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Post-Impact Studies of an Inland Oilfield in South-Western Nigeria: A Bacteriological Perspective

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Abstract: The study was designed to determine the post-impact effect of operation of Shell Petroleum Development Corporation (SPDC) in Afiesere oilfields for both rainy and dry seasons using bacteriological indices. Soil samples from transects, villages and water (surface and underground) from Rivers and boreholes, as well as sediments from Rivers were cultured using appropriate media for heterotrophic and hydrocarbonoclastic bacteria, respectively. The hydrocarbonoclastic bacteria consistently isolated from samples were *Pseudomonas, Bacillus, Aeromonas, Chromobacterium, Corynebacterium, Flavobacterium, Citrobacter, Micrococcus* and *Alcaligenes* species. In general, heterotrophic counts were more than hydrocarbonoclasts. Hydrocarbonoclasts were preponderant in rainy than dry seasons along transects. The percentage hydrocarbonoclasts were lower than 1.0% except in T3, 5 and 6 with values of 1.06, 1.06 and 3.2 respectively. Lower counts were obtained for water and sediments analyzed. *Pseudomonas, Aeromonas* and *Chromobacterium* used crude oil better with optical density of 0.105 after 10 days of fermentation. Analysis with SPSS Version 17 shows there is no significant seasonal difference in the distribution of hydrocarbonoclastic bacteria in Afiesere oilfield ($P<0.05$) except for transect 5. We conclude that Afiesere oilfield has not suffered from petroleum crude oil pollution and that the indigenous hydrocarbonoclastic bacteria genera in the oilfield can respond appropriately in the event of a spill. More information may be needed from chemical analysis to validate this finding. The oilfield consists of numerous bacteria that can be cultured and used as inocula for bioremediation of petroleum crude impacted soils and water.

Key words: Bacteriological · Hydrocarbonoclastic · Oilfield · Preponderant · Post-impact · Transects · Post-impact studies of Afiesere oilfield in Southwestern Nigeria

INTRODUCTION

Microorganisms capable of utilizing and deriving energy from hydrocarbons are widespread in nature and are not confined to hydrocarbon-bearing soils [1-4]. A wide range of studies have dealt with biotransformation, biodegradation and bioremediation of petroleum hydrocarbons from the environment [5-8] and interest in exploiting petroleum-degrading organisms for environmental clean-up has become central to petroleum microbiology [9]. A lot has been learnt about cellular and other physiological adaptations to the presence of hydrocarbons as well as biochemical mechanisms involved in hydrocarbon accession and uptake [10, 11].

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Microbial hydrocarbon transformations have long been recognized as important from a fundamental and applied viewpoint and lists of hydrocarbon-degrading organisms are available [5,12]. MacNaughton *et al.* [13] in their microbial community-based site assessment obtained a consortium of microbes responsible for hydrocarbon degradation consisting mainly of bacteria.

Increasing petroleum exploration, refining and other allied industrial activities in the Niger Delta have led to wide-scale contamination of most of its creeks, swamps, rivers and streams [14] with hydrocarbons and dispersant products. The contamination of these habitats constitutes public health and socio-economic hazards [15]. The xenobiotics released has serious aquatic consequence [16-19]. Microbial degradation is the major mechanism for the elimination of spilled oil from the environment [20, 9].

Several studies have documented a broadly distributed and diverse collection of microorganisms capable of hydrocarbon utilization around the world [21-26].

Afiesere oilfield is operated by Shell Development Corporation of Nigeria. The agitation of the Niger Delta over pollution of their arable lands and bodies of water has become issue of public concern. The study was designed to determine the occurrence and distribution of hydrocarbon-utilizing bacteria in the Afiesere oilfield along transect lines, as well as random soil, water and sediment samples, the *in vitro* crude oil degradation potential of isolates and give comprehensive information based on bacteriological indices on the post-impact effect of oil exploration in Afiesere Oilfield. In addition, statistical inferences will be made to determine if there is/no significant difference between hydrocarbon – utilizers in rainy and dry season samples and between transects.

MATERIALS AND METHODS

Study Area: This study was conducted in Afiesere oilfield, which is the oldest oilfields in petroleum - rich Delta region of Southern Nigeria.

Sources of Soil, Water and Sediment Samples: Soil samples were obtained from transect lines (500m) and a control was cut into the vegetation in both trips. The control was cut into virgin vegetation 3 kilometres away from the Shell operational area. Random soil samples were collected from build up areas/villages and isolated buildings or huts. They were marked RS1 - RS15 and RSC, which serves as the control. Surface water (SW) and Groundwater (GW) were collected within the Afiesere oilfields with SWC as control. Sediment samples were collected from all the Rivers within the location and Designated SD1¯SD15 with SDC as control. All samples were obtained aseptically and kept in sterile McCartney bottles, marked appropriately, refrigerated and transported by flight to University of Lagos for analysis.

Assessment of Bacterial Population

Total Count: The method of Amund and Igiri [27], Amund and Akangbou [26] were employed. Total heterotrophic counts (THC) were carried out by plating aliquot (0.1ml) of appropriate dilutions of samples on nutrient agar and incubating at room temperature (28±2.0°C) for 48h and counting the resulting colonies (Colony Counter Model CC-1, BOECO, Germany). Total counts for hydrocarbon-utilizing (HU) bacteria were similarly determined on minimal salt agar plates as previously described by Amund *et al.* [28] and Amund and Igiri [27]. Crude oil used (Bonny Light) as carbon sources was introduced by vapour transfer by placing filter discs impregnated with oil into the lids of Petri dishes [29]. Subsequent procedures were as described by these authors. The isolates were identified by the identification scheme of Bergey's Manual of determinative Bacteriology as reviewed by Buchanam and Gibbons [30] and Cowan [31]. Diagnostic process were as described by Odokuma and Okpokwasili [14].

Hydrocarbon Fermentation: Aliquots containing approximately 1.0×10^6 cells of an overnight grown culture of each isolate were inoculated into 40ml of synthetic mineral salt agar (MSA) to which 1ml of crude oil (Bonny Light) was incorporated in Erlenmeyer flasks. Growth was continued for 10 days at room temperature (25°C). On each sampling day, aliquot of appropriate dilution was aseptically spread onto the surface of standard plate count agar (SPC) to assess the colony-forming unit (cfu/ml) of viable hydrocarbon utilizers. At intervals, aliquots of 5ml were withdrawn and the cell density determined turbidimetrically using UV/VIS Spectrophotometer Model UV-9200 (Beijing Rayleigh Analytical Instrument Corp, China Beijing 100016, PR China) at 550nm.

RESULTS

Soil Microbiology

Analysis of Soil Samples from Transects: Total percentage hydrocarbon – utilizers count was determinant relative to their heterotrophic counterparts. Fig. (1,2) shows the percentage distribution of oil-eating bacteria

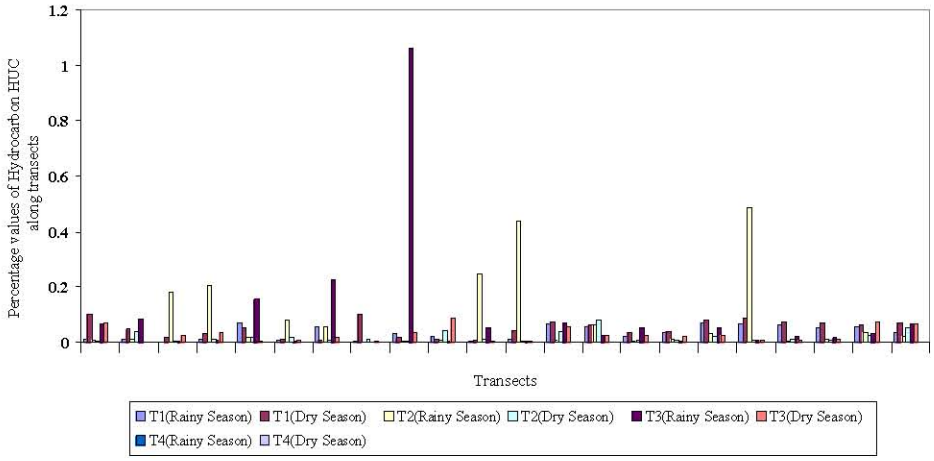

Fig. 1: Composite Bar Chart of percentage hydrocarbon utilizers counts for both Rainy and Dry seasons for soil samples obtained along Transect1, 2, 3, 4

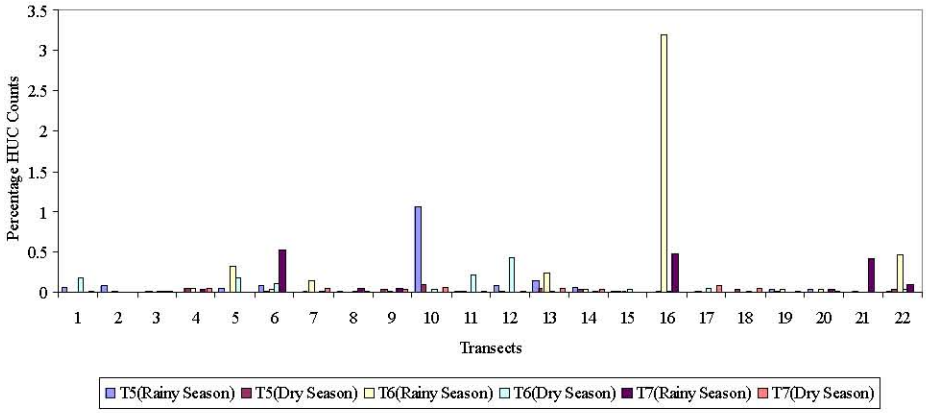

Fig. 2: Composite Bar Chart of percentage hydrocarbon utilizers counts for both Rainy and Dry seasons for soil samples obtained along Transect 5, 6, 7

relative to their heterotrophic counterparts for both seasons along the transects. In general, the percentage incidence of HU vis a vis their heterotrophic counterparts were low and in most cases less than 1.0%. There were points along transects T3, T5 and T6 rainy season where the occurrence of HU were relatively high with values of 1.06%, 1.06% and 3.2% respectively.

For seasonal distribution of HU, there was a preponderant of oil-eating microbes during rainy season. In few cases percentage HU were more in dry than in rainy season. Hydrocarbonoclastic bacteria were more in rainy than dry season in four transects (T2, T3, T4 and T5), while they were more in dry than rainy season in T1 and T7. There were equal distribution of this class of bacteria for both seasons in transect 6. The maximum percentage hydrocarbonoclastic bacteria for control for both seasons was less than 1.0 (0.12) (Fig. 6).

Random Soil Analysis: Results of random soil samples (Fig. 3) from isolated villages revealed that oil-eating bacteria were more in dry than in rainy season with a peak value of 0.12%, but less than 1.0%. The values for the control soil samples were relatively lower than most of the experimental samples.

Water Microbiology

Surface Water and Groundwater Analysis: Analysis of surface and groundwater samples from the community (Fig. 3) shows a higher percentage HU in surface than groundwater. The peak value was 0.53. Percentage hydrocarbon utilizers were preponderant in samples collected in rainy than dry season for all river samples processed except for sample 8 where percentage HU was more for dry than rainy season. The percentage HU counts obtained from control samples from streams (C1 and C2) (these serves as alternative source of drinking

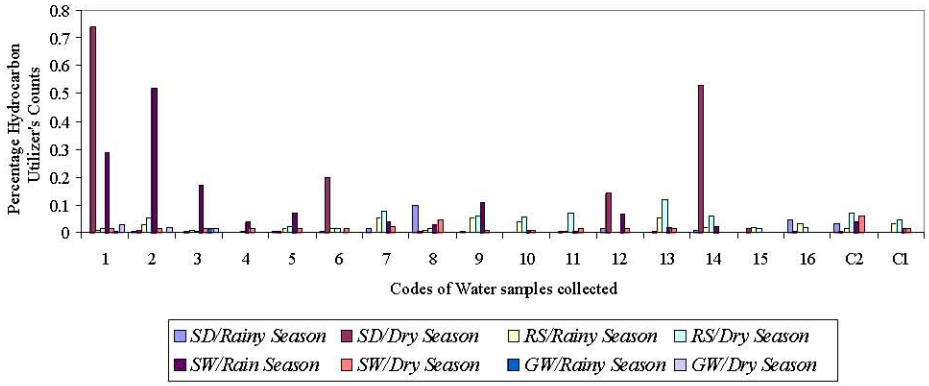

Fig. 3: Composite Bar Chart of percentage hydrocarbon utilizers counts for isolates from SD (Sediments), RS(Random soil), SW(Surface water) and GW (Groundwater) for Rainy and Dry seasons

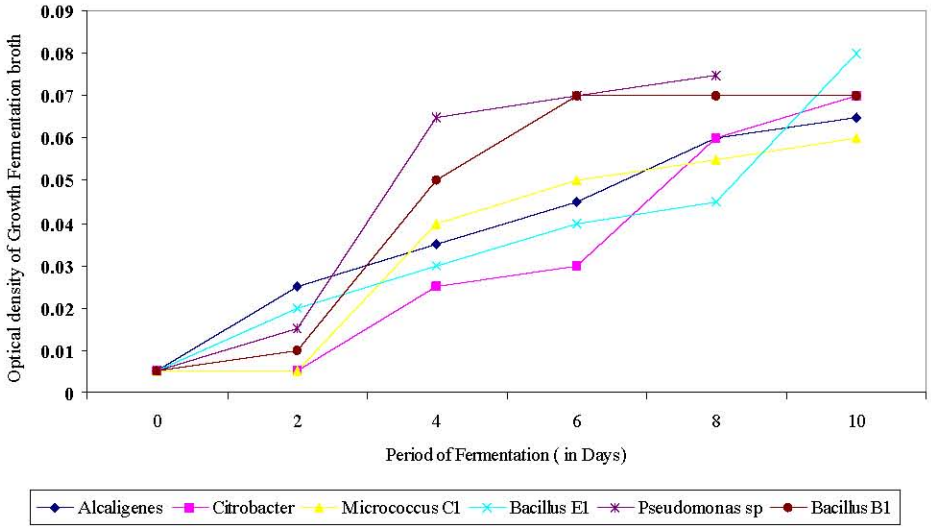

Fig. 4: Curves of optical densities of Fermentation broth of bacterial isolates against days of incubation

water for the community as some prefer it to borehole water) were relatively low compared with river water. In groundwater (borehole), the percentage HU's were less than those obtained from rivers in dry than rainy season with a value of 0.03.

Sediment Analysis: Results of sediment samples from rivers (Fig. 3) show that the percentage HU's was more in dry season for sediments samples than in rainy season with peak value of 0.74. However, the HU's value for control (SD/C2) (0.034) was very low with higher values obtained in rainy than dry season.

Bacterial Isolates from Afiesere Oilfield: After cultural studies of isolates and identification, hydrocarbonoclastic bacteria consistently isolated from soil, water and sediment samples belong to the following genera – *Pseudomonas, Bacillus, Aeromonas, Acinetobacter, Chromobacterium, Micrococcus, Alcaligenes, Corynebacterium, Flavobacterium, Citrobacter and Clostridium* spp.

Crude Oil Fermentation: The bacterial strains utilized petroleum crude -oil as sole source of carbon and energy, which was evident in the increase in the optical density in growth broth after 240 h of incubation (Fig. 4,5). Results from fermentation tests for all bacterial isolates revealed collectively that *Pseudomonas* E5, *Aeromonas, Chromobacterium* sp, *Corynebacterium* sp. and *Bacillus* sp utilizes petroleum crude oil (Bonny light) more efficiently than other isolates with peak optical densities of 0.105, 0.105, 0.105, 0.09 and 0.08 respectively.

Statistical Analysis of Data: In analysis, the statistical program SPSS Version 17 was used to compare the preponderance of hydrocarbon-utilizers in all transects

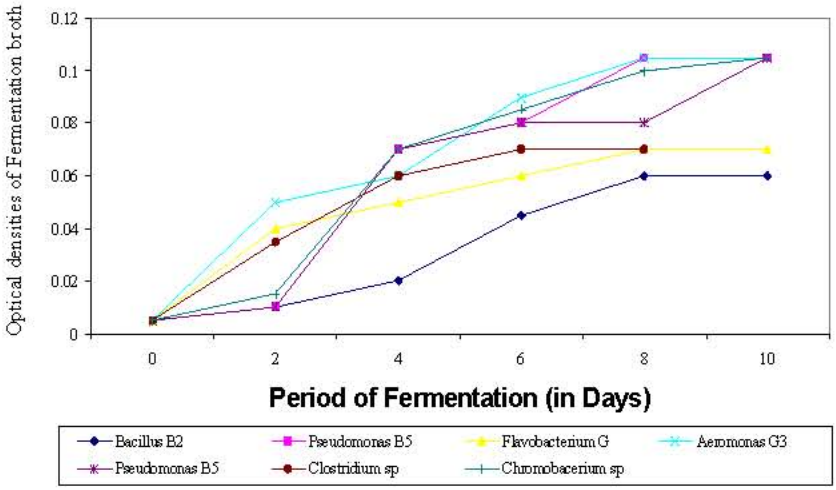

Fig. 5: Curves of optical densities of Fermentation broth cultures of bacterial isolates against days of incubation

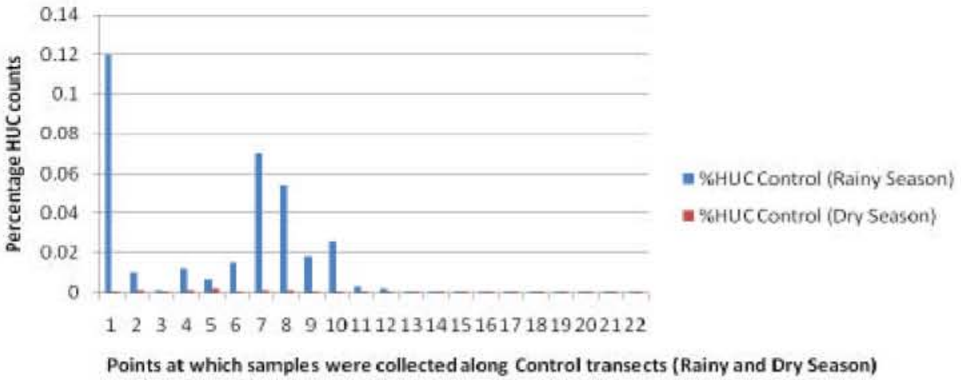

Fig. 6: Percentage Hydrocarbon utilizers count of control samples collected during rainy and dry seasons

for both dry and rainy seasons. Analysis using *t*-test shows that differences in percentage hydrocarbon utilizers distribution are not significant for rainy and dry seasons at $p<0.05$ in transects 1, 3, 5, 6 and 7, while those of transects 2 and 4 are significant at $p<0.05$. Correlation test shows there is no correlations in the preponderance of hydrocarbonoclastic bacteria for both seasons in each transect for hydrocarbon utilizers except for transect 5.

Analysis of variance (ANOVA) shows there is no significant difference in the distribution of hydrocarbon utilizers during rainy season. However, there was significant difference in hydrocarbon utilizers distribution during dry season ($p<0.05$). Paired sample correlation shows there was no significant association between the control and the rest transects for both seasons except for T3 rainy season which cannot be explained by available data. Using independent t-test for 2 samples revealed there was no significant difference in the mean for control for both seasons.

DISCUSSION

The information presented in this study originate from samples collected from transects, isolated buildings, rivers, streams in Afiesere oilfield. Prior to this study, no attempt was made to determine the post-impact effect of the activities of SPDC in this oilfield. In the post-impact studies we employed bacteriological parameters to determine the extent of pollution, if any, of the Afiesere oilfield environment. After exhaustive analysis of all samples, heterotrophic and hydrocarbonoclastic counts were noted to be more for rainy than dry seasons.

There were more heterotrophic and hydrocarbonoclasts in water samples relative to their sediment counterparts. This finding however, contrast the observation of Zobell [32] who noted that organic transformation involving members of the hydrocarbon-utilizers are more pronounced in sea sediments than water. This contrary observation may have stemmed from the fact that Afiesere oilfield is mainly freshwater.

In all samples analysed lower percentages of hydrocarbonoclasts were observed compared with heterotrophic communities.

Relative proportions of hydrocarbonoclasts within the location were lower than 1.0%, exceptions occurring in points along transects 3,4,5,6 with values of 1.06, 1.06 and 3.2% respectively. The low percentages observed in transects, soil, water and sediment samples demonstrate that Afiesere oilfield had not been polluted by crude oil.

The consortia of hydrocarbonoclastic bacteria isolated in this study have consistently been isolated from virgin and oil-impacted environments – soil, water and sediments by various workers - *Flavobacterium, Pseudomonas* and, *Micrococcus* spp, *Bacillus* spp [33, 34]. These workers observed in fermentation experiments that the isolates utilized whole crude oil efficiently over a period of time. Though some of these workers isolated *Chromobacterium,* attempts were not made to determine the hydrocarbon utilizing potential of this bacterium.

However, this is the first report to our knowledge of a hydrocarbon-utilizing *Chromobacterium sp* that grew on petroleum crude oil efficiently. Austin *et al.* [35] working in the USA isolated petroleum-degrading strains from polluted sediments and 18 strains from unpolluted sediments which included *Pseudomonas* and *Micrococcus* sp. from environmental samples and reported that they are highly hydrocarbonoclastic.

The implication of these findings suggests that hydrocarbonoclastic microbes are present both in polluted and unpolluted environments but are more preponderant in polluted than unpolluted environments. Jones and Edington [1], Jones *et al.* [2] reported that microorganisms capable of utilizing hydrocarbons are widespread in nature and are not confined to hydrocarbon bearing soils. The report of these workers further substantiates our findings that these isolates are hydrocarbonoclastic.

Amongst the isolates *Pseudomonas, Aeromonas and Chromobacterium spp.* showed more promise as oil – users compared with the rest. This is confirmed by the high optical density observed for these isolates during the growth period under study (Fig. 5, 6). This finding is in line with the observations of several other studies [36, 33, 38, 39, 40, 41, 42]. The growth pattern observed for each isolate is due to the rapid utilization of the growth-supporting components of the crude within the first few days of fermentation.

The degradation of crude oil by the isolates in this report is in line with previous report on microbial degradation of Nigerian crude oil by *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from crude oil-polluted soils in Nigeria [38].

Christopher and Kitts [43] in a study on bacteria succession in a Land treatment unit in the USA, observed that specific microbes were associated with different phases of petroleum degradation. They observed that *Pseudomonas* and *Flavobacterium* were dominant in samples during rapid total petroleum degradation. The data they present suggests that specific bacteria were associated with the different phases of petroleum degradation in the environment during petroleum spill breakdown.

Dott *et al.* [40] in their study of adapted commercially available mixed bacterial cultures observed that strains of *Pseudomonas aeruginosa* mineralized hydrocarbons more efficiently. Ghazali *et al.* [42] in their study noted enhance crude oil degradation by strains of *Pseudomonas, Bacillus* and *Micrococcus* in soil samples at room temperature. They noted that normal strains of microbes are potent at degrading crude oil than indigenous microbes.

Recently, Ijah and Antai [41] reported *Bacillus* sp being predominant isolate of all crude oil utilizing bacteria characterized from highly polluted soil samples in the Delta Region of Nigeria. From their 5 soil isolates they observed that one *Bacillus* sp. was best oil degrader compared with isolates belonging to *Micrococcus varians, P. aeruginosa, Vibrio* sp and *Alcaligenes* sp. Their findings are also in line with our observation, although the *Bacillus* isolates in this study were not as efficient as *Pseudomonas, Aeromonas* and *Chromobacterium* spp.

Several factors may have attributed to this, which could among others be, the species of *Bacillus*, their genetic status, including presence or absence of extra genetic elements that harbours degradative genes. There is growing evidence that isolates belonging to *Bacillus* sp. could be effective in cleaning oil spills [42]. Medaura and Encoli [44] posited after their study of petroleum-impacted soils that microbial intervention was responsible for the transformation and reduction of petroleum fractions. Ghazali *et al.* [42] also observed that background degradation by indigenous population of contaminated soil also occurred; however, the extent of alkane removal by the microbial consortium was greater and that removal of hydrocarbons from soils could be attributed to the combined actions of indigenous microbial population of the polluted soil as well as abiotic weathering.

Das and Mukherjee [39] reported efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains in the biodegradation of crude petroleum hydrocarbons in soils and shake flask studies. Groudeva *et al.* [45] working in Sofia Bulgaria isolated very active oil-degrading *Pseudomonas, Bacillus* and *Corynebacterium* spp. from crude oil-impacted waters and reported their efficiency in the bioremediation of oil – impacted environments. Cubitto *et al.* [46] reported the production of high concentration of biosurfactants by *Bacillus subtilis* and its use in bioremediation of crude oil-impacted soils.

Statistical analysis using SPSS Version 17 shows that there is no significant difference in the percentage distribution of hydrocarbon utilizers between rainy and dry seasons $P<0.05$) in Transects 1, 3, 5, 6 and 7, while Transects 2 and 4 are significant at $P<0.05$. Correlation test shows there is no significant difference in the percentage hydrocarbon utilizers for both rainy and dry seasons in each transect except for transects 5.

ANOVA test shows that there is no significant difference in the distribution of hydrocarbon utilizers during rainy season. On the other hand there was significant difference in the distribution of hydrocarbon utilizers in dry season at $P<0.005$. Paired sampled correlation shows there is no significant difference between control samples for both seasons and the rest transects except for T3 rainy season, which cannot be explained from the present data.

The low percentage hydrocarbon counts relative to the heterotrophic population along transect, water, soils and sediments may suggest that the Afiesere oilfield and its environment had not suffered from oil pollution, rather than from possible minor accidental discharges. Although isolates could degrade crude oil to different degrees, the two *Pseudomonas*, *Aeromonas* and *Chromobacterium* spp. showed more promise as hydrocarbon-utilizers, which are reflected in the high optical densities recorded during the 10-day period of investigation. High percentages of the hydrocarbon-utilizers vis a vis their heterotrophic counterpart would have indicated that the environment have been severely exposed to hydrocarbon contaminants. However, the low value obtained proved otherwise.

The occurrence and distribution of these hydrocarbon-degraders in Afiesere oilfield is not an indication of pollution, rather it demonstrates that the indigenous microbes are capable of responding appropriately in the event of a spill. These microbial isolates could be cultured and used for environmental cleaning of petroleum crude oil in the event of a spill as well as in bioremediation of petroleum crude oil-impacted soils and water. Hence the continuous outcry of environmental pollution by communities around this oilfield should be put to rest as bacteriological data has proved that the environment has not suffered from crude petroleum hydrocarbon pollution.

Probably chemical, anatomical and physiological studies of the animals indigenous to the Afiesere community may add credence to these findings before comprehensive judgment can be made. The noticeable diminution of low molecular weight hydrocarbon content and hence environmental risk by the hydrocarbon mineralization process in soils and water, are promising results with regards to future application at large-scale.

CONCLUSION

In summary, we describe the distribution of hydrocarbon degrading bacteria in the Afiesere oilfields in the Delta Regions of Southern Nigeria. Prior to this study, efforts had not been made by Shell Petroleum Development Corporation of Nigeria, to assess the post-impact studies of their operations in the communities of their operation. The results from the bacteriological analysis show that the Afiesere oilfields have not suffered from petroleum crude pollution and that the indigenous microbes present are capable of responding appropriately in the event of a spill. However, it is important that other parameters such as chemical analysis of soil, plants and animals indigenous to the environment are done to augment the results from the bacteriological analysis to give a better assessment of the pollution status of Afiesere oilfields.

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