

Original Research Article

Enumeration of total heterotrophic and petroleum degrading bacteria counts in water and sediments from Diobu Creek, Port Harcourt, Nigeria.

Introduction: Bacteria play major role in biochemical cycling of nitrogen, carbon, sulphur, and oxygen in aquatic environments. Hydrocarbons are released naturally from oil seeps and incidental discharges which represents significant source of pollution. Estuarine systems are particularly susceptible to anthropogenic hydrocarbon contamination. Although oil contaminants are weathered by photooxidation and evaporation, complete degradation is dependent on the metabolic activities of the microbial population inherent to the area.

Aim: The aim of this work was to determine the total heterotrophic bacterial counts (THBCs) and petroleum degrading bacteria counts (PDBs) 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 and petroleum degrading bacteria agar and isolated bacteria were identified.

Results: The THBCs in water were from 6.3×10^3 CFU/mL and 6.33×10^3 CFU/mL, the highest THBCs were recorded in June (wet season). The THBCs in sediments was from 1.7×10^5 CFU/g to 1.85×10^6 CFU/g. The highest THBCs were recorded in the month of June. The PDBCs in water were from 0.2×10^3 CFU/mL and 3.9×10^3 CFU/mL, high counts were obtained in samples collected during or after rain, whereas the PDBCs in sediments ranged from 3.4×10^5 to 9.5×10^5 CFU/g, high counts were obtained if sampling were carried out in rain or after rain. The bacteria isolated were *Bacillus sp.*, *Pseudomonas sp.*, *Corynebacterium sp.*, *Acinetobacter sp.*, *Alkaligenes sp.*, *Escherichia coli*, *Micrococcus* and *Flavobacterium sp.*

Conclusions: The increased counts of PDBs in aquatic environments were stimulated by the presence of pollutant hydrocarbons or chemicals discharged into creek which were degraded by bacteria. The activities of PDBs in detoxifying polluted environments are eco-friendly.

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Keywords: Enumeration, Total heterotrophic, Petroleum degrading, polluted, Creek.

1. INTRODUCTION

The ubiquity of bacteria confers on them the ability of inhabiting any habitat on planet earth surface, having greater biomass than any other group of organisms. -This is achieved by large surface area to volume ratio, metabolically versatile and obtaining energy by oxidizing carbon, parasitism, chemoautotrophy and photo-autotrophy [1]. -Bacteria play major role in biochemical cycling of nitrogen, carbon, sulphur, and oxygen in aquatic environments [2, 3]. About 470 thousand and 8.3 million ~~tones of tons of~~ petroleum hydrocarbons are released into aquatic environments each year globally [4]. -Half of these are released naturally from oil seeps, incidental discharges which represents 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~~ ~~1mg/Kg~~ sediment in UK location at Melford Haven [6].

Although oil contaminants are weathered by photooxidation and evaporation, complete degradation is dependent 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 the consequence of various natural (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, fresh water run-off, rivers and sewage systems). Of particular concern is the accumulation of low molecular weight PAHs such as naphthalenes, which have been found at concentration of up to 2.4 ~~mg~~ ~~kg~~ ~~1mg/Kg~~ dry weight sediment in the Tyne estuary [6], and are acutely toxic to aquatic invertebrates at concentrations as low as 8 mg L⁻¹ [8]. In addition, many high molecular weight PAHs such as chrysene, which has been found at concentrations of up to 6.94 ~~mg~~ ~~kg~~ ~~1mg/Kg~~ dry weight sediment at Milford Haven [6], are 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 most of the hydrocarbon contaminants are released into the environment as a result of human activities [10,11, 11]. -The presence of these hydrocarbons in an environment is known to stimulate the presence of hydrocarbon degrading bacteria in the affected environments. -The total heterotrophic bacterial counts and petroleum degrading bacteria obtained from oil polluted Bodo Creek in Rivers State, Nigeria; Gokana, Rivers State and Ennore Creek, India, [1, 15, 16] were similar to the bacterial counts and genera isolated by other researchers [17, 13, 12].

The hydrocarbon utilizing bacteria isolated and identified from 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 [17].

About 247 strains of bacteria had been isolated by other workers from hydrocarbon polluted environments. The bacteria isolated were predominantly gram-negative bacteria with prevalence of 62.34% and gram-positive bacteria 35.63%. The gram negatives isolated were four genera namely, *Pseudomonas sp*, *Vibrio sp*, *Achromobacter spp*, and *Serratia spp* with prevalences 39.68%, 15.78%, 3.64% and 3.24% respectively; whereas the gram-positive bacteria were two genera, *Bacillus sp* and *Micrococcus sp* with prevalence of 27.94% and 7.69% respectively [-1, 17-]

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

Other workers also isolated *Bacillus sp.*, *Proteus*, *Pseudomonas*, *Flavobacterium*, *Conynebacterium*, *Serretia*, *Micrococcus*, *Klebsiellaea*, *Enterobacter* and *Azotobacter* from polluted water and sediments [11,16, 16]. In Ennore Creek, India, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Vibrio*, *Acenetobacter*, *Serretia* were isolated from hydrocarbon polluted water and sediments [13]. The aims of this work are to determine the total heterotrophic bacterial counts (THBCs) and petroleum degrading bacterial counts (PDBC) in water and sediments from Diobu Creek in Port Harcourt.

2. MATERIALS AND METHODS

2.1 Study area

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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-old~~ Government Reserved Area (GRA). -Numerous activities were carried out by the creek and the most prominent are the mechanic workshops and car engine washing, 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 were at least 200 meters apart, while station one was marked as control station upstream, with relatively little or no human activities. 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 (twelve calendar months) 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 in a sterile container 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 opposite direction to the water current into a sterile universal bottle.

About 20g of sediment was collected at low tide from the oxidized thin layer (1 – 5cm) surfaces of exposed mud flats along transects across intertidal zone into a wide mouth sterile glass container. -These samples were collected once monthly from November, 2016 – October, 2017 from each of the sampling stations. -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 mixture 3:1 ratio 5mL, ammonium chloride 0.5g/l, dipotassium hydrogen phosphate 0.5g/l, disodium hydrogen phosphate 2.5g/l, agar 15g [18].The media was sterilized 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 were made by adding 1mL (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 1ml of the last dilution to 9.0mL of fresh sterile saline. -Lastly, 0.1mL 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 25-30°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

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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, pourges prausker, oxidase, and carbohydrate fermentation tests etc [18].

2.8 Statistical analysis

Statistical analysis was carried out using statistical package for social sciences (SPSS) version .

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3. RESULTS

3.1 Total heterotrophic bacterial counts in water and sediments (10^5 CFU/g)

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The THBCs from water was high in ~~the months~~ March, April, and May which were 6.33×10^3 , 5.38×10^3 and 5.96×10^3 CFU/mL respectively. The highest count was in the month of June, while the lowest counts ~~are were~~ recorded in November. High THBCs from sediments obtained were in the months of November, 1.76×10^6 CFU/g, January, 1.6×10^6 CFU/g February, 1.7×10^6 CFU/g, June, 1.84×10^6 CFU/g and September, 1.65×10^6 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 Figures 1 and 2.

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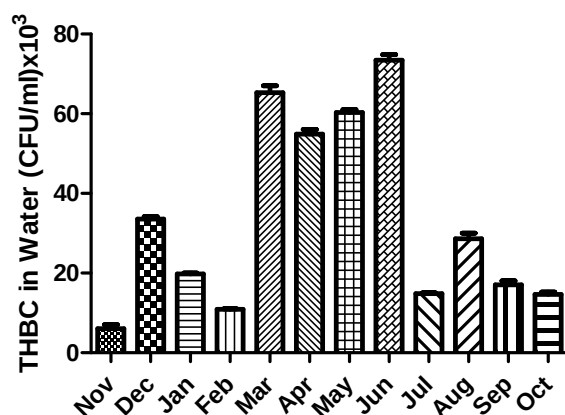


Figure 1: THBCs in water (monthly) 10^3

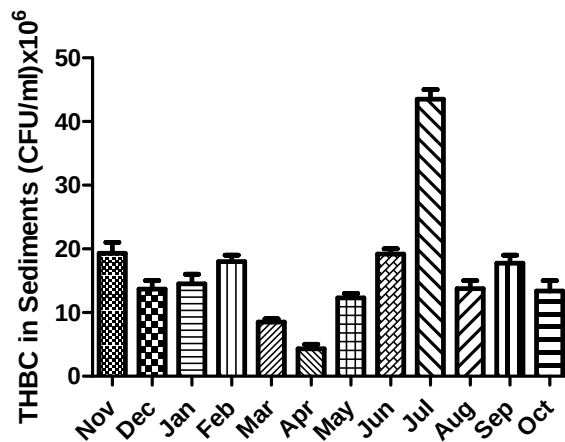


Figure 2: THBCs in sediments (monthly)

3.2 Petroleum degrading bacterial counts in water and sediments

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

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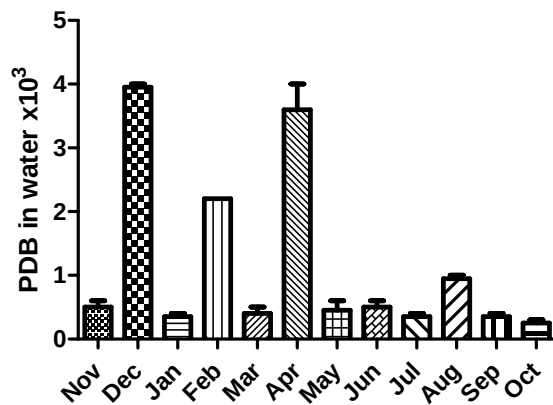


Figure 3: Monthly PDBC water

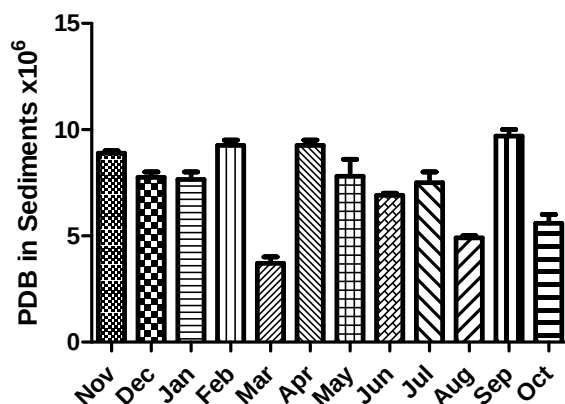


Figure 4: Monthly PDBs in sediments

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 [17, 1, 13-13]. Similarly the current work was in line of the previous study having The work of [20], agreed with this finding, they had also more counts in wet seasons; the difference was attributed to increase in water content of the soil in 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 8.67×10^5 CFU/g were obtained in sediments [21]. In Uppanar Estuary (habour), Cuddalore coast [22] THBCs 8×10^2 CFU/mL in April (summer) and 4.56×10^2 CFU/mL in December (monsoon-wet season) showing that THBCs are were season driven or influenced and from this results, sediment had higher counts of THBCs and PDBCs 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 sediment 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 significant deference in the counts THBCs and PDBs in water and sediment.

In a research conducted in Awash River mouth in Ethiopia, higher counts were observed in the (Monsoon) wet season in comparison with (summer) dry season [23]. In the above research, the highest THBCs in water was 2.6×10^4 CFU/mL and lowest was 0.98×10^4 CFU/mL whereas, THBCs in sediment was between 3.0×10^5 to 11.3×10^6 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 range of 1.0×10^6 to 5.0×10^6 CFU/mL in coastal water and 5×10^4 to 1.0×10^6

CFUcfu/mL in estuaries were obtained [24]. The counts obtained by other workers were similar to what was obtained from ~~this the current~~ study. Other researchers also had analogous results in their studies [25, 26, 27, 27]. The THBCs were in the range of 3.6×10^3 to 1.47×10^3 CFUcfu/mL in water, and THBCs 2.5×10^3 to 5.1×10^3 CFUcfu/g were obtained in water and sediment of Persian Gulf [28]. In Padma River, Bangladesh, THBCs in sediments was 2.1×10^3 CFUcfu/g in dry season (summer) and 3.46×10^6 CFUcfu/g in wet season; ~~it was concluded that seasonal variation effects on THBCs, especially in tropical countries was a key factor influencing THB counts,~~ [23, 13]. In the Niger Delta Region where this work was conducted, rainfall is experienced almost all through the year, ~~this may which might~~ be why there was no significant difference in the THB and PDB counts between the seasons but there was significant difference in THBC and PDB isolated from water and sediments. The most prevalent bacteria in environments highly polluted with hydrocarbons are those stimulated by their presence. –Diobu creek is subject to daily pollution with petroleum hydrocarbons and other pollutants discharged into the creek by mechanics and other allied shops situated along the creek. The presence of the pollutant hydrocarbons and other chemicals in the creek may have stimulated the boost of PDBs that were capable of degrading or mineralizing these pollutants. It is always very difficult to remove PAHs from environment due to their high hydrophobicity which 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]. PAHs consist of two or more than two fused benzene rings which are arranged in linear, angular or clustered forms. PAHs are 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, 31, 31]. There are over 175 bacteria genera that have been known to utilizing different crude oil components as source of carbon and energy, thus petroleum hydrocarbon contaminants in aquatic environments were commonly biodegraded by insitu microbial communities [32]. PAHs are potential threat to the environment due to their toxic, mutagenic, and carcinogenic properties [33]. Moreover some PAHs have been listed as great concern by the US Environmental Protection Agency [29, 34, 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.

4. CONCLUSION

The THBCs and PDBCs in sediment were higher compared to those isolated from water, both THB and PDB may be influenced by the seasons, especially in tropical countries. The counts of THB and PDB were also increased when sampling were carried out in rain or after rainfall irrespective of the season. The increased counts of PDB in an aquatic environment were possibly stimulated by the presence of pollutant hydrocarbons or chemicals discharged into creek. 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

All author declared there was no conflict of interest in the course of the research and during write-up.

CONSENT (WHERE EVER APPLICABLE)

This was not applicable in this research.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

This was not applicable in this research.

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