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Physicochemical Indices of Phyto Remediated Hydrocarbon Polluted Soil and the Effect of Bio-nutrient Amendment

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JODA, DNO, TKSA and AEG designed the study, Author JODA performed the statistical analysis, Author JODA wrote the protocol and the first draft of the manuscript. Authors JODA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the Physicochemical indices of Phytoremediated Crude Oil polluted amended soil using grass plant *Cyperus esculentus* (Cyp) and *Phyllanthus amarus* (Phy).
Study Design: The study employs experimental design, statistical analysis of the data and interpretation.
Place and Duration of Study: Rivers State University demonstration farmland in Nkpolu-Oroworukwo, Mile 3 Diobu area of Port Harcourt, was used for this study. The piece of land is situated at Longitude 4°48'18.50" N and Latitude 6°58'39.12" E measuring 5.4864 m x 5.1816 m with a total area of 28.4283 square meter. Phytoremediation process monitoring lasted for 240 days; analyses were carried out monthly at 30 days' interval.
Methodology: The study was carried out on Crude Oil Polluted soil (PS) amended with bio-nutrient supplements (Spent Mushroom Substrate (SMS) and selected fungi (*Aspergillus niger*(AN) and*Mucor racemosus* (MR)) used to stimulate and augment the indigenous microbial population

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present in a crude oil polluted soil thereby enhancing hydrocarbon reduction in *pari per sue* with phytoremediation (uptake of Crude oil by test plants) over a period of 240 days. Ten (10) experimental plots (two Control (Unpolluted and polluted soil without amendment) and eight polluted amended/treated plots) employing Randomized Block Design (each having dimensions: 100 x 50 x 30 cm LxBxH); formed and mapped out on agricultural soil and left fallow for 6 days before contamination on the seventh day; after which it was allowed for 21 days for proper contamination and exposure to natural environmental factors (to mimic soil crude oil spill site); thereafter nutrients/organics (biostimulating agents) and bioaugmenting organisms were applied. Baseline studies were carried out on soil profile before and after contamination, major parameters monitored and assayed were Total Petroleum Hydrocarbon (TPH) uptake by plant roots and stem, Polycyclic Aromatic Hydrocarbons (PAHs) and TPH reduction in soil. Other physicochemical properties analyzed in the soil from different plots were pH, Electrical Conductivity, Moisture Content, Total Nitrogen, Available Phosphorus, Potassium, Total Organic Carbon, Plant Height, Iron, Lead and Zinc at regular intervals; days 1, 60, 90, 120, 150, 180, 210 & 240. The rate of phytoremediation was estimated from percentage (%) uptake of Total petroleum hydrocarbon (TPH) in plant roots and stem from day 1 -240; while percentage (%) reduction of TPH and PAHs in soil was estimated from day 1 to the residual at day 240.

Results: The test plants decreased significant amount of crude oil as revealed in TPH uptake in their roots and Stem. Mean amount and percentage Total Petroleum Hydrocarbon (TPH) uptake by *Cyperus esculentus r*oots and stem were; 152.33±50.34mg/kg, 12.57±4.16% and 201.13±8.80mg/kg, 13.27±0.58% respectively; while that of *Phyllanthus amarus*roots and stem were 141.50±35.62mg/kg, 11.68±2.94% and 174.44±19.98mg/kg, 11.51±1.32% respectively; revealing higher Uptake of TPH in plant stem than roots. From the initial TPH contamination value of 5503.00mg/kg, it was observed that plots planted with *Cyperus esculentus* (TPH 5492.75±76.36mg/kg) showed higher reduction of TPH from soil than those planted with *Phyllanthus amarus*(TPH 5449.72±18.27mg/kg); while PAHs degradation/reduction showed a reverse trend with plots planted with *Phyllanthus amarus* (PAHs 28.72±2.74mg/kg; 60.46±5.77%) higher than plots planted with *Cyperus esculentus*
s (PAHs 25.77±2.12mg/kg, 54.24±4.47%).

Conclusion: Plots planted with *Cyperus esculentus* showed higher reduction of TPH from soil than those planted with *Phyllanthus amarus* while PAHs degradation/reduction in plots planted with *Phyllanthus amarus* was higher than plots planted with *Cyperus* esculentus. TPH uptake was higher in plant stems than roots; more so, plots amended with nutrient supplements showed significant higher percentage reduction in hydrocarbon in the polluted soil than unamended polluted soil. It is therefore recommended that *Cyperus esculentus* is a suitable plant species for phytoremediation of crude oil contaminated soil with high TPH value while *Phyllanthus amarus*is the best option in phytoremediation of polluted soil with high PAHs value, both in combination with bio-nutrient supplement.

Keywords: Oil spills; polluted soil; phytoremediation; PAHs.

1. INTRODUCTION

Crude oil spills occur in most regions of the world where its exploration takes place. This very often results from poorly maintained pipelines, none observance of operating procedures, from sabotage and to a very negligible source natural disaster.The leakages flow into the surrounding environment affecting land, flora and fauna and into the surrounding water bodies killing aquatic life [1]; the entire ecosystem is therefore, destroyed. A well canvassed system of remediation that has proven successful is the employment of bio and phytoremediation. It has been used extensively and the results are in its favour [2].

Odogwu and Onianwa [3], Dan-Kalio and Samuel- Allasseh [2] identified some plants that are resistant to crude oil pollution.These include*Oenanthe lachenalii, Cochlearia spp; Kyllinga* and *Commelina spp, Cyperus spp, Phyllanthus amarus spp.* These plants where chosen because they possess the appropriate qualities of a decontaminating plant as given by Dan-Kalio and Samuel-Allasseh [1] such as:

i. Plants that can be found around and at no financial cost.

ii. Plants with a growth pattern that can be monitored easily and without any sophisticated instrument.
iii. Plants that do not need tendering and any special treatment to grow when cultivated.
iv. Plants that are appropriate for the climate, soil condition of the contaminated sites, Pivetz [4].
v. Plants that should be able to tolerate stree, Sicilliano and Germida [5].
vi. Plants that have fibrous root.

Some of the plants were able to colonize the oil impacted area a few months after the spill. Phytoremediation; uses plants and associated microorganisms to restore soil and water bodies contaminated with hydrocarbon and other heavy metals.Phytoremediation is more environmentally friendly than most conventional clean-up methods used in the remediation of contaminated soil.

Schnoor [6] observed that phytoremediation is more effective in vigorously growing plant that have the ability to accumulate large concentration of contaminates in their body parts (root, stem and leaves).

Abogidi and Ejemta [7] noted that hydrocarbons from oil contaminated soils accumulates in the chloroplast of leaves. Huesemann et al [8] are of the opinion that the main mechanism of phytoremediation include the following; direct up-take of contaminates and their subsequent metabolism in plant tissues, transpiration of volatile organic hydrocarbons through the stem and leaves, discharge of exudates microbial activity and the enhancement of mineralization at the root-soil interface. The phytotoxic effect of crude oil has been investigated by Ikhajiagbe *et al* [9].

Effect of oil spill on plants may vary according to the type and amount of oil involved, the degree of its weathering, the time of the year, the species and age of plants concerned, soil type, drainage characteristics and climatic factors [10].

In Nigeria the exploration of crude oil in the Niger Delta has led to the pollution of land and waterways. The agricultural lands have become less productive [11,4,5] and the creeks and fishing water have become more or less dead [12,13,14]. Also, several civil unrests are witnessed in the Niger Delta region due to exploration of oil [15]. Efe and Elenwo [16] noted that crude oil exploration has massively threatened the subsistent peasant economy and the entire livelihood and basic survival of the people. Abii and Nwosu [17] in their study of two communities in Eleme, Ogali and Agbonchia, used Aleto community as control because they share geographical similarities. The study analyzed soil nutrient and soil fertility with the following parameters K, Ca, Mg, C, P, pH, carbon exchange capacity and soil structure using standard methods indicated that the soil at the sites were adversely affected by the soil nutrient and fertility which necessitated the inclusion of Eleme in the Ogoni remediation for soil Clearing in Rivers State.

Oil compounds react with the inorganic nitrogen and phosphorus in the soil limiting the nitrification process and removal of phosphoric acid is critical, thus nitrogen and phosphorus in the soil decreases and the absorption of crops is affected [18,19,20]. *Commelina benyhalensis* is more effective in water logged oil polluted soil. Dan-Kalio and Samuel- Allasseh [2] evaluated the effect of Bonny light crude oil contamination of soil, soil amendment by the successive plantings at four-week intervals on the health and growth of *Phyllanthus amarus* and reported some improvement of growth of crops in oil contaminated soil.

Chiara *et al*; [21] observed that in contaminated soil micro-organism can produce certain enzyme system and gradually form a dominant pollution with symbiotic or synergy effect. Peng, *et al* [22] reported that the population of living microorganisms was highly dependent on the concentration of petroleum contaminates in the soil. Studies have shown that hydrocarbon pollution can change the microbial Population in the soil, the composition of the community structure and enzymes system in soil. Prior to inhibitory action [23,24].

Heavy metal usage are widespread in industrial applications such as in the manufacture of batteries, alloys, pesticides, textile dyes, steel, electroplated metal parts. Soil may be contaminated by the accumulation of Heavy Metals and Metalloids through emissions from the rapidly expanding industrial area, disposal of Heavy Metal wastes, paints and leaked gasoline, animal manure, sewage sludge, pesticides, combustion residues, spillage of petrochemicals, application of fertilizers and atmospheric deposits [25,26].

Jingehum Tang *et al* [27] in their work on Bioremediation of petroleum polluted soil by combination of Rye Grass with effective micro-organism reported that degradation rate of 58% using combined treatment of plant and micro-organism after 162 days enhanced total petroleum hydrocarbon (TPH) drgradation by 17% as compared to the control.

This study is aimed at assessing the Physicochemical indices of Phyto-remediated Crude Oil polluted amended soil using grass plant *Cyperus esculentus* (Cyp) and *Phyllanthus amarus* (Phy) and effect of bio-nutrient amendment in remediating crude oil polluted soils in coastal areas of the Niger Delta region of Nigeria

2. MATERIALS AND METHODS

2.1 Area/Scope of the Study

The piece of land used for the study is situated at Longitude $4^{o}48'18.50''N$ and Latitude $6^{o}58'39.12''E$ measuring 5.4864m x 5.1816m with a total area of $28.4283m^2$. in an experimental land that lies within the Rivers State University Demonstration farmland in Nkpolu-Oroworukwo, Mile 3 Diobu area of Port Harcourt, Rivers State.The piece of land was cleared and sub-partitioned into 10 blocks of 100cm x 50cm x 30cm giving $150,000cm^3$ of soil in each plot.

The study tends to ascertain the level of efficacy in the use of the test plants (*Cyperus esculentus* and *Phyllanthus amarus)* in the remediation of crude oil (Bonny light) polluted soil amended with Spent mushroom substrate and augumented with *Aspergillus niger* and *Mucor racemusus*.

2.2 Research Design

This study adopted the experimental research design of Montegomery [28]. The Randomized Complete Block Design approach was used. Experimental design is the process of planning a study to meet specified objectives. Planning an experiment properly is very important in order to ensure that the right type of data, sufficient sample size and power are available to answer the research questions of interest as clearly and efficiently as possible.

2.3 Experimental Set-up

The materials used for the experiment were the test plants *Cyperus esculentus* (Cyp) and *Phyllanthus amarus*(Phy), Crude oil, soil amendment bioorganics - Spent Mushroom Substrate (SMS) and Augmenting microbes – *Aspergillus niger*(Asp), *Mucor racemosus* (Muc) served as treatments.

The top soil (0-15cm depth) at the experimental site was tilled with the aid of a shovel to loosen the soil. The experimental site was then divided into plots of 100cm x 50cm x 30cm. Crude oil of 2500ml was added to each plot except the control.

Soil amendment, 300g Spent mushroom substrate (SMS) and augumemting microbes *Asperigillus niger* and *Mucor racemosus* were added to some of the plots, then mixed properly and left to fallow for 56 days within which period is expected for the process of bioremediation to take place. On the 57th day Rake was used to mix the soil further to harmonize the soil to provide favorable condition for plant growth and obtain a near uniform concentration of Petroleum hydrocarbon, soil amendement and augumentation in the experimental plots. Ridges were dug to a dimension of 100/50/30. (Table 1).

Uniform test plant seedlings was obtained and transplanted immediately into the plots including the control plots. Each experimental plot received 10 seedlings, Sixty (60) test plants were planted in the experiment. The duration of the experiment was twelve months (one year) as to cover both dry and wet sessions.

2.4 Tilling

The experimental plots including the control plots were tilled once every week within the 56days of fallow period. This practice is to optimize the transfer of oxygen into polluted soils and promote aerobic degradation of organic contaminants.

2.5 Watering

Watering of the experimental plots started after preparation of plots for planting.

The plots were watered once weekly with about 300ml of water per plot for the first 56days and 600ml once daily later from the 57th day (After the planting) as required [29].

2.6 Weeding

Weeding was done at the interval of every 7 days during the fallow period (first 56days) and from the 57th day at 14days interval.

2.7 Experimental Data

Experimental data monitored, collected and analysed using standard methods were the following variables:Physical parameter: Particle size analysis, Plant Height (cm), pH, Temperature, Electrical Conductivity (EC), Acidity,Soil Moisture Content.

Chemical parameters: Nitrogen (N), Phosphorus (P), Potassium (K), Soil Organic Matter (SOM), Sodium (Na), Calcium (Ca), Magnesium (Mg), TPH, PAHs

Heavy metal: Zinc (Zn), Iron (Fe), Lead (Pb)

2.8 Source of Data

The source of data for the study was the primary source which was gotten through field work and laboratory analysis of samples from experimental plot set up to showcase the timeline of phytoremediation from events of oil spillage to full recovery of soil for agricultural production.

2.9 Method of Data Collection

The major data collection was done through the collection of plant roots, stems and soil sample to ascertain the efficacy of the test plant used for the experiment. This was done with the use of a soil auger which is an instrument used in collecting soil samples. Samples were collected within the range of 0-15cm (top soil) was analyzed. All the soil samples taken were analyzed monthly. Safety measures were ensured that the Auger after each use was properly washed before use in another plot. To preserve the sample's integrity, samples from the field to the laboratory was taken within 2-4 hours and was carried in foil containers. The experiments were set and measurements carried out.

Soil sample was collected from each plot including the control before (commencement of experiment), during and at the end of the experiment. During the experiment soil samples were collected monthly and tested in the laboratory. Plant height (cm) was measured in the field weekly.

2.10 Paticle Size Analysis

This was carried out on soil at the experimental site to determine the texture of the soil and soil type at the experimental site.

Particle size analysis of soils consists of separation of the soil particles into various sizes and determining the percentage of each by weight. Particle size analysis was done by hydrometer method modified by Juo., [30]. Soil samples was dispersed with 5% sodium hexametaphosphate (calgon) solution.

The mixture was stirred for 30 minutes in a mechanical shaker and transferred into a 1000ml volumetric flask and allowed to stand overnight and then made up to the mark on the volumetric flask, hydrometer was inserted, the mixture was then inverted up side down by covering the mouth of the flask; the first hydrometer and thermometer readings were taken after 40 seconds and the second hydrometer and thermometer readings taken after 2 hours.

The percentage sand, silt and clay were determined based on gravitational sedimentation as governed by stokes law. Soil textures was established by using a standard textural triangle.

2.11 Plant Height

This was done with the aid of a measuring tape. The essence of this was to reveal the rate of growth in each of the test plant as to show its susceptibility to the effects of soil pollution. This was done weekly till the end of the experimentation period.

2.12 Soil pH

pH meter was used for the measurement of pH. The meter was first calibrated with buffers;

pH was determined following the protocol outlined by Eckerts and Sims [31] the soil was air dried and sieved to remove large particles and debris. 5g of sieved soil was mixed with 5ml of distilled water and stirred very well. The mixture was allowed to stand for 30 minutes. The electrode of the pH meter was inserted into the soil water mixture and pH read off.

2.13 Electrical Conductivity

Soil sample was collected, 10g soil was weighed into 100ml polyethylene tube, 20ml of distilled H_2O was added, then tube closed with a stopper and agitated on a mechanical shaker for 15minutes'then Allowed to stand for 1hour then returned back into the shaker for 2hrs.

Centrifuges were used to decant the supernatant solution and its conductivity was then measured. Salt concentration in mg can be approximated by multiplying the conductivity reading expressed as $1X10^2$µmhos/cm, (µS/cm) a factor of 8.

2.14 Moisture Content

Soil was air dried and 5g of the air-dried soil was put in a beaker and weighed. The can was placed in a drying oven at 105°c then the can was removed and put in a desiccator to cool and weighed.

%Moisture content was calculated as;

$$\%\text{moisture content} = \frac{\text{A} - \text{B}}{\text{B} - \text{tare can}} \text{x } 100$$

Where:

A = Moist soil,
B= Dry soil
Tare can= 0

Moisture correction factor is obtained as follows

$$\text{Mcf} = \frac{100 + \text{moisture content}}{100}$$

2.15 Total Petroleum Hydrocarbons (TPH)

TPH was analyzed for the root, stem and soil Residual Total Petroleum (TPH) was extracted from the soil samples, the root and the stem were quantified using Gas Chromatography - Flame Ionization Detector (GC-FID) Agilent 7890A according to the methods of ASTDM 3921 and US EPA 8015 [32] analytical protocol (API, [33]) as reported by Chikere*et al*., [34] and in accordance with Nigerian requirements of Department of Petroleum Resources (DPR), National Oil Spill Detection Responses Agency (NOSDRA) and Federal Ministry of Environment (FME). Samples were collected in aluminum foil container. Labeled appropriately and sent to the laboratory for analysis. All samples were analyzed in duplicates while ensuring precision and reliability of results through standard quality assurance and control measures

2.16 Acidity

pH meter was used for the measurement of acidity The meter was first calibrated with buffers; acidity was determined following the protocol outlined by Eckerts and Sims [31] the soil was air dried and sieved to remove large particles and debris. 5g of sieved soil was mixed with 5ml of distilled water and stirred very well. The mixture was allowed to stand for 30 minutes. The electrode of the pH meter was inserted into the soil water mixture and acidity read off.The pH gives a measure of acidity, the lower the value of pH, the higher the acidity.The pH has a value range from 1- 14, with 7 as neutral. Values above 7 indicate increasing alkalinity, while values below 7 indicate increasing acidity.

2.17 Temperature

A dowel was used to make a hole in the top soil (0-15cm). This was followed by pushing the thermometer into the hole ensuring thet it gets to the bottom of the hole for accurate reading. The thermometer was then removed and the reading taken The reading was taken in the afternoon in the different plots.

2.18 Polycyclic Aromatic Hydrocarbons – PAHs

Chromatography Spectrometer - Flame Ionization Detector (GC-FID) Agilent 7890A was used to measure the concentration and performance of PAHs

2.19 Percentage (%) Phytoremediation Analysis

The method of Nrior and Mene [35] were modified and used in calculating the percentage of phytoremediation in the experiment. The process followed the steps stated.

Step 1: The Amount of pollutant uptake in Roots (Px) equals Final Concentration of pollutant (Last day or Week of experiment) (Fx) minus the Initial Concentration of pollutant at day or Week 1(Ix).

Px = Fx – Ix …equation 1

Where:

Px = Amount of pollutant uptake by Root or Stem
Ix = Initial Concentration of pollutant in Roots or Stem (day or week 1 which is usually zero)
Fx = Final Concentration of pollutant in Roots or Stem (last day or week of experiment)

Step 2: The percentage (%) Phytoremediation (%PR) equals Amount of pollutant uptake (Px)

divided by the Initial Concentration of pollutant in the soil at day or week 1(Initial pollutant contamination value), multiplied by 100

%PR = (Px/Ics) x 100 ….equation 2

Where;

%PR = Percentage (%) Phytoremediation
Px = Amount of pollutant uptake by Root or Stem
Ics = Initial Concentration of pollutant in the soil at day or week 1(Initial pollutant contamination value) (Nrior and Mene [35] modified),

2.20 Determination of Percentage (%) Crude Oil Reduction (% Bioremediation) in polluted soil

The method of Nrior and Inweregbu [36] was used in calculating the percentage (%) bioremediation in the experiment at day 240. The process followed the steps stated below;

Step 1: The amount of pollutant remediated equals to Initial Concentration of pollutant (Week 1) minus the Final Concentration of pollutant at the end of experiment (Last day or Week of experiment).

Bc = Ic - Fc …equation 3

Step 2: The percentage (%) Bioremediation equals Amount of pollutant divided by the Initial Concentration of pollutant (week 1), multiplied by 100.

Rc= (Bc /Ic) x 100 …equation 4

Where:

Bc = Amount of pollutant remediated
Ic = Initial Concentration of pollutant (week 1)
Fc = Final Concentration of pollutant (week8)
% Reduction OR % Bioremediation (%Rc)

2.21 Statistical Analysis

Results were subjected to statistical analysis using Analysis of Variance (Two-way ANOVA) to test whether the different nutrient amendments given to the crude oil polluted plots were statistically significant in relation to the uptake by plant roots and stem. Regression analysis of Physiochemical parameters during phytoremediation of crude oil polluted soil showing regression equation of each parameter and their R^2 values were also evaluated.

3. RESULTS AND DISCUSION

Evaluatory data from the baseline and phytoremediation analysis in the 10 Randomized Complete Block Design (RCBD) plots field studies are represented below as illustrative tables, figures, graphs and charts.

3.1 Identification of Seedlings used in Phytoremediation Studies

The seedlings used as test plants for the experiment were identified as *Cyperus esculentus* (Cyp) and *Phyllanthus amarus*(Phy) in the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt.

3.2 Molecular Identification of Microbial Isolates Used for Augmentation

The fungal isolates used as bioaugmenting organism were identified using molecular analysis technique by Polymerase Chain Reaction (PCR) and genomic sequencing; Identifier classification of the two augmenting fungi identify them as A*spergillus niger,* and *Mucor racemosus*.

The obtained 16S rRNA sequence from the isolate produced an exert match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database.

3.3 Physicochemical Parameters

Selected physicochemical parameters monitored were pH, Electrical Conductivity, Moisture Content, Plant Height, Total Petroleum Hydrocarbon (TPH) absorbed on Roots, TPH absorbed in stems, TPH Reduction in soil, Polycyclic Aromatic Hydrocarbon (PAHs).

Table 2-4 shows the mean, standard deviation and regression values, and significant differences of the selected monitored physicochemical parameters: Petroleum Hydrocarbon (TPH); Polycyclic Aromatic Hydrocarbon (PAHs), Total Nitrogen, Available Phosphorus, Potassium, Organic Carbon (OC), Plant Height, Hydrogen ion concentration (pH), Electrical Conductivity (EC), Heavy Metals -Iron (Fe), Lead (Pb), Zinc (Zn) monitored during the phytoremediation study.

There were no significant differene ($p<0.05$) in Hydrogen ion concentration (pH) in the various treatment plots (Table 4) but there were variation with highest value observed in Control - Unpolluted Soil (US) + *Cyperus esculentus* (7.57±0.26) and least in Polluted soil + Spent Mushroom Substrate + *Phyllanthus amarus*(7.41±0.30) (Table 3-4). The result from this study indicate that under normal pH, oxygen and sufficient nutrients, phytoremediation of crude oil contaminated soil increases in each plot compared with the controls. The seedlings used as test plants for the experiment were identified as *Cyperus esculentus* Lin (Yellow Nut Sedge) And *Phyllanthus amurus* (BhumiAmla).

Electrical Conductivity (EC) (µS/cm) showed highest value in Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ *Phyllanthus amarus*(340.29±40.32) with least value in Polluted soil + *Aspergillus niger*+ *Phyllanthus amarus*(233.86±38.61). Moisture Content (%) showed two equal highest value; Control - unpolluted soil (US) + *Phyllanthus amarus*= Polluted soil + *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanthus amarus*(1.25±0.32) with least value in Polluted soil + *Aspergillus niger*+ *Phyllanthus amarus*(0.90±0.28) (Table 2).

The values for Total Nitrogen (%) shows Control Unpolluted Soil (US) + *Cyperus esculentus* has the highest (0.23±0.01) and Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanthus amarus*(0.18±0.02) with the lowest while Available Phosphorus (%) value showed that Polluted soil + Spent Mushroom Substrate + *Phyllanthus amarus* had the highest (9.77±0.85) and Control -Unpolluted Soil (US) + *Cyperus esculentus* (5.41±0.48) the lowest. Potassium (%) had highest value in Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanthus amarus*(0.36±0.07) with least value in (Polluted Soil + *Cyperus esculentus* (0.28±0.04). The value for TOC shows two treatment plots having equal highest value; Polluted soil + Spent Mushroom Substrate + *Phyllanthus amarus*= Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ *Cyperus esculentus* (3.02±0.13) while Control- unpolluted soil (US) + *Phyllanthus amarus* (1.99±0.21) has the lowest percentage (Table 2-3). Similar trend was observed by Ogbonna *et al* [37] during bioremediation of Crude oil polutted soil using fish waste and goat manure as bioorganics and bacteria as bioaugmenters.

Evaluation of Heavy metals reduction in soil in this study showed significant difference ($p<0.05$) between control plots (unpolluted soil) and the polluted soil. This could be attributed to content of the crude oil having some amount of heavy metals as contaminants; moreso the action of crude oil in soil chemical properties and that of amendment nutrient could result to the elevated value of heavy metals found in the crude oil polluted soil/plots.The value of Iron (mg/kg) showed highest concentration in Polluted soil + *Mucor racemosus*+ *Cyperus esculentus*(53.88±11.38) and least in Control – Unpolluted soil + *Phyllanthus amarus* (0.01±0.00) = Unpolluted soil + *Cyperus esculentus*(0.01±0.00)while Zinc had highest concentration in Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ *Phyllanthus amarus*(4.78±2.64) with least value recorded in Control - Unpolluted soil + *Phyllanthus amarus* (1.00±0.00) = Unpolluted soil + *Cyperus esculentus*(1.00±0.00). Lead (mg/kg) result showed low values compared to other heavy metals with the consortium of two or more amendment items having same higher valves (0.06±0.05) in four treatment plots: Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ *Phyllanthus amarus*=Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ *Cyperus esculentus*= Polluted soil + *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanth usamarus*= ; Polluted soil + *Aspergillus niger*+ *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanthus amarus.* The least Lead values were found in Control - unpolluted soil (US) + *Phyllanthus amarus*= unpolluted soil + *Cyperus esculentus*(0.01±0.00) (Table 2-3). There was significant difference ($p<0.05$) in Lead concentration between Control – Unpolluted plot and Polluted treatment plots; which could be attributed to Lead (Pb) residual contaminant in the Crude oil used in contaminating/ polluting the experimental plots. Similar observations were made by Ule *et al* [38].

Comparative average reduction in Total Petroleum Hydrocarbon (TPH-mg/kg) in soil during the phytoremediation evaluation. The plots show that: Polluted soil + *Aspergillus* niger+ *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanthus amarus*(1155.60±2430.40; 99.43%) >Polluted soil + *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanthus amarus*(1159.86±2427.99, 99.23%)>Polluted soil

+*Aspergillus* niger + *Mucor racemosus*+ *Phyllanthus amarus*(1166.08; 99.06%) > Polluted soil + *Mucor racemosus*+ *Cyperus esculentus*(1178.78±2417.45; 99.01%) > Polluted soil + Spent Mushroom Substrate + *Phyllanthus amarus*(1171.98±2421.19; 98.91%)> Polluted soil + *Aspergillus* niger +*Mucor racemosus*+ *Cyperus esculentus* (1178.16±2417.76, 98.86%) >Control 3 – Polluted soil + *Cyperus esculentus* (no amendment) (1216.22±2396.45, 97.78%).

Experimental transplants had an initial height of 16.7 cm. In the first 60 days of growth, plant showed reduced growth whereas; plants in uncontaminated soil were in good condition. *Phyllanthusamarus*indicated a high potential of adaptation in the contaminated soil as shown by the growth during 120 and 210 days regardless of the bioorganics 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 *Phyllanthus amarus* were 52.47±27.50, 55.40±29.98, 55.83±35.31, 58.15±34.04 and 57.70±33.26 cm respectively in P4, P6, P8, P9 and P10 in comparison to 36.40±13.03 cm in (uncontaminated plots) during 210 days; while *Cyperus esculentus* were 39.77±16.22, 42.67±22.07, and 51.37±31.23 cm respectively in P3, P5 and P7 in comparison to 41.13±18.20 cm in (uncontaminated plots). There was no significant difference of plant height between the contaminated and uncontaminated soil (Table 2-4).

Root structure is considered just as important as root biomass concerning degradation process [39]. Notably, 75% to 85% of the root surface in contaminated soil belonged to fine roots compared to 91% in uncontaminated soil. Generally, the roots growing in uncontaminated soil were longer, and covered more surface area than those growing in contaminated soil.

Total Petroleum Hydrocarbon (TPH) uptake in plant Roots within 240 days period across the plots were:PS+Cyp (210.4 mg/kg, 17.36%) >PS+AN+Phy (200.1 mg/kg, 16.51%) >PS+AN+MR+SMS+Phy (150.7 mg/kg, 12.44%) >PS+MR+Cyp (125.6 mg/kg, 10.36%) >PS+MR+SMS+Phy (121.6 mg/kg, 10.03%) >PS+AN+MR+Cyp (121.0 mg/kg, 9.98%) >PS+SMS+Phy (120.1 mg/kg, 9.91%) >PS+AN+MR+Phy (115.0 mg/kg, 9.49%); least in control - uncontaminated soil US+Cyp (24.2mg/kg, 2.0%) >US+Phy (23.19 mg/kg, 1.91%) (Table 5 and Fig. 1).

TPH uptake in plant stem within 240 days period across the plots were: PS+Cyp (210.0mg/kg, 13.86%) >PS+MR+Cyp (201.0 mg/kg, 13.26%) >PS+AN+MR+Cyp (192.4 mg/kg, 12.70%) >PS+SMS+Phy (191.1 mg/kg, 12.61%) >PS+AN+MR+Phy (171.3 mg/kg, 11.30%) >PS+AN+Phy (161.7 mg/kg, 10.67%) >PS+MR+SMS+Phy (150.1 mg/kg, 9.90%) and lower values in Uncontaminated Control plots US+Cyp (20.01 mg/kg, 1.32%) >US+Phy (19.8mg/kg, 1.31%) (Table 5 and Fig. 1)

The highest uptake was found with *Cyperus esculentus* both in roots and stem analysis of the test plants; this could be attributed to its root system moreover the mechanism of its xylem vessels. Similar observations were seen in experiments done by Lopez-Martinez *et al*. [40], who also found significant reduction of TPH by *Cyperuslaxus* Lam. in 24 months when plants were cultivated on hydrocarbon-contaminated soil and spiked perlite.

Basumatary *et al* [41] observed Total Oil and Grease TOG (Total Hydrocarbon Content THC) decreased up to 50.01% in TI(Treatment 1) 46.13% in TII, 42.59% in TIII, 38.79% in TIV and 32.65% in TV during 180 days. Whereas, the average TOG decrease in unplanted pots were 4.4%, 5.6%, 6.6%, 7.6% and 9.6% respectively in TA, TB, TC, TD and TE. However, TOG degradation was significantly more in vegetated pots in comparison to unvegetated pots (P=0.05).

From the initial TPH contamination value of 5503.00mg/kg , Total Petroleum Hydrocarbon Reduction and % Hydrocarbon Reductionin soil at 240 days in the different treatment plots in a decreasing order were as follows: PS+AN+MR+SMS+Phy(5470.9 mg/kg; 99.43%) >PS+MR+SMS+Phy(5460.60 mg/kg; 99.23%) >PS+AN+MR+Phy(5451.30 mg/kg; 99.06%) >PS+MR+Cyp(5448.30 mg/kg; 99.01%) >PS+AN+MR+Cyp (5440.00 mg/kg; 98.86%) >PS+AN+Phy(5422.905 mg/kg; 98.54%) >PS+Cyp (5380.90 mg/kg; 97.78%).

The differences in Total Petroleum Hydrocarbon (TPH) decrease in crude oil polluted and unpolluted soil/ plot treatments were significant (Table 4-5).

Evaluation of Ploycyclic Aromatic Hydrocarbons (PAHs) from initial contamination value 47.5mg/kg , reduction in PAHs (amount remediated) and % Bioremediation in soil at 240

days in the different treatment plots in a decreasing order were: PS+AN+MR+SMS+Phy (31.3 mg/kg; 65.89%) >PS+MR+SMS+Phy (33.1 mg/kg; 65.47%) >PS+AN+MR+Phy (29.5 mg/kg; 62.11%) >PS+AN+MR+Cyp (27.5 mg/kg; 57.89%) >PS+MR+Cyp (26.40 mg/kg; 55.58%) = PS+SMS+Phy (26.40 mg/kg; 55.58%) >PS+AN+Phy (25.3 mg/kg, 53.26%) >PS+Cyp (no amendment) (23.40 mg/kg; 49.26%) (Table 5).

However, the presence of plants resulted in significant decrease in TPH and PAHs concentration at day 240. Merkl *et al*. [42] showed enhanced degradation of crude oil under the influence of a tropical grass after only a few months. Muratova et al. [43] showed total petroleum hydrocarbon (TPH) reduction up to 52% during 3 years of rye cultivation. Diab [9] recorded 30%, 16.8% and 13.8% reduction of TPH in rhizosphere soil of broad bean, corn and wheat respectively.

In the present study, both test plants decreased significant amount of crude oil as revealed in TPH uptake in their roots and Stem. Mean amount and percentage (%) Total Petroleum Hydrocarbon (TPH) uptake by *Cyperus esculentus* Roots and Stem were; 152.33±50.34 mg/kg, 12.57±4.16% and 201.13±8.80 mg/kg, 13.27±0.58% respectively; while that of *Phyllanthus amarus* Roots and Stem were 141.50±35.62 mg/kg, 11.68±2.94% and 174.44±19.98 mg/kg, 11.51±1.32% respectively (Table 6, Fig. 2). Similar trend was observed in the control plots where TPH uptake by *Cyperus esculentus* Roots and Stem were; 24.2 mg/kg, 2.00% and 20.01 mg/kg, 1.32% respectively while in control plot of *Phyllanthus amarus*TPH uptake by Roots and Stem were 23.19 mg/kg, 1.91% and 19.80 mg/kg, 1.31% respectively (Table 6).

Moreso, it was observed that plots planted with *Cyperus esculentus* (TPH 5492.75±76.36mg/kg) showed higher reduction of TPH from soil than those planted with *Phyllanthus amarus*(TPH 5449.72±18.27mg/kg) (Table 6 and Fig. 3).

In the study reported here, the maximum degradation was found during 240 days. This might be due to increased interaction between roots and rhizosphere microorganisms as microbial population increase utilizing both hydrocarbon and bio-organics (SMS) over 240days time compared to 60 and 120 days. Basumatary et al [41] also found similar result though theirs was at day 120.Kulakow *et al* [44]; Yateem *et al*. [45] also found enhanced degradation of petroleum hydrocarbons (PHCs) by using the plant-microbe interaction.

The study also revealed that TPH absorbed/stored in plant stem are higher than that of plant roots. From initial contamination value of 5503 mg/kg in soil, plant stem absorbed/stored 905.6 mg/kg, 16.46% while plant roots absorbed/stored 711.7 mg/kg, 12.93% (Fig. 4).

Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) reduction in soil during the phytoremediation process showed distinctive significance in relation to initial value, amendment and test plant uptake potential. Comparative evaluation revealed higher reduction in PAHs in soil (plot) planted with *Phyllanthus amarus.* Highest PAHs removal from soil was seen in Polluted soil + *Aspergillus* niger + *Mucor racemosus*+ Spent Mushroom Substrate + *Phyllanthus amarus*(31.3 mg/kg; 65.89%) while least was recorded in Polluted soil + *Cyperus esculentus* (no amendment) (23.4 mg/kg, 49.26%). It was observed in this study that PAHs degradation/reduction in plots planted with *Phyllanthus amarus* (PAHs 28.72±2.74 mg/kg; 60.46±5.77%) was higher than plots planted with *Cyperus esculentus* (PAHs 25.77±2.12 mg/kg, 54.24±4.47%)(Table 6).

Apart from biodegradation, a potential weathering process Petroleum Hydrocarbon in soil is volatilization of low molecular weight, aliphatic, and aromatic compounds [46]. In the study, there was PAHs degradation in both amended and unamended plots but amended (amended with Bio-organic SMS and augumentting fungi – *Aspergillus niger* and *Mucor racemosus* plots showed significantly more PAHs degradation.

Amount of TPH degraded in soil far exceeds PAHs values. Aromatic and polar compounds are less biodegradable than aliphatic [47] and asphaltene group is the least biodegradable of all [48]. However, degradation study of separate hydrocarbon components (saturates, aromatics, asphalthins, and resins) will require long term monitoring of soil and plant development.

Table 1. Experimental set-up for phytoremediation of crude oil polluted bioaugmented soil

S/N	Plot Code	Volume of Soil 100x50x30cm (150,000cm^3)	Crude Oil (2500ml) (2122.25g)	Test Plants Cyperusesculentus (Cyp)	Phyllanthusamarus (Phy)	Augmenting Microbes Aspergillusniger (Asp) broth(ml)	Mucorracemosus (Muc) Broth(ml)	Bioorganics Spent Mushroom Substrate (SMS) (g)
P1	US+ Phy	+	-	-	+	-	-	-
P2	US+Cyp	+	-	+	-	-	-	-
P3	PS+Cyp	+	+	+	-	-	-	-
P4	PS+AN+Phy	+	+	-	+	750ml	-	--
P5	PS+MR+Cyp	+	+	+	-	-	750ml	-
P6	PS+SMS+Phy	+	+	-	+	-	-	+
P7	PS+AN+MR+Cyp	+	+	+	-	375ml	375ml	-
P8	PS+AN+MR+Phy	+	+	-	+	375ml	375ml	-
P9	PS+MR+SMS+Phy	+	+	-	+	-	750ml	+
P10	PS+AN+MR+SMS+Phy	+	+	-	+	375ml	375ml	+

Key: US = Uncontaminated soil, PS = Crude Oil Polluted soil, Phy = Phyllanthus amarus, Cyp = Cyperus esculentus, AN = Aspergillus niger, MR = Mucor racemosus

Table 2. Mean and standard deviation of physicochemical parameters during phytoremediation of crude oil polluted soil

Plot	Treatments	Physicochemical parameters							
		pH	Electrical Conductivity (EC)(S/cm)	Plant Height (cm)	Total Nitrogen (%)	Available Phosphorus (%)	Potassium (%)	% Organic Carbon	Moisture Content (%)
P1	US+ Phy	7.49±0.31^a	255.29±6.32^a	36.40±13.03^a	0.23±0.00^a	7.74±0.12^c	0.31±0.00^a	1.99±0.21^a	1.25±0.32^b
P2	US+Cyp	7.57±0.26^a	281.57±24.58^a	41.13±18.20^a	0.23±0.01^a	5.41±0.48^b	0.31±0.00^a	2.31±0.14^a	0.99±0.22^b
P3	PS+Cyp	7.55±0.30^a	276.71±44.11^a	39.77±16.22^a	0.22±0.01^a	6.85±1.49^a	0.28±0.04^b	2.41±0.28^{ab}	0.99±0.24^b
P4	PS+AN+Phy	7.54±0.22^a	233.86±38.61^a	52.47±27.50^a	0.22±0.04^a	7.04±1.59^a	0.29±0.04^a	2.09±0.43^a	0.9±0.289^b
P5	PS+MR+Cyp	7.46±0.32^a	240.71±38.20^a	42.67±22.07^a	0.22±0.05^a	7.26±1.42^a	0.30±0.04^a	2.27±0.56^a	0.99±0.26^b
P6	PS+SMS+Phy	7.41±0.30^a	265.43±23.09^a	55.40±29.98^a	0.22±0.05^a	9.77±0.85^c	0.32±0.05^a	3.02±0.13^c	1.00±0.23^b
P7	PS+AN+MR+Cyp	7.48±0.21^a	293.57±59.67^a	51.37±31.23^a	0.22±0.04^a	6.76±2.26^a	0.32±0.05^a	3.02±0.11^c	1.04±0.22^b
P8	PS+AN+MR+Phy	7.55±0.45^a	340.29±40.32^b	55.83±35.31^a	0.22±0.02^a	6.56±1.83^a	0.34±0.05^a	2.89±0.05^c	0.98±0.19^b
P9	PS+MR+SMS+Phy	7.49±0.29^a	272.29±29.15^a	58.15±34.04^a	0.21±0.01^b	6.89±1.56^a	0.33±0.07^a	2.74±0.31^b	1.25±0.32^b
P10	PS+AN+MR+SMS+Phy	7.55±0.30^a	254.57±28.02^a	57.70±33.26^a	0.18±0.02^b	7.03±1.50^a	0.36±0.07^a	2.10±0.57^a	0.99±0.22^b
Plot	Treatments	TPH absorbed in Plant Roots	TPH absorbed in Plant Stem	TPH in Soil (mg/kg)	PAH in Soil (mg/kg)	Iron (Fe) (mg/kg)	Lead (Pb) (mg/kg)	Zinc (Zn) (mg/kg)	

Plot	Treatments	Physicochemical parameters							
		pH	Electrical Conductivity (EC)(S/cm)	Plant Height (cm)	Total Nitrogen (%)	Available Phosphorus (%)	Potassium (%)	% Organic Carbon	Moisture Content (%)
		(mg/kg)	(mg/kg)						
P1	US+ Phy	16.43±9.43^{a}	14.12±8.04^{a}	27.46±22.68^{a}	8.47±9.93^{a}	0.01±0.00^{c}	0.01±0.00^{c}	1.00±0.00^{c}	
P2	US+Cyp	16.98±9.76^{a}	14.49±8.25^{a}	28.206±22.53^{a}	3.98±0.75^{a}	0.01±0.00^{c}	0.01±0.00^{c}	1.00±0.00^{c}	
P3	PS+Cyp	145.62±83.37^{b}	156.62±88.22^{b}	1216.22±2396.45^{a}	37.18±9.74^{c}	37.31±19.05^{b}	0.05±0.05^{a}	4.28±2.83^{b}	
P4	PS+AN+Phy	136.56±81.12^{b}	99.28±60.71^{b}	1184.86±2413.99^{a}	32.98±9.36^{b}	44.88±7.88^{b}	0.05±0.05^{ab}	4.34±2.62^{b}	
P5	PS+MR+Cyp	78.20±48.16^{b}	133.40±80.31^{b}	1178.78±2417.45^{a}	34.22±10.37^{b}	53.88±11.38^{b}	0.05±0.05^{ab}	4.51±2.54^{b}	
P6	PS+SMS+Phy	61.54±47.58^{a}	88.84±71.86^{ab}	1171.98±2421.19^{a}	34.96±10.59^{b}	42.21±8.49^{b}	0.05±0.05^{a}	4.63±2.62^{b}	
P7	PS+AN+MR+Cyp	71.10±49.00^{b}	90.58±71.40^{ab}	1178.16±2417.76^{a}	33.30±10.77^{b}	45.89±9.01^{b}	0.06±0.05^{b}	4.67±2.65^{b}	
P8	PS+AN+MR+Phy	62.10±47.01^{a}	79.08±65.47^{a}	1166.08±2424.51^{a}	32.32±10.84^{b}	38.34±8.49^{b}	0.06±0.05^{b}	4.78±2.64^{b}	
P9	PS+MR+SMS+Phy	66.72±48.34a^{b}	60.94±56.52^{a}	1159.86±2427.99^{a}	29.60±11.99^{b}	44.95±14.76^{b}	0.06±0.04^{b}	3.72±2.69^{b}	
P10	PS+AN+MR+SMS+Phy	79.78±58.88a^{b}	141.22±80.36^{b}	1155.6±2430.40^{a}	30.36±12.01^{b}	32.29±12.10^{b}	0.06±0.04^{b}	3.27±2.85^{b}	

***means with the same superscript along the columns are not significantly different (p>0.05).*

Key: US = Uncontaminated soil, PS = Polluted soil, Phy = Phyllanthus amarus, Cyp = Cyperus esculentus, AN = Aspergillus niger, MR = Mucor racemosus, SMS = Spent Mushroom Substrate

Table 3. Regression analysis of Physiochemical parameters during Phytoremediation of crude oil polluted soil

Plot	Treatments	pH		Electrical Conductivity (EC)(S/cm)		Moisture Content (%)		Total Nitrogen (%)	
		Regression equation (Y)	R²	Regression equation (Y)	R²	Regression equation (Y)	R²	Regression equation (Y)	R²
P1	US+ Phy	0.1168x + 7.0257	0.6428	-2.5x + 265.29	0.7309	0.1304x + 0.73	0.7663	0.0002x + 0.2277	0.75
P2	US+Cyp	0.1082x + 7.1343	0.8057	9.7857x + 242.43	0.7399	0.0771x + 0.6857	0,5645	0.0019x + 0.2177	0.1374
P3	PS+Cyp	0.1175x + 7.0771	0.7353	5x + 256.71	0.06	0.0836x + 0.6557	0.5499	0.0058x + 0.1969	0.6884
P4	PS+AN+Phy	0.0893x + 7.1829	0.7352	-12.857x + 285.29	0.5176	0.0918x + 0.6186	0.5075	0.015x + 0.1584	0.5545
P5	PS+MR+Cyp	0.1146x + 7.0043	0.6016	-14.607x + 299.14	0.6825	0.09x + 0.6286	0.5506	0.0166x + 0.15	0.5873
P6	PS+SMS+Phy	0.1064x + 6.9814	0.5814	-3.75x + 280.43	0.1231	0.0814x + 0.6743	0.6107	0.0175x + 0.1516	0.6125
P7	PS+AN+MR+Cyp	0.0814x + 7.1514	0.7357	56.329x	-7.446	0.0761x + 0.7357	0.5534	0.0117x + 0.1697	0.4813
P8	PS+AN+MR+Phy	0.1725x + 6.8643	0.6938	15.857x + 276.86	0.7219	0.0404x + 0.8171	0.2165	0.0086x + 0.1903	0.8524
P9	PS+MR+SMS+Phy	0.11x + 7.0529	0.6618	-7.2143x + 301.14	0.2858	0.1304x + 0.73	0.7663	0.0016x + 0.2034	0.103
P10	PS+AN+MR+SMS+Phy	0.1225x + 7.0629	0.7632	-9.1071x + 291	0.4931	0.0771x + 0.6857	0.5645	0.0034x + 0.1651	0.1048
Plot	**Treatments**	**Available Phosphorus (%)**		**Potassium (%)**		**% Organic Carbon**		**Plant Height(cm)**	
		Regression equation (Y)	R²	Regression equation (Y)	R²	Regression equation (Y)	R²	Regression equation (Y)	R²
P1	US+ Phy	0.05x + 7.5429	0.8627	0.311	0.0	-0.0602x + 2.232	0.3748	8.2x - 1.6	0.9487
P2	US+Cyp	0.0432x + 5.2357	0.0376	0.311	0.0	-0.0039x + 2.3283	0.0036	10.371x - 6.2286	0.9694
P3	PS+Cyp	-0.4496x + 8.6514	0.4256	0.0085x + 0.242	0.2177	-0.043x + 2.577	0.1136	9.6x - 4.3143	0.966

P4	PS+AN+Phy	-0.3543x + 8.46	0.2305	0.0115x + 0.246	0.4014	-0.1606x + 2.7304	0.6481	14.682x - 13.757	0.9829
P5	PS+MR+Cyp	-0.2964x + 8.4443	0.2028	0.0143x + 0.2437	0.4918	-0.0759x + 2.5754	0.0866	11.546x - 9.6143	0.9341
P6	PS+SMS+Phy	-0.0286x + 9.8886	0.0053	0.0201x + 0.2364	0.641	0.0413x + 2.8516	0.4773	15.829x - 15.829	0.9847
P7	PS+AN+MR+Cyp	-0.1014x + 7.17	0.0094	0.0198x + 0.2434	0.7008	0.0223x + 2.9323	0.1925	15.432x - 17.7	0.9342
P8	PS+AN+MR+Phy	-0.2964x + 8.4443	0.2028	0.022x + 0.2506	0.8108	-0.0005x + 2.8944	0.0004	17.343x - 21.514	0.9455
P9	PS+MR+SMS+Phy	-0.475x + 8.7943	0.4345	0.0224x + 0.2443	0.5029	-0.0181x + 2.8146	0.0164	17.275x - 19.257	0.9614
P10	PS+AN+MR+SMS+Phy	-0.5732x + 9.3257	0.6845	0.0259x + 0.2529	0.5583	-0.1997x + 2.8941	0.563	16.986x - 18.486	0.9636
Plot	**Treatments**	**TPH in Plant Roots (mg/kg)**		**TPH in Plant Stem(mg/kg)**		**TPH in Soil (mg/kg)**		**PAH in Soil (mg/kg)**	
		Regression equation (Y)	**R²**	**Regression equation (Y)**	**R²**	**Regression equation (Y)**	**R²**	**Regression equation (Y)**	**R²**
P1	US+ Phy	5.039x + 1.309	0.7143	4.2x + 1.522	0.6829	-12.189x + 64.027	7221	-0.552x + 5.41	0.944
P2	US+Cyp	5.259x + 1.207	0.7257	4.313x + 1.555	0.6833	-12.387x + 65.367	0.7554	-0.468x + 5.38	0.9826
P3	PS+Cyp	44.2x + 13.02	0.7026	44x + 24.62	0.6219	-889.3x + 4924.7	0.5946	-6.09x + 55.45	0.9772
P4	PS+AN+Phy	45.82x - 0.9	0.7977	35.49x - 7.19	0.8542	-975.4x + 4869.4	0.5951	-5.66x + 49.96	0.914
P5	PS+MR+Cyp	28.55x - 7.45	0.8786	46.41x - 5.83	0.8349	-1011.2x + 4916	0.614	-6.55x + 53.87	0.9973
P6	PS+SMS+Phy	30.08x - 28.7	0.9992	44.61x - 44.99	0.9635	-1005x + 4829.4	0.5889	-6.68x + 55	0.9941
P7	PS+AN+MR+Cyp	30.02x - 18.96	0.9385	44.16x - 41.9	0.9563	-1000.1x + 4876.5	0.6014	-6.8x + 53.7	0.9969
P8	PS+AN+MR+Phy	29.42x - 26.16	0.979	40.74x - 43.14	0.968	-1034.2x + 4858	0.6025	-6.65x + 52.27	0.9408
P9	PS+MR+SMS+Phy	30.38x - 24.42	0.9875	34.09x - 41.33	0.9096	-1048.8x + 4839.6	0.6	-7.43x + 51.89	0.9597
P10	PS+AN+MR+SMS+Phy	36.73x - 30.41	0.9728	42x + 15.22	0.6829	-1072.7x + 4868.7	0.6122	-7.52x + 52.92	0.9807
Plot	**Treatments**	**Iron (mg/kg)**		**Lead (mg/kg)**		**Zinc (mg/kg)**			
		Regression equation (Y)	**R²**	**Regression equation (Y)**	**R²**	**Regression equation (Y)**	**R²**		
P1	US+ Phy	2E-18x + 0.01	-1E-15	2E-18x + 0.01	-1E-15	1.0	#N/A		
P2	US+Cyp	2E-18x + 0.01	-1E-15	2E-18x + 0.01	-1E-15	1.0	#N/A		
P3	PS+Cyp	-9.2911x + 69.827	0.833	-0.0022x + 0.0589	0.0078	-1.4278x + 9.2735	0.8887		
P4	PS+AN+Phy	-4.032x + 58.989	0.9166	0.0008x + 0.0513	0.001	-1.3379x + 9.0223	0.9102		
P5	PS+MR+Cyp	2.6629x + 44.563	0.1915	-0.0059x + 0.0728	0.0589	-1.2696x + 8.955	0.8758		
P6	PS+SMS+Phy	-4.5177x + 58.019	0.9913	-0.0043x + 0.0623	0.0314	-1.3052x + 9.2007	0.8675		
P7	PS+AN+MR+Cyp	-1.2554x + 50.284	0.068	-0.0157x + 0.1167	0.3407	-1.313x + 9.2644	0.8568		
P8	PS+AN+MR+Phy	-3.6163x + 50.992	0.6346	-0.0157x + 0.1167	0.3407	-1.2443x + 9.13	0.7749		
P9	PS+MR+SMS+Phy	1.7889x + 38.687	0.0514	-0.0237x + 0.1467	0.9958	-1.6614x + 10.366	0.9516		
P10	PS+AN+MR+SMS+Phy	-6.1451x + 53.798	0.9022	-0.0236x + 0.1443	0.9961	-1.7565x + 10.294	0.9523		

Key: US = Uncontaminated soil, PS = Polluted soil, Phy = Phyllanthus amarus, Cyp = Cyperus esculentus, AN = Aspergillu sniger, MR = Mucor racemosus, SMS = Spent Mushroom Substrate

Table 4. Analysis of Variance (ANOVA) for parameters monitored during Phytoremediation

Parameter	Source of Variation	SS	df	MS	F	P-value	F crit
Treatment*pH	Between Groups	0.166663	9	0.018518	0.201887	0.993053	2.040098
	Within Groups	5.503514	60	0.091725			
Treatments*Electrical conductivity (µs/cm)	Between Groups	58092.29	9	6454.698	5.006174	4.96E-05	2.040098
In soil	Within Groups	77360.86	60	1289.348			
Treatments*Moisture content (%)	Between Groups	0.74548	9	0.082831	1.283587	0.264676	2.040098
in soil	Within Groups	3.871857	60	0.064531			
Treatments*Total nitrogen (%)	Between Groups	0.012528	9	0.001392	1.524148	0.160292	2.040098
in soil	Within Groups	0.054797	60	0.000913			
Treatments*Available phosphorus (%)	Between Groups	76.72396	9	8.524884	4.091485	0.000391	2.040098
in soil	Within Groups	125.014	60	2.083567			
Treatments*Potassium (%) in soil	Between Groups	0.03473	9	0.003859	1.633464	0.126255	2.040098
	Within Groups	0.141743	60	0.002362			
Treatments*Organic carbon (%) in soil	Between Groups	10.06262	9	1.118069	10.24213	2.28E-09	2.040098
	Within Groups	6.549823	60	0.109164			
Treatments*Plant height (cm)	Between Groups	3145.924	9	349.5471	0.451365	0.90089	2.040098
	Within Groups	46465.32	60	774.422			
Treatments*TPH (mg/kg) uptake	Between Groups	80078.08	9	8897.564	3.098773	0.006446	2.124029
in plant roots	Within Groups	114852.7	40	2871.318			
Treatments*TPH (mg/kg) uptake	Between Groups	107047.7	9	11894.18	2.8151	0.011636	2.124029
in plant stem	Within Groups	169005.5	40	4225.138			
Treatments*TPH (mg/kg) Reduction	Between Groups	28075215	9	3119468	0.926977	0.512526	2.124029
in soil	Within Groups	1.35E+08	40	3365206			
Treatments*PAHs (mg/kg) Reduction	Between Groups	7053.518	9	783.7242	8.471887	6.02E-07	2.124029
in soil	Within Groups	3700.353	40	92.50882			
Treatments*Iron (mg/kg) variation	Between Groups	19113.19	9	2123.688	18.56413	4.32E-13	2.073351
in soil	Within Groups	5719.871	50	114.3974			
Treatments*Lead (mg/kg) variation	Between Groups	0.022552	9	0.002506	1.418506	0.205688	2.073351
in soil	Within Groups	0.088324	50	0.001766			
Treatments*Zinc (mg/kg) variation	Between Groups	115.5984	9	12.84426	2.198441	0.037751	2.073351
in soil	Within Groups	292.1221	50	5.842441			

Table 5. Comparative Total Petroleum Hydrocarbon (TPH) (mg/kg) uptake by plants roots and stem during Phytoremediation of crude oil polluted soils

Plots	Treatments	TPH (mg/kg) uptake In plant roots	% phyto-remediation (in roots)	TPH (mg/kg) uptake In plant stem	% phyto-remediation (in stem)	TPH (mg/kg) Reduction in soil	% TPH Reduction	PAHs (mg/kg) Reduction in soil	% PAHs Reduction
P1	US+ Phy	23.19	1.91	19.8	1.31	54.4	0.12	2.38	1.06
P2	US+Cyp	24.2	2.00	20.01	1.32	54.3	0.12	1.99	0.88
P3	PS+Cyp	210.4	17.36	210	13.86	5380.9	12.33	23.4	10.39
P4	PS+AN+Phy	200.1	16.51	161.7	10.67	5422.9	12.43	25.3	11.23
P5	PS+MR+Cyp	125.6	10.36	201	13.26	5448.3	12.49	26.4	11.72
P6	PS+SMS+Phy	120.1	9.91	191.1	12.61	5442.9	12.48	26.4	11.72
P7	PS+AN+MR+Cyp	121	9.98	192.4	12.70	5440	12.47	27.5	12.21
P8	PS+AN+MR+Phy	115	9.49	171.3	11.30	5451.3	12.50	29.5	13.10
P9	PS+MR+SMS+Phy	121.6	10.03	150.1	9.90	5460.6	12.52	31.1	13.81
P10	PS+AN+MR+SMS+Phy	150.7	12.44	198	13.07	5470.9	12.54	31.3	13.89

Key: US = Uncontaminated soil, PS = Polluted soil, Phy = Phyllanthus amarus, Cyp = Cyperus esculentus, AN = Aspergillus niger, MR = Mucorr acemosus, SMS = Spent Mushroom Substrate

Table 6. Summary of Phytoremediation (Uptake by Plant roots and Stem) and Reduction of Hydrocarbon in soil

Test plants	TPH uptake by plants roots	% Phyto-remediation	TPH uptake by plants stem	% Phyto-remediation	TPH (mg/kg) Reduction in soil	% Reduction	PAHs (mg/kg) Reduction in soil	% Reduction
Cyperusesculentus	152.33±50.34	12.57±4.16	201.13±8.80	13.27±0.58	5492.73±76.36	98.55±0.67	25.77±2.12	54.24±4.47
Phyllanthusamarus	141.50±35.62	11.68±2.94	174.44±19.98	11.51±1.32	5449.72±18.27	99.03±0.34	28.72±2.74	60.46±5.77

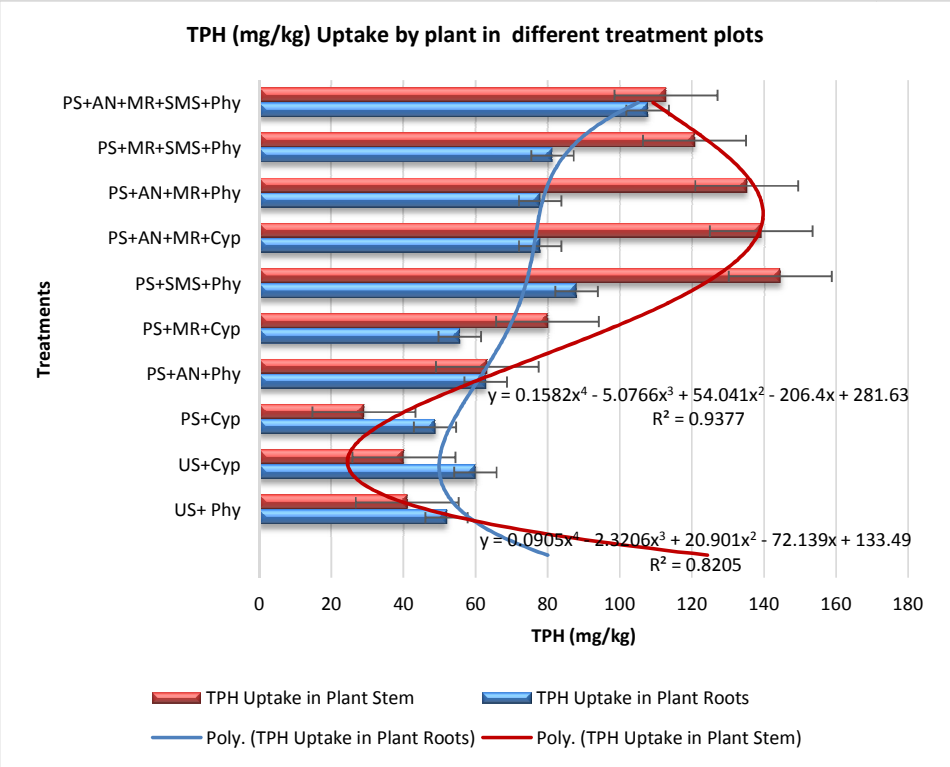

Fig. 1. Comparative Total Petroleum Hydrocarbon (TPH) (mg/kg) uptake in Plant Stem and Roots during phytoremediation of crude oil polluted soils in the different treatment pots

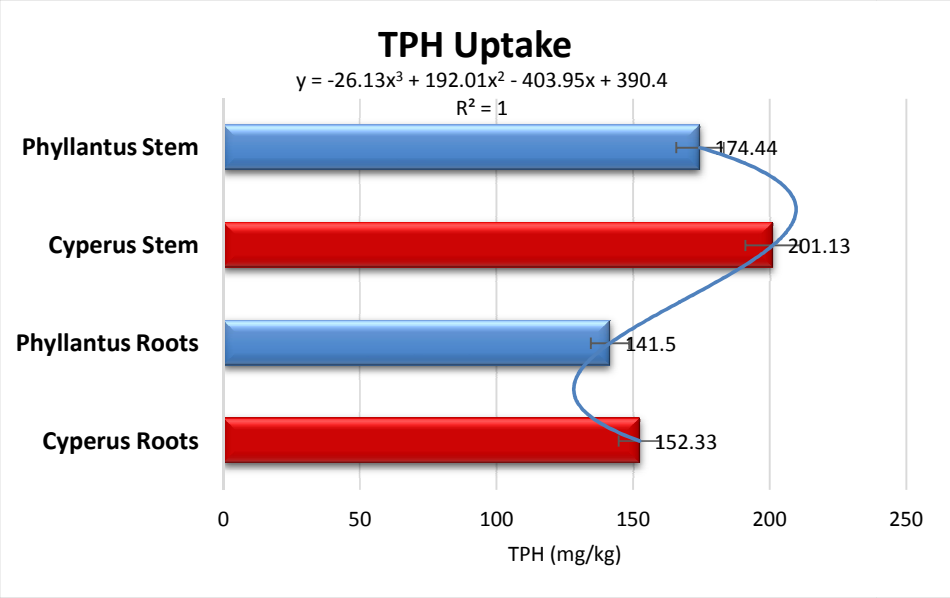

Fig. 2. Total Petroleum Hydrocarbon (TPH)(mg/kg) uptake by roots and stem of Test plants (*Phyllanthus amarus*and *Cyperus esculentus*) during phytoremediation in crude oil polluted soil

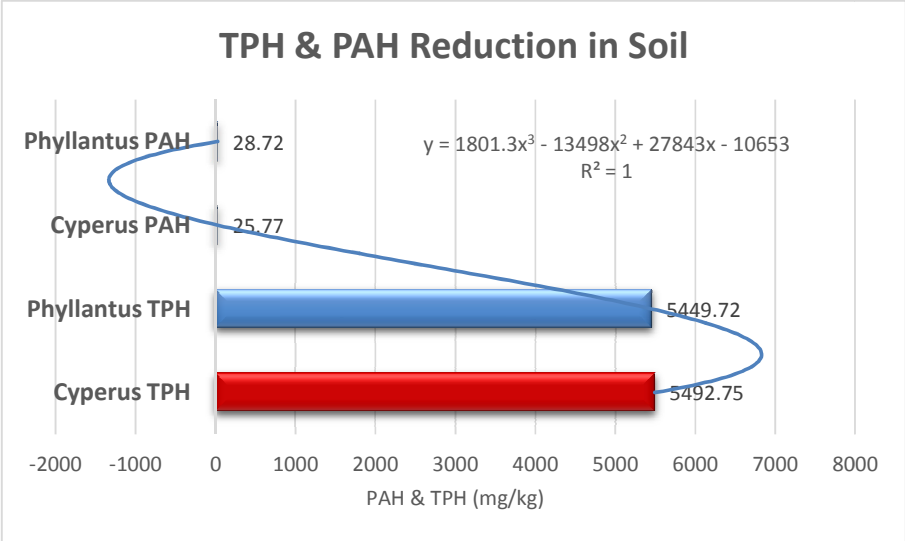

Fig. 3. ComparativeTotal Petroleum Hydrocarbon (TPH) (mg/kg) uptake in Plant Roots and stem during phytoremediation of crude oil polluted soils

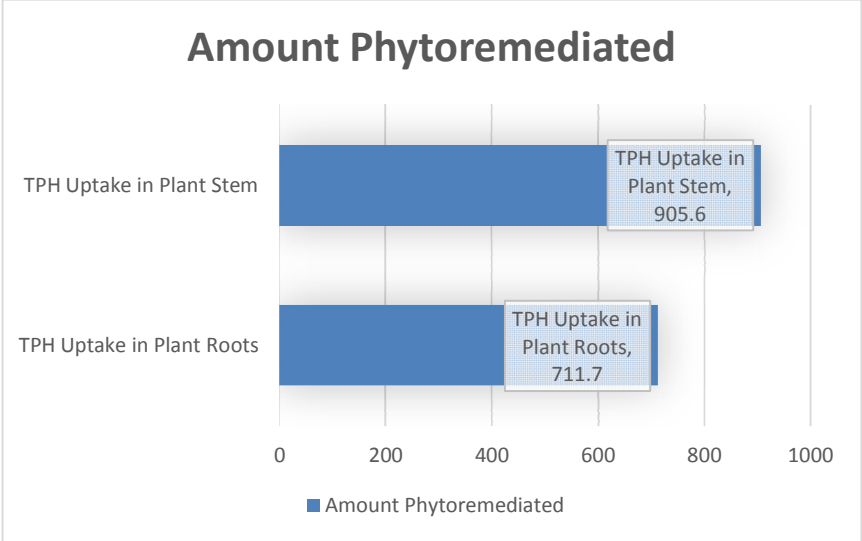

Fig. 4. Total Petroleum Hydrocarbon (TPH)(mg/kg) uptake by plant stem and roots

4. CONCLUSION

It is concluded that TPH uptake was higher in plant stems than roots; more so, plots amended with nutrient supplements showed significant higher percentage reduction in hydrocarbon in the polluted soil than unamended polluted soil. The present study recommended that *Cyperus esculentus* is a suitable plant species for phytoremediation of crude oil contaminated soil with high TPH value while *Phyllanthus amarus*is the best option in phytoremediation of polluted soil with high PAHs value, both in combination with bio-nutrient supplement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kinika PDS. Short-term effects of oil pollution of species umber productivity of a

simple territorial ecosystem. Environ. Pollution. 1981;26:187-189.
2. Daniel-KalioLA, Samuel-Allaseh JO. Effect of bonny light crude oil contamination of soil, soil amendment and successive planting at four-week intervals on the health and growth of phyllanthusamarus schum & thonn. Journal of Biological Science Research. 2006;1
3. Odogwu EC, Onianwa. Environmental impact assessment. a case study of utorogu 1984. 16th deliveryline spillage. The petroleum industry and the Nigerian environment. Proc 1987, int.sem. 1987;288-301.
4. Wang J, Zhang ZZ, Whe YM, Fhe SU, Song HG. Phytoremediation of petroleum polluted Soil. Petroleum Science. 2008; 5(2):167- 169.
5. Efe SI, Okpali AE. Management of petroleum impacted oil with phytoremediation and soil amendment in Ekpan, Delta State Nigeria. Journal of Environmental Protectionvol 3 article ID 19508; 2012.
6. Schnoor JL. Phytoremediation of soil and groundwater: Technology evaluation report TE-02-01. Groundwater Remediation Technologies Analysis Centre {GWRTAC}; 2002.
7. Agbogidi OM, Ejemeta OR. Qan assessment of the effect of crude oil pollution on soil properties, germination and growth of *Gambayaalbida* (L) Uniswa Research Journal of Agricultural Science and Technology. 2005;8(2):148-155.
8. Husemann M. Hausmann T, Fortman T, Thom R, Cullin V. In situ phytoremediation of PAHs and PCB eelgrass (Zosteramarina). Ecol. Eng. 2009;35:1395-1404.
9. Ikhajiagbe B, Chijioke OC, Obonoji N, Osaruese U. Effects of soil ammendments on the intensic qualities and development of soil seed bank of a monitored naturally attenuated petroleum hydrocarbon polluted soil. Journal of Natural Sciences Research ISSN 2224, 3186 (paper) ISSN 2225, 0921. 2014;4.
10. Crone TI, Tolstoy M. Magnitude of the 2010 Gulf of Mexico oil leak Science, 631. Cunningham, S.D. & Ow, D.W. (1996). Promises and prospect of phytoremediation.Plant Physiol. 2010;100:715-719.
11. Diab EA. Phytoremediation of oil contaminated desert soi using the rhizosphere effects. Global J. Environ. Res. 2008;2(2):66-73
12. Okpokwasili GC, Odokuma LO. Effect of salinity on biodegradation of oil spill dispersant, waste. Manage. 1990;10:141-146.
13. Odokuma Lo, Ibor MN. Nitrogen fixing bacterial enhanced bioremediation of crude oil polluted soil, Global Journ. Pure Appl. Sci. 2002;8(4);455-468.
14. Daniel-Kalio LA, Samuel-Allasseh JO. The sensitivity of yellow Nutsedge (*Cyperus esclentus*) Bonny Light Crude Oil in Niger Delta, Nigerian Environment Society (JNES), 2006,3:115
15. Inoni OE, Omotor DG, Adun FN. The effects of oil spillage on crop yield and farm income in Delta Sate, Nigeria. Journal of Central European Agriculture. 2006;7(1):41-49.
16. Efe SI,Elenwo EI. Phytoremediation of crude oil contaminated soil with *Axonopus compressus* in the Niger Delta Region Nigeria Effects of oil spill/green living tips; 2014.
17. Abii T, Nwosu TA. The effect of oil spillage on the soil ofEleme in Rivers State of the Niger Delta area of Nigeria. Research Journal Environmental Science. 2009;3(3);316-320.
18. Liao JQ, Wang J, Haung Y. Bacterial community features are shaped by geographic location, physichemical properties, and oil contamination of soil in main oil fields of China. MicrobEcol, 2015;70:380-389.
19. Pinshin HE, Williams JB, May E. Insitu and microcosm investigations into phytoremediation of hydrocarbon-contaminated lagoon sediments using phragmite saustralis. Journal of Environmental Engineering. 2013;139(4):488–495
20. Singh R. Heavy metals and living systems: An overview – NCBI; 2011.
21. Chiara A, Rosario M, Flavia T. Bioremediation of diesel oil in a contaminated soil by bioaugmentation with a microbial formula taiored with native strains elected for heavy metals resistance. Science of the Total Environment. 2009;407(8):3024-3032.
22. Peng SW, Zhou QX, Zhang H, Shi RG. Responses of seed germination of 8 ornamental plants to petroleum contamination. Chinese J. Environ. Sci. 2009;29:786-790.

23. Deng D, Deng J, Li J, Zhang J, Hu M, Lin Z. Accumulation of zinc, cadmium, and lead in four populations of Sedum alfredi growing on lead/zinc mine spoils. J Integr. Plant Biol. 2008;50:691-698.
24. Uzoije AP, Agunwamba JC. Physiochemical properties of soil in relation to varying rates of crude oil pollution. Journal of Environmental Science and Technology. 2011;4(3):313–323.
25. Bohn HL, MCneal BL, Connor GA. Soil Chemistry. Second Edition. 2001; 430.
26. Allison C, Oriabure G, Ndimele PE, Shittu JA. Chapter 23 – Dealing with oil spill scenarios in the Niger Delta: Lessons from the past. The Political Ecology of Oil and Gas Activities in the Nigerian Aquatic Ecosystem, P. E. Ndimele, ed., Academic Press. 2018;351-368.
27. Jingchun T, Xiawoei N, Rugang W, Qixing Z. Bioremediation of petroleum polluted soil by combination of rye grass with effect microorganisms; 2009.
28. Montgomery DC. Design and analysis of experiments (4th edition), New York: John Wiley and Sons; 1997.
29. Ogbonna DN. Application of Biological methods in the remediation of oil polluted environment in Nigeria Journal of Advances in Biology and Biotechnology. 2018;1119.
30. Juo ASR, Fox RL. Phosphorus sorption characteristics of some bench-mark soils of West Africa. Soil Sci. 1977;124:370-376.
31. Eckert D, Sims JT. "Recommended soil pH and lime requirement tests ",In: J.T. Sims and A. Wolfe, Eds., Recommended Soil Testing Procedures for the Northeastern United States. Northeast Regional Bullentin 493, Agriculture Experiment Station, Universityof Delaware, Newark. 1995;11-16.
32. USEPA (U.S. Environmental Protection Agency). Test methods for evaluating solid and hazardous waste. USEPA, Washington, DC; 1994.
33. API Publication Number 4599: "Interlaboratory study of three methods for analyzing petroleum hydrocarbons in soils, diesel-range organics (DRO), gasoline-range organics (GRO), petroleum hydrocarbons (PHC); 1994.
34. Chikere CB, Chikere BO, Okpokwasili GC. Bioreactor-based bioremediation of hydrocarbon-polluted Niger Delta marine sediment, Nigeria. 3 Biotech. 2012; 2(1):53–66.
Available:https://doi.org/10.1007/s13205-011-0030-8
35. Nrior RR, Mene GB. Assessment of bioaugmentation efficiency of *Penicillium chrysogenum* and Aspergillus nudilans in bioremediation of crude oil spill soil. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT). 2017;11(8):01–09.
36. Nrior RR and Inweregbu AO. Bioremediation potential of *Pseudomonas aeruginosa* KX828570 on crude oil spill polluted marshland and terrestrial soil treated with oil spill dispersant. Journal of Advances in Microbiology2019,15(2):1-15
37. Ogbonna DN, Ngah, SA, Okparanma, RN, Ule O, Nrior RR. percentage bioremediation assessment of Spent Mushroom Substrate (SMS) and mucorracemosus in hydrocarbon contaminated soil. Journal of Advances in Microbiology(JAMB). 2020;20(12):1-21.
38. Ule O, Ogbonna DN, Okparama RN and Nrior RR. Myco-enhanced bioremediation in open field crude oil contaminated soil using mucor racemosusand aspergillus niger. Current Journal of Applied Science and Technology. 2021;40(1):119-141.
39. Wiltse CC, Rooney WL, Chen Z, Schwab AP, Banks MK. Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. Journal of Environmental Quality. 1998;27:169-173.
40. Lopez-Martinez S, Gallegos-Martinez ME, Perez-Flores LJ, Gutierrez-Rojas M. Contaminated soil phytoremediation by Cyperuslaxus Lam. cytochrome P450 Erod-activity induced by hydrocarbons in roots. 2008;10:289-301.
41. Basumatary B, RubulS,Sabitry B. Phytoremediation of crude oil contaminated soil using nut grass, Cyperusrotundus. Journal of Environmental Biology. 2012;33:891-896.
42. Merkl N, Schultze-Kraft R, Infante C. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. Water Air Soil Pollut. 2005;165:195-209.
43. Muratova A, Dmitrieva TV, Panchenko LV, Turkovskaya OV. Phytoremediation of oil-sludge contaminated soil.International Journal of Phytoremediation. 2008,10:486-502.

44. Kulakow PA, Schwab AP, Banks MK. Screening plant species for growth on weathered, petroleum hydrocarbon contaminated soils. International Journal of Phytoremediation. 2000;2:297-317.
45. Yateem A, Al-Sharrah T, Bin-Haji A. Investigation of microbes in the rhizosphere of selected trees for the rhizoremediation of hydrocarbon-contaminated soils. International Journal of Phytoremediation. 2008;10:311-324.
46. Potter TL, Simmons KE. Composition of petroleum mixtures. Total Petroleum Hydrocarbon Criteria Working Group Series. Amherst, MA, Amherst Sci; 1998,2.
47. Diaz-Ramirez IJ, Saad HR, Rojas MG, Favela TE. Biodegradation of Maya crude oil fractions by bacterial strains and a defined mixed culture isolated from Cyperus laxus rhizosphere soil in a contaminated site. Can. Journal of Microbiology. 2003;49:755-761.
48. Garcia-Rivero M, Saucedo-Castaneda G, Flores de Hoyos S, Gutierrez-Rojas M. Mass transfer and hydrocarbon biodegradation of aged soil in slurry phase. Biotechnol. Progr. 2002;18:728-733.

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