Original Research Article

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

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The total heterotrophic bacterial counts (THBCs) and petroleum degrading bacterial counts (PDBCs) in water and sediments obtained from Diobu Creek Port Harcourt, Rivers State, Nigeria were examined by serial dilution and plating on nutrient and petroleum degrading bacteria agar. The THBCs in water were from 63.3 x 103 CFU/mL and 63.3 x103 CFU/mL, the highest THBCs were recorded in June (wet season). The THBCs in sediments was from 17.0 x 106 CFU/g to 18.5 x106 CFU/g. The highest THBCs were recorded in the month of June. The PDBCs in water were from 0.2 x 103 CFU/mL and 3.9 x103 CFU/mL , high counts were obtained in samples collected during or after rain, whereas the PDBCs in sediments were from 3.4 x106 to 9.5 x 106 CFU/g, high counts were obtained if sampling were carried out in rain or after rain. The highest count was recorded in February. The bacteria isolated were: B*acillus spp, Pseudomonas spp, Corynebacterium spp, Acenetobacter spp, Alkaligenes spp. Escherichia coli, Micrococcus, Flavobacterium spp* respectively.

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 petroleum hydrocarbons are released into aquatic environments each year globally (4). Half of these are released naturally from oil seeps from 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_1 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 concentrations of up to 2.4 mg kg_1 dry weight sediment in the Tyne estuary (6), and which 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_1 dry weight sediment at Milford Haven (6), they 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). The presence of these hydrocarbons in an environment is known to stimulate the presence of hydrocarbons degrading bacteria in the affected environment.

The total heterotrophic bacterial counts from water and sediments in hydrocarbons polluted Creek in Bodo, Rivers State, Nigeria were 3.0 – 70 x 10^3 CFU/mL for surface water and 1.6 – 5.6 x 10^4 CFU/mL in sediments (1). In a similar work conducted in Bodo, the total heterotrophic bacterial counts was 0.7 to 1.37 x 10^6 CFU/g and hydrocarbons degrading bacteria was 0.8 – 8.0 x 10^6 CFU/g in sediments (12). In Ennore Creek, India, the total heterotrophic bacterial counts were 12.3 x 10^6 CFU/g and hydrocarbon degrading bacteria of 5.7 – 15.10^4 CFU/g in sediments (13). The average number of hydrocarbon degraders during the study period was 10.75x10^4 CFUg-1, with the highest count 15.8x10^5 CFUg-1 being recorded in the post monsoon, August and June, and the lowest 5.7x104CFUg-1 in March. Similarly HDBC counts are between 2.0x10^7 – 1.8x10^8 CFU mL-1 was enumerated from petroleum effluent discharge **(**11**).** The average number of heterotrophic bacteria during the study period was 22.32 x 10^5 CFUg-1, with the highest count 32.4x10^5 CFUg-1 recorded in the post monsoon, August, and the lowest 12.21x10^4 CFUg-1 in the beginning of summer, March. Similar studies recorded that the THB counts ranged from 9.0x10^3 to 2.6x10^6 CFUg-1 in the hydrocarbon contaminated surface sediment samples of Gokana, River state (15)**.** THB population ranged from 10^5 to 9.0x10^8 CFUg-1 on sediment samples collected from Cuddalore fishing harbor over a period of 8 months from October. The counts of cultivatable hydrocarbon-degrading bacteria in oil-contaminated sands (2.4 × 10^{10} cells ml^{-1}) exceeded those from "clean" sands sampled in parallel by 3 to 4 orders of magnitude (2.4 × 10^6 to 9.3 × 10^6 cells ml $^-$ (16). The samples, mean total heterotrophic bacterial count of 6.6 × 107cfu g-1 and hydrocarbon utilizing bacteria of 8.22 × 10^2 cfu g-1. The hydrocarbon utilizing bacteria isolated and identified were of the following genera; *Bacillus*, *Nocardia*, *Staphylococcus*, *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Acinetobacter* and *Enterobacter*, *Bacillus spp*. The gram positive bacteria (rod) *Bacillus* were the most predominant, followed by gram negative *Pseudomonas;* these isolates have also been isolated from hydrocarbon polluted environment by other investigators (17).

A total of 247 strains of heterotrophic bacteria had been isolated. The bacterial flora in all the tested samples was predominated by gram-negative bacteria. In the samples collected 62.34% were gram negative and 35.63% were found to be gram-positive. The gram negative isolates were mainly 4 genera namely, *Pseudomonas spp* , *Vibrio spp, Achromobacter spp* and *Serratia spp* with prevalence of 39.68%, 15.78%, 3.64% and 3.24% respectively. The gram-positive isolates are 2 genera namely, *Bacillus* s*pp* and *Micrococcus spp* with percentage prevalence of 27.94% and 7.69% respectively

The bacteria isolated from the brackish polluted water of Bodo Creek were, *Bacillus spp*. *Alkaligenes, Entrobacter, Cetrobacter, Myroides, Instnibacillus, Pseudomonas, Escherichia coli* (1).

Other workers also isolated *Bacillus spp. Proteus, Pseudomonas, Flavobacterium, Conynebacterium, Serrathia, Micrococcus, Klebsiellea, Enterobacter and Azotobacter* from water and semtrents(1). 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 (PDBCs) in water and sediments from Creek (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 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 (4) sampling stations were established along the creek, while station one was marked as control station upstream, 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 (twelve calendar months).

2.3 Collection of Water and Sediments for Bacteriological Analysis

The water samples for bacteriological examinations were collected 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.
Sediment samples (about 20 g) were collected at low tide from the oxidized thin layer (1 – 5cm) surfaces of exposed mud flats along the transects across intertidal zone into a wide mouth sterile container. These samples were collected monthly from November, 2016 – October, 2017 from each sampling station. 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 incorporation method (18).The media was sterilized and dispensed into disposable petri-dishes, allowed to solidify and stored in the refrigerator for subsequent use.

2.6 Isolation

The method of isolation used was the ten-fold serial dilution technique of (19).Decimal dilution of the samples were made by adding 1m (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 in duplicate on agar medium and evenly spread with a sterile glass rod spreader.
The plates for total heterotrophic counts were incubated at 37°C for 18-24 hours on nutrient agar and the plates for petroleum degrading bacteria incubated at ambient temperature for about one week in petroleum agar before discarding.

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.

3. RESULTS

3.1 Total heterotrophic bacterial counts in water and sediments (10^5cfu)

The THBCs from water was high in the months March, April, and May which are 63.3 x $10^{3,}$ 53.8 x 10^3 and 59.6 x 10^3 CFU/mL respectively. The highest count was in the month of June, while the lowest counts are recorded in November. High THBCs from sediments obtained were in the months of November, 17.6 x 10^6CFU/g, January, 16.0 x 10^6 CFU/g February, 17 x 10^6 CFU/g, June, 18.4 x 10^6 CFU/g and September, 16.5 x 10^6 CFU/g respectively. The highest THBC was in July. The total heterotrophic bacteria counts in sediments were high compared with results obtained in water. Increase were noticed when sapling in rain or immediately after rainfall.

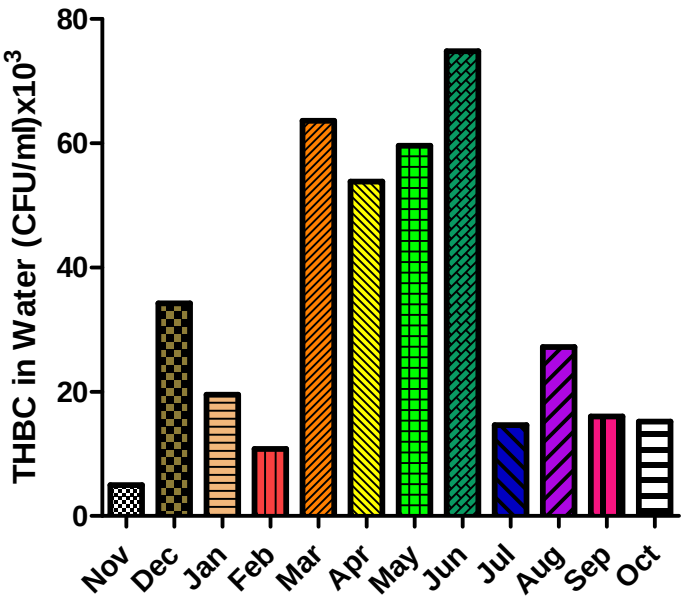

Figure 1: THBCs in water (monthly) 10^3

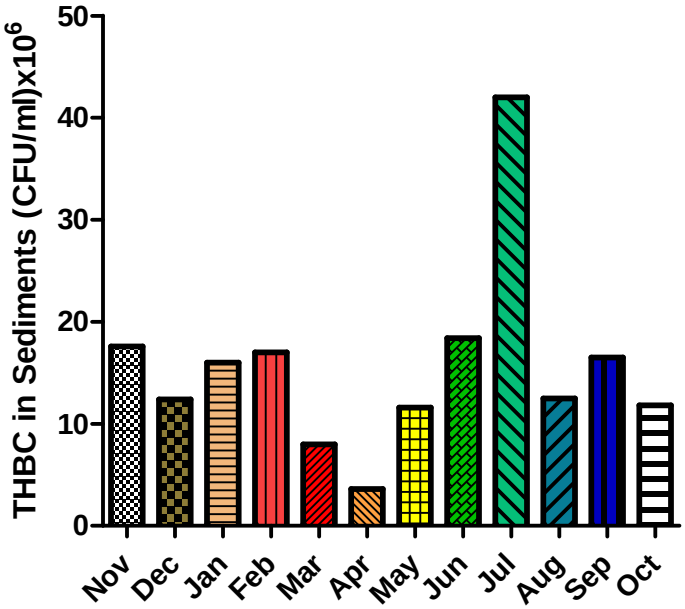

Figure 2: THBCs in sediments (monthly)

3.2 Petroleum degrading bacterial counts in water and sediments

The monthly petroleum degrading bacterial counts (PDBCs) in water was between 0.2 x 10^5 CFU/mL and 3.9 x 10^5 CFU/mL , the count were high in December, April and February respectively as shown in fig, 3. In sediment samples PDBCs range from 3.4 x 10^5CFU/g to 9.5 x 10^5CFU/g, the highest PDBCs was recorded in February, whereas the lowest was in March. Figures: 3 and 4.

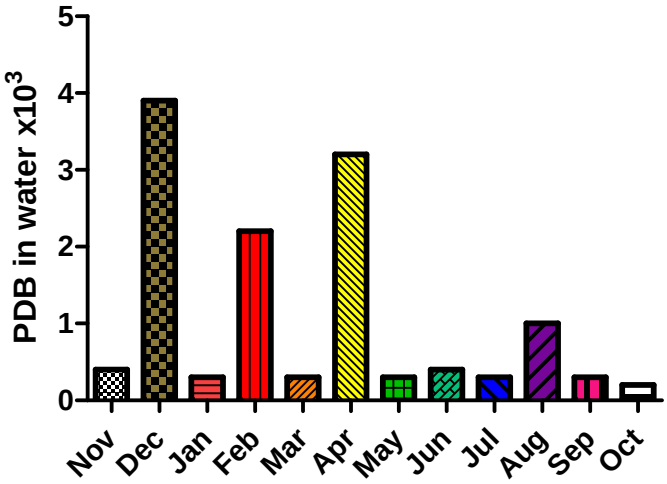

Figure 3: Monthly PDBCs *water*

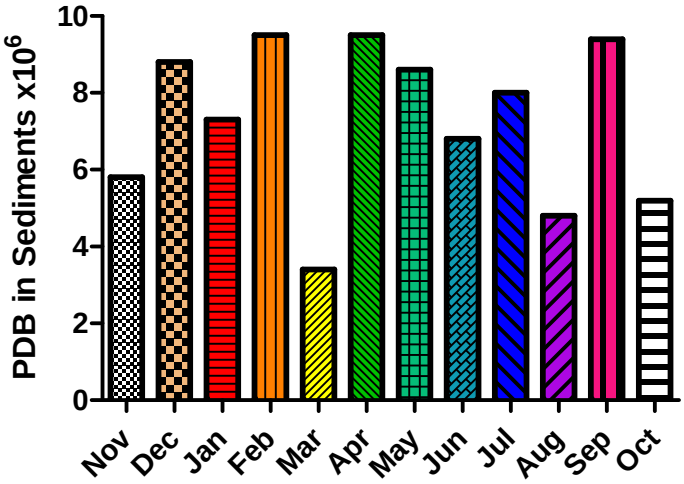

Figure 4: Monthly PDBCs in sediments

4. DISCUSSION

Besides the factors that influence the presence and proliferation of bacteria in aquatic environments such as nutrient availability, temperature, pH or salinity etc, the presence of THBCs and PDBCs appears to be season influenced. High counts were obtained in the wet season (Monsoon) as compared to the dry season. As per Fig.4, the distribution of PDBC in sediments was random independent of the season. During rainfalls, the drains from various locations within the municipality were emptied into the creek thereby boosting its THBCs and PDBCs respectively. The wash-off from the soil with their bacterial loads may contribute to the high counts obtained in water and sediments, in addition to other factors. From the results, sediment had higher counts of THBCs and PDBCs compared to the values obtained in water. The interaction of bacteria with benthic organisms, their chemoatractant of bacteria to nutrients at the base or bottom of the creek (on sediment) and the gentle ebbing of water which may have deposited most of these bacteria on the sediment might have account for the difference of counts observed in sediment and water. Diobu creek is subjected 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 these pollutant hydrocarbons and other chemicals in sediments may have stimulated higher boost of PDBs that are capable of degrading these pollutants. This could be another reason why higher counts of PDBs were recorded in sediments. When samples were collected after rainfall or in the rain, the THBCs and PDBCs are increased because the water drained into creek from the municipality may have increased bacterial population in the creek. Bacteria isolated from water and sediments from Diobu creek were: *Bacillus spp, Pseudomonas spp, Conynebacterium spp. Acentobacter spp, Vibrio spp, Alcaligenes spp. Escherchhia coli, Micrococcus, Chromobacterium spp, Klesiella spp, Flavobacterium spp*.

The bacterium with the highest percentage occurrence was *Bacillus* spp. The bacteria isolated from this work were similar to the isolates obtained by other workers (17), (1), 13). The work of (20), agreed with this finding, they had more counts in wet seasons; this difference was attributed to increased water content of the soil in wet season. The results of a similar works showed that THBCs and PDBCs were more in wet season as compared to dry season and the bacterium with the highest prevalence isolated from the oil polluted water

and sediments was *Bacillus spp,* (1). In a comparable work in River Nun at Amasoma, Bayelsa State, Nigeria, THBCs of1.5 to 8.67 x 10^5 cfu/g was recorded in sediments (21). In Uppanar Estuary (habour), Cuddalore coast (22) had THBCs 8 x 10^2 cfu/mL in April (summer) and 45.6 x 10^2 cfu/mL in December (monsoon-wet season) showing that THBCs are season driven or influenced as noted in this study

In a research conducted by (23) in Awash River mouth in Ethiopia, higher counts were observed in the (Monsoon) wet season in comparison with (summer) dry season. In the above research, the highest THBCs in water was 2.6 x 10^4 cfu/mL and lowest was 0.98 x 10^4 cfu/mL whereas, THBCs in sediment was between 3.0 x 10^6 to 11.3 x 10^6 cfu/g. They also noted that the populations of bacteria were actually influenced by the physio-chemical parameters of creek and THBCs was more in sediments. In an investigation of THB counts and human pathogens in Cuddalore fishing habour after Tsunami, counts in range of 1to 5 x 10^6 cfu/mL in coastal water and 5 x 10^4 to 1 x 10^6 cfu/mL in estuaries were recorded (24). These counts were similar to what was obtained from this study. Other researchers also had analogous results in their studies (25), (26), (27). The THBCs in the range of 36 x 10^3 to 147.5 x 10^3 cfu/mL, and THBCs 25 x 10^3 to 51 x 10^3 cfu/g were obtained in water and sediment of Persian Gulf (28). In Padma River, Bangladesh, THBCs in sediments was 20.60 x 10^3 cfu/g in dry season (summer) and 3.46 x 10^6 cfu/g in wet season; it was concluded that seasonal variation effects on THBCs, especially in tropical countries as a key factor influencing THB counts (23,13) This sentence does not make any sense.. The most prevalent bacteria in environments highly polluted with PAHs are those stimulated by the presence PAHs. It is very difficult to remove PAHs from the environment due to their high hydrophobicity which increases with increasing molecular weight and it results in high toxicity and long persistence in the environment (29). But there were variety of microbes capable of degrading certain PAHs, therefore there is 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 form. PAHs are present in the environment due to natural and mostly anthropogenic activities as it is in Diobu Creek, this was also noted by (6,31). There are over 175 bacteria genera that were able to utilize different crude oil compounds as source of carbon and energy, thus petroleum hydrocarbons that contaminate aquatic environments were commonly biodegraded by in situ microbial communities (32). PAHs are potential threat to the environment due to their toxic, mutagenic, and carcinogenic properties (33). Moreover the ecotoxicity of some PAHs have been listed as great concern by the US Environmental Protection Agency (29), (34). The counts of total heterotrophic bacteria and petroleum degrading bacteria counts are crucial to ascertain their presence and activities.

4. CONCLUSION

The THBCs and PDBCs in sediment were higher compared what were obtained in water, both THB and PDB are seem to be influenced by season 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 which these bacteria mineralize. The activities of PDBs in detoxifying polluted environments are eco-friendly.

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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