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Remediation of petroleum polluted soils with organic manures and inorganic fertilizer and its effect on fluted pumpkin (*Telefeira occidentalis*) in Bayelsa State

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ABSTRACT

Growth parameters of fluted pumpkin (*Telefeira occidentalis*) were examined to assess the effectiveness of remediating crude oil-polluted soils on application of organic manures and inorganic fertilizer in enhancing hydrocarbon-degrading bacteria (HDB) used in the bioremediation process in Bayelsa State. Two locations, Imiringi and Koloama, in Ogbia and Southern Ijaw Local Government Areas, respectively, known for crude oil spills were chosen for this study. Soil samples were collected from a depth of 0–30 cm, bulked for pot experiment in a screen house. Twenty-four plastic buckets, each holding 5 kg of soil and 0.5 kg of each of the treatments, were applied to each location, totaling 48 buckets. The experimental design was a 2 x 8 factorial arranged in a Completely Randomized Design (CRD), replicated three times. The data were statistically analyzed using Tukey's Test to distinguish significant differences at 5% probability level. The analyzed soil samples aimed to assess the impact of organic manures (cow dung and poultry droppings), inorganic fertilizer (NPK), and their combinations (CD + PD, PD + NPK, NPK + CD, and CD + PD + NPK) on the growth of fluted pumpkin (*Telefeira occidentalis*) and on the population of hydrocarbon-degrading bacteria (HDB) in the crude oil-polluted soils. In Imiringi, the HDB population (expressed in CFU/g) ranged from 8.30 x 10^5a to 1.14 x 10^6a, while Koloama, ranged from 5.74 x 10^5b to 7.36 x 10^5b, indicating significant difference between the two locations. The overall results showed high significant difference in growth parameters of fluted pumpkin (*Telefeira occidentalis*) with a few variations and HDB populations at different time (30, 60 and 90 days) suggesting the effectiveness of the application of these amendment materials.

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1.0 Introduction

Petroleum pollution poses significant environmental concern, exerting widespread effects on soil quality and the overall health of the ecosystem. The introduction of petroleum hydrocarbons into the soil leads to extensive pollution, thereby presenting threats to both plant growth, animals and human health. In view of the adverse impacts of petroleum pollutants on the soil, various remediation strategies have been employed, among which the application of organic manures and inorganic fertilizers plays a crucial role (Johnson and Smith, 2021). The ongoing study of remediation of petroleum-polluted soils, utilizing these amendments, underscores their potential influence on the growth and development of fluted pumpkin (*Telefeira occidentalis*), a crop of substantial versatility and commercial value.

Soils of Bayelsa State are affected adversely with ecological issues associated with the activities carried out on the land along with oil exploration, spillage and disposal of petroleum products ensuing in the pollution of the aquatic and soil environment (Teknikio *et al.*, 2018). Thus, Oil spillage have detrimental effects on both plants and animals. Many studies have been carried out on environmental pollution in the Niger Delta, but very little clean up takes place (Sojinu *et al.*, 2010). For example, in Bayelsa State where pollution is high, little or no research has been carried out on how to remedy the polluted soils with the available resources. The result is that more and more farmlands have being lost to crude oil pollution incidents. Research interests has been directed towards developing new

techniques and environmentally friendly methods for the remediation of soils polluted with petroleum hydrocarbons leading to bioremediation processes using biological means as an alternative remedy to pollutants elimination from the soil without causing any deleterious effects on the surroundings. This method is cost effective, environmentally friendly and materials are easily and readily available (April *et al.*, 2000). This type of biological treatment is seen as a viable option because of the capacity of the organisms to breakdown pollutants. The search for inexpensive and environmentally friendly options for enhancing petroleum hydrocarbons degradation through bio-stimulation has been the focus of research in recent times (Danjuma *et al.*, 2012; Nyankanga *et al.*, 2012). One of such option is the use of organic wastes derived from animals.

The objectives of this study are to (1) examine the influence of organic manures and inorganic fertilizer amendments on hydrocarbon-degrading bacteria (HDB) within the study areas; and (2) assess the effectiveness of these amendments or treatments on the growth of fluted pumpkin (*Telfairia occidentalis*).

2.0 Materials and Methods

Two (2) crude oil polluted locations were selected for this research namely; Imiringi with latitudes 4º.52' – 4º.85' N and longitudes 6º.23' – 6º.37' E in Ogbia Local Government Area and Koloama with latitudes 4º.58' - 52.8'N and longitudes 6º.06' - 27.2' E in Southern Ijaw Local Government Area both in Bayelsa State.

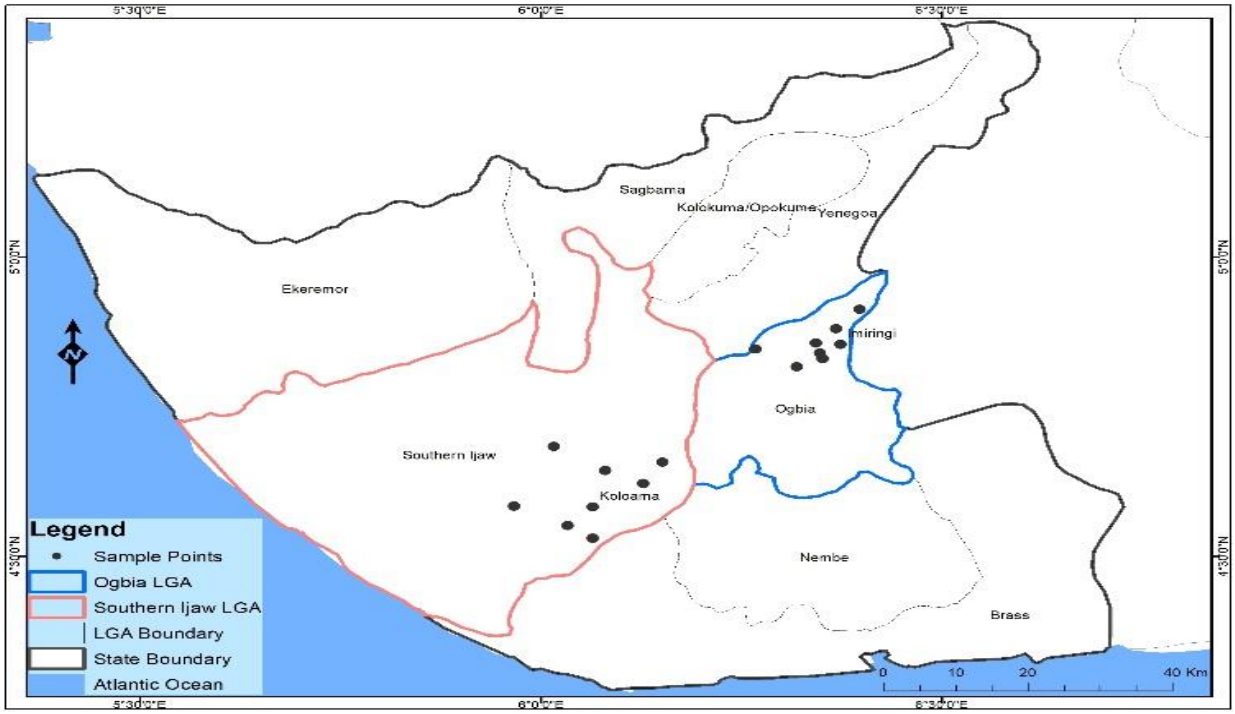


Fig. 1 Map of Bayelsa State showing the sampling points in the two study locations (Imiringi and Koloama) of the crude oil polluted soils

2.1 Quantification of Total Petroleum Hydrocarbons (TPHs)

Total Petroleum Hydrocarbon content was quantified utilizing a Gas Chromatography – Flame Ionization Detector Model HP 5890 series II, originating from the United States.

Methodology: Extraction Process, Purification and Separation were used in this process. The determination of total petroleum hydrocarbon content was further executed through a standard solvent extraction approach. A gram of sieved soil sample was dissolved in chloroform within a test tube. Subsequently, the clear lower layer was collected using a clean test tube, followed by dehydration with anhydrous sodium sulfate. The resulting clear extract was then subjected to quantification using the Gas Chromatography – Flame Ionization Detector Model HP 5890 series II from the United States to obtain the reading which in turn was calculated in mg/kg of the soil to ascertain the reduction in the level of TPH in the soil, during the remediation process.

2.2. Hydrocarbon degrading bacteria count Pour plate method

1 g of each soil sample in three replicates was placed in 9 ml of normal saline water in a beaker to make a 10 ml solution (stock). The mixture was thoroughly stirred for 3 minutes and 1ml of the stock solution was mixed with 9 ml of normal saline water to make a 10^{th} fold serial dilution and 1 ml of the 10^{4th} one served as the inoculum and the media (nutrient agar) was poured on the petri-dish plate for bacterial colony count and was incubated at 37ºC for 24 hours to 48 hours. Later, 1 ml of the suspension was also transferred into 10 ml of Bushelnel Haas (BH) broth containing 1 ml of crude oil as the sole carbon source and was incubated at 37ºC for 21 days for degrading hydrocarbon bacterial count. After incubation, the culture suspension was poured plated using sterile nutrient agar (NA) and incubated at 37ºC for 48 hours. Predominant bacterial

colonies grown on the NA plates were selected and sub-cultured on a freshly prepared nutrient agar. The sub culture was done repeatedly until a pure culture was obtained. This formula was used for calculating the colony-forming unit of the bacteria;

$$\text{CFU/g/of soil} = \frac{\text{C x DF}}{\text{V}}$$

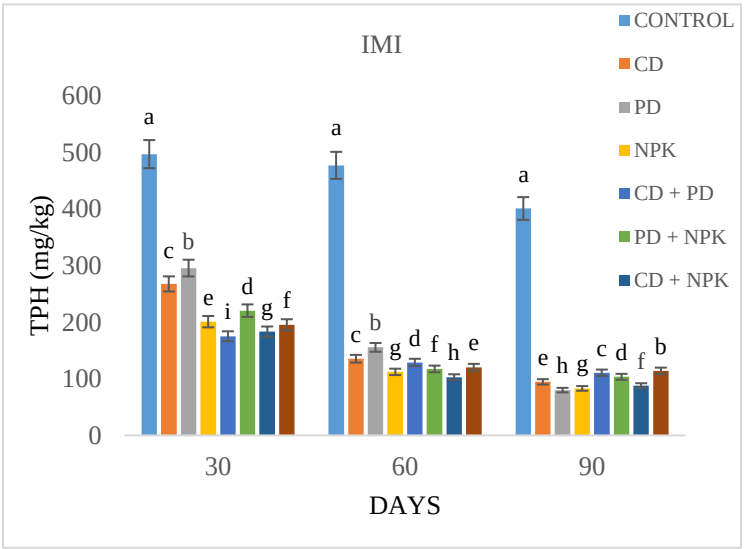
Where; C is number of colonies, DF is dilution factor and V is Volume of cultured sample. Numbers of colonies formed were used to estimate the hydrocarbon degrading bacteria population (Ameh and Kawo, 2017).

2.3 Respiratory activity of hydrocarbon degrading bacteria Acid/Base titration method

The microbial respiratory activities in the soil were estimated from the amount of C-CO_2 released in an interval of 10-days incubation period. 10 g of soil samples (polluted and treated soils) were mixed with 20 g of glucose as substrate (served as the sole energy in the experiment) and placed in an air-tight glass jar of 1000 ml for incubation. 50 ml beaker containing 5 ml of NaOH (1mol) alkaline was placed in each jar containing the soil sample (polluted and treated) to trap or capture the CO_2 that is released by the bacteria during respiration after 10-days incubation period respectively. The jars were sealed and maintained at room temperature of an average of 28^{o}C for the experimental period. After each incubation period the beakers containing the NaOH (base) were collected and 2.5 ml of $BaCl_2.2H_2O$ (1 mol) was added to it to precipitate the carbonates and 3-drops of phenolphthalein indicator was added giving it a pink colour and back titrated with HCl (0.25 mol) and the end-point a clear colourless colour indicates the amount of C-CO_2 released. The amount of C released was estimated in mg/g of C-CO_2 of the soil.

2.4 Characterization and identification of hydrocarbon degrading bacterial isolates

Eight (8) hydrocarbons degrading bacteria isolated from the locations (IMI and KLM) were characterized based on their morphological and biochemical test as outlined in Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1994).



2.5 Experiments / Material Used

The study was carried out in the screen house. The materials used for the experiment were sourced locally and included the remediation materials (cow dung, poultry droppings and NPK) and their combinations. The soils were randomly collected at a depth of 0 – 30 cm from the oil spilled polluted sites for the experiment. A total of 48 samples comprising of the control, treatments and their combinations were analyzed in this experiment. The soils were bulked and 5kg of soil were measured into 48 plastic buckets of 7 liters each according to the controls, treatments and treatment combinations. A fixed rate of 0.5 kg of the amendments (cow dung, poultry dropping and NPK 20 -10 - 10) were applied to the polluted soils 3 weeks before planting. A total of 48 plastic buckets of 7 liters each were filled with soils collected from the two (2) polluted locations (Imiringi and Koloama) at a fixed weight of 5 kg into each bucket, 24 plastic buckets per location. A fixed rate of 0.5 kg of the amendments and their combinations (CD, PD, NPK, CD + PD, PD + NPK, CD + NPK and CD + PD + NPK) was applied to the polluted soils except for the control bucket.

Fluted pumpkin (*Telfaria occidentalis*) seeds were planted 2 seeds per pot to access the effect of soil microbial biomass on the growth of fluted pumpkin (*Telfairia occidentalis*) in petroleum polluted soils amended with organic manures and inorganic fertilizers.

Soils were collected at different times (30, 60 and 90 days) for analysis in the laboratory, while the growth parameters of the plants were measured at different times (4, 8 and 12 weeks) after planting.

2.6 Experimental design and data collection

Experimental design was a 2 x 8 factorial experiment in a completely randomized design (CRD) where locations and treatments are factors replicated three (3) times. Soil samples were collected at 30 days, 60 days and 90 days respectively during the research period.

2.7 Statistical Analysis

All data collected were subjected to statistical analysis of variance (ANOVA) and data analysis of General Linear Model (GLM) was used to evaluate the effects of treatments on crude oil polluted soils. Tukey test was used to separate all the means. All analyses were performed using Minitab Statistical Software Release 17.1, and significance reported at 5% probability level and graphs plotted on Excel 2016 for windows.

3.0. Results and Discussion

3.1. Impacts of treatments on the level of total petroleum hydrocarbons (TPHs) concentration in soils of IMI and KLM locations at different times during the remediation process

The level of total TPH in the soils of Imiringi (IMI) and Koloama (KLM) were assessed on application of the different treatments and measured at different times in comparison with the control (untreated polluted soil) and significant differences were observed as gradual decline in concentration in the level of TPH were seen as time passed in both locations (IMI and KLM) as seen in the graphs below.

Figure 2. Level of Total Petroleum Hydrocarbons at Imiringi Location at 30, 60 and 90 days. (Vertical bars show standard errors of the means considered (n = 3). Bars with the same alphabet (s) within treatments of the same parameters are not significant different ($p \leq 0.05$)

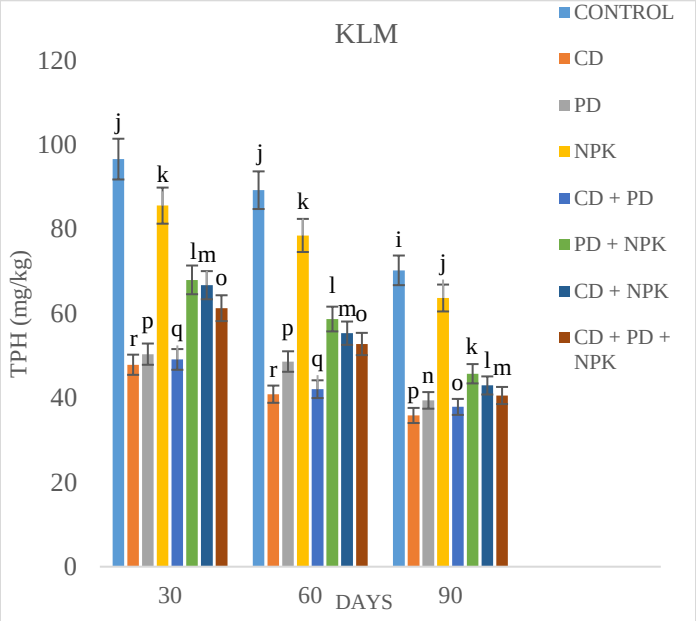


Figure 3. Level of Total Petroleum Hydrocarbons at Koloama Location at 30, 60 and 90 days.
(Vertical bars show standard errors of the means considered (n = 3). Bars with the same alphabet (s) within treatments of the same parameters are not significant different ($p \leq 0.0$))

3.2. Effects of locations and treatments on the hydrocarbon degrading bacterial population (CFU/g x10^4) during the remediation period

The effects between locations and treatments on the hydrocarbons degrading bacterial population (HDB) as express in CFU/g x 10^4 of soil showed significant differences ($p \leq 0.05$) at 30 and 60 days while at 90 days no significant difference ($p \leq 0.05$) was observed as seen in Table 1 indicating that the treatments resulted in increase in population of the HDB.

3.3. Respiratory activities of hydrocarbons degrading bacteria (HDB) in the locations in relation to the treatments applied at different times during the remediation period

Figures 4 and 5 shows the biological activities of the hydrocarbons degrading bacteria of the two locations (IMI and KLM) on application of the treatments (CD, PD, NPK, CD + PD, PD + NPK, NPK + CD and CD + PD + NPK) compared with the control (untreated polluted soil) at different times (30, 60 and 90 days). The rate of respiration (C-CO_2) was observed at every 10 days of incubation of the bacteria at an average room temperature of 28^{o}C in which at the end of each incubation period, the residual substance was titrated and the values were obtained. The graphs below showed the response of the hydrocarbons degrading bacteria as they react on application of the treatments, at different times (30, 60 and 90 days) and competition taking place between the HDB.

Table 1. Main and Interaction effects of locations and treatments on the soil hydrocarbon degrading bacteria population

HDB Count (CFU/g)			
Factors		Days	
Locations	30	60	90
IMI	8.30 x 10^5a	1.14 x 10^6a	2.39 x 10^5a
KLM	5.74 x 10^5b	7.36 x 10^5b	3.06 x 10^5a
Treatments			
CONTROL	1.21 x 10^5c	1.75 x 10^5e	1.94 x 10^5a
CD	1.86 x 10^5c	9.88 x10^5bc	5.47 x 10^5a
PD	1.63 x 10^6a	1.35 x 10^6a	1.84 x 10^6a
NPK	3.27 x 10^5c	2.52 x 10^5e	2.19 x 10^5a
CD + PD	9.33 x 10^5b	1.18 x 10^6ab	2.01 x 10^6a
PD + NPK	1.00 x 10^6b	3.62 x 10^5e	2.37 x 10^6a
CD + NPK	2.57 x 10^5c	6.38 x 10^5d	2.53 x 10^5a
CD + PD + NPK	7.30 x 10^5b	8.70 x 10^5c	2.30 x 10^6a
P value	0.00	0.00	0.62
Locations	*	*	NS
Treatments	*	*	NS
Locs. x Trts	*	*	NS
CV (%)	20.70	22.40	35.97
R^2 (%)	90.85	97.70	31.89

The means with same letters in the columns separated using Tukey's Test are not significantly different at $p \leq 0.05$ level test. Same letters, NS = Not Significantly different and different letters, * = Significantly different.

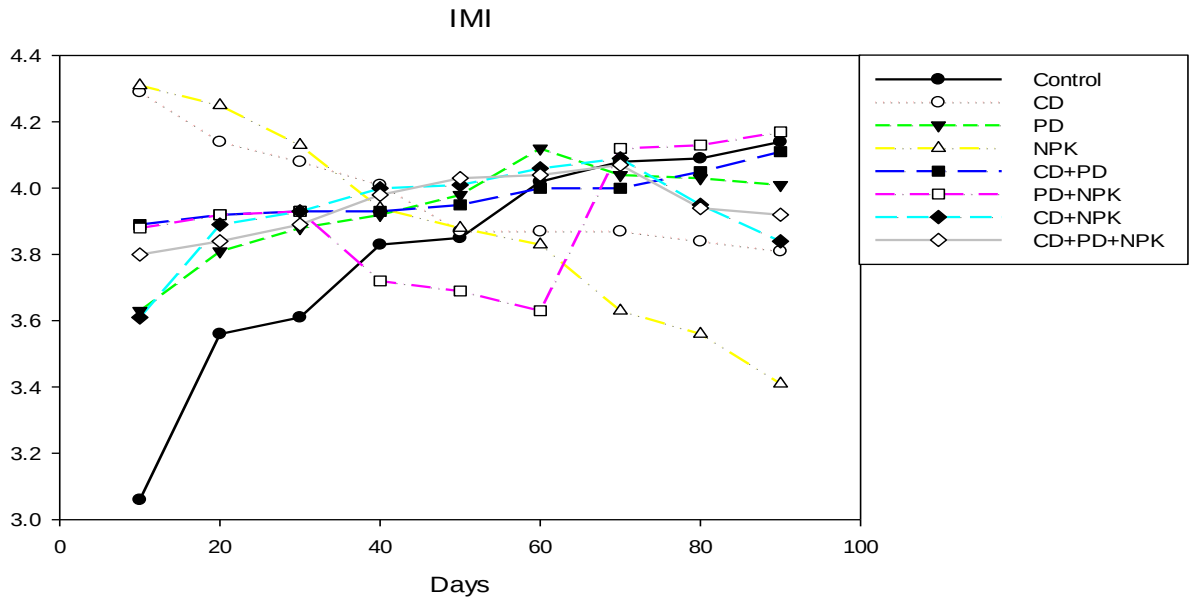


Fig. 4. Rate of hydrocarbons degrading bacterial respiration (C-CO_2) incubated at 10- day intervals in Imiringi soils

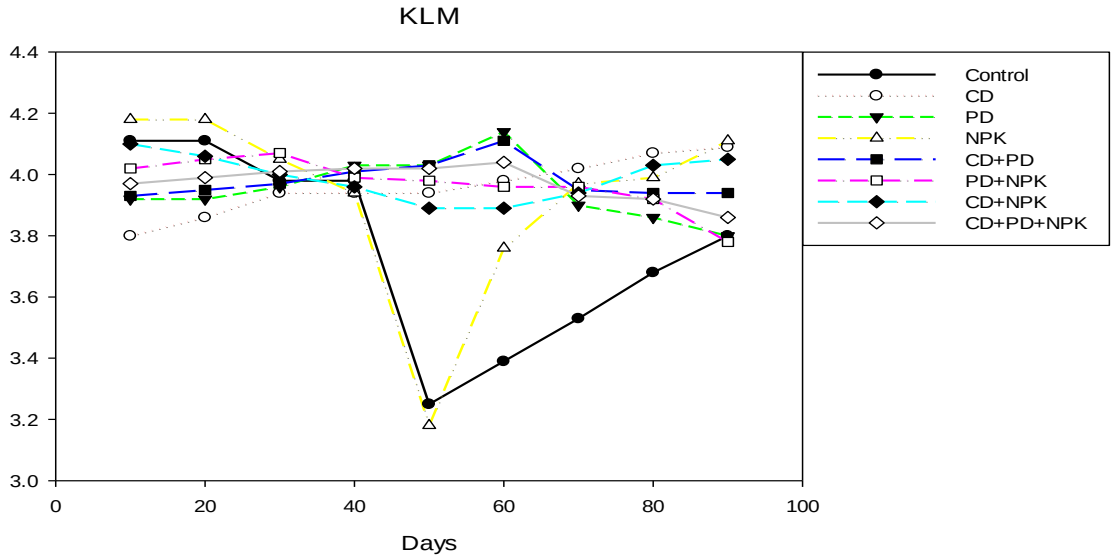


Fig. 5. Rate of hydrocarbons degrading bacterial respiration (C-CO_2) incubated at 10- day intervals in Koloama soils

3.4. Morphological and Biochemical characteristics of hydrocarbons degrading bacteria isolated from the treated soils in the locations (IMI and KLM)

Table 2, shows the morphological and biochemical characteristics of eight (8) bacterial isolates found in the two study areas. The eight hydrocarbon degrading bacteria isolated in the soil samples were *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Lysinibacillus macroides*, *Bacillus altitudinis*, *Priestia flexa*, *Staphylococcus arlettae*, *Bacillus cereus and Bacillus licheniformis.* They are gram positive and negative. There were six grams' positive and two grams' negative hydrocarbons degrading bacteria in the study locations. Plate 1, shows a hydrocarbon degrading bacteria viewed under the Microscope (*Bacillus spp.*)

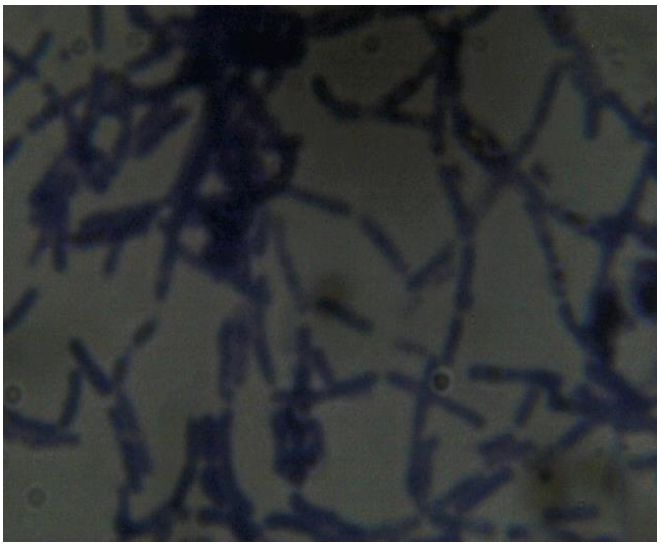


Plate 1. Hydrocarbon Degrading Bacteria viewed under the Microscope (*Bacillus spp.*)

Table 2. Morphological and Biochemical test of eight hydrocarbon degrading bacteria isolates found in the soils of the two study locations (IMI and KLM)

Bacteria Isolates	Morphological Tests					Biochemical Tests									
Organisms	Color	Shape	Colony form	Margin	Elevation	Gram Stain	Indol	Methyl red	Motility	Catalase	Oxidase	H_2S	Citrate	Urease	Nitrate
Pseudomonas aeruginosa	Diffusible green	Rods	Oval	Wavy	Umb-onate	-	-	-	+	+	+	-	+	-	+
Acinetobacter baumannii	Pale yellow to greyish white	Rods	Circular	Entire	Con-vex	-	-	-	-	+	-	-	+	-	-
Lysinibacillus macrolides	Creamy white	Rods	Round	Wavy	Flat	+	-	-	+	+	+	-	+	+	-
Bacillus altitudinis	White	Rods	Circular	Regular	Con-vex	+	-	-	+	+	+	-	+	+	-
Priestia flexa	Opaque to creamish	Rods	Round	Regular	Flat	+	-	-	+	+	+	-	+	-	+
Staphylococcus arlettae	Whitish	Rods	Round	Irregular	Con-vex	+	-	-	-	+	-	+	+	+	+
Bacillus cereus	Pink- Orange	Rods	Circular	Irregular	Con-vex	+	-	-	+	+	-	-	+	+	-
Bacillus licheniformis	Pale	Rods	Round	Irregular	Flat	+	-	-	+	+	-	-	+	-	+

Source: Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1994).

3.5. Effects of locations and treatments on growth parameters of Fluted pumpkin (Telfairia occidentalis) plant during the remediation period

The Table below, shows the effect of organic manures and inorganic fertilizer on the growth parameters of Fluted pumpkin *(Telfairia occidentalis*), showing significant differences ($p \leq 0.05$) with few variations not significantly different ($p \leq 0.05$).

3.6. Discussion

The locations as shown in the map of the State above, revealed that the level of concentration of TPH decreased progressively as the polluted soils were treated with the various amendments of both organic manures and inorganic fertilizer. The results highlight that among the various treatments used, soils treated with poultry droppings (PD) proved most effective in reducing TPH levels in Imiringi soils, with the order of efficacy being PD > NPK > CD + NPK > CD > PD + NPK > CD + PD > CD + PD + NPK. While, at Koloama location, soils treated with cow dung (CD) demonstrated the highest effectiveness, following the sequence CD > CD + PD > PD > CD + PD + NPK > CD + NPK > PD + NPK > NPK. These variations underscore the significant differences between both locations.

Increased population of the hydrocarbon degrading bacteria (HDB) was observed in Imiringi location more than in Koloama location on application of the treatments (organic manures and inorganic fertilizer). It could be as a result of the soil recovering from the toxic effect of crude oil pollution after a long time in this location (Imiringi) while Koloama location experienced a recent pollution due to an oil spillage resulting in reduction in microorganisms. This is in agreement with previous findings that the proportion of hydrocarbons degrading bacteria utilizers generally increased as a result of exposure to petroleum over time (MacNaughton *et al.,* 1999). The result also supports those reported by Obire *et al.,* (2008).

The differences in bacterial counts could be as a result of pH and organic matter content present in the soils which aid in the proliferation of microorganisms (hydrocarbon degrading bacteria). At 30 and 60 days, a significant difference was observed between locations Imiringi and Koloama, but at 90 days both locations (IMI and KLM) showed no significant difference. This indicates a decline in the population of the hydrocarbons degrading bacteria in these locations as a result of competition among the organisms or nutrients depletion due to some environmental factors resulting in death of some of the organisms.

The high amount of hydrocarbon degrading bacteria observed

Table 3. Effects of locations and treatments on the growth parameters of Fluted pumpkin *(Telfairia occidentalis*).

Factors	Vine Length (cm)			Girth (cm)			No. of Branches			No. of Leaves			Leaf Area (cm^2)		
	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP	4 WAP	8 WAP	12 WAP
Locations															
IMI	17.44c	82.79a	77.81b	0.37b	0.49b	0.52b	0.04b	0.96c	1.62b	8.56b	15.40b	23.53c	13.55b	18.78b	26.28b
KLM	48.39a	44.96a	74.18b	0.48ab	0.56b	0.54b	0.81a	3.11a	3.00a	19.15a	30.29a	35.92b	20.20a	25.67ab	34.64ab
Treatments															
CONTROL	13.33d	32.56a	51.56d	0.51ab	0.54ab	0.61a	-0.00a	0.33d	1.22c	8.78c	15.56d	20.44c	11.67cd	12.82c	21.14cd
CD	33.78bc	56.89a	88.88bc	0.45ab	0.71ab	0.75a	0.33a	1.11bc	1.77c	11.67bc	23.11cd	28.67b	14.28c	21.78c	32.06bc
PD	57.67a	106.33a	151.88a	0.63a	0.80a	0.80a	0.55a	3.22a	3.33ab	20.44ab	43.22a	52.66a	34.08a	48.49a	62.42a
NPK	5.44e	35.22a	66.00cd	0.11c	0.49b	0.54a	0.00a	0.67cd	2.22bc	2.89d	16.22d	23.33c	3.28d	14.77c	19.34cd
CD + PD	49.22ab	84.77a	122.66ab	0.63a	0.68ab	0.66a	0.55a	3.11a	4.44a	23.78a	37.22ab	53.77a	23.46ab	37.79ab	50.40ab
PD + NPK	41.89ab	89.77a	102.11bc	0.56a	0.69ab	0.70a	0.33a	3.11a	3.55ab	23.44a	34.55ab	46.11a	28.26ab	25.14bc	38.12bc
CD + NPK	40.67abc	49.83a	80.00bc	0.18bc	0.63ab	0.48a	0.00a	2.00ab	1.50c	18.67ab	22.67bc	28.00bc	7.62cd	26.21bc	27.87cd
CD + PD + NPK	43.22ab	136.00a	93.33bc	0.59a	0.62ab	0.65a	0.89a	2.44ab	3.33ab	25.44a	25.67b	42.55ab	18.60bc	28.64bc	30.81cd
P value	0.00	0.44	0.00	0.21	0.16	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.15	0.
Locations	*	NS	*	*	NS	NS	*	*	*	*	*	*	*	*	*
Treatments	*	NS	*	*	*	NS	NS	*	*	*	*	*	*	*	*
Locs. x Trts	*	NS	*	*	*	*	NS	*	*	*	*	*	*	*	*
CV (%)	51.97	53.15	36.79	40.43	37.09	37.06	42.57	30.16	31.15	48.08	43.57	39.53	47.16	51.63	53.75
R^2 (%)	81.47	35.84	83.51	76.51	52.11	54.10	69.01	82.19	81.09	88.16	77.96	83.42	77.57	73.71	74.94

The means with same letters in the columns separated using Tukey's Test are not significantly different at $p \leq 0.05$ level test. Same letters, NS = Not Significantly different and different letters, * = Significantly different.

in the soils of Imiringi could also be attributed to their ability to survive the toxicity of hydrocarbons overtime and their capacity to utilize crude oil as carbon and energy source.

The significant differences ($p \leq 0.05$) that occurred among the treatments applied to the polluted soils were also due to the presence and availability of more nitrogen and phosphorus from the organic and inorganic manures that contributed to the stimulation of the microbial flora in the soils. At 30 and 60 days, the treatments differ significantly ($p \leq 0.05$) while at 90 days it showed no significant difference at all because the hydrocarbon degrading bacteria utilized the nutrients from the bio-stimulants (organic manures and inorganic fertilizer) which affected the growth of the bacteria leading to either increase or decrease in the population of the degrading bacteria. The presence of eight hydrocarbons degrading bacterial isolates (*Pseudomonas aeruginosa, Acinetobacter baumannii, Lysinibacillus macroides, Bacillus altitudinis. Priestia flexa, Staphylococcus arlettae, Bacillus cereus, Bacillus licheniformis*) in the polluted soils were due to their ability to utilize crude oil as their carbon source.

The results presented for Imiringi and Koloama location respectively, showed that the rate of respiration ($C\text{-}CO_2$) was not linear as the bacteria competes for nutrients as energy source. The changes observed in the control (untreated polluted soil) as well as the treated soils throughout the remediation period, tells the state of the organisms, as they utilized the substrate (glucose) causing an ecological association resulting in either a decrease or increase in their population as they struggle to survive (Teknikio *et al.,* 2018). The rate of respiration of the bacteria was unstable because of the population of the organisms present in the soil. $C\text{-}CO_2$ evolution from the soil is thus a measure of the total soil biological activity. Therefore, the higher the population, the lower the rate of respiration and vice versa.

Based on morphological examination and biochemical tests, eight (8) hydrocarbon degrading isolates reflected different biochemical features as observed in this study. These characteristics were used to identify the hydrocarbons degrading isolates based on traditional identification methods (Krieg and Holt, 1994; Udgire *et al.,* 2015). A major HDB (*Bacillus*) was seen under the microscope.

Fluted pumpkin *(Telfairia occidentalis*), a fast-growing and highly adaptable plant, was found to have a remarkable ability to tolerate drought and stress and so was used as a test plant in the remediation of petroleum-polluted soils to access the effect on addition of organic manures and inorganic fertilizer to the petroleum polluted soils. Vine length, number of branches, number of leaves, and leaf area increased with organic manure application (Aderi *et al.,* 2011). The incorporation of organic manures and inorganic fertilizer into the soil fosters heightened microbial activity and diversity, resulting in increased decomposition of organic matter and efficient nutrient cycling. The resultant soil conditions create a conducive environment for the flourishing growth and development of fluted pumpkin *(Telfairia occidentalis*) plant. Consequently, the combined application of organic manures and inorganic fertilizers accelerates the remediation process by facilitating the transformation of petroleum pollutants into less toxic forms.

4.0 Conclusion

The results from this study proved that local materials such as poultry droppings (PD), cow dung (CD), inorganic fertilizers (NPK) and their combinations provided necessary nutrients required for the bioremediation process as these amendment materials increased the degradation of total petroleum hydrocarbons (TPHs) in the soils by the hydrocarbons degrading bacteria (HDB). Thus, this bio-stimulating treatment strategy enhanced petroleum hydrocarbons microbial degradation. A similar observation was reported for crude oil degradation using poultry manure (Okolo *et al.*, 2005). However, poultry and cow dung manure treatments showed greater petroleum hydrocarbon reductions than NPK fertilizer treatment alone. This could be as a result of high nutrient level and hydrocarbon-utilizing bacterial species found in poultry and cow dung manures than in inorganic fertilizer (NPK), hence, poultry droppings and cow dung manures act as both nutrient and microbe carriers (bio-stimulating and bio-augmenting agent). Eight (8) hydrocarbons degrading bacteria isolates were identified in this study, showed high performance in terms of growth on hydrocarbons for degradation and were found to be good degraders. *Bacillus spp*. isolates grew well on the polluted soils.

Finally, the results obtained from both locations (Imiringi and Koloama), showed that the integration of both organic manures and inorganic fertilizer had a positive effect on the growth of fluted pumpkin *(Telfairia occidentalis*) and could lead to improve soil fertility resulting in increased agricultural productivity in these areas. Therefore, farmers should be encouraged to use these remediation materials in the cleaning of crude oil polluted soils in their areas.

4.0 References

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