



## Screening Various Native Plants and their Associate Microorganisms for Potential Bioremediation of Heavy Metals and Crude Oil-Polluted Soil

Ikeanumba, Michae Okwudiri<sup>1</sup>, Orji, Justina Chibuogwu<sup>2</sup>, Nweke, Christian Okechukwu<sup>3</sup>, Adieze, Ifechukwu Enyinnaya<sup>4</sup>

<sup>1</sup> Biology Department, Alvan Ikoku Federal University of Education, Owerri, Nigeria

<sup>2</sup> Microbiology Department, Federal University of Technology, Owerri, Nigeria

<sup>3</sup> Microbiology Department, Federal University of Technology, Owerri, Nigeria

<sup>4</sup> Microbiology Department, Federal University of Technology, Owerri, Nigeria

### ABSTRACT

The study investigates the potential of native plants and their associated microorganisms for tolerance to different concentrations of crude oil (3%, 7%, and 10%) and heavy metals (Zn = 39.4, Ni = 10.20, Cu = 29.40 and Pb = 11.20) polluted soils. A total of eight (8) native plants species (*Brachiaria distachyoides* Stapf, *Cyperus dichrostachyus* Hochst. ex A. Rich, *Kalanchoe pinnata* (Lam.) Pers, *Panicum maximum* Jacq, *Mimosa pudica* L, *Paspalum conjugatum* P.J. Bergius., *Mariscus rotundus* and *Mariscus ligularis* L.) were sourced from crude oil-impacted sites and assayed for tolerance to different concentrations of pollutants). Results obtained showed that *Paspalum conjugatum* P.J. Bergius gave the best result, thriving in 10% crude oil and heavy metal-contaminated soil for the five months' duration of the study, followed by *Mariscus ligularis* L. and *Brachiaria distachyoides* Stapf which survived for 30 days under the same conditions. Other species were only able to tolerate 7% crude oil heavy metals. Results obtained from microbiological assay of soil sample shows showed that total heterotrophic bacterial (THB) count was  $3.4 \times 10^8$  CfU/g soil, HUB =  $4.6 \times 10^6$  CfU/g soil, percentage heterotrophic bacterial that are hydrocarbon utilizers = 1.35%. Molecular analysis of the microbial isolates identified *Pseudomonas xiamenensis*, *Acinetobacter baumannii*, *Alcaligenes cloacae*, *Enterobacter cloacae*, *Pantoea dispersa*, *Lysinibacillus fusiformis*, and *Kocuria palustris* as hydrocarbon-degrading bacterial species while the hydrocarbon degrading fungi species are *Penicillium* spp, *Aspergillus* spp, and *Fusarium* This study shows that plants differ in their susceptibility or otherwise to crude oil and heavy metals polluted soil.

Keywords: Bioremediation, Phytoremediation, heterotrophic bacterial and hydrocarbon utilizing Bacteria

### 1. Introduction

Over the past 50 years, the petroleum and gas industries in Nigeria have released significant quantities of hydrocarbons and associated pollutants, including heavy metals, into the Niger Delta environment from both refined and unrefined petroleum products (Obot et al., 2006; UNEP, 2011). Pollution levels have severely impacted the environment, particularly in soil and water bodies, leading to a significant decline in both terrestrial and aquatic biodiversity, as well as disruptions to public health and the life support systems of local communities. The dynamic field of bioremediation has emerged as a mainstream method for repairing and restoring contaminated environments, driven by the global demand for environmentally friendly solutions. Over the years, numerous studies have been published in this area, showcasing significant advancements in the treatment of various contaminants through both laboratory and field research (Barker & Bryson 2002; Ceccanti, Masciandaro, Garcia, Macci, Doni, 2006). Phytoremediation involves the use of plants and their associated microorganisms to assimilate, transform, metabolize, detoxify, and degrade various toxic inorganic and organic compounds, such as petroleum hydrocarbons (PHCs), pesticides, dyes, and solvents, in soil, water, groundwater, and air (Kabra, Khandar, Waghmode, & Govindwar, 2012; Prasad, Freitas, Fraenzle, Wuenschmann, & Markert, 2010). Native plants, well-adapted to their local environmental conditions, offer a viable resource for phytoremediation in polluted soils. Bacteria equipped with catabolic genes and enzymes can utilize complex compounds present in petroleum mixtures as energy sources, enabling the decomposition of petroleum hydrocarbons (PHCs) (Das & Chandran, 2011; Rojo, 2009). Several bacterial strains, such as *Pseudomonas*, *Acinetobacter*, *Mycobacterium*, *Haemophilus*, *Rhodococcus*, *Paenibacillus*, and *Ralstonia*, have been extensively studied for their capacity to degrade hydrocarbons (Tyagi, da Fonseca, & de Carvalho, 2011).

This study aims to identify native plant species and associated microbial communities that demonstrate strong phytoremediation potential in soils contaminated by heavy metals and crude oil.

## 2. Materials and Methods

### Soil and Soil Pollution

The soil samples for this study are (i) uncontaminated soil (ii) contaminated soil through simulation. Uncontaminated soil sample was collected from the back of Okemini Naval barrack, Rumuolumini, in Obio Akpor Local Government area of Rivers State (this site is not known to be polluted with petroleum hydrocarbon or by any other industrial pollutants), dried and sieved and stored in plastic bottles and covered with stoppers until analysis. The crude oil type that was used for this work, was Bonny light crude oil obtained from the Port Harcourt Refinery Company Limited Eleme, in Rivers State. Uncontaminated soil obtained as stated above was polluted using method adopted by Adieze, Orji, Nwabueze, & Onyeze, (2012).

### Water sample pollution with heavy metals

Soil samples were polluted with solution of heavy metals' respective salts with concentrations greater than Federal Ministry of Environment (FME) maximum permissible limits for discharge waste water effluent. into Nigerian environment. The following were the salts of heavy metals used for the pollution process with fixed concentrations of heavy metals (Zn, Cu, Ni and Pb) by dissolving their respective hydrated salts ( $ZnSO_4 \cdot 7H_2O$ ,  $CuSO_4 \cdot 5H_2O$ ,  $Ni(NO_3)_2 \cdot 6H_2O$  and  $Pb(NO_3)_2$ ) in water to achieve concentrations above the maximum Nigerian Federal Ministry of Environment permissible limits for discharge waste water effluent into Nigerian environment as (Federal Ministry of Environment (FME), 1992).

### Soil pollution

Uncontaminated soil obtained as stated above was polluted using method adopted by Adieze, Orji, Nwabueze, & Onyeze, (2012). The crude oil was dissolved in acetone (3:1), and mixed with 10% of total soil. The crude oil laddered soil which served as the stock was added to the bulk of the soil and mixed with heavy metals laddered distilled water to obtain the final concentrations of 3% (30 g/kg), 7% (70 g/kg) and 10% (100 g/kg) crude oil and heavy metals (Zn = 39.4 mg/l, Ni = 10.2 mg/l, CU = 29.4 mg/l and PB = 11.2 mg/l) in the soil. The mixed crude oil enriched soil and heavy metals was stirred several times for 2 days to remove acetone (Adieze et al., 2012).

### Plant selection

Several native plant species were selected based on their prevalence and survival in crude oil contaminated sites. A total of eight (8) different plant species were screened for their ability to tolerate crude oil (i.e., experimental concentrations), heavy metals pollution (concentration as shown in table 1) and for their tolerance to wetland environment. They were screened for their ability to tolerate 3%, 7% and 10% crude oil and heavy metals.

### Plant identification

Selected plants for preliminary studies were taken to University of PortHarcourt, Faculty of Science, Department of Plant Science and Biotechnology. The plants were identified by Dr. Ekeke, Chimezie a specialist in plant taxonomy in charge of University of Port Harcourt reference herbarium for research and germplasm conservation section of the department.

### Measured criteria for plants species for tolerance to crude oil and heavy metals in wetland soil

The screening was carried out in a greenhouse and involved the screening of eight (8) different species of plants for tolerance to different concentrations of crude oil and heavy metals pollutants in the CW soil. The protocols for selection of heavy metals and crude oil tolerant plant species involved the determination of the following plant growth indices;

Plants' shoot height,

Plants' leaf width and,

Plants' total biomass (wet weight)

### Seedlings preparation

Seedlings of plants to be screen for their heavy metals accumulation and phytoremediation of crude oil pollution abilities were planted into an uncontaminated soil (100 g) in a perforated transparent polythene bag of 10.5 x 25 cm and then transferred into a black ornamental polyethylene bag measuring 29 x 59 cm containing 3 kg of soil contaminated with heavy metals and crude oil of various experimental concentrations. The concentrations of crude oil pollution were 3%, 7% and 10% and while that of heavy metals were as stated in table 1 respectively. The eight plants species under investigation were planted each in a separate ornamental polythene bag containing soil of different concentrations of crude oil pollutant but the same concentrations of heavy metals as stated in Table 1 above. The total experimental setup was made up of twenty-six (26) ornamental polythene bag with twenty-four (24) containing eight (8) plants in three (3) different treatments with different concentrations (3%, 7% and 10%) of crude oil and heavy metal pollutants, while eight (8) were unpolluted control for each of the eight (8) plants and three (3) containing polluted soil only without any plant and one control (1) ornamental bag without polluted soil). This set up served to determine the effect of microorganisms and natural attenuation on the pollutants.

### Determination of crude oil and heavy metal concentrations' tolerance limit for the selected plants

The plant that exhibited the best traits as listed in section 3.3.3 from experiment in Table 4 was further subjected to 12% concentrations of crude oil with the fixed concentrations of heavy metals remaining the same. The experimental protocols are shown below in Table 5.

## Sample analysis

Estimation of plant's performance

### Estimation of plant's performance

(i) Measurement of root length

Plant with the highest tolerance to the pollutants was destructively harvested and the shoot severed from the base of the plant. The root was washed three times with tap water and allowed to dry at atmospheric temperature for two hours. Root lengths was measured using a meter rule. All analysis in this work were done in duplicates.

(ii) Measurement of plants' height

Plant's shoot height was measured from the shoot base to the apical tip using a meter rule at intervals of 30 to 150 DAP. All the measurements were carried out at the same intervals and concurrently.

(ii) Measurement of plants' biomass

Plants whose shoot height had been measured were from the soil level carefully uprooted and washed with tap water. The wet weight of the plants were measured and recorded using CW-X (0.1g accuracy) electronic weighing machine, made in China. (Merkl et al., 2005). This exercise was performed using two (2) replicate potted plants of each treatment chosen randomly.

(iv) Plant's susceptibility to crude oil (phytotoxicity)

The plant's susceptibility to crude oil phytotoxicity was estimated by a comparison of test plant's biomass (biomass of plants grown in various concentrations of crude oil and heavy metals soil) and their control plant's biomass (biomass in unpolluted soils). Test plant's biomasses were recorded as percentages of their control plant's biomasses.

(v) Evaluation of plant's response to stress

The method adopted by Kage, Kochler, & Stützel, (2004) was employed in evaluating the response of the plants to the stress from the crude oil and heavy metals contaminants soil. Plants whose weights were less than 25% of their control plant's weight were considered strongly susceptible, and higher than 50% as tolerant.

### Percentage performances of shoot length, root length, wet weight and leaf size of test plants

Plant growth characteristics/ performance was determined every four (4) weeks for twenty-four (24) weeks for shoot length, root length, wet weight and leaf size.

The formula below was used to calculate the percentage performance of the measured parameters in comparison to that of the seedlings of the different plants at day 0;

$$\frac{X_i - X_o}{X_o} \times 100$$

Where  $X_o$  – seedling (shoot length, root length, wet weight and leaf size) at day 0

$X_i$  - plant (shoot length, root length, wet weight and leaf size) at day t

### Physicochemical and microbiological analysis of soil samples

The soil samples for physicochemical analysis were first air dried after collection, and sieved through a 2 mm mesh, stored in covered plastic bottles until ready for analysis.

(i) Particle size

Particle size was carried out using standard sieves for sand and gravel fractions and pipette analysis for the mud (silt and clay) fraction according to the procedures outlined by Folk (1974).

(ii) Soil pH

pH of the soil was determined based on the modified method of Mc Lean (1982).

(iii) Phosphorus

Available phosphorus was determined using the modified Bray No.1 method Olsen & Sommers, (1982).

(iv) Total nitrogen

Total nitrogen was determined using the macro Kjeldahl method of Walkley & Black (1934).

(v) Organic carbon

Organic carbon was measured using modified Walkley & Black method (1934)

#### Microbiological analysis

Microbial populations in the soil samples were assayed by standard plate count technique. The total aerobic heterotrophic culturable microbial populations present in the soil samples during the study were estimated by spread plate techniques of Pelczar & Chan, 1977.

Enumeration and isolation of hydrocarbon utilizing species was determined by the modified method of Okpokwasili & Amanchukwu, (1988). Characterization of the isolates followed the procedures in the Bergey's manual of determinative bacteriology (Holt, 1994).

#### Molecular identification of isolated hydrocarbon utilizing strains

##### Bacterial genomic DNA extraction

Total DNA isolation and isolates identification were carried out at Nucleomatrix Molecular Laboratory, Yengoa, Bayelsa State. The method as described by Saitou & Nei, (1987), was adopted for molecular identification of isolates

##### DNA quantification

The extracted genomic DNA as described above was quantified using the Nanodrop 1000 spectrophotometer.

##### 16S rRNA Amplification

The 16s RRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles.

##### Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

##### Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN.

**Table 1: Respective concentrations of heavy metals in constituted polluted soil samples and their FME permissible limits in soil.**

Metals	Exp Conc. (mg/l)	Limit for agric. land (mg/l)* (NFME, 1992)	Limit for nonagric. land (mg/l)* (NFME, 1992)
Zn	39.4	5.0	0.03
Ni	10.2	0.2	0.2
Cu	29.4	1.0	4.0µ/l
Pb	11.2	0.05	1.8µ/l

**Table 2: Experimental set up for the for the selection of the plant that shows the highest tolerance to crude oil and heavy metals pollution**

Treatment	Purpose	Number of pots
Polluted soil only	Effect of microorganisms on pollutants	1
Unpolluted soil +plants	Control	1
Crude oil polluted soil (3%) + plants+ heavy metals	Effect of 3% crude oil and heavy metals on the different species of plants	8
Crude oil polluted potted soil (7%) + plants+ heavy metals	Effect of 7% crude oil and heavy metals on the different species of plants	8
Crude oil polluted potted soil (10%) + plants+ heavy metals	Effect of 10% crude oil and heavy metals on the different species of plants	8

**Table 3: Plant species used for the study and their scientific and English name**

Scientific name	English name
<i>Brachiaria distachyoides</i> Stapf,	Signalgrass
<i>Cyperus dichrostachyus</i> Hochst. ex A. Rich	African nut sedge
<i>Kalanchoe pinnata</i> (Lam.) Pers	Cathedral bells, Air plant, Life plant, Miracle leaf, Goethe plant or love bush
<i>Panicum maximum</i> Jacq	Guinea grass
<i>Mimosa pudica</i> L	Sensitive Plant, Sleepy plant, Touch-me-not or Shameplant
<i>Paspalum conjugatum</i> P.J.Bergius	Crab grass
<i>Mariscus rotundus</i>	Purple nutsedge, Nut grass or Red nut sedge
<i>Mariscus ligularis</i> (L.)	Swamp flat sedge

**Table 4: Experimental set up for the preliminary study for the selection of test plants in response to hydrocarbon and heavy metals pollution**

Treatment	Purpose	Number of pots
Polluted soil only	Effect of microorganisms on pollutants	1
Unpolluted soil +plants	Control	1
Crude oil polluted soil (3%) + plants+ heavy metals	Effect of 3% crude oil and heavy metals on the different species of plants	8
Crude oil polluted potted soil (7%) + plants+ heavy metals	Effect of 7% crude oil and heavy metals on the different species of plants	8
Crude oil polluted potted soil (10%) + plants+ heavy metals	Effect of 10% crude oil and heavy metals on the different species of plants	8

**Table 5: Experimental set up for determination of crude oil and fixed heavy metal tolerance limits for the selected plants**

Treatment	Purpose
Unpolluted soil + plants	Control
Crude oil polluted soil (12%) + heavy metals + plants	The effect of 12% crude oil and heavy metals on the 2 plant species with the best growth performances in 3,7and 10% as described in table 2.

### 3.0 Results

**Table 6: Physicochemical and microbiological characteristics of unpolluted soil sample**

Soil parameters	Values in soil
(i) physicochemical	
Sand	47.3%
Silt	27.7%
Clay	23.0%
Texture	Loamy soil
Organic matter	45,000 mg/kg or 0.45%
Organic carbon	65,000 mg/kg or 0.65%
Total nitrogen	18,000mg/kg or 0.18%

Available phosphorous	79.82 mg/kg or 0.80%
pH	7.16
(ii) microbiological	
Total heterotrophic bacterial count	$3.4 \times 10^8$ Cfu/g soil
Hydrocarbon utilizing bacterial count	$4.6 \times 10^6$ Cfu/g soil
% heterotrophic bacterial that are hydrocarbon utilizers	1.35%
Total fungal count	$1.5 \times 10^4$ Cfu/g soil
Hydrocarbon utilizing fungal count	$0.9 \times 10^3$ Cfu/g soil
% fungi that are hydrocarbon utilizers	6%

#### Plant of choice for the study

Based on the outcome of the preliminary study, *Paspalum conjugatum* P.J. Bergius was the plant of choice. Table 7-9 showed that although the experimental conditions resulted in significantly lowering growth rates across all measured parameters compared to the control group. However, the persistent green color of the leaves indicated that these conditions are not causing severe nutrient deficiencies though measured parameters were affected but their tolerance to the maximum pollutant concentration used for this study superseded that of other plants species used for this study.

*Arthrobacter* spp., *Agrobacterium* spp., *Flavobacterium* spp., *Corynebacterium* spp., *Mycobacterium* spp., *Rhodococcus* spp., *Arthrobacter* spp., *Acinetobacter* spp., *Enterobacter* spp., *Rhizobium* spp., *Alcaligenes* spp., *Micrococcus* spp., *Pseudomonas* spp., *Bacillus* spp., *Erwinia* spp.

#### Biochemical identification of bacterial and fungal isolates

Biochemical characterization and probable identity of hydrocarbon utilizing bacterial (HUB) isolates

*Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp., *Alcaligenes* spp., *Erwinia* spp., *Bacillus* spp., *Micrococcus* spp., *Microsporium* spp.

#### Morphological and biochemical characteristics and probable identity of fungal isolates

*Penicillium* spp., *Mucor* spp., *Aspergillus* spp., *Fusarium* spp., *Candida* spp., *Microsporium* spp.

Table 7: Morphological and biochemical characteristics and probable identity of hydrocarbon utilizing fungal isolates from pollutes soil sample

S/N	Isolate	Type of hyphae	Type of spore	Sucrose fermenter	Maltose fermenter	pigmentation
G1	<i>Penicillium</i> spp.	sepatate	Smooth conidiophore	–	–	green
G2	<i>Aspergillus</i> spp.	septate	Smooth chain conidiophore	–	–	black
G3	<i>Fusarium</i> spp.	septate	Oval	–	–	white

Table 8: Percentage occurrence of different heterotrophic bacteria isolates

S/N	Organism	Frequency	% Occurrence
1	<i>Arthrobacter</i> spp.	iiii	14.29
2	<i>Agrobacterium</i> spp.	iii	8.57
3	<i>Flavobacterium</i> spp.	iiii	11.42
4	<i>Corynebacterium</i> spp.	iii	8.63
5	<i>Mycobacterium</i> spp.	i	2.86
6	<i>Rhodococcus</i> spp.	iiii	11.42
7	<i>Acinetobacter</i> spp.	i	2.85
8	<i>Enterobacter</i> spp.	ii	5.71
9	<i>Pseudomonas</i> spp.	ii	5.71

10	<i>Alcaligenes</i> spp.	ii	5.71
11	<i>Erwinia</i> spp.	i	2.85
12	<i>Bacillus</i> spp.	i	2.85
13	<i>Micrococcus</i> spp.	ii	5.71
14	<i>Rhizobium</i> spp.	iiii	11.42
	<b>Total</b>	<b>35</b>	<b>100.00</b>

Table 9: Molecular equivalent of biochemical identified hydrocarbon utilizing bacteria (HUB) isolates

S/N	Probable identity of isolates from biochemical tests	Molecular identity of isolate
1	<i>Pseudomonas</i> spp.	<i>Pseudomonas xiamenensis</i>
2	<i>Acinetobacter</i> spp.	<i>Acinetobacter baumannii</i>
3	<i>Alcaligenes</i> spp.	<i>Alcaligenes cloacae</i>
4	<i>Enterobacter</i> spp.	<i>Enterobacter cloacae</i>
5	<i>Erwinia</i> spp.	<i>Pantoea dispersa</i>
6	<i>Bacillus</i> spp.	<i>Lysinibacillus fusiformis</i>
7	<i>Micrococcus</i> spp.	<i>Kocuria palus</i>

Percentage performances of shoot length, root length, wet weigh and leave size of study plants in 10% crude oil with fixed concentration of heavy metals

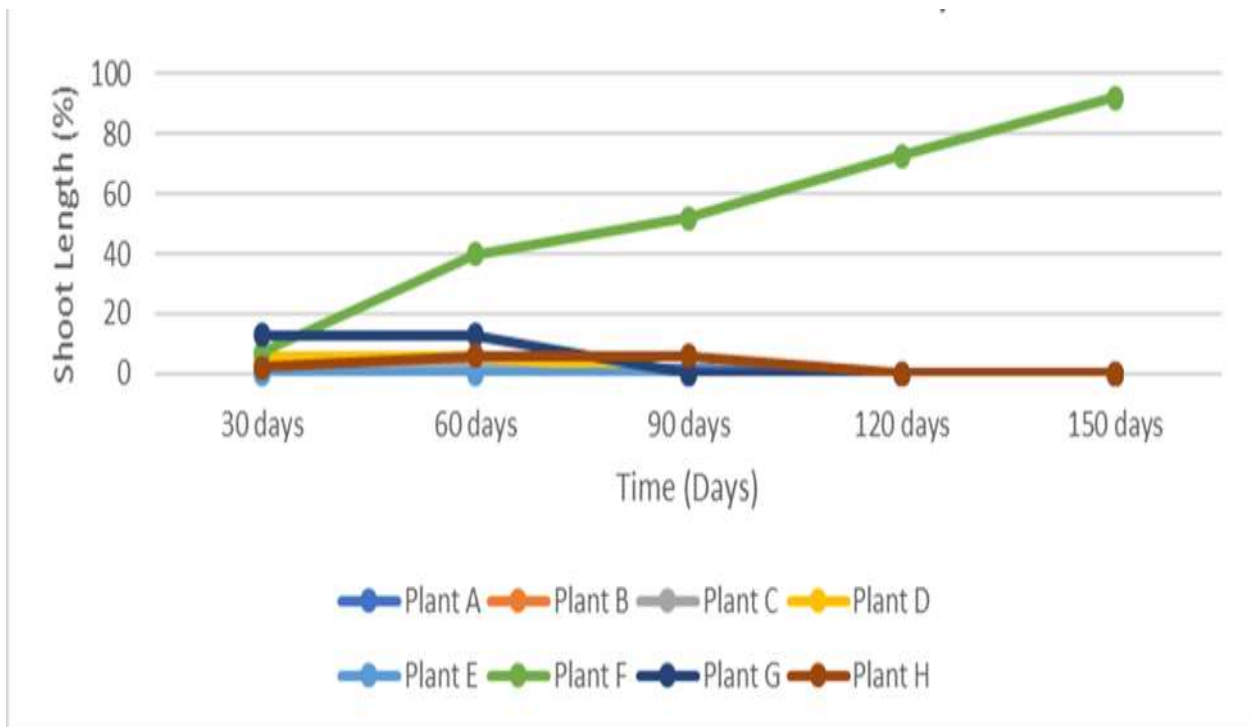


Fig. 1 - Figure 1: Shoot length (%) of the study plants' performance in 10% crude oil with fixed concentration of heavy metals.

Key: Plant (A–*Brachiaria distachyoides* Stapf., B – *Cyperus dichrostachyus* Hochst. ex A. Rich, C–*Kalanchoe pinnata* (Lam.) Pers, D–*Panicum maximum* Jacq, E – *Mimosa pudica* L, F – *Paspalum conjugatum* P.J.Bergius, G – *Mariscus rotundus*, H – *Mariscus ligularis* (L.)

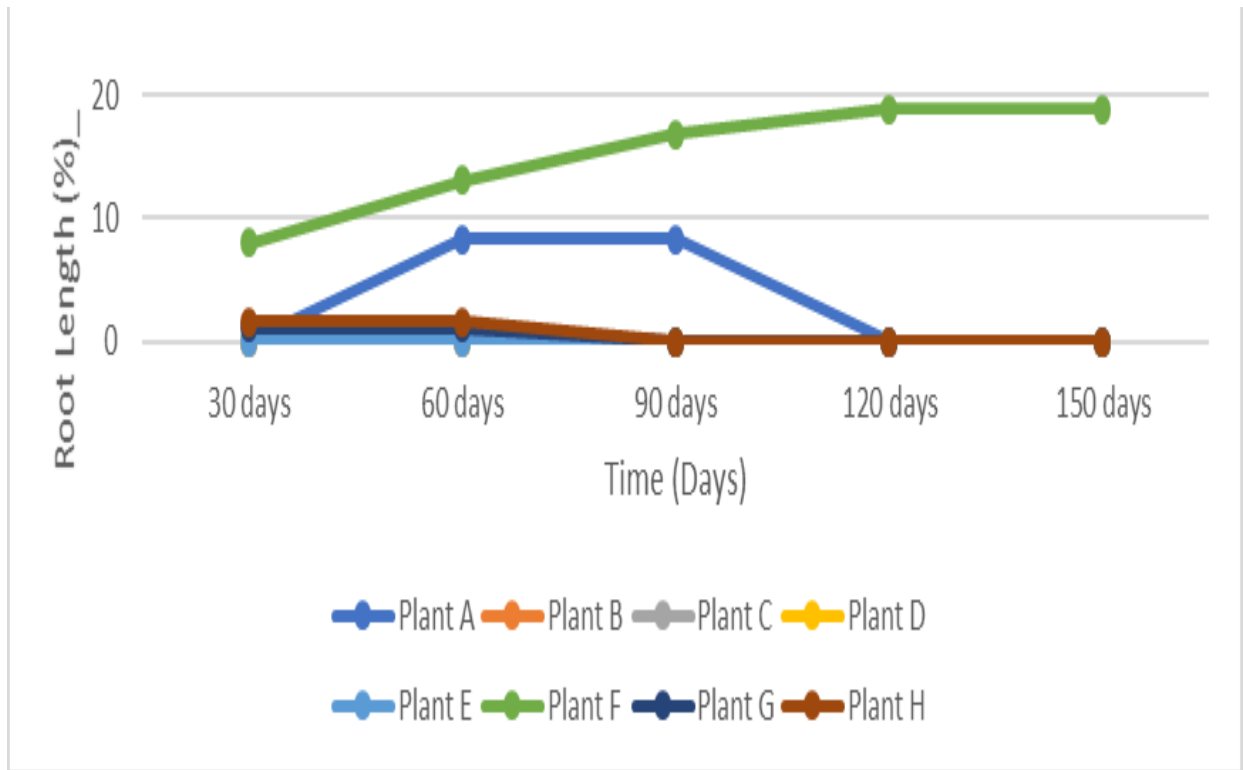


Figure 2: Root length (%) of the study plants performance in 10% crude oil with fixed concentration of heavy metals.

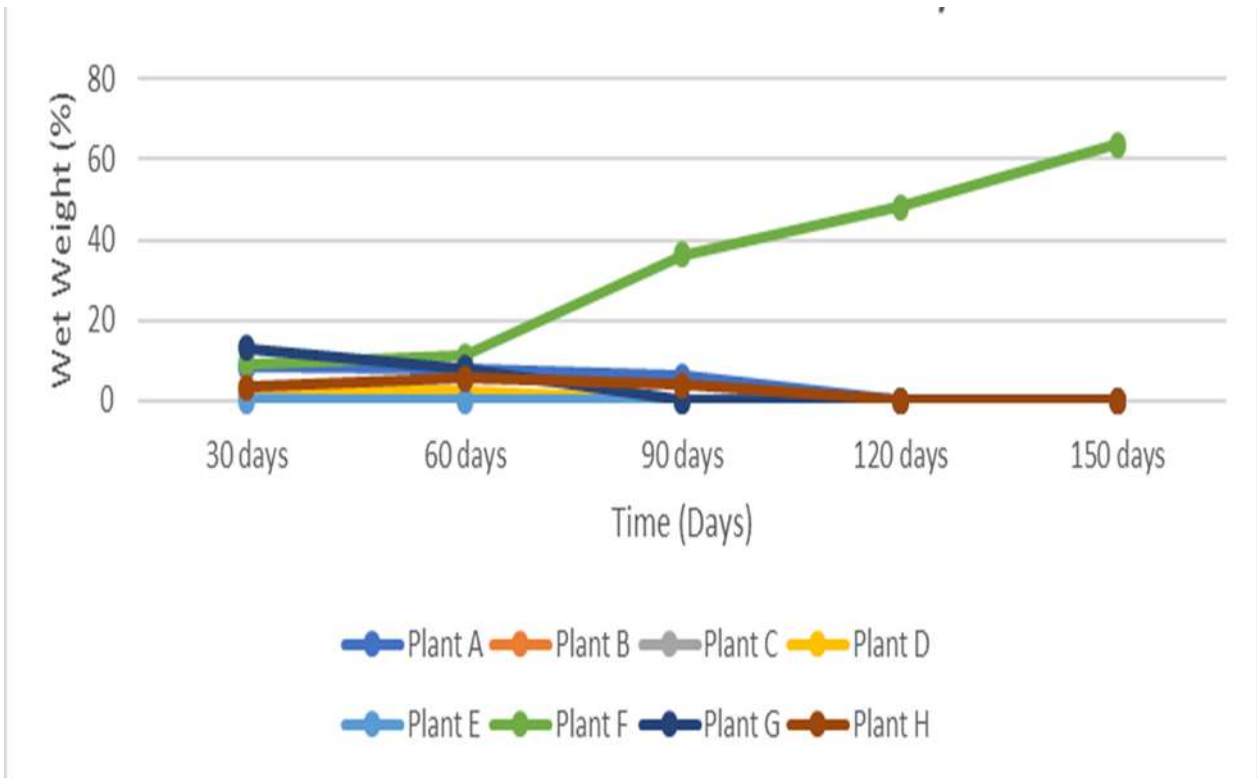


Figure 3: wet weigh (%) of the study plants performance in 10% crude oil with fixed concentration of heavy metals



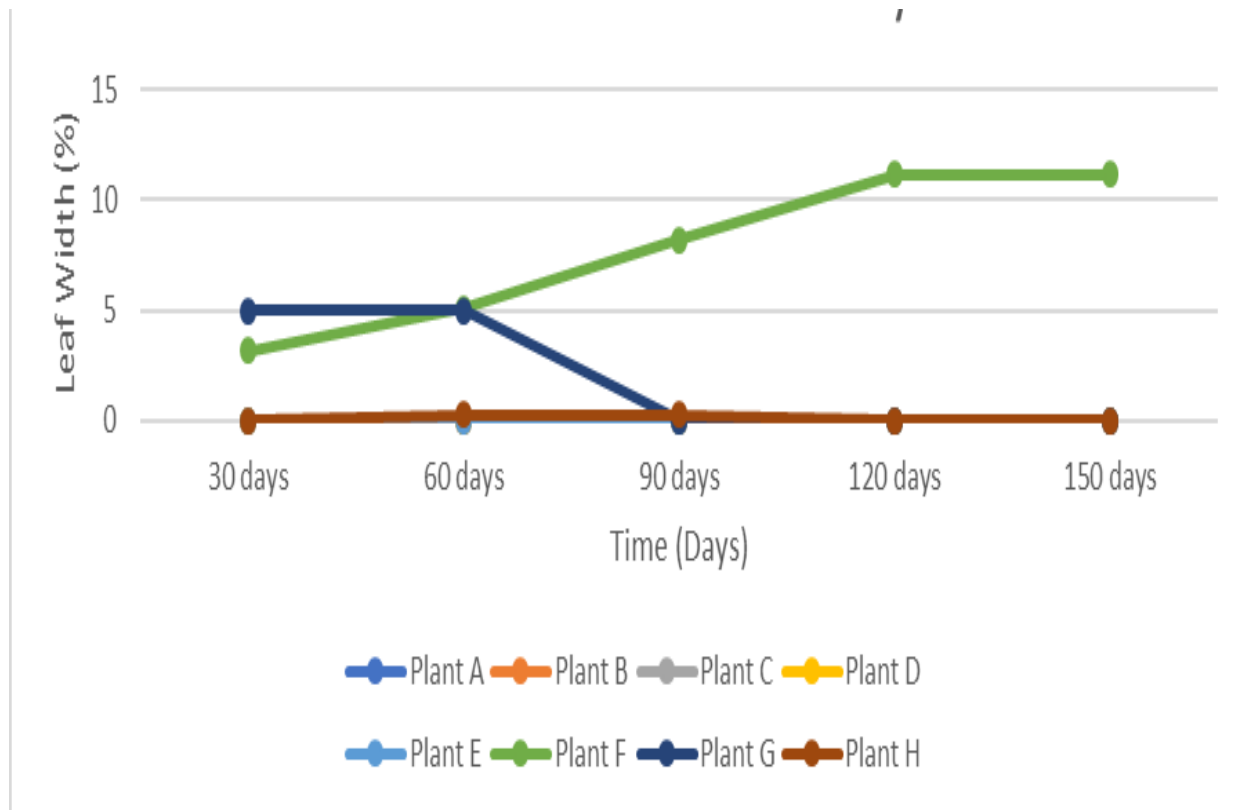


Figure 4: Leaf width (%) of the study plants performance in 10% crude oil with fixed concentration of heavy metals (1)

#### 4. Discussion

The analysis of the unpolluted soil sample used in this study revealed the following composition: sand content of 47.3%, silt content of 27.3%, and clay content of 23%. Additionally, the soil had an organic carbon content of 0.65%, organic matter content of 0.45%, total nitrogen of 0.18%, available phosphorus content of 0.80%, and a pH level of 7.16.

These findings align with previous reports by Ogundola, Bvenura & Afolayan, (2018); Lindsay, (2001); Umeugokwe, Ugwu, Umeugochukwu, Uzoh, Obalum, Ddamulira, & Alenoma (2021); Jin, Hu, Khan, Zhang, Yang, Jia & Sun (2021). They have all documented varying levels of sand, silt, clay, organic matter, organic carbon, total nitrogen, and available phosphorus in loamy soil. The study also indicates that the percentage of hydrocarbon-utilizing bacteria in the soil sample is 1.35%. This result is consistent with the earlier work of Ogbonna, Douglas, & Awari (2020), who observed that unpolluted soils generally have a lower percentage of hydrocarbon-utilizing bacteria compared to polluted soils.

The soil's average total heterotrophic bacteria (THB) count was  $3.4 \times 10^8$  cfu/g, while the average hydrocarbon-utilizing bacteria (HUB) count was  $4.6 \times 10^6$  cfu/g. Additionally, the total fungal count was  $1.5 \times 10^4$  cfu/g, and the average hydrocarbon-utilizing fungi (HUF) count was  $0.9 \times 10^2$  cfu/g. These findings align with the previous studies by Soludo, Orji, Anaukwu, Anyaoha, Ajogwu, & Eze (2024) and Eze, Owunna, & Avoaja (2013). They reported that heterotrophic bacterial and fungal counts in unpolluted soil were higher than those in polluted soil. Both studies, along with others, have documented varying average THB, HUB, THF, and HUF counts in unpolluted and hydrocarbon-polluted soils.

The result from this study shows that among the eight (8) plants assayed for their phytoremediation and phytoaccumulation potentials, *Paspalum conjugatum* P.J.Bergius (Crab grass) significantly exhibited more tolerance to 10% crude oil and heavy metals (Zn = 39.4 mg/l, Ni = 10.2 mg/l, Cu = 29.4 mg/l, Pb = 11.2 mg/l), followed by *Mariscus ligularis* L. and *Brachiaria distachyoides* Stapf which survived for 30 days under the same conditions. Other species were able to tolerate maximum of 7% crude oil and heavy metals, when compared to other plants used for this study in terms of measured shoot length, root length, wet weight and leave size.

The results show that they were tolerant to the toxic effect of the pollutants at the applied study concentrations although at 10% crude oil and heavy mental concentration, there were impairment to growth indices but observation from the study, showed that *P. conjugatum* showed evidence of growth throughout the study period but could not grow when subjected to a higher concentration of 12%. These observations are in agreement with the previous studies by Fadliah, Yadi, Didy, & Mohamad, (2020); Adesuyi, Njoku, Akinola, & Jolaoso, (2018); in their comparative studies involving different plants they found that *Paspalum conjugatum* significantly reduced the total petroleum hydrocarbons (TPH) in the soil even at a 15% crude oil concentration. The plant showed good growth and resilience, contributing to the degradation of hydrocarbons. The study also observed that *Paspalum conjugatum* is a good phytoaccumulator of heavy metals. Also the study by Ogbo, Zibigha, & Odogu, (2009); showed that at varying degrees of crude oil contamination (0.00, 2.50, 5.00, 7.50, 10.00, 12.50, and 15.00%) on the growth of *Paspalum scrobiculatum*, a prevalent weed in Nigeria. Plant height, fresh weight, and

leaf area were significantly decreased as a result of the varying degrees of crude oil pollution. The effect grew as the contamination level rose (for example, the leaf area decreased from 68.47 cm<sup>2</sup> in the control to 34.07 cm<sup>2</sup> in the 15.00% level of contamination). The plant's dry weights did not significantly decrease as a result of the pollution. Erute, Zibigha, & Odogu, (2009), reported that 15% crude oil pollution had insignificant reduction in the dry weights of *Paspalum scrobiculatum*. In contrast, the study by Paz-Alberto, Sigua, Bauí, & Prudente, (2007) reported that *Paspalum conjugatum* L. was the least phytoaccumulator of Pb amongst four plants studied, while the study by Adesuyi et al. (2019) who carried out a comparative study involving many plant species to monitor the distribution of Cd, Cr, Cu, Ni, Pb and Zn in plants of Lagos lagoon wetlands in Nigeria reported that *Paspalum vaginatum*'s root had the highest Cu concentration and also a good phytoaccumulator plant for the above mentioned heavy metals. *Paspalum conjugatum* P.J. Bergius was the plant of choice for this study because of its ability to tolerate 10% crude oil and heavy metal concentration as applied to the study.

---

## 5. Recommendation

The study suggests the use of *P. conjugatum* P.J. Bergius as a phytoremediator plant in the course of soil pollution with 10% crude oil and heavy metals, due to its numerous advantages over some grasses which includes ability to tolerate high concentration (10%) crude oil and heavy metals pollution, high growth rate, easy to propagate and ability to tolerate dual environmental conditions because it can thrive well in both normal and waterlogged soils and requiring little or no fertilizer for its proliferation.

### Acknowledgements

This work has been supported by Tertiary Education Trust Fund (TETFund). Special thanks to Professor Orji, Justina Chibuogu, Professor Nweke, Christain Okechukwu and Professor Adieze, Ifechukwu Enyinnaya for their invaluable guidance and supervision throughout the study. Their expertise and encouragement were pivotal to the successful completion of this work.

### References

---

- Adesuyi, A.A., Njoku, K.L., Akinola, M.O. & Jolaoso, A.O. (2018). Biomonitoring of Heavy Metals Level in Wetland Plants of Lagos Lagoon, Nigeria. *J. Appl. Sci. Environ. Manage.*22:1489–1498
- Adieze, I. E., Orji, J. C., Nwabueze, R. N., & Onyeze, G. O. C. (2012). Hydrocarbon stress response of four tropical plants in weathered crude oil contaminated soil in microcosms. *Inter. J. of Environ. Studies*, 69:490-500.
- Barker, A.V. and Bryson, G.M. (2002) Bioremediation of heavy metals and organic toxicants by Composting. *The Scientificworld J.* 2:407–420
- Ceccanti, B., Masciandaro, G., Garcia, C., Macci, C., Doni, S. (2006) Soil bioremediation: a combination of earthworms and compost for the ecological remediation of a hydrocarbon polluted soil. *Water Air Soil Pol.* 177:383–397
- Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnol. Res. Inter.* 1:941810.
- Erute, M. O., Zibigha, M. & Odogu, G (2009). The effect of crude oil on growth of the weed (*Paspalum scrobiculatum* L.) –phytoremediation potential of the plant. *African J. of Environ. Sci. and Technol.* 3:229-233
- Eze, V. C., Owunna, N. D., & Avoaja, D. A. (2013). Microbiological and physicochemical characteristics of soil receiving palm oil mill effluent in Umuahia, Abia state, Nigeria.
- Fadliah, S., Yadi, S., Didy, S. & Mohamad, Y. (2020). Adaptation Selection of Plants for Utilization in Phytoremediation. *Hayati J. of Biosci.*27: 45-56
- Folk, R.L. (1974) *Petrology of Sedimentary Rocks*. Hemphill Publishing Co., Austin, 170 p.
- Holt, J.G. (1994) *Bergey's manual of determinative bacteriology*. 9th Edition, Lippincott Williams and Wilkins, Baltimore.
- Jin, X., Hu, C., Khan, A., Zhang, S., Yang, X., Jia, L., & Sun, R. (2021). Comparison of fractionation methods for soil phosphorus with soils subjected to various long-term fertilization regimes on a calcareous soil. *PeerJ Inorganic Chem.* 3:132-141
- Kabra, A. N., Khandar, R. V., Waghmode, T. R. and Govindwar, S. P. (2012). Phytoremediation of textile effluent and mixture of structurally different dyes by *Glandularia pulchella* (Sweet) Tronc. *Chemosphere* 87 265–272.
- Lindsay E. (2001). National Heritage Trust funded project Save Our Soils - Empowering the Advisers, a joint project carried out by NSW Agriculture and the Department of Land and Water Conservation. <https://www.environment.nsw.gov.au/media/OEH/CorporateSite/Documents/Land-and-soil/assessing-texture-of-your-soil.pdf>
- McLean, E.O., 1982. Soil pH and lime requirement. In: *Methods of soil analysis, Part 2*. (Edited by A.L. Page, R.H. Miller and D.R. Keeney). American Society of Agronomy, Madison, Wisc, pp: 199-224.
- Nigeria Federal Ministry of Environment (NFME) (2002) <https://www.placng.org/lawsofnigeria/laws/F10.pdf>

- Obot, E., Antonio, Q.B., Braide, S., Dore, M., Wicks, C. and Steiner, R. (2006) Niger delta natural resource damage assessment and restoration project, phase 1 – scoping report Federal Ministry of Environment, Abuja, Nigeria Conservation Foundation, Lagos, WWF UK, CEESP-IUCN Commission on Environmental, Economic, and Social Policy
- Ogbo, E. M., Zibigha, M., & Odogu, G. (2009). The effect of crude oil on growth of the weed (*Paspalum scrobiculatum* L.)–phytoremediation potential of the plant. *African Journal of Environmental Science and Technology*, 3:229-233
- Ogbonna, D. N., Douglas, S. I., & Awari, V. G. (2020). Characterization of hydrocarbon utilizing bacteria and fungi associated with crude oil contaminated soil. *Microbiol. Res. J. Inter.* 30:54-69.
- Ogundola, A. F., Bvenura, C., & Afolayan, A. J. (2018). Nutrient and antinutrient compositions and heavy metal uptake and accumulation in *S. nigrum* cultivated on different soil types. *The Sci. World J.* 570-579.
- Okpokwasili, G. C. & Amanchukwu, S.C. (1988). Petroleum hydrocarbon degradation by *Candida* species. *Environ. Int.*, 14: 243-247.
- Olsen, S.R. and Sommers, L.E. (1982) Phosphorus. In: Page, A.L., Ed., *Methods of Soil Analysis Part 2 Chemical and Microbiological Properties*, American Society of Agronomy, Soil Science Society of America, Madison, 403-430.
- Paz-Alberto, A.M., Sigua, G.C., Bauj, B.G. & Prudente, J.A. (2007) Phytoextraction of lead-contaminated soil using vetivergrass (*Vetiveria zizanioides* L.), cogongrass (*Imperata cylindrica* L.) and carabao grass (*Paspalum conjugatum* L.). *Environ. Sci. Pollut. Res. Int.* 14:498-504
- Pelczar, M. J. & Chan, E.C. 1977. *Laboratory Exercises in Microbiology*, 4th edition, McGraw Hill, Inc
- Prasad, M. N. V., Freitas, H., Fraenzle, S., Wuenschmann, S. and Markert B. (2010). Knowledge explosion in phytotechnologies for environmental solutions. *Environ. Pollut.* 158 18–23.
- Royo, F. (2009). Degradation of alkanes by bacteria. *Environ. Microbiol.* 11:2477–2490
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biol. and evol.*4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Soludo, O. C., Orji, M. U., Anaukwu, C. G., Anyaoha, V. I., Ajogwu, T. M. C., & Eze, H. C. (2024). Microbial Populations of Agricultural Soil Polluted with Crude Oil. *J. of Advances in Biol. & Biotechnol.* 27:149–158.
- Tyagi, M., da Fonseca, M. M. R. and de Carvalho, C. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegrad.* 22:231–241
- Umeugokwe, C. P., Ugwu, V. U., Umeugochukwu, O. P., Uzoh, I. M., & Obalum, S. E., Ddamulira, G., Karwani G.M. and Alenoma, G. (2021). Soil fertility indices of tropical loamy sand as influenced by bambara groundnut variety, plant spacing and fertilizer type. *Agro. Sci.* 20:65-71.
- UNEP, (2011) *Environmental Assessment of Ogoniland*, United Nations Environment Programme. ISBN: 978-92-807-3130-9. <http://www.unep.org/nigeria>. Accessed 12 Oct 2014
- Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil sci.*37:29-38