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Research Article

# Hydrocarbonoclastic Bacteria and Polycyclic Aromatic Hydrocarbon Profile of Surface Water in Borikiri Wetlands, Port Harcourt, Nigeria

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## Abstract

The presence of Polycyclic Aromatic Hydrocarbons (PAHs) in territorial water is of great concern due to the carcinogenicity. In this study, monthly collection of surface water from four artisanal petroleum marketing points was made from November 2019 - October 2020 using standard methods. Hydrocarbon utilizing (Hydrocarbonoclastic) bacteria were isolated by vapour phase transfer method using mineral salts medium, isolates were identified based on cultural, morphological and biochemical characteristics. The mean HUB count was 1.2 x $10^4$ $cfuml^{-1}$. The HUB isolates were *Pseudomonas,* 12.4%; *Chromobacterium,* 1.8%; *Serratia,* 5.3%; *Corynebacterium,* 6.25%; *Escherichia,* 12.1%; *Bacillus,* 12.4%; *Staphylococcus,* 10.6%; *Micrococcus,* 3.5%; *Citrobacter,* 7.1%; *Enterobacter,* 3.5%; *Acinetobacter,* 3.5%; *Alcaligens,* 4.4%; *Nocardia,* 3.5%; *Streptococcus,* 6.2%; and *Shigella.* 6.2%. The PAHs level in the surface water samples were determined used Gas Chromatography - Flame Ionization Detector, GC-FID. The PAHs level observed was a mean of 0.004$mgl^{-1}$ which is below the EU permissible limit of 0.007$mgl^{-1}$; which can be attributed to activities of the polycyclic aromatic hydrocarbon mineralizing bacteria present as a natural consortium in the ecosystem. If the presence of these strains of bacteria continues in the ecosystem, the continual stability of the ecosystem can be ensured. This study reveals a natural bacterial consortium that can degrade PAH in the surface water within a short time.

**Keyword**: Wetland; Crude Oil Pollution; Hydrocarbonclastic Bacteria; PAHs; Borikiri

## Introduction

Petroleum hydrocarbons are frequently discharged into the water bodies in the oil producing communities in Rivers State due to improper regulated crude oil refining and marketing activities. Polycyclic aromatic hydrocarbons are of great significance among the petroleum hydrocarbons making up the crude oil [1-3].

PAHs are hydrocarbons of 2-6 rings and are 16 in number. PAHs of 2-3 rings include Naphthalene, Acenaphthene and Acenaphthylene. PAHs of 4-6 rings include Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo (a) anthracene, chrysene, Benzo (b) fluoranthene, Benzo (k) Fluoranthene, Benzo (a) pyrene, indeno (1,2,3-cd) pyrene, dibenzo (a,h) anthracene and Benzo (g,h,i) phyrelene. The presence of PAHs in the ecosystem is of great concern due to the carcinogenicity of some e.g. Benz (a) pyrene metabolite is rated group 1 Carcinogen in IARC (International Agency for Research on Cancer). Some are lipophilic and can be easily taken into tissues of fishes when exposed, there become sequestered in lipid droplets become in accessible to microbial enzyme which could easily biodegrade them [1].

Apart from their carcinogenicity, they can easily be bioaccumulated and eventually biomagnified across the food chain [4]. The ecosystem however is equipped with keystone organisms and bioengineers that are equipped with genetic makeup that

enables them to enzymatically oxidize these environmental threats. *Pseudomonas acruginosa* grows and reduces surface tension under a wide range of pH, salinities and temperature. The biosurfactant production is also recorded for *Bacillus* spp*., Micrococcus spp., Arthroacter* spp and *Staphylococcus* spp [5].

Hydrocarbonoclastic bacteria are those equipped with genetic systems that enable them oxidize hydrocarbons and use them as energy source. This research aims at evaluating the PAH level of the research area and also the extent to which the natural environment "purifiers" - aerobic HUBs are present to match the PAHs contamination.

## Materials and Methods

### Study area

The selected sample stations were four artisanal petroleum marketing points along the creeks of between Bieama and Pereama of Ikpukulu Creek, Borikiri, Port Harcourt. The study location is an Estuary with salinity ranging from 10% - 30% and pH ranging 6.7-6.9 and a mean temperature of $28^0$C - $32^0$C [6]. It is used for recreation, sewage and refuse disposal as well as transportation and fishing activities. The vegetation is mangrove dominated by *Rhizophora racemosa, Rhizophora nitida, Nypa fruticans, Ipomoea aquatic, Nymphea lotus, Mimosa pigra and Eichornia nataris* with subsoil characterized by typical fibrous clayey mud that shows a large value of compressibility [7]. The sediment is lateritic clay with intertidal mudflats. The study area is characterized by diurnal ebb and flow tides.

The four sampling points and their geographic coordinates were: Station 1: Bieama - Ikpukulu jetty (Lat $4^0$ $43^1$ N, Long $7^0$ $01^1$ E), Station 2: MTN mast (Lat $4^0$ $44^1$ N, Long $7^0$ $1^1$ E), Station 3: Island (Lat N $4^0$.744065, Long E 7.035092), Station 4: Okilopolo (Lat N $4^0$ $45^1$, Long E $7^0$ $2^1$).

### Sampling

A total of 240 surfaces water samples were collected from November 2019 - October 2020 (5 from each of the 4 stations for 12 months). The area of study was not affected by the lockdown Covid-19 pandemic. Sterile 1 liter plastics bottles carefully opened under the water within a depth of 10 cm were used for the collection for bacterial analysis. Samples from each station were homogenized to obtain bulk composite [8]. Samples were labeled and transported in Ice-Chest to the microbiology laboratory of the Rivers State University of Port Harcourt for microbial analysis [9].

Water samples for PAH analyses were collected with one (1) litre glass bottles. Water samples were filtered and preserved with hydrochloric acid to prevent microbial oxidation on site [8]. Labeled samples were transported ice-chest and transported to the laboratory of the Institute of Pollution studies (IPS) Rivers State University Nkpolu Oruworukwo Port Harcourt for analysis.

### Isolation and enumeration of hydrocarbon utilizing bacteria (HUB)

Collected water samples 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 nitrate to prevent fungal contamination. Isolation and enumeration were done using spread plate techniques [10-12] using vapour phase transfer technique on mineral salts agar for HUB. The plates were incubated at $30^o$C for 7 days for HUB, while for THB, incubation was at $28^o$C for 24hours. Enumeration of isolates was done and expressed as $cfuml^{-1}$ using

$$\text{cfu ml}^{-1} = \frac{\text{Plate counts (No.of Colonies)}}{\text{Dilution x Volume plated}}$$

### Identification of bacterial isolates

Bacterial isolate were identified based on cultural morphological and biochemical and characteristics using Bergey's Manual of Determinative Bacteriology [11,13].

### PAH analysis of surface water

From each water sample, 250 ml of water samples were measured into a separating funnel rinsed with dichloromethane. To the 250 ml water sample, 25 ml 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 get and anhydrous sulphate for cleaning and dehydration. The organic extract obtained was injected into gas chromatograph. A 5µl of the concentrated sample was injected by means of hypodermal syringe through a rubber septum into the column. The vapour fractions of the PAHs were automatically detected as it emerges from the column by the flame ionization detector (FID). The results were expressed in $mgl^{-1}$.

## Results and Discussion

The hydrocarbon utilizing bacteria counts (HUBC) and the hydrocarbonoclastic bacteria isolated from the surface water as analyzed are summarized in table 1, figure 1 respectively.

The total heterotrophic bacterial counts for the sampling stations are as follows: Station 1 ranged from 1.0 x $10^7$ to 2.1 x $10^7$cfu/ml, station 2 ranged from 1.4 x $10^7$ to 1.5 x $10^7$ cfu/ml, Station 3 ranged from 1.3 x $10^7$ to 1.5 x $10^7$ cfu/ml, while Station 4 ranged from 1.4 x $10^7$ to 1.9 x $10^7$ cfu/ml. Hydrocarbon utilizing

| Station | Month (Nov. 2019 - Oct. 2020) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nov | Dec | Jan | Feb | Mar | Apr | May | June | Jul | Aug | Sep | Oct |
| 1 | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^3$ | $1.0x10^3$ | $1.0x10^5$ | $1.0x10^5$ | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^4$ |
| 2 | $1.2x10^4$ | $1.2x10^4$ | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^4$ | $1.3x10^4$ | $1.4x10^4$ | $1.2x10^4$ | $1.4x10^4$ | $1.2x10^4$ | $1.4x10^4$ | $1.0x10^4$ |
| 3 | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^4$ | $1.0x10^3$ | $1.0x10^4$ | $1.0x10^5$ | $1.0x10^5$ | $1.0x10^5$ | $1.0x10^3$ | $1.0x10^5$ | $1.0x10^3$ |
| 4 | $1.5x10^4$ | $1.4x10^4$ | $1.5x10^4$ | $1.5x10^4$ | $1.6x10^4$ | $1.7x10^4$ | $1.4x10^4$ | $1.6x10^4$ | $1.7x10^4$ | $1.3x10^4$ | $1.5x10^4$ | $1.4x10^4$ |

**Table 1:** Hydrocarbon Utilizing Bacteria Count (cfu $ml^{-1}$)in Surface Water From Borikiri Wetlands.

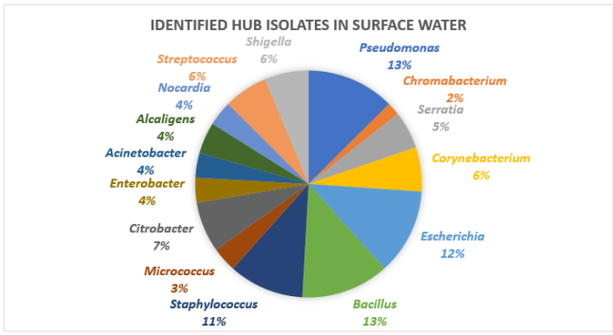


**Figure 1:** Frequency of Hydrocarbonoclastic Bacterial Isolates from Crude Oil Polluted Surface Water in Borikiri Wetlands.

bacterial counts for Station 1 ranged from 1.0 x $10^3$ to 1.0 x $10^5$ cfu/ml, Station 2 ranged from 1.0 x $10^4$ to 1.4 x $10^4$ cfu/ml, Station 3 ranged from 1.0 x $10^3$ to 1.0 x $10^5$ cfu/ml and Station 4 ranged from 1.3 x $10^4$ to 1.7 x $10^4$ cfu/ml. These results indicate the presence of active population of indigenous organisms that may play a role in the degradation of PAHs.

The following hydrocarbonoclastic bacteria were identified with their frequency of occurrences *Pseudomonas* spp 13%, *Chromobacterium* spp 2%, *Serraria* spp. 3%, *Corynebacterium* spp. 6%, *Escherichia* 12%, *Bacillus* spp 13%, *Staphylococcus* spp.11%, *Micrococcus* spp.5%, *Citrobacter* spp 7%, *Enterobacter* spp.4%, *Acinetobacter* spp. 4%, *Nocardia* spp. 4%, *Streptococcus* spp. 4% and *Shigella* spp 4%. Members of these genera have been identified from hydrocarbon polluted surface water. *Pseudomonas* and *Bacillus* spp. had the highest frequency of isolation. This observation has been made by previous researchers, the dominance of *Pseudomonas* spp may be due to its metabolic versatility while *Bacillus* spp. may be due to its ability to form endospores, which enables it to survive even in very harsh environments. Chikere *et al.*[14] identified the following genera of bacteria; *Bacillus, Nocardia, Staphylococcus, Pseudomonas, Flavobacterium, Escherichia, Acinetobacter* and *Enterobacter* from marine sediment. Ariyo and Obire [15] also reported by *Bacillus* sp., *Pseudomonas* sp., *Escherichia coli*, *Staphylococcus* sp., *Streptococcus* sp, *Alcaligenes* sp., *Salmonella* sp, *Shigella* sp and *Vibrio* sp in Abattoir wastewaters.

*Pseudomonas, Bacillus, Alcaligenes, Chromobacterium,* and *Arthrobacter* were also identified from soil polluted by hydrocarbon with high concentrations of PAH [16]. Edlund and Jansson [17] reported *Pseudomonas* and *Flavobacterium* as the most dominant species of bacteria in an environment with very high concentrations of PAH. Chikere *et al.*[14] has also reported the ability of some *(Pseudomonas, Bacillus, Acinetobacter, Staphylococcus, Nocardia)* of these isolates identified as having the ability to biodegrade PAH in sediments. The presence of *Escherichia* is an indication of the pollution of the surface water by feacal contamination. This may be due to the direct disposal of untreated sewage into the water body.

*Pseudomonas* spp are reported to reduce PAH components of high molecules weights fractions (Chrysene, Benzo (a) pyrene, incleno 1, 2, 3, (cd) pyrene and benzo (g,h,l) phrylene) [5]. In a consortium of five strains of bacteria, Temitayo., *et al.* [5] reported complete removal of ideno (1,2,3 cd) pyrene, Benzo (k) flouranthere, Benzo (b) fluoranthene and dibenzo (a,h) anthracene as well as significant reduction of all PAHs. Three strains of the hydrocarbonoclastic bacteria used in that research; *Pseudomonas* spp, 15%, *Bacillus* spp, 15% *Micrococcus* sp 2.4%. Their presence in this research could be the reason for such small quantity of PAHs inspite of the extent of crude oil marketing in the research site. *Pseudomonas* spp, *Acinetobacter* spp; *Nocardia* spp and *Bacillus* spp possess nahH genes which codes for catechol 2, 3-dioxygenase for degradation of aromatic via meta-cleavage pathway. This however is a incomplete mineralization. *Micrococcus* spp, *Pseudomonas* spp and *Bacillus* spp Cat. A gene which codes for Catechol 1, 2-dioxygenase for the complete mineralization of the aromatics via otho-cleavage pathway producing metabolites that enter the TCA cycles [4].

| PAH Components | Concentration ($mgl^{-1}$) |
|---|---|
| Naphthalene | 0.000016 |
| Acenaphthylene | 0.00006 |
| Acenaphthene | 0.000075 |
| Fluorine | 0.00009 |
| Phenanthrene | 0.00003 |
| Anthracene | 0.00006 |
| Fluoranthene | 0.00006 |
| Pyrene | 0.0003 |
| Benzo (a) anthracone | 0.00019 |
| Chemsene | .0000099 |
| Benzo (b) fluoranthene | 0.0024 |
| Benzo (k) Fluorathene | 0.00015 |
| Indeno (1,2,3) (d) pyrene | 0.0002 |
| Dibenes (a, k) anthracene | 0.00024 |
| Total | 0.004 |

**Table 2:** PAH Components in Surface Water (Nov 2019 - Oct 2020).

The components of PAH in the Borikiri wetland surface water is presented in table 2 while the levels of PAH in the surface water samples as analyzed are as shown in figures 2 and 3.

In nature biodegradation typically involves succession of species in the consortia of microbes present with a single bacteria strain with the metabolic capacity to biodegrade all the components found in the crude oil [18].

The means PAH level of the surface water was 0.004 mg/l which is lower than the DPR standard of 0.007 mg/l. 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  1 at 0.05 probability ($t_{cal}$ = 11.11 and $t_{tab}$ = 12). Daka., *et al.* [15] recorded 0.0058 - 0.009 $mgl^{-1}$ in surface water of Ogboinbiri and 0.009 - 0.0122 gmg/l in Olugbobiri in Bayelsa State. PAHs levels in abattoir soils reported by Ariyo and Obire [20] were also below the EU permissible limits. The PAH level in the research site shows a contamination factor $c^1f$ of 0.571 $\left(\frac{0.004 mgl^{-1}}{0.007 mgl^{-1}}\right)$ which implies low contamination since $c^1f < 1$. The highest PAH component recorded is Benzo (b) fluoranthene (0.0024 $mgl^{-1}$) and constitutes 60% of the PAH contamination of the surface water. Benzo (b) fluoranthene is one of the carcinogens reported in ecosystems [5]. Its affinity with plasma membrane makes it cause cell death by simple osmosis and turgidity as the cell attempts to dilute its plasma content by allowing in water by osmosis. Among the least contaminating PA components are: Chrysene > Fluorene > acenapthene > acenaphthylene > fluoranthene > nepthalene. Naphthalene is the least contamination PAH (0.000176 $mgl^{-1}$) which is 0.44%. The low quantity of PAH could be attributed to photooxidation, volatility of the components and activities of hydrocarbononoclastic bacteria.

The levelest of the low molecular weight (LMW) PAHs in the surface water recorded is 0.0007 mg/l (Table 3). LMW PAHs include (Naphthalene, acenaphthylene, acenaphthene, fluorine, phenanathrene, fluoranthene) while the HMW PAHs include (Chrysene, benz (b) fluoranthene, benz (a) anthracene, benz (a) pyrene, benzo (k) fluoranthene, indeno (1,2,3 cd) pyrene, dibene z (a,h) anhracene)

| Station | Month (Nov. 2019 – Oct. 2020) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct |
| 1 | 0.004 | 0.004 | 0.0035 | 0.0045 | 0.004 | 0.003 | 0.005 | 0.005 | 0.003 | 0.005 | 0.004 | 0.004 |
| 2 | 0.003 | 0.003 | 0.004 | 0.005 | 0.005 | 0.004 | 0.005 | 0.005 | 0.003 | 0.003 | 0.004 | 0.004 |
| 3 | 0.004 | 0.004 | 0.005 | 0.003 | 0.004 | 0.003 | 0.004 | 0.004 | .005 | 0.004 | 0.004 | 0.004 |
| 4 | 0.004 | 0.004 | 0.004 | 0.005 | 0.005 | 0.004 | 0.004 | 0.003 | 0.003 | 0.005 | 0.005 | 0.004 |

**Table 3:** PAHs Levels ($mgl^{-1}$) in Surface Water From Borikiri Wetlands, Port Harcourt.

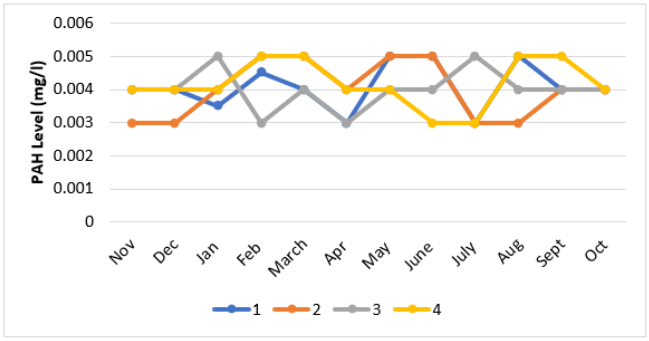


**Figure 2:** Comparative PAH Levels in Surface Water (mg/l) in Crude Oil Polluted Stations in Borikiri Wetlands, Port Harcourt (Stations 1, 2, 3 and 4).

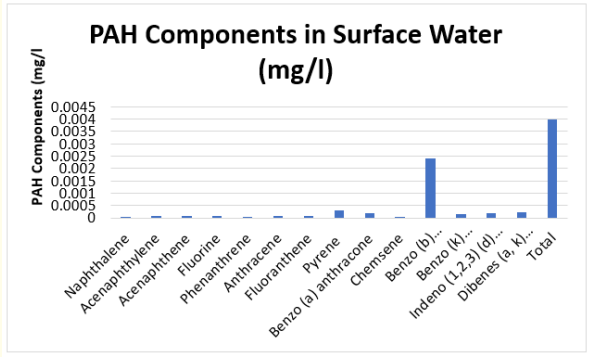


**Figure 3:** Comparative PAH Components in Surface Water (mg/l) in Crude Oil Polluted Stations in Borikiri Wetlands, Port Harcourt.

The HMW PAHs concentration is 0.0033 mg/l (Table 3). The ratio of LMW to HMW in the surface water hence is 0.2 (0.0007 mg/l/.0033 mg/l). Since this is < 1, it implies that PAH source in this ecosystem is pyrogenic. This falls in line with the reported ratio of <1 for Nigeria Delta ecosystem [21]. Recorded maximum PAH level for Niger Delta Surface Water is 0.9 mg/l. Going by the Phenanthrene/anthracene ratio for surface water, CP1 (Carbon preference index) is 0.5 (00006 $mgl^{-1}$) Fluoranthene/Pyrene is 0.2 (0.00006 $mgl^{-1}$/0.0003 $mgl^{-1}$).

The CP1 calculated is < 1 indicating that the PAHs in the surface water of this research site is predominately pyrogenic (products of incomplete combustion). It could be explained that, petrogeic PAH contaminants are faster mineralized than the pyrogenic PAHs due to the significant presence of hydrocarbonclastic bacteria in the ecosystem.

## Conclusion

The surface water of the Ikpukulu Creek has a low contamination of Polycyclic Aromatic Hydrocarbons which can be attributed to activities of the polycyclic aromatic hydrocarbon mineralizing bacteria present as a natural consortium in the ecosystem. If the presence of these strains of bacteria continues in the ecosystem, the continual stability of the ecosystem can be ensured.

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