Evaluation of Phytoremediation, Physicochemical and Heavy Metal Assessment of Crude Oil Polluted Soil Using *Pseudomonas* and *Bacillus spp,* Rivers State, Nigeria

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Original Research Article

ABSTRACT

Crude oil exploration has been beneficial to our economy but detrimental to our environment with the artisanal `refineries further compounding the challenge. This research was aimed at investigating the phytoremediation, physicochemical and heavy metal assessment of crude oil polluted soil using *Peudomonas* spp. and *Bacillus* spp. as augmenting microorganism, in Rivers state, Nigeria. This study was carried out in in south-south Nigeria (B-dere in Gokana Local government of `Rivers State). Contaminated Soil were collected and subjected to standard microbiological methods. Contaminated Soil samples were collected from two different area of Rivers state with sterilized soil auger at two depths of 0-15cm and 15-30cm. The contaminated soil sampled were analyzed for heavy metal (Cadium Chromium, Lead and Zinc) using Atomic Absorption spectrophotometric method. Microbiological analysis was carried out on the soil samples.

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Ten (10) treatments consisiting of contaminated soil (CS), uncontaminated soil (US), uncontaminated soil(US), *Panicum subalbidum* and *Schoenoplectus senegalensis* were setup for a period of 28 days. Physicochemical parameters were analyzed for uncontaminated soil and contaminated soil. The physicochemical parameters analyzed were pH, Temperature, Nitrogen, Phosphorus, Potassium and Total Hydrocarbon Content. The physiochemical parameters of the uncontaminated soil were pH (5.43), temperature (27°C), Electrical conductivity (9uS/cm), Moisture content (7.80%), Total organic carbon (0.93%), Soil organic matter (1.60%), total Nitrogen (56.695mg/kg), available phosphorus (0.621mg/kg), potassium (7.125mg/kg) and total hydrocarbon content (700mg/kg). Results revealed that the amount of hydrocarbon remediated and percentage (%) bioremediation in the soil after 28 days of monitoring from the initial THC value of (4050 mg/kg), is higher in set up with CS+PSE+SCH (3454mg/kg; 85.28%) and lowest in set up with US+SCH (434mg/kg: 62%) and the amount uptake of phytoremediation in the root after 28 days of monitoring to be higher in set up with CS+BAC+SCH (632Mg/kg; 15.6%) and lowest in set up with US+SCH (12.2mg/kg; 1.74%). The heavy metals assessed were cadium (Cd), chromium (Cr), lead (Pb) and zinc (Zn). This research revealed and recommend that *Schoenoplectus senegalensis* as a suitable plant species for phytoremediation of crude oil contaminated soil. Hydrocarbon utilizing bacteria identified in this study were; *Pseudomonas* and *Bacilus* spp.

1. INTRODUCTION

Soil pollution with crude oil and its products has become a major global environmental concern. Crude oil spill arises from vandalism of oil installations, corrosion of over aged oil facilities and uncontrolled spillage in oil refineries and storage tanks [1]. Crude oil is a complex mixture containing thousands of hydrocarbons that can be divided into four classes, namely the saturates, the aromatics, the asphaltenes and the resins. It is physically, chemically and biologically harmful to soil because it contains many toxic compounds in relatively high concentrations, and is thus classified as environmental pollutant by the US Environmental Protection Agency [2]. When crude oil is released on the ground surface, it gradually penetrates the soil and at a depth of around 10-20 cm, it results in soil fertility loss. Other effects are environmental degradation, groundwater pollution, biodiversity loss and threat to environmental sustainability [3-5]. Under normal conditions, crude oil in soil persists much longer than most conventional carbon sources (e.g. carbohydrate and proteins) which take only weeks to be degraded, while under extreme conditions (e.g. drought) it persists much longer [6].

Remediation of petroleum hydrocarbon contaminated sites is a real-world problem. Over the years, several methods have been developed and investigated for the remediation of petroleum hydrocarbons contaminated sites [7-10]. Some of the major methods are physicochemical, thermal and biological techniques [11]. The choice of the method to use depends on the chemical, physical and biological properties of both contaminant and soil. The physicochemical and thermal techniques have been found to be expensive [12]. Bioremediation (biological technique) has appeared as the most desirable method due to its simplicity, cost-effectiveness and ecofriendliness [13,14]. Bioremediation is a treatment process that uses microorganisms to breakdown or degrades hazardous substances into less toxic or nontoxic substances [15-19]. Critical conditions for effective bioremediation include the presence of contaminants, microbes that feed on the contaminants, sufficient oxygen, suitable soil moisture, right temperature, nutrients to support microbe growth, and suitable pH. Naturally, bioremediation can be slow due to the presence of high molecular weight compounds with very low solubility [20-23]. More so, the oxidizing microorganisms may not be present in contaminated soil in the numbers required for effective bioremediation. In order to improve the natural tendency of soil microorganisms to decompose hydrocarbons from crude oil, many techniques have been proposed and tested [24,25,26]. These techniques include the use of amendments and microorganism immobilization [27]. Accordingly, bioremediation could be achieved either as biostimulation (addition of nutrients/amendments) or bio augmentation (addition of oxidizing

microorganisms), depending on the pollution situation and type of microorganisms being used. But biostimulation has been proven to be a promising bioremediation technique for the treatment of polluted soil aerobically [28].

Thus, this research is aimed at understanding the phytoremediation, physicochemical and heavy metal analysis level of soil polluted with crude oil using *Pseudomonas* and *bacillus spp* as augmenting organism capable of delivering nutrients in order to enhance microbial degradation.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Rivers State university school farm in Port Harcourt Local Government (4°48'3.59496" N6°58'46.09848"E) and B-Dere in Gokhana Local Government of Rivers state (32N 305238 518350 29° E) all in Rivers state, Nigeria. These two sites are located in the South-South geopolitical zone of Nigeria where crude oil exploration take place. The B-Dere location have been implicated for crude oil spills as a result of the activities of antisanal refineries. The locations were selected due to the fact that they are sites known for various activities including illegal bunkering/illegal local refining of crude oil.

2.2 Sampling Technique

Simple random sampling technique was used to collect the soil samples. From each soil samples, one bag was contaminated soil and the other bag was uncontaminated were collected by simple random sampling technique.

2.3 Sample Collection, and Processing

The collection of samples was done aseptically using auger apparatus. Soil samples were collected by adopting the Food and Agriculture Organization (FAO), 2002 guideline using a sterile soil auger to make a depth of topsoil. The soil samples for analysis were collected into fresh unused black polythene bags. The samples were transported within 2hours of collection to the laboratory of Microbiology Department, Rivers state university Port Harcourt.

2.4 Physicochemical Analysis of soil Sample

The soil samples were tested for different parameters like pH, temperature, Electrical conductivity, Moisture content, total organic carbon, Soil organic matter, Nitrogen, Phosphorous, Potassium and Total hydrocarbon content. The pH of the samples was analyze with the aid of pH meter S-901.

2.5 Heavy Metal Analysis for Soil and plant (Grass)

The soil and plant samples were air dried. While the dried soil was crushed and sieved, the grasses were grinded to powder. Five hundred milligram (500mg) of each sample (crushed soil and growth plants were weighed into conical flasks and 20ml of aqua regia (comprising 15ml HCl and 5ml HNO_3) were added. The mixture was digested until the volume was reduced to about 5ml and about 20ml distilled water was added. The mixture was filtered through a Whattman No 42 filter paper into a 50ml volumetric flask and diluted to volume with distilled water [29]. Subsequently, analysis for metals (cadium chromium, lead and zinc) were done using GBC XplorAA Atomic Absorption Spectrophotometer instrument as stated in the operational manual (GBC 2016). A Set of three standards were analyzed alongside the samples with one serving as quality control.

2.6 Bacteriological Analysis of Samples

Serial tenfold dilution was done on the weighed soil sample with dilution factor from10^{-1} to 10^{-6}. Aliquot (0.1ml) of appropriate dilutions were spread plated in duplicates onto Nutrient Agar, and Mineral salt agar. The plates was incubated at 37°C for 24 hours. The colonies formed on the plates were counted and described morphologically. Colonies formed on Nutrient Agar was used to estimate the total heterotrophic bacterial counts (THBC). Representative distinct colonies was purified by sub-culturing on freshly prepared sterile nutrient agar plates and incubated at 37°C for 24hours to obtain pure cultures.

2.7 Isolation and Enumeration of Crude oil Utilizing Bacteria

For the isolation of hydrocarbon utilizing bacteria, Mineral salt agar medium was used. The composition (g/L) of the mineral salt media are

0.2 MgSO4, 0.02 CaCl$_2$, 1.0 KH$_2$PO$_4$, 1.0 KH$_2$PO$_4$, 1.0 NH$_4$NO$_3$, 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. Colonies that appeared on the agar plates was counted after a week and resulted as the count of total hydrocarbon degrading bacteria for the four soil samples. The colonies counted were expressed as the colony forming unit (CFU) per gram soil.

2.8 Preparation of Bacterial suspension for Bioremediation setup

Suspension of *Pseudomonas* and *Bacillus spp* was prepared from 24hrs sub-cultured Petri plate. Two hundred milliliter (200ml) of nutrient media broth was transfer into Two hundred and fifty milliliter (250ml) conical flask and sterilized using an autoclave at 121°c for 15minutes at 15psi, and allowed to cool at room temperature. Cicatrin 0.8g was added to the broth. Pure cultures of the organism (*Pseudomonas* and *Bacillus spp*) were picked from the culture plate and then transported to the 250ml nutrient broth in conical flask until a turbid was form. The flask was cap with cotton wool. This was incubated at room temperature 28°C for 48hrs.

2.9 Treatment of the Soil for Bioremediation

The soil sample was treated for bioremediation as described by [30] shows the experimental set up. In this method, 10 setups were made. Each basin contained;

1. 2500g of uncontaminated soil + *Panicum subalbidum* which served as control
2. 2500g of uncontaminated soil + *Schoenoplectus senegalensis* which served as control
3. 2500g of contaminated soil + *Panicum subalbidum* + 250ml of bonny light crude oil
4. 2500g of contaminated soil + *Panicum subalbidum* + 250ml of bonny light crude oil + 50ml of *Pseudomonas spp* broth.
5. 2500g of contaminated soil + *Panicum subalbidum* + 250ml of bonny light crude oil + 50ml of *Bacillus spp* broth.
6. 2500g of contaminated soil + *Panicum subalbidum* + 250ml of bonny light crude oil + 25ml of *Pseudomonas spp* broth + 25ml of *Bacillus spp* broth

7. 2500g of contaminated soil + *Schoenoplectus senegalensis* sediment + 250ml of bonny light crude oil.
8. 2500g of contaminated soil + *Schoenoplectus senegalensis* + 250ml of bonny light crude oil + 50ml of *Pseudomonas spp* broth.
9. 2500g of contaminated soil + *Schoenoplectus senegalensis* + 250ml of bonny light crude oil + 50ml of *Bacillus spp* broth.
10. 2500g of contaminated soil + *Schoenoplectus senegalensis* + 25ml of *Pseudomonas spp* broth + 25ml of *Bacillus spp* broth

This bioremediation set up was monitored for selected microbiological and physicochemical parameters from day 1 to 28 days, such as Hydrocarbon Utilizing Bacterial (HUB), Total Heterotrophic Bacterial (THB), Total Hydrocarbon Content (THC), Nitrogen, Potassium, Phosphorus, Soil Organic Matter, Moisture Content, Temperature and pH, respectively at 14 days' interval. One Hundred milliliter (100ml) of sterilized water was added to the set up two times weekly and agitated for proper aeration and adequate distribution of microorganisms.

2.10 Plant Selection and Cultivation

Two plant species common in the Oduoha in Emohua 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.

2.11 Phytoremediation Set Up

Five pots of soil contaminated with crude oil were prepared by mixing the soil uniformly with crude oil. Each pot contained 2500g of soil mixed with 250ml of crude oil. The soil was commercially available compost soil from Rivers state university school farm while the crude oil was Bonny light crude.

2.12 Soil Preparation and Application of Crude Oil and Nutrients

Soils were collected in two places, one batch is a hydrocarbon-contaminated soil while the other batch is uncontaminated soil. Two thousand five hundred grams (2500g) of the contaminated soil were weighed into 8 batches while 2500g of the uncontaminated soil were weighed into 2 batches. However, different treatments were considered for each soil batch. In order to ensure easy interpretation of these results, according to different bioremediation strategies, the uncontaminated soil was named as control

2.13 Soil Analysis

Soil sampling was conducted on the first week after planting and weekly subsequently for another 5 weeks during which soil samples were collected from each pot at a fixed radius from the plant. The samples collected were sieved with 2mm mesh to separate organic materials and particulate maters. Sixteen (16 g) of soil was collected after sieving for subsequent analysis. The soil was tested for the moisture content, pH and the crude oil concentration. Soil moisture content was tested to maintain a sufficient level of soil moisture for phytoremediation. pH was tested as plants were known to alter the pH of surrounding soil as phytoremediation occurred.

2.14 Data Processing and Analysis

"Statistical Package for Social Sciences (SPSS) version 25 was used to statistically analyse the data obtained from counts and the measurement of the zones of inhibition. Descriptive statistics was used to summarize all data obtained. Analysis of variance (ANOVA) was carried out to test for significant difference ($p \leq 0.05$) in the bacterial counts from the various locations. Duncan multiple range test was used to separate the means where difference existed" [31].

3. RESULTS AND DISCUSSION

Natural attenuation, which is a variety of physical, chemical, or biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil also set in [32]. After a few months, microorganism which are not able to utilize the hydrocarbons present in the soil either mutate or completely die off due to environmental stress. After a few years, the organisms in the environment fully adapt and reproduce, most of the crude oil components are also completely broken down. "Due to these factors, sites with older spills have lesser total petroleum hydrocarbon (TPH) content, lesser TPH contents implies higher chances for the proliferation of microorganisms" [33]. "Microbes make the major contribution to mineralization of crude oil pollutants [34]. Bioremediation utilizes the metabolic versatility of microorganisms to degrade hazardous pollutant for the ecological recovery of petroleum waste contaminated sites [35]. Among the microorganisms, bacteria are usually the choice because of their rapid metabolic rates and because the fellow numerous degradation pathways and can be genetically manipulated to improve their bioremediation capabilities" [36].

Results obtained from this study has shown that *Panicum subalbidum* and *Schoenoplectus senegalensis* 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 [37].

The physicochemical analyses of the bioremediation soil set up was dully conducted and the results are presented in Table 2. The following physicochemical parameters: pH, Temperature, Nitrogen, Phosphorus, potassium, soil total hydrocarbon content and Root total hydrocarbon content were all carried out. The results revealed that pH, Temperature, Nitrogen, phosphorus increased slightly after treatment of contaminated soil. The pH value increased from 5.43 to 6.69. This indicates that the soil used in this study can support the growth of bacterial according to the report of Williams, [38] who

Table 1. 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

Table 2. 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 (Bacillus spp), Pse (Pseudomonas spp), PAN (Panicum subalbidum), SCH (Schoenoplectus senegalensis)

Table 3. Heavy metals analysis of soil

Treatment code	Caduim (cd)	Chromium (Cr)	Lead (pb)	Zinc (Zn)
US+ PAN-G	3.62 ± 3.19^a	9.43 ± 8.17^b	8.62 ± 7.46^b	30.33 ± 2.31^b
U S+ SCH-G	1.32 ± 1.14^a	3.93 ± 3.41^a	4.34 ± 4.04^a	21.17 ± 4.55^a
CS + PAN-G	4.52 ± 3.93^a	0.003 ± 5.31^b	0.01 ± 0^a	34.87 ± 15.93^b
CS + SCH-G	3.10 ± 2.70^a	0.003 ± 5.31^b	31.14 ± 26.97^{ab}	60.07 ± 6.07^{ab}
CS + Pse+ PAN-G	4.97 ± 4.30^a	0.004 ± 0.001^a	22.67 ± 19.65^a	28.47 ± 15.66^a
CS + Pse+ SCH-G	6.07 ± 6.87^b	0.003 ± 0.001^a	4.19 ± 3.64^a	42.5 ± 24.5^a
CS +Bac + PAN-G	8.11 ± 0.30^a	106.97 ± 92.63^{ab}	13.47 ± 11.66^a	19.23 ± 7.07^a
CS +Bac + SCH-G	4.93 ± 3.23	7.97 ± 6.91^a	44.25 ± 38.58^b	33.93 ± 24.20^a
CS + Pse + Bac + PAN-G	12.45 ± 0.35^a	22.68 ± 19.69^b	37.47 ± 32.45^{ab}	13.43 ± 5.49^a
CS + Pse + Bac + SCH-G	12.23 ± 1.45	17.05 ± 14.77^b	30.4 ± 5.80^{ab}	26.7 ± 11.18^a

Table 4. Heavy metals analysis of plant root

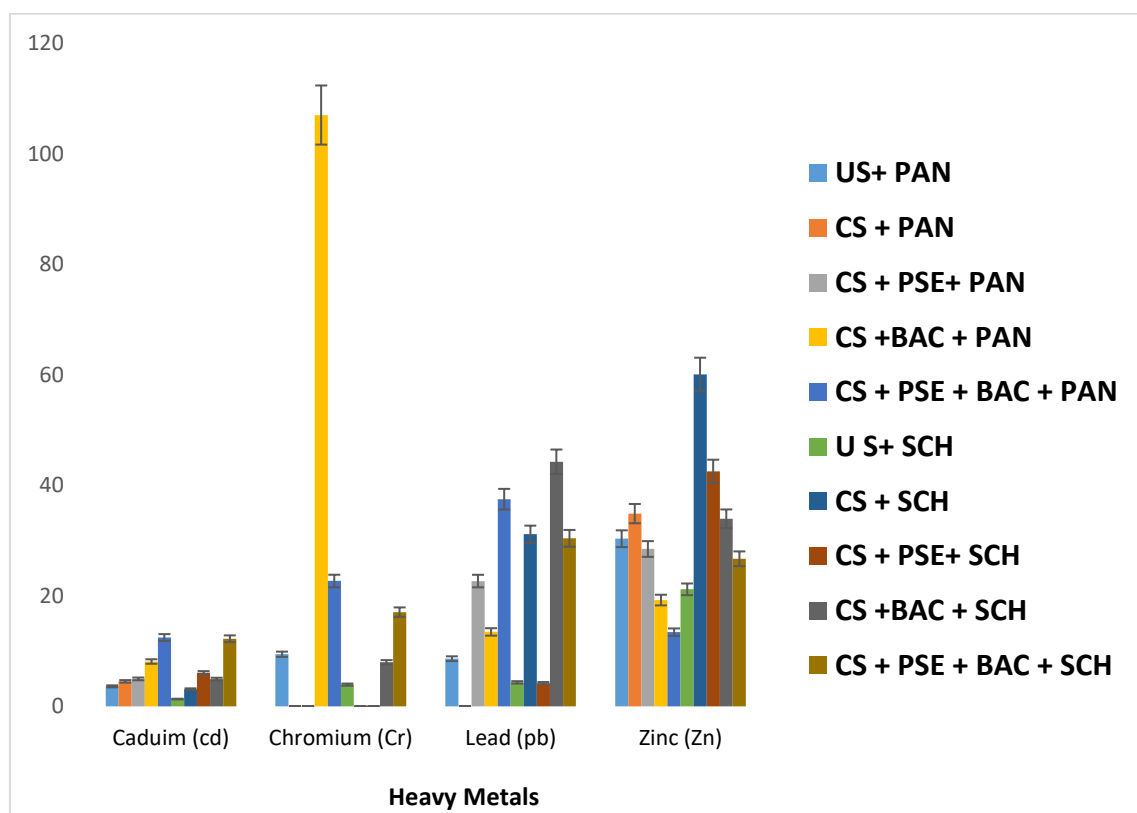| Treatment Code	Caduim (Cd)	Chromium (Cr)	Lead (Pb)	Zinc (Zn)
US+ PAN-G	1.93+2.31a	16.6+5.05a	35.83+15.88a	21.53+9.08a
U S+ SCH-G	4.07+1.05a	11.83+20.49a	28.3+13.19a	18.27+4.20a
CS + PAN-G	7.83+3.84a	14.2+19.23a	12.77+11.08a	23.57+0.75a
CS + SCH-G	6.52+2.85a	0.002+0.001a	68.4+10.81a	38.13+2.98a
CS + Pse+ PAN-G	6.17+0.85a	19.50+33.77a	20.1+13.27a	12.97+8.20a
CS + Pse+ SCH-G	4.67+1.50a	30.57+52.94a	33.4+31.75a	8.93+1.12a
CS +Bac + PAN-G	9.83+2.36b	9.50+16.45ab	34.9+27.11a	16.2+5.07a
CS +Bac + SCH-G	1.70+1.57a	41.10+71.19b	11.28+5.23a	45.77+22.24a
CS + Pse + Bac + PAN-G	7.48+0.71b	27.13+47.00a	46+7.08a	15.92+8.85a
CS + Pse+ Bac + SCH-G	2.88+0.73a	35.43+61.37b	65.13+43.85a	25.88+7.39a

Fig. 1. Heavy metals analysis of soil

Table 5. Total hydrocarbon content in soil (mg/kg)

S/N	SET UP CODE	DAY 1	DAY 14	DAY 28	Amount Remediated	Percentage Bioremediation %
1	US+ Pan (G)	700	400	226	474	67.71
2	U S+ Sch (G)	700	506	266	434	62
3	CS + Pan (G)	4050	2140	1098	2952	72.89
4	CS + Sch (G)	4050	1860	1128	2922	77.15
5	CS + PSE+ Pan (G)	4050	1392	1176	2874	70.96
6	CS + PSE+ Sch (G)	4050	1182	596	3454	85.28
7	CS +BAC + Pan (G)	4050	1218	858	3192	78.81
8	CS +BAC + Sch (G)	4050	804	786	3264	80.59
9	CS + PSE + BAC + Pan (G)	4050	1584	1048	3002	74.12
10	CS + PSE + BAC + Sch (G)	4050	1170	840	3210	79.26

Key: US: Uncontaminated Soil, CS: Contaminated Soil, BAC: Bacillus spp, PSE: Pseudomonas spp, Grass 1: Elbow buffalo Grass 1 (Panicum subalbidum), Grass 2: Sedge Plant (Grass) (Schoenoplectus senegalensis)

Table 6. Total hydrocarbon content in root (mg/kg)

S/N	SET UP CODE	DAY 1	DAY 14	DAY 28	AMOUNT UPTAKE	PERCENTAGE UPTAKE %
1	US+ Pan (G)	0	10.4	19.2	19.2	2.74
2	US+ Sch (G)	0	6	12.2	12.2	1.74
3	CS + Pan (G)	0	170	184	184	4.54
4	CS + Sch (G)	0	262	610	610	15.6
5	CS + PSE+ Pan (G)	0	168	200	200	4.94
6	CS + PSE+ Sch (G)	0	224	506	506	12.49
7	CS +BAC + Pan (G)	0	170	318	318	7.85
8	CS +BAC + Sch (G)	0	190	632	632	15.6
9	CS + PSE + BAC + Pan (G)	0	180	322	322	7.95
10	CS + PSE + BAC + Sch (G)	0	188	192	192	4.74

Key: US: Uncontaminated Soil, CS: Contaminated Soil, BAC: Bacillus spp, PSE: Pseudomonas spp, Grass 1: Elbow buffalo Grass 1 (Panicum subalbidum), Grass 2: Sedge Plant (Grass) (Schoenoplectus senegalensis)

stated clearly that bacteria can proliferate in the soil that has a pH ranging from 5.0 to 8.5. The crude oil contaminated pots had relatively lower pH; this implies that crude oil had a reductive effect on the soil pH tending toward acidity. Typically, petroleum hydrocarbon are complex substances formed from hydrogen and carbon molecules and sometimes containing other impurities such as oxygen, surphur and nitrogen. They are highly viscos (e.g tar and motor oil), and are generally readily absorbed through skin and intact mucosae [39].

pH had a notably steady reduction during 28 days of monitoring period as metabolites were produced by the organism during the remediation process. pH levels were shown to decrease tending toward acidity. The pH value increased after treatment. There was no significant difference in the pH value across the set up. The reduction in pH value may be due to release of organic acid in the medium. Generally, alkaline or slightly acidic soil pH enhances bioremediation, while acidic environments pose limitation to biodegradation [40]. The result of temperature increased after treatment. The value of temperature was highest in uncontaminated soil + Panicum subalbidum (27.83+0.83). The concentration of Nitrogen also increased after treatment while phosphorus and potassium value decreased after treatment.

The result showed that the total hydrocarbon content in the root decreased with an increased in time (Day) from Day 1 to 28 of the study. This is because on the first day, there was suitable feeding materials available for these microorganisms to feed on, but with increasing time (Day), the lack of organic matter appeared little by little limiting the growth of the microorganism. Saeki et al. [41] made a similar observation and concluded that hydrocarbon microbial population increased rapidly on the first Day of the 28 days testing period. They proposed these findings may be considered as an indicator

for the feasibility study of oil contaminated soil bioremediation.

Safdari et al. [42] showed that between the remediation periods, there exists a negative relationship. However, in this present study, in the first day, because of a suitable environmental condition and appropriate feeding, oil degradation was high, but on day 14 to 28, lack of nutritional element caused the decrease of bioremediation process especially in those uncontaminated soil samples. The treated soil showed a continuous phase of remediation, and this could be due to nutrient and acclimatization of the degraders.

Results of the heavy metals analysis showed variation in their concentration of the bioremediation monitoring. Heavy matals such as Caduim, Chromium, Lead and Zinc were all considered in the study. Evaluation of heavy metal reduction in soil and plant root in this study showed significant difference (p<0.05) between control pots (uncontaminated soil and the contaminated soil). This could be attributed to the content of the crude oil having some amounts of heavy metals as contaminants [43]; Moreso the

action of crude oil in the chemical properties of soil and that of amendment nutrient could result to the elevated value of heavy metals found in the crude oil contaminated soil [44]. The value of Caduim (Mg/kg) showed highest concentration in contaminated soil + *Pseudomonas* + *Baccilus* + *Panicum subalbidum* (12.45±0.35mg/kg) in soil and contaminated soil + *Baccilus* + *Panicum subalbidum* (9.83±2.36mg/kg) in plant root. The value of Chromium showed highest concentration in contaminated soil + *Baccilus* + *Panicum subalbidum* (106.97±92.65mg/kg) and contaminated soil + *Baccilus* + *Schoenoplectus senegalensis* (41.10±71.19mg/kg) for soil and plant root respectively. The value of lead showed highest concentration in contaminated soil + *Baccilus* + *Schoenoplectus senegalensis* (44.25±38.58mg/kg) in soil and contaminated soil + *Schoenoplectus senegalensis* (68.4±10.81mg/kg) in plant root. The value of Zinc showed highest concentration in contaminated soil + *Schoenoplectus senegalensis* (60. 07±6.07mg/kg) in soil and contaminated soil + *Baccilus* +*Schoenoplectus senegalensis* (45.77±22.24mg/kg) in plant root. The value of Caduim showed low value to other heavy metals.
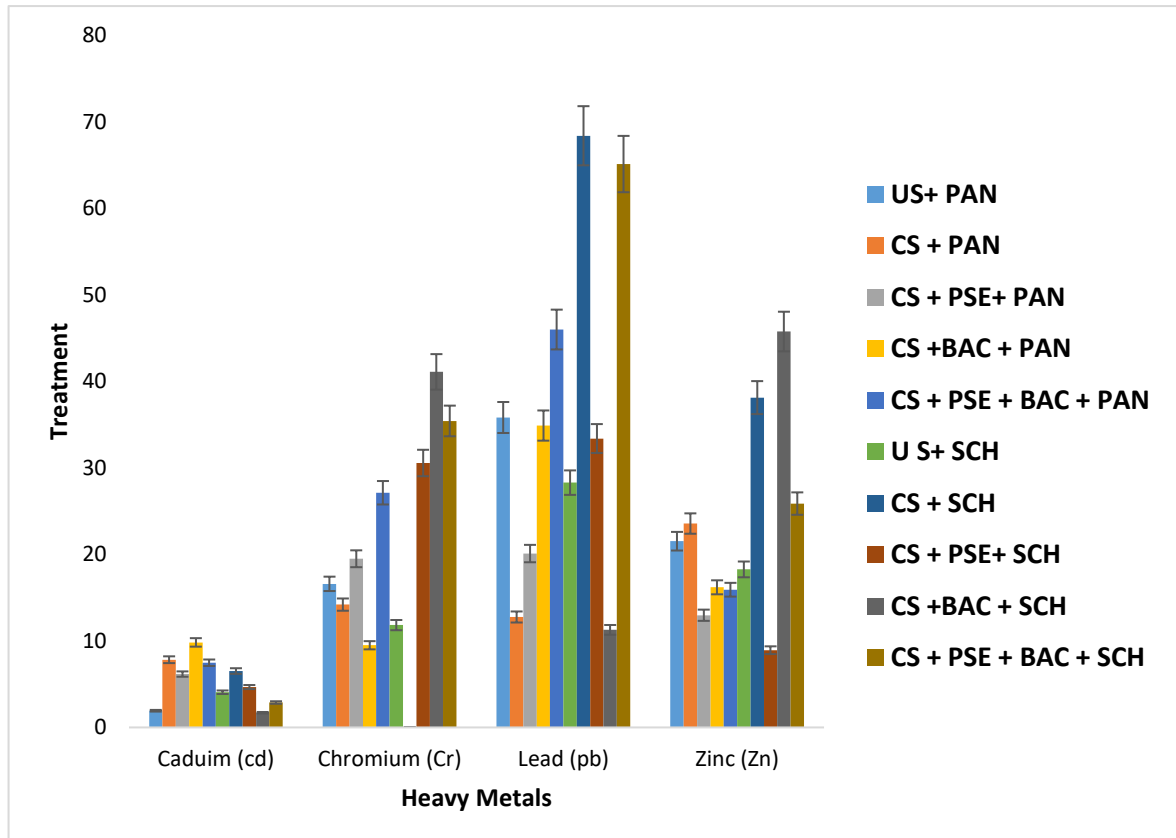

Fig. 2. Heavy metals analysis of plant root

Phytoremediation using grass plant *Panicum subalbidum* (Elbow buffalo grass) and Sedge plant (*Scoenoplectus senegalensis*) were carried out on Crude Oil contaminated soil. Some isolated microorganisms – *Bacillus spp* and *Pseudomonas spp* 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 (*Scoenoplectus senegalensis*) survive the first screening stage with crude oil contamination and absorbed the crude oil. The Elbow buffalo grass (*Panicum subalbidum*) survive after monitoring of 28 days with crude oil contamination.

Experimental transplants had an initial height of 16.7cm on the first 7 days of growth, Plant showed reduced growth whereas; plant in uncontaminated soil were in good condition. *Panicum subalbidum* (Elbow buffalo grass) indicated a high potential of adaptation in the contaminated soil as shown by the growth during 14 to 28 days regardless of the bio-organic in the contaminated soil compensating for the higher C/N ratio. The plant height increased significantly with time (p=0.05). The average plant height of *Panicum sbalbidum* (Elbow buffalo grass) were 52.46 and 55.82cm respectively in pot 4 and pot 6 in comparison to 36.88cm in (uncontaminated plots) during the 28 days. 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 [45]. Generally, the roots growing in uncontaminated soil were longer, and covered more surface area than those growing in contaminated soil [46]. 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.

4. CONCLUSION

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. [47]. It has been revealed that heavy metals accumulate in the soil, especially when there is an oil spillage. The absorption of these heavy metals is facilitated by low soil pH, which can be accelerated by bacteria products of metabolism and organic matter [48].

This research revealed and recommend *Panicum subalbidum* (Elbow buffalo grass) and *Scoenoplectus senegalensis* as suitable plant species for phytoremediation of crude oil polluted soil with high total hydrocarbon content value.

In the present study, the test plant *Panicum subalbidum* (Elbow buffalo grass) and *Scoenoplectus senegalensis*) promoted degradation of hydrocarbon which may be due to the complexity of plant roots-microorganism interaction which is similar to the findings of Ogbonna et al.

Generally, the study revealed microbial counts with respect to physicochemical parameters, heavy metals and total petroleum hydrocarbon content. This information is useful in understanding microbiology of crude oil polluted soil and inference can be made on the health of the environment as well.

Heavy metals such as lead (pb), zinc (Zn), Cadmium (cd), copper (cu) and iron were also considered in this study. The study revealed that the soil with the highest total petroleum hydrocarbon content had the highest concentration of the heavy metals except leads (Pb). However, all the heavy metals were present in all the set up soil. More so, based on my findings, I recommend the use of ecofriendly and augmenting microbes as amendment option with phytoremediation plants to facilitate pollutant removal/clean up.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Borah D, Yadav RNS. Bioremediation of petroleum based contaminants with biosurfactant produced by a newly isolated petroleum oil degrading bacterial strain Egypt. Journal of Petroleum. 2016;26:181-188

2. Brooijmans RJ, Pastink MI, Siezen RJ. Hydrocarbon-degrading bacteria: the oil-spill clean-up crew. Microbial Biotechnology. 2009;2(6):587-594.

3. Vogel T. Bioaugmentation as a soil bioremediation approach. Current Opinion in Biotechnology 1996;7:311-316.

4. Wang J. Compositional changes of hydrocarbons of residual oil in contaminated soil during ozonation. ozone: Science and Engineering 2013;35(5):366-374

5. Wang Q, Zhang S, Li Y, Klassen W. Potential approaches to improving biodegradation of hydrocarbons for bioremediation of crude oil pollution. Journal of Environmental Protection. 2011;2:47–55.

6. Budhadev B, Rubul S, Sabitry B, Hari SP. Phytoremediation of petroleum hydrocarbon (PHC) contaminated soil by using Mimosa pudica. Journal of Environmental Science and Engineering. 2014;56(3):327-332.

7. Muratova A, V Panchenko L, V Semina D, Golubev S, Turkovskaya O. New strains of oil- degrading microorganisms for treating contaminated soils and wastes. In IOP Conference Series: Earth and Environmental Science. 2018;107:012-066.

8. Pi Y, Chen B, Bao MT, Fan F, Cai Q, Ze L. Microbial degradation of four crude oil by biosurfactant producing strain Rhodococcus sp. Bioresources Technology. 2017;232:263-269.

9. Rahman P, Rahman T, Mc Clean S, Marchant R, Banat I. Rhamnolipid biosurfactant production by Strains of pseudomonas aeruginosa using low-cost raw materials. Biotechnology Progress. 2002;18(6):1277-1281.

10. Williams JO, Hakam K. Microorganism associated with dump sites in Port Harcourt metropolis. Journal of Ecology and Natural Environment. 2016;8(2):9-12.

11. Chebbi A, Hentati D, Zaghden H, Baccar N, Rezgui F. Polycyclic aromatic hydrocarbon degradation and biosurfactant production by a newly isolated Pseudomonas sp. strain from used motor oil-contaminated soil. International Biodeterioration and Biodegradation. 2017;122:128-140.

12. Marinescu M, Toti M, Veronica T, Carabulea V, Georgiana P. An Assessment of the effects of crude oil production on soil properties. Annals: Food Science and Technology. 2010;11.

13. Sverdrup LE, Nielsen T, Krogh PH. Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility. Environmental Science and Technology. 2002;36:2429–2435

14. Tang J, G Wang R, W Niu X, MW, R. Chu H, Zhou Q. Characterisation of the rhizoremediation of petroleum-contaminated soil: Effect of different influencing factors. Biogeosciences. 2010; 7(12):3961-3969.

15. Milić JS. Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium. Journal of the Serbian Chemical Society. 2009;74:455–460.

16. Westlake DWS, Jobson A, Phillippe R, Cook FD. Biodegradability and crude oil composition. Canadian Journal of Microbiology. 1974;20(7):915-928.

17. Williams JO, Barisi S. Bioremediation potential of *Aspergillus clevatus* and *Pichia spp.* On oil spill dispersant polluted marshland. Journal of Advances in Biology and Biotechnology. 2018;19(4):1-4

18. Xenia M, Refugio R. Microorganism's metabolism during bioremediation of oil contaminated soils. Journal of Bioremediation and Biodegredation. 2016; 7:340.

19. Yadav BK, Hassanizadeh SM. An Overview of Biodegradation of LNAPLs in Coastal (Semi)-arid Environment. Water Air Soil Pollution. 2011;220(1):225-239.

20. Muratova A, Turkovskaya OP Antonyuk LE Makarov OI Pozdnyakova L. Oil- oxidizing potential of associative rhizobacteria of the genus Azospirillum. Journal of Microbiology. 2005;74(2):210-215.

21. Urum K, Pekdemir T. Evaluation of biosurfactants for crude oil contaminated soil washing. Chemosphere. 2004; 57(9):1139-1150.

22. Varjani SJ, Upasani VN. A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. International Biodeterioration and Biodegradation. 2017;120:71–83.

23. Varjani SJ, Rana DP, Jain AK, Bateja S, Upasani VN. Synergistic ex-situ biodegradation of crude oil by halotolerant bacterial consortium of indigenous strains isolated from on shore sites of Gujarat, India. International Biodeterioration and Biodegradation. 2015; 103:116-124

24. Ndimele PE, Saba AO, Ojo DO, Ndimele CC, Anetekhai MA. Remediation of crude oil spillage. In the Political Ecology of Oil

and Gas Activities in the Nigerian Aquatic Ecosystem. 2018:369-384.

25. Yalaoui Guellal D, Brahmi F, Touati A, De Champs C, Banat I. Production of biosurfactants by hydrocarbons degrading bacteria isolated from soummam watershed sediments of bejaia in Algeria. Environmental Progress and Sustainable Energy. 2017;37(1):189-195.

26. Zhao X, Fan F, Zhou H, Zhang P, Zhao G. Microbial diversity and activity of an aged soil contaminated by polycyclic aromatic hydrocarbons. Bioprocess and Biosystems Engineering 2018;41(6): 871-883.

27. Nguemté PM, Wafo GD, Djocgoue P, Noumsi IK, Ngnien AW. Potentialities of six plant species on phytoremediation attempts of fuel oil-contaminated soils. Water, Air, and Soil Pollution. 2018; 229(3):88.

28. Njoku K, Akinola M, Oboh B. Phytoremediation of crude oil contaminated soil using glycine max (Merril); Through Phytoaccumulation or Rhizosphere Effect. Journal of Biological and Environmental Sciences. 2016;10(30):115-124.

29. Jones BR, Laslett RE. Methods for analysis of trace metals in marine and other samples. Directorate of Fisheries Research; 1994.

30. Nrior RR, David NO. Evaluation of organic nutrient supplements and Bioaugmenting microorganism on crude oil polluted soil. Current Journal of Applied Science and Technology 2019;38(6):1-19

31. Bewick V, Cheek L, Ball J. Statistics review 12: survival analysis. Critical care. 2004 Oct;8:1-6.

32. Parthipan P, Preetham E, Machuca LL, Rahman PK, Murugan K. Biosurfactant and degradative enzymes mediated crude oil degradation by bacterium bacillus subtilis A1. Frontier Microbiology. 2017;8:193.

33. Panchenko L, Muratova A, Turkovskaya O. Comparison of the phytoremediation potentials of Medicago falcata L. And Medicago sativa L. in aged oil-sludge-contaminated soil. Environmental Science and Pollution Research International. 2016;24(3):3117-3130.

34. Peng S, Zhou Q, Cai Z, Zhang Z. Phytoremediation of petroleum contaminated soils by Mirabilis Jalapa L. in a greenhouse plot experiment. Journal of

Hazardous Materials. 2009;168(2-3):1490-1496.

35. Prince RC, McFarlin KM, Butler JD, Febbo EJ, Wang FCY, Nedwed TJ. The primary biodegradation of dispelled crude oil in the sea. Chemosphere. 2013;90(2):521-526.

36. Radwan S. Microbiology of oil-contaminated desert soils and coastal areas in the Arabian gulf region in Microbiology of Extreme Soils-Soil Biology. 2008;13:275–298.

37. Rahman KSM, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat I M. Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresource Technology. 2003;90(2):159-168

38. Williams JO, Agunkwo M. Remediation of crude oil polluted River using Nostoc and Oscillatoria species, Journal of Biology and Genetic Research. 2018;4(1):1-9

39. Rabus R, Boll M, Heider J, Meckenstock R, Buckel W. Anaerobic microbial degradation of hydrocarbons: From enzymatic reactions to the Environment. Journal of Molecular Microbiology and Biotechnology. 2016;26(1-3):5-28.

40. Roy A, Dutta A, Pal S, Gupta A, Sarkar J. Biostimulation and bioaugmentation of native microbial community accelerated bioremediation of oil refinery sludge. Bioresources Technology. 2018;253:22-32.

41. Saeki H, Sasaki M, Komatsu K, Miura A, Matsuda H. Oil spill remediation by using the remediation agent JE1058BS that contains a biosurfactant produced by Gordonia sp. Strain JE-1-58 Bioresources Technology; 2009.

42. Safdari MS, Kariminia HR, Rahmati M, Fazlollahi F, Polasko A. Development of bioreactors for comparative study of natural attenuation, biostimulation, and bioaugmentation of petroleum-hydrocarbon contaminated soil. Journal of Hazardous Material. 2018;342:270-278.

43. Segura A, Luis Ramos J. Plant–bacteria interactions in the removal of pollutants. Current opinion in biotechnology. 2012; 24(3):467-473.

44. Siles J, Margesin R. Insights into microbial communities mediating the bioremediation of hydrocarbon-contaminated soil from an Alpine former military site. Applied Microbiology and Biotechnology. 2018; 102(10):4409-4421.

45. Silva A, Delerue Matos C, Fiúza A. Use of solvent extraction to remediate soils contaminated with hydrocarbons. Journal of Hazardous Material. 2005;124(1-3):224-229.

46. Smith MBJ. Keystone Pipeline Leaks 210,000 Gallons of Oil in South Dakota. The New York Times; 2017.

47. Steffen K, Hatakka A, Hofrichter M. Degradation of benzo-pyrene by the litter-decomposing basidiomycete stropharia coronilla: Role of Manganese Peroxidase. Applied and Environmental Microbiology 2003;69(7):3957-3964.

48. Soleimani M, Majid A, Hajabbasi M, Nourbakhsh F, Sabzalian M, Christensen J. Phytoremediation of an aged petroleum contaminated soil using endophyte infected and non- infected grasses. Chemosphere. 2010;81(9):1084-1090.