Journal of Pollution Monitoring, Evaluation Studies and Control
J Pollut Monit Eval Stud Contr, 2(1): 15-29 (2023)
DOI: https://doi.org/10.54117/jpmesc.v2i1.5

Post-Reclamation Evaluation of Residual Hydrocarbons in Crude Oil Contaminated Soil Using Gas Chromatographic Techniques and Plant Growth Indices

Marcel C. Ifediegwu[1], Bright O. Uba[2*], Victoria G. Awari[3], Anulika G. Chukwujekwu[1] and Ifeanyi P. Akaun[4]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambra State, Nigeria.
[2]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, P.M.B.02 Uli, Anambra State, Nigeria.
[3]Department of Microbiology, Faculty of Natural and Applied Sciences, Tansian University, Umunya, Anambra State, Nigeria.
[4]Department of General Studies, Delta State Polytechnic, Ogwashi-Uku, Delta State, Nigeria.
*Correspondence: bo.uba@coou.edu.ng; +2348069693773

Abstract

The present study was undertaken to evaluate the post-reclamation of crude oil contaminated soil using gas chromatographic technique and plant growth indices. Enrichment techniques, turbidometric test, plasmid curing test, classical identification method, gas chromatographic flame ionization detection technique and plant growth indices were carried out using standard procedures. The results indicated that 22 of the isolates were observed to have high pollutant degrading potentials (A600nm > 0.3) due to their crude oil utilization ability. The results also indicated that isolates which were able to degrade crude oil belonged to genera *Bacillus*, *Pseudomanas* sp., *Ochrobacterium* sp. and *Enterobacter* sp. Moreso, the remediation percentages recorded were 98 %, 80 %, 21 % and 98 %, 78 % and 22 % for the respective crude oil pollution (3 %, 5 % and 10 %) for soil inoculated plasmid borne bacteria whereas soil inoculated with plasmid cured bacterial cultures had 65 %, 52 %, 11 % and 65 %, 50 % and 20 % degradation of the same concentration of the total petroleum hydrocarbon after 60 days for both *Phaseolus vulgaris* and *Glycine max* plant seeds, respectively. The remediation percentages in the uninoculated controls include 3 %, 5 %, 10 % and 12 %, 9 % and 5 % across the pollutions for both plant seeds. A significant ($P < 0.05$) increase in the growth parameters of the leguminous plants like percentage germination, mean plant shoot length, mean plant root length, plant fresh weight and number of root nodules were seen more in the soil microcosms inoculated with plasmid borne bacteria than microcosm inoculated with culture of plasmid cured bacteria across all crude oil dilutions. Crude oil had a dose-dependent negative effect on the mean bacterial counts of each of the polluted soil containing the two different plants. Thus, the study clearly suggests that the selected hydrocarbon bacterial degraders could be effectively exploited in the remediation of crude oil polluted sites.

Keywords: *Bioremediation, Plant growth index, Crude Oil, Pollution, Phaseolus vulgaris, Glycine max.*

Article History
Received: 19 Sept 2023
Accepted: 24 Sept 2023
Published: 09 Oct 2023

Scan QR code to view•

How to cite this paper: Ifediegwu, M. C, Uba, B. O., Awari, V. G., Chukwujekwu, A. G., & Akaun, I. P. (2023). Post-Reclamation Evaluation of Residual Hydrocarbons in Crude Oil Contaminated Soil Using Gas Chromatographic Techniques and Plant Growth Indices. *Journal of Pollution Monitoring, Evaluation Studies and Control*, *2*(1), 15–29. https://doi.org/10.54117/jpmesc.v2i1.5.

1. Introduction

Petroleum hydrocarbons are extensively used worldwide as fuel. It has caused a huge amount of contamination and relevant adverse effects on the environment and living organisms though social and economic development largely depend on petroleum hydrocarbon as it is a dominant source of energy (Fowzia and Fakhruddin, 2018). Discovery of oil by multinational corporations set Nigeria on an interesting path of wealth and controversies (Oka, 2017). The environmental consequences from oil and gas exploration activities in the Niger Delta region are yet unquantifiable and not so well documented (Oka, 2017). According to Francis *et al* (2011), 546 million gallons of crude oil were spilled from 1958 – 2010, averaging about 300 spills or nearly 10.8 million barrels per annum and about 50,000 acres of mangrove forest disappeared between 1986 – 2003. United Nations Development Programme (UNDP) report (2011) also estimated spillage of 3 million barrels from 6,817 incidents, comprising 6 % on land, 25 % on the swamp and 69 % on the offshore environment between 1976 and 2000.

In Nigeria, most of the terrestrial ecosystems and the shores in oil producing communities are important agricultural land under continuous cultivation. Contact with crude oil results in

the damage of the soil condition of this agricultural land, and of microorganisms and plants (Ijah *et al*., 2013). Crude oil has a coagulatory effect on soil; it binds the soil particles and hence, reduces aeration. Therefore, seed sown on such soils will fail to germinate. Heavily contaminated soils may remain unproductive for months or years until the oil has been degraded to tolerable levels (Ijah *et al*., 2013). Crude oil contamination of agricultural soils has greatly affected food production particularly in oil producing areas. Oil contamination in soils results in an imbalance of the carbon to nitrogen ratios. This causes a nitrogen deficiency, which not only retards the growth of agriculturally important microorganisms but also plants grown on such soils (Yerima *et al.,* 2011). When an oil spill occurs, oil floats being less dense than water. It also pollutes air since the most volatile hydrocarbons start to evaporate initially after the oil spills (Mulder and Spierings, 2017).

There are fewer literature reports on the importance of plasmid borne soil bacteria on the reclamation crude oil polluted soil ecosystems and the available reports contained paucity of information on the exploration of the treatment success using plant growth indices and therefore necessitates the present research study. This research study was aimed at post-reclamation evaluation of residual hydrocarbons in crude oil contaminated soil using Gas chromatographic techniques and plant growth indices.

2. Materials and Methods

2.1 Materials

2.1.1 Procurement of Bonny light crude oil

Bonny light crude oil (API gravity= 32.15) was obtained from the Nigerian National Petroleum Corporation (NNPC) Port Harcourt Refinery, Alesa - Eleme, Rivers State, Nigeria was used in this research.

2.2 Experimental Design

2.2.1 Sampling site

Soil samples were obtained from four sites of 15 year old hydrocarbon contamination at Awka, Aguleri, Onitsha and Ekwulobia in Anambra State. The Awka sampling site lies within latitude N06.22677º and Longitude E007.07602º with a mean elevation of 133 meters above sea level and located at Aroma Junction, Awka South Local Government Area of Anambra State.

The sampling point at Aguleri lies between latitude N6.33231° and longitude E6.87444° with mean elevation of 45 metres above sea level and located at Aguleri Junction in Anambra East Council Area. The sampling site at Onitsha lies within latitude N6.13378º and longitude E6.79393º with mean elevation of 43 meters above sea level and situated at Upper Iweka Axis of Onitsha South Local Government of Anambra State while the Ekwulobia area lies within latitude N5.99053º and longitude E7.17018º with a mean elevation of 88 meters, with Ekwulobia Motor Park in Aguata Local Government Area of Anambra State as the sample site.

2.3 Sampling Method

Soil samples were collected from depths of 0 - 10 cm at georeferenced point at the centre of the site and two other points 8 m away, and also, 3 subsamples 6 m away from each other were collected from each point (Nakamura *et al*., 2014). A total of 75 composite samples were collected from the 4 sampling sites. The sampling was performed with soil auger and transferred into a polyethylene bag. The polyethylene bag containing the composite sample was stored in a plastic bucket with dry ice, then, immediately sent to the Microbiology Laboratory of Nnamdi Azikiwe University, Awka, Anambra State and kept below 4 °C in a refrigerator for microbial and chemical analyses**.**

2.4 Isolation of Hydrocarbon Degrading Bacteria

The enrichment culture technique was used for the isolation of bacterial strains capable of utilizing crude oil as a sole source of carbon and energy as described by Gayathri *et al*. (2014). Crude oil mixed media were prepared by thoroughly mixing different concentrations of crude oil (1 %, 2 %, 5 % and 10 %) with 100 mL media (LB, MS and Nutrient broth) when the media were about to solidify (45 – 50 ºC). One gram of each soil sample was suspended in 9 mL of distilled water and kept at room temperature for 24 hours. On the next day, 250 µL of the supernatant was spread on crude oil containing Nutrient agar, LB and MS Petri-dishes, for the isolation of hydrocarbon degrading bacteria and incubated at room temperature. The Petri dishes were observed on the next day to till fourth day for the appearance of bacteria colonies (Hyina *et al*., 2003). The cultures were randomly selected with series of seven transfers. The purified bacterial strains were stored on agar slants and kept at 4 °C under refrigeration for further analysis.

2.5 Preliminary Screening of Isolate for Hydrocarbon Degradation

2.5.1 Preparation of inoculum

Inoculum was prepared as described by Nwanyanwu *et al*. (2016). The test isolates were grown in nutrient broth medium contained in Erlenmeyer flasks (100 mL) at room temperature for 48 hr. Thereafter, the cells were harvested by centrifugation at 6000 rpm for 10 min and washed in sterile de-ionized water. The cell suspensions were standardized by adjusting the turbidity to optical density of 0.1 at absorbance of 540 nm and used throughout the study unless otherwise stated.

2.5.2 Turbidometric test

The MS medium was also used in assessing the ability of the bacterial isolates to utilize hydrocarbon fraction which contained 4 % (v/v) of the hydrocarbon fraction. The medium was made out in 100 mL conical flask containing 50 mL of the MS medium and sterilized. The 60 bacterial isolates were inoculated individually into separate flasks. The flasks were incubated at room temperature on an orbital shaker at 120 rpm for 7 days. Cell growth measured as optical density at 600 nm (OD600) was used as a parameter for crude oil degradation capability (Nwanyanwu *et al*., 2016). Crude oil degrading bacteria that have high degrading potentials were selectively picked and used for further research.

2.6 Plasmid Curing

After screening test for presence or absence of plasmid, 8 out of 22 isolates were plasmid borne and they were subjected to plasmid curing. The plasmid curing was performed using the method described by Isiodu *et al.* (2016). Bacterial cells were grown in broth overnight. Five (5) mL of Nutrient broth supplemented with 0.1 mg/mL acridine orange was prepared. Zero point one millilitre (0.1 mL) of freshly prepared culture

suspension of the test strains from 10^{-5} dilution was inoculated into Nutrient broth containing the acridine orange, incubated at 37 ºC (pH 7.6) for 4 days in dark and plated out on nutrient agar. Similarly, control cultures were prepared without acridine orange in Nutrient broth. Colonies that were able to grow on nutrient agar but not on modified solid mineral salt medium were isolated and considered cured.

2.7 Characterization and Identification of Crude Oil Degrading Organism

The selected pure cultures were identified using colonial description, Gram Staining, spore test, motility test, indole test, Voges-Proskauer-Methyl Red test, urease test, coagulase test, starch hydrolysis, citrate utilization test and hydrogen sulphide test, catalase test, oxidase test, nitrate reduction, sugar fermentation and growth on MacConkey agar.

3.13.4 Reclamation of polluted soil

3.13.4.1 Effect of mixed culture on crude oil degradation and plant growth

a. Inoculum preparation for microbial consortium

The bacterial consortium was prepared using method of Nwanyanwu *et al.* (2016). Individual cultures of *Pseudomonas aeroginosa* and *Ochrobacterium intermedium* were grown in nutrient broth medium contained in Erlenmeyer flasks (100 mL) for 48 hour. Thereafter, the cells were harvested by centrifugation at 6000 rpm for 10 minutes and washed in sterile deionized water. The cell suspensions were standardized by adjusting the turbidity to optical density of 0.5 at absorbance of 540 nm and used throughout the study.

b. Experimental Design

Agricultural soil (1 kg) sample was air-dried, sieved and added into 24 different plastic buckets (4cm deep x 5 cm diameter). The soil in each plastic bucket was grouped into 2 and each group has 3 sets; set A (A_0, A_1, A_2, A_3); set B (B_0, B_1, B_2, B_3) and set C (C_0, C_1, C_2, C_3). The soil microcosms were spiked with Bonny light crude oil (0 %, 3 %, 5 %, and 10 % (v/w)) concentrations, labeled and thoroughly mixed together to achieve complete artificial contamination. The experiment was set up in triplicates.

Twenty miliiliters (20 mL) of standardized inoculum culture of plasmid borne consortium was inoculated in A_1, A_2 and A_3; B_1, B_2 and B_3 were individually seeded with 20 mL of standardized inoculum culture of plasmid cured consortium while C_1, C_2 and C_3 were uninoculated (controls). Inaddition, A_0, B_0 and C_0 were neither contain crude oil nor seeded with isolates. Therefore, two types of controls were prepared. One control contained crude oil but not inoculated with organisms while the other has neither crude oil nor organism. After 20 days of starting the experiment, ten seeds of *Phaseolus vulgaris* (common white beans) were planted in each pot in set A, B, C and *Glycine max* (soya beans) were planted in the second set of pot in A, B and C at a depth of 2 cm and kept under partial shade in a greenhouse at the demonstration farm, Ministry of Agriculture, Awka, Anambra State. Sterile water was added to the pots every 48 hour for the period of 45 days. Seeds of soya beans and common white beans were obtained as a single batch from Agricultural Input Department, Ministry of Agriculture, Awka, Anambra State. The viability of the seeds was determined by floatation test using water. The seeds which remained at the bottom of the water were considered as potentially viable and those that floated on water were discarded (Eze *et al.*, 2014 and Aboaba *et al.*, 2007).

At 5th, 10th, 20th, 30th, 40th and 60th day, soil samples were removed and analyzed for total viable count of bacteria. At 40th day, % seed germination, shoot length, root length and plant fresh weight were evaluated while % TPH degradation was determined at 60th day. The % seed germination was calculated using the formula:

% Seed Germination = $\frac{Number\ of\ seedlings\ that\ emerged\ from\ the\ soil}{Number\ of\ seeds\ sown}\ x\ 100$

Seeds which failed to sprout were considered as non germinatable. Plant samples were collected from each bucket by carefully uprooting the plants. The weights of the fresh plants were measured using digital weighing balance. Shoot length was determined by measuring the plant from the base of each plant (above soil level) to the tip, root length was measured from the part of the stem buried in the soil to the longest rootlet. Bacterial populations in the soil microcosms were determined by standard spread plate technique. One gram of the soil sample was aseptically added into 10 ml of sterile water, shaken vigorously to dislodge the cells from the soil particles and allowed to stand for about ten minutes, after the supernatant was serially diluted (Okoh, 2013) .The population of viable cells was determined using this formula:

CFU/g = $\frac{Number\ of\ colonies\ x\ dilution\ factor}{Volume\ of\ culture}$

The experiment was performed in triplicate. Nodulation was measured by physically counting the nodules in the uprooted plant at the end of the study as described by Ogbo *et al.* (2010) and Roy *et al.* (2014).

c. Analysis of Crude Oil Samples with Gas Chromatography (GC)

This test was done using method described by Malik and Ahmed (2012). The samples (2 mL) were extracted ultrasonically with mixture of methanol, di-chloromethane (DCM) and water (1:5:4) and centrifuged for 15 min, concentrated in rotary evaporator to final volume of 5 mL. A 1 mL aliquot was separated and was diluted to concentration (1µL) appropriate for Gas chromatography (GC).Individual components of the aliphatic hydrocarbon were used as standards (standard match method).

3.14 Statistical Analyses

Statistical calculations were made using SPSS version 25 software (SPSS Inc., Chicago, IL, USA).The data obtained was subjected to descriptive statistics using mean and standard deviation of mean. One-way analysis of variance (ANOVA) test under Completely Randomnized Design (CRD) was used in interpreting the results. Post-Hoc test using Duncan Multiple Range Test (DMRT) was used to determine the averages that are conspicuously different from the other. *P* value of less than 0.05 was considered to indicate statistical significance. Excel plots were used for the charts.

3. Results

3.1 Preliminary Screening of Isolate for Hydrocarbon Degradation

The preliminary screening test showed that the isolates exhibited variations in their growths in the growth medium containing crude oil and the optical density (A600 nm) ranged between 0.10 to 0.42 as shown in Table 1. Twenty-two isolates exhibited pronounced growths (OD600 nm > 0.30) viz: A_1, A_2, B_1, C_1, C_3, D_1, E_1, E_3, F_2, F_5, G_2, H_2, H_4, I_3, I_5, I_6, J_3, K_1, K_4, L_2, M_1 and N_4, indicated in table 4.3 and were selected for further studies. Isolate designated as C3 had the highest growth with optical density of 0.42 while the isolate with least growth was designated as K_5 and had optical density of 0.10.

3.2 Identification of Crude Oil Degrading Organism

The colony and biochemical properties characteristics of the bacterial isolates shown in Tables 2 and 3 indicated that isolates which were able to degrade crude oil belonged to genera *Bacillus*, *Pseudomonas* sp., *Ochrobacterium* sp. and *Enterobacter* sp. **3.3 Reclamation of Polluted Soil**

3.3.1 Effect of mixed culture of isolates on crude oil degradation and plant growth

Effect of the hydrocarbon remediation potentials of the mixed cultures of plasmid borne and plasmid free *Pseudomonas aeruginosa and Ochrobacterium intermedium* in the contaminated soil was presented in Figures 1 - 12. The data indicated that plasmid borne bacterial mixed cultures were better bioaugmenters of petroleum hydrocarbon degradation. The percentage of remediation of the crude oil varies across all level of concentrations in each of the microcosms. In the soil microcosms polluted with 3 %, 5 % and 10 % crude oil and treated with consortium of plasmid borne bacteria, the remediation percentages recorded were 98 %, 80 % and 21 % for the respective crude oil pollution (3 %, 5 % and 10 %) whereas soil inoculated with plasmid cured bacterial cultures had 65 %, 52 % and 11 % degradation of the same concentration of the total petroleum hydrocarbon after 60 days. Also within the time under review, the percentage losses of total petroleum hydrocarbon in the un-inoculated controls was 24 %, 15 % and 24 % for the respective hydrocarbon concentrations of 3 %, 5 % and 10 % in the polluted soil in which *Phaseolus vulgaris* plant was grown.

However, remediation of the hydrocarbon followed the same patterns in the *Glycine max* plant-soil microcosms, inoculated with plasmid borne bacterial mixed culture and polluted with 3 %, 5 % and 10 % crude oil. In this experiment, the percentages decontamination of the crude oil polluted soil by the co-culture of plasmid borne cells were 98 %, 78 % and 22 % in the respective oil pollution while remediation percentages in the microcosm inoculated with co-culture of plasmid cured bacteria were 65 %, 50 % and 20 % in the same environmental conditions. The remediation percentages in the uninoculated controls include 12 %, 9 % and 5 % across the pollutions (Figures 6 - 10).

It was also observed that seed germination was highest on the control 1 which contains neither crude oil nor the isolate as compared to microcosms inoculated with plasmid borne bacteria, plasmid cured bacteria and un-inoculated control 2 across the three levels of crude oil concentrations of 3 %, 5 % and 10 %. *Glycine max and Phaseolus vulgaris* showed improved growths in soil microcosm treated with plasmid borne isolates as compared to soil microcosms treated with plasmid free isolates and oil contaminated un-inoculated control 2. It was noted that seed germination nearly doubled as compared to un-inoculated controls polluted with 3 %, 5 % and 10 % crude oil.

In the crude oil polluted soil microcosms, inoculation with plasmid borne isolates led to 80 % and 90 % germinations of *Phaseolus vulgaris and Glycine max* seeds, respectively while inoculation with plasmid cured isolates had 50 % and 60 % seed germinations of the experimental plants respectively. Germination of *Phaseolus vulgaris (*common white beans) was ceased in the soil inoculated with plasmid cured bacteria at 10 % level of pollution and totally inhibited in un-inoculated control 2 at 5 % and 10 % crude oil pollution percentages. *Glycine max* germinated in microcosm inoculated with plasmid borne bacterial mixed culture at 10 % crude oil concentration but withered after 12 days of germination.

Table 1: Preliminary screening of isolates for hydrocarbon degradation

Isolate code	OD600	Isolate code	OD600	Isolate code	OD600	Isolate code	OD600
A1	**0.35**	**E1**	**0.31**	G4	0.18	J2	0.12
A2	**0.37**	E2	0.28	G5	0.11	**J3**	**0.40**
A3	0.28	**E3**	**0.35**	H1	0.15	J4	0.28
B1	**0.35**	E4	0.18	**H 2**	**0.34**	J5	0.20
B2	0.27	E5	0.19	H3	0.12	**K1**	**0.33**
B3	0.29	F1	0.31	**H4**	**0.31**	K2	0.12
B4	0.19	**F2**	**0.36**	I1	0.25	K3	0.14
C1	**0.31**	F3	0.29	I2	0.11	**K4**	**0.30**
C2	0.27	F4	0.15	**I3**	**0.30**	K5	0.10
C3	**0.42**	**F5**	**0.32**	I4	0.17	L1	0.13
D1	**0.38**	G1	0.16	I5	**0.31**	**L2**	**0.37**
D2	0.22	**G2**	**0.37**	**I_6**	**0.36**	L3	0.15
D3	0.24	G3	0.14	J1	0.10	L4	0.14
M1	**0.35**	M2	0.21	M3	0.17	M4	0.23
N1	0.28	N2	0.19	N3	0.15	**N4**	**0.32**

Table 2: Colonial morphology of the crude oil degrading bacterial isolates

Code	Shape	Elevation	Margin	Optics	Texture	Appearance	Colour	Size
C3	Circular	Flat	Undulate	Translucent	Smooth	Shinny	Creamy	small
D1	Circular	Flat	Entire	Translucent	Smooth	Dull	Creamy	Large
H4	Circular	Flat	Undulate	Translucent	Smooth	Dull	Creamy	Small
I6	Circular	Raised	Irregular	Translucent	Rough	Shinny	Golden	Small
J3	Circular	Flat	Entire	Translucent	Smooth	Shinny	Creamy	Large
K4	Circular	Flat	Undulate	Translucent	Smooth	Shinny	Creamy	Small
L2	Circular	Flat	Irregular	Translucent	Rough	Shinny	Yellow	Small
G2	Circular	Flat	Entire	Translucent	Smooth	Dull	Creamy	Large

Table 3: Morphological and biochemical characteristics of isolates

Property	Isolates Codes							
	D_1	C_3	L_2	J_3	H_4	G_2	K_4	I_6
Gram reaction	+	-	+	-	+	+	+	-
Shape	SR	SR	SR	SR	SR	SR	SR	SR
Arrangement	S	S	S	S	S	S	S	S
Spore test	-	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+
Indole test	-	-	-	-	-	-	-	-
Motility test	+	+	+	+	+	+	+	+
Methyl Red	-	-	-	-	-	-	-	-
Voges-Proskauer	+	-	+	-	+	+	+	+
Citrate test	-	+	-	-	-	-	-	+
Urease test	-	-	-	-	-	-	-	-
Starch hydrolysis	+	-	+	-	+	+	+	-
Growth on MacConkey	-	+	+	+	+	+	+	-
$N0_3$ Reduction	+	+	+	+	+	+	+	+
Coagulase	-	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-
Oxidase	-	+	-	+	-	-	-	-
Acid from:								
Mannitol	-	-	+	-	+	-	+	+
Glucose	+	-	+	-	+	+	+	-
Xylose		-	-	-		-	-	-
Lactose	+	-	+	-	+	+	+	+
Sucrose	+	-	+	-	+	+	+	+
Arabinose	-	-	+	-	+	+	+	+
Galactose	-	-	-	-	-	-	-	-
Saccharose	-	-	-	-	-	-	-	-

Key: SR = Short rod; S = Single

An increase in the growth parameters of the leguminous plants like mean plant shoot length, mean plant root length, plant fresh weight and number of root nodules was seen more in the soil microcosms inoculated with plasmid borne bacteria than microcosm inoculated with culture of plasmid cured bacteria across all crude oil dilutions (Figures 3.8 - 3.12). In the polluted soil microcosm, inoculation with plasmid borne isolates recorded 45 % increment in shoot length of *Phaseolus vulgaris* while inoculation with plasmid cured isolates had 41 % increment when compared with uninoculated control 2 which were contaminated with crude oil. Treatment of the polluted soils with plasmid borne isolates significantly increased the plant fresh weight to 2.5 folds ($p < 0.05$) when compared with plant fresh weights of plants grown in the soil microcosms treated with plasmid cured isolates (Figures 5 and 10).

Comparatively, *Phaseolus vulgaris* had more nodules than *Glycine max* cross all crude oil level of pollutions and nodulation decreases in increase in concentrations of petroleum hydrocarbons in all studies (Figures 11 - 12). The gas chromatograms of extracted residual oils indicated the existence of a relationship between crude oil degradation and soil recovery. The gas chromatograms of the test samples (polluted samples) showed evidence of crude oil degradation when compared to the control chromatograms. This evidence was shown by the reduction or disappearance of some peaks. When the test sample chromatograms were examined vis-à-vis the plant growth responses, it was seen that oil remediation evidenced by reduction in the GC peaks led to soil recovery (confirmed by increase in crop shoot lengths, root length, seed germination, fresh plant weight and nodulation).Generally, both the lower and higher molecular weight hydrocarbons were amenable to biodegradation as evidenced in the reduction of some peaks in the chromatograms, though lower molecular weight hydrocarbons were more responsive to degradation than higher molecular weights.

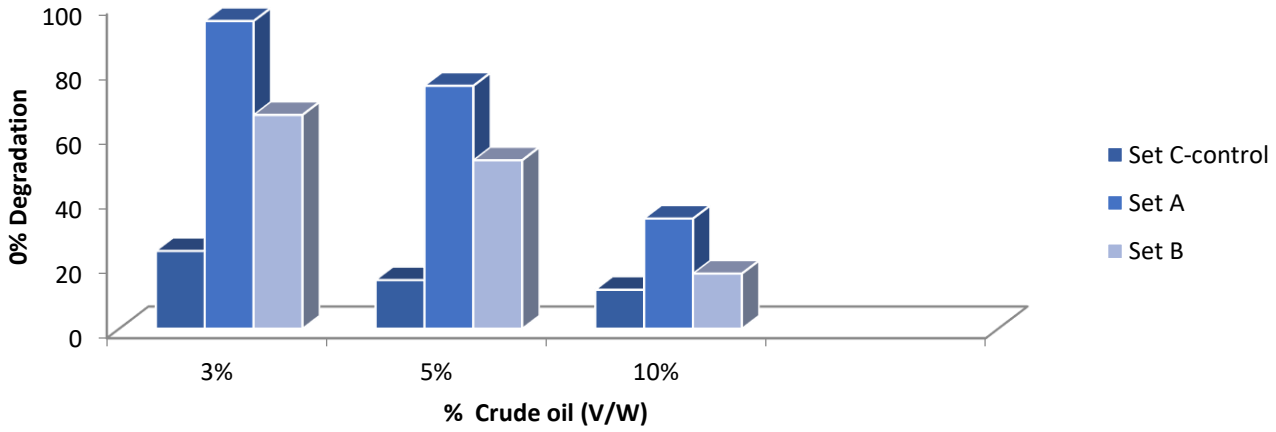

Figure 1: Degradation percentages of crude oil in soil microcosm inoculated with plasmid borne and plasmid cured bacterial mixed cultures and grown with *Phaseolus vulgaris*. **Key:** Set A = Soil + crude oil + consortium of plasmid borne isolates + common white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + common white beans; Set C- control = Soil + crude oil + common white beans.

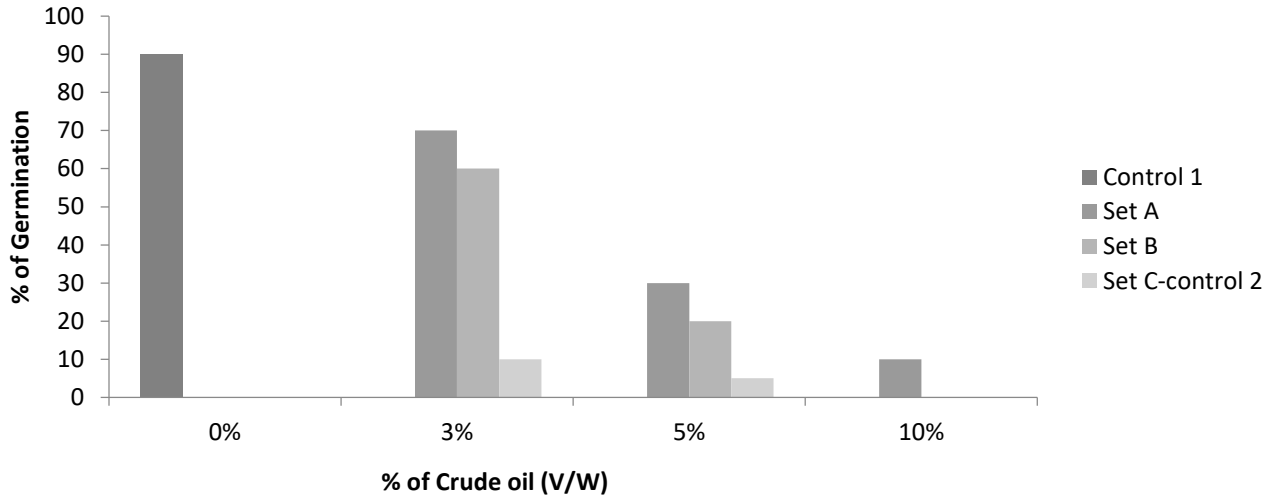

Figure 2: Germination percentage of *Phaseolus vulgaris* (common white beans) grown in soil with different concentrations of crude oil.
Key: Control 1 = Soil + white beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + common white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + common white beans; Set C- control 2 = Soil + crude oil + common white beans

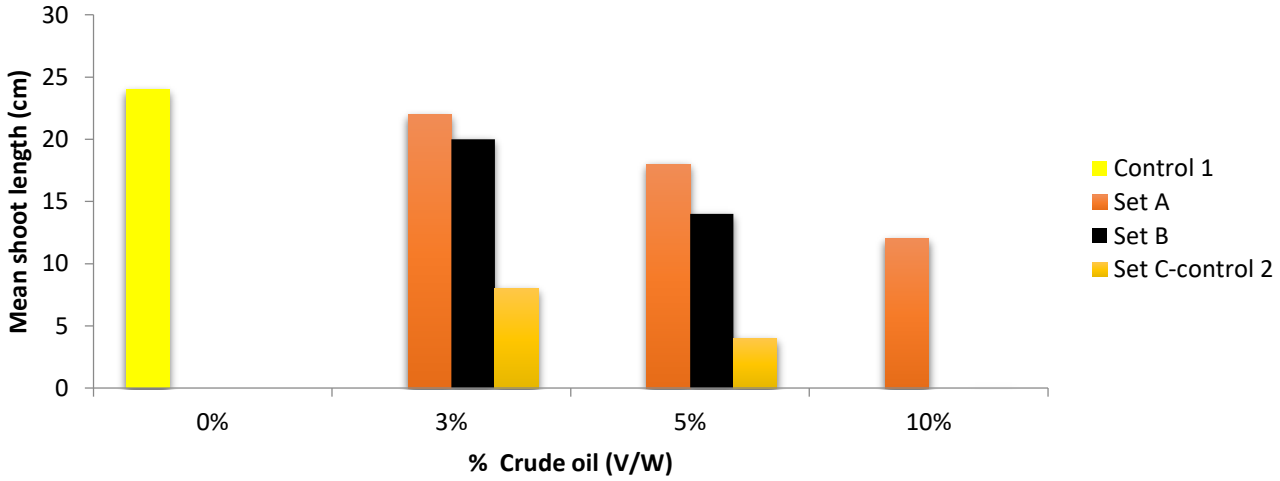

Figure 3: Effect of varying levels of crude oil concentration on shoot length of *Phaseolus vulgaris* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1 = Soil + white beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + common white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + common white beans; Set C- control 2 = Soil + crude oil + common white beans

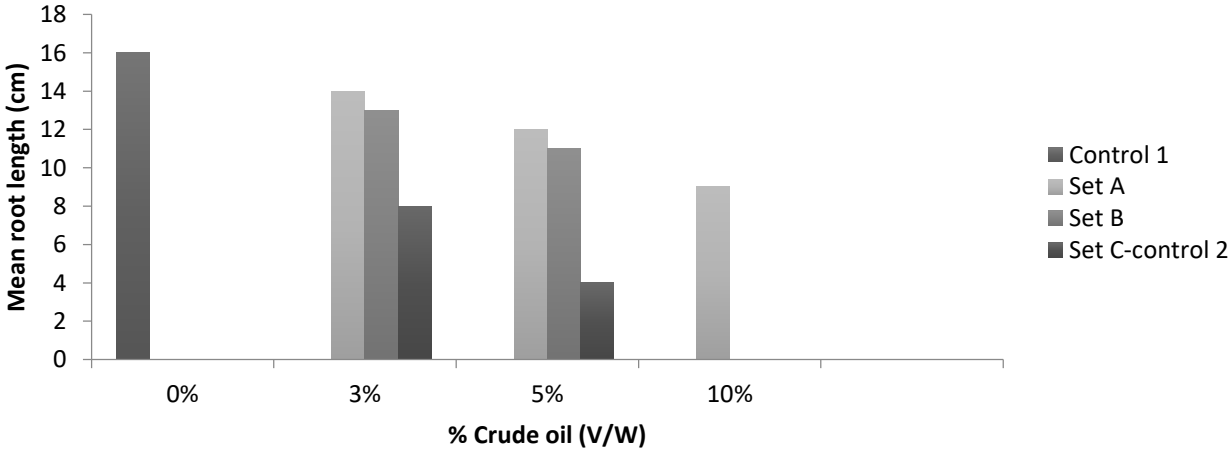

Figure 4: Effect of varying levels of crude oil concentration on root length of *Phaseolus vulgaris* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1 = Soil + white beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + common white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + common white beans; Set C- control 2 = Soil + crude oil + common white beans.

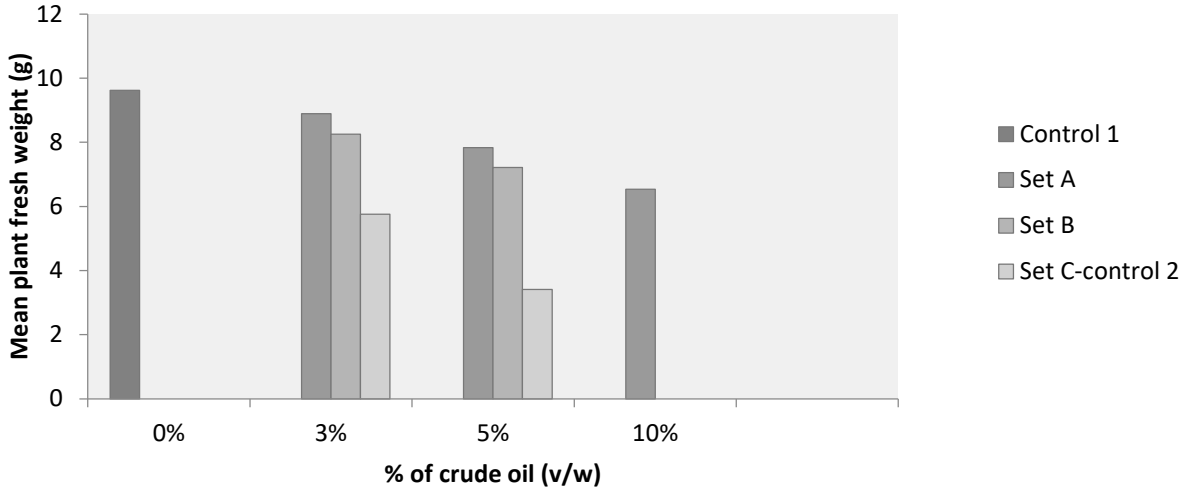

Figure 5: Effect of varying levels of crude oil concentration on fresh weight of *Phaseolus vulgaris* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1 = Soil + white beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + common white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + common white beans; Set C- control 2 = Soil + crude oil + common white beans

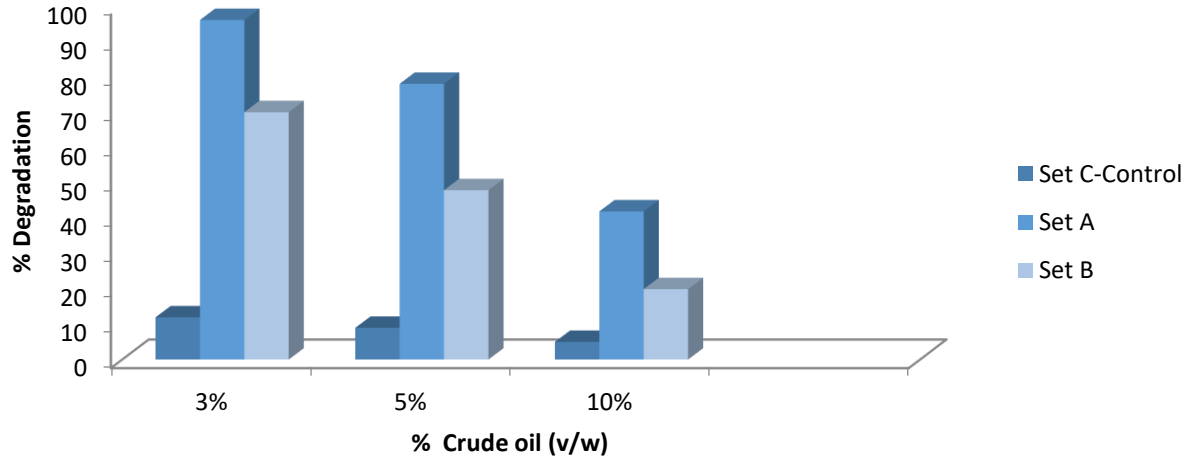

Figure 6: Percentage degradation of crude oil in soil microcosm planted with *Glycine max* (soy beans).
Key: Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control = Soil + crude oil + soy beans.

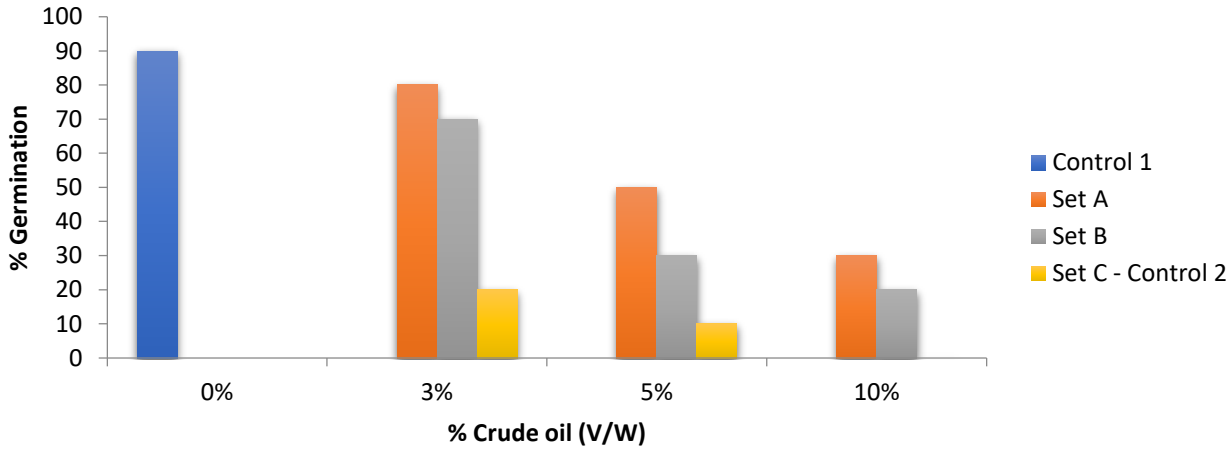

Figure 7: Percentage germination of *Glycine max* (soy beans).
Key: Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control = Soil + crude oil + soy beans.

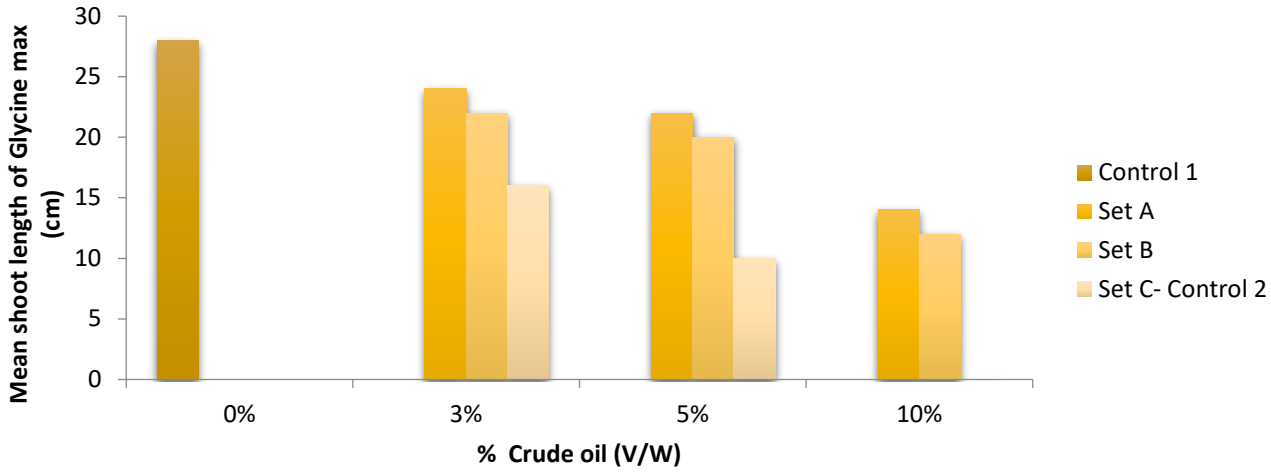

Figure 8: Effect of varying levels of crude oil concentration on shoot length of *Glycine max* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control = Soil + crude oil + soy beans.

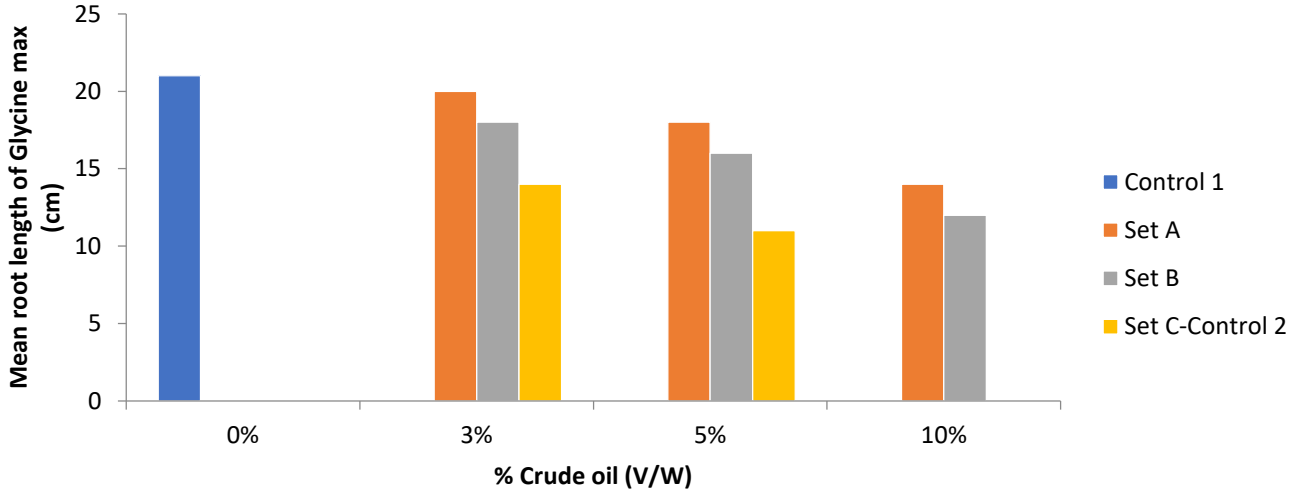

Figure 9: Effect of varying levels of crude oil concentration on root length of *Glycine max* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control = Soil + crude oil + soy beans.

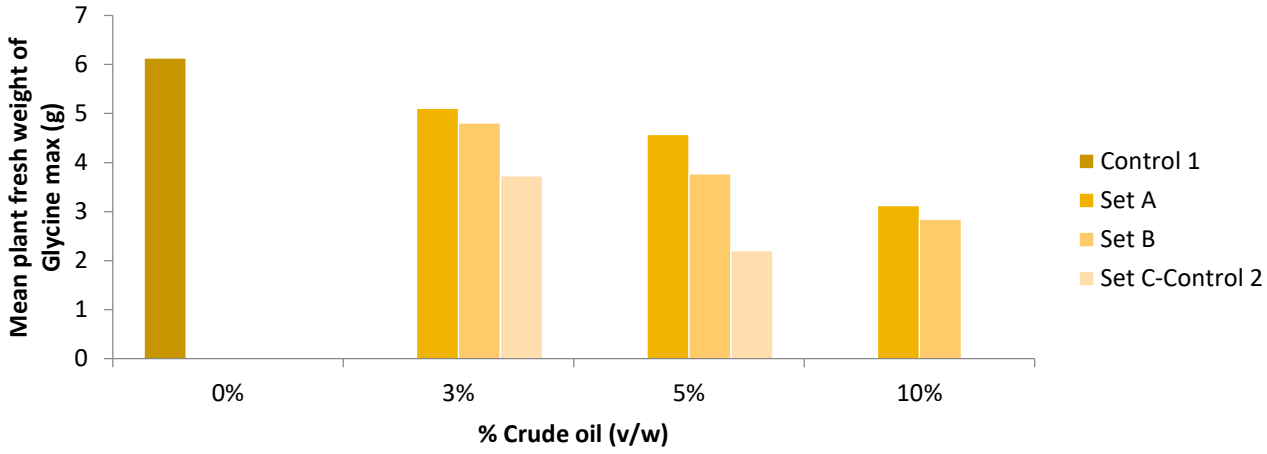

Figure 10: Effect of varying levels of crude oil concentration on fresh weight of *Glycine max* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control = Soil + crude oil + soy beans

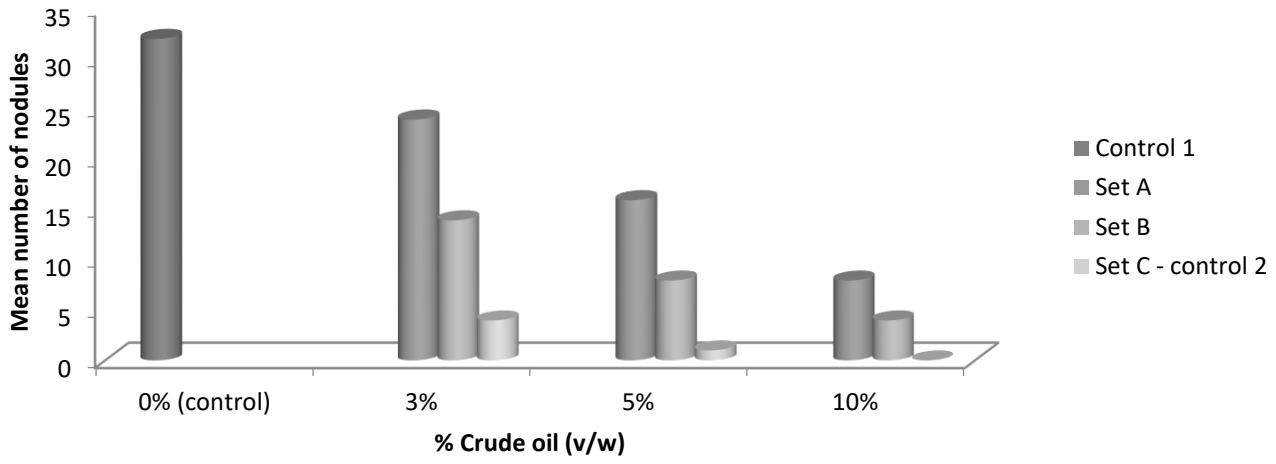

Figure 11: Effect of varying levels of crude oil concentration on nodulation of *Phaseolus vulgaris* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control = Soil + crude oil + soy beans

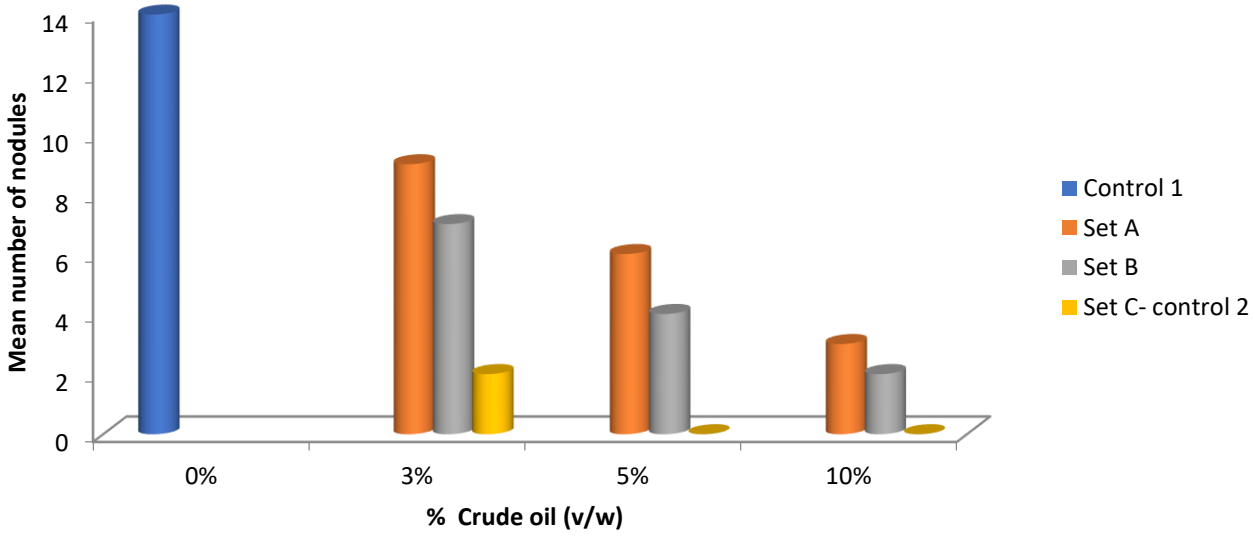

Figure 12: Effect of varying levels of crude oil concentration on nodulation of *Glycine max* grown in soil microcosm treated with mixed culture of plasmid borne and cured isolates.
Key: Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control = Soil + crude oil + soy beans.

3.3.2 Determination of bacterial population

The inoculation size of the mixed bacterial culture was 2.4 x 10^6 cells/mL at zero incubation and total mean bacteria viable counts of the experimental soil was 2.8 x 10^5 CFU/g before inoculation of the isolates .The mean bacterial viable count showed that the isolates utilized crude oil as sole source of carbon and energy, which was evident from the increase in mean viable counts from 2.8 ×10^5 CFU/g to 3.4 x 10^7 CFU/g and from 2.8 ×10^5 CFU/g to 3.5 x 10^7 CFU/g after 40 days incubation at 3 % pollution of crude oil by plasmid borne and cured bacterial isolates respectively (Figure 13). It was observed that plasmid borne isolates utilized the substrates more than the plasmid cured mutants which was evidently shown by their growth responses in all the studies (Figures 13 – 18). The maximum mean bacteria counts for plasmid borne bacteria in crude oil (3%) polluted soils in which common white beans were grown was 3.2 x 10^7 CFU/g and mean viable counts for polluted soil with soy bean plants was 3.4 x 10^7 CFU/g, whereas those for plasmid cured bacteria were 3.5 x 10^6 CFU/g and 3.7 x 10^6 CFU/g for the respective soil microcosms. The original mean count of bacteria in the control devoid of crude oil was 2.8×10^5 CFU/g and it remained in the range of 1.8 - 3.1 × 10^6 CFU/g over the period of 60 days. In the un-inoculated control 2, the bacterial count was drastically reduced from 2.8×10^5 CFU/g to 1.6 ×10^3 CFU/g at 3% crude oil, 2.4 ×10^6 CFU/g to 1.2 x 10^3 CFU/g at 5 % and 2.8 ×10^5 CFU/g to 1.1 x 10^2 and at 10 % crude oil (Figures 13 – 18). Crude oil had a dose-dependent negative effect on the mean bacteria counts of each of the polluted soil containing the two different plants. The greatest growth was seen at 3 % crude oil polluted soils while the least growth observed at 10 % crude oil polluted soils. There was continuous decrease in mean bacteria counts in the un-inoculated control. There was a rise in population of the isolates after the growth of the plants at forty-fifth day.

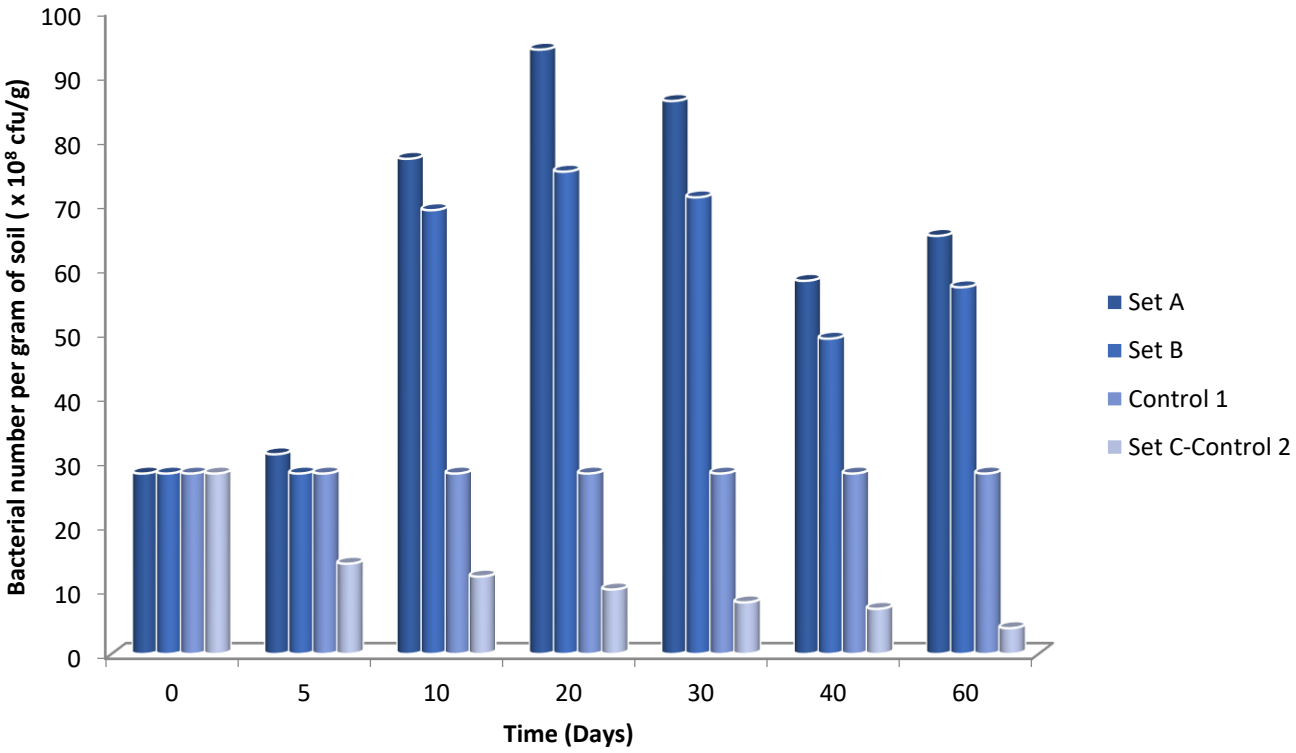

Figure 13: Changes in total viable bacterial count of *Phaseolus vulgaris* grown in soil microcosms that contained 3 % crude oil concentrations (v/w).
Key: Control 1= Soil + white beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + white beans; Set C- control = Soil + crude oil + white beans

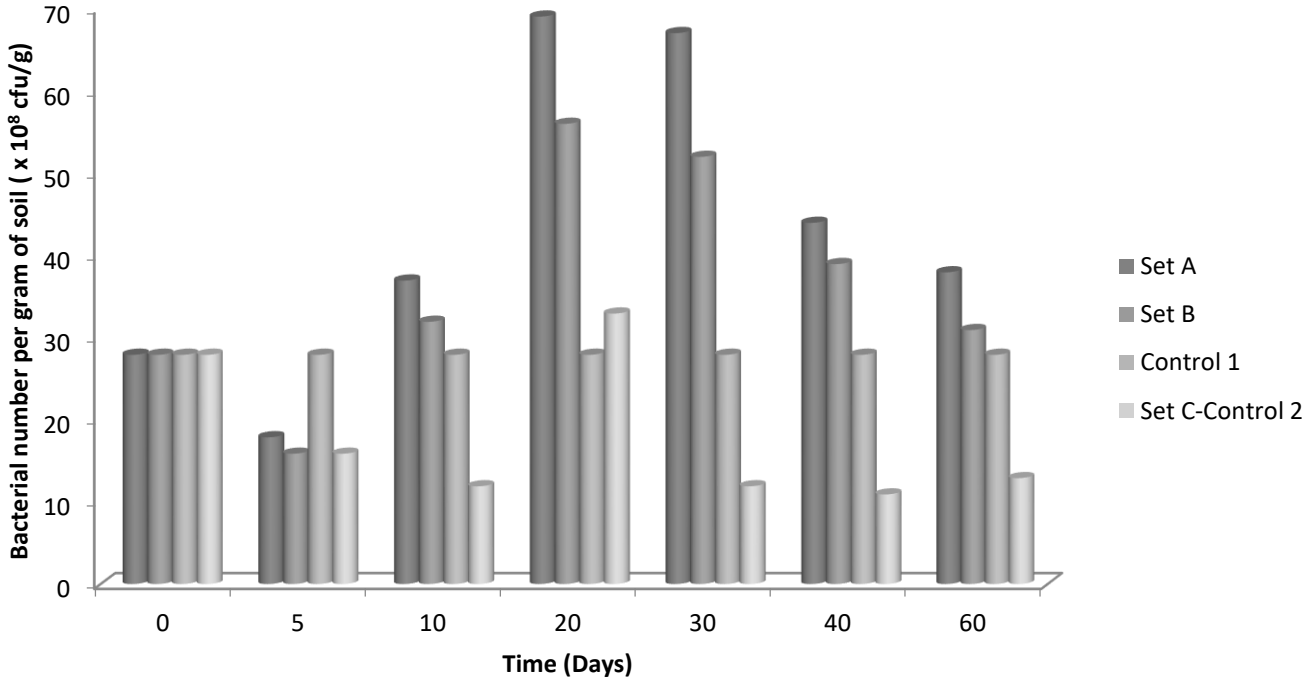

Figure 14: Changes in total viable bacterial count of *Phaseolus vulgaris* grown in soil microcosms that contained 5 % crude oil concentrations (v/w).
Key: Control 1= Soil + white beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + white beans; Set C- control 2 = Soil + crude oil + white beans

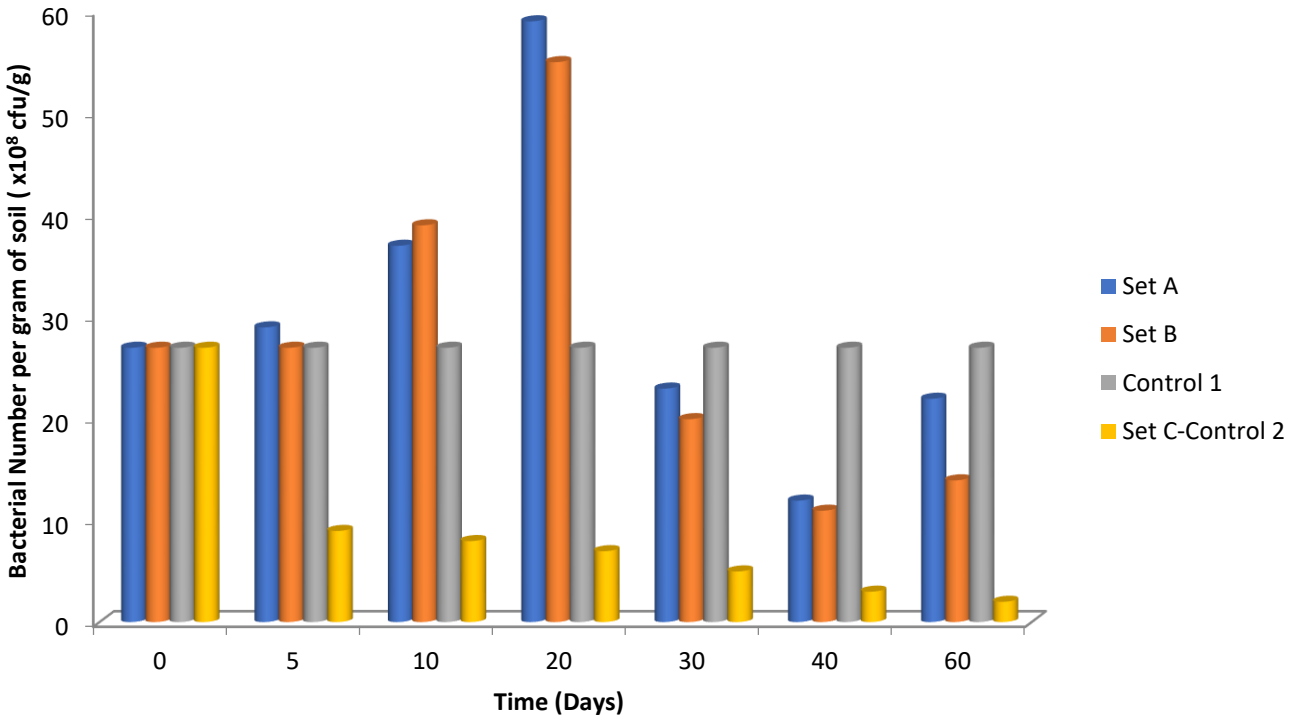

Figure 15: Changes in total viable bacterial count of *Phaseolus vulgaris* grown in soil microcosms that contained 10 % crude oil concentrations (v/w). **Key**: Control 1= Soil + white beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + white beans; Set B = Soil + crude oil + consortium of plasmid free isolates + white beans; Set C- control 2 = Soil + crude oil + white beans

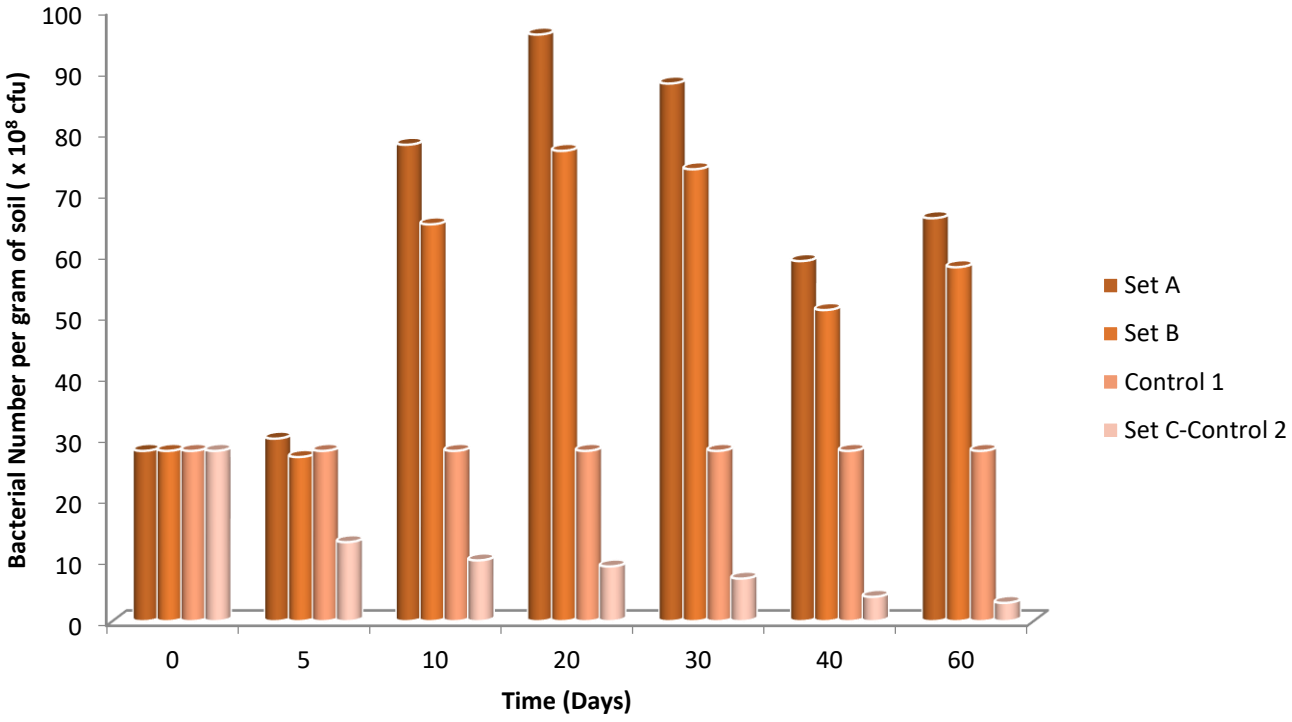

Figure 16: Changes in total viable bacterial count of *Glycine max* grown in soil microcosms that contained 3 % crude oil concentrations (v/w). **Key:** Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control 2 = Soil + crude oil + soy beans

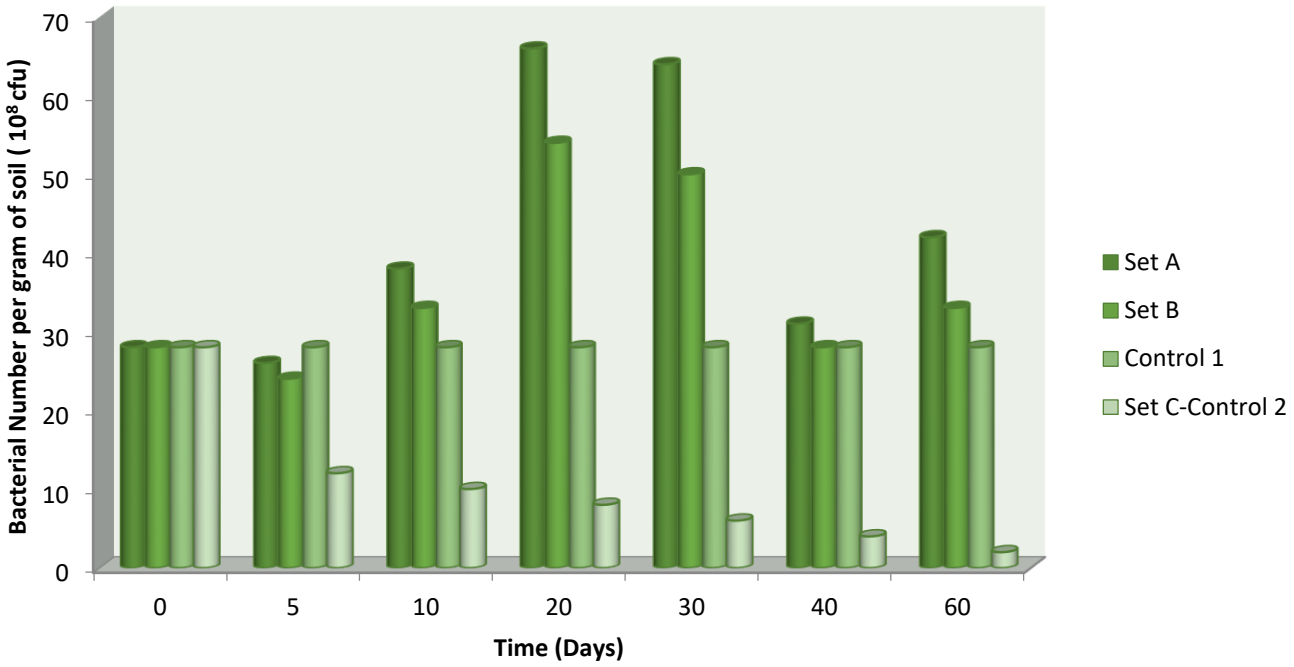

Figure 17: Changes in total viable bacterial count of *Glycine max* grown in soil microcosms that contained 5 % crude oil concentrations (v/w). **Key:** Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control 2 = Soil + crude oil + soy beans

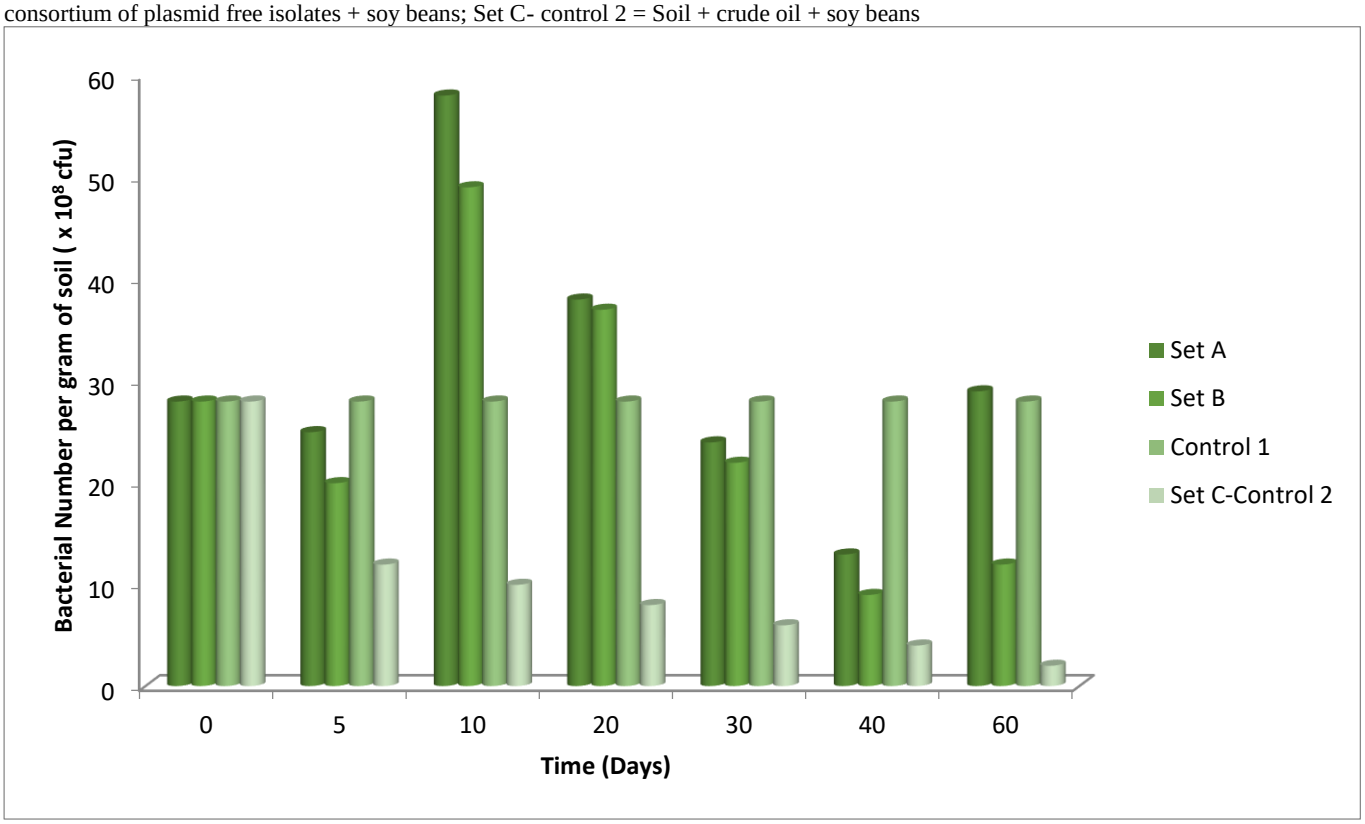

Figure 18: Changes in total viable bacterial count of *Glycine max* grown in soil microcosms that contained 10 % crude oil concentrations (v/w). **Key**: Control 1= Soil + soy beans; Set A = Soil + crude oil + consortium of plasmid borne isolates + soy beans; Set B = Soil + crude oil + consortium of plasmid free isolates + soy beans; Set C- control 2 = Soil + crude oil + soy beans.

Discussion

Sixty hydrocarbon utilizing bacteria were isolated from crude oil supplemented mineral salt agar medium (Table 1). Since all the bacteria in the present study were isolated from a petroleum contaminated soil sample, they survived in the oil-supplemented media very easily as also reported by other authors (Rahman *et al.*, 2003). Table 1 indicated that 22 of the isolates were observed to have high pollutant degrading potentials (A600 nm > 0.3) due to their crude oil utilization ability. Absorbance (A600 nm) of the cells grown in a medium

with petroleum as a sole carbon source has been used as an index of PHC biodegradation potential (Binazadeh *et al.*,2009; Celik *et al.*, 2008; Ciric *et al.*, 2010; Husain *et al.*, 2011). The other isolates showed variations in their growth on the substrate and the isolate with least growth has optical density of 0.11.Ciric *et al.* (2010) had differentiated growth of the alkane degraders based on A600nm using the following criteria: No growth = A600 nm 0.00 – 0.019; +, minimal growth = A600 nm 0.02 – 0.099; ++, moderate growth = A600 nm 0.1 – 0.2; +++, maximum growth = A600 nm > 0.2. Nwanyanwu *et al.* (2016) isolated *Micrococcus* sp. RS38 which showed impressive level of growth during screening in crude oil and other petroleum products where the organisms grew at equal optical densities of > 0.2 within 14 days of incubation. Vinothini *et al.* (2015) reported optical density of 0.55 by *Pseudomonas putida* which crude oil degradation ability was screened based on the growth efficiency on 2 % crude oil at the 7th day of incubation period.

The results in Tables 2 and 3 indicated that 75 % (6 out of 8) of plasmid-borne bacteria belonged to the genera *Bacillus*. Several species of Gram positive bacteria carry multiple plasmids which serve as adaptive mechanism especially those belonging to the genera *Staphylococcus, Streptococcus, Lactobacillus, Bacillus* and *Corynebacterium* (Kunninalaiyan *et al*., 2001, Igwilo-Ezikpe *et al*., 2010).

All the results in this study clearly pointed out that the remediation percentages of crude oil in the soil-plant microcosms inoculated with plasmid borne organisms was more than the degradation percentages in the microcosms seeded with the plasmid cured isolates. Consequently, there was increase in the growth parameters of the leguminous plants such as total mean plant shoot and root length, plant fresh weight and number of nodules in the uprooted plants in the microcosms in which plasmid borne organisms were applied than that of plasmid cured organisms across all crude oil dilutions (Figures 3.1 – 3.12). There was also an observation of doubling of germinated seeds in the microcosms treated with bacteria as compared to un-inoculated controls in 3 % and 5 % crude oil containing soils. This observation affirmed that plasmid confers biodegradation potentials on microorganisms. Johnson *et al*. (2005) reported that many genes involved in the degradation are often located on plasmids. Similarly, Lin and Cai (2008) reported that *Escherichia coli* cells transformed with the plasmids of the enriched consortium degraded 85.7 % of pyrene in three weeks. Mukherjee and Bordoloi (2011) demonstrated bioremediation and reclamation of 20 % (v/w) PHC contaminated soils using combination of *Bacillus subtilis* DM-04 and *Pseudomonas aeruginosa*. The authors reported 76 % TPH degradation in 180 days and observed that the reclaimed soil supported the germination and growth of crop plants *Cicer aretinum* and *Phaseolus mungo*.

The results also clearly showed that low concentration (3 %) of the crude oil did not have significant negative effects on the population of both the plasmid borne and plasmid cured soil bacteria. This is evidenced by the enormous growth of the experimental plants and when compared to the control (0 %), there is no remarkable difference between them. On the other hand, crude oil at high concentrations (5 % and 10 %) had more significant ($P < 0.05$) negative effect on plasmid cured bacterial isolates than that of plasmid borne organisms. This observation is based on the parameters of growth of the test plants such as mean plant shoot and root length, plant fresh weight and number of nodules in the uproot which were more pronounced in the soil-plant microcosms added with plasmid borne microorganisms (Figures 3.3 – 3.12). This poor growth response may be attributed to the exclusion of air from the soil due to the oil interfering with soil/water relationships, and also to the depletion of oxygen by microbial degradation of the contaminants. This corroborates with work of Onuoha *et al. (*2014) which documented that concentration of crude oil of > 3 % in an environment, crude oil becomes increasingly deleterious to soil biota and crop growth.

Generally, crude oil retarded some plant growth indices and the degree of retardation was dependent on bioremediation capacities of the inoculated bacteria (plasmid borne and cured bacteria) and crude oil doses. However, in some hydrocarbon contaminated soil which original soil conditions that permitted plant germination was totally restored by the bacteria, plant shoot growth did gradually increased with time. *Glycine max* exhibited a remarkable degree of tolerance for the oil by germinating in higher levels of pollution. Though *Glycine max* grew in all the levels of crude oil, there was significant suppression of growth at higher doses and some germinated seeds of *Glycine max* withered after some days of germination. Based on available literature (Adams *et al*, 2014), oil contamination reduces the ability of soil to support the growth of plants, seeps into ground to contaminate ground water, and increases the presence of heavy metals which can bioaccumulate and biomagnify causing adverse health effects. The inhibition of germination observed in *Phaseolus vulgaris* at high doses of crude oil is in line with the finding of Malek-Hossein *et al.* (2007). They studied the effect of light crude oil on the germination of alfalfa (*Medicago sativa*) and observed germination inhibition at high doses. Their observation confirmed the report of Amadi *et al.* (1996) that high doses of petroleum hydrocarbon can inhibit germination in some plants.

Nodules in the control plants significantly ($P<0.05$) outnumbered those of the test plants (plants grown in the polluted soils). Moreso, leguminous crops nodulated more in the microcosms seeded with plasmid borne bacterial cultures than in microcosms bio-augmented with plasmid cured bacteria. The number of nodules in the test samples decreased with increase in crude oil pollution. The bioaugmentation agent, plasmid borne organism caused better growth enhancement at the all crude oil dilution level than plasmid cured counterpart in all the plants. Conclusively, plasmid borne bacterial culture is a better option for biodegradation of polluted soil since it restored the polluted soils agents used and enhanced germination and growth of *Phaseolus vulgaris* and *Glycine max* up to 10 %. This study confirmed that treatment of oil-impacted agricultural land with a selected culture of plasmid borne *Pseudomonas aeroginosa* and *Ochrobacterium intermedium* can produce soils which are capable of growing healthier plants than plasmid free isolates. Moreover, from the results of this study, planting of *Phaseolus vulgaris* and *Glycine max* seeds seems to be a good biological index that can be used in evaluating the level of recovery of agricultural land upon pollution with crude oil.

The mean bacterial viable count showed that the isolates utilized crude oil as sole source of carbon and energy, which was evident from the increase in mean viable counts from 2.8 $\times 10^5$ CFU/g to 3.4 x 10^7 CFU/g and from 2.8 $\times 10^5$ CFU/g to 3.5 x 10^7 CFU/g after 40 days incubation at 3 % pollution of crude oil in soil treated with plasmid borne and cured bacterial

isolates, respectively (Figure 13). It was observed that plasmid borne isolates utilized the substrates more than the plasmid cured mutants which was evidently shown by their growth responses in all the studies (Figures 3.13 – 3.18). This higher number of the organisms in the soil treated with plasmid borne bacteria can be attributed to the ability of these organisms to drastically reduce the pollutant to a less toxic levels. The maximum mean bacteria counts for plasmid borne bacteria in crude oil (3%) polluted soils in which common white beans were grown was 3.2 x 10^7 CFU/g and mean viable counts for polluted soil with soy bean plants was 3.4 x 10^7 CFU/g, whereas those for plasmid cured bacteria were 3.5 x 10^6 CFU/g and 3.7 x 10^6 CFU/g for the respective soil microcosms.

The original mean count of bacteria in the control devoid of crude oil was 2.8×10^5 CFU/g and it remained in the range of 1.8 - 3.1 × 10^6 CFU/g over the period of 60 days. In the un-inoculated control 2, the bacterial count was drastically reduced from 2.8×10^5 CFU/g to 1.6 ×10^3 CFU/g at 3 % crude oil, 2.4 ×10^6 CFU/g to 1.2 x 10^3 CFU/g at 5 % and 2.8 ×10^5 CFU/g to 1.1 x 10^2 and at 10 % crude oil (Figures 3.13 – 3.18). Rahman *et al*. (2002) also reported that population level of hydrocarbon utilizers and their populations within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons. The growth in microbial population was also fostered by the crude oil as it served as carbon and energy source as asserted by Akpoveta *et al*. (2011). There was slight increase in the bacteria population towards the 60 days period. Subathra *et al*. (2013) reported similar observation in their work. High heterotrophic bacteria counts in the contaminated plot towards the end of the experiment have been attributed to the fact that more nutrients are now available for the indigenous bacteria present in the soil due to the breakdown of hydrocarbons to the simpler forms by the oil degraders.

Conclusion

The whole study has revealed that indigenous hydrocarbon bacterial biodegraders existed in the sampling sites. The selected bacterial degraders possess both plasmid and chromosomal mechanisms in the reclamation of crude oil polluted soil samples. There were significant increases in the plant growth indices of *Phaseolus vulgaris* and *Glycine max* plant seeds relative their controls. The plasmid borne selected bacterial agents showed more promising bioremediation potentials than the plasmid free selected bacterial counterparts under the same treatment conditions.

References

Aboaba, O.A., Aboaba, O.O., Nwachukwu, N.C., Chukwu, E.E. and Nwachukwu, S.C.U. (2007).Evaluation of bioremediation of agricultural soils polluted with crude oil by planting beans seeds, *Phaseolus vulgaris*. Nature and Science, 5 (4):1-8.

Adams, G. O., Tawari-Fafeyin, P.; Igelenyah, E. and Odukoya, E. (2014). Assessment of heavy metals bioremediation potential of microbial consortia from poultry litter and spent oil contaminated site. *International Journal of Environmental Bioremediation and Biodegradation*, 2 (2): 84-92.

Akpoveta, O.V, Egharevba, F., Medjo, O.W.(2011). A pilot study on the hydrocarbon and its kinetics on kerosene simulated soil.*International Journal of Environmental Science 2(1): 54 - 67*

Amadi, A., Abbey, S. D.And Nma, A. (1996). Chronic Effects Of Oil Spill On Soil Properties And Microflora Of A Rainforest Ecosystem In Nigeria. *Water Air Soil Pollution, 86*, 1-11.

Binazadeh, M., Karimi, I. A. and Li, Z. (2009).Fast biodegradation of long chain *n*-alkanes and crude oil at high concentrations with *Rhodococcus* Sp. Moj-3449.Enzyme *Microbial Technology*, 45: 195-202.

Celik, G. Y., Aslim, B. and Beyatli, Y. (2008).Enhanced crude oil biodegradation and rhamnolipid production by *Pseudomonas stutzeri* strain g11 in the presence of tween-80 and triton x-100. *Journal of Environmental Biology*, 29: 867-870.

Ciric, L., Philp, J. C. and Whiteley, A. S. (2010).Hydrocarbon utilization within a diesel degrading bacterial consortium. *Fems Microbiology Letter*, 303: 116 – 122.

Eze, C.N.,Ugwu, C.C .,Eze,E.A.and.Eze, U.S. (2014). Evaluation of germination, shoot growth and rhizofungal flora of *Zea mays* and S*orghum bicolor* in soil contaminated with varying levels of Bonny light crude oil. *International Journal of Current Microbiology and Applied Sciencies*, 3 (1): 253-263.

Fowzia, A. and Fakhruddin, A.N.M. (2018). A review on environmental contamination of petroleum hydrocarbons and its biodegradation. International Journal of Environmental Science and Natural Resource, 11(3): 555-811.

Francis, P., Lapin, D. and Rossiasco, P. (2011).Securing development and peace in the niger delta: a social and conflict analysis of change, Washington, Woodrow Wilson International Centre For Scholar Africa Programme, 90-118.

Gayathri E, Bharathim B, Natarajan S. Isolation, identification and characterization of diesel degrading bacteria. International Journal of Current Research 2014; 6(6): 7295-300.

Husain, I. A. F., Alkhatib, M., Zahangir, A. M. D. and Muyibi, S. A. (2011). Kinetic study of a bacterial consortium isolated from soil contaminated with crude oil. *Australian Journal of Basic Applied Science*, 5: 925-930.

Hyina, A., Castillo, S.M.I., Villarreal, S.J.A., Ramirez, E.G and Candelas, R.J., 2003. Isolation of soil bacteria for bioremediation of hydrocarbon contamination. Oceania, 44(1), 88-91.

Igwilo-Ezikpe,M.N.,Okpuzor,J.,Awodele,O.,Nwaokorie,F.O.,Fowora, M.A.andAkinbo, M.O. (2010). Prevalence of polycyclic aromatic hydrocarbon: involvement of plasmid in degradation .*International Journal Of Biology and Chemical Science*, 4 (6): 2133 – 2145.

Ijah, U.J.J., Auta, S. H. and Olanrewaju, R. K. (2013). Biostimulation of crude oil contaminated soil using soybean waste. *Advanced Science Focus*, 1: 1–7.

Isiodu, G.G., Stanley, H. O., Ezebuiro, V. and Okerentugba, P.O. (2016). Role of plasmid-borne genes in the biodegradation of polycyclic aromatic hydrocarbons (PAHS) by consortium of aerobic heterotrophic bacteria. *Journal of Petroleum and Environmental Biotechnology*, 7:264.

Johnsen, A. R.,Wick, L,Y. and Harms, H. (2005).Principles of microbial PAH-degradation in soil. *Environmental Pollution*, 133 (1):71-84.

Kunnimalaiyan, M., Steveson, D.M., Zhou, Y. and Vary, P. S. (2001). Analysis of the replicon region and identification of an RNA operon on pbm 400 of *Bacillus megaterium* Qm B1551. *Molecular Microbiology*, 39:1010 – 1021.

Lin, Y. and Cai, L.X. (2008). PAH-Degrading microbial consortium and its pyrene-degrading plasmids from mangrove sediment samples in Huian.China.*Marine Pollution Bulletin*, 57 (6-12):703-706.

Malek-Hossein, S. and Gholamreza, S. (2007). Study of growth and germination of *Medicago sativa* (Alfalfa) in light crude oil-contaminated soil. *Journal of Agriculture and Biological Sciences*, 3(1): 46-51.

Mukherjee, A. K. and Bordoloi, N. K. (2011).Bioremediation and reclamation of soil contaminated with petroleum oil

hydrocarbons by exogenously seeded bacterial consortium: a pilot-scale study. *Environmental Sciences and Pollution Research*,18: 471-478.

Mulder, M.J.H.L.and Spierings, E.L.H. (2017).Treatments of orofacial muscle pain: a review of current literature. *Journal of Dentistry and Oral Disorders* .3: 1075-1080.

Nakamura, F.M., Germano, M. G. and Tsai, S.M. (2014). Capacity of aromatic compound degradation by bacteria from amazon dark earth. *Diversity, 6*: 339 – 353.

Nwanyanwu, C.E., Opurum, C.C. and Nweke, C.O. (2016). Crude oil biodegradation potential of bacterial strains isolated from mangrove ecosystem. *Futo Journal Series (Futojnls)*, 2(1): 244 - 254.

Ogbo, E. M., Tabuanu, A. and Ubebe, R. (2010).Phytotoxicity assay of diesel fuel-spiked substrates remediated with *Pleurotus tuberregium* using *Zea mays*. *International Journal of Applied Research and National Productivity*, 3: 12-16.

Oka, N.O. (2017). "The spate of environmental degradation and its impacts on Niger Delta communities' sustenance". *International Journal of Development and Sustainability*, 6 (10):1461-1485.

Okoh, A.I. (2013). Biodegradation of Bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. *African Journal of Microbiology*, 1 (3):45 - 48.

Onuoha, S.C., Chukwura,E.I.and Fatokun,K. (2014) .Stimulated biodegradation of spent lubricating motor oil in soil amended with animal droppings. *American Journal of Bioscience*, 2(1): 19-27.

Rahman, K.S.M., Thahira-Rahman, J., Kourkoutas, Y., Petsas, I., Marchant,R., Banat, I.M.(2003). Enhanced bioremediation of *n*-alkanes in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. *Bioresource Technology*, 90: 159–168.

Rahman, K.S.M., Thahira-Rahman. 1., Lakshmanaperumalsamy. P. and Banat, I.M. (2002).Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresource Technology*, 85: 257-261.

Roy, A.S., Baruah,R., Borah, M., Singh,A.K, Boruah,H.P.D., Saikia,N., Deka,M., Duta,N.and Bora,T.C.(2014). Bioremediation potential of native hydrocarbon degrading bacterial strains in crude oil contaminated soil under microcosm study. *International Biodeterioration and Biodegradation*, 94:79-89.

Subathra, M. K.; Immanuel, G. and Suresh, A. H. (2013). Isolation and identification of hydrocarbon degrading bacteria from Ennore Greek. *Bioinformation*, 9(3): 150-157.

UNDP Report.(2006)."Nigeria Niger Delta Development Report",UNDP Abuja, Www.Undp.Org (Accessed July 10, 2017).

Yerima, M.B.,Agina, S.E.,Zuru, A.A.,Venil, K.,Maishanu,H.M.,Shinkafi,A.L. and Kashim, Z.A.(2011).*Journal of Sustainable Devevelopment and Environment Protection*,1:69

Vinothini, C., Sudhakar, S. and Ravikumar, R. (2015). Biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus*. *International Journal of Current Microbiology and Applied Sciences*, 4(1): 318 – 329.