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Enumeration of Total Heterotrophic and Petroleum-degrading Bacteria Counts in Water and Sediments from Diobu Creek, Port Harcourt, Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Introduction: Bacteria play a significant role in the biochemical cycling of nitrogen, carbon, sulphur, and oxygen in aquatic environments. Hydrocarbons are released naturally from oil seeps and incidental discharges which represents a significant source of pollution. Estuarine systems are particularly susceptible to anthropogenic hydrocarbon contamination. Although oil contaminants are weathered by photo-oxidation and evaporation complete degradation is dependent on the metabolic activities of the microbial population inherent to the area.
Aim: This work aims to determine the total heterotrophic bacterial counts (THBCs) and petroleum-degrading bacteria counts (PDBCs) in water and sediment from Diobu creek.
Methodology: The THB and PDB in water and sediments from a polluted creek (Diobu Creek) were determined by serial dilution and plating on nutrient agar and petroleum-degrading bacteria agar and the isolated bacteria were identified.
Results: The THBCs in water were from 6.3 x 10^3 CFU/mL and 6.33 x10^3 CFU/mL, the highest THBCs were recorded in June (wet season). The THBCs in sediments was from 1.7 x 10^6 CFU/g to 1.85 x10^6 CFU/g. The highest THBCs were recorded in the month of June. The PDBCs in water was

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from 0.2 x 10^3 CFU/mL and 3.9 x10^3 CFU/mL, whereas the PDBCs in sediments ranged from 3.4 x10^6 to 9.5 x 10^6 CFU/g, high counts were obtained if sampling were carried out in rain or after rain. The bacteria isolated were B*acillus sp, Pseudomonas sp., Corynebacterium sp., Acenetobacter sp., Alkaligenes sp., Escherichia coli, Micrococcus, Klebsiella sp.* and *Flavobacterium sp*.
Conclusions: The increased counts of PDBs in aquatic environments might be stimulated by the presence of pollutant hydrocarbons or chemicals discharged into the creek which was degradable by bacteria. The activity of PDBs in detoxifying polluted environments is the most eco-friendly.

Keywords: Enumeration; total heterotrophic; petroleum degrading; polluted; Creek.

1. INTRODUCTION

The ubiquity of bacteria confers on them the ability to inhabit any habitat on planet earth surface, having greater biomass than any other group of organisms. This is achieved by a large surface area to volume ratio, metabolically versatile and obtaining energy by oxidising carbon, parasitism, chemoautotrophy and photo-autotrophy [1]. Bacteria play a major role in the biochemical cycling of nitrogen, carbon, sulphur, and oxygen in aquatic environments [2,3]. About 470 thousand and 8.3 million tons of petroleum hydrocarbons are globally released into aquatic environments each year [4]. Half of these are released naturally from oil seeps and incidental discharges, which represents a significant source of pollution [3]. From 2000 to 2013, 43 large marine oil spills and 167 medium sized marine oil spills were reported [5]. Estuarine systems are particularly susceptible to anthropogenic hydrocarbon contamination. The concentrations of poly-aromatic hydrocarbons (PAHs) were shown to exceed 100 mg/ Kg sediment in UK location at Melford Haven [6].

Although oil contaminants are weathered by photo-oxidation and evaporation, complete degradation depends on the metabolic activities of the microbial population inherent to the area [7]. Petroleum hydrocarbons are the most widespread contaminants within the marine environment. Pollution by hydrocarbons in marine environments may be caused by various natural seepages and/or anthropogenic activities (discharge during tanks and/or ships transportation and/or pipeline failures) coupled with chronic pollution (ships, harbours, oil terminals, freshwater run-off, rivers and sewage systems). Of particular concern is the accumulation of low molecular weight PAHs such as naphthalene, which had been found at a concentration of 2.4 mg/Kg dry weight sediment in the Tyne estuary [6], and are acutely toxic to aquatic invertebrates at concentrations as low as 8 mg/L [8]. In addition, much high molecular weight PAHs such as chrysene, which was found at concentrations of about 6.94 mg/Kg dry weight sediment at Milford Haven [6], were classed as carcinogens and can cause chronic toxic effects in fish and invertebrates [9].

Different hydrocarbon degrading bacteria have been isolated from hydrocarbons polluted environments as hydrocarbon contaminants are released into the environment by human activities [10,11]. The presence of these hydrocarbons in an environment was known to stimulate the presence of hydrocarbons degrading bacteria in the affected environment. The total heterotrophic bacterial and petroleum degrading bacteria counts obtained from oil polluted Bodo Creek in Rivers State, Nigeria; Gokana, Rivers State and Ennore Creek, India, [1,12,13] were similar to the bacterial counts and genera isolated by other researchers [14,15,16,17].

The hydrocarbon utilising bacteria isolated and identified from the above studies were; *Bacillus*, *Nocardia*, *Staphylococci*, *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Acinetobacter Enterobacter*, and *Bacillus sp*. The gram-positive bacteria (rod) *Bacillus* were the most predominant, followed by the gram-negative *Pseudomonas;* these bacteria have also been isolated from hydrocarbons polluted environments by other investigators [14].

About 247 strains of bacteria were isolated by other workers from hydrocarbon polluted environments. The bacteria isolated were predominantly gram-negative bacteria with the prevalence of 62.34% and gram-positive bacteria 35.63%. The gram negatives isolated were four genera namely, *Pseudomonas sp.*, *Vibrio sp., Achromobacter sp.,* and *Serratia sp.* with prevalence 39.68%, 15.78%, 3.64% and 3.24% respectively; whereas the gram-positive bacteria were two genera, *Bacillus* s*p* and *Micrococcus sp* with prevalence of 27.94% and 7.69% respectively [1,14].

The bacteria isolated from the brackish polluted water of Bodo Creek were, *Bacillus sp*. *Alkaligenes, Entrobacter, Cetrobacter, Myroides, Instnibacillus, Pseudomonas,* and *Escherichia coli* [1].

Other workers also isolated *Bacillus sp. Proteus, Pseudomonas, Flavobacterium, Corynebacterium, Serretia, Micrococcus, Klebsiellea, Enterobacter and Azotobacter* from polluted water and sediments [11,13]. In Ennore Creek, India, *Bacillus, Micrococcus, Pseudomonas, Vibrio, Acenetobacter, Serretia* were isolated from hydrocarbon polluted water and sediments [15]. The aims of this study is to determine the total heterotrophic bacterial counts (THBCs) and petroleum degrading bacterial counts (PDBCs) in water and sediments from Diobu Creek in Port Harcourt.

2. MATERIALS AND METHODS

2.1 Study Area

Diobu creek transects Port Harcourt metropolis and it originates from behind Mile four (4) in Rumueme Port Harcourt, Rivers State, Nigeria. It empties into Amadi Creek in the old Government Reserved Area (GRA). Numerous activities were carried out by different individuals beside the creek and the most prominent are the mechanic workshops and car engine wash, coupled with municipal wastes discharging into the creek. The creek is constantly exposed to petroleum hydrocarbons and other pollutants. For the purpose of this study, four sampling stations were established along the creek which was at least 200 meters apart. Station one was marked as control station upstream which was about 1Km from station 2, with relatively little human activities and pollution. The coordinates for the stations were established using Gram 76GPS.

2.2 Collection of Samples

Samples of water and sediments were collected monthly during low tide at the established sampling stations. This was possible by using the tidal data published by the Nigerian Navy Hydrographic School as a guide. Samples were collected from November 2016 to October 2017 from each of the sampling stations.

2.3 Collection of Water and Sediments for Bacteriological Analysis

The water samples for bacteriological examinations were collected once monthly aseptically to avoid contamination. Detailed quality assurance and quality control procedures were followed for sample collection, holding and analysis (APHA, 1976). The water samples were collected at a depth of about 15 – 25 cm in the opposite direction of water current into commercial sterile universal bottles.

About 20g of sediment were collected at low tide from the oxidised thin layer (1 – 5 cm) surfaces of exposed mud flats along transects across intertidal zone into a wide mouth sterile glass containers sterilised by autoclaving at 121°C for 15 minutes. The water and sediment samples were immediately taken to the laboratory for bacteriological examinations.

2.4 Preparation of Media

Nutrient agar was prepared according to the manufacturer's instruction and stored in the refrigerator for total heterotrophic bacterial counts.

2.5 Media for the Isolation of Petroleum-degrading Bacteria

The isolation of petroleum-degrading bacteria were carried out using engine oil/diesel 3:1 ratio 5 mL, ammonium chloride 0.5 g/L, dipotassium hydrogen phosphate 0.5 g/L, disodium hydrogen phosphate 2.5 g/L, agar 15 g [18]. The media was sterilised by autoclaving at 121°C for 15minutes and dispensed into disposable Petri-dishes, allowed to solidify and stored in the refrigerator for subsequent uses.

2.6 Isolation

The method of isolation used was the ten-fold dilution technique. Decimal dilution of the samples was made by adding 1 mL (water) or 1g (sediment) of the sample to 9.0 mL sterile normal saline to give an initial dilution of 1:10. Subsequent serial dilutions were made by adding 1 mL of the last dilution to 9.0 mL of fresh sterile saline. Lastly, 0.1 mL of appropriate dilution were plated out in duplicate on agar medium and evenly spread with a sterile glass rod spreader [19].

The plates for total heterotrophic counts were incubated at 30 - 35°C for 18-24 hours on nutrient agar and the plates for petroleum-degrading bacteria incubated at ambient temperature.

2.7 Identification of Isolated Bacteria

Series of tests including Gram's stain, chemical and biochemical tests were used for the identification of isolated bacteria such as catalase, coagulase, indole, citrate, methyl red, hydrogen sulphide production, Vorges Prauskouer, oxidase, and carbohydrate fermentation tests etc [18].

2.8 Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 5.07.

3. RESULTS

3.1 Total Heterotrophic Bacterial Counts in Water (10^3 CFU) and Sediments (10^6 CFU)

The THBCs from water was high in March, April, and May which were 6.33 x 10,^{3} 5.38 x 10^3 and 5.96 x 10^3 CFU/mL respectively. The highest count was in June, while the lowest counts were recorded in November. High THBCs from sediments obtained were in November, 1.76 x 10^6 CFU/g, January, 1.60 x 10^6 CFU/g February, 1.70 x 10^6 CFU/g, June, 1.84 x 10^6 CFU/g and September, 1.65 x 10^6 CFU/g respectively. The highest THBC was obtained in July. The total heterotrophic bacteria counts in sediments were high compared to results obtained in water, as shown in Figs. 1 and 2.

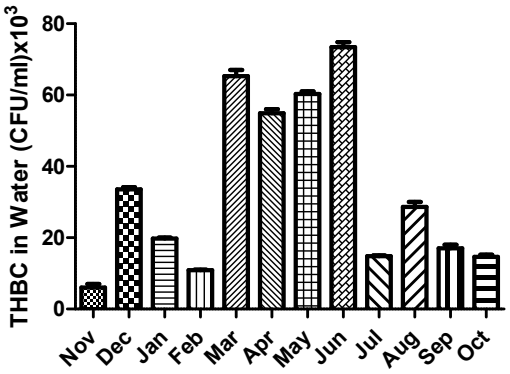

Fig. 1. Monthly THBCs in water

3.2 Petroleum Degrading Bacterial Counts in Water and Sediments

The monthly petroleum degrading bacterial counts (PDBCs) in water were between 0.2 x 10^3 CFU/mL and 3.9 x 10^3 CFU/mL, the counts were high in December, April and February as shown in Fig. 3. In sediment samples PDBCs range from 3.4 x 10^6 CFU/g to 9.5 x 10^6 CFU/g, the highest PDBCs were recorded in February, whereas the lowest was in March as shown in Fig. 4.

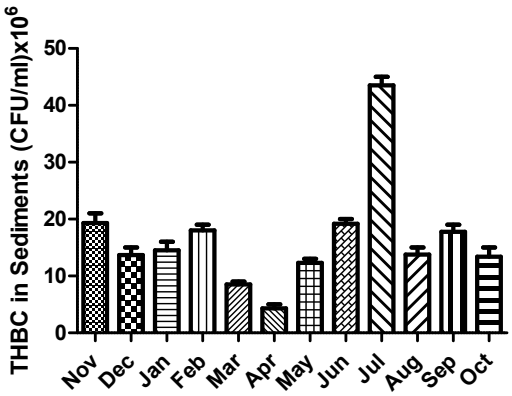

Fig. 2. Monthly THBCs in sediments

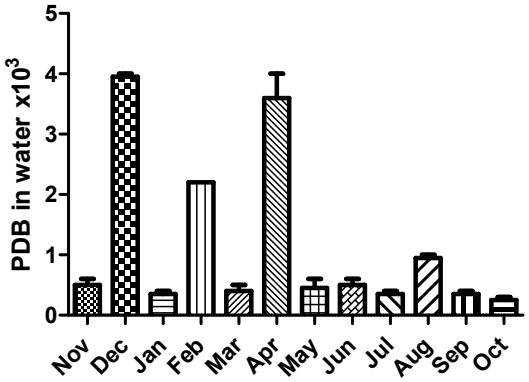

Fig. 3. Monthly PDBCs in water

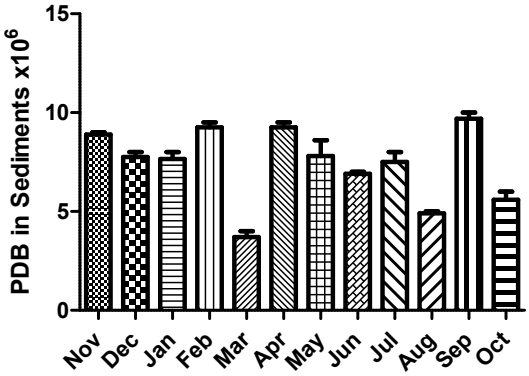

Fig. 4. Monthly PDBCs in sediments

Table 1. Identification of isolated bacteria

S/No	Colour	Surface	Edge	Translucency	Texture	Gram Rxn	Size	Shape	Motility	Methyl Red	Voges Proskauer	Oxidase	H2S Production	Indole	Coagulase	Catalase	Citrate	Urease	Starch Hydrolysis	Glucose	Lactose	Sucrose	Maltose	Galactose	Mannitol	Arabinose	Oxidative	Fermentative	Bacteria
1	M	R	E	C	D	+	Md	Rd	+	-	+	-	-	-	-	+	-	-	+	A	A	A	A	A	±	-	+	+	***Basillus sp.***
2	G	S	E	C	Mt	-	Md	Rd	+	-	-	+	-	-	-	+	+	-	-	A	A	A	A	A	A±	A±	+	-	***Pseudomonas sp.***
3	Cr	S	Sr	O	D	-	CB	Rd	+	-	-	+	-	-	-	+	-	-	-	A±	--	-	-	A±	-	A±	+	-	***Alcaligenes Sp.***
4	W	S	E	O	D	+	L	Rd	-	-	-	-	-	-	-	+	-	+	-	A	A	A	-	A	A	A	-	-	***Corynebcterium Sp.***
5	Y	S	E	C	Mt	-	St	Rd	-	-	-	+	-	+	-	+	-	-	-	-	-	A	-	-	A	-	+	-	***Flavobacterium Sp.***
6	V	Dl	E	O	Mt	-	Sm	Rd	+	+	-	-	+	-	-	-	+	+	-	A	-	-	A	-	-	A	+	+	***Chromobacteria Sp.***
7	Cr	S	E	C	Mt	-	Md	Rd	+	-	-	-	-	+	-	+	-	-	-	AG	AG	AG	AG	AG	AG	AG	+	AG	***Escherichia coli***
8	O	S	E	C	Mt	+	Sm	Co	-	-	-	-	-	-	-	+	-	-	-	AG	-	-	A	AG	-	-	+	-	***Micrococus Sp.***
9	Cr	S	E	O	Mt	-	Sm	Cv	+	-	+	+	-	-	+	+	-	-	-	A	-	-	-	-	-	-	+	-	***Vibro Sp.***
10	Cr	S	E	O	Mt	-	Sm	Rd	-	-	+	-	-	-	-	+	+	+	-	AG	AG	A or AG	AG	A or AG	AG	AG	+	+	***Klebsiella Sp.***
11	Cr	S	E	C	Mt	-	St	Rd	+	-	-	-	+	-	-	+	+	-	-	-	-	A	-	-	A	-	+	-	***Acenetobacter Sp.***

Key:
Cultural Characteristics
M = Milky, G = Green, Cr = Creamy, W = Whitish, S = Smooth, Or. = Orange, V = Violet, Y = Yellow, Dl = dull, O = Opaque, C = Clear, E= Entire, Sr. = Serrated, Mt. = Moist, D = Dry, Md. = Muccoid, R = Rough, Mo = Moderate, Rd = Rod, St. = Short, CB = Coco bacillary, Co = Cocci, Cv = Curved, Sm = Small, L = Large

4. DISCUSSION

Bacteria isolated from water and sediments from Diobu creek were: *Bacillus sp, Pseudomonas sp, Corynebacterium sp. Acentobacter sp, Vibrio sp, Alcaligenes sp. Escherchhia coli, Micrococcus, Chromobacterium sp, Klesiella sp, Flavobacterium sp*. The bacterium with the highest percentage occurrence was *Bacillus sp*. The bacteria isolates from this work were similar to the isolates obtained by other workers. [14,1,15]. Similarly, the current work was in line with the previous study had also more counts in wet seasons [20], the difference was attributed to increasing the water content of the soil in the wet season. The results of similar works showed that THBCs and PDBCs were more in wet season compared to dry season and the bacterium with the highest prevalence from oil-polluted water and sediments was *Bacillus sp*. [1]. In a comparable work in River Nun at Amasoma, Bayelsa State, Nigeria, THBCs 1.5 to 8.67 x 10^5 CFU/g were obtained in sediments [21]. In Uppanar Estuary (harbour) Cuddalore coast [22] THBCs 8 x 10^2 CFU/mL was obtained in April (summer) and 4.56 x 10^2 CFU/mL in December (monsoon-wet season) which showed that THBCs were season driven or influenced and sediment had higher counts of THB and PDB compared to the values obtained in water. The interaction of bacteria with benthic organisms, the chemoattractant of bacteria to nutrients at the base or bottom of the creek (on sediment) and the gentle ebbing of water which deposits most bacteria on the sediments may be responsible for the difference in counts observed in sediments and water in this study. Using chi-square $p>0.05$, statistical analysis showed a significant difference in the counts of THB and PDB in water and sediment.

In a research conducted in Awash River mouth in Ethiopia, higher counts were observed in the (Monsoon) wet season when compared with (summer) dry season [23]. In the above research, the highest THBCs in water was 2.6 x 10^4 CFU/mL and lowest was 9.8 x 10^3 CFU/mL whereas, THBCs in sediment was between 3.0 x 10^6 to 1.13 x10^7 CFU/g. They also noted that the populations of bacteria were actually influenced by the physio-chemical parameters of the creek and THBCs were more in sediment. In an investigation of THB counts and human pathogens in Cuddalore fishing harbour after Tsunami, counts in the range of 1.0 x 10^6 to 5.0 x 10^6 CFU/mL were recorded in coastal water and 5 x 10^4 to 1.0 x 10^6 CFU/mL in estuaries [24]. The results of other workers were similar to what was obtained from this study. Other researchers also had analogous results in their studies [25,26,27]. The THBCs were in the range of 3.6 x 10^3 to 1.47 x 10^3 CFU/mL, and THBCs 2.5 x 10^3 to 5.1x10^3 CFU/g were obtained in water and sediment of Persian Gulf [28]. In Padma River, Bangladesh, THBCs in sediments were 2.1 x 10^3 CFU/g in dry season (summer) and 3.46 x 10^6 CFU/g in wet season [23,15]. In the Niger Delta Region where this work was carried out, rainfall is experienced through the year which might be why there was no significant difference in THB and PDB counts between the seasons. The most prevalent bacteria in environments highly polluted with hydrocarbons are those stimulated by their presence. The pollutant hydrocarbons and other chemicals in the creek may stimulate the boost of PDBs that were capable of degrading or mineralising these pollutants. It is always very difficult to remove PAHs from environment due to their high hydrophobicity that increases with increasing molecular weight and this may result in high toxicity and long persistence in the environment [29]. Variety of microbes capable of degrading certain PAHs stimulates significant interest in studying microorganisms in contaminated creeks as a means of bioremediation [30,31]. PAHs consist of two or more than two fused benzene rings which are arranged in linear, angular or clustered forms. PAHs were present in the environment due to natural and mostly anthropogenic activities as the case in Diobu Creek, this was also noted by other researchers [6,15]. There are over 175 bacteria genera that have been known to utilising different crude oil components as a source of carbon and energy, thus petroleum hydrocarbon contaminants in aquatic environments were commonly biodegraded by in-situ microbial communities [32]. PAHs are a potential threat to the environment due to their toxic, mutagenic, and carcinogenic properties [33]. Moreover, some PAHs have been listed as a great concern by the US Environmental Protection Agency [29,34]. The counts of total heterotrophic bacteria and petroleum-degrading bacteria are crucial to ascertain their presence and activities. The mineralization of hydrocarbons by microorganisms is the only eco-friendly means of detoxifying the environment from these pollutants.

5. CONCLUSION

The THBCs and PDBCs isolated from sediments were higher compared to the results obtained in water. The counts of THB and PDB were also increased when sampling was carried out in rain

or after rainfall irrespective of the season. The increased counts of PDB in this aquatic environment were possibly stimulated by the presence of the pollutant hydrocarbons or chemicals discharged into the creek which they mineralise. The activities of PDBs in a polluted environment is the most efficient and eco-friendly means of riding off pollutants from (detoxifying) the environment.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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