Current Journal of Applied Science and Technology

40(33): 32-49, 2021; Article no.CJAST.77222
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

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.

Article Information

DOI: 10.9734/CJAST/2021/v40i3331562
Editor(s):
(1) Dr. Orlando Manuel da Costa Gomes, Lisbon Polytechnic Institute, Portugal.
Reviewers:
(1) Theerawut Phusantisampan, King Mongkut's University of Technology, Thailand.
(2) Shaimaa Jassim AlSultany, University of Babylon, Iraq.
Complete Peer review History: https://www.sdiarticle4.com/review-history/77222

Original Research Article

Received 08 September 2021
Accepted 17 November 2021
Published 19 November 2021

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-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 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 physicochemical 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* (Phy). The rate of phytoremediation was estimated from percentage (%) uptake of Total petroleum hydrocarbon (TPH) in plant roots and stem from day 1 - 240; while percentage (%) reduction of TPH and PAHs in soil was estimated from day 1 to the residual at day 240.

Results: The test plants decreased significant amount of crude oil as revealed in TPH uptake in their roots and Stem. Mean amount and percentage Total Petroleum Hydrocarbon (TPH) uptake by *Cyperus esculentus r*oots and stem were; 152.33±50.34mg/kg, 12.57±4.16% and 201.13±8.80mg/kg, 13.27±0.58% respectively; while that of *Phyllanthus amarus* roots and stem were 141.50±35.62mg/kg, 11.68±2.94% and 174.44±19.98mg/kg, 11.51±1.32% respectively. Similar trend was observed in the control plots were TPH uptake by *Cyperus esculentus r*oots 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; 99.01%) >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%). 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 least was recorded in PS+ Cyp (no amendment) (23.4mg/kg, 49.26%).

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 *Phyllantus 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 augmentating 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-Kalio 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 sedge of the family *Cyperaceae* with a perennial life that grows up to 9m (3ft) at a fast rate. This specie 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 90^0 from each other. It is a common weed of cultivated and fallow ground, and an early colonize. 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 Euphobiaccea. 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, carbazoles, sulfoxides 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.

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 coastlands of southern Nigeria.it lies at the intersection of latitude $5^0$33N and longitude $5^0$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-Oroworukwo, Mile 3 Diobu area of Port Harcourt, Rivers State. The piece of land is situated at Longitude 4^o48'18.50''N and Latitude 6^o58'39.12''E measuring 5.4864m x 5.1816m with a total area of 28.4283m^2. This was cleared and sub-partitioned into seven blocks of 100cm x 50cm x 30cm giving 150,000cm^3 of soil in each plot.

The study tends to ascertain the level of efficacy in the use of the test plants in the remediation of crude oil polluted, bio-augumented soils.The test plants were *Cyperus esculentus* and *Phyllanthus amarus* and the soil augumented with *Aspergillus niger* and *Mucor racemusus* 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 remedy 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 & Germida [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-augmemting microbes *Asperigillus niger* and *Mucor racemosus* were added to some of the plots, the set-up were, 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 esculemtus*), Plot 4: PS + Asp+ Phy (Pollluted soil + *Asperigillus niger* + *Phyllanthus amarus*), Plot 5: PS + Muc+ Cyp (Polluted soil+ *Mucor racemosus*+ *Cyperus esculentus*), Plot 6: PS+ Asp+ Muc+ Cyp (Polluted soil+ *Asperigillus niger*+ *Mucor racemosus* + *Phyllanthus amarus*), Plot 7: PS+ Asp+ Muc+ Phy (Polluted soil + *Asperigillus 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 (150,000cm^3)	Crude Oil (2500ml) (2122.25 g)	Test Plants		Augmenting Microbes	
				Cyperus esculentus (Cyp)	*Phyllanthus amarus (Phy)*	*Aspergillus niger (Asp) broth (ml)*	*Mucor racemosus (Muc) Broth (ml)*
P1	*US+ Phy*	+	-	-	+	-	-
P2	*US+Cyp*	+	-	+	-	-	-
P3	*PS+Cyp*	+	+	+	-	-	-
P4	*PS+AN+Phy*	+	+	-	+	*750ml*	-
P5	*PS+MR+Cyp*	+	+	+	-	-	*750ml*
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 14days 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 Paticle 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 H_2O was added, then tube closed with a stopper and agitated on a mechanical shaker for 15minutes'then Allowed to stand for 1hour then returned back into the shaker for 2hrs.

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

$$\%\text{moisture content} \quad \frac{A-B}{B\text{-tare can}} \times 100\%$$

Moisture correction factor is obtained as follows

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

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 ASTDM 3921 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 $K_2Cr_2O_7$ was added, swirled gently and 20ml of Conc. H_2SO_4 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 1minute. Flask was allowed to stand for 30minutes while 200ml of Distilled water and 10ml Conc. H_3PO_4 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.

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).

$$Px = Fx - Ix \quad \ldots (1)$$

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

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

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

$$\%PR = (Px/Ics) \times 100 \quad \ldots. (2)$$

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, [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).

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

$$Bc = Ic - Fc \quad \ldots (3)$$

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

$$Rc = (Bc /Ic) \times 100 \quad \ldots (4)$$

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 sue 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 Physiochemical 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, 590uS/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 A*spergillus niger,* and *Mucor racemosus*.

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

3.4 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 23.19 mg/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).

Table 3. Sumary of Phytoremediation - TPH (mg/kg) Uptake by Plant roots and Stem

Test plants	TPH uptake by plant	% phyto-remediation
Cyperus esculantus roots	152.33±50.34	12.57±4.16
Phyllanthus amaus roots	141.50±35.62	11.68±2.94
Cyperus esculantus stem*	201.13±8.80	13.27±0.58
Phyllanthus amarus stem	174.44±19.98	11.51±1.32

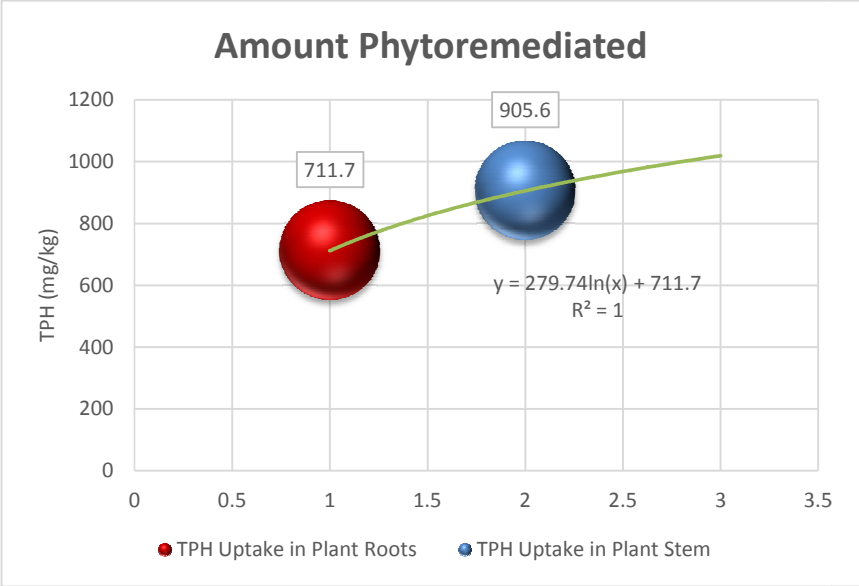

Fig. 1. Mean Total Petroleum Hydrocarbon (TPH) (mg/kg) uptake in Plant Stem and Roots during phytoremediation of crude oil poll

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 (19.8mg/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

Comparative % Phytoremediation in Plant Roots and Stem					
Plots	**Treatments**	**TPH (mg/kg) Uptake in Plant Roots**	**% Phytoremediation (in Roots)**	**TPH (mg/kg) Uptake in Plant Stem**	**% 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

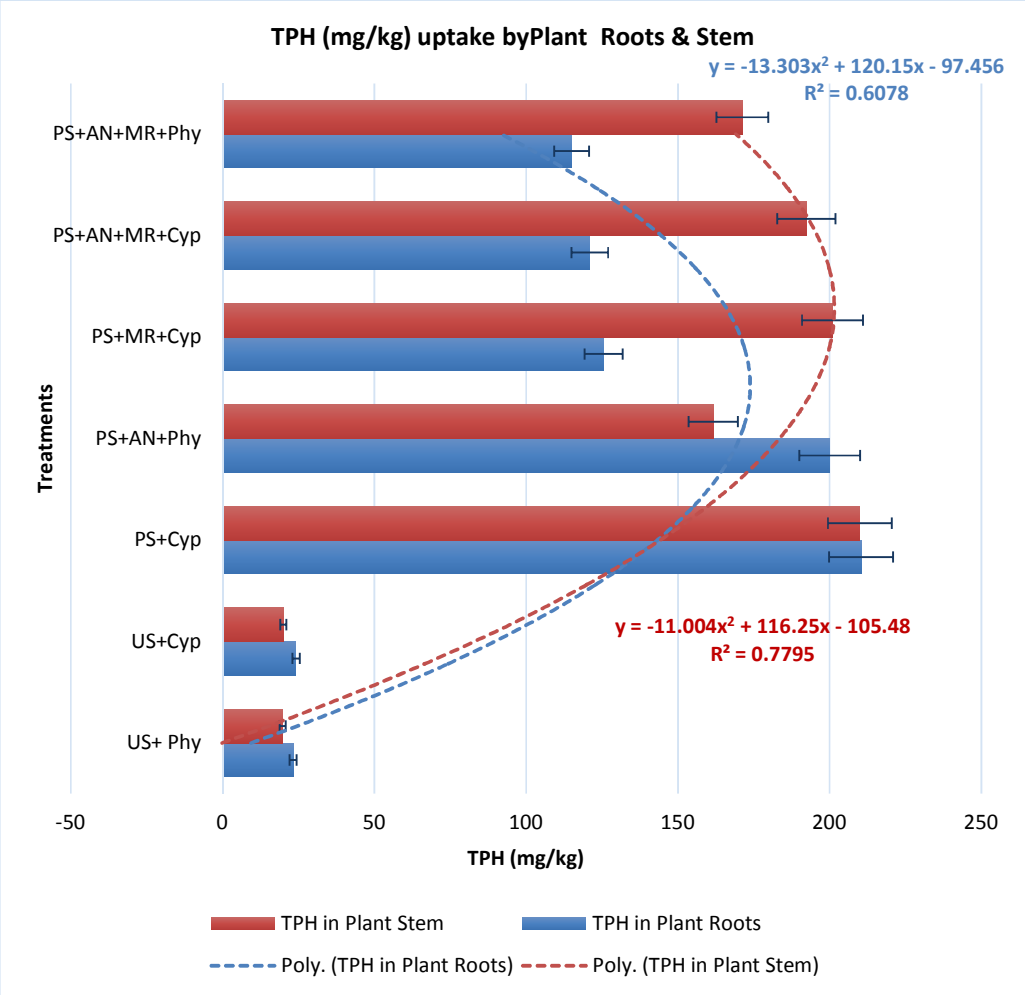

Fig. 2. Total Petroleum Hydrocarbon (TPH) (mg/kg) uptake by Plant Roots and Stem during phytoremediation of crude oil polluted soil

Table 5. Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAHs) (mg/kg) reduction in Soil during Phytoremediation of crude oil polluted soils

Comparative %TPH &%PAH reduction IN SOIL					
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	1.06
P2	US+Cyp	54.3	0.12	1.99	0.88
P3	PS+Cyp	5380.9	12.33	23.4	10.39
P4	PS+AN+Phy	5422.9	12.43	25.3	11.23
P5	PS+MR+Cyp	5448.3	12.49	26.4	11.72
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 esculantus	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 240days 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 Ploycyclic 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 aumentting 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 andP7 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+Cyp (7.57±0.26) and least in PS+MR+Cyp (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+Cyp having the highest (0.23±0.01) and Polluted plots PS+Cyp, PS+AN+Phy, PS+MR+Cyp, PS+AN+MR+Cyp 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 of 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 valves (0.06±0.05) in two treatment plots: PS+AN+MR+Cyp 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 rhizophere characteristics, interaction between plant roots and soil organisms viv-a vis nutrient present, environmental factors such as soil pH, Ranfall, 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].

Table 7. Mean and Standard deviation of Physicochemical parameters (pH, EC, MC, N, P & K) during Phytoremediation of crude oil polluted soil

Plot	Treatments	Physicochemical parameter					
		pH	Electrical conductivity (ec)(s/cm)	Moisture content (%)	Total nitrogen (%)	Available phosphorus (%)	Potassium (%)
P1	US+ Phy	7.49±0.31^{a}	255.29±6.32^{a}	1.25±0.32^{b}	0.23±0.00^{a}	7.74±0.12^{c}	0.31±0.00^{a}
P2	US+Cyp	7.57±0.26^{a}	281.57±24.58^{a}	0.99±0.22^{b}	0.23±0.01^{a}	5.41±0.48^{b}	0.31±0.00^{a}
P3	PS+Cyp	7.55±0.30^{a}	276.71±44.11^{a}	0.99±0.24^{b}	0.22±0.01^{a}	6.85±1.49^{a}	0.28±0.04^{b}
P4	PS+AN+Phy	7.54±0.22^{a}	233.86±38.61^{a}	0.9±0.289^{b}	0.22±0.04^{a}	7.04±1.59^{a}	0.29±0.04^{a}
P5	PS+MR+Cyp	7.46±0.32^{a}	240.71±38.20^{a}	0.99±0.26^{b}	0.22±0.05^{a}	7.26±1.42^{a}	0.30±0.04^{a}
P7	PS+AN+MR+Cyp	7.48±0.21^{a}	293.57±59.67^{a}	1.04±0.22^{b}	0.22±0.04^{a}	6.76±2.26^{a}	0.32±0.05^{a}
P8	PS+AN+MR+Phy	7.55±0.45^{a}	340.29±40.32^{b}	0.98±0.19^{b}	0.22±0.02^{a}	6.56±1.83^{a}	0.34±0.05^{a}

***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

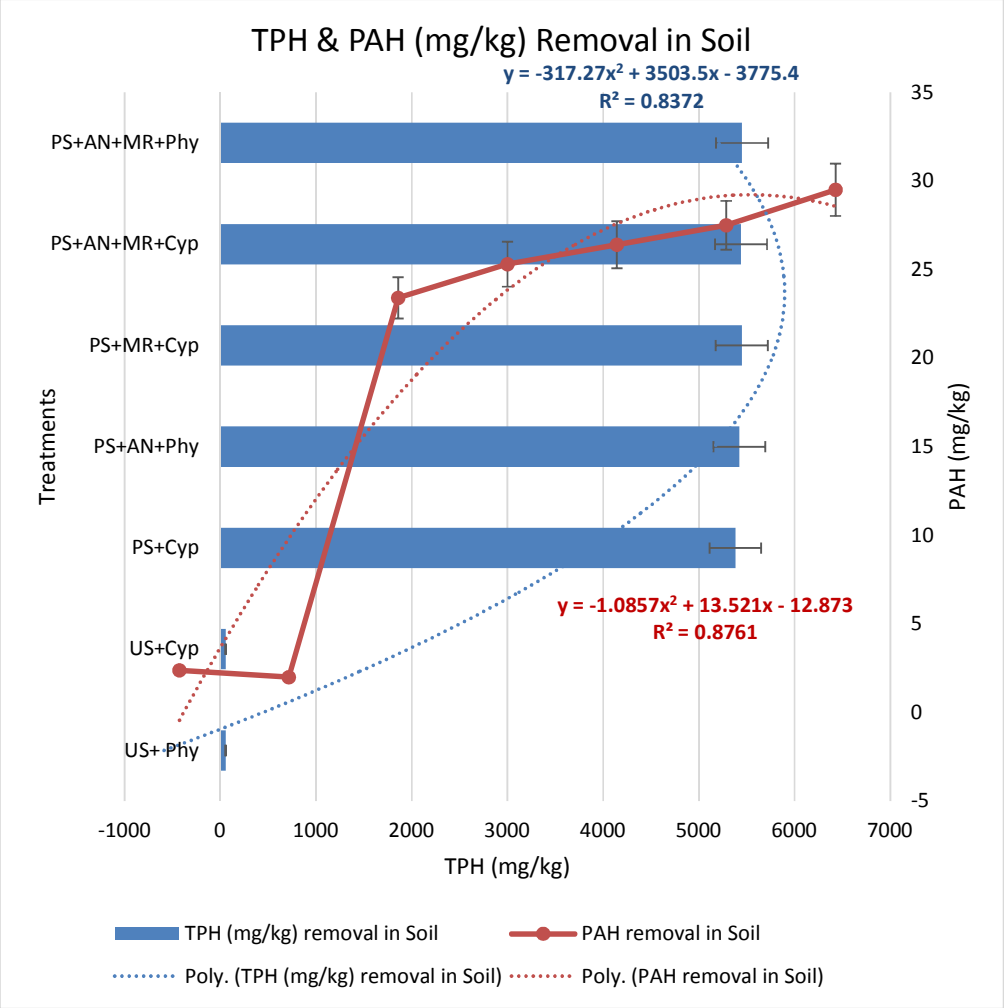

Fig. 3. Comparative analysis of Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbon (PAHs) (mg/kg) reduction in Soil during Phytoremediation of crude oil polluted amended soils

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±0.21^{a}	36.40±13.03^{a}	0.01±0.00^{c}	0.01±0.00^{c}	1.00±0.00^{c}
P2	US+Cyp	2.31±0.14^{a}	41.13±18.20^{a}	0.01±0.00^{c}	0.01±0.00^{c}	1.00±0.00^{c}
P3	PS+Cyp	2.41±0.28^{ab}	39.77±16.22^{a}	37.31±19.05^{b}	0.05±0.05^{a}	4.28±2.83^{b}
P4	PS+AN+Phy	2.09±0.43^{a}	52.47±27.50^{a}	44.88±7.88^{b}	0.05±0.05^{ab}	4.34±2.62^{b}
P5	PS+MR+Cyp	2.27±0.56^{a}	42.67±22.07^{a}	53.88±11.38^{b}	0.05±0.05^{ab}	4.51±2.54^{b}
P6	PS+AN+MR+Cyp	3.02±0.11^{c}	55.40±29.98^{a}	45.89±9.01^{b}	0.06±0.05^{b}	4.67±2.65^{b}
P7	PS+AN+MR+Phy	2.89±0.05c	51.37±31.23^{a}	38.34±8.49^{b}	0.06±0.05^{b}	4.78±2.64^{b}

***means with the same superscript along the columns are not significantly different (p>0.05).*
Key: US = Uncontaminated soil, PS = Polluted soil, Phy = Phyllanthus amarus, Cyp = Cyperus esculentus, AN = Aspergillus niger, MR = Mucor racemosus, SMS = Spent Mushroom Substrate

Table 9. Mean and Standard deviation of Physicochemical parameters (TPH & PAH) during Phytoremediation of crude oil polluted soil

Plot	TREATMENTS	PHYSICOCHEMICAL PARAMETER			
		TPH absorbed in Plant Roots (mg/kg)	TPH absorbed in Plant Stem (mg/kg)	TPH in Soil (mg/kg)	PAH in Soil (mg/kg)
P1	US+ Phy	16.43±9.43[a]	14.12±8.04[a]	27.46±22.68[a]	8.47±9.93[a]
P2	US+Cyp	16.98±9.76[a]	14.49±8.25[a]	28.206±22.53[a]	3.98±0.75[a]
P3	PS+Cyp	145.62±83.37[b]	156.62±88.22[b]	1216.22±2396.45[a]	37.18±9.74[c]
P4	PS+AN+Phy	136.56±81.12[b]	99.28±60.71[b]	1184.86±2413.99[a]	32.98±9.36[b]
P5	PS+MR+Cyp	78.20±48.16[b]	133.40±80.31[b]	1178.78±2417.45[a]	34.22±10.37[b]
P6	PS+AN+MR+Cyp	71.10±49.00[b]	90.58±71.40[ab]	1178.16±2417.76[a]	33.30±10.77[b]
P7	PS+AN+MR+Phy	62.10±47.01[a]	79.08±65.47[a]	1166.08±2424.51[a]	32.32±10.84[b]

***means with the same superscript along the columns are not significantly different (p>0.05).*
Key: US = Uncontaminated soil, PS = Polluted soil, Phy = Phyllanthus amarus, Cyp = Cyperus esculentus, AN = Aspergillus niger, MR = Mucor racemosus, SMS = Spent Mushroom Substrate

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.

REFERENCES

1. Daniel-Kalio LA, Samuel-Allaseh JO. Effect of Bonny Light Crude Oil Contamination of Soil, Soil Amendment and Successive Planting at Four-Week Intervals on the Health and Growth of Phyllantusamarus Schum & Thonn. Journal of Biological Science Research, 2006;1
2. Nrior RR, Jirigwa CC. Comparative bioremediation potential of Mucor racemosus and Paecilomyces variotii on crude oil spill site in Gio Tai, Ogoni land. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 2017;11(10):49-57
3. Nrior RR, Inweregbu AO. Bioremediation Potential of Pseudomonasaeruginosa KX828570 on Crude Oil Spill Polluted Marshland and Terrestrial Soil Treated with Oil Spill Dispersant. Journal of Advances in Microbiology 2019;15(2):1-15
4. Daniel-Kalio LA, Samuel-Allasseh JO. The sensitivity of yellow Nutsedge (Cyperus esclentus) Bonny Light Crude Oil in Niger Delta, Nigerian Environment Society (JNES), 2006;3:115
5. Sanchez-Zapata E, Fernandes-Lopez J, Angel PJ. "Tiger Nut (Cyperus esculentus) Commercialization: Health Aspects,

Composition, Properties, and Food Applications". Comprehensive Reviews in Food Science and Food Safety; 2012.
6. Ite AE, Semple KT. Biodegradation of Petroleum Hydrocarbons in Contaminated Soils. Microbial Biotecgnology Energy and Environment, R. Arora, ed., 2012;250-278,
7. Abii T, Nwosu A. The effect of oil spillage on the soil of Eleme in Rivers State of the Niger Delta area of Nigeria. Res. J. Environmental sci. 2009;3(3):316-320.
8. Achi SS, Shide EG. Analysis of Trace Metals by Wet Ashing And Spectrophotometric Techniques of Crude Oil Samples. Journal of Chemical Society of Nigeria 2004;29(11): 1-4
9. US DOD. Remediation technologies screening matrix and reference guide. DOD Environmental Technology Transfer Committee. MK01RPT, 2281012; 1994
10. Pivetz BE, "Phytoremediation of contaminated Soil and Ground Water at Hazardous Waste Sites. ManTech Environmental Resources Services Corporation, Ada, 2001;36.
11. Siciliano SD, Germida JJ. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria. Environ. Rev., 1998;6: 65-79.
12. Shen HZ, Haung Y, Wang R, Zhu D, Li W, Shen GF, Tao S. Global atmospheric Emissions of Polycyclic Aromatic Hydrocarbons from 1960 to 2008 and future Predictions. Environmental Science and Technology, 2013; 47:6415-6424.
13. Ayotamuno MJ, Kogbara RB, Ogaji SOT, Probert SD. Bioremediation of Crude Oil Polluted Agricultural Soil Port Harcourt, Nigeria. Journal of Applied Energy 2006; 83(2):1249-1259
14. Efe SI, Elenwo EI Phytoremediation of Crude Oil Contaminated Soil with Axonopus compressus in the Niger Delta Region Nigeria Effects of oil spill/green living tips; 2014.
15. Montgomery DC. Design and Analysis of Experiments (4th edition), New York: John Wiley and Sons; 1997.
16. Ogbonna DN. Application of Biological Methods in the Remediation of Oil Polluted Environment in Nigeria Journal of Advances in Biology and Biotechnology 2018;1119
17. Juo ASR, Fox RLPhosphorus sorption characteristics of some bench-mark soils of West Africa. Soil Sci. 1977;124:370-376.
18. Eckert D, Sims JT. "Recommended Soil pH and Lime Requirement Tests ",In: J.T. Sims and A. Wolfe, Eds., Recommended Soil Testing Procedures for the Northeastern United States. Northeast Regional Bullentin 493, Agriculture Experiment Station, University of Delaware, Newark, 1995;11-16.
19. Bremmer JM, Mulavaney CS. Nitrogen-Total. In Methods of soil analysis, American Society of Agronomy, Madison, Wsc. USA. NO9, Part2 1982;595-624
20. USEPA (U.S. Environmental Protection Agency): Test Methods for Evaluating Solid and Hazardous Waste. USEPA, Washington, DC; 1994.
21. API Publication Number 4599: "Inter-laboratory study of three methods for analyzing petroleum hydrocarbons in soils, diesel-range organics (DRO), gasoline-range organics (GRO), petroleum hydrocarbons (PHC), June; 1994
22. Chikere CB, Chikere BO, Okpokwasili GC. Bioreactor-based bioremediation of hydrocarbon-polluted Niger Delta marine sediment, Nigeria. 3 Biotech, 2012;2(1): 53–66.
https://doi.org/10.1007/s13205-011-0030-8
23. Nrior RR, Mene GB. Assessment of bioaugmentation efficiency of Penicillium chrysogenum and Aspergillus nudilans in bioremediation of crude oil spill soil. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 2017; 11(8):01–09.
24. Ogbonna DN, Ngah, SA, Okparanma, RN, Ule O, Nrior RR. Percentage Bioremediation Assessment of Spent Mushroom Substrate (SMS) and Mucor racemosus in Hydrocarbon Contaminated Soil. Journal of Advances in Microbiology (JAMB), 2020;20(12):1-21.
25. Lopez-Martinez S, Gallegos-Martinez ME, Perez-Flores LJ, Gutierrez-Rojas M. Contaminated soil phytoremediation by Cyperus laxus Lam. cytochrome P450 Erod-activity induced by hydrocarbons in roots. Int. J. Phytorem., 2008;10: 289-301.
26. Basumatary B, Saikia R, Bordoloi S. Phytoremediation of crude oil contaminated soil using nut grass, Cyperus rotundus Journal of Environmental Biology 2012;33:891-896
27. Kulakow PA, Schwab AP, Banks MK. Screening plant species for growth on weathered, petroleum hydrocarbon

contaminated soils. Int. J. Phytorem. 2000; 2:297-317.
28. Radwan SS, Dashti N, El-Nemr IM, Khanafer M. Hydrocarbon utilization by nodule bacteria and plant growth promoting rhizobacteria. Int. J. Phytorem., 2006,9:475-486.
29. Yateem A, Al-Sharrah T, Bin-Haji A. Investigation of microbes in the rhizosphere of selected trees for the rhizoremediation of hydrocarbon-contaminated soils. Int. J. Phytorem. 2008; 10:311-324.
30. Potter TL, Simmons KE. Composition of petroleum mixtures. Total Petroleum Hydrocarbon Criteria Working Group Series. Amherst, MA, Amherst Sci. Publ. 1998;2
31. Diaz-Ramirez IJ, Saad HR, Rojas MG, Favela TE. Biodegradation of Maya crude oil fractions by bacterial strains and a defined mixed culture isolated from Cyperus laxus rhizosphere soil in a contaminated site. Can. J. Microbiol. 2003; 49:755-761.
32. Harayama S. Polycyclic aromatic hydrocarbon bioremediation design. Curr. Opin. Biotechnol., 1997;8:268-273.
33. Garcia-Rivero M, Saucedo-Castaneda G, Flores de Hoyos S, Gutierrez-Rojas M. Mass transfer and hydrocarbon biodegradation of aged soil in slurry phase. Biotechnol. Progr., 2002;18:728-733.
34. Wiltse CC, Rooney WL, Chen Z, Schwab AP, Banks MK. Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. J. Environ. Qual., 1998;27:169-173.
35. Ule O, Ogbonna DN, Okparama RN and Nrior RR. Myco-enhanced Bioremediation in Open Field Crude Oil Contaminated Soil Using Mucor racemosus and Aspergillus niger. Current Journal of Applied Science and Technology 2021; 40(1):119-141.
36. Merkl N, Schultze-Kraft R, Infante C. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. Water Air Soil Pollut., 2005;165:195-209.
37. Muratova AY, Dmitrieva TV, Panchenko LV, Turkovskaya OV. Phytoremediation of oil-sludge contaminated soil. Int. J. Phytorem. 2008; 10:486-502.
38. Diab IA. Phytoremediation of oil contaminated desert soil using the rhizophere effects of some plants. Res. J. Agric. Biol. Sci., 2008; 4:604-610.
39. Peng SW, Zhou QX, Zhang H, Shi RG. Responses of seed germination of 8 ornamental plants to petroleum contamination. Chinese J. Environ. Sci. 2009; 29:786-790.
40. Liste HH, Prutz I. Plant performance, dioxygenase-expressing rhizosphere bacteria, and biodegradation of weathered hydrocarbons in contaminated soil. Chemosphere. 2006; 62:1411-1420.

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