Phytoremediation of Crude Oil Polluted Microbial Augmented Soil Using *Cyperus esculentus* and *Phyllanthus amarus*

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Authors' contributions

This work was carried out in collaboration between both authors. Authors JODA and DNO designed the study. Author JODA performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author JODA also managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

**Aim:** To assess the phytoremediation potential of *Cyperus esculentus* and *Phyllanthus amarus* in crude oil polluted soil and ascertain the enhancement of augmented microbes (fungi).

**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-Oworukwo, Mile 3 Diobu area of Port Harcourt, 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 weekly at 30 days’ interval.

**Methodology:** Seven (7) experimental plots (two Control (Unpolluted and polluted soil) and five polluted amended/treated plots) employing Randomized Block Design (each having dimensions: 100 x 50 x 30 cm LxBxH) were formed and mapped out on agricultural soil and left fallow for 6 days.

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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 bioaugmenting organisms were applied. Baseline studies were carried out on the top soil before and after contamination, major parameters monitored and assessed were Total Petroleum Hydrocarbon (TPH) uptake by plant roots and stem, Polycyclic Aromatic Hydrocarbon (PAHs) and TPH reduction in soil. Other physiochemical analyzed in the soil of different plots were pH, Electrical Conductivity, Moisture Content, Total Nitrogen, Available Phosphorus, Potassium, Total Organic Carbon, Plant Height, Iron, Lead at regular intervals; days 1, 60, 90, 120, 150, 180, 210 & 240. Application of augmenting organisms was to enhance phytoremediation by test plant Cyperus esculentus (Cyp) and Phyllanthus amarus (Ph). 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. Similar trend was observed in the control plots were TPH uptake by Cyperus esculentus roots and stem were; 24.2mg/kg, 2.00% and 20.01mg/kg, 1.32% respectively while in control plot of Phyllanthus amarus TPH uptake by roots and stem were 23.19mg/kg, 1.91% and 19.80mg/kg, 1.31% respectively. Comparatively, uptake of TPH was higher in plant stem than roots. From the initial TPH contamination value of 5503.00mg/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 (5470.9mg/kg; 99.43%) >PS+MR+SMS+Phy (5460.60mg/kg; 99.23%) >PS+AN+MR+Phy (5451.30mg/kg; 99.06%) >PS+MR+Cyp (5448.30mg/kg; 99.86%) >PS+AN+Phy (5422.905mg/kg; 98.54%) >PS+Cyp (no amendment) (5380.90mg/kg; 97.78%). Comparative evaluation revealed higher reduction of PAHs in soil (plot) planted with Phyllanthus amarus. Highest PAHs reduction in soil was seen in PS+AN+MR+SMS+Phy (31.3mg/kg; 65.89%) while PAHs reduction in soil was 25.77±2.12mg/kg, 54.24±4.47%). More so, plots amended with augmenting microbes 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 for phytoremediation of polluted soil with high PAHs value, in combination with augmenting microbes.

Conclusion: 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 in plots planted with Phyllanthus amarus (PAHs 28.72±2.74mg/kg; 60.46±5.77%) was higher than plots planted with Cyperus esculentus (PAHs 25.77±2.12mg/kg, 54.24±4.47%). More so, plots amended with augmenting microbes 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 for phytoremediation of polluted soil with high PAHs value, in combination with augmenting microbes.

Keywords: Phytoremediation; crude oil; TPH uptake; cyperus esculentus and phyllanthus amarus, aspergillus niger; mucor racemosus.

1. INTRODUCTION

A major reason why crude oil spillage causes so much damage is, it spreads and cuts off oxygen supply from the living systems and this results to death. The land becomes useless as aeration is reduced for underground living things. Water in such areas become contaminated with dangerous organic compounds and as such is unfit for human consumption[1]. Crude oil however, is the kingpin in the economies of most nations where it is found. In these countries, for instance, Nigeria, there have been reported cases of crude oil spills and a noted destruction of land and aquatic life. The livelihood of persons in the Niger Delta where the oil is has been battered nearly unabatedly. One fact remains clear, the exploration for oil will continue as far as there is still oil in such areas, and given the imperfections of our technologies, crude oil spills will continue to be discussed. It is therefore expedient to have a system to enable decontamination or remediation in place at all times. 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 [1,2,3].

Daniel-Katio and Samuel-Allasseh [4] 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. Some of the plants were able to colonize the oil impacted area a few months after the spill.

Cyperus esculentus Lin (Yellow nutsedge) is a sedge of the family Cyperaceae with a perennial life that grows up to 9m (3ft) at a fast rate. This species is a hermaphrodite and is pollinated by wind. It has tall, erect, triangular ribbed and glabrous stem. Some of the fibrous roots occasionally bear small bulbs (nuts). The leaves are 3mm-10mm wide, basal, linear, finch ribbed and glabrous with terminal, brown, linear, flat spikelet flowers arranged in loose terminal panicles subtended by leafy tracts that are usually longer than the inflorescence. The fruits are brown trigourous nutlets surrounded by four hanging leaves positioned 90° from each other. It is a common weed of cultivated and fallow ground, and an early colonizer. It is found in most of the eastern hemisphere including southern Europe, Africa and Madagascar as well as the middle east and the Indian sub-continent (cool temperate zones to the tropics) [4].

Phyllanthus amarus Schum & Thonn (Bhumi amla) belong to the family Euphobiaceae. It is an annual herb, 15-50cm high, branched from the base with an erect, glabrous and profusely branched stem. The leaves are small (about 6-8cm) auxiliary, solitary and five green central regions. The fruits are calaboose, 6 seeded capsule minutely putrescent brown seed. The flowers are mainly hidden behind the stems. It is a common weed of cultivation and fallow and an early colonizer [5].

Petroleum is a naturally occurring complex mixture made up predominantly of hydrocarbon compounds and frequently contains significant amounts of nitrogen, sulphur, and oxygen together with smaller amounts of nickel, vanadium, and various elements. Petroleum compounds can occur in solid form as asphalt, liquid form as crude oil and / or gaseous form as natural gas. Petroleum hydrocarbons could be divided into four classes: saturates (pentane, hexadecane, octacosane, cyclohexane), aromatics (phenols, fatty acids, ketones, esters, and porphyrins), and resins (pyridines, quinolines, carbazole, sulfides and amides) [6]. Solids and sediments are the ultimate sink for most petroleum contaminants, such as benzene, toluene, ethyl benzene, and xylenes (BTEX), aliphatic and polycyclic aromatic hydrocarbons (PAHs). Petroleum hydrocarbon contamination of soils and sediment is a global concern because of the toxicity [6]. Although predominant oil pollution in the United Kingdom contains high volumes of aliphatic hydrocarbons [7]. Petroleum pollution in the tropical region like Nigeria’s Niger Delta contains complex of both the aliphatic and aromatic hydrocarbons [8].

The inadvertent discharges of petroleum hydrocarbons into the environment often pose threats to human health, safety and the environment, and have significant socio-economic consequences.

Remediation removes, degrades or transforms contaminants to harmless or less harmful substances. It also reduces the mobility and migration of contaminants and prevents their spread to all contaminated areas [9].

Phytoremediation is the use of plants/and or associated microorganisms to remove or render harmful material harmless. It is the use of plants to detoxify, restore or purify the environment; phytoremediation presents several major advantages compared to other remediation technique. Phytoremediation can be applied to both organic and inorganic pollutants present in solid or liquid substrate. Its application is for a broad range of organic pollutants and heavy metals from spillage sites. Numerous treatment systems have been established and biological and engineering strategies designed to improve and optimize phytoremediation. The knowledge of the physical, biological molecular mechanism of woody plants species, shrubs and annual plants with agricultural background have been found suitable for phytoremediation. Plants for phytoremediation should be appropriate for the climate and soil conditions of the contaminated sites [10]. The plants should also have the ability to tolerate stress [11]. The plants/weeds should be selected because of its features e.g. fibrous root enhance the ability of the plant/weed to survive adverse phytostabilization. Many authors reported phytoremediation as a cost-effective method with high public acceptance and also environmentally friendly [12]. For removal of petroleum from soil, and a great potential in remediation of soil contaminated with petroleum.
2.1 Area/Scope of the Study

Ayotamuno [13] experimented with zea maize (corn) and *Pennisetum purpureum* (elephant grass) for phytoremediation in crude oil polluted soil in Port Harcourt for six (6) weeks. It was observed that degradation of petroleum hydrocarbon on agricultural soil took place and there was an average hydrocarbon loss of 77.5% (Zea maize) and 83% (*p* purpureum) within the first two (2) weeks.

These values decreased to 67% and 55% after the six (6) weeks remediation period for corn and elephant grass respectively. Also, in the study at Ekpan which lasted for three (3) months Efe and Elenwo used *Axonopus spp*, *Cyperus spp*, and soil amendments. It was found that the combined effect of *Axonopus spp*, *Cyperus spp* and soil amendments accounted for 59% reduction in hydrocarbon. However, *Axonopus spp* and *Cyperus spp* accounted for 47% and 48% reduction in hydrocarbon respectively.

With these enormous potential achievements of phytoremediation, there is need to evaluate its potential in the tropics especially in Niger Delta region of Nigeria where crude oil pollution in soil is very high.

This study is aimed at assessing the efficacy of phytoremediation technique using *Cyperus esculentus* Lin and *Phyllanthus amarus* in crude oil polluted soil augmented with microbes in coastal areas of the Niger Delta region and to determine the most effective test plant in the phytoremediation process between *Cyperus* and *Phyllanthus*.

2. MATERIALS AND METHODS

2.1 Area/Scope of the Study

The study was done in the coastal part of the Niger Delta which falls within the central coastslands of southern Nigeria. It lies at the intersection of latitude 5°33N and longitude 5°32E. The region is divided into two subdivisions, western and eastern region and one - third (1/3) of the region is made up of wetlands and houses, the third largest mangrove forest in the world [14].

The experimental land lies within the Rivers State University Demonstration farmland in Nkpolu-Oworukwo, Mile 3 Diobu area of Port Harcourt, Rivers State. The piece of land 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. This was cleared and sub-partitioned into seven 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 in the remediation of crude oil polluted, bio-augmented soils. The test plants were *Cyperus esculentus* and *Phyllanthus amarus* and the soil augmented with *Aspergillus niger* and *Mucor racemus* while the contaminating crude oil specifically was Bonny light crude.

2.2 Choice of Technique and Test Plants for the Study

The adoption of Phytoremediation technic in the decontamination process stands out as this is a biological approach which employs plants to render harmful materials harmless. It has an edge over other processes as it can be applied on organic and inorganic pollutants as well. There are species of plants that can grow on contaminated soil and aggressively extract pollutants from the growth medium. Phytoremediation is nondestructive and could remedry the soil structure and recover the biological environment.

Choices of test plants – *Cyperus esculentus* and *Phyllanthus amarus* for the study: These plants were chosen because they possess the appropriate qualities of a decontaminating plant as given by Dan- Kalio and Samuel-Allasseh, [1]. These qualities include;

i. Plant that can be found around and at no financial cost.
ii. Plant with a growth pattern that can be monitored easily and without any sophisticated instrument.
iii. Plant that does not need tendering and any special treatment to grow when cultivated.
iv. Plant appropriate for the climate, soil condition of contaminated sites Pivets [10]
v. The plant should have ability to tolerate stress. Siciliano & Gemida [11]
vi. The plant should be selected for its fibrous root.

2.3 Research Design

This study adopted the experimental research design. Montgomery [15] defined Experimental design as 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 and a sufficient sample size and power are available to answer the research questions of interest as clearly and efficiently as possible.

2.4 Experimental Set-up

The materials used for the experiment were the test plants *Cyperus esculentus* Lin (Cyp) and *Phyllanthus amarus* (Phy), Crude oil and soil 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 25000ml concentration was added to each plot. Crude oil was added to the plots except the control while bio-augmenting microbes *Aspergillus niger* and *Mucor racemosus* were added to some of the plots, the set-up was, Plot 1: Control US + Phy (Unpolluted soil + *Phyllanthus amarus*), Plot 2: Control US + Cyp (Unpolluted soil + *Cyperus esculentus*), Plot 3: PS + Cyp (Polluted soil + *Cyperus esculentus*), Plot 4: PS + Asp + Phy (Polluted soil + *Aspergillus niger* + *Phyllanthus amarus*), Plot 5: PS + Muc + Cyp (Polluted soil + *Mucor racemosus* + *Cyperus esculentus*), Plot 6: PS + Asp + Muc + Phy (Polluted soil + *Aspergillus niger* + *Mucor racemosus* + *Phyllanthus*), then mix 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 seedling 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.

2.5 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.

2.6 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 [16].

### Table 1. Experimental Set-up for Phytoremediation of Crude Oil Polluted Bioaugmented Soil

| S/ N | Plot Code       | Volume of Soil 100x50x30cm (150,000cm³) | Crude Oil 25000ml (2122.25 g) | Test Plants | Augmenting Microbes |
|------|----------------|----------------------------------------|-------------------------------|-------------|---------------------|
|      |                |                                        |                               | *Cyperus esculentus* (Cyp) | *Phyllanthus amarus* (Phy) | *Aspergillus niger* (Asp) | Mucor racemosus (Muc) |
| P1   | US + Phy       | +                                      | -                             | -           | +                   | -                        | -                        |
| P2   | US + Cyp       | +                                      | -                             | +           | -                   | -                        | -                        |
| P3   | PS + Cyp       | +                                      | +                             | +           | -                   | -                        | -                        |
| P4   | PS + AN + Phy  | +                                      | +                             | -           | +                   | 750ml                    | -                        |
| P5   | PS + MR + Cyp  | +                                      | +                             | +           | -                   | 750ml                    | 375ml                    |
| P7   | PS + AN + MR + Cyp | +                             | +                             | +           | -                   | 375ml                    | 375ml                    |
| P8   | PS + AN + MR + Phy | +                             | +                             | -           | +                   | 375ml                    | 375ml                    |

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

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

2.8 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, PAH

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

2.9 Source of Data

The primary type of data was used for this study. 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.

2.10 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 were taken within 2-4 hours and were carried in foil containers. The experiments were set and measurement 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 was measured in the field (cm) weekly.

2.11 Particle Size Analysis

This was carried out on soil to determine the texture of the soil and soil type at the experimental site. Particle size analysis was done by hydrometer method modified by Juo [17]. Soil samples were 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 and 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 were established by using a standard textural triangle.

2.12 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.

2.13 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 [18]

2.14 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 15 minutes. Then allowed to stand for 1 hour 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 1X10² µmhos/cm (µs/md) a factor of 8.
2.15 Moisture Content
Soil was air dried and 5g of the air dried soil was put in moisture 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:
\[ \text{% moisture content} = \frac{A-B}{B-\text{tare can}} \times 100\% \]

Moisture correction factor is obtained as follows:
\[ \text{MCF} = 100 + \frac{\text{moisture content}}{100} \]

2.16 Total Nitrogen (TN)
The total nitrogen content of the soil was determined by the macro-kjeldahl method (Bremer and Mulavaney [19]).

2.17 Available Phosphorus (P)
Available phosphorus was determined by the Bray No. 1 method as modified as by Olsen et al. (1982).

2.18 Exchangeable Cations (Ca, Mg, Na, and K)
Exchangeable K of the soil sample was extracted with neutral normal ammonium acetate buffered at pH 7 after shaking for 2 hours. Exchangeable Ca and Mg were determined by EDTA complexometric titration while Na was determined by flame photometry (Knudsen et al., 1982).

2.19 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 and quantified using Gas Chromatography – Flame Ionization Detector (GC-FID) Agilent 7890A according to the methods of ASTM D3921 and US EPA 8015 [20] analytical protocol (TPI, [21]) as reported by Chikere et al., [22] 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).

2.20 Polycyclic Aromatic Hydrocarbons – PAHs
Chromatography Spectrometer was used to measure the concentration and performance of PAHs.

2.21 Heavy Metals in Soil
Heavy metals were determined using Atomic Absorption Spectrophotometer (AAS) with the specific wavelengths for each metal.

2.22 Total Organic Carbon (TOC) in Soil Sample
The soil sample, 0.2g soil measured into a 500ml conical flask and 10ml of 0.5M K₂Cr₂O₇ was added, swirled gently and 20ml of Conc. H₂SO₄ was then added rapidly and directly into suspension but with care to avoid splashing. Immediately the conical flask with its content was swirled gently until the reagents are mixed for 1 minute. Flask was allowed to stand for 30 minutes while 200ml of Distilled water and 10ml Conc. H₃PO₄ was added cautiously to avoid splashing and mixture was cooled. Three drops of Ferrous Indicator Solution added. Solution was titrated to get a deep green 0.25M FAS (Ferrous Ammonium Sulphate) solution.

2.23 Percentage (%) Phytoremediation Analysis
The method of Nrior and Mene [23] were modified and used in calculating the percentage of phytoremediation in the experiment. The process followed the steps stated.

\[ \text{Step 1: The Amount of pollutant uptake in Roots (Pₓ) equals to Final Concentration of pollutant (Last day or Week of experiment) (Fₓ) minus the initial concentration of pollutant at day or Week 1(Iₓ).} \]

\[ \text{Pₓ} = \text{Fₓ} - \text{Iₓ} \]

Where:
\[ \text{Pₓ} = \text{Amount of pollutant uptake by Root or Stem} \]
\[ \text{Iₓ} = \text{Initial Concentration of pollutant in Roots or Stem (day or week 1 which is usually zero)} \]
\[ \text{Fₓ} = \text{Final Concentration of pollutant in Roots or Stem (last day or week of experiment)} \]

\[ \text{Step 2: The percentage (%) Phytoremediation (%PR) equals Amount of pollutant uptake (Pₓ)} \]
divided by the Initial Concentration of pollutant in
the soil at day or week 1 (Initial pollutant
contamination value), multiplied by 100

\[
\%PR = \frac{P_x}{I_{cs}} \times 100
\]  

(2)

Where;

\%PR = Percentage (%) Phytoremediation

\[P_x = \text{Amount of pollutant uptake by Root or Stem}\]

\[I_{cs} = \text{Initial Concentration of pollutant in the soil at day or week 1 (Initial pollutant contamination value)}\]

(Nrior and Mene, [23] modified),

2.24 Determination of Percentage (%)

Crude Oil Reduction (%

Bioremediation) in Polluted Soil

The method of Nrior and Mene [23] 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).

\[
B_c = I_c - F_c
\]  

(3)

Where:

\[B_c = \text{Amount of pollutant remediated}\]

\[I_c = \text{Initial Concentration of pollutant (week 1)}\]

\[F_c = \text{Final Concentration of pollutant (week 8)}\]

\[\%Rc = \frac{B_c}{I_c} \times 100
\]  

(4)

2.25 Statistical Analysis

Results were subjected to statistical analysis using Analysis of Variance (Two-way ANOVA) to test whether the different 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 DISCUSSION

Phytoremediation using grass plant *Cyperus esculentus* and *Phyllanthus amarus* was carried out on Crude Oil Polluted soil. Some isolated microorganisms – *Aspergillus niger* (AN) and *Mucor racemosus* (MR) were used to augment the indigenous microbial population present in a crude oil polluted soil to enhance microbial remediation in pari per suse with phytoremediation (uptake of Crude oil by test plants) over a period of 240 days.

Evaluation data from the baseline and phytoremediation analysis in the 7 Randomized Complete Block Design (RCBD) plots field studies are represented below as illustrative tables, figures, graphs and charts.

3.1 Physico-chemical Properties of the Soil Prior to Application of Various Treatments for Phytoremediation Evaluations

The Physicochemical parameters of the unpolluted and polluted soil before experimental treatment with different bioaugmenting microorganisms showed: pH (7.01, 6.80), Particle size (0.88, 0.28%), Electrical Conductivity (500, 590μS/cm), Moisture Content (15.95, 18.67%), Total nitrogen (0.229, 0.203%), Available Phosphorus (6.58, 10.20%), Potassium (0.311, 0.312%), Sulphate (0.025, 0.020mg/kg), Phosphate (0.00156, 0.00167mg/kg), Total Petroleum Hydrocarbon (66.8, 5503mg/kg), Polycyclic Aromatic Hydrocarbon (5.0, 47.5 mg/kg), Iron (0.01, 52.62 mg/kg), Zinc (1.00, 6.90 mg/kg), Lead (0.01, 0.12 mg/kg). Most of the values of polluted soil were higher than that of Control (unpolluted soil) (Table 2).

3.2 Identification of Seedlings Used in Phytoremediation Studies

The seedlings used as test plants for the experiment were identified as *Cyperus esculentus* Lin (Cyp) and *Phyllanthus amarus* (Phy) in the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt.
### Table 2. Baseline Result of Physico-chemical parameters of Uncontaminated and Contaminated soil before Phytoremediation

| S/N | Parameters                              | Unit          | Uncontaminated soil | Contaminated/Polluted soil |
|-----|-----------------------------------------|---------------|---------------------|---------------------------|
|     | Physical parameter                      |               |                     |                           |
| 1   | Particle size (>75µm)                   | %             | 0.88                | 0.28                      |
| 3   | pH                                      | -             | 7.01                | 6.80                      |
| 4   | Electrical Conductivity (EC)            | µS/cm         | 500.00              | 590.00                    |
| 5   | Soil Moisture Content                   | %             | 15.95               | 18.67                     |
|     | Chemical parameters                     |               |                     |                           |
| 6   | Total Nitrogen (N)                      | %             | 0.229               | 0.203                     |
| 7   | Available Phosphorus (P)                | %             | 6.58                | 10.20                     |
| 8   | Potassium (K)                           | %             | 0.311               | 0.312                     |
| 9   | Soil Organic Matter (SOM)               | %             | 0.88                | 0.28                      |
| 10  | Sulphate SO\(_{4}\)\(^{2-}\)           | mg/kg         | 0.026433            | 0.020025                  |
| 11  | Phosphate PO\(_{4}\)\(^{3-}\)          | mg/kg         | 0.00156             | 0.00167                   |
| 12  | Total Petroleum Hydrocarbon (TPH)       | mg/kg         | 66.8                | 5503                      |
| 13  | Polycyclic Aromatic Hydrocarbon (PAHs)  | mg/kg         | 5.0                 | 47.5                      |
|     | Heavy metals                            |               |                     |                           |
| 14  | Zinc (Zn)                               | mg/kg         | 1.00                | 6.90                      |
| 15  | Iron (Fe)                               | mg/kg         | 0.01                | 52.62                     |
| 17  | Lead (Pb)                               | mg/kg         | 0.01                | 0.12                      |

### 3.3 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.

### 3.4 Total Petroleum Hydrocarbon (TPH) Uptake in Plant Roots and Stem

Sampling for Plant roots and stem assay, physicochemical and chromatographic analysis of the polluted soil and Control (Unpolluted soil), Plant roots and stem were carried out at regular intervals (days 1, 60, 90, 120, 150, 180, 210 & 240) respectively. Ogbonna et al. [24].

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±50.34mg/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 (Table 3). Similar trend was observed in the control plots where TPH uptake by **Cyperus esculentus** Roots and Stem were; 24.2mg/kg, 2.00% and 20.01mg/kg, 1.32% respectively while in control plot of **Phyllanthus amarus** TPH uptake by Roots and Stem were 23.19mg/kg, 1.91% and 19.80mg/kg, 1.31% respectively (Table 3).

The highest uptake was found with **Cyperus esculentus** both in roots and stem analysis of the test plants (Table 3); 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. [25], who also found significant reduction of TPH by **Cyperus laxus** Lam. in 24 months when plants were cultivated on hydrocarbon-contaminated soil and spiked per litre.

The study also revealed that TPH absorbed/stored in plant stem are higher than that of plant roots. From initial contamination value of 5503mg/kg in soil, plant stem absorbed/stored 905.6mg/kg, 16.46% while plant roots absorbed/stored 711.7mg/kg, 12.93% (Fig. 1).
3.5 Sequential Analysis of TPH Uptake by Plant Roots and Stem in the Different Treatments Per Plot

TPH uptake in plant stem within 240 days period across the plots were: PS+Cyp (210.0mg/kg, 13.86%) > PS+MR+Cyp (201.0mg/kg, 13.26%) > PS+AN+MR+Cyp (192.4mg/kg, 12.70%) > PS+AN+MR+Phy (171.3mg/kg, 11.30%) > PS+AN+Phy (161.7mg/kg, 10.67%) > and lower values in Uncontaminated Control plots US+Cyp (20.01mg/kg, 1.32%) > US+Phy (19.81mg/kg, 1.31%) (Table 4, Fig. 2).

Basumatary et al [26] 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 not vegetated pots (P≤0.05).

3.6 Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAH) (mg/kg) Reduction in Soil

From the initial TPH contamination value of 5503.00mg/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+Phy (5451.30mg/kg; 99.06%) > PS+MR+Cyp (5448.30mg/kg; 99.01%) > PS+AN+MR+Cyp (5440.00mg/kg; 98.86%) > PS+AN+Phy (5422.905mg/kg; 98.54%) > PS+Cyp (no amendment) (5380.90mg/kg; 97.78%) (Table 5).
Table 4. 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) | % Phytoremediation (in Roots) | TPH (mg/kg) | % Phytoremediation (in Stem) |
|-------|-------------|-------------|-------------------------------|-------------|-------------------------------|
| P1    | US+ Phy     | 23.19       | 1.91                          | 19.8        | 1.31                          |
| P2    | US+Cyp      | 24.2        | 2.00                          | 20.01       | 1.32                          |
| P3    | PS+Cyp      | 210.4       | 17.36                         | 210         | 13.86                         |
| P4    | PS+AN+Phy   | 200.1       | 16.51                         | 161.7       | 10.67                         |
| P5    | PS+MR+Cyp   | 125.6       | 10.36                         | 201         | 13.26                         |
| P6    | PS+AN+MR+Cyp| 121         | 9.98                          | 192.4       | 12.70                         |
| P7    | PS+AN+MR+Phy| 115         | 9.49                          | 171.3       | 11.30                         |

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

Fig. 2. Total Petroleum Hydrocarbon (TPH) (mg/kg) uptake by Plant Roots and Stem during phytoremediation of crude oil polluted soil
Table 5. Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAHs) (mg/kg) reduction in Soil during Phytoremediation of crude oil polluted soils

| Plots   | Treatments | TPH (mg/kg) removal in Soil | % TPH Reduction in Soil | PAHs (mg/kg) removal in Soil | % PAHs Reduction in Soil |
|---------|------------|-----------------------------|-------------------------|----------------------------|--------------------------|
| P1      | US+ Phy    | 54.4                        | 0.12                    | 2.38                       | 0.86                     |
| P2      | US+Cyp     | 54.3                        | 0.12                    | 1.99                       | 0.88                     |
| P3      | PS+Cyp     | 5380.9                      | 12.33                   | 25.3                       | 11.23                    |
| P4      | PS+AN+Phy  | 5422.9                      | 12.43                   | 27.5                       | 12.21                    |
| P5      | PS+MR+Cyp  | 5448.3                      | 12.49                   | 29.5                       | 13.10                    |
| P7      | PS+AN+MR+Cyp | 5440                      | 12.47                   | 27.5                       | 12.21                    |
| P8      | PS+AN+MR+Phy | 5451.3                    | 12.50                   | 29.5                       | 13.10                    |

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

Table 6. Summary TPH & PAHs Removal and their %Reduction in soil

| Test plants          | TPH (mg/kg) removal in soil | %TPH Reduction in soil | PAHs (mg/kg) removal in soil | %PAHs reduction in soil |
|----------------------|----------------------------|------------------------|----------------------------|-------------------------|
| Cyperus esculentus   | 5492.73±76.36              | 98.55±0.67             | 25.77±2.12                 | 54.24±4.47              |
| Phyllanthus amarus   | 5449.72±18.27              | 99.03±0.34             | 28.72±2.74                 | 60.46±5.77              |

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)

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 240 days time compared to 60 and 120 days. Basumatary et al [26] also found similar result though theirs was at day 120. Kulakow et al. [27]; Radwan et al. [28] and Yateem et al. [29] also found enhanced degradation of petroleum hydrocarbons (PHCs) by using the plant-microbe interaction.

Assessment of Polycyclic Aromatic Hydrocarbon (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* + *Phyllanthus amarus* (29.5mg/kg; 62.11%) while least was recorded in Polluted soil + *Cyperus esculentus* (no amendment) (23.4mg/kg; 49.26%) (Table 5, Fig. 3)

Sequence evaluation of Polycyclic Aromatic Hydrocarbon (PAHs) from initial contamination value 47.5mg/kg , reduction in PAHs (amount remediated) and % PAHs Reduction in soil at 240 days in the different treatment plots in a decreasing order were: PS+AN+MR+Phy (29.5mg/kg; 62.11%) >PS+AN+MR+Cyp (27.5mg/kg; 57.89%) >PS+MR+Cyp (26.40mg/kg; 55.58%) >PS+AN+Phy (25.3mg/kg; 53.26%) >PS+Cyp (no amendment) (23.40mg/kg; 49.26%) (Table 5, Fig. 3).

Apart from biodegradation, a potential weathering process of Petroleum Hydrocarbon in soil is volatilization of low molecular weight, aliphatic, and aromatic compounds [30]. In the study, there was PAHs degradation in both amended and unamended plots but amended with augmenting 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 [31] and asphaltene group is the least biodegradable of all [32,33]. However, degradation study of separate hydrocarbon components (saturates, aromatics, asphalthins, and resins) will require long term monitoring of soil and plant development.
It was observed in this study that PAHs degradation/reduction in plots planted with *Phyllanthus amarus* (PAHs 28.72±2.74mg/kg; 60.46±5.77%) was higher than plots planted with *Cyperus esculentus* (PAHs 25.77±2.12mg/kg, 54.24±4.47%) (Table 6, Fig.3).

### 3.7 Other Monitored Physicochemical Assay

Table 7-9 shows the mean, standard deviation and regression values for the selected physicochemical parameters: Petroleum Hydrocarbon (TPH); Polycyclic Aromatic Hydrocarbon (PAHs), Total Nitrogen, Available Phosphorus, Potassium, Organic Carbon (TOC), Plant Height, Hydrogen ion concentration (pH), Electrical Conductivity (EC), Heavy Metals -Iron (Fe), Lead (Pb), Zinc (Zn) monitored during the phytoremediation study.

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 and 55.83±35.31 cm respectively in P4 and P7 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 P6 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 8)

Root structure is considered just as important as root biomass concerning degradation process [34]. 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.

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. Statistically there were no significant difference (p<0.05) in Hydrogen ion concentration (pH) in the various treatment plots but there were variation with highest value observed in Control -US+Cy (7.57±0.26) and least in PS+MR+Cy (7.46±0.32) while Electrical Conductivity (EC) (µs/cm) showed highest value in PS+AN+MR+Phy (340.29±40.32) with least value in PS+AN+Phy (233.86±38.61). Moisture Content (%) showed highest value in Control – US+Phy (1.25±0.32) with least value in PS+AN+Phy (0.90±0.28) (Table 7).

The values for Total Nitrogen (%) shows the unpolluted plots US+Phy and US+Cy having the highest (0.23±0.01) and Polluted plots PS+Cy, PS+AN+Phy, PS+MR+Cy, PS+AN+MR+Cy and PS+AN+MR+Phy having lowest value (0.22±0.05).Available Phosphorus (%) value showed that US+Phy had the highest (7.74±0.12) and US+Cyp (5.41±0.48) the lowest. Potassium (%) had highest value in PS+AN+MR+Phy (0.34±0.05) with least value in PS+Cyp (0.28±0.04). The value for TOC showed highest value in PS+AN+MR+Cyp (3.02±0.11) while Control- US+Phy (1.99±0.21) has the lowest percentage (Table 8). Similar trend was observed by Ogbonna *et al* [24] during bioremediation of Crude oil polluted soil using fish waste and goat manure as bio-organics and bacteria as bio-augmenters.

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; more so 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 for Iron (mg/kg) showed highest concentration in PS+MR+Cyp(53.88±11.38) and least in Control – US+Phy = US+Cyp (0.01±0.00) while Zinc had highest concentration in PS+AN+MR+Phy(4.78±2.64) with least value recorded in Control –US+Phy = US+Cyp (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 values (0.06±0.05) in two treatment plots: PS+AN+MR+Cy and PS+AN+MR+Phy. The least Lead values were found in Control - unpolluted soil (US) + *Phyllanthus amarus* = unpolluted soil + *Cyperus esculentus* (0.01±0.00) (Table 8). 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. [35].

Comparative average reduction in Total Petroleum Hydrocarbon (TPH-mg/kg) in soil during the phytoremediation evaluation, the plots showed: 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 + *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%). (Table 9). The differences in Total Petroleum Hydrocarbon (TPH) decrease in crude oil polluted and unpolluted soil/ plot treatments were significant (Table 9). However, the presence of plants resulted in significant decrease in TPH concentration at day 240. Merkl et al. [36] showed enhanced degradation of crude oil under the influence of a tropical grass after only a few months. Muratova et al. [37] showed total petroleum hydrocarbon (TPH) reduction up to 52% during 3 years of rye cultivation. Diab [38] recorded 30%, 16.8% and 13.8% reduction of TPH in rhizosphere soil of broad bean, corn and wheat respectively. In addition, Peng et al. [39] noted 41.61-63.2% removal of TPH by *Mirabilis jalapa*.

The variation may be due to various factors including Plant roots rhizosphere characteristics, interaction between plant roots and soil organisms, viscosity, nutrient present, environmental factors such as soil pH, Temperature etc. Basumatary et al (2012) also noted that, plant root exudates control the quality and quantity of microbial populations in the soil, therefore an altered plant metabolism caused by pollutants may have an effect. On the other hand, microorganisms also have a strong influence on the health conditions of plants.

In the present study, the test plants (*Phyllanthus amarus* and *Cyperus esculentus*) promoted degradation of hydrocarbon which may be due to the complexity of plant roots-microorganism interactions which is similar to the findings of Liste et al. [40] and Muratova et al. [37].

| Plot | Treatments | Physicochemical parameter |
|------|------------|---------------------------|
|      |            | pH                        | Electrical conductivity (ec) (s/cm) | Moisture content (%) | Total nitrogen (%) | Available phosphorus (%) | Potassium (%) |
| P1   | US+ Phy    | 7.49±0.31<sup>a</sup>     | 255.29±56.32<sup>b</sup>            | 1.25±0.32            | 0.23±0.00<sup>a</sup> | 7.74±0.12<sup>a</sup>   | 0.31±0.00<sup>a</sup> |
| P2   | US+Cyp     | 7.57±0.26<sup>a</sup>     | 281.57±24.58<sup>b</sup>            | 0.99±0.22            | 0.23±0.01<sup>a</sup> | 5.41±0.48<sup>b</sup>   | 0.31±0.00<sup>a</sup> |
| P3   | PS+Cyp     | 7.55±0.30<sup>a</sup>     | 276.71±44.11<sup>b</sup>            | 0.99±0.24            | 0.22±0.01<sup>a</sup> | 6.85±1.49<sup>b</sup>   | 0.28±0.04<sup>b</sup> |
| P4   | PS+AN+Phy  | 7.54±0.22<sup>a</sup>     | 233.86±38.61<sup>b</sup>            | 0.9±0.289            | 0.22±0.04<sup>a</sup> | 7.04±1.59<sup>b</sup>   | 0.29±0.04<sup>a</sup> |
| P5   | PS+MR+Cyp  | 7.46±0.32<sup>a</sup>     | 240.71±38.20<sup>b</sup>            | 0.99±0.26            | 0.22±0.05<sup>a</sup> | 7.26±1.42<sup>a</sup>   | 0.30±0.04<sup>a</sup> |
| P7   | PS+AN+MR+Cyp | 7.48±0.21<sup>a</sup>   | 293.57±59.67<sup>b</sup>            | 1.04±0.22            | 0.22±0.04<sup>a</sup> | 6.76±2.26<sup>a</sup>   | 0.32±0.05<sup>a</sup> |
| P8   | PS+AN+MR+Phy | 7.55±0.45<sup>a</sup>  | 340.29±40.32<sup>b</sup>            | 0.98±0.19            | 0.22±0.02<sup>a</sup> | 6.56±1.83<sup>a</sup>   | 0.34±0.05<sup>a</sup> |

**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
Fig. 3. Comparative analysis of Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAHs) (mg/kg) reduction in Soil during Phytoremediation of crude oil polluted amended soils

Table 8. Mean and Standard deviation of Physicochemical parameters (OC, Plant Height, Fe, Pb & Zn) during Phytoremediation of crude oil polluted soil

| Plot | Treatments | Physicochemical parameters |
|------|------------|---------------------------|
|      | % Organic Carbon | Plant Height (cm) | Iron (Fe) (mg/kg) | Lead (Pb) (mg/kg) | Zinc (Zn) (mg/kg) |
| P1   | US+ Phy    | 1.99±0.21a           | 36.40±13.03a      | 0.01±0.00c     | 1.00±0.00c      |
| P2   | US+Cyp    | 2.31±0.14a           | 41.13±18.20a      | 0.01±0.00c     | 1.00±0.00c      |
| P3   | PS+Cyp    | 2.41±0.28ab          | 39.77±16.22a      | 0.05±0.05a     | 4.28±2.83a      |
| P4   | PS+AN+Phy | 2.09±0.43a           | 52.47±27.50a      | 0.05±0.05ab    | 4.34±2.62a      |
| P5   | PS+MR+Cyp | 2.27±0.56a           | 42.67±22.07a      | 0.05±0.05ab    | 4.51±2.54a      |
| P6   | PS+AN+MR+Cyp | 3.02±0.11c       | 55.40±29.98a      | 0.06±0.05b     | 4.67±2.65a      |
| P7   | PS+AN+MR+Phy | 2.89±0.05c        | 51.37±31.23a      | 0.06±0.05b     | 4.79±2.64b      |

**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
4. CONCLUSION AND RECOMMENDATION

The phytoremediation rate of TPH and biodegradation of TPH in soil was higher in plots planted *Cyperus esculentus* than plots planted with *Phyllanthus amarus*. PAHs had the reverse trend with plots planted with *Phyllanthus amarus* having higher phytoremediation rate than plots planted with *Cyperus esculentus*.

It can also be concluded from this study that the amendment aided the degradation of the pollutant (Crude oil) which is readily available in the Niger Delta region, thus offers a potential option for crude oil remediation measure as plots with its application/treatment had 98.29% hydrocarbon reduction. The organic nutrients and test plants used in this study are readily available, natural, cost effective, eco-friendly and effective. This research provides a baseline data in crude oil pollution remediation and clean-up of polluted sites.

This research revealed and we recommend *Cyperus esculentus* as a suitable plant species for phytoremediation of crude oil contaminated/polluted soil with high TPH value while *Phyllanthus amarus* is the best option for phytoremediation of polluted soil with high PAHs value.

More so, based on our findings we recommend the use of ecofriendly bio-organic (biostimulants) and augmenting microbes as amendment option with phytoremediating plants to facilitate pollutant removal/clean up.

The use of *Cyperus esculentus* and *Phyllanthus amarus* as efficiency phytoremediation agents should be encouraged.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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