and Environmental Microbiology*. *72 (2006)*: 6800-6807.

Edori, O. S., and Edori E. S. (2021). Profile of total petroleum hydrocarbons in the water and sediment of the Orashi River Engenni, Rivers State, Niger Delta, igeria. Journal of Global Ecology and Environment. 11(2): 12 – 21.

Eisler, R. (1987). Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates: *A Synoptic Review. Contaminant Hazard Reviews Report.11; Biological Report 85(1.11). U.S. Department of Interior, Fish and Wildlife Service, MD.*, USA.

Ekweozor, I.K.E. (1996). The impact of chronic discharge of petroleum on litoralmacrofauna of Elechi creek, Nigeria. *Niger Delta*. *Biologia*. *I*: 55-60.

European Union Environmental protection Agency (EUEPA,2009). *Framework for the use of rapid measurement techniques (RMT) in the risk management of sediment and water contamination* .UK.

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

FME (2006) Niger Delta natural resource damage assessment and restoration project: phase 1- scoping report *Res. J. Environ. Toxicol. 10*: 29-37.

Holt, J.G., *et al*. (1994). "*Bergey's Manual of Determinative Bacteriology*". Williams and Wilkins, Baltimore, Maryland, USA. Pp 151- 157.

Ibim, A. T and Bongili, B. (2018). Fish stock status of the middle reach of the sombreiro River of the Niger Delta Basin, Nigeria. *London Journal of Research in Science: Natural and Formal. 18(1): Compilation 1.0.* 37 – 51.

Ideriah, T.J.K., David-Omiema, D., Ogbonna, D.N. (2012): Distribution of heavy metals in water and sediment along Abonnema shoreline, Nigeria. *Resources and Environment*. *2(1)*: 33 – 40.

Isaac, O.A., Gabriel, A.D., Ukinebo, I.E., and Mry, K.E. (2018). Proximate composition and heavy metal analysis of three aquatic foods in Makoko River, Lagos, Nigeria. *Hindawi Journal of Food Quality vol. 2018, Article ID 2362843, 6 pages http://doi.org/10.1155/2018/2362843.*

Jih, Gong Y, Duan J. *et al*. (2018). Degradation of petroleum hydro carbons in seawater by simulated surface level atmospheric zone: reaction kinetics and effect of oil dispersation. *Mar. Pollut. Bull. 135*: 427-440.

Nrior, R.R., Odokuma, L.O., Tete, E. (2017). Ultimate Biodegradation of Industrial Detergent Used in the Upstream Sector of the Nigeria Petroleum Industry in Freshwater, Brackish and Marine Water. *International Journal of Ecotoxicology and Ecobiology. 2(4)*:134-144.

Nrior, R.R., Ogbonna, D.N., Alabo, A.E. (2017). Biodegradation of Drilling Fluid used in Upstream Sector of the Nigeria Petroleum Industry in Marine Water Environment. *Int. J. Waste Resour. 7(4)*: 302-306.

Obire. 0., and Nwaubeta, O. (2002). Effect of refined petroleum hydrocarbons on soil Physiochemical and bacteriological characteristics. *Journal of Applied Environmental Management. 6(1)*: 39-44.

Obiukwu, C.E., and Otokunefor, T.V. (2014). Microbial Community Diversity of a water body in Niger Delta affected by refinery effluent. *Universal Journal of Microbiology Research. 2(1)*: 1 – 14.

Oboh, B.O, llori, O.M., Akinyemi, J.O *et al*. (2006). Hydrocarbon degrading potentials of bacteria isolated from a Nigerian Bitumen (Tarsand) deposit. *Nat Sci. 4*: 51-57.

Okpokwasili, G.C., and Amanchukwu, S.C. (1998). Petroleum hydrocarbon degradation by Candida species. *Environmental International. 14*:243-247.

Prescott, L.M., Harley, J.P., Klein, D.A. (2005). *Microbiology*. 6th Ed. McGraw Hill, London.

RPI/NNPC (1985a): *Institute of pollution studies for the establishment of control criteria and standards against petroleum related pollution in Nigeria (RP/IR84/ 4/15-7).*

RPI/NNPC (1985b): *Environmental baseline studies for the establishment of control criteria and standards against petroleum related pollution in Nigeria (rpi/ir84/4/15 – 7).*

Temitayo, O.O., Oluyege J.O., Aderiye B.L., Oluwole, O.A. (2019). degradation of polyaromatic fractions of crude oil and detection of catabolic genes in hydrocarbon-degrading bacteria isolated from Agbabu bitumen sediments in Ondo State. *AIMS Microbiology*. *5(4)*: 308-323.

Temitayo, O.O., Oluyege, J.O., Aderiye, B.L., Oluwole, O.A. (2019). Degradation of polyaromatic fractions of crude oil and detection of catabolic genes in hydrocarbon-degrading bacteria isolated from Agbabu bitumen sediments in Ondo State. *AIMS Microbiology*. *5(4)*: 308-323.

Unimke, A.A., Antais, P., Agbor, R.B., Nseabasi, N.O., Agbo, B.E. (2014): Evaluation of seasonal variation in the microbial and heavy metal concentration of Imo River Estuary of the Niger Delta. *Advance Research in Agriculture and Verteterinary Science*. *1 (2)*: 88 – 94.

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.

Vincent- Akpu, I., and Okosiemiema, I.J. (2019). Trace elements in water, sediments and Biota from Ikpukulu Creek, Port Harcourt River State. *doi: 10. 2044/ preprints 2019 040145. 1*.

Vinithin, C., Sudhakar, S., Ravir Kumar, R. (2015) Biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus*. *Int J. Curr. Microbiol. Appl. Sci. 4*: 318-329.

Wemedo, S.A., Nrior, R.R., and Ike, A.A. (2018). Biodegradation Potential of Bacteria Isolated from Crude Oil Polluted Site in South South, Nigeria. *Journal of Advances in Microbiology. 12(2)*:1-13

Zobell, C.E. (1971) Sources and biodegradation of carcinogenetic hydrocarbons. *Proc. Conf, prevent, controls spills. Washington*, USA. *Pp*. 441-451.

Citation: Benibo *et al*. (2023). Microbiological and polycyclic aromatic hydrocarbon profile of *Pomadasys commersonnii* harvested from crude oil impacted creeks in Borikiri, Port Harcourt, Nigeria. *International Journal of Microbiology and Applied Sciences*. *1(1)*: 32 - 45.