Original Research Article

Enhanced Phytoremediation and Physicochemical parameter of Crude oil polluted soil using *Pseudomonas fluorescen*s and *Bacillus substilis.*

ABSTRACT

There has been worry over the Niger Delta's environmental contamination. Bacteria and other microorganisms have shown to be very helpful in the breakdown of hydrocarbons generated from petroleum. The goal of this research is to use elbow bufallow grass and sedge plants for phytoremediation of soil affected by crude oil. Standard microbiological techniques were applied to the contaminated soil once it was gathered. Using a hand auger, contaminated soil samples were taken twice a month for three months from two separate locations in Rivers State at two distinct depths: 0–15 cm and 15–30 cm. Two plant species common in the Ogoni region of Rivers state, i.e. Sedge plant (Schoenoplectus), Elbow buffalo grass (*Panicum subalbidum*) were used for phytoremediation monitoring. A combination of treatment consisting of the application *of Pseudomonas fluorescensBacillussubstilis*, *Panicum subalbidum and Schoenoplectus senegalensis* was evaluated during a period of 28 days of remediation.Each pot contained crude oil mixture in the soil as a sole source of carbon and energy. THB counts ranged from2.35 to 4.15cfu/g. The statistical analysis revealed that there was no significant difference ($p>0.05$) in the total heterotrophic bacteria counts between the samples. HUB counts range from 0.7to 1.45cfu/g. The total bacterial population counts obtained from soil sample during bioremediation monitoring ranged from 17$\pm$1.41 (CS+PAN) to 40$\pm$1.412cfu/g (CS+PSE+BAC+PAN) in Day 1. Results of Day 14 range from 13$\pm$1.41 (CS+PAN) to 35.5$\pm$3.542cfu/g (CS+BAC+SCH). Results of Day 28 ranged from 8.5$\pm$0.71 (CS+PAN) to 27$\pm$1.412cfu/g (CS+PSE+BAC+PAN).The presence of microbial activity was determined by the enumeration and isolation of total heterotrophic and hydrocarbon utilizing bacteria. Three (3) most occurring hydrocarbon utilizing bacterial isolates were isolated and identified culturally and phenotypically from the soil samples these bacteria isolates were confirmed to be *Peudomonas*, *Priestia megaterium* and *Bacillusspp* molecularly via sequencing of the 16SrRNA gene. The most common bacteria isolated were *Bacillus spp* at a dilution of 10^4. This research revealed and recommend that *Panicumsubalbidum* as a suitable plant species for phytoremediation of crude oil contaminated soil.

Keyword: Phytoremediation, Physicochemical, polluted soil, crude oil, Enhanced,*Pseudomonasfluorescen*s**,** *Bacillussubstilis***,** Sedge plant and Elbow bufallow grass.

INTRODUCTION

Crude oil is highly complex mixture, containing hundreds of thousands of hydrocarbons. Compounds in crude oil can be divided into three classes consisting of saturated hydrocarbons, aromatic hydrocarbons and polar organic compounds. Soil which are contaminated by

hydrocarbons have extensive damage of local ecosystem since accumulation of pollutants in animals and plants tissues, may cause progency's death or mutation. Crude oil is physically, chemically and biologically harmful to soil because it contains many toxic compounds in relatively high concentrations (e.g polycyclic aromatic hydrocarbons, benzene and its substituted, cycloalkane rings). The presence of high molecular weight compounds with very low solubility in water prevents natural biodegradation process from working efficiently in hydrocarbon contaminated soils. These compounds also penetrate macro and microspores in soil and thus limit water and air transport that would be necessary for organic matter conversion. Generally, petroleum hydrocarbon compounds bind to soil components and are difficult to remove or degrade

Oil pollution is the term used to describe the entrance of crude oil into the environment, which can either partially or fully impair its aesthetic value. With an increase in the need for crude oil as a source of energy and a crucial raw material for businesses, its production, transportation, and refining have all increased, which has resulted in significant environmental damage (Anikwe*et al*., 2017). Both aquatic and terrestrial creatures have faced a serious threat from environmental contamination. According to Borah *et al.* (2016), one of the contaminants that humans release into the environment during oil extraction and transportation is crude oil. One of the continent of Africa's top oil producers is Nigeria. The components of crude oil are deposited in the soil and nearby water bodies when it is discharged into the environment, changing the ecosystem's usual composition of both biotic and abiotic elements (De Boer *et al.,* 2016).

Crop productivity and aquatic life in the water bodies are both impacted by reduced agricultural land as a result of soil and water contamination. The health of the animals may be at risk and the plants may become hazardous when agricultural techniques are performed on polluted soil

(Diphare et al., 2014). When there is a crude oil contamination, the microbes in these ecosystems respond. Crude oil contamination drastically enhances heavy metal concentration in soil and water bodies. Heavy metals such as zinc, chromium, nickel, mercury, iron and copper are components of crude oil, though in low concentrations (Escobar *et al.,* 2018). In the respect of oil pollution, soil remediation methods aim preventing the further spread of pollutant and also its removal from the soil.

In the present study, we aimed to summarize the knowledge used in the remediation of oil polluted soil and to underline importance of bioremediation as a fresh study area in environmental and soil sciences

MATERIALS AND METHODS

Study Area

The study was carried out in Rivers State. Two local government in Rivers state were selected for the study; Port Harcourt Local Government and B-Dere in Gokhana Local Government of Rivers state all in Rivers state, Nigeria. Crude oil exploration takes place in these two locations in Nigeria's geopolitical South-South zone. Crude oil spills at the B-Dere area have been linked to antisanal refineries' operations. The locations were selected due to the fact that they are sites known for various activities including bunkering/Local refining of crude oil.

Sampling technique

To get the soil samples, a straightforward random sampling procedure was employed. Using a straightforward random sampling procedure, one bag of contaminated soil and one bag of uncontaminated soil were taken from each soil sample.

Sample Collection, and Processing

Using an auger device, samples were collected aseptically. Following the Food and Agriculture Organization's (FAO) 2002 guidelines, soil samples were taken using a sterile soil auger to measure the topsoil's depth. The soil samples for analysis were collected into fresh unused black polythene bags perforated for aeration. The samples were transported within 2hours of collection to the Postgraduate laboratory of Microbiology Department, Rivers state university Port Harcourt.

Bacteriological Analysis of Samples.

The weighed soil sample was subjected to a serial tenfold dilution with a dilution factor ranging from 10-1 to 10-6. Onto Nutrient Agar, an aliquot (0.1 ml) of the suitable dilutions was spread plated in duplicate. For twenty-four hours, the plates were incubated at 37°C.The total heterotrophic bacterial counts (THBC) were estimated from the colonies formed on nutrient agar, which were counted and described morphologically. To obtain pure cultures, representative distinct colonies were purified by sub-culturing on newly prepared sterile nutrient agar plates and then incubated at 37 °C for 24 hours.

Isolation and Enumeration of Crude oil Utilizing Bacteria

In Mineral Salt Agar Medium, hydrocarbon-degrading bacteria were isolated. The mineral salt media's composition (g/L) is as follows: 0.2 $MgSO_4$, 0.02 $CaCl_2$, 1.0 KH_2PO_4, 1.0 NH_4NO_3, 0.05 $FeCl_3$, and pH adjusted to 7–7.2. The Mineral salt agar (MSA) plates were inoculated in duplicate with 0.1ml aliquots of 10^{-6} dilution of each soil samples and incubated at 35 ^{o}C for 7 days. After a week, colonies on the agar plates were counted, yielding the total number of hydrocarbon-degrading bacteria for each of the four soil samples. The colony forming unit (CFU) per gram of soil used to represent the counted colonies.

Preparation of Bacterial suspension for Bioremediation setup

Bacillus substilis and *Pseudomonas fluorescens* suspension was made from a 24-hour subcultured Petri plate. After being transferred into a 250 ml conical flask, 200 ml of nutritional media broth was autoclaved at 121 oC for 15 minutes at 15 psi, and the mixture was allowed to cool at room temperature. Cicatrin0.8g was added to the broth. *Pseudomonas fluorescens* and *Bacillus substilis* were isolated as pure cultures on a culture plate, and they were subsequently transferred to a 250 ml nutrient broth in a conical flask until turbidity formed. The flask was cap with cotton wool. This was incubated at room temperature 28°C for 48hrs.

Treatment of the Soil for Bioremediation.

The soil sample was treated for bioremediation as described by (Nrior, 2014) shows the experimental set up. In this method, 10 setups were made. Each basin contained;

1. 2500g of uncontaminated soil + *Panicumsubalbidum* which served as control
2. 2500g of contaminated soil + *Panicumsubalbidum*+ 250ml of bonny light crude oil
3. 2500g of contaminated soil +*Panicumsubalbidum*+ 250ml of bonny light crude oil + 50ml of *Pseudomonasfluorescens*broth.
4. 2500g of contaminated soil + *Panicumsubalbidum* + 250ml of bonny light crude oil + 50ml of *Bacillussubstilis*broth.
5. 2500g of contaminated soil + *Panicumsubalbidum*+ 250ml of bonny light crude oil + 25ml of *Pseudomonasfluorescens* broth + 25ml of *Bacillussubstilis*broth
6. 2500g of uncontaminated soil + *Schoenoplectussenegalensis*.
7. 2500g of contaminated soil + *Schoenoplectussenegalensis* sediment + 250ml of bonny light crude oil.

8. 2500g of contaminated soil + *Schoenoplectussenegalensis*+ 250ml of bonny light crude oil + 50ml of *Pseudomonasfluorescens* broth.

9. 2500g of contaminated soil +*Schoenoplectussenegalensis*+ 250ml of bonny light crude oil + 50ml of *Bacillussubstilis*broth.

10. 2500g of contaminated soil + *Schoenoplectussenegalensis*+ 25ml of *Pseudomonasfluorescens* broth + 25ml of *Bacillussubstilis*broth

Table 1: Sample Label for Bioremediation Set-Up

Sample label	Soil batches
Sample A	Uncontaminated soil + *Panicumsubalbidum*
Sample B	Contaminated soil + *Panicumsubalbidum*
Sample C	Contaminated soil + *Pseudomonas* + *Panicumsubalbidum*

Sample D	Contaminated soil + *Bacillus*sp+ *Panicumsubalbidum*
Sample E	Contaminated soil + *Pseudomonas*+ *Bacillus*sp+ *Panicumsubalbidum*
Sample F	Uncontaminated soil + *Schoenoplectussenegalensis*
Sample G	Contaminated soil + *Schoenoplectussenegalensis*
Sample H	Contaminated soil + *Pseudomonas* + *Schoenoplectussenegalensis*
Sample I	Contaminated soil + *Bacillus*sp+ *Schoenoplectussenegalensis*
Sample J	Contaminated soil + *Pseudomonas* + *Bacillus spp* + *Schoenoplectussenegalensis*

Phytoremediation of Plant.

The soil was evenly mixed with crude oil to create five pots of contaminated soil containing 5% crude oil. There were 2500g of soil and 250ml of crude oil in each pot. The crude oil was Bonny light crude (API = 32.3o; sulfur content: 0.08%), and the soil was commercially available compost soil from Rivers State University school farm.

Soil preparation and application of crude oil and nutrients

Two batches of soils were collected; one batch had soil contaminated with hydrocarbons, and the other batch contained uncontaminated soil. Eight batches of two thousand five hundred grams (2500g) of contaminated soil and two batches of two thousand five hundred grams (2500g) of uncontaminated soil were weighed. But for every batch of soil, a new set of treatments was taken into account. The uncontaminated soil was designated as the control in accordance with various bioremediation techniques to facilitate simple interpretation of these results.

Plant Selection and Cultivation

Two plant species common in the Port Harcourt Local Government in Rivers state were used. They are Elbow buffalo grass (*Panicum subalbidum*) and Sedge plant (Schoenoplectus), were chosen for the study because they were readily available and locally widespread while being easy and inexpensive to cultivate. The plants were identified by Dr M.G Ajuru of Plant Science Department of Rivers State University. The plants have also been observed to proliferate in the vicinity of petrol stations and crude oil storage facilities, and their ability to phytoremediate crude oil has not been characterized. The plants were screened for uniformity of fresh weight before planting. The fresh weight of the plants was approximately 0.5kg. Healthy-looking plants with profuse roots were selected to ensure higher success of cultivation in the crude oil-contaminated soil. The roots were trimmed to reduce variability of roots' abundance among the plants. The plants were planted directly in potted soil. One pot served as the control. All the pots were watered twice daily by spraying to maintain sufficient moisture of the soil. The pots were placed in area shaded from rain but with access to sunlight.

Soil Analysis

Soil samples were taken from each pot at a set distance from the plant during the first week following planting and then every week for an additional five weeks. 2 mm mesh was used to sift the collected samples in order to separate the organic materials from the particulate matter. After sieving, sixteen (16 g) grams of soil were gathered for further examination. The pH, moisture content, and concentration of crude oil in the soil were all measured. To ensure that there was enough soil moisture for phytoremediation, the soil's moisture content was measured. pH was measured since it was known that during phytoremediation, plants would change the pH of the surrounding soil.

Data Processing and Analysis

The data from counts and the measurement of the zones of inhibition were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 25. The collected data was all summarized using descriptive statistics. To determine whether there were any significant differences ($p \leq 0.05$) between the bacterial counts from the various locations, analysis of variance (ANOVA) was used. Duncan multiple range test was used to separate the means where difference existed (Bewick *et al*., 2004).

RESULTS

The presence of microbial activity was determined by the enumeration and isolation of total heterotrophic and hydrocarbon utilizing bacteria carried out and presented in previous work of Williams *et al*.2018

Results of the bacterial population of soil samples are presented in Table 1. The result of analysis showed that the mean total heterotrophic bacterial count ranged from 2.35 to 4.15cfu/g. The statistical analysis revealed that there was no significant difference ($p>0.05$) in the total heterotrophic count between the samples.

Results of the hydrocarbon utilizing bacterial ranged from 0.7 to 1.45cfu/g. There was no significant difference ($p>0.5$) in the total hydrocarbon degrading microorganism count.

Results of the total bacterial population count obtained from soil sample during Bioremediation monitoring are presented in table 3.

Table 2. Bacterial Population of soil samples.

Location	THB $X10^6$cfu/g	HUB $X10^5$cfu/g
A	3.65 ± 0.7^a	1.15 ± 7.8^a

B	2.35±3.5[a]	1.45±9.2[a]
C	3.7±5.7[a]	0.7±5.7[a]
D	4.15±7.8[a]	1.15±3.5[a]

Key: THB (Total Heterotrophic Bacterial), HUB (Hydrocarbon utilizing Bacterial)

Table 3: Baseline Results of Physicochemical Parameters of Uncontaminated Soil before Phytoremediation

S/N	Parameters	Uncontaminated soil
1.	Ph	5.43
2.	Temperature (^{o}C)	27
3.	Electrical Conductivity (µS/cm)	9
4.	Moisture Content (%)	7.80
5.	Total Organic Carbon (%)	0.93
6.	Soil Organic Matter (%)	1.60
7.	Nitrogen (Mg/kg)	56.695
8.	Phosphorus (Mg/kg)	0.621
9.	Potassium (Mg/kg)	7.125
10.	Total Hydrocarbon Content (Mg/kg)	700

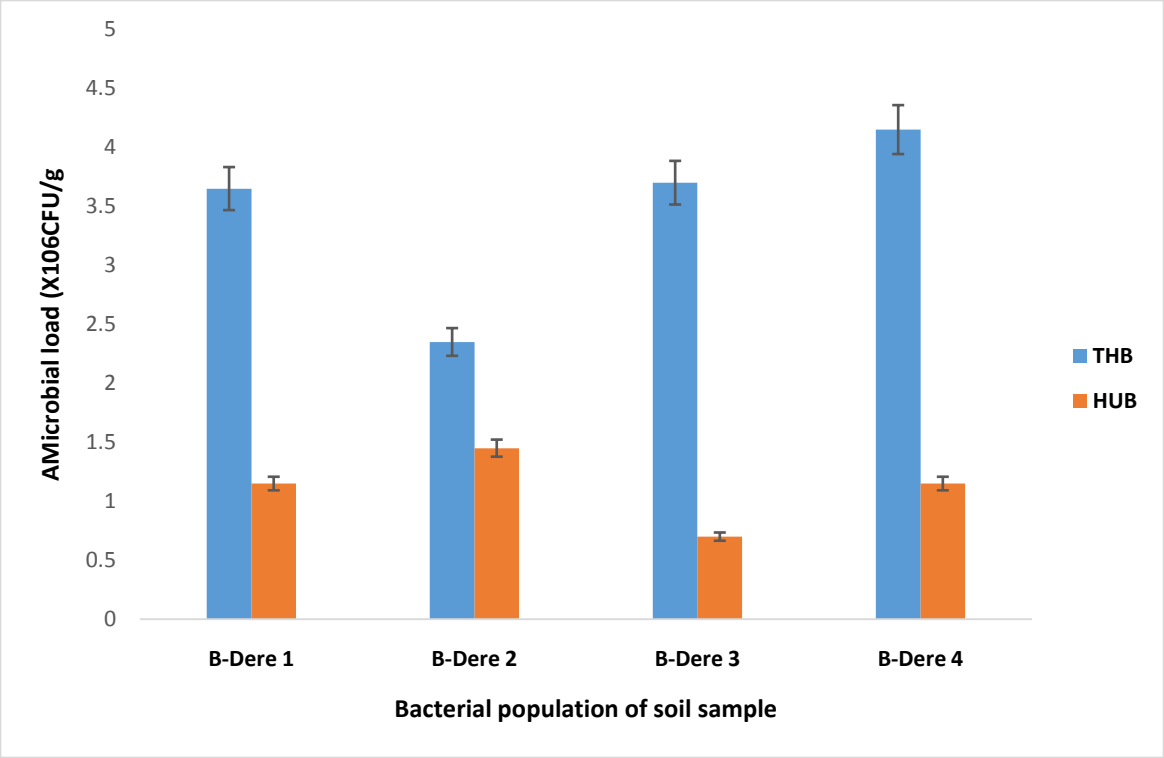

Fig. 1 Mean Bacterial population of soil sample

Table 4. Mean Physicochemical Parameter of the Soil Sample during Bioremediation Monitoring

Treatment code	pH	Temperature	Nitrogen	Phosphorus	Potassium	Soil THC	Root THC
US+ PAN	6.48±1.01^{ab}	27.83±0.83^{b}	66.18±23.59^{a}	0.48±0.22^{a}	5.38±1.96^{b}	442±239.77^{ab}	9.87±9.61^{ab}
U S+ SCH	6.69±1.44^{b}	27.47±0.67^{ab}	65.51±33.30^{b}	0.47±0.21^{a}	4.13±2.94^{a}	490.67±217.41^{ab}	6.07±6.10^{a}
CS + PAN-G	6.07±0.73^{b}	27.43±0.43^{b}	35.27±20.82^{a}	0.45±0.24^{a}	3.38±1.98^{b}	2429.33±1497.12^{ab}	118±107.43^{b}
CS + SCH-G	6.01±0.64	27.3±0.44^{b}	34.15±8.61^{ab}	0.49±0.28^{a}	2.36±1.57^{a}	2346±1520.42^{ab}	290.67±306.01^{ab}
CS + Pse+ PAN-G	6.07±0.70^{b}	27.3±0.3^{b}	32.36±12.15^{ab}	0.46±0.25^{a}	2.54±1.38^{a}	2206±1600.60^{b}	122.67±107.43^{b}
CS + Pse+ SCH-G	6.11±0.74^{ab}	27.37±0.55^{a}	40.40±20.03^{b}	0.45±0.24^{a}	5.54±3.36^{b}	1942.67±1848.37^{ab}	243.33±253.55^{ab}
CS +Bac + PAN -G	5.96±0.61^{b}	27.43±0.67^{b}	38.83±22.39^{a}	0.44±0.22^{a}	5.63±5.05^{a}	2042±1748.27^{cd}	162.67±159.13^{b}
CS +Bac + SCH-G	6.14±0.76^{b}	27.3±0.44^{b}	45.06±4.17^{ab}	0.39±0.29^{a}	4.46±3.39^{ab}	1880±1879.30^{ab}	274±324.27^{ab}
CS + Pse + Bac + PAN-G	6.22±0.83^{b}	27.33±0.49^{b}	118.87±116.31^{b}	0.45±0.24^{a}	2.23±1.65^{a}	2227.33±1601.07^{ab}	167.33±161.37^{ab}
CS + Pse + Bac + SCH-G	5.99±0.64b	27.4±0.61^{b}	158.21±221.08^{ab}	0.44±0.22^{a}	3.11±1.75^{a}	2020±1765.76^{ab}	126.67±109.71^{ab}

KEY: US (uncontaminated soil), CS (contaminated soil), Bac (*Bacillusspp*), Pse (*Pseudomonasspp*), PAN (*Panicumsubalbidum*), SCH (*Schoenoplectussenegalensis*

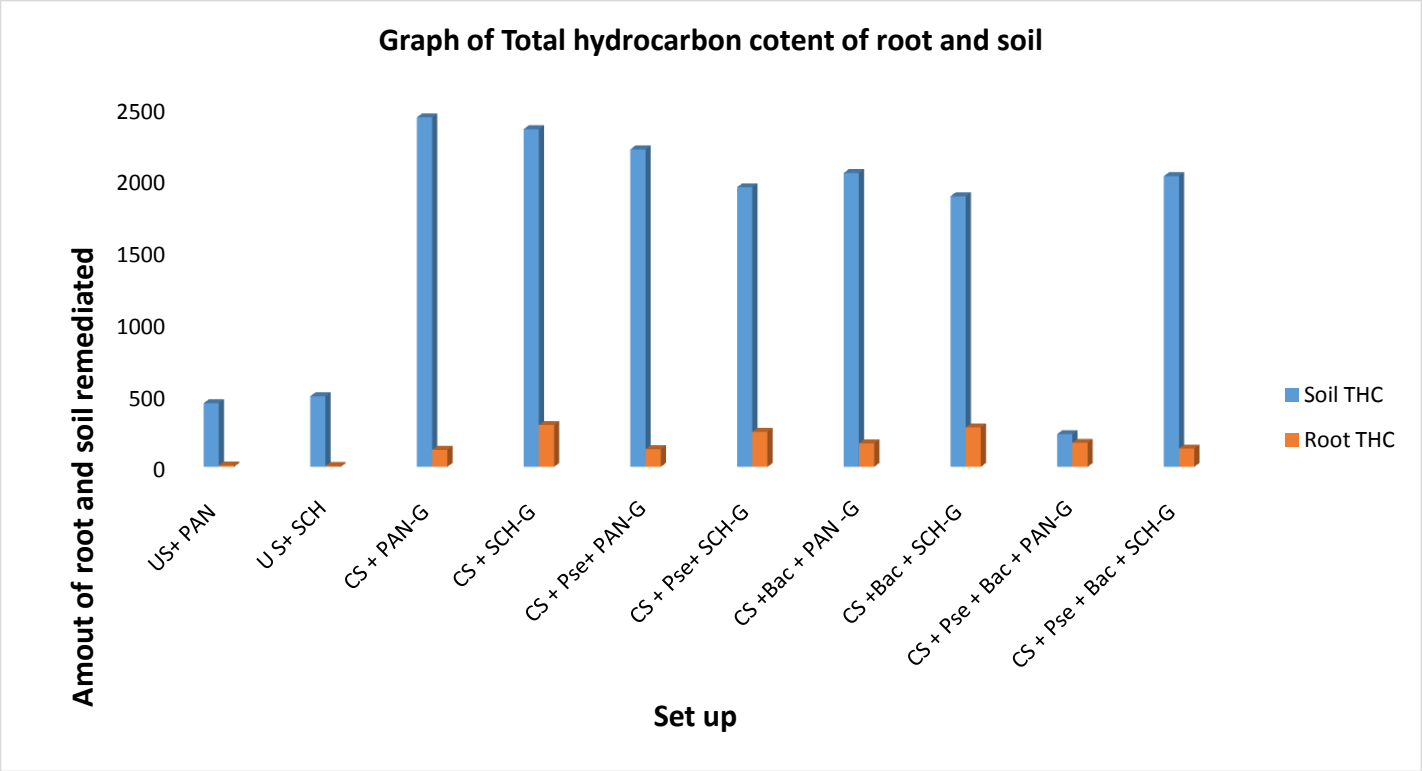

Figure 2. Total hydrocarbon content of Root and Soil

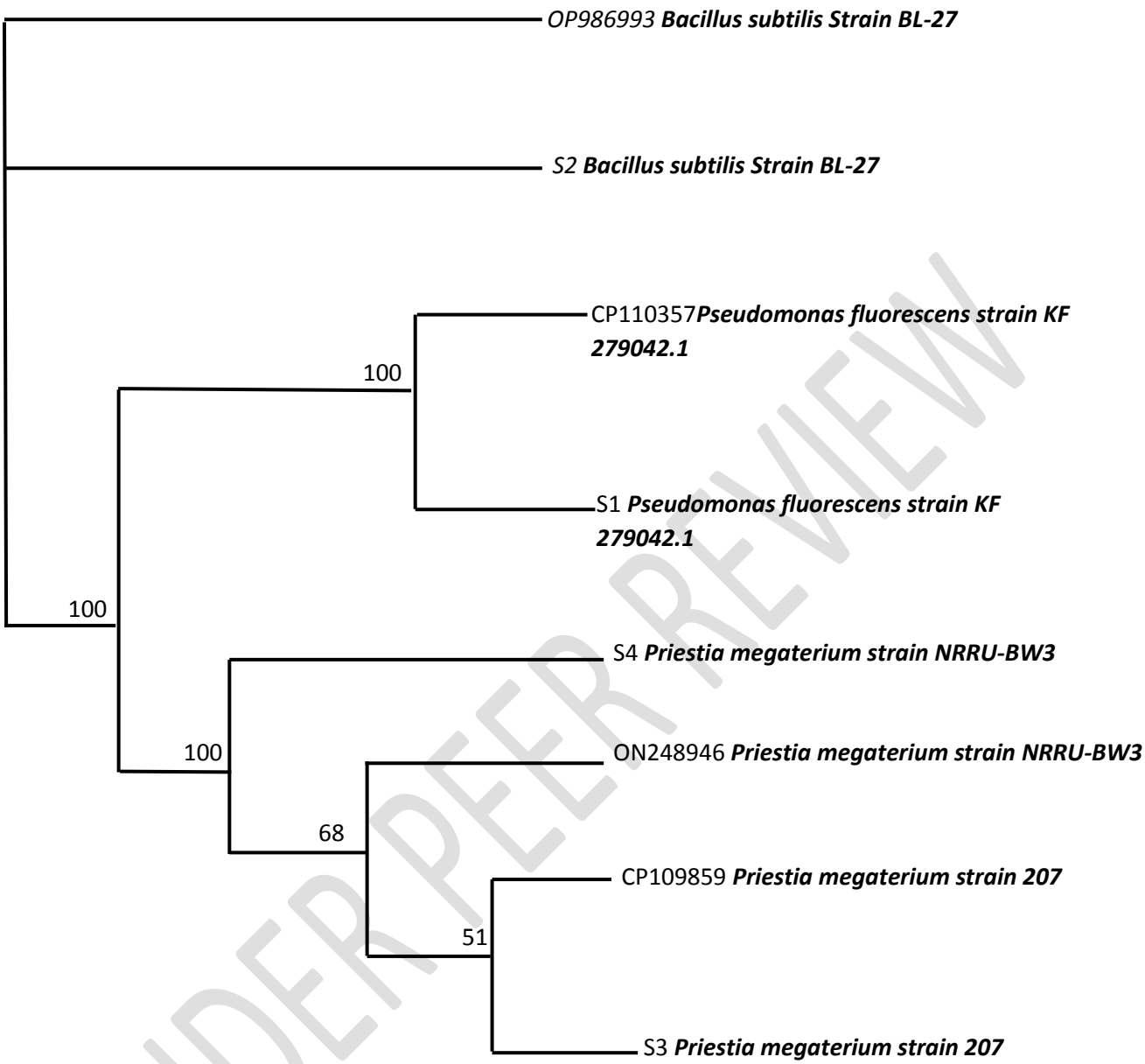

Plate 1: Phylogenetic Tree Showing Evolutionary Distance between Bacterial Isolates

DISCUSSION

Crude oil contamination is one of the major environmental problems affecting aquatic and terrestrial environments. An ecosystem's native microorganisms can differ in composition as a result of crude oil contamination. Microbes in such ecosystem react when there is contamination

with crude oil. Either a good or negative response is possible. In positive response, the microorganism especially bacteria maintain their ecological niche due to their ability to withstand the introduced stress. This adaptive measure enables the organism to source their nutrients from the composition of the crude oil. When the response is negative, the bacterial species are sensitive to the component of the crude oil, so they cannot withstands the stress, which may result to their complete elimination from the habitat (Anwar *et al.*, 2017).

According to the study, total heterotrophic bacteria were more prevalent in sample 4 (the school farm at Rivers State University) at both depths (0–15 cm and 15–30 cm), which is consistent with research by Williams and Hakam (2016).The fact that total heterotrophic bacteria are the most abundant organisms at both soil depths may be related to their ability to withstand large fluctuations in the characteristics of the soil, including its nutrient and moisture contents, oxygen concentration, and many other parameters that are significant to this investigation. The findings of this investigation confirmed the pattern seen by Nrior and Ogbonna (2019) for soil bacterial populations. The isolate from soil samples has a notable capacity to use crude oil as the only source of carbon and energy, and other researchers, including Williams and Hakam (2016), have reported the existence of these organisms. A number of things happen in an environment that lead to the degradation of that environment when crude oil or other petroleum products are spilled into it. Twenty to forty percent of the oil mass evaporates during the first few days, leaving the heavier components behind as the volatile gasses evaporate (Williams *et al.*, 2018). According to Williams *et al.* (2018), natural attenuation refers to a range of physical, chemical, or biological processes that, in the presence of suitable circumstances, function naturally to lessen the bulk, toxicity, mobility, volume, or concentration of pollutants in soil. A few months later, environmental stress causes microorganisms that are unable to use the hydrocarbons in the

soil to either mutate or go extinct. After a few years, most of the components of crude oil also totally decompose and the organisms in the environment fully adapt and multiply. These characteristics contribute to the lower total petroleum hydrocarbon (TPH) content in sites with older spills, which increases the likelihood of microorganism proliferation (Nriore*t al.,* 2019). The study's findings also indicated that as soil depth grew, the numbers of heterotrophic bacteria decreased. This might be because there are more growth-promoting elements available at the surface soil (0–15 cm) than at the subsurface soil layers (15–30 cm), such as utilizable organic matter and oxygen.

The results of this investigation showed that, as predicted, the majority of the bacteria in the soil sample were heterotrophic, whereas the bacteria that used hydrocarbons were the least prevalent (Table 2). Microorganisms that break down hydrocarbons are widely found in soil environments contaminated with crude oil. Williams and Barisi (2018) state that whereas the population of hydrocarbon degraders typically makes up less than 1% of all microbial communities, in environments where oil pollution are present, this population typically increases to 10%.According to this study, there was a decrease in the number of creatures that used hydrocarbons compared to the number of heterotrophic microbial species because not all members of the heterotrophic population could use the crude oil and petroleum products that were spilled in the soil environment (Anwar *et al.,* 2017). The high hydrocarbon utilizing bacterial counts could be attributed to the utilizable organic matter present in crude oil.

In this study, a soil sample contaminated with crude oil was used to identify approximately three species of bacteria by genetic analysis, with Bacillus being the most prevalent. In this investigation, *Peudomonas, Priestia megaterium,* and *Bacillus species* were isolated and identified. These organisms are able to get all of the carbon and energy they need from crude oil.

And the dominance of these organisms have been reported by different researchers as crude oil degraders (Nrior and Ogbonna, 2019; Williams and Barisi, 2018).

The primary factor in the mineralization of contaminants from crude oil is microbes. Utilizing the metabolic adaptability of microorganisms, bioremediation employs the degradation of hazardous pollutants to facilitate the ecological restoration of sites affected by petroleum waste. Bacteria are commonly selected among microorganisms due to their swift metabolic rates, ability to participate in several degradation pathways, and capacity to undergo genetic manipulation for enhanced bioremediation (Anwar *et al.,* 2017).

Results obtained from this study has shown that *Panicum subalbidum* and *Schoenoplectussenegalensis* plant due to their high moisture and nutrient content properties makes them appropriate agents for enhanced bioremediation. It further revealed that a combination of phytoremediation and Bioaugmentating agents creates more favorable conditions for biological activity to thrive and has shown to be effective, economical, eco-friendly and sustainable in remediating organic contaminants from contaminated soil.

The bacterial from the experimental soil used in this study belong to the genera; *Pseudomonas fluorescens* and *Bacillussubstilis*. This is in line with the observations of various research who reported similar bacterial from crude oil contaminated soil. The results of the microbial evaluation of the study are shown in Table 2. Significant microbial counts for total heterotrophic bacteria counts were recorded. The results of bacterial population counts revealed that the total heterotrophic bacterial generally increased during the study as the treatment progressed resulting in corresponding bioremediation with time (Day). The result is consistent with the reports of Nrior and Ogbonna (2019) who observed that total heterotrophic bacterial and Hydrocarbon utilizing bacterial increased over time in a nutrient amended crude oil contaminated soil

undergoing bioremediation with time (Day). This may also be as a result of increase in microbial activities in soil as a consequence of added nutrient. Temperature generally increases the rates of chemical reactions, microbiological activity, and biodegradation. One of the main processes used to mineralize and extract petroleum hydrocarbons from polluted environments is biodegradation, which is mediated by native microbial communities. Thus, microbial oil biodegradation is recognized as one of the most important methods for petroleum hydrocarbon remediation.

Phytoremediation using grass plant *Panicumsubalbidum* (Elbow buffalo grass), Sedge plant (*Scoenoplectussenegalensis*) was carried out on Crude Oil contaminated soil. Some isolated microorganisms – *Bacillus substilis* and *Pseudomonas fluorescens* were used to augment the indigenous microbial population present in a crude oil contaminated soil to enhance microbial remediation in pari per sue with phytoremediation (uptake of Crude oil by test plants) over a period of 28 days. The Sedge plant (*Scoenoplectussenegalensis*) survive the first screening stage with crude oil contamination but died during the monitoring period of 28days. The Elbow buffalo grass (*Panicumsubalbidum*) survive after monitoring of 28 days with crude oil contamination.

During the first seven days of growth, the experimental transplants reached a height of 16.7 cm. The plants displayed decreased growth, while the seedlings in uncontaminated soil grew well.Regardless of the bio-organic in the contaminated soil compensating for the greater C/N ratio, *Panicum subalbidum* (Elbow buffalo grass) showed a strong potential for adaption in the contaminated soil as demonstrated by the growth between 14 to 28 days. With time, the plant's height dramatically grew ($p=0.05$).While Scoenoplectus senegalensis did not survive the crude oil contamination after 28 days of monitoring, the average plant height of Panicum sbalbidum (Elbow buffalo grass) was 52.46 and 55.82 cm, respectively, in pots 4 and 6, compared to 36.88

cm in (uncontaminated plots) during the 28 days of observation. There was no significant difference of plant height between the contaminated and uncontaminated.

Root structure is considered just as important as root biomass concerning degradation process. Generally, the roots growing in uncontaminated soil were longer, and covered more surface area than those growing in contaminated soil. The result from this study indicates that under normal pH, oxygen and sufficient nutrients, phytoremediation of crude oil contaminated soil increased in each pot compared to the controls. Statistically there was no significant difference ($p<0.05$) in hydrogen ion concentration (pH) in various treatment pots.

CONCLUSION AND RECOMMENDATIONS

Environmental research has significant challenges in the repair of oil-contaminated soils, as petroleum hydrocarbon pollution poses a global hazard to the ecosystem. The biological treatment method known as "bioremediation" is used to eliminate or significantly lower the amount of hazardous waste present in contaminated areas. Crude oil contamination drastically enhances heavy metal concentration in soil and water bodies. Findings showed the percentage (%) and amount of soil hydrocarbon extracted. Following a 28-day period of monitoring, bioremediation was found to be more effective when using CS+PSE+SCH (3454 mg/kg; 85.28%) and less effective when using US+SCH (434 mg/kg; 62%) in terms of the amount of root hydrocarbon content eliminated and the percentage (%) After 28 days of observation, bioremediation was shown to be lower in the setup with US+SCH (12.2 mg/kg; 1.74%) and greater in the setup with CS+BAC+SCH (632 mg/kg; 15.6%).

According to this study, Panicum subalbidum is a good plant species for phytoremediation of soil contaminated with crude oil. The microorganisms in this study that used hydrocarbons were *Pseudomonas, Priestia megaterium,* and *Bacillus sp*.This study also suggests that the organism

and test plant utilized are naturally occurring, easily accessible, affordable, eco-friendly, and efficient.

It is recommended to promote the use of Panicum subalbidum and Schoenoplectussenegalensi as effective phytoremediation agents. The results of this study suggest that, in order to aid in the removal and cleanup of pollutants, the use of environmentally friendly bioorganic (or biostimulants) and boosting microorganisms as amendment choices with phytoremediation plants should be promoted. This study showed that *Panicumsubalbidum* should be used as a suitable plant species for phytoremediation of crude oil contaminated soil.

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