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Rhizosphere Associated Bacteria in Bioremediation of Crude Oil Polluted Aquatic Environment

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Authors' contributions

This work was carried out in collaboration between both authors. Author TLA designed the study, wrote protocol, managed the analyses and performed the statistical analysis. Author FUO wrote the first draft of the manuscript and managed the literature searches. Both authors read and approved the final manuscript.

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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.

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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. Residual oil and grease values were 1.308mg/l, 2.344mg/l and 2.578mg/l for *Pseudomonas*, *Proteus* and *Bacillus* in set-up; suggesting they could degrade crude oil using it as carbon source. *Pseudomonas* species performed the most while the control had a value of 3.533mg/l at the end of study. Hydrocarbon utilizing bacterial counts during the assay increased. The bacterial counts were in the increasing sequence; control (3.5×10^5 CFU/ml), *Bacillus* (2.05×10^6 CFU/ml), *Proteus* (2.28×10^6 CFU/ml) and *Pseudomonas* (2.85×10^6 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 use of oil and petroleum products are increasing worldwide, and the threat of oil pollution increases accordingly" (Singha and Pandey, 2021, Chettri et al., 2021). "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" (Jia et al., 2017, Ali et al., 2020). "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" (Yu et al., 2020). 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" (Novianty et al., 2021, Ataikiru et al., 2020).

"Soil, an integral component of the environment, inhabited by heterogeneous microorganisms, including: bacteria, fungi, algae, viruses and protozoa" (Ataikiru and Okorhi, 2024). "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" (Ataikiru et al., 2018, Aragaw, 2021, Sayara and Sánchez, 2020). "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"* (Ataikiru et al., 2020, Ataikiru et al., 2018, Tudararo-Aherobo et al., 2017, Okorhi-Damisa and Tudararo-Aherobo, 2023).

"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" (Kebede et al., 2021). 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" (Ataikiru and Ajuzieogu, 2023). "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" (Dhote et al., 2017, Fatima et al., 2017).

"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" (Kuzyakov and Razavi 2019, Ismail et al., 2021, Vescio et al., 2021, Fu et al., 2023). "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" (Yu et al., 2020, Jiao et al., 2017, Ebadi et al., 2018, Mamarasulov and Davranov, 2024). This investigation assessed the efficiencies of bacteria isolated from rhizosphere of plants found in an oil polluted site in biodegradation and bioremediation.

2. MATERIALS 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. The pla*n*ts were *Axonopus compressus* (carpet grass or cow grass) a*n*d *Grona triflora* (creeping tick trefoil or three-flower beggarweed or èpà-ilè).

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, the coordinates of the location were 5°34'49"N, 5°46'56"E (polluted soil) and 7°23'N; 3°51'E (unpolluted samples). Unpolluted samples were collected using a soil auger at 0-15cm depth randomly to form composite samples. The soil samples were aseptically collected using sterile labelled plastic bags, transported to the laboratory in an ice chest and stored at 4 ±1°C, for further analyses.

2.2 Physicochemical a*n*d Biological Analysis

Fresh soil samples was characterized for pH, electrical conductivity, moisture content, nitrogen, sulphur, total organic carbon and phosphates were analyzed following standard methods by American Public Health Association (2018). 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 (2018). Also, total heterotrophic bacterial and hydrocarbon utilizing bacterial counts were done following standard procedures (Okorhi-Damisa and Tudararo-Aherobo, 2023).

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 (10^{-3} and 10^{-4}) were plated out using appropriate media. Solid media plates were then inverted and incubated for 24hours at 28^0C. Discrete colonies were sub cultured onto nutrient agar plates for further purification which was done twice (using streak method) and transferred into agar slants for storage in the refrigerator at 4ºC and identified using biochemical tests (Tudararo-Aherobo and Okorhi-Damisa, 2023).

2.4 Isolation and Identification of Hydrocarbon Utilizing Bacteria

The vapour phase method of Ataikiru et al., (2020) was adopted. Ten-fold serial dilutions with physiological saline were done using one gram of soil samples. Each tube was vortexed and 0.1ml aliquot (10^{-4} and 10^{-5}) were inoculated onto replicate sterile petri dishes containing mineral salt agar (MSA) using the spread plate method. Plates were inverted and incubated at 28±2ºC for 5 days. The MSA contained KH_2PO_4 (1g), K_2HPO_4 (1g), NH_4NO_3 (1g), $MgSO_4$ (0.2g), $FeCl_3$ (0.05g), $CaCl_2$ (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 slants and stored in the refrigerator at 4ºC for identification.

2.5 Screen Test for Selection of Hydrocarbon Degraders

The bacterial isolates were inoculated into a prepared nutrient broth, which were incubated for 24hours at 28±2^{0}C. A 0.1ml of the fresh 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^{o}C. Broth containing hydrocarbon utilizing bacteria turned turbid as against broth without bacterial growth (control) (Ukaegbu-Obi and Mbakwem-Aniebo, 2014).

2.6 Standardization of Bacterial Isolates

McFarland standard was used as a reference to adjust the turbidity of bacterial suspension (Ajuzieogu et al., 2022). This was done by sub culturing isolated colonies from a pure culture plate into peptone water and incubating at 28±2^{0}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 ($BaCl_2$.$2H_2O$) with 9.95ml of 1% sulphuric acid (H_2SO_4). The approximate cell density of 0.5% McFarland standard is 1.5 x 10^8/ml (Ajuzieogu et al., 2022).

2.7 Bioremediation studies

The bioremediation studies were carried out according to the method of Ukaegbu-Obi and Mbakwem-Aniebo (2014). 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 (Tudararo-Aherobo et al., 2020).

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 (Tudararo-Aherobo et al., 2017). Bacterial growth was also determined via total heterotrophic count employing the standard plate count technique (Ataikiru and Okorhi, 2024). A serial dilution of 1ml of the crude oil contaminated broth samples were carried out. Plate count agar was prepared according to the manufacturer`s instruction. Aliquots (0.1 ml) of the serially diluted samples (10^{-4}) 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 (fungicide) was added to the media to inhibit fungal growth. The plates were inverted and incubated at 28 ± 2^{o}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 ad unpolluted soil samples. Microorganisms as well as their enzyme activities are inhibited by soil pH, though most microbes thrive in neutral pH for growth and remediation (Wang et al., 2016). Most microorganisms prefer neutral or low-alkaline conditions to decompose hydrocarbon compounds (Muangchinda et al., 2018, Snehal 2014). 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 (2014) 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 (Ibiene et al., 2011). The EC values recorded were 64ϒs/cm (polluted) and 70ϒs/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., (2019), 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., (2016) 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.

3.2 Identification of Bacterial Isolates

The cell morphology, Gram's reaction and biochemical characteristics of bacteria isolated from rhizosphere soil 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 (Tudararo-Aherobo et al., 2020, Aloo et al., 2020). Okoye et al., (2020) isolated *Pseudomonas* and *Bacillus* among viable indigenous hydrocarbon degrading bacteria in a chronically polluted soil in Gio Community, Niger Delta. Again, species of *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Xathomonas*, *Proteus*, *Acetobacter*, *Burkholderia*, *Erwinia*, *Serretia*, *Achromobacter*, *Agrobacterium*, *Halomonas*, *Azospiriillum* among others have *b*een 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 (Khatoon et al., 2020, Saeed et al., 2021, Okorhi-Damisa and Tudararo-Aherobo, 2023).

Table 1. Physicochemical and Microbiological characteristics of the polluted soil and control soil samples

Parameters	Polluted soil sample	Control soil sample
pH	6.60	7.50
Electrical conductivity(ϒs/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 ($x10^5$ CFU/g)	6.20	9.80
Hydrocarbon utilizing bacterial counts ($x10^4$ CFU/g)	9.50	8.70

Table 2. Cell morphology, gram's reaction and biochemical characteristics of bacteria isolated from rhizosphere soil

Isolates'code	Gram stain	Shape	Motility	Catalase	Oxidase	Glucose	Sucrose	Gas	Acid	Indole	Citrate	Hydrogen Sulphide	Vogues Proskeur	Methyl red	Urease	Probable microorganisms
H1	–	R	+	+	+	+	–	–	–	+	–	–	+	–	+	*Pseudomonas sp*
H2	+	R	+	+	–	+	+	+	+	+	–	+	–	–	–	*Bacillus sp*
H3	–	R	+	+	–	+		+	+	+	–	+	–			*Enterobacter sp*
H4	–	R	+	+	+	+	+	+	+	+	+	–	–	+	–	*Escherichia coli*
H5	–	R	+	+	+	+	+	+	+	+	+	–	–	+	–	*Escherichia coli*
H6	–	R	+	+	–	+		–	+	+	+	–	+	+	+	*Proteus sp*
H7	–	R	+	+	–	+	–	–	+	+	+	–	+	+	+	*Proteus sp*
H8	–	R	+	+	–	+	–	–	–	+	–	–	+	–	–	*Pseudomonas sp*
H9	+	R	+	+	–	+	+	+	+	+	–	+	–	–	–	*Bacillus sp*
H10	+	C	–	+	–	+	+	+	+	+	–	+	–	+		*Staphylococcus sp*

Key: R: Rods, C: Cocci,+: Positive, -: Negative

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

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

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

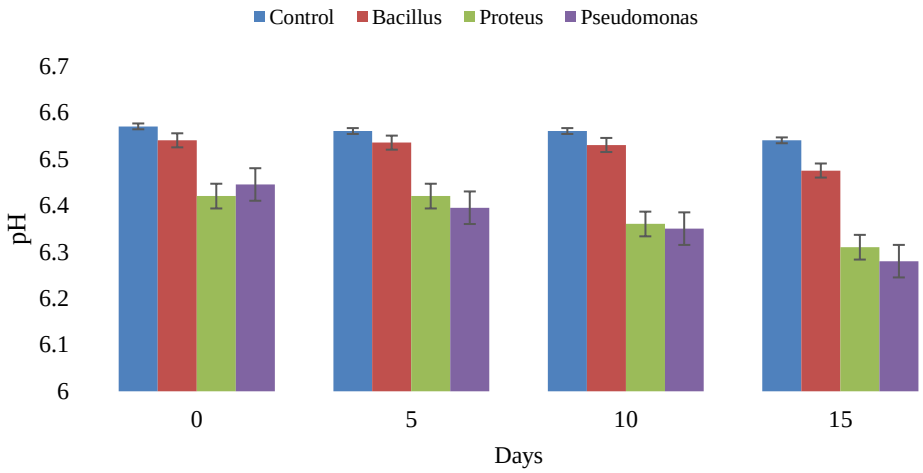

Fig. 1. Average pH values for the biodegradation set-up

3.3 Screening Test

The screening test for petroleum hydrocarbon bacteria 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 (Okorhi-Damisa and Tudararo-Aherobo, 2023, Iheanacho et al., 2014).

3.4 Changes in pH During the Study

Fig. 1 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 (Ejileugha et al., 2015, Wang et al., 2021). Acidity and alkalinity are noteworthy influences in petroleum hydrocarbon breakdown. Unlike soils, most aquatic ecosystems pH, are rarely extremely variable (Wang et al., 2021). The pH inhibits microorganisms and enzyme activity, whereas most microbes prefer neutral pH for growth and hydrocarbons remediation (Wang et al., 2016). Majority of microorganisms prefer neutral or low-alkaline conditions to degrade hydrocarbon compounds (Snehal, 2014). Al-Hawash et al. (2018) 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 areas. Hence, pH

values between 6 and 9 are optimum for microbial growth rate and hydrocarbons biodegradation even if adequate nutrient and oxygen supplies are lacking (Mekonnen et al., 2024).

3.5 Turbidity

Fig. 2 shows the average turbidity during the period the assay was monitored. The turbidity values at the end of the incubation period (day15) 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 (Okorhi-Damisa and Tudararo-Aherobo, 2023, Vasudevan et al., 2010).

The existence of active hydrocarbon utilizing microorganisms is key for bioremediation. Omenna et al., (2023), opined that an effective bioremediation process hinges on the tolerance, characteristics and biodiversity of the microbial isolates. Microbial groups such as bacteria, fungi, algae and viruses have been extensively used as degraders of hydrocarbon contaminants (Ubani et al., 2016). 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 (Mekonnen et al., 2024).

3.6 Bacterial Counts during Study

Fig. 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 x 10^6 - 2.05 x 10^6 CFU/ml (*Bacillus* sp.), 1.37 x 10^6 - 2.28 x 10^6 CFU/ml (*Proteus* sp) and 1.46 x 10^6 - 2.85 x 10^6 CFU/ml (*Pseudomonas*), *Pseudomonas* species recorded the highest bacterial count of 2.85 x 10^6 CFU/mL while the control was the least (3.5 x 10^5 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 (Tudararo-Aherobo et al., 2020). 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., (2022) 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 (Mekonnen et al., 2024). Also, Sui et al., (2021) unequivocally reported microbial remediation for polluted environs due to its ease of use, absence of secondary contamination and cost value but that researchers essentially must understand the components and cause of pollutants, metabolic genes and pathways for the microbial degradation as well as the internal and external features influencing remediation to select prime treatment strategy.

3.7 Oil and Grease

Fig. 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).

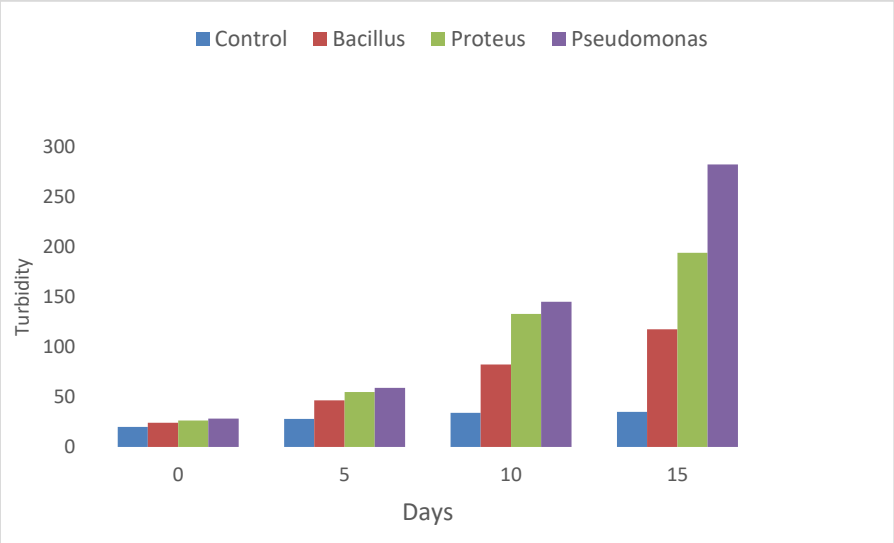

Fig. 2. Average turbidity values of the set-up during the study

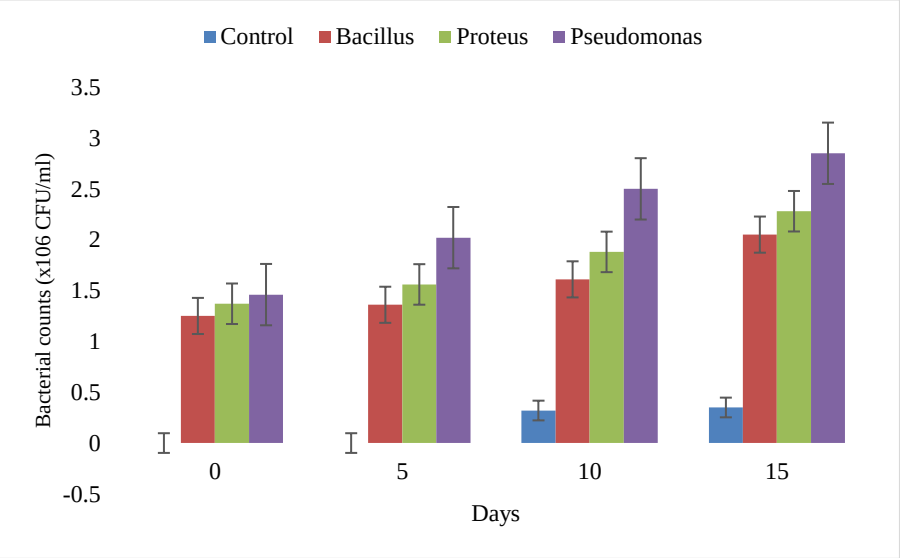

Fig. 3. Average hydrocarbon utilizing bacterial counts during assay

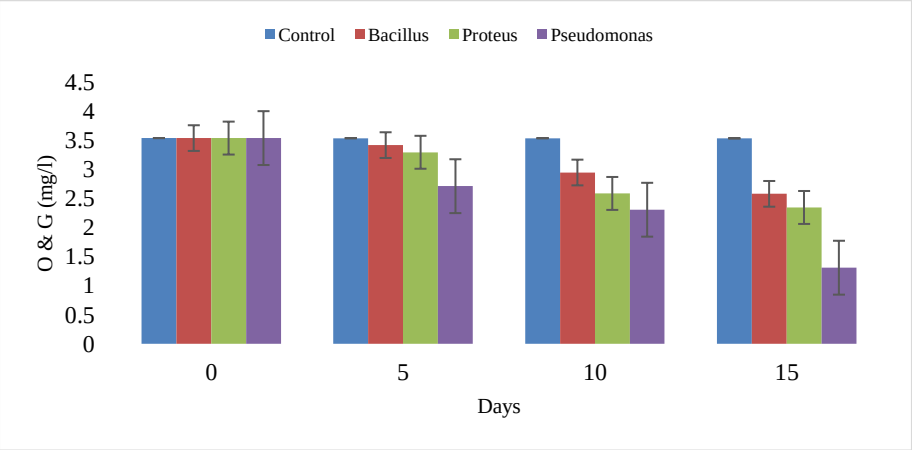

Fig. 4. Average oil and grease values during assay

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 (Agrawal and Shahi, 2017, Agrawal et al., 2018, Ajona and Vasanthi, 2020, Chaudhary et al., 2021, Bala et al., 2022, Popoola et al., 2022). 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 (Yu et al., 2020, Ukaegbu-Obi and Mbakwem-Aniebo, 2014). Yu et al., (2020) reported a substantial rhizosphere bacterial community which was responsible for a remarkable crude oil degradation.

Most scientists have interpreted microbes as the main players in petroleum degradation and plants stimulating microbial growth and ameliorating the soil. Plants release root exudates with organic compounds (e.g. sugars and amino acids) that are metabolized by microbes and help to degrade the pollutants. Plant roots loosen the soil and release oxygen into the soil, so boosting microbial bioenergetics and increasing the number of aerobic microbes. Furthermore, they may increase the availability of hydrophobic compounds by selectively releasing organic acids to different organic pollutants (Stepanova et al., 2022).

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 (Mekonnen et al., 2024).

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 restoration of hydrocarbon polluted environment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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