

Comparative Study of the Phytoremediation Activity of the Rhizobacterial Flora Of *Vigna Unguiculata* (Cowpea) And *Arachis Hypogaea* (Groundnut) On Hydrocarbon Contaminated Soil

Manga, S. S.¹, Nwosu, C. O.¹, Bazata, Y. A.², Jabaka R. D.¹ and M. I. Ribah³

¹Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria

²Department of Microbiology, Federal University Birnin-Kebbi, Birnin-Kebbi, Nigeria

³Department of Animal Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria

Abstract:

Background: Since the discovery of crude oil as an energy source, it has tremendously stepped up the rate of civilization. However, with its enormous benefits notwithstanding, it has wreaked much havoc and damage on the ecosystem due to its toxicity.

Materials and Methods: This study evaluated hydrocarbon degradation potentials by the rhizobacterial flora of two legumes *Vigna unguiculata* (Cowpea) and *Arachis hypogaea* (Groundnut) grown in potted sandy-loamy soil samples in the screen house of Kebbi State University of Science and Technology, Aliero, Nigeria. Crude oil concentrations of 0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0, and 20.0mls were used to contaminate the soil samples respectively. All soil samples apart from the control were polluted.

Results: Groundnut germinated after seven days at concentration of 0.0 to 2.5% but at higher concentration of 5.0% of the contaminant, the germination time increased to nine days and at concentration 20%, it increased to ten (10) days. Cowpea germinated on the fourth day at concentration 0.0 to 2.0%. At concentration of 2.5%, it germinated around the fifth day, but at higher doses (5-20.0%), germination of cowpea seeds were totally inhibited. Even though groundnut germination was observed in all concentrations of crude oil tested, significant shoot retardation still occurs in both legumes consequent on crude oil toxicity. Rhizobacterial population also diminished with increase in crude oil concentration. The rhizobacterial population diminished with increase in crude oil concentration. The rhizobacteria isolated from the soil sample include *Bacillus subtilis*, *Clostridium tetani*, *Staphylococcus aureus*, *Proteus vulgaris*, and *Enterobacter aerogenes*. Rhizobacterial population also diminished with increase in crude oil concentration.

Conclusion: The study reveals both the vulnerability of cowpea and the resistance of groundnut to crude oil ($p < 0.05$), marking groundnut out as a promising phytoremediation plant.

Key word: *Arachis hypogaea*, Bacteria flora, Hydrocarbon-Contaminated Soil, Oil Spillage, Phytoremediation, *Vigna unguiculata*.

Date of Submission: 08-01-2020

Date of Acceptance: 23-01-2020

I. Introduction

Hydrocarbon spills from petroleum products both on land and in water have been a problem since the discovery of crude oil as a fuel source. This can have devastating effects on the biota of an environment. Oil spills and oil wastes discharged into the sea or land from refineries, factories or ships contain poisonous compounds that constitute potential danger to plants and animals. The poisons can pass through the food web of an area and may eventually be eaten by humans¹. Environmental contamination by hydrocarbons and petroleum products constitutes nuisance to the environment due to their persistent nature and tendency to spread into ground and surface water's, this has attracted much attention in recent decades. It was reported by^{2,3} that used motor oils such as diesel or jet fuel contaminate natural environment with hydrocarbon.

The hydrocarbons may spread horizontally on the groundwater surface thereby causing extensive groundwater contamination. Aromatic hydrocarbons are considered to be the most acute, toxic component of petroleum products, and are also associated with chronic and carcinogenic effects⁴. Lighter mono aromatics (one ring) compounds include benzene, toluene, ethyl benzene and xylenes (BTEX). Aromatics with two or more rings are referred to as polycyclic aromatic hydrocarbons (PAHs)⁵. Motor oil had concentrations of benzene up to 29 to 66 mg/L but those of other BTEX compounds were higher, typically 500 to 2000 mg/L⁶. Hydrocarbon contamination of the air, soil, freshwater (surface water and groundwater) especially by PAHs has drawn public

concerns because many PAHs are toxic, mutagenic and carcinogenic⁷. Clinical studies have shown that exposure of a mixture of highly concentrated PAHs may cause skin, lung, stomach and liver cancers⁸.

Phytoremediation (from Ancient Greek (phyto), meaning "plant", and Latin *remedium*, meaning "restoring balance") describes the treatment of environmental problems by using plants without the need to excavate the contaminant material and dispose of it elsewhere⁹.

One of the indices of loss of biological activity of soils as a result of crude oil pollution is the reduction or inhibition of microbial activity. Microorganisms of particular interest in this study are the rhizobacterial flora (rhizosphere bacteria), due to their many beneficial roles. They have been shown to be important in the degradation of pollutants, biofertilization through nitrogen fixation, phytostimulation and biocontrol of soil-borne plant diseases¹⁰. This area of soil around plant roots, known as the rhizosphere contains higher populations and greater diversity of microorganisms than soil with no plant¹¹. This is because plant roots release exudates into the soil that increase microbial activity by supplying nutrients to the organism. These exudates consist of enzymes, aliphatics, aromatics, amino acids, sugars and low molecular weight carbohydrates¹².

Phytoremediation is an eco-friendly approach for remediation of contaminated soil and wastewater using plants. It consists of two components, one by the root colonizing microbes and the other by plants themselves, which accumulate the toxic compounds to further change to non-toxic metabolites. Various compounds viz: organic synthetic compounds, xenobiotics, pesticides, hydrocarbons, heavy metals and radionuclides are among the contaminants that can be effectively remediated by plants^{13,14}. Different mechanisms are employed in phytoremediation of petroleum hydrocarbons. Both plants and microorganisms are involved directly or indirectly in the degradation or transformation of petroleum hydrocarbons into products that are generally less toxic and less persistent in the environment than the parent compound¹⁵.

The primary mechanisms for plant-mediated remediation of soils contaminated with petroleum hydrocarbons as outlined by⁴ are: phytodegradation (rhizodegradation), phytostabilization, phytoextraction (phytoaccumulation), phytovolatilization and rhizofiltration. The success of phytoremediation at a given site cannot always be attributed to just one of these mechanisms because a combination of mechanisms may be at work¹⁶.

II. Material And Methods

Study area: This study was conducted in the screen house of Kebbi State University of Science and Technology, Aliero, Nigeria.

Sample Collection

Crude oil (specific gravity = 0.81; API gravity = 43.2°) was obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt Refinery, Alesa – Eleme, Rivers State, Nigeria, on 2nd July, 2017. The crude oil was unweathered, having been obtained fresh from the production plant.

Plant Seeds: Seeds of *Vigna unguiculata* (cowpea) were sourced from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria, and *Arachis hypogaea* (groundnut) were purchased at Kebbi Central Market, Birnin Kebbi, Kebbi State, Nigeria, and stored at ambient temperature.

Soil Sample: Fifty kilogram (50kg) of sandy loam soil was collected from Fadama Teaching and Research Centre Jega, Kebbi State, Nigeria, by clearing the top soil to reduce contaminants. It was dug to the depth of 2m and collected using clean polythene bags in the morning.

Soil Processing and Sowing of Plant Seeds: Completely randomized design (CRD) was adopted in this study. This is because the test plants (*Vigna unguiculata* and *Arachis hypogaea*) were allocated randomly to the hydrocarbon-contaminated soils (treatment) and uncontaminated soils (control). This study lasted for twelve (12) weeks. The soil sample was air-dried, sieved and dispensed in 3 kg weights into eighteen (18) plastic pots (20 cm deep × 20 cm diameter) perforated at their bases for aeration with three replicates. Each pot in a group, apart from the control, was contaminated with one of eight different levels of crude oil (0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0 or 20.0% v/w) (Eze *et al.*, 2013). All control samples were not contaminated. Thereafter, seeds of the plants (cowpea and groundnut) were sown, which consisted of three seeds of each plant sown in triplicate pots. All pots were kept in a Green house at the Faculty of Agriculture, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria and watered every twenty four hours by spraying.

Enumeration of Bacteria

Enumeration of bacteria population in the rhizosphere of the contaminated samples and that of the control was carried out using the standard plate count technique¹⁷. Soil samples (0 to 3 cm deep) were collected as described by¹⁸ both from the contaminated samples and control at two-weekly intervals from each pot and put into sterile labeled polythene bags. A sterilized spatula was used to dig the soil to collect soil sample from

the root area. The samples were immediately taken to the laboratory for analysis. One gram of each sample was serially diluted using 9ml of sterile distilled water and up to 10⁻⁸ dilution to reduce the bacterial load. Using a sterile micropipette, 0.1ml was inoculated by spread plating on sterile nutrient agar plates for 24 hours at 37°C. After 24hrs, the plates that had 30-300 colonies were counted and recorded.

Isolation and Identification of the Test Bacteria

The colonies observed were sub-cultured onto nutrient agar and were incubated at 37⁰C for 24hours in order to obtain pure cultures of the bacterial cell. From the colonies that developed on Nutrient agar, a smear was made on a clean glass slide using sterile wire loop. It was dried and heat fixed. The smear was flooded with crystal violet solution for 60 seconds and washed, tipped off and covered with Lugol’s iodine for 2 minutes. The stain was decolorized with acetone and washed off immediately with distilled water. It was counter stained with safranin for 2 minutes and rinsed with distilled water. The back of the slide was wiped clean; the smear was placed on a draining rack and allowed to air dry. The smear was viewed under the microscope using oil immersion objective x100. Further biochemical tests such as (catalase, coagulase, oxidase, indole, motility and urease test) to confirm the isolates to species level was carried out as described by^{19,20}.

Biochemical Characterization of the Bacterial Isolates

Using standard methods adopted by²⁰, the following test were carried out: Catalase, Coagulase, Citrate, Motility, Indole, Urease, Triple sugar iron, Methyl red, Voges-Proskauer, Mannitol, Spore formation, Oxidase tests.

Seed Germination

Germination of seeds was observed daily for 60 days as positive or negative; it was positive if there was a visible cracking of the seed coat with measurable root or shoot production²¹. The germination time (in days) was observed and recorded for seeds in every pot.

Plant Growth Evaluation

Plant shoot growth was with meter rule (cm) initially fourteen days after seed sowing and subsequently done weekly throughout the eight-week experiment. Measurement was carried out using a calibrated 30 cm transparent plastic rule.

Statistical Analysis

Data analysis was carried out using a one–way analysis of variance (ANOVA), and the difference was done to determine statistical significance differences (P< 0.05).

III. Result

Total Bacteria Count of Soil Samples Where Cowpea Was Grown

Table 1 represent the total bacteria count of soil samples contaminated with crude oil and control where cowpea was grown. The result indicated that at the control sample, the number of bacteria count were high, but gradually decreased as the concentration of crude oil increased. At the concentration of 5.0% to 20%, the number of bacterial count from the soil sample declined. The mean in the bacterial count in soil samples where cowpea was grown was greater in the second replication and lower in the third replication. Where the cowpea was grown the mean in the bacterial count in soil samples was highest in 0.0 – 0.5 concentration of crude oil and decreased down as a concentration increases.

Table 1: Mean bacteria counts (log10 cfu/g) of soil samples were cowpea was grown

Replication	Concentration								
	CO	C 0.5	C 1.0	C 2.0	C 2.5	C 5.0	C 10.0	C 15.0	C 20.0
1 st	9.90	9.78	9.62	9.56	9.26	9.00	8.90	8.78	8.70
2 nd	9.95	9.81	9.60	9.54	9.30	9.08	8.78	8.60	8.48
3 rd	9.89	9.79	9.59	9.51	9.32	8.95	8.78	8.70	8.52
\bar{X}	9.91 ^b	9.79 ^b	9.60 ^{ab}	9.54 ^{ab}	6.29 ^a	9.01 ^{ab}	8.82 ^{ab}	8.69 ^{ab}	8.57 ^{ab}
SD±	0.03	0.02	0.02	0.03	5.19	0.07	0.07	0.09	0.11

CO = Control Sample

Means with the same superscript are not significantly different at (p<0.05)

Total Bacteria Count of Soil Samples Where Groundnut was Grown

Table 2 represent the total bacteria count of soil sample polluted with crude oil where groundnut was grown. The result indicated that at the concentration of 0.0%, the number of bacterial count were high but gradually decreases as the concentration of crude oil increases. At the concentration 5.0% to 20%, the number of bacteria count from the soil declined significantly. The mean amount of bacteria in soil samples were groundnut was grown peaked at 0.0 concentration of crude oil and consistently decline in a regular manner as the concentration increases.

Table 2: Mean Bacteria Counts (log₁₀ cfu/g) of Soil Samples where Groundnut was Grown.

Replication	Concentration								
	GO	G 0.5	G 1.0	G 2.0	G 2.5	G 5.0	G 10.0	G 15.0	G20.0
1 st	9.88	9.70	9.60	9.54	9.48	9.43	9.40	9.30	9.18
2 nd	9.90	9.65	9.54	9.40	9.36	9.30	9.28	9.26	9.00
3 rd	9.93	9.60	9.48	9.43	9.40	9.36	9.32	9.23	9.00
X	9.90 ^g	9.65 ^f	9.54 ^e	9.46 ^{de}	9.41 ^{cd}	9.36 ^{bcd}	9.33 ^{bc}	9.26 ^b	9.06 ^a
SD±	0.03	0.05	0.06	0.07	0.06	0.07	0.06	0.04	0.10

GO = Control sample

Means with the same superscript are not significantly different at (P<0.05)

Bacteria Identified from Soil Samples where Cowpea was Grown

Table 3 indicates the bacteria isolated from the contaminated soil where cowpea was grown. After the isolates has been subjected to various morphological and biochemical test the following bacteria genera were identified: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Clostridium tetani*, *Bacillus subtilis*, *Proteusvulgaris*,

Table 3: Bacteria Identified from Soil Samples where Cowpea was Grown

Gram React.	Shape	Cat	Coa	Man	Cit	Ure	MR	VP	Oxi	Ind.	Trp	Mot	Spo	Bacteria Isolates
+	Cocci	+	+	+	+	-	+	-	-	-	-	-	-	<i>Staphy. Aureus</i>
-	Rod	+	-	+	+	-	-	-	+	-	+	+	-	<i>Pseudo. aeruginosa</i>
+	Rod	+	-	+	+	-	-	+	-	-	-	+	+	<i>Bacillus subtilis</i>
+	Rod	+	-	-	-	+	+	-	-	+	+	+	-	<i>Proteus vulgaris</i>
-	Rod	+	-	+	+	-	+	+	-	-	-	+	-	<i>Entero. aerogenes</i>
+	Rod	-	-	-	-	-	-	-	-	-	-	+	+	<i>Clostridium tetani</i>

KEY: Cat = catalase test, Coa = Coagulase test, Man = Mannitol test, Cit = Citrate Test, Ure = Urease Test, Vp = Voges-Proskauer, Oxi = Oxidase test, Ind = Indole test, Trp = Triple Sugar test, MR = Methyl Red Test, Mot = Motility test, Spo = Spore formation

Bacteria Identified From Soil Samples Where Groundnut Was Grown

Table 4 indicates the bacteria isolated from the contaminated soil where groundnut was grown. After the isolates has been subjected to various morphological and biochemical test the following bacteria genera were identified: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Clostridium tetani*, *Bacillus subtilis*,

Table 4: Bacteria Identified from Soil Samples where Groundnut was Grown

Gram React.	Shape	Cat	Coa	Man	Cit	Ure	MR	VP	Oxi	Ind.	Trp	Mot	Spo	Bacteria Isolates
+	Rod	+	-	+	+	-	-	+	-	-	-	+	+	<i>Bacillus subtilis</i>
-	Rod	+	-	+	+	-	-	-	+	-	+	+	-	<i>Pseudo. aeruginosa</i>
-	Rod	+	-	+	+	-	+	+	-	-	-	+	-	<i>Entero. aerogenes</i>
+	Rod	-	-	-	-	-	-	-	-	-	-	+	+	<i>Clostridium tetani</i>
+	Cocci	+	+	+	+	-	+	-	-	-	-	-	-	<i>Staphy. Aureus</i>

KEY: Cat = catalase test, Coa = Coagulase test, Man = Mannitol test, Cit = Citrate Test, Ure = Urease Test, Vp = Voges-Proskauer, Oxi = Oxidase test, Ind = Indole test, Trp = Triple Sugar test, MR = Methyl Red Test, Mot = Motility test, Spo = Spore formation

Germination of Cowpea and Groundnut Seeds on the Crude Oil Contaminated Soil

Table 5 represents the germination of the legumes (cowpea and groundnut). From the table, groundnut germinated at different level of contamination with crude oil but the table indicates that at concentration 5.0 to 20%, the germination of cowpea was inhibited.

Table 5: Germination of Cowpea and Groundnut Seeds on the Crude Oil contaminated Soil

Crop plant	Crude oil level (%)								
	0.0	0.5	1.0	2.0	2.5	5.0	10.0	15.0	20.0
Groundnut	+	+	+	+	+	+	+	+	+
Cowpea	+	+	+	+	+	-	-	-	-

Key: + = There is germination
- = No germination

Germination in Days of Groundnut and Cowpea on Crude Oil Contaminated Soil

Table 6 represents the germination time in days of legume. The table indicates that groundnut germinated after seven days (7) of sowing at concentration 0.0 to 2.5% but at higher concentration of 5.0% of the contaminant, the germination time increase to nine days and at concentration 20.0% it increased to 10 days. The table indicates that cowpea germinated on the fourth day at concentration 0.0 to 2.0%. At concentration 2.5, it germinated around the fifth day but cease to germinate as a concentration increase to 5.0.

Table 6: Germination Time (days) of Seeds at Different Level of Crude Oil Contaminated Soil

Crop Plant	Germination Time (days)								
	Crude oil level (%)								
	0.0	0.5	1.0	2.0	2.5	5.0	10.0	15.0	20.0
Groundnut	7±0.5	7±0.5	7±0.5	7±0.5	7±0.5	9±0.5	9±0.6	9±0.6	10±0.6
Cowpea	4±0.5	4±0.5	4±0.5	4±0.5	5±0.5	*	*	*	*

Key: * = No germination

Shoot Growth of Cowpea Plant at Weekly Intervals on Crude Oil Contaminated Soil

Table 7 indicates the shoot growth of cowpea measured with a metre rule from the second week to eight week. From the table the cowpea in the control test (co) shows a progressive increase in the shoot growth as the week progresses, but as the contaminant were introduced at different concentration there was a progressive decline in the shoot growth as the percentage of the crude oil contamination increases to 2.5%. At concentration 5.0 to 20.0 there was no germination at all.

Table 7: Shoot Growth of Cowpea Plant at Weekly Intervals on Crude Oil Contaminated Soil

Test Sample	Sampling weeks							
	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	Mean total (cm)
Co	16	18	20	24	30	35	40	26
0.5	13	14	18	21	25	28	32	22
1.0	13	12	14	16	19	20	25	17
2.0	10	11	12	14	17	18	21	15
2.5	8	9	11	11	12	14	19	12
5.0	*	*	*	*	*	*	*	*
10.0	*	*	*	*	*	*	*	*
15.0	*	*	*	*	*	*	*	*
20.0	*	*	*	*	*	*	*	*

Co = Control
* = No growth

Shoot Growth of Groundnut at Weekly Interval on Crude Oil Contaminated Soil

Table 8 indicates the shoot growth of groundnut measured with a metre rule from the second week to eight week. From the table, the groundnut in the control test (GO) shows a progressive increase in the shoot growth as the week progresses, but as the contaminant were introduced at different concentration there was a progressive decline in the shoot growth as the percentage of the crude oil contamination increases. In groundnut, the mean maximum shoot lengths at the 8th week, of the control plants and plants grown in soils with 2.5, 5, 10, 15 and 20% crude oil contamination varied from 17cm to 15 cm, 13 cm, 12cm and 10 cm respectively. Even though groundnut germinated and grew in all the levels of crude oil pollution, there was growth depression and subsequent stagnation at high doses.

Table 8: Shoot Growth of Groundnut at Weekly Interval on Crude Oil Contaminated Soil

Test Sample	Sampling weeks							
	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	Mean total (cm)
Go	14	15	17	19	22	24	26	20
0.5	13	13	15	17	20	22	23	18
1.0	11	11	13	16	17	19	20	15
2.0	9	10	11	14	15	17	18	13
2.5	9	10	11	13	14	15	17	13
5.0	8	9	10	11	12	13	15	11
10.0	7	7	9	10	11	12	13	10
15.0	6	7	8	9	10	11	12	9
20.0	4	5	6	7	8	9	10	7

Go = Control

IV. Discussion

A total of six bacteria species were identified in this study. The dominant bacterium was *Bacillus subtilis*. Several reports of bioremediation of petroleum contaminants by the action of *Bacillus subtilis* in extreme environments have been recorded. ²² reported *Bacillus subtilis* as being the dominant bacteria of all the petroleum oil utilizing bacteria characterized from highly polluted soil samples. This is consistent with the present study since *Bacillus* was the most dominant bacteria isolated. There is growing evidence that isolates belonging to the *Bacillus subtilis* could be effective in cleaning oil spills²³.

Bacteria belonging to the *Alcaligenes* and *Enterobacter* genera are also widely reported to be implicated in petroleum hydrocarbon utilization. According to²⁴, bacteria of the genera *Alcaligenes* and *Enterobacter* had been isolated from petroleum oil contaminated soils. The presence of *Staphylococcus aureus* in the present study also agrees with the study conducted by²⁵ who isolated *Staphylococcus hominis* from petroleum oil contaminated soils and²⁶ who also isolated *Staphylococcus aureus* from a diesel contaminated soil.

The ability of these bacteria to survive in crude oil contaminated soil agrees with previous reports that there is increased microbiological activity within the rhizosphere^{11,27,28}. This increase could be caused by exudates and sloughed-off tissues from the plants, which served as nutrients to the microorganisms.

In this research work, groundnut seed was able to germinate and grow at all level of crude oil contamination, but the cowpea stops germination and growing at concentration of 5.0%. A similar effect of petroleum on germination was reported by^{29,30}. They reported reduction in germination rate in several plant species caused by petroleum contamination. The decrease in germination as diesel concentration increased might not just be due to the contaminant concentration but also to the hydrocarbon type, plant species and reduction in oxygen transfer between the seed and the surrounding environment as reported by³¹. The negative effect of diesel oil on germination with increased diesel oil concentration might also be due to their hydrophobic properties as reported by^{29,32}. Hydrocarbons may coat the seed, preventing or reducing gas and water exchange; they may also enter the seeds and alter the metabolic reactions and/or kill the embryo by direct toxicity^{29,33}.

The depression of germination of seeds by crude oil is in line with previous reports on related research^{34,35,36,37,38}. Crude petroleum is able to interfere with seed germination by coating the seeds with oily substances thereby limiting water-air movement within the seed and directly through toxic actions.

This study revealed that groundnut seeds had higher percentage germination than cowpea at 1.0% crude oil level and above. This was probably caused by innate resistant qualities of the groundnut seeds. The remarkably low phytate content of groundnut seed might have enhanced its germination and growth at all the levels of crude oil used, since high levels of phytate (an anti-nutrient) inhibit mineral nutrients absorption in both plants and animals as reported by^{39,40}. Some of these mineral elements (for example, Ca, P and Mg) are needed for seed germination. When the phytate level is low, seeds sown in crude oil polluted soil will probably have only the external crude oil factor to contend with during germination.

Shoot growth retardation in plants due to petroleum pollution as observed in this work had been reported by different workers on related studies^{35,34,41,18}. According to¹⁸ during their study with three vascular plants (fluted pumpkin, maize and okro) reported retardation in their shoot growth as a result of crude oil contamination. When crude oil coats plant parts with hydrophobic substances, it reduces respiration and cell membrane permeability in the affected parts. Reduction in cell membrane permeability consequently reduces nutrient absorption, metabolism and growth in the plants.

V. Conclusion

The result from this research work clearly demonstrate that phytoremediation effect or strength is higher for groundnut grown on soil sample polluted with crude oil at a specified concentration value ranges from 0.0 to 20.0 to still grow. I.e. despite the pollution of the soil sample, reduction in bacteria count of the soil, growth depression and unfavorable soil condition yet groundnut still beat restrictions to grow and survive, but for the other legume (cowpea) it germinated and grew at the following level of crude oil pollution (0.0, 0.5, 1.0, 2.0 and 2.5 respectively) but at high concentration value of 5.0 and above it stopped growing. And as a result of this, there was growth depression with subsequent stagnated at high doses of crude oil pollution. According to results of this study, groundnut resisted the toxic effects of crude oil more than cowpea. This is evidenced by its ability to germinate and grow in crude oil concentrations high enough to cause mortality in cowpea. This property marks it out as a promising candidate for the phytoremediation of crude oil-polluted soils since the usefulness of any plant in the phytoremediation of a polluted habitat is determined by its ability to grow in the polluted habitat in question. Six bacteria genera were identified; *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Clostridium tetani*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* in the soils used in the research work. The dominant and most effective bacteria, *Bacillus subtilis* can be isolated and packaged for future phytoremediation of crude oil contaminated soil.

Acknowledgement

My profound appreciation goes to the TETFund for sponsoring this research work.

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Manga, S. S, et.al. "Comparative Study of the Phytoremediation Activity of the Rhizobacterial Flora Of *Vigna unguiculata* (Cowpea) And *Arachis hypogaea* (Groundnut) On Hydrocarbon Contaminated Soil." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(1), (2020): pp. 36-43.