

Original Research Article

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

The total heterotrophic bacterial counts (THBCs) and petroleum degrading bacterial counts (PDBC) 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 63.3×10^3 CFU/mL and 63.3×10^3 CFU/mL, the highest THBCs were recorded in June (wet season). The THBCs in sediments was from 17.0×10^6 CFU/g to 18.5×10^6 CFU/g. The highest THBCs were recorded in the month of June. The PDBC 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 PDBC in sediments were from 3.4×10^6 to 9.5×10^6 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: *Bacillus spp*, *Pseudomonas spp*, *Corynebacterium spp*, *Acinetobacter 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 dependant 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 10³ CFU/mL for surface water and 1.6 – 5.6 x 10⁴ CFU/mL in sediments (1). In a similar work conducted in Bodo, the total heterotrophic bacterial counts was 0.7 to 1.37 x 10⁶ CFU/g and hydrocarbons degrading bacteria was 0.8 – 8.0 x 10⁶ CFU/g in sediments (12). In Ennore Creek, India, the total heterotrophic bacterial counts were 12.3 x 10⁶ CFU/g and hydrocarbon degrading bacteria of 5.7 – 15.10⁴ CFU/g in sediments (13). The average number of hydrocarbon degraders during the study period was 10.75x10⁴ CFUg⁻¹, with the highest count 15.8x10⁵ CFUg⁻¹ being recorded in the post monsoon, August and June, and the lowest 5.7x10⁴CFUg⁻¹ in March. Similarly HDBC counts are between 2.0x10⁷ – 1.8x10⁸ CFU mL⁻¹ was enumerated from petroleum effluent discharge (11). The average number of heterotrophic bacteria during the study period was 22.32 x 10⁵ CFUg⁻¹, with the highest count 32.4x10⁵ CFUg⁻¹ recorded in the post monsoon, August, and the lowest 12.21x10⁴ CFUg⁻¹ in the beginning of summer, March. Similar studies recorded that the THB counts ranged from 9.0x10³ to 2.6x10⁶ CFUg⁻¹ in the hydrocarbon contaminated surface sediment samples of Gokana, River state (15). THB population ranged from 10⁵ to 9.0x10⁸ 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 x 10¹⁰ cells ml⁻¹) exceeded those from “clean” sands sampled in parallel by 3 to 4 orders of magnitude (2.4 x 10⁶ to 9.3 x 10⁶ cells ml⁻¹) (16). The samples, mean total heterotrophic bacterial count of 6.6 x 10⁷cfu g⁻¹ and hydrocarbon utilizing bacteria of 8.22 x 10² 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 spp* 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*, *Klebsielllea*, *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 (PDBC) 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 (10^5 cfu)

The THBCs from water was high in the months March, April, and May which are 63.3×10^3 , 53.8×10^3 and 59.6×10^3 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, 17.6×10^6 CFU/g, January, 16.0×10^6 CFU/g February, 17×10^6 CFU/g, June, 18.4×10^6 CFU/g and September, 16.5×10^6 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.

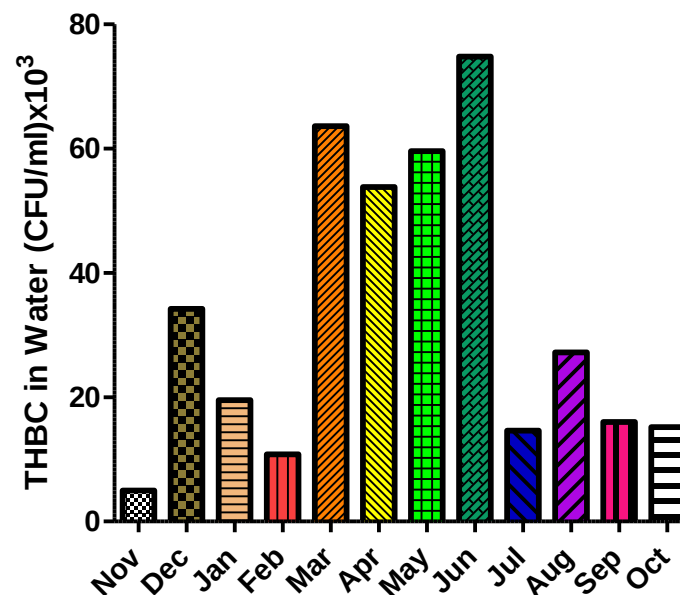


Figure 1: THBCs in water (monthly) 10^3

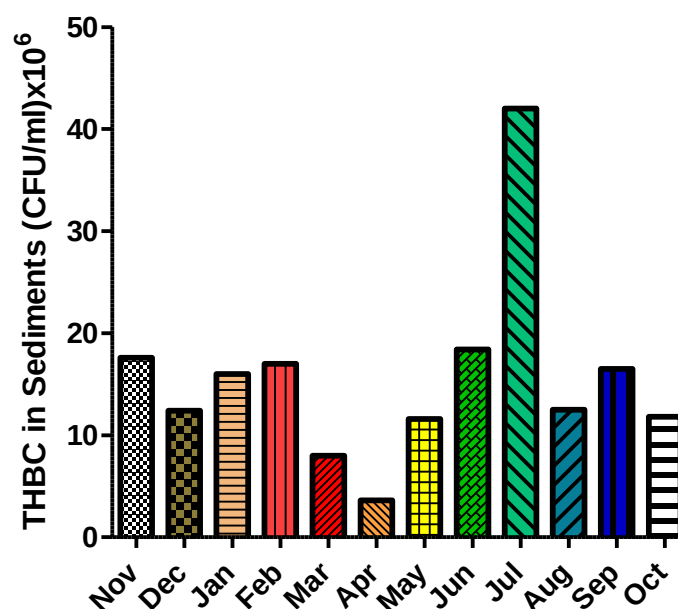


Figure 2: THBCs in sediments (monthly)

3.2 Petroleum degrading bacterial counts in water and sediments

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

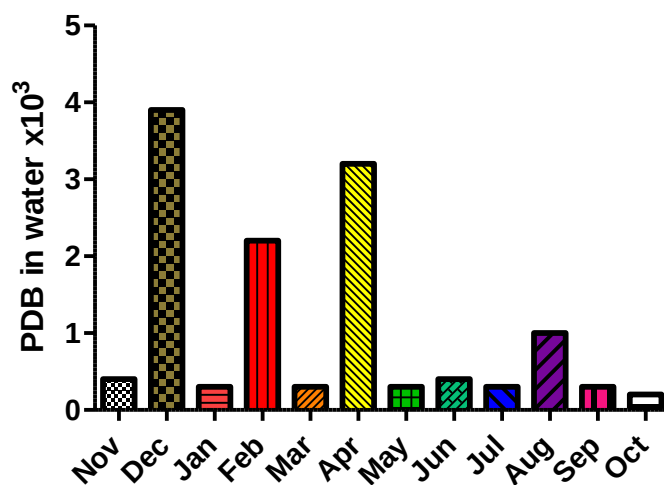


Figure 3: Monthly PDBC water

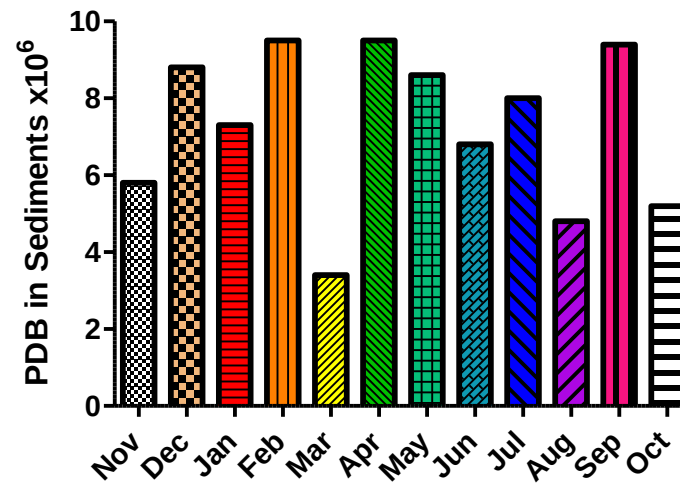


Figure 4: Monthly PDBs in sediments

4. DISCUSSION

Aside 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. 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 chemoattractant 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,

211 Bayelsa State, Nigeria, THBCs of 1.5 to 8.67×10^5 cfu/g was recorded in sediments (21). In
212 Uppanar Estuary (habour), Cuddalore coast (22) had THBCs 8×10^2 cfu/mL in April
213 (summer) and 45.6×10^2 cfu/mL in December (monsoon-wet season) showing that THBCs
214 are season driven or influenced as noted in this study

215 In a research conducted by (23) in Awash River mouth in Ethiopia, higher counts were
216 observed in the (Monsoon) wet season in comparison with (summer) dry season. In the
217 above research, the highest THBCs in water was 2.6×10^4 cfu/mL and lowest was 0.98×10^4
218 cfu/mL whereas, THBCs in sediment was between 3.0×10^6 to 11.3×10^6 cfu/g. They also
219 noted that the populations of bacteria were actually influenced by the physio-chemical
220 parameters of creek and THBCs was more in sediments. In an investigation of THB counts
221 and human pathogens in Cuddalore fishing harbour after Tsunami, counts in range of 1 to $5 \times$
222 10^6 cfu/mL in coastal water and 5×10^4 to 1×10^6 cfu/mL in estuaries were recorded (24).
223 These counts were similar to what was obtained from this study. Other researchers also had
224 analogous results in their studies (25), (26), (27). The THBCs in the range of 36×10^3 to
225 147.5×10^3 cfu/mL, and THBCs 25×10^3 to 51×10^3 cfu/g were obtained in water and
226 sediment of Persian Gulf (28). In Padma River, Bangladesh, THBCs in sediments was 20.60
227 $\times 10^3$ cfu/g in dry season (summer) and 3.46×10^6 cfu/g in wet season; it was concluded that
228 seasonal variation effects on THBCs, especially in tropical countries as a key factor
229 influencing THB counts (23), (13). The most prevalent bacteria in environments highly
230 polluted with PAHs are those stimulated by the presence PAHs. It is very difficult to remove
231 PAHs from the environment due to their high hydrophobicity which increases with increasing
232 molecular weight and it results in high toxicity and long persistence in the environment (29).
233 But there were variety of microbes capable of degrading certain PAHs, therefore there is
234 significant interest in studying microorganisms in contaminated creeks as a means of
235 bioremediation (30). PAHs consist of two or more than two fused benzene rings which are
236 arranged in linear, angular or clustered form. PAHs are present in the environment due to
237 natural and mostly anthropogenic activities as it is in Diobu Creek, this was also noted by
238 (6), (31). There are over 175 bacteria genera that were able to utilizing different crude oil
239 compounds as source of carbon and energy, thus petroleum hydrocarbons that contaminate
240 aquatic environments were commonly biodegraded by in situ microbial communities (32).
241 PAHs are potential threat to the environment due to their toxic, mutagenic, and carcinogenic
242 properties (33). Moreover the ecotoxicity of some PAHs have been listed as great concern
243 by the US Environmental Protection Agency (29), (34). The counts of total heterotrophic
244 bacteria and petroleum degrading bacteria counts are crucial to ascertain their presence and
245 activities.

246 4. CONCLUSION

247 The THBCs and PDBC in sediment were higher compared what were obtained in water,
248 both THB and PDB are seem to be influenced by season especially in tropical countries. The
249 counts of THB and PDB were also increased when sampling were carried out in rain or after
250 rainfall irrespective of the season. The increased counts of PDB in an aquatic environment
251 were possibly stimulated by the presence of pollutant hydrocarbons or chemicals discharged
252 into creek which these bacteria mineralize. The activities of PDBs in detoxifying polluted
253 environments are eco-friendly.

258 **COMPETING INTERESTS**

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

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264 **CONSENT (WHERE EVER APPLICABLE)**

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266 This was not applicable in this research.

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269 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

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271 This was not applicable in this research.

272

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