Original Research Article

Enhanced Phytoremediation, Physicochemical and Heavy Metal Assessment of Crude Oil Polluted Soil Using *Pseudomonas* and *Bacillus spp,* Rivers state.

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~~investigated the phytoremediation, physicochemical, and heavy metal assessment of crude ~~oil polluted~~oil-polluted soil using Peudomonasspp and Bacillusspp as an ~~a~~ augmenting microorganism~~,~~ in Rivers ~~state~~State, Nigeria. This study was carried out in ~~in~~ south-south Nigeria (B-dere in Gokana Local government of `Rivers State). Contaminated Soil ~~were~~ wascollected and subjected to Standard microbiological methods. Contaminated Soil samples were collected from two different ~~area~~ areasof Rivers state with hand auger at two depths of 0-15cm and 15-30cm twice monthly for three months. The contaminated soil sampled were analyzed for heavy metal (~~Cadium~~Cadmium Chromium, Lead,and Zinc)using Atomic Absorption spectrophotometric method with microbiological analysis ~~involved~~ involvingisolation and enumeration of microbial population of the soil samples. Ten (10) experimental plots (two controls (uncontaminated soil with Panicum subalbidumand uncontaminated soil with Schoenoplectus senegalensis) and eight contaminated soil amended/treated plots employing Randomized Block design were formed. . A combination of treatment consisting of the application of Pseudomonas sppand Bacillusspp, 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. Physicochemical parameters were analyzed for uncontaminated soil and contaminated soil with treatments in the soil of different plots. The physicochemical parameters analyzed are pH, Temperature, Nitrogen, Phosphorus, Potassium, Soil total hydrocarbon content, and Root total hydrocarbon content. Application of augmenting organisms was to enhance phytoremediation by test ~~plant~~plantsPanicum subalbidum and Schoenoplectus senegalensis. The rate of phytoremediation was estimated from the percentage (%) uptake of total petroleum hydrocarbon (TPH) and percentage (%) reduction of TPH in plant and root from day 1 to day 28.The physiochemical parameters of the uncontaminated soil before experimental treatment with different Bioaugmenting microorganism showed~~:~~ pH (5.43), temperature (27oC), 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 the amount of soil hydrocarbon removed and percentage (%) Bioremediation remediated after 28 days of monitoring to be 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 of root hydrocarbon content removed and percentage (%) Bioremediation remediated after 28 days of monitoring to be higher in set up with CS+BAC+SCH (~~632Mg~~632 mg/kg; 15.6%) and lowest in set up with US+SCH (12.2mg/kg; 1.74%). The heavy metals (~~cadium~~cadmium (Cd), chromium (Cr), lead (Pb), and zinc (Zn))were below the Department of

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petroleum resourcesPetroleum Resources (DPR) permissible limit.This research revealed and recommend —recommendedthat Panicumsubalbidum as a suitable plant species for phytoremediation of crude oil contaminated soil. Hydrocarbon utilizing bacterial identified in this study were; Pseudomonas and Bacilussp.

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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. 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 an environmental pollutant by the US Environmental Protection Agency. 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. Under normal conditions, crude oil in soil persists much longer than most conventional carbon sources (e.g.e.g.,carbohydrate carbohydratesand proteins), which take only weeks to be degraded, while under extreme conditions (e.g.e.g., drought), it persists much longer.

Remediation of petroleum hydrocarbons contaminated sites is a real-world problem. Over the years, several methods have been developed and investigated for the remediation of petroleum hydrocarbons hydrocarbon-contaminatedcontaminated sites. Some of the major methods are physicochemical, thermal, and biological techniques. 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 and labouriouslaborious. Bioremediation (biological technique) has appeared as the most desirable method due to its simplicity, cost-effectiveness and ecofriendlinesseco-friendliness. Bioremediation is a treatment process that uses microorganisms to breakdown break downor degrades hazardous substances into less toxic or nontoxic substances. 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. 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. These techniques include the use of amendments and microorganism immobilization. Accordingly, bioremediation could be achieved either as biostimulation (addition of nutrients/amendments) or ~~bioaugumentation~~ bioaugmentation(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.

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*~~*Bacillus*~~*spp*~~ as augmenting ~~organism~~organismscapable of delivering nutrients in order to enhance microbial degradation.

MATERIALS AND METHODS

Study Area

The study was carried out in Rivers State ~~university~~ Universityschool farm in Port Harcourt Local Government (4^{o}48'3.59496" N6^{o}58'46.09848"E) and B-Dere in Gokhana Local Government of Rivers ~~state~~ State(32N 305238 518350 29^{o} E) all in Rivers state, Nigeria. These two sites are located in the South-South geopolitical zone of Nigeria, where crude oil exploration ~~take~~ takesplace. The B-Dere location ~~have~~ hasbeen implicated ~~for~~ incrude oil spills ~~as a result of~~due to the activities of ~~antisanal~~ Antisanalrefineries. 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

~~Simple~~ A simplerandom sampling technique was used to collect the soil samples. From each soil ~~samples~~sample, one bag was contaminated soil, and the other bag was uncontaminated and ~~were~~ wascollected by a simple random sampling technique.

Sample Collection~~,~~ and Processing

The collection of samples was done aseptically using an 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 perforated for aeration. The samples were transported within 2hours of collection to the laboratory of Microbiology Department, Rivers ~~state university~~State University Port Harcourt.

Physicochemical Analysis of ~~soil~~ 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~~was analyzed with the aid of pH meter S-901.

Heavy Metal Analysis for Soil and ~~plant~~ Plant (Grass)

The soil and plant samples were ~~air dried~~air-dried. While the dried soil was crushed and sieved, the grasses were grinded to powder. Five hundred ~~milligram~~ milligrams(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~~ WhatmanNo 42 filter paper into a 50ml volumetric flask and diluted to volume with distilled water (Jones &Laslett, 1994).Subsequently, analysis for metals (~~cadium~~cadmium chromium, lead and zinc) ~~were~~ wasdone 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.

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~~ were incubated at 37^{o}C for 24 hours. The colonies formed on the plates were counted and described morphologically. Colonies formed on Nutrient Agar ~~was~~ were used to estimate the total heterotrophic bacterial counts (THBC). Representative distinct colonies ~~was~~ were purified by sub-culturing on freshly prepared sterile nutrient agar plates and incubated at 37^{o}C for 24hours to obtain pure cultures.

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_2PO_4, 1.0 KH_2PO4, 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~~ sample and incubated at 35^{o}C for 7 days. Colonies that appeared on the agar plates ~~was~~ were counted after a week and resulted as the count of total ~~hydrocarbondegrading~~hydrocarbon-degrading bacteria for the four soil samples. The colonies counted were expressed as the colony forming unit (CFU) per gram soil.

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~~was transferred into Two hundred and fifty milliliter (250ml) conical flask ~~and sterilized~~, sterilized using an autoclave at ~~121^{o}c~~ 121^{o}Cfor 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~~formed. The flask was cap with cotton wool. This was incubated at room temperature 28^{o}C for 48hrs.

Treatment of the Soil for Bioremediation~~.~~

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

1. 2500g of uncontaminated soil + *Panicumsubalbidum* which served as control
2. 2500g of uncontaminated soil + *Schoenoplectussenegalensis*which served as control
3. 2500g of contaminated soil + *Panicumsubalbidum*+ 250ml of bonny light crude oil
4. 2500g of contaminated soil +*Panicumsubalbidum*+ 250ml of bonny light crude oil + 50ml of *Pseudomonasspp*broth.
5. 2500g of contaminated soil + *Panicumsubalbidum* + 250ml of bonny light crude oil + 50ml of *Bacillusspp*broth.
6. 2500g of contaminated soil + *Panicumsubalbidum*+ 250ml of bonny light crude oil + 25ml of *Pseudomonasspp* broth + 25ml of *Bacillusspp*broth
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 *Pseudomonasspp* broth.
9. 2500g of contaminated soil +*Schoenoplectussenegalensis*+ 250ml of bonny light crude oil + 50ml of *Bacillusspp*broth.
10. 2500g of contaminated soil + *Schoenoplectussenegalensis*+ 25ml of *Pseudomonasspp* broth + 25ml of *Bacillusspp*broth

This bioremediation ~~set up~~setup 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~~ milliliters(100ml) of sterilized water

~~was~~were added to the ~~set up~~setup two times weekly and agitated for proper aeration and adequate distribution of microorganisms.

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~~ phytoremediationcrude 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.

Phytoremediation setup

Five pots of soil contaminated with 5% 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 (API = 32.3o; sulfur content: 0.08%).

Soil preparation and application of crude oil and nutrients

Soils were collected in two places~~, one~~; one batch is ~~a~~ hydrocarbon-contaminated soil, while the other ~~batch is uncontaminated soil~~is uncontaminated. Two thousand five hundred grams (2500g) of the contaminated soil were weighed into 8 batches while 2500g of the uncontaminated soil ~~were~~ waseighed 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.

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~~matter. 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~~soil moisture level for phytoremediation. pH was tested as plants were known to alter the pH of surrounding soil as phytoremediation occurred.

Data Processing and Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used to statistically ~~analyse~~ analyzethe 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~~ differences($p \leq 0.05$) in the bacterial counts from the various locations. Duncan multiple range test was used to separate the means where ~~difference~~ differencesexisted (Bewick*et al*., 2004).

RESULTS

TABLE 1: Baseline Results of Physicochemical Parameters of Uncontaminated Soil Before Phytoremediation

S/N	Parameters	Uncontaminated soil
1.	~~Ph~~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

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7. Nitrogen (~~Mg~~mg/kg)	56.695
8. Phosphorus (~~Mg~~mg/kg)	0.621
9. Potassium (~~Mg~~mg/kg)	7.125
10. Total Hydrocarbon Content (~~Mg~~mg/kg)	700

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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}
US+ 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*)

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]

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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

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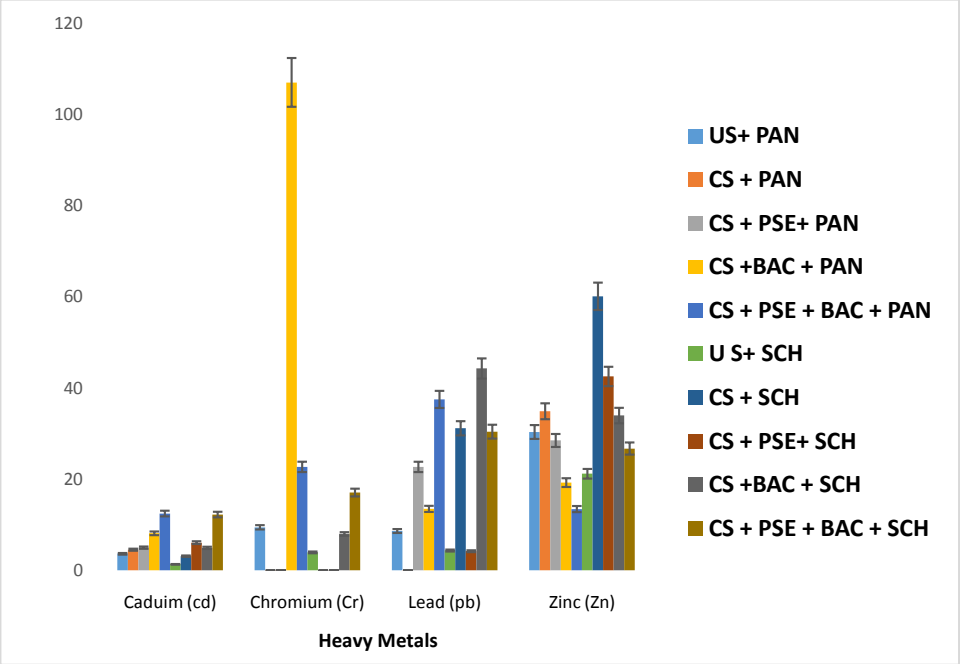

Fig 1. Heavy Metals Analysis of ~~soil~~Soil.

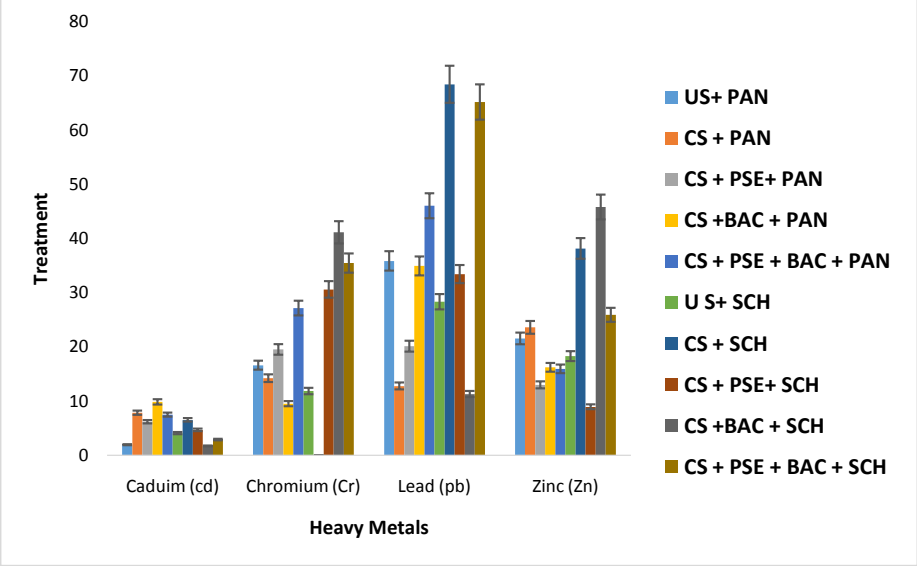

Fig 2. Heavy Metals Analysis of Plant Root.

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~~soil contaminants' mass, toxicity, mobility, volume, or concentration ~~also set in~~. After a few months, ~~microorganism~~ microorganisms~~which~~ that~~are not able to~~cannot 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, and 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, and lesser TPH ~~contents~~ contentimplies higher chances for the proliferation of microorganisms (Nrior*et al*., 2019).Microbes make ~~the~~ amajor contribution to the mineralization of crude oil pollutants. Bioremediation utilizes the metabolic versatility of microorganisms to degrade hazardous ~~pollutant~~ pollutantsfor the ecological recovery of petroleum ~~waste contaminated~~waste-contaminated sites. ~~Among the microorganisms, bacteria are usually the choice~~ Bacteria are usually the choice among the microorganisms because of their rapid metabolic rates and because ~~the fellow~~they follow numerous degradation pathways and can be genetically manipulated to improve their bioremediation capabilities (Anwar *et al.*, 2017).

Results obtained from this study ~~has~~ haveshown that *Panicum* ~~*subalbidum*~~*subpallium*and *Schoenoplectussenegalensis*plant, due to their high moisture and nutrient content properties,~~makes~~ makethem appropriate agents for enhanced bioremediation. It further revealed that ~~a combination of~~combining phytoremediation and ~~Bioaugmentating~~ Bioaugmentationagents creates more favorable conditions for biological activity to thrive~~ and~~. It has shown to be

effective, economical, eco-friendly, and sustainable in remediating organic contaminants from contaminated soil.

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, and 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~~bacteria, according to the report of Ogbonna~~,~~ (2016), who stated ~~clearly that bacteria can proliferate in the soil that has~~that bacteria can proliferate in soil with a pH ranging from 5.0 to 8.5. The crude ~~oil contaminated~~oil-contaminated pots had a 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~~Petroleum hydrocarbons are complex substances formed from hydrogen and carbon molecules and sometimes contain other impurities such as oxygen, ~~surphur~~ sulfur,and nitrogen. They are highly ~~viscos~~ viscous(e.g tar and motor oil), and are generally readily absorbed through the skin and intact mucosae.

pH had a notably steady reduction during 28 days of the monitoring period as ~~metabolites were produced by the organism~~the organism produced metabolites 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~~setup. 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 a limitation to biodegradation. 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 root total hydrocarbon content 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. Adams *et al.* (2015) 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.

Adams *et al.*, (2015) 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~~metals such as ~~Caduim~~Cadmium, 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~~;~~ . More~~over~~ ~~so~~, the action of crude oil in the chemical properties of soil and that of amendment ~~nutrient~~ nutrients could result ~~to~~ in the elevated value of heavy metals found in the crude oil contaminated soil. The value of ~~Caduim~~Cadmium (~~Mg~~mg/kg) showed the highest concentration in contaminated soil + *Pseudomonas* + ~~*Baccilus*~~*Bacillus* + *Panicum subalbidum* (12.45±0.35mg/kg) in soil and contaminated soil + ~~*Baccilus*~~*Bacillus* + *Panicum subalbidum* (9.83±2.36mg/kg) in plant root. The value of Chromium showed highest concentration in contaminated soil + ~~*Baccilus*~~*Bacillus* + *Panicum subalbidum* (106.97±92.65mg/kg) and contaminated soil + ~~*Baccilus*~~*Bacillus* + *Schoenoplectus senegalensis* (41.10±71.19mg/kg) for soil and plant root respectively. The value of lead showed highest concentration in contaminated soil + ~~*Baccilus*~~*Bacillus* + *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*~~*Bacillus* +*Schoenoplectus senegalensis* (45.77±22.24mg/kg) in plant root. The value of ~~Caduim~~Cadmium showed low value to other heavy metals.

Phytoremediation using grass plant *Panicumsubalbidum* (Elbow buffalo grass), Sedge plant (*Scoenoplectussenegalensis*) and Primrose plant (*Ludwigiaerecta*) was 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. Evaluation data from the baseline and phytoremediation analysis in the 10 Randomized Complete Block Design (RCBD) pots field studies shows that

Primrose plant (*Ludwigiaerecta*)does not survive the first screening stage with crude oil contamination. 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~~ survivedafter monitoring ~~of~~ for28 days with crude oil contamination.

Experimental transplants had an initial height of 16.7cm on the first ~~7~~ sevendays of growth~~,~~ ~~Plant~~. Plant showed reduced growth, whereas~~;plant~~ plantsin uncontaminated soil were in good condition. *Panicumsubalbidum* (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 *Panicumsbalbidum* (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; while the *Scoenoplectussenegalensis* did not survive the crude oil contamination after 28days of monitoring. There was no significant difference of plant height between the contaminated and uncontaminated.

Root structure is considered just as important as root biomass concerning the 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~~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

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. (Adams *et al.*, 2015). 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~~bacteria products of metabolism and organic matter can accelerate.

This research revealed and ~~recommend~~ recommended*Panicumsbalbidum* (Elbow buffalo grass) and *Scoenoplectussenegalensis*~~as~~ assuitable plant species for phytoremediation of crude ~~oil polluted~~oil-polluted soil with high total petroleum hydrocarbon content value.

In the present study, the test plant (*Panicumsbalbidum* (Elbow buffalo grass) and *Scoenoplectussenegalensis*) 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* [12]

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 setup soil. More so, based on my findings, I recommend ~~the use of ecofriendly and augmenting microbes as~~ using ecofriendly and

augmenting microbes as an amendment option with phytoremediation plants to facilitate pollutant removal/clean up.

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