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.
present in a crude oil polluted soil thereby enhancing hydrocarbon reduction in pari per sua 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* roots 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* (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, Pivotz [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 uptake 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 Ilchajagbe 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 benghalensis is more effective in water logged oil polluted soil. Dan-Kallo and Samuel- Alasseh [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 microorganism reported that degradation rate of 58% using combined treatment of plant and microorganism after 162 days enhanced total petroleum hydrocarbon (TPH) degradation 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°48’18.50”N and Latitude 6°58’39.12”E measuring 5.4864m x 5.1816m with a total area of 28.4283m² in an experimental land that lies within the Rivers State University Demonstration farmland in Nkpoul-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 150,000cm³ 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 augmenting bacteria – *Aspergillus niger* and *Mucor racemosus*.

**2.2 Research Design**

This study adopted the experimental research design of Montgomery [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 augmenting microbes *Aspergillus 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 amendment and augmentation 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 0-15 cm (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 Particle 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 H2O 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 $1 \times 10^2 \mu$mhos/cm, $(\mu$S/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$^\circ$C then the can was removed and put in a desiccator to cool and weighed.

%Moisture content was calculated as:

$$\text{Moisture content} = \frac{A - B}{B - \text{tare can}} \times 100$$

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 ASTM D3921 and US EPA 8015 [32] analytical protocol (API, [33]) as reported by Chikerete 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 that 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).

$$Px = Fx - Ix \ldots \text{equation 1}$$

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

\[
\%PR = \left( \frac{Px}{Ics} \right) \times 100 \quad \text{..equation 2}
\]

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).

\[
Bc = Ic - Fc \quad \text{..equation 3}
\]

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

\[
Rc= \left( \frac{Bc}{Ic} \right) \times 100 \quad \text{..equation 4}
\]

Where:

\( Bc \) = Amount of pollutant remediated
\( Ic \) = Initial Concentration of pollutant (week 1)
\( Fc \) = Final Concentration of pollutant (week 8)
\% Reduction OR % Bioremediation (%Rc)

3. RESULTS AND DISCUSSION

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 *Aspergillus 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 differences (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 amarus (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(9.36±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 polluted 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; moreover 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 + Phyllanthus amarus= ; 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(1159.86±2427.99; 99.23%) >Polluted soil + Mucor racemosus+ Spent Mushroom Substrate + Phyllanthus amarus(1159.86±2430.40; 99.43%) >Polluted soil
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. *Phyllanthus amarus* 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, 58.15±35.31, 57.70±33.26 cm respectively in P4, P6, P8, P9 and P10 in comparison to unvegetated pots. 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 (210.0 mg/kg, 13.86%) >PS+AN+MR+Cyp (201.0 mg/kg, 13.26%) >PS+AN+MR+Cyp (192.4 mg/kg, 12.70%) >PS+AN+MR+Cyp (191.1 mg/kg, 12.61%) >PS+AN+MR+Cyp (171.3 mg/kg, 11.30%) >PS+AN+MR+Cyp (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 (19.8 mg/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 *Cyperus laxus* 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 T1(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 5503.00 mg/kg, Total Petroleum Hydrocarbon Reduction and % Hydrocarbon Reduction in 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+AN+MR+SMS+Phy (5460.60 mg/kg; 99.23%) >PS+AN+MR+SMS+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; 97.75%).

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

Evaluation of Polycyclic Aromatic Hydrocarbons (PAHs) from initial contamination value 47.5 mg/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 phyto remediation 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 + Phyllanthus 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 augumenting 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, asphalhtins, and resins) will require long term monitoring of soil and plant development.
### Table 1. Experimental setup for phytoremediation of crude oil polluted bioaugmented soil

| S/N | Plot Code          | Volume of Soil (100x50x30cm³) | Crude Oil (2500ml) (2122.23g) | Test Plants Cyperus esculentus (Cyp) | Phyllanthus amarus (Phy) | Augmenting Microbes Aspergillus niger (Asp) broth/ml | Mucor racemosus (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       | +                             | +                             | +                                   | -                        | -                                                  | -                               | -                                           |
| P8  | PS+AN+MR+Phy       | +                             | +                             | +                                   | -                        | -                                                  | -                               | -                                           |
| P9  | PS+MR+SMS+Phy      | +                             | +                             | +                                   | -                        | -                                                  | -                               | -                                           |
| P10 | PS+AN+MR+SMS+Phy   | +                             | +                             | +                                   | -                        | -                                                  | -                               | -                                           |

*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                  | pH                   | Electrical Conductivity (EC) (S/cm) | Plant Height (cm) | Total Nitrogen (%) | Available Phosphorus (%) | Potassium (mg/kg) | % Organic Carbon | Moisture Content (%) |
|---------|-----------------------------|----------------------|------------------------------------|-------------------|---------------------|--------------------------|-------------------|------------------|---------------------|
| P1      | US+ Phy                     | 7.49±0.31a           | 255.29±6.32a                       | 36.40±13.03a      | 0.23±0.00a          | 7.74±0.12a               | 0.31±0.00a         | 1.99±0.21a       | 1.25±0.32b          |
| P2      | US+Cyp                      | 7.57±0.26a           | 281.57±24.58a                      | 41.13±18.20a      | 0.23±0.01a          | 5.41±0.48a               | 0.31±0.00a         | 2.31±0.14a       | 0.99±0.22a          |
| P3      | PS+Cyp                      | 7.55±0.30a           | 276.71±44.11a                      | 39.77±16.22a      | 0.22±0.01a          | 6.85±1.49a               | 0.28±0.04a         | 2.41±0.28a       | 0.99±0.24a          |
| P4      | PS+AN+Phy                   | 7.54±0.22a           | 233.86±38.61a                      | 52.47±27.50a      | 0.22±0.04a          | 7.04±1.59a               | 0.29±0.04a         | 2.09±0.43a       | 0.9±0.285a          |
| P5      | PS+MR+Cyp                   | 7.46±0.32a           | 240.71±38.20a                      | 42.67±22.07a      | 0.22±0.05a          | 7.26±1.42a               | 0.30±0.04a         | 2.27±0.56a       | 0.99±0.26a          |
| P6      | PS+SMS+Phy                  | 7.41±0.30a           | 265.43±23.09a                      | 55.40±29.98a      | 0.22±0.05a          | 9.77±0.85a               | 0.32±0.05a         | 3.02±0.13a       | 1.00±0.23a          |
| P7      | PS+AN+MR+Cyp                | 7.48±0.21a           | 293.57±59.67a                      | 51.37±31.23a      | 0.22±0.04a          | 6.76±2.26a               | 0.32±0.05a         | 3.02±0.11a       | 1.04±0.22a          |
| P8      | PS+AN+MR+Phy                | 7.55±0.45a           | 340.29±40.32a                      | 55.83±35.31a      | 0.22±0.02a          | 6.56±1.83a               | 0.34±0.05a         | 2.89±0.05a       | 0.98±0.19a          |
| P9      | PS+MR+SMS+Phy               | 7.49±0.29a           | 272.29±29.15a                      | 58.15±34.04a      | 0.21±0.01a          | 6.89±1.56a               | 0.33±0.07a         | 2.74±0.31a       | 1.25±0.32a          |
| P10     | PS+AN+MR+SMS+Phy            | 7.55±0.30a           | 254.57±28.02a                      | 57.70±33.26a      | 0.18±0.02a          | 7.03±1.50a               | 0.36±0.07a         | 2.10±0.67a       | 0.99±0.22a          |

*TPH = Total Petroleum Hydrocarbons*
| Plot | Treatments | pH | Available Phosphorus (%) | Potassium (%) | % Organic Carbon | Moisture Content (%) |
|------|-------------|----|--------------------------|--------------|-----------------|---------------------|
| P1   | US+Phy      | 7.68 | 0.6428 | 7.9875x + 242.43 | 0.7399 | 0.3104x + 0.73 | 0.7683 | 0.0002x + 0.2277 | 0.75 |
| P2   | US+Cyp      | 7.10 | 0.0507 | 9.857x + 242.43 | 0.7399 | 0.0771x + 0.6857 | 0.5645 | 0.0019x + 0.2177 | 0.1374 |
| P3   | PS+Cyp      | 7.35 | 0.0753 | 5x + 256.71 | 0.06 | 0.0836x + 0.6557 | 0.5499 | 0.0058x + 0.1969 | 0.6884 |
| P4   | PS+AN+Phy   | 7.35 | 0.0893 | -12.857x + 285.29 | 0.5176 | 0.0918x + 0.6186 | 0.5075 | 0.015x + 0.1584 | 0.5545 |
| P5   | PS+MR+Cyp   | 7.10 | 0.1146 | -14.607x + 299.14 | 0.6825 | 0.09x + 0.6286 | 0.5506 | 0.0166x + 0.15 | 0.5873 |
| P6   | PS+SMS+Phy  | 7.10 | 0.0581 | -3.75x + 280.43 | 0.1231 | 0.0814x + 0.6743 | 0.6107 | 0.0175x + 0.1516 | 0.6125 |
| P7   | PS+AN+MR+Cyp| 7.35 | 0.0814 | 56.329x | 0.7446 | 0.0761x + 0.7357 | 0.5534 | 0.0117x + 0.1697 | 0.4813 |
| P8   | PS+AN+MR+Phy| 7.10 | 0.1725 | 15.857x + 276.86 | 0.7219 | 0.040x + 0.8171 | 0.2165 | 0.0086x + 0.1903 | 0.8524 |
| P9   | PS+MR+SMS+Phy | 7.05 | 0.11x | -7.2413x + 301.14 | 0.2858 | 0.1304x + 0.73 | 0.7663 | 0.0016x + 0.2034 | 0.103 |
| P10  | PS+AN+MR+SMS+Phy | 7.06 | 0.1225 | -9.1071x + 291 | 0.4931 | 0.0771x + 0.6857 | 0.5645 | 0.0034x + 0.1651 | 0.1048 |

**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 Physicochemical parameters during Phytoremediation of crude oil polluted soil
| Plot | Treatments | TPH in Plant Roots (mg/kg) | TPH in Plant Stem (mg/kg) | TPH in Soil (mg/kg) | PAH in Soil (mg/kg) |
|------|-------------|---------------------------|--------------------------|--------------------|---------------------|
| P4   | PS+AN+Phy   | -0.3543x + 8.46           | 0.2305                   | 0.0115x + 0.246    | 0.4814              |
|      |             |                           |                          |                    | -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 - 6.9143   | 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 + 0.2568    | 0.8108              |
|      |             |                           |                          |                    | -0.0005x + 2.8944  | 0.0004              |
|      |             |                           |                          |                    | 17.343x - 21.514   | 0.9455              |
| P9   | PS+MR+SMS+Phy| -0.475x + 8.7843         | 0.4335                   | 0.0224x + 0.2443   | 0.5029              |
|      |             |                           |                          |                    | -0.0161x + 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 - 18.468   | 0.9636              |

**Plot Treatments**

**Regression equation (Y)**

- PS+AN+Phy: $0.5039x + 1.309$
- PS+MR+Cyp: $5.259x + 1.207$
- PS+Cyp: $4.42x + 13.02$
- PS+AN+Phy: $45.82x - 0.9$
- PS+MR+Cyp: $28.55x - 7.45$
- PS+SMS+Phy: $30.08x - 28.7$
- PS+AN+MR+Cyp: $30.02x - 18.96$
- PS+AN+MR+Phy: $29.42x - 26.16$
- PS+MR+SMS+Phy: $30.38x - 24.42$
- PS+AN+MR+SMS+Phy: $36.73x - 30.41$

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

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**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    | 5.006174      | 4.96E-05    | 2.040098  |
| Treatments*Electrical conductivity (µs/cm)    | Between Groups      | 588092.29   | 9  | 6454.698    | 0.082831      | 1.283587    | 0.264676  |
| In soil                                       | Within Groups       | 77360.86    | 60 | 1289.348    | 1.001392      | 1.524148    | 0.160292  |
| 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    | 1.001392      | 1.524148    | 0.160292  |
| Treatments>Total nitrogen (%)                 | Between Groups      | 0.012528    | 9  | 0.001392    | 1.001392      | 1.524148    | 0.160292  |
| In soil                                       | Within Groups       | 0.054797    | 60 | 0.000913    | 1.001392      | 1.524148    | 0.160292  |
| Treatments*Available phosphorus (%)           | Between Groups      | 76.72396    | 9  | 8524884     | 4.091485      | 0.000391    | 2.040098  |
| In soil                                       | Within Groups       | 125.014     | 60 | 2.083567    | 1.633464      | 0.126255    | 2.040098  |
| Treatments*Potassium (%) in soil              | Between Groups      | 0.03473     | 9  | 0.003859    | 1.633464      | 0.126255    | 2.040098  |
|                                               | Within Groups       | 0.141743    | 60 | 0.002362    | 1.633464      | 0.126255    | 2.040098  |
| Treatments*Organic carbon (%) in soil         | Between Groups      | 10.06262    | 9  | 1.116069    | 10.24123      | 2.28E-09    | 2.040098  |
|                                               | Within Groups       | 6.549823    | 60 | 0.109164    | 10.24123      | 2.28E-09    | 2.040098  |
| Treatments*Plant height (cm)                  | Between Groups      | 3145.924    | 9  | 349.5471    | 0.451365      | 0.90089     | 2.040098  |
|                                               | Within Groups       | 46465.32    | 60 | 774.422     | 3.098773      | 0.066446    | 2.124029  |
| Treatments*TPH (mg/kg) uptake in plant roots  | Between Groups      | 80078.08    | 9  | 8897.564    | 3.098773      | 0.066446    | 2.124029  |
|                                               | Within Groups       | 114852.7    | 40 | 2871.318    | 2.8151        | 0.011636    | 2.124029  |
| Treatments*TPH (mg/kg) uptake in plant stem   | Between Groups      | 107047.7    | 9  | 11894.18    | 2.8151        | 0.011636    | 2.124029  |
|                                               | Within Groups       | 169005.5    | 40 | 4225.138    | 2.8151        | 0.011636    | 2.124029  |
| Treatments*TPH (mg/kg) Reduction in soil     | Between Groups      | 28075215    | 9  | 3119468     | 0.926977      | 0.512526    | 2.124029  |
|                                               | Within Groups       | 1.35E+08    | 40 | 3365206     | 8.471887      | 6.02E-07    | 2.124029  |
| Treatments*PAHs (mg/kg) Reduction in soil    | Between Groups      | 7053.518    | 9  | 763.7242    | 8.471887      | 6.02E-07    | 2.124029  |
|                                               | Within Groups       | 3700.353    | 40 | 92.50882    | 92.50882      | 3700.353    | 2.124029  |
| Treatments*Iron (mg/kg) variation in soil    | Between Groups      | 19113.19    | 9  | 2123.688    | 18.56413      | 4.32E-13    | 2.073351  |
|                                               | Within Groups       | 5719.871    | 50 | 114.3974    | 18.56413      | 4.32E-13    | 2.073351  |
| Treatments*Lead (mg/kg) variation in soil    | Between Groups      | 0.022552    | 9  | 0.002506    | 1.418506      | 0.205688    | 2.073351  |
|                                               | Within Groups       | 0.088324    | 50 | 0.001766    | 1.418506      | 0.205688    | 2.073351  |
| Treatments*Zinc (mg/kg) variation in soil    | Between Groups      | 115.5984    | 9  | 12.84426    | 2.198441      | 0.037751    | 2.073351  |
|                                               | Within Groups       | 292.1221    | 50 | 5.842441    | 2.198441      | 0.037751    | 2.073351  |
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 = Mucor 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 | % Phytoremediation | TPH uptake by plants stem | % Phytoremediation | TPH (mg/kg) Reduction in soil | % Reduction | PAHs (mg/kg) Reduction in soil | % Reduction |
|---------------------|---------------------------|--------------------|---------------------------|--------------------|-------------------------------|-------------|-----------------------------|-------------|
| Cyperus esculentus  | 152.33±50.34              | 12.57±4.16         | 201.13±28.80              | 13.27±0.56         | 5492.73±76.36                 | 98.55±0.67 | 25.77±2.12                  | 54.24±4.47   |
| Phyllanthus amarus  | 141.50±35.62              | 11.69±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
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.

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