

# RHIZOSPHERE ASSOCIATED BACTERIA IN BIOREMEDIATION OF CRUDE OIL POLLUTED AQUATIC ENVIRONMENT

## ABSTRACT

**Aim:** This study was conducted to examine the biodegradation of crude oil by microorganisms isolated from rhizosphere soil of plants found in an oil polluted aquatic environment.

**Study design:** Laboratory scale broth microcosms was adopted.

**Place and Duration of Study:** Study was done in Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun/six months.

**Methodology:** Hydrocarbon utilizing bacterial species were isolated from the rhizosphere soil of plants from a crude oil polluted soil. They were screened for hydrocarbon degradative abilities and identified. Isolates with the best degradative capabilities were used for the bioremediation studies. Microcosms contained 5mls of crude oil (5% volume per volume (V/V)), 100mls of mineral salt and 10mls of isolates (10% V/V). Degradation was evaluated by changes in pH, microbial counts, residual oil and grease.

**Results:** Ten bacterial isolates were isolated and identified as *Pseudomonas*, *Bacillus*, *Proteus*, *Enterobacter*, *Escherichia* and *Staphylococcus*. *Pseudomonas*, *Bacillus*, *Proteus* were used for the biodegradation experiment. Results from the oil and grease analysis showed that *Pseudomonas* (1.308mg/l), *Proteus* (2.344mg/l) and *Bacillus* (2.578mg/l) could degrade crude oil in set-up using it as carbon source with *Pseudomonas* species performing the most while the control had 3.533mg/l. Bacterial counts during the assay increased. The bacterial counts were in the increasing sequence; control ( $0.35 \times 10^6$  CFU/ml), *Bacillus* ( $2.05 \times 10^6$  CFU/ml), *Proteus* ( $2.28 \times 10^6$  CFU/ml) and *Pseudomonas* ( $2.85 \times 10^6$  CFU/ml).

**Conclusion:** Results indicated that rhizosphere soil of plants in an oil polluted environment could be a ready source of hydrocarbon utilizing bacteria. Microbial communities exposed to hydrocarbons become adapted, exhibiting selective enrichment and genetic changes, resulting in increased proportions of heterotrophic hydrocarbon-degrading bacteria.

*Keywords: Rhizosphere bacteria, crude oil, biodegradation, oil polluted aquatic environment*

## 1. INTRODUCTION

Petroleum is a naturally occurring unrefined mixture of hydrocarbons, generally in a liquid state, that may also include compounds of sulphur, nitrogen, oxygen. The exploration, production and consumption of oil and petroleum products are increasing worldwide, and the threat of oil pollution increases accordingly [1,2]. The movement of petroleum from the oil fields to the consumer involves as many transfers among different modes of transportation including tankers, pipelines, railcars, and tank trucks [3,4]. Oil is stored at transfer points, terminals, and refineries along the route. Accidents can happen during any of these

exploration, production, and transportation steps or storage times, Disposal often lead to release of hydrocarbon pollutants into the environment with serious ecological problems, including air pollution issues, global climate change [5]. More so, oil spillage greatly influences soil microbial population and alter biogeochemical cycles.

Long-term presence of petroleum contaminants in the environment can bring about microorganisms that are able to use organic pollutants as sole carbon and energy source. The ability to isolate high numbers of certain oil-degrading microorganisms from oil polluted environment is commonly taken as evidence that these microorganisms are active degraders of crude oil in the environment [6,7].

Soil, an integral component of the environment, inhabited by heterogeneous microorganisms, including: bacteria, fungi, algae, viruses and protozoa [8]. The toxicity of crude oil or its products is a function of different factors such as: its composition, concentration, environmental factors and biological state of organisms at the time of exposure. Degradation of hydrocarbons by natural populations of microorganisms is a phenomenon in bio-remediating hydrocarbon polluted environment [9,10,11]. Numerous genera of bacteria are known to degrade hydrocarbons. They tolerate high concentrations of hydrocarbon and have a high capability for their degradation. Most of these bacterial strains belong to species of *Pseudomonas*, *Sphingomonas*, *Aeromonas*, *Alcaligenes*, *Acinetobacter*, *Arthrobacter*, *Brevibacterium*, *Xanthomonas*, *Mycobacterium*, *Rhodococcus* and *Bacillus* [7,9,12,13].

Biodegradation of oil contaminated environments, which use the ability of microorganisms to degrade and/or detoxify organic contamination has been established as one of the efficient, economic, versatile and environmentally sound treatment. Nonetheless, energy sources, bioavailability, bioactivity, biochemistry, oxygen, nutrient availability, pH, temperature, and metabolite inhibition are influences on the biological activities of microorganisms [14]. However, the effectiveness of bioremediation is often a function of the extent to which a microbial population can be enriched and maintained in the environment [15]. One of such environment is the rhizosphere. Some compounds in hydrocarbons may not be degraded by

microorganisms, others may be degraded into carbon dioxide, water and fatty acids while others may be transformed into other compounds [16,17].

Rhizosphere, the area of soil under the direct influence of roots, could be an appropriate microsite for *in situ* degradation of petroleum contaminated soils. Microbial density, diversity and activity in the rhizosphere soil are greater and more effective for bioremediation than non-rhizosphere soils, likely due to the growth stimulation by the exudation of chemical compounds from the roots [18,19,20,21]. It has been shown that microbial populations in the rhizosphere may enhance a plant's adaptation to petroleum hydrocarbons through detoxifying contaminated soils as a result of direct mineralization of these organic contaminants [5,22,23,24]. This investigation assessed the efficiencies of bacteria isolated from rhizosphere of plants found in an oil polluted site in biodegradation and bioremediation.

## **2. MATERIAL AND METHODS**

### **2.1 Sample collection**

The soil samples were collected from Effurun roundabout, Uvwie Local Government Area, Delta state, Nigeria. Rhizosphere soil from plants found in a site with history of crude oil pollution were collected using sterile labelled bags. Also, composite soil samples were collected from the Federal University of Petroleum Resources Effurun, Delta state, a site with no previous history of crude oil contamination. A Global Positioning System (GPS) was used to determine the coordinates of the area where samples were collected. Samples were transported to the laboratory for further analysis in ice packs.

### **2.2 Physicochemical analysis**

Fresh soil samples was characterized for pH, electrical conductivity, moisture content, nitrogen, sulphur, total organic carbon and phosphates were analyzed following the standard methods by American Public Health Association [25]. Cation exchange capacity (CEC: calcium, magnesium, potassium, sodium) were ascertained by flame analysis methodology via atomic adsorption spectrophotometer Model AA500 (PG instruments) following digestion of samples adopting the protocol described by APHA [25]. Also, total heterotrophic bacterial and hydrocarbon utilizing bacterial counts were done following standard procedures [13].

### 2.3 Isolation and Identification of total culturable heterotrophic bacteria

All media were prepared according to manufacturer's specification and autoclaved at 121°C for 15 minutes at 15 psi (pounds per square inch). Serial dilution was done aseptically using sterile physiological saline. Aliquots (0.1ml) of the dilutions were plated out using appropriate media. Solid media was then inverted and incubated for 24 hours at 28°C. Discrete colonies were sub cultured onto nutrient agar for further purification which was done twice (using streak method) and transferred into agar slant for storage and identified using biochemical test [26].

### 2.2 Isolation and identification of hydrocarbon utilizing bacteria

The vapour phase method of Ataikiru et al. [7] was adopted. Serial dilutions were done using one gram of soil samples. Each tube was vortexed and one ml aliquot was inoculated onto replicate sterile petri dishes containing mineral salt agar (MSA). Plates were inverted and incubated at 28±2°C for 5 days. The MSA contained KH<sub>2</sub>PO<sub>4</sub> (1g), K<sub>2</sub>HPO<sub>4</sub> (1g), NH<sub>4</sub>NO<sub>3</sub> (1g), MgSO<sub>4</sub> (0.2g), FeCl<sub>3</sub> (0.05g), CaCl<sub>2</sub> (0.02g), agar (15g) and distilled water (1000ml). Discrete colonies were sub cultured onto MSA impregnated with sterile filter paper soaked in crude oil for further purification (using streak method) and transferred into agar slant for storage and identification.

### 2.5 Screen test for selection of hydrocarbon degraders

The bacterial isolates were inoculated into a prepared nutrient broth, which was incubated for 24 hours at 28±2°C. A 0.1ml of the young broth culture was inoculated into test tube containing 9.9mls sterile mineral salt broth and 0.1ml of crude oil. A control test tube containing 9.9mls sterile mineral salt broth with 0.1ml of crude oil remained un-inoculated. The cultures were incubated for seven days at 28±2°C. Broth containing hydrocarbon utilizing bacteria turned turbid as against a clear control [27].

### 2.6 Standardization of bacterial isolates

McFarland standard was used as a reference to adjust the turbidity of bacterial suspension [28]. This was done by sub culturing isolated colonies from a pure culture plate into peptone

water and incubating at  $28 \pm 2^\circ\text{C}$  for 12hrs. Turbidity of the growth was adjusted by dilution with sterile distilled water until equal to turbidity of a 0.5% McFarland standard. The 0.5% McFarland standard was prepared by mixing 0.05mm of 1.175% dehydrated barium chloride ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) with 9.95ml of 1% sulphuric acid ( $\text{H}_2\text{SO}_4$ ). The approximate cell density of 0.5% McFarland standard is  $1.5 \times 10^8/\text{ml}$ .

## 2.7 Bioremediation studies

The bioremediation studies were carried out according to the method of Mbakwem-Aniebo and Ukaegbu-Obi [27]. Microcosms were set up as;

Control = 5mls of crude oil + 100mls of mineral salt with no isolate

A = 5mls of crude oil + 100mls of mineral salt + 10mls of *Proteus* species

B = 5mls of crude oil + 100mls of mineral salt + 10mls of *Bacillus* species

C = 5mls of crude oil + 100mls of mineral salt + 10mls of *Pseudomonas* species

The pH, turbidity, bacterial counts, oil and grease were assessed by collecting samples at day 0, 5, 10 and 15, respectively following standard procedures [29].

## 2.8 Determination of bacterial growth

This was determined by measuring the turbidity of the inoculated and un-inoculated cultures on day 0, 5, 10, and 15 using a Hach Ratio Turbimeter [12]. Bacterial growth was also determined via total heterotrophic count employing the standard plate count technique [8]. A serial dilution of 1ml of the crude oil contaminated samples was carried out. Plate count agar was prepared according to the manufacturer's instruction. Aliquots (0.1 ml) of the serially diluted samples were introduced into sterile petri dishes and 15-20 ml of sterilized and cooled agar was poured into the petri dish. The plate was gently swirled to homogenized and evenly dispense the medium. A 0.1 mL of fungizone was added to the media to inhibit fungal growth. The plates were inverted and incubated at  $28 \pm 2^\circ\text{C}$  for 24hours. Counts were expressed as colony forming units (CFU/ml).

# 3. RESULTS AND DISCUSSION

## 3.1 Physicochemical analysis

Table 1 shows the physicochemical characteristics of both polluted and unpolluted soil samples.

Microorganisms as well as their enzyme activities are inhibited by soil pH, though most microbes desire neutral pH for growth and remediation [30]. Most microorganisms prefer neutral or low-alkaline conditions to decompose hydrocarbon compounds [31,32]. The pH of soil samples were 6.60 and 7.50 for the polluted and unpolluted soils, respectively. The pH of both polluted and unpolluted soil samples were within the optimum range for bacterial growth and bioremediation. This could be a pointer to the fact of the presence of hydrocarbon utilizers with degrading capabilities. However, the moisture content of crude oil polluted soil was lower than that of control, with values of 11.53% for the control, and 9.17% for polluted samples. Snehal [33] reported that either crude oil can coat the soil and consequently prevent the penetration of water, or the microorganisms utilize the water for their activities. Electrical conductivity (EC), a determinant of the total ionized constituent of water, is directly proportional to the sum of the cations and anions [34]. The EC values recorded were 64  $\mu\text{s}/\text{cm}$  (polluted) and 70  $\mu\text{s}/\text{cm}$  for the unpolluted soil sample. Phosphate content was found to be higher (0.98mg/kg) in unpolluted than polluted soil (0.72mg/kg), and the phosphate content. The measured values for nitrates were 0.009mg/kg (polluted soil) and 17.24mg/kg (unpolluted soil). It has been established that hydrocarbon pollution has an adverse impact on nitrates and phosphates concentration thus making them limiting nutrients. Ibiene et al. [35], reported the absence of nitrate in the polluted soil as an indicator that the limiting nutrients were not released to the microorganisms involved. Similarly, the release of petroleum pollutants lead to the drop in the amounts of soil mineral nutrients. These elements are crucial for biodegradation process to take place, therefore, limiting nutrients (nitrogen) must be made available to speed up the process. Oualha et al. [36] examined the biodegradation of weathered oil hydrocarbons via combining biopile technology system, bioaugmentation with indigenous *Bacillus sonorensis* strain and optimum nutrients. The study was done under these optimized conditions; carbon/nitrogen/phosphorus (100/10/1), temperature (37°C), surfactant Tween 80 (0.12% (v/w)), and moisture (10%). The percentage removal of the diesel range organics and polycyclic aromatic hydrocarbon (PAH) from the soil were 39.2% and 32.4%, respectively after

160 days with ammonium nitrate as the nitrogen source. Total heterotrophic bacteria were  $9.80 \times 10^5$  CFU/g (unpolluted) and  $6.20 \times 10^5$  CFU/g (polluted) while hydrocarbon utilizing bacterial counts were  $8.70 \times 10^4$  CFU/g (unpolluted) and  $9.50 \times 10^4$  CFU/g (polluted), respectively.

Table 1: Physicochemical parameters of the polluted soil and control soil samples.

Parameters	Polluted sample	Control sample
pH	6.60	7.50
Electrical conductivity( $\mu\text{s}/\text{cm}$ )	64	70
Total organic carbon (%)	0.41	0.22
Moisture content (%)	09.17	11.53
Cation Exchange Capacity (meq/100g)	22.56	24.76
Sulphur (mg/kg)	4.85	1.61
Nitrate (mg/kg)	0.009	17.24
Phosphate (mg/kg)	0.72	0.98
Total heterotrophic bacterial counts ( $\times 10^5$ CFU/g)	6.20	9.80
Hydrocarbon utilizing bacterial counts ( $\times 10^4$ CFU/g)	9.50	8.70

### 3.2 Identification of bacterial isolates

The cultural and morphological characteristics of bacterial isolates are shown in Table 2. A total of ten (10) isolates were identified. The following hydrocarbon utilizing bacteria genera were identified: *Pseudomonas*, *Bacillus*, *Proteus* and *Enterobacter*. Heterotrophic bacteria

isolated were: *Escherichia coli* and *Staphylococcus* species. Similar species like *Pseudomonas*, *Bacillus*, *Streptococcus* and *Staphylococcus* have been isolated by different researchers [29,37]. Again, species of *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Xanthomonas*, *Proteus*, *Acetobacter*, *Burkholderia*, *Erwinia*, *Serratia*, *Achromobacter*, *Agrobacterium*, *Halomonas*, *Azospirillum* among others have been reported to inhabit the plant rhizosphere contributing to the plant in different ways. These organisms secrete indole acetic acid (IAA) that elevate the size and surface area of root system in contact with the soil increasing plants ability for nutrient and water uptake improving their growth and yields amongst others [38,39,40].

Isolates	Gram stain	Shape	Motility	Catalase	Oxidase	Glucose	Sucrose	Gas	Acid	Indole	Citrate	Hydrogen Sulphide	Voges Proskauer	Methyl red	Urease	Probable microorganisms
H1	-	R	+	+	+	+	-	-	-	+	-	-	+	-	+	<i>Pseudomonas sp</i>
H2	+	R	+	+	-	+	+	+	+	+	-	+	-	-	-	<i>Bacillus sp</i>
H3	-	R	+	+	-	+	-	+	+	+	-	+	-	-	-	<i>Enterobacter sp</i>
H4	-	R	+	+	+	+	+	+	+	+	+	-	-	+	-	<i>Escherichia coli</i>
H5	-	R	+	+	+	+	+	+	+	+	+	-	-	+	-	<i>Escherichia coli</i>
H6	-	R	+	+	-	+	-	-	+	+	+	-	+	+	+	<i>Proteus sp</i>
H7	-	R	+	+	-	+	-	-	+	+	+	-	+	+	+	<i>Proteus sp</i>
H8	-	R	+	+	-	+	-	-	-	+	-	-	+	-	-	<i>Pseudomonas sp</i>
H9	+	R	+	+	-	+	+	+	+	+	-	+	-	-	-	<i>Bacillus sp</i>
H10	+	C	-	+	-	+	+	+	+	+	-	+	-	+	-	<i>Staphylococcus sp</i>

Table 2: Bacterial isolates from the Rhizosphere soil

Key:

R: Rods

C: Cocci

+: Positive

-: Negative .

### 3.3 Screening test

The screen test for petroleum hydrocarbon utilization presented in Table 3, showed the *Pseudomonas* grew best (\*\*\*), *Bacillus* and *Proteus* species grew moderately (\*\*), while, *Enterobacter* and *Staphylococcus* grew minimally on the crude oil growth medium. These organisms were isolated from crude oil contaminated rhizosphere soil of plants and as such have developed capacities to use hydrocarbons (crude oil) as a source of carbon and energy. The variation in the ability of these isolates to utilize hydrocarbons, could be due to differences in competencies of their crude oil degrading enzyme system. Some isolates stand the chance to withstand toxic components of the oil and thrive while others may be inhibited. There are other reports that microorganisms acquire such capabilities when exposed to such environmental stressors [13,41].

Table 3: Growth test for the different isolates in the set-up

Isolate	Tentative Identification	Growth in crude
H1	<i>Pseudomonas sp</i>	***
H2	<i>Bacillus sp</i>	**
H3	<i>Enterobacter sp</i>	*
H6	<i>Proteus sp</i>	**
H10	<i>Staphylococcus sp</i>	*

Key: \*\*\*= heavy growth, \*\*= moderate growth, \*= little growth

### 3.4 Changes in pH during the study

Figure1 shows the average pH values in the set up for the experimental period. The pH results of the assay monitored were 6.28 (*Pseudomonas sp.*), 6.31 (*Proteus sp.*), 6.475 (*Bacillus sp.*) and control having a pH of 6.54 at the end of the study. The pH values for the biodegradation set-up were in a decreasing sequence from the start of experiment. The decrease in pH value might be due to an increase in the production of acidic metabolites by the inoculated

microorganisms. Hence, the medium turned more acidic than usual [42]. Acidity and alkalinity are noteworthy influences in petroleum hydrocarbon breakdown. Unlike soils, most aquatic ecosystems pH, are rarely extremely variable [43]. The pH inhibits microorganisms and enzyme activity, whereas most microbes prefer neutral pH for growth and HC remediation [30]. Majority of microorganisms prefer neutral or low-alkaline conditions to degrade hydrocarbon compounds [32]. Al-Hawash et al. [44] reported that most heterotrophic bacteria and fungi prefer a pH near neutral, with fungi being more tolerant to acidic environments. For that reason, the capability of the microbial population to degrade hydrocarbons is likely to be deleteriously affected by a pH rise observed in some regions. Hence, pH values between 6 and 9 are optimum for microbial growth rate and HC biodegradation even if adequate nutrient and oxygen supplies are lacking [45].

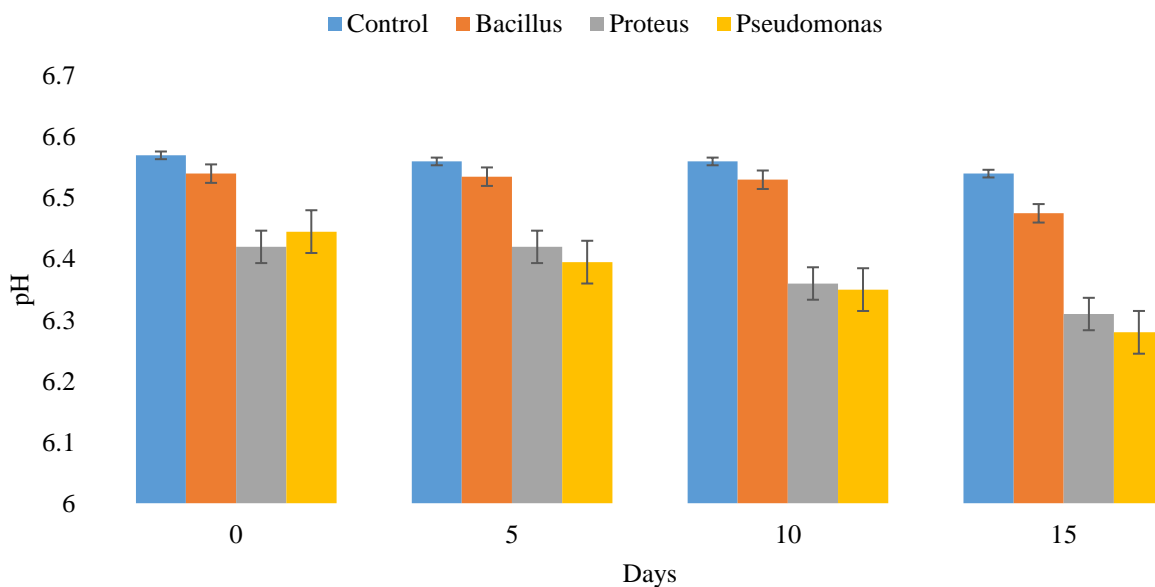


Fig.1: Average pH values for the biodegradation set-up

### 3.5 Turbidity

Figure 2 shows the average turbidity during the period the assay was monitored. The turbidity values at the end of the incubation period (day 15) for the set up were 35 NTU (control), 117.5

NTU (*Bacillus* species), 194 NTU (*Proteus* species) and 282.5 NTU (*Pseudomonas* species). *Pseudomonas* species recorded the highest turbidity value of 282.5 NTU. Bacterial growth can be measured using turbidity as the amount of light absorbed by the bacterial cells is directly proportional to cell concentration. Hence, the higher the number of cells, the higher the turbidity [13,46].

The existence of active hydrocarbon utilizing microorganisms is key for bioremediation. Microbial groups such as bacteria, fungi, algae and viruses have been extensively used as degraders of hydrocarbon contaminants [47]. These strains can persist and use pollutants as sources of growth and metabolism in contaminated soils. Consequently, polluted soils have an ample number of active hydrocarbon utilizing microorganisms. In contrast, the microbial inoculum size affects the breakdown of pollutants. For instance, it has been reported that  $10^6$  CFU/g soil microbes are optimal for degradation [45].

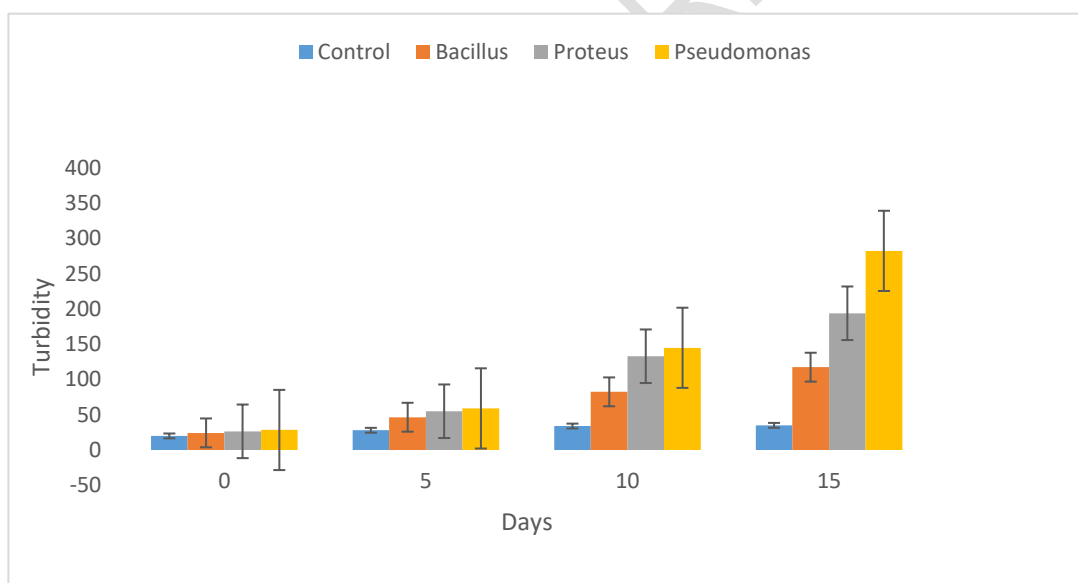


Fig.2. Average turbidity values of the set-up during the study

### 3.6 Bacterial counts during study

Figure 3 shows the bacterial counts during the research. The bacterial counts increased throughout the study. The control showed the least counts and *Pseudomonas* species had the highest counts at all concentrations of crude oil contamination during study (day 15). The bacterial counts were in the increasing sequence; control > *Bacillus* sp. > *Proteus* sp. >

*Pseudomonas* sp. Increases were from  $1.25 \times 10^6$  -  $2.05 \times 10^6$  CFU/ml (*Bacillus* sp.),  $1.37 \times 10^6$  -  $2.28 \times 10^6$  CFU/ml (*Proteus* sp) and  $1.46 \times 10^6$  -  $2.85 \times 10^6$  CFU/ml (*Pseudomonas*), *Pseudomonas* species recorded the highest bacterial count of  $2.85 \times 10^6$  CFU/mL while the control was the least ( $0.35 \times 10^6$  CFU/ml) after the study period (day 15).

There are several reports on accurate management of environmental conditions, crude oil concentrations, time, temperature and the suitable microorganisms in mass and range in resolving cases of oil spill. Endogenous hydrocarbon utilizing microorganisms are readily present in hydrocarbon impacted soils and water in the Niger Delta which can be harnessed in a commercial scale for prompt cleanup of crude oil contaminated aquatic and terrestrial environments [29]. Bioaugmentation involves low cost, small amounts of added biomass, and do not necessitate extreme management of natural conditions for introduced microorganisms to be effective. An instantaneous rise in the population of the microorganisms may well guarantee the swift removal of the pollutants. Gupta et al. [48] in their report, stated that native and exogenous microbial inocula during laboratory study increases the number of viable counts with the bioaugmentation strains. However, biodegradation metabolic pathways and routes are poorly understood, in addition to inefficient transformation results in the accumulation of toxic intermediates that can resist further breakdown via prevailing pathways. For that reason, applicable inoculum selection, optimized inoculation time, reaction conditions (aerobic or anaerobic) and physicochemical parameters are crucial for the far-reaching and effective elimination of these contaminants [45].

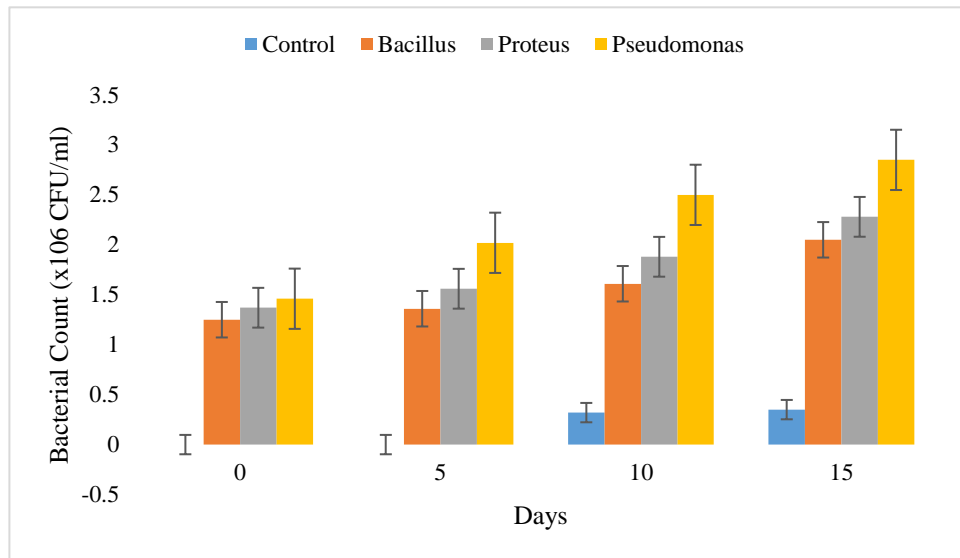


Fig.3. Average bacterial counts during assay.

### 3.7 Oil and Grease

Figure 4 presents the values for residual oil and grease during the study. The oil and grease values at the end of the study period (day 15) for the set up were; 3.533mg/l (control), *Bacillus* species (2.578mg/l), *Proteus* species (2.344mg/l) and *Pseudomonas* species (1.308mg/l).

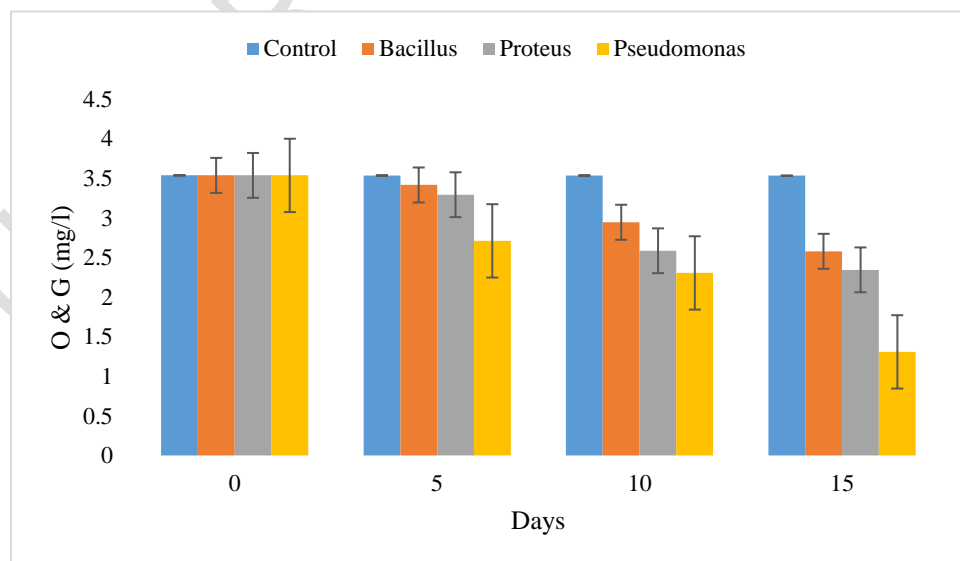


Fig.4. Average oil and grease values during assay.

The oil and grease decreased as the bacterial isolates used the crude oil as their sole carbon source, hence, degraded the crude oil content in the set up. *Pseudomonas* species recorded the least residual oil and grease at the end of the experiment (day 15). A reverse trend was observed for the control. There was a progression in the decrease of the residual oil and grease for the 5% crude oil from day 0 to 15 in the test set up. The degradation sequence at day 15 were 27.11% (*Bacillus*), 33.73% (*Proteus*), 63.02% (*Pseudomonas*) and the control had the least degradation (0.11%).

*Pseudomonas* species degraded the crude oil most in the experimental set up. *Pseudomonas* species which recorded the highest percentage degradation at the end of the period, recorded 34.82% degradation on day 10 and progressed to 63.02% degradation by day 15. There are reports by different researchers that the amount/percentage degradation of crude oil decreases with increased oil concentration possibly due to the presence of highly persistent aromatic alkanes [49,50,51,52,53,54]. Bacterial growth is slower on insoluble hydrocarbon substrates, due to less bioavailability and is one of the major constraints in bioremediation experiments. Our findings were in corroboration with other researchers' reports [5,27]. Yu et al [5] reported a substantial rhizosphere bacterial community which was responsible for a remarkable crude oil degradation. Crude oil contaminated sites can be restored in a sustainable and eco-friendly manner by utilizing natural microbial processes via a diverse range of microorganisms. These microbes can adapt to a target/specific hydrocarbon pollutants, thereby improving the efficiency of remediation processes. Methods/techniques like bioaugmentation and biostimulation, are promising paths for accelerated degradation of hydrocarbons and providing solutions to defined contamination scenarios. Nonetheless, microbial activity, nutrient availability and environmental conditions are pivotal in the success of bioremediation [45]

#### **4. CONCLUSION**

The results of this study indicated that hydrocarbon utilizing bacteria can be found in the rhizosphere of plants. In this research, species of *Pseudomonas*, *Bacillus*, *Proteus* amongst others were isolated and were able to utilize and/or degrade crude oil, using it as carbon source. *Pseudomonas* species performed best amongst the study isolates. Crude oil, even if a very important resource to the economy of Nigeria has posed serious threat to the

environment. This threat is now being overcome by environmental scientists and researchers who adopt bioremediation techniques which is cheap and more environmentally friendly as opposed to other forms of remediation. It is however evident from this study that hydrocarbon-utilizing/degrading microorganisms could readily be isolated from the plant rhizosphere soil of oil-contaminated sites and possibly used for bioremediation of hydrocarbon-polluted environment.

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