EFFECTS OF BIOSTIMULATION AND BIOAUGUMENTATION ON MICROBIAL GROWTH AND BIOREMEDIATION OF HYDROCARBON CONTAMINATED SOILS.

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Abstract- **Fossil oil spillage is a common source of soil contamination in Nigeria. In severe cases, it can lead to loss of lives, damage to farmlands and reduction of bio density and biodiversity of the biotic components of the impacted environment. While natural breakdown of pollutants occurs, it is important to control hydrocarbon pollution in order to prevent an increase in the contamination leading to destruction of the ecosystems. However, the aim of this study is to identify the effects of bio stimulation and bioaugmentation on microbial growth and bioremediation of hydrocarbon contaminated soils. Pristine garden soil was contaminated with three concentrations of Bonny light crude oil (100,00mg/kg or 10%,10,000mg/kg or 1% and 1,000mg/kg or 0.1%), while food wastes compost was used as the bio stimulant to enhance the bioremediation of the contaminated soils. Three identified indigenous bacteria species (*Bacillus velezensis, Lysinibacillus pakistanensis and Stenotrophomonas species)* were used as a consortium to bio augment the bioremediation process. Microbial and physicochemical analyses were conducted using standard methods, while metals were analysed using Inductively Coupled plasma Atomic emission spectroscopy (ICP-AES) and atomic absorption spectrometry (AAS) analytical techniques that measure the concentrations of elements qualitatively and quantitatively. The hydrocarbon impacted and unimpacted garden soil samples had mean pH values of 5.75±1 and 7.84±1respectively, while the compost had a pH value of 8.00±1.0. The values of nitrate, phosphate, calcium (ca) magnesium (Mg) and potassium (k) present in the various soils are; 0.091ppm, 0.244ppm, 0.803545%, 0.020542% and 0.269008% for the impacted soil sample and for the unimpacted 1.335ppm 0.287%, 0.441135%, 0.01256% and 0.357033% was recorded respectively. Composite microbial culture (CMC) isolated from the hydrocarbon impacted soil and organic compost from food wastes were combined in three different concentrations of Bonny light crude oil as follow: 10 %(100,000mg/kg), 1 %(10,000mg/kg) and 0.1 %(1000mg/kg). For the control, 10% and 1% of crude oil was used to monitor the process. The set up was monitored for 16 weeks with GC-FID to monitor the total petroleum hydrocarbon (TPH) removal. The data obtained from the study shows that bio stimulation using composite microbial culture and organic compost could lead to a drastic reduction of the TPH in the hydrocarbon polluted soil. The data obtained from the GC-FID revealed that concentration A (10%) recorded 13.00±00.71mg/kg, B (1%) recorded11.56±0.01mg/kg, while C (0.1%) recorded1.77±0.01mg/kg. 1% of the control recorded 27.84±0.01mg/kg, whereas 10% recorded 200.08±0.16mg/kg by the end of the experiment. However, the microbiological analyses of the impacted soil revealed that the soil had appreciable mean population of hydrocarbon utilizing bacteria counts (HUB) is 106.67±4.51 (cfu/g), 93.00±4.00 (cfu/g) and 87.67±7.51 (cfu/g) and total heterotrophic bacteria count (THB) 110.00±20.00, 109.33±1.53 and 105.00±15.00 (cfu/g) with the fossil oil concentrations of 10%, 1% and 0.1% respectively. However, the difference between THB and HUB counts was observed to be minimally insignificant, which suggest that most of the microorganisms present in the various polluted sample sites are hydrocarbon degraders that can withstand the concentrations of hydrocarbons and also use them as source of carbon. The impacted soil amended with CMC and Compost has shown from the study to stimulate the growth of indigenous oil degrading micro biota and enhance the remediation of hydrocarbon polluted soils. This is a win-win situation of proper management of organic wastes while enhancing removal of hydrocarbons from polluted soils.**

Keywords: **Hydrocarbon polluted Soil, Hydrocarbon utilizing bacteria, Remediation**

INTRODUCTION:

The main components of petroleum hydrocarbons are only carbon and hydrogen; therefore, the environment must have enough other nutrient elements to ensure growth of bacterial degraders. It is estimated that approximately 150g of nitrogen and 30 g of phosphorous are consumed to convert 1kg of hydrocarbons in bacterial cells (Ron and Rosenberg, 2014).Soluble and non-soluble nutrients suffer from problems in the actual remediation, leading to low bioremediation efficiencies (Ron & Rosenberg, 2014). Researchers have found that using nitrogen-fixing hydrocarbon-degrading bacteria to improve the bioremediation efficiency was another good strategy instead of providing nitrogen sources (Thavasi *et al.*, 2006). For aerobic degradation processes, using oxygen as an electron acceptor is quite important, but it is usually not adequate in petroleum oil-contaminated environments because of the limited air permeability. (Gogoi *et al*., 2003) reported that up to 75% of the hydrocarbon contaminants were degraded within 1 year in field tests by controlling and regulating aeration. However, providing a sufficient oxygen supply to stimulate the bioremediation of petroleum pollutants in the environment is rather expensive and not feasible. Hence, the application of bulking agents such as

saw dust into the soil to increase permeability or other electron acceptors (NO_3^-, Fe^{3+}, or Mn^{2+}) into anoxic environments to stimulate anaerobic microorganisms is often more economical than oxygen supplementation (Brown *et al*., 2017; Altomare,*et al*., 2021).
Soils are the most complex and diverse ecosystem in the world. In addition to providing humanity with 98.8% of its food, soils provide a broad range of other services, from carbon storage and greenhouse gas regulation, to flood mitigation and providing support for our sprawling cities (Kopittke *et al*., 2019).
Soil microorganisms are a very important part of the environmental ecosystems, which could adjust energy flow and cycle of matter by digesting animal, plant and oil residues, and play a pivotal role in growth and development of agricultural crops, balance of the soil ecosystem, organic matter transfer and bioremediation. Furthermore, the diversity of the microbial community in soil is closely related to the function and structure of its surrounding ecosystem, and is one of the components to maintain soil productivity (Thorsten *et al.,* 2015).

Several studies have revealed that the bacteria community composition in hydrocarbon polluted soil tends to comprise mostly bacteria that are specially adapted to use hydrocarbon as carbon source. Due to the abilities of certain microbes to mineralize hydrocarbon components into environmentally friendly substances such as carbon dioxide and water, the ability of bacteria in breaking down hydrocarbons has gained growing attention in modern day research (Kadali *et al.*, 2012; Zhang, *et al*., 2019). Biodegradation by microbes is the key removal process of hydrocarbons which is controlled by hydrocarbon physico-chemistry, environmental conditions, bioavailability and the presence of catabolically active microbes.(Chorom *et al.*, 2010; Agarry,2019).

For many years, microbiologists have tried to culture beneficial microorganisms for use as soil inoculants to overcome the harmful effects of phytopathogenic organisms, including bacteria, fungi, and nematodes. Natural fermentation composting by using mixed cultures of beneficial microorganisms have considerable potential for suppressing and controlling the phytopathogenic organisms and thus providing a more favourable soil environment for plant growth and protection. (Par *et al*., 1994). Organic compost, when applied to the top level of the soil, can retain and release its nutrients slowly to the roots of the crops unlike inorganic fertilizer which can dissolve quickly when come in contact with water and thus travel deep into the ground, resulting in most of it not being captured by the roots of the crops. Therefore, the organic compost has beneficial effect on soils by improving the soil quality and producing crops with better yield and quality. The organic compost, when applied correctly, does not contaminate and pollute the groundwater sources, rivers and seas and thus it is a preferred alternative to conventional NPK based chemical fertilizers (Beffa *et al*., 1996)..

In order to achieve hydrocarbon utilization by bacteria, a number of rate limiting nutritional requirements need to be provided. Hydrocarbons as their name implies are composed of hydrogen and carbon; therefore there is a need to supply all other elements essential for growth in the growth medium.
Bio-stimulation by addition of organic and inorganic nutrients to hydrocarbon polluted matrices has been shown to supply limiting nutrients to indigenous hydrocarbon degraders to ensure that microbial activity is enhanced for biodegradation of pollutants to occur (Chikere *et al.*, 2012). Hydrocarbon pollution usually results in nutrient depletion and destruction of viable microorganisms causing a shift in the microbial community composition of such sites.

Materials and methods

Site Description/Sample study area

The study location is situated in the integrated institute of Environment and Development (IIED), Hydrocarbon Pollution Research/Training site, Obi-Ayagha, Ughelli South, Delta State. Soil samples from the surface horizons (0-15m) were collected from sites with the history of crude-oil contamination from artisanal refinery activities, using a soil auger (polluted soil sample). It was collected within the first quarter of the year (dry season). Delta covers 20,000km2(27,000sq m) within wetlands of 70,000km2(27,000sq m) formed primarily by sediment deposition, the Delta environment can be broken down into four ecological zones. Coastal barrier islands, mangrove swamp forests, freshwater swamps and lowland rainforests fresh water swamps and lowland rainforests. The Niger Delta generally have an equatorial climate on its southern coast and subequatorial climate in the North, the monthly mean temperature ranges between 25^0c and 29^0c, while the annual precipitation ranges between 2000mm and 4000mm, with relative humidity being above 70%.
Environmental contamination is central to the Niger Delta, as it has hampered rural economic activities.

GPS MAP OF THE STUDY AREA

The Coordinates of the sampling locations:

Sample Collection Site	Type of Sample	Latitude	Longitude	Temperature/ Soil texture
Obi-Ayagha abandoned artisanal refinery site	Hydrocarbon Contaminated Soil	5.3674330	5.8499400	Loamy/clay 28.3^0C-29.0^0C
FUPRE Garden	Natural pristine soil	5.570334.5	5.840970	Loamy/clay 28.3^0C-29.0^0C

Materials used:

- Fossil Oil (Light crude) was sourced from Bonny and used for isolating hydrocarbon utilizing bacterial (HUB). It was obtained from Port Harcourt Refinery Company, Eleme, in Rivers State Nigeria.
- Organic compost was purchased at the integrated waste management facility (FOMAS Agro Care Facility) situated in Jeddo, in Okpe local Government area, in Delta State.

Microbiological analysis

Isolation of hydrocarbon utilizing bacteria (using spread plate method

The total heterotrophic bacterial (THB) and hydrocarbon utilizing bacterial (HUB) counts were carried out using ten-fold serial dilution with normal saline. One gram of the impacted soil was weighed into a test tube containing 10ml of normal saline. Then 1ml was transferred from the stock into another test tube containing 9ml normal saline giving 10-1 dilution. This procedure was repeated up to 10^{-3}. Aliquots of dilution (0.1ml) were inoculated onto nutrient agar (NA) plates in triplicates using spread plate method (APHA, 2012). The plates were incubated at 30ºC for 24hours. The HUB count was carried out in triplicates on mineral salt agar (MSA) of (Mills *et al*., 1978) as modified by Okerentugba *et al*. (2016). Aliquots of 0.1ml of dilution was inoculated onto MSA plates in triplicates by spread plate method (APHA, 2012). Vapour phase method was used to isolate the hydrocarbon utilizing bacteria (HUB), adopting the method of Okerentugba *et al*. (2016). (The filter paper was saturated with crude oil and served as a sole source of carbon and energy). MSA comprises of KH2PO4 (1g), K_2HPO_4 (1g), NH_4NO_3 (1g), $MgSO_4$ (0.2g), $FeCl_2$ (0.05g), CaCl (0.02g), agar agar (15g) all dispensed into 1litre distilled water. Culturable bacteria isolates from the HUB plates were subcultured onto NA plates and incubated at 28^0C for 24h. Distinct colonies were further sub-cultured onto slant NA in Bijou bottles and incubated at 28^0C for 24h. The NA slant cultures were preserved in the refrigerator at 4ºC and served as pure stock culture for subsequent characterization and identification of the isolates. Standard characterization tests were performed as described by Cheesbrough, (2006) (using the Culture dependent and culture independent method to identify the isolates).

Screening and Identification of Bacterial Isolates

High through put screening using Turbidometric and spectrophotometric approach was used to quantify the growth of the isolates and the quantity of hydrocarbon present, following the method of (Habib, *et al.*, 2017).

Molecular Identification of Isolates:

Using pure bacterial isolates, Genomic deoxyribonucleic acid (DNA) extraction, sequencing and bioinformatics were done in International Institute of Tropical agriculture (IITA) a federal research institute located at Ibadan, in Nigeria, to identify the bacteria isolates with high hydrocarbon utilizing capability.

DNA extraction was carried out by using a ZR **Bashing™ Lysis Tube**, bacterial DNA Miniprep extraction kit obtained from Inquaba, South Africa. The DNA extraction protocol was based on manufacturer's instruction. Heavy growth of the pure bacteria isolates were subcultured on Nutrient agar plates and suspended in 200µl of isotonic buffer into ZR bashing bead lysing tubes, 750ul of ZymoBIOMICS™ Lysis Solution was added into the tube Lysing solution was added. The tube was secured in a bead beater (Disruptor Genie) and processed for 20minutes.

They were processed at maximum speed for 5mins.The ZR Bashing bead lysing tubes were centrifuged at 10,000 xg for 1min. Four hundred (400) µl of the supernatants were transferred into ZYMO-spin IV spin filter in collection tubes and centrifuged at 8000xg for 1min. The amount of 1,200µl of fungal/bacterial DNA binding buffer was added to each filtrate in the collection tubes bringing the final volume to 1,600µl, 800µl was then transferred into ZYMO-spin11C column in a collection tube and centrifuged at 10,000xg for 1min, the flow through were discarded from the collection tubes. The remaining volumes were transferred to the same ZYMO-spin and spun at 10,000xg for 1min. Two hundred (200) µl of the DNA pre-wash buffer were added to the ZYMO-spin 11C in new collection tubes and spun at 10,000xg for 1min followed by addition of 500µl of fungal/bacterial DNA wash buffer and centrifuged at 10,000xg for 1min. The ZYMO-spin 11C column were transferred to clean 1.5µl centrifuge tubes and 100µl of DNA elution buffer were added to the column matrix and centrifuged at 10,000xg for 30 seconds to elute the DNA. The ultra-pure DNA of each isolate properly labeled were then stored at -200C for use. After extraction, the DNA samples were quantified using NANODROP (ND1000). PCR amplification of 16S rRNA: The 16S of the rRNA genes of the isolates were amplified using the 27F and 1492R primers on a PCR System (9700 Applied Bio system thermal cycler) at a final volume of 25µl for 40 cycles. The PCR mix include: the x2 Dream tag master mix supplied by Inquaba, South Africa (tag polymerase, DNTPs, MgCl2, the primers at a concentration of 0.4M and extracted DNA as a template.

General layout of the 16s metagenomics workflow

Briefly the genomic DNA samples were sequenced and the PCR was amplified using a universal primer pair 341F and 805R - targeting the V3 and V4 region of the bacterial 16S rRNA gene. (C − e bron *et al*., 2008) Resulting amplicons were purified, and repaired, illumina specific adapter sequences were ligated to each amplicon (NEBNext Ultra II DNA library prep kit).

Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1), and another AMPure XP bead-based purification step was performed. Amplicons were then sequenced on illumina'sMiSeq platform, using a MiSeq v3 (600 cycle) kit.20Mb of data (2x300bp long paired end reads) were produced for each sample. The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

PCR PRODUCT PURIFICATION

The PCR conditions were as follows:2vol (20ul) of absolute ethanol was added to the PCR product, the tube was then incubated at room temperature for 15minutes,and Spinned down at 10,000rpm for 15minutes. The Decant supernatant was Spin down as well at 10,000rpm for 15minutes,then add 2vol (40ul) of 70% ethanol Decant supernatant Air dry Add about 10ul of ultrapure water Check for amplicon on 1.5% agarose then the PCR product is ready for sequence reaction. As displayed below:

PRIMER: 27F: AGAGTTTGATCCTGGCTCAG
1492R: GGTTACCTTGTTACGACTT

Physical properties

The soil samples collected from the field was air dried, homogenized with a sledge hammer (except those for organic analysis) and sieved with a 2.3mm mesh. Portions of the samples were thoroughly homogenized and stored for organic carbon as well as other parameters for physic-chemicals.

Analysis of soil properties using ICP-AES and AAS

Inductively Coupled plasma Atomic emission spectroscopy (ICP-AES) and atomic absorption spectrometry (AAS) analytical techniques that measures the concentrations of elements qualitatively and quantitatively were used to analyse the metals and the various samples used in this study. ICP Analysis also called ICP testing is performed to identify and measure a range of chemical elements necessary for the analysis of metal samples. It calculates quantitative and qualitative data that can be included in an ICP test report. It is the latest technology for major, minor and trace element analysis, it detects the atomic emissions produced as light.

Total Petroleum Hydrocarbon (TPH)

Gas chromatograph- flame ionization detector (GC-FID), was used to achieve the analysis was carried out in thermo steel laboratory situated in Warri, Delta State, Nigeria

Ten grams (10g) of soil samples (polluted sample and control sample) was transferred into an extraction bottle, spiked with known amount of the internal standard (0.1ml of squalene) and dried with anhydrous sodium sulphate, then the dried sample was then extracted with a known volume of a mixture of n-hexane and dichloromethane in the ratio of 3:1 by shaking with a sonicator. The extract was cleaned in a silica gel column and the final volume of extract is taken, 1.0µl of the final volume of the extract was injected into an already calibrated Gas Chromatograph equipped with capillary column and identification with data processing software (DATA APEX CLARITY). Total petroleum, hydrocarbon (TPH) was extracted and quantified using the gas chromatography- flame ionization detection (GC-FID) Agilent 7890. ASTM-D2862, (2016).

pH

This is a figure expressing the acidity or alkalinity of a solution on a logarithmic scale on which 7 is neutral, lower values are more acidic and higher values are more alkaline. The pH is equal to $-\log_{10}c$, where c is the hydrogen ion concentration in moles per litre. This was measured in 1:1 soil to water suspension with a glass electrode pH meter (handheld/portable) with a sensitivity of ±0.01. Twenty grams (20g) of air dried soil samples (polluted sample and control sample) was put in a 50ml beaker; 20ml of distilled water was added and the content was stirred occasionally with a glass rod. The electrode of the pH meter was inserted into the mixture and the reading was taken (Dora, 2019).

Result and Discussion

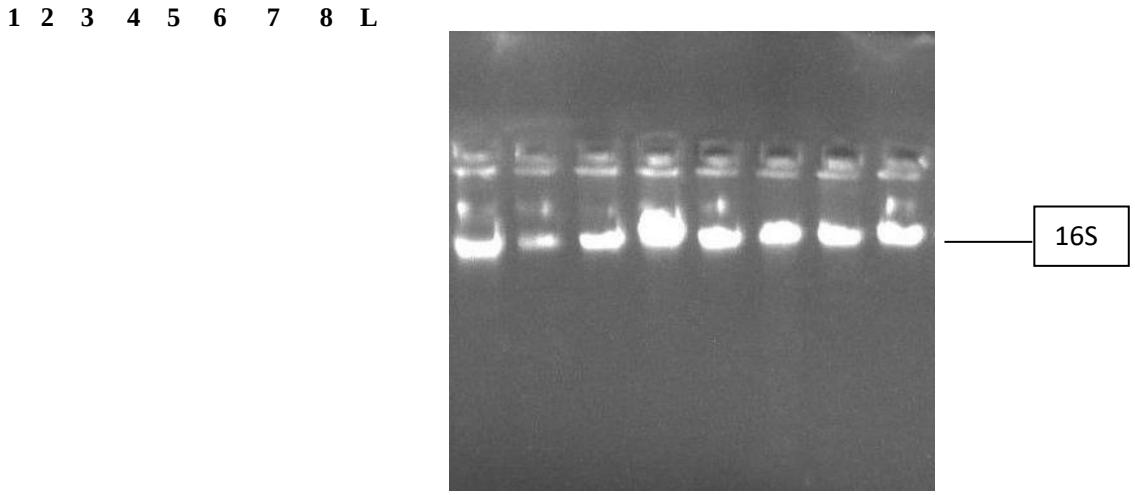

Fig. 1: Agarose gel electrophoresis showing the DNA gel
L: represents the 1kb ladder, 1-6 represents 16 S gene bands of the isolates.

Table 1: Identity of Bacteria Isolates used as consortium

S/N	SAMPLE IDENTITY	PERCENTAGE	**GenBank ACCESSION NUMBER**
1	***Lysinibacillus pakistanensis***	*97.18%*	FJ418643.1
2	***Bacillus velezensis***	*93.06%*	Cp1016121
3	***Stenotrophomonas species***	*98.30%*	JNOOO347.1

Table 2: Physicochemical characteristics of pristine soil, Fossil Oil contaminated soil and Fossil (crude) oil used for the study

PARAMETERS ANALYZED/UNITS	FOSSILOIL CONTAMINATED SOIL SAMPLE	PRISTINE SOIL (CONTROL)	FOSSIL OIL
TOC%	2.44	0.669	2.969
Nitrate, PPM	0.091	1.335	NIL
Phosphate, PPM	0.244	0.287	NIL
Ca %	0.863545	0.441133	0.23786
Mg %	0.020542	0.012565	0.002699
K %	0.269008	0.357037	0.123716
Na %	0.005853	0.004552	0.008581
Cr %	0.000086	0.000132	0.000086
Co %	0.000106	0.000072	0.000072
Cd %	0.000054	0.000043	0.000031
Se %	0.000403	0.000297	0.000197
Pb %	0.00056	0.00323	0.000273
Mn %	0.001103	0.001924	0.000288
Cu %	0.000369	0.000862	0.000271
Zn %	0.00072	0.000343	0.000199
Fe %	0.008012	0.022919	0.001233
Ni %	0.000228	0.000164	0.000114
Ph	5.75	7.84	
Electrical Conductivity, µs/m	56.90	102.80	
Temperature, ^{o}C	28.00	28.00	
Cation exchange capacity	9.60	3,30	
Total petroleum hydrocarbon, mg/kg	7488	8.54	

Table 3: Organic compost and its composition

PARAMETERS	RESULTS
pH	8.00
Conductivity(µs/m)	13830
Temperature, ^{o}C	27.90
Nitrate mg/kg	309.93
Total organic carbon%	118.08
Moisture content%	1.70

Table 4: Experimental Design

Bioaugumentation/Biostimulation (Nutrient)	16g Consortium CMC(8) Compost(8)	1.0g CMC(0.8) Compost(1.2)	0.12g CMC(0.04) Compost(0.16)
Light fossil oil	10% (100,000mg/kg)	1% 10,000mg/kg	0.1% (1000mg/kg)
NS	184g	198g	199.8g
TOTAL	**200g**	**200g**	**200g**

Key: CMC (Composite microbial culture)
Compost (Organic compost from waste)
NS (Natural soil)

Figure 1 shows the phylogenetic analysis of the 16S rRNA of the isolates showing their percentage similarities to other species at *98.30%* being most closely related *Lysinibacillus* ***pakistanensis, Bacillus velezensis and Stenotrophomonas species.***
Results of physicochemical parameters are shown in Table 2. The pH value of crude oil polluted soil is lower as compared to that of the control sample. The highest pH value (7.84) was recorded for the control soil sample which is neutral/slightly alkaline while the lowest soil pH (5.75) was recorded for the polluted soil sample. The decrease in pH value may be due to increase in degradation of crude oil by microorganisms in the soil resulting in accumulation of acidic metabolites (Ejileugha, 2015). As shown in Table 1, the electrical conductivity (EC) of the control sample was 102.80µs/cm and the polluted sample was 56.90µs/cm i.e the electrical conductivity of control sample is higher. EC is a function of level of contamination at the polluted site, the higher the level of spill the lower the EC (Pathak *et al.*, 2011). The total organic carbon (TOC) of control sample is 2.44% and for the polluted soil sample was 0.669% and for the fossil oil used it recorded 2.969% the reason is that the soil polluted with crude oil which contain hydrocarbon has more carbon content compared to that of the control. (Sulaiman, 2015). The amount of nitrate in the control soil sample was 0.000164mg/kg and that of polluted soil sample was<0.000228 which means that there is no presence of nitrate in the polluted soil sample, and for the fossil oil we have 0.000114%.The total absence of nitrate in the polluted soil is an indication that the limiting nutrients were released to the microorganism involved (Ibiene *et al.*, 2011). Also, crude oil pollution leads to the deterioration of soil mineral nutrients. For phosphate, the control soil sample was 0.42mg/kg while the polluted soil sample was 0.75mg/kg. Sulphate control soil sample was 1.61mg/kg while for the polluted soil sample was 4.85mg/kg. The result indicated that the phosphate and sulphate levels of the polluted sample seems to be higher than that of the control sample because the pollution exerts adverse effects on soil conditions, microorganisms and plants, however phosphate present in the fossil oil was not analysed (Uche *et al.*, 2011).
Control soil sample for calcium reads 0.863545% and for the polluted soil sample it was 0.441133% and that of potassium for the control soil sample was 0.357037% while that of the polluted soil sample was 0.269008%. The control soil sample for sodium was 0.004552% and the polluted soil sample was 0.005853% while that of magnesium , the polluted soil sample was 0.020542% and control soil sample was 0.012565% this due to the deterioration of soil structure (Snehal, 2014).
The cation exchange capacity (CEC) which is the calculation from the absorbance obtained from the various cations and the activities of the individual ions. For the control soil sample it was 3.30meq/100g while the polluted soil sample was9.60meq/100g. It is observed that the lower concentrations of calcium, potassium, sodium and CEC were found in the control soil sample e.t.c and higher for the polluted sample except for magnesium potassium and the increase depends on the concentration of the contamination. The concentration of lead in the polluted soil sample was higher than the concentration in the control sample. Lead (Pb) concentration in polluted soil was 0.00056% and the control soil sample was 0.00323%, the concentration of copper in the two soils tested however showed that the control soil sample had the percentage of 0.000369% while the lower concentration was observed in polluted soil sample as %. The polluted soil sample concentration for zinc is 0.00072% which was higher than that of the control soil sample which was 0.000343% as the lowest. According to Tanee & Eshalomi-Mario, (2015) the increase in the concentration of heavy metals in the polluted soil may be due to the hydrocarbon pollution which altered the **physico-chemical** parameters of the soil as well as increasing the concentration of heavy metals.

composting can hasten the transformation of biodegradable food waste into very rich organic compost which can nourish plants and soils and thus be very useful for farming of crops for human consumption, (Pleissner *et al*., 2014) in Table 3 the **moisture content** of the organic compost used recorded (1.70%) this may be due to the fact that it is free of toxicity and cannot coat the soil and consequently it couldn't prevent the penetration of water that can be caused by microorganism which utilize water for their activities (Snehal, 2014). Soil pH controls the solubility, mobility, and bioavailability of trace elements, which determine their translocation in plants (Dora, 2019), hence the pH recorded for the compost recorded 8.00, whereas the Nitrate constituent i the compost recorded 309.93 mg/kg The presence of nitrate in the polluted soil is an indication that the limiting nutrients were released to the microorganism involved (Ibiene *et al.*, 2011).The solubility of soil micro and macro nutrients are influenced by the soil pH (Dora, 2019) these are: pH, conductivity(µS/cm), Temperature, Nitrate (%), Total organic carbon and moisture content, sulphate(%), Nitrate(%) and phosphates(%) .Total organic carbon recorded118.08% and the temperature 27^0c; however organic compost (food waste) is an amendment used in biostimulation.

The total petroleum hydrocarbon (TPH) concentration for the control sample was 8.54mg/kg and polluted sample was 7488mg/kg, the polluted sample is extremely higher than that of control sample because it was contaminated with hydrocarbon found in crude oil and this petroleum hydrocarbon released tends to increase toxicity in the soil. (Cermak *et al.*, 2010).

Table 5: Mean Concentration of Total Heterotrophic Bacteria Count (THBC) in Natural attenuation (Natural soil) setup

Exposure Duration(days)	**(1%)x(10^3)** (CFU/g) THBC	**(0.1%) (10^3)** (CFU/g) THBC
0	35.00±5.00	33.33±1.53
28	44.33±4.51	43.00±1.00
56	53.33±3.51	58.00±12.00
84	57.67±2.52	69.00±13.00

A (1%=10,000mg/kg) B (0.1%=1000mg/kg)

Table 6: Mean Concentration of Total Heterotrophic Bacteria Count (THBC) in Biostimulation and Bioaugumentation Amendments

Exposure duration (days	A	B	C
0	49.33±3.79	54.67±3.06	46.67±10.41
28	82.67±10.21	81.33±6.11	73.67±5.51
56	87.33±8.50	89.33±11.02	79.67±8.50
84	110.00±20.00	109.33±1.53	105.00±15.00

A (1%=10,000mg/kg) B (1%=1000mg/kg) C (0.1%=1000mg/kg)

Table 4 shows how the set up was designed and Table 5 displays the Mean Concentration of the control experiment (Natural attenuation) with two concentrations of crude oil (1.0% and 0.1%). Total Heterotrophic Bacteria Count (THBC) in cfu/g (**10^3**) was carried out and their result represented in mean standard deviation. The following was achieved within the first and last week of the experimental design for the THBC, for the natural attenuation (Control) (0-84days). The values ranged between 31.33±1.53 *cfu/g* and 119.00±9.64 *cfu/g*, whereas Table 6 shows the Biostimualtion and Bioaugumentation set up which was augmented and stimulated with compost and composite microbial culture (CMC). A showed 49.33±3.79 and 110.00±20.00 whereas for B we have 54.67±3.06 *cfu/g* and 109.33±1.53 *cfu/g*, for C we have 46.67±10.41 *cfu/g* and 105.00±15.00 *cfu/g*. It is known that *cfu/g* counts are higher in polluted soil than unpolluted soil, and microbial counting of a contaminated site is the simplest method to monitoring microbial activities that can be used for bioremediation.

In polluted sample the THB had a mean value of a similar observation as reported by (Chikere& Ekwuabu, 2014). Petroleum hydrocarbon degrading bacteria are ubiquitous in nature and can utilize these compounds as sources of carbon and energy. Bacteria displaying such capabilities (Pseudomonas species, Bacillus species etc) are often exploited for the bioremediation of petroleum oil contaminated environment, recent studies have identified bacteria from more than 79 genera that are capable of degrading petroleum hydrocarbon (Trembley, *et al*.,2017).

Table 7: Mean Counts of Hydrocarbon Utilizing Bacteria (HUB) in Natural soil (Natural Attenuation)

Exposure duration (days)	**A(1%)(10^3)**(CFU/g) **HUB**	**B (0.1%)(10^3)** (CFU/g) **HUB**
0	36.67±5.77	31.33±1.53
28	49.33±2.08	51.67±33.29
56	81.33±5.51	90.33±1.53
84	103.00±3.00	97.67±2.52

A (1%=10,000mg/kg) B (0.1%=1000mg/kg)

Table 8: Mean Concentration of Hydrocarbon Utilizing Bacteria (HUB) Count **(10^3)** in Bioaugumentation and Biostimulation set up

Exposure duration (days)	**A (10%)(10^3)** (CFU/g)	B **B(1%)(10^3)** (CFU/g)	C **B(0.1%)(10^3)** (CFU/g)
0	32.33±2.08	31.67±1.53	33.00±2.00
28	73.67±3.51	69.00±1.00	53.00±19.67
56	80.00±10.00	72.67±2.52	61.33±1.53
84	106.67±4.51	93.00±4.00	87.67±7.51

A (1%=10,000mg/kg) B (1%=1000mg/kg) C (0.1%=1000mg/kg)

For the Hydrocarbon utilizing bacteria (HUB), the log cfu/g of control were in two concentrations as well, for 1% we have 30.67±1.15 and 108.67±1.53 for 0.1% we have 31.33±1.53.However for the Biostimulation and Bioaugumentation which was augmented and stimulated as well with compost and composite microbial culture(CMC), we have in A 32.33±2.08 and 106.67±4.51,for B we have 31.67±1.53 and 93.00±4.00, for C we have 33.00±2.00 and 87.67±7.51 all recorded within the initial and the final duration of the experimental set up. The difference between THB and HUB counts was observed to be minimally insignificant which suggest that most of the microorganisms present in the various polluted sample sites are hydrocarbon degraders that can withstand the concentrations of hydrocarbons and also use them as source of carbon.(Jones *et al*., 2021;Chikere& Ekwuabu,2014). By the end of the study, population of indigenous oil degrading microbiota increased rapidly, which corresponds to high availability of hydrocarbons during these periods (Al-Kindi, *et al* .,2016). Crude oil polluted soil amended with organic matter may stimulate growth of the indigenous oil degrading micro biota in it (Abioye, *et al*., 2010). Similar observations have been reported using organic amendment (Jones *et al*., 2021).

Table 9: EXPERIMENTAL DESIGN OF GC-FID RESULT PRESENTED IN MEAN STANDARD DEVIATION

Exposure Period (Days)	**A (1%) Mg/kg**	**B (10%) Mg/kg**
0	1071.62±0.54	12504.50±0.71
28	423.76±0.03	756.39±0.19
56	119.79±0.26	267.41±0.24
84	27.84±0.01	200.08±0.16

Natural attenuation (Natural and Contaminated soil) 200g (100,000mg/kg,and 10,000mg/kg

Table 10: EXPERIMENTAL DESIGN OF GC-FID RESULT PRESENTED IN MEAN STANDARD DEVIATION

Exposure Period (Days)	**A Mg/kg**	**B Mg/kg**	**C Mg/kg**
0	1506.21±0.01	455.81±0.01	46.00±0.00
28	1089.73±0.00	105.36±0.01	7.96±01
56	209.63±0.52	49.26±0.36	19.17±0.01
84	13..00±00.71	11.56±0.01	1.77±0.01

Biostimulation (Compost and CMC combined) Biostimulation and Bioaugumentation =200g A=20;180=100,000mg/kg B=2;198=10,000mg/kg , C=0.2;199.9=1000mg/kg

The set up was designed in triplicate but the control wad in duplicate, it was monitored in the laboratory in a room temperature (25-27^{0}C) for a period of 0-84days. During this period some parameters were analysed and result obtained from the GC-FID shows that the control (Natural attenuation) in Table 9 shows that were within the range of 1071.62±0.54 and 27.84±0.01, for the 1.0% whereas 10% recorded 12504.50±0.71 and 200.08±0.16 within the period of exposure, drastic reduction of TPH was observed within the initial and the final stage of the laboratory experiment.

In Table 10 For the biostimulation set up, it revealed that Compost has the concentration A recorded 1506.21±0.01 and 13..00±00.71, for B they were within the range of 455.81±0.01 and 11.56±0.01 and for concentration C they were within the range of 46.00±0.00 and 1.77±0.01,the reduction of the TPH was so drastic that it was almost within detectable limit especially with concentration A as this is a true fact that microbes has catalytic effect on hydrocarbons.This can be attributed to the fact that different indigenous bacteria have different catalytic enzymes; thus, their roles in oil-contaminated sites also vary widely. This also implies that the remediation of petroleum hydrocarbon contamination requires the joint action of multiple functional bacteria to achieve the best environmental purification effect (Dombrowski *et al.*, 2016). Taken together, these studies indicate that improving the biodegradation potential via the application of bacterial consortia possessing multiple catabolic genes and application of other organic ammendments is a reasonable and feasible strategy for accelerating the removal efficiency of petroleum hydrocarbons from contaminated environments. Organic compost, when applied to the top level of the soil, can retain and release its nutrients slowly to the roots of the crops unlike inorganic fertilizer which can dissolve quickly when come in contact with water and thus travel deep into the ground, resulting in most of it not being captured by the roots of the crops. Therefore, the organic compost has beneficial effect on soils by improving the soil quality and producing crops with better yield and quality. The organic compost, when applied correctly, does not contaminate and pollute the groundwater sources, rivers and seas and thus it is a preferred alternative to conventional (Pleissner, *et al*.,2013)

CONCLUSION

Globally it is generally observed that hydrocarbon pollution is highly prevalent, in severe cases, it can lead to loss of lives, and damage to the entire ecosystem. While natural breakdown of pollutants occurs, it is important to control hydrocarbon pollution in order to prevent an increase in the contaminated area, and to destroy micro organisms resistant to natural degradation. The core element of bioremediation is useful microorganisms that need sufficient nutrients and suitable environmental conditions. In general, petroleum oil hydrocarbons are not necessary substrates for hydrocarbon-degrading bacteria, and they utilize these compounds as alternative carbon and energy sources, especially in the absence of their preferable substrates.Taken together, these studies indicate that improving the biodegradation potential via the application of organic amendment through biostimulation and bacterial consortia possessing multiple catabolic genes is a reasonable and feasible strategy for accelerating the removal efficiency of petroleum hydrocarbons from contaminated environments.

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