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EFFECT OF ORGANIC AND INORGANIC AMENDMENTS ON HYDROCABONS DEGRADING BACTERIA IN THE REMEDIATION OF CRUDE OIL SOILS IN BAYELSA STATE, NIGERIA

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Abstract: **Soil samples were examined to assess the impact of applying both organic and inorganic amendments on hydrocarbon-degrading bacteria (HDB) in remediating crude oil-polluted soils in Bayelsa State. Two locations, Imiringi and Koloama, in Ogbia and Southern Ijaw Local Government Areas, respectively, which experienced crude oil spills wer0e chosen for the study. The samples were collected from a depth of 0–30 cm, bulked for pot experiment in the screen house. Twenty-four plastic buckets, each holding seven liters of soil (5 kg), were used for each location, totaling 48 buckets. The experimental design was a 2 x 8 factorial arrangement in a Completely Randomized Design (CRD), replicated three times. The data were statistically analyzed using Tukey's Test to distinguish significant differences at a 5% probability level. The analysis of the 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 total petroleum hydrocarbons (TPH) levels and the population of hydrocarbon-degrading bacteria (HDB) in the crude oil-polluted soils. For Imiringi, the HDB population (expressed in CFU/g) ranged from 8.30 x 10^5a to 1.14 x 10^6a, while for Koloama, it ranged from 5.74 x 10^5b to 7.36 x 10^5b, indicating a significant difference between the two locations. The results demonstrated a highly significant difference in TPH levels and HDB populations among the locations and treatments at 30, 60, and 90 days, suggesting the effectiveness of the application tof hese amendment materials.**

Keywords: **Hydrocarbons Degrading Bacteria, Organic manures, Inorganic Fertilizer (NPK), Crude Oil Polluted Soils.**

1. INTRODUCTION

Pollution from petroleum hydrocarbon products generates great environmental concern, since they are poisonous to the environment (Mishra *et al.*, 2020; Ramadass *et al.*, 2018). The pollution of the soil by crude oil and its products presents a global concern for its potential consequences on the ecosystem and human health (Onwurah *et al.*, 2007). Aliphatic and aromatic hydrocarbons are two primary petroleum hydrocarbons known for their recalcitrant and harmful effects to health. Straight chains aliphatic hydrocarbons are easily degraded by microorganisms, while branched chains aliphatic are not easily degraded and therefore, they persist in the soils (Hasanuzzaman *et al.*, 2007).

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Soils of Bayelsa State are affected adversely with ecological issues associated with the activities being carried 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 of 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.

Bio-stimulation (the addition of nutrients to support microbial increase) utilizes indigenous microbial population to remediate polluted soils. It is a sort of biological remediation that may enhance pollutant degradation by optimizing conditions together with aeration, addition of nutrients, pH and temperature control (Margesin, *et al.*, 2007). Crude oil bioremediation in soil may be promoted through the stimulation of the indigenous microbial population, by introducing nutrients into the soil through bio-stimulation (Seklemova *et al.*, 2001).

The objectives of this study are to (1) quantify total available petroleum hydrocarbons (TPHs) present in the soils of the study area;(2) identify and quantify hydrocarbon degrading bacteria (HDB) present in the soils: of the study area and (3) study the effect of organic manures and inorganic fertilizer (amendments) in the conservation HDB in the study area.

2. MATERIALS AND METHODS

Two (2) crude oil polluted locations were selected for this study namely; Imiringi with latitudes 4. 8519 N and longitudes 6.3745 E in Ogbia Local Government Area and Koluama with 4.582222 N and longitudes 6.064722 E in Southern Ijaw Local Government Area of Bayelsa State respectively. Soils were randomly collected at a depth of 0 – 30 cm from the oil spilled polluted area for the experiment. A total of 48 samples were analyzed in this experiment. The soils were bulked and a fixed weight of 5 kg of soil were measured into 48 plastic buckets of 7 liters each according to control and treatments and a fixed weight of 0.5 kg of the amendment materials (treatments) were added to the soils three (3) weeks after collection from the field.

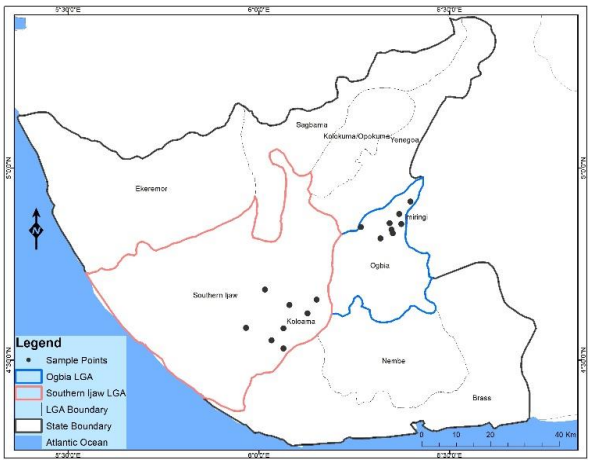

Fig. 1 Map of Bayelsa State showing the sampling points in the two study locations (Imiringi and Koloama) of the crude oil polluted soils

Determination of Petroleum Hydrocarbons content in the soils

The total petroleum hydrocarbon content was analyzed using standard solvent (hexane) extraction method. 1 g of the sieved soil sample was dissolved in chloroform in a test tube. Thereafter, the clear lower layer was collected with a clean test tube upon which, it was dehydrated by the addition of a spoonful of anhydrous sodium sulphate. The clear extracted solution

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was measured using a Gas Chromatography – Flame Ionization Detector (FID) and the level of the total petroleum hydrocarbon (TPH) was recorded accordingly.

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 37°C 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 37°C for 21 days for degrading hydrocarbon bacterial count. After incubation, the culture suspension was poured plated using sterile nutrient agar (NA) and incubated at 37°C 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).

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°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.

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).

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. 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.

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.

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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. RESULTS

KEY:

IMI = Imiringi

KLM = Koloama 1

C = Control (Polluted Soil Not Treated)

CD = Polluted Soil Treated with Cow Dung Manure

PD = Polluted Soil Treated with Poultry Dropping Manure

NPK=Polluted Soil Treated with Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

CD + PD = Polluted Soil Treated with Cow Dung Manure + Poultry Dropping Manure

PD + NPK = Polluted Soil Treated with Poultry Dropping Manure + Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

CD + NPK = Polluted Soil Treated with Cow Dung Manure + Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

CD + PD + NPK = Polluted Soil Treated with Cow Dung Manure + Poultry Dropping Manure + Nitrogen Phosphorous Potassium Fertilizer (Inorganic Manure)

HDB = Hydrocarbons Degrading Bacteria

TPH = Total Petroleum Hydrocarbon

Effects of treatments on the level of total petroleum hydrocarbons (TPH) in the soils of the locations (IMI and KLM) at 30 days, 60 days and 90 days during the remediation period

The chart in figures 1 to 2, shows the effect of locations (IMI and KLM) and treatments (C, CD, PD, NPK, CD + PD, PD + NPK, NPK + CD and CD + PD + NPK) on the level of total petroleum hydrocarbons (TPH) found in the soils at 30 days, 60 days and 90 days. From the results, significant differences were seen among the treatments in all three locations as compared to their control. The level of TPH in the locations (IMI, KLM and FUTA) tends to reduce in concentration as time passed by across the treatments (CD, PD, NPK, CD + PD, PD + NPK, NPK + CD and CD + PD + NPK) as compared to the control (C) during the remediation period.

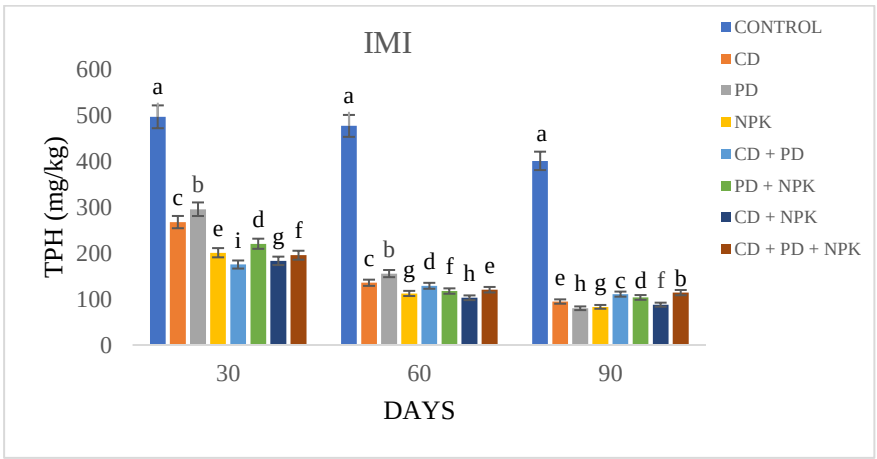

Fig. 2. Level of Total Petroleum Hydrocarbons at Imiringi Location at 30, 60 and 90 days. Vertical bars show standard errors of the means considered (n = 3). Bars with the same alphabet (s) within treatments of the same parameters are not significant different ($p \leq 0.05$)

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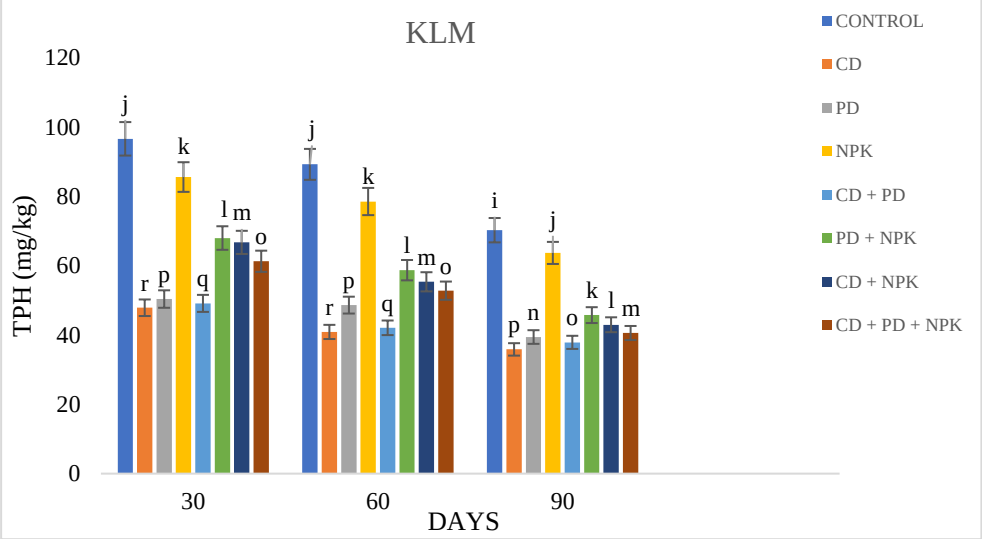

Fig. 3. Level of Total Petroleum Hydrocarbons at Koloama Location at 30, 60 and 90 days. Vertical bars show standard errors of the means considered (n = 3). Bars with the same alphabet (s) within treatments of the same parameters are not significant different ($p \leq 0.05$)

Interaction effects of locations and treatments on the hydrocarbon degrading bacterial population (CFU/g x10^6) during the remediation period

The interaction effect between the locations and treatments on the hydrocarbons degrading bacterial population (CFU/g x 10^4) in the soil shows that there are significant differences ($p \leq 0.05$) among the locations and treatments at 30 days and 60 days while at 90 days no significant differences ($p \leq 0.05$) was seen from the results.

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

Factors	HDB Count (CFU/g x 10^4)		
Locations	30 Days	60 Days	90 Days
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			
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.

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

Figures 3 and 4 shows the biological activities of the hydrocarbons degrading bacteria of the two (2) locations (IMI and KLM) under study before and after the application of the treatments (CD, PD, NPK, CD + PD, PD + NPK, NPK + CD and CD + PD + NPK) at different times (30 days, 60 days and 90 days). The rate of respiration 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 of 10 days the residual substance was titrated and the values were obtained. The graphs for each location showed the response of the hydrocarbons degrading bacteria as they respire on application of the treatments at different times (30 days, 60 days and 90 days) and it could be seen that there were intra and extra competition taking place between the bacteria during the research period.

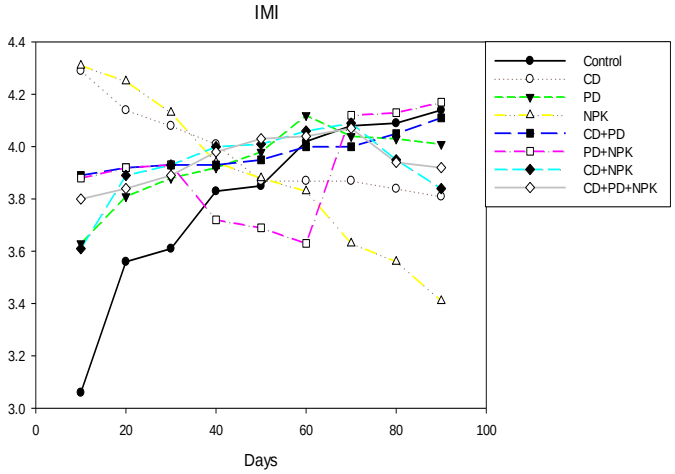

Fig. 4. Rate of hydrocarbons degrading bacterial respiration incubated at 10 days intervals in Imiringi soils

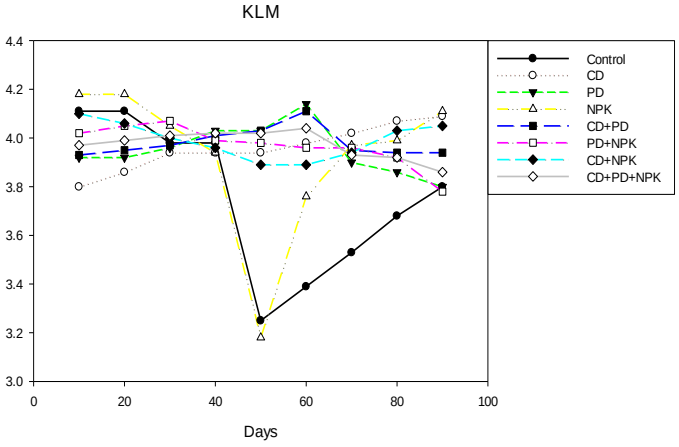

Fig. 5. Rate of hydrocarbons degrading bacterial respiration incubated at 10 days intervals in Koloma soils

Biochemical, Morphological and Molecular identification of hydrocarbons degrading bacteria isolated from the treated soils in the locations at different times during the remediation period

Table 2, shows the morphological and biochemical characteristics of eight (8) bacterial isolates found in the three 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.*)

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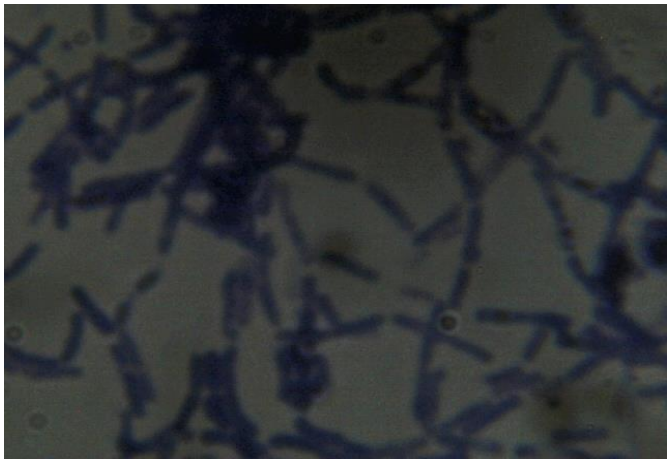

Plate 1. Hydrocarbon Degrading Bacteria viewed under the Microscope (*Bacillus spp.*)

Table 2. Morphological and Biochemical test of eight hydrocarbon degrading bacteria isolates found in the soils of the three 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).

4. DISCUSSION

The locations as shown in the map of the State as seen in Fig.1, for the study, revealed that the polluted soils had significant increase in hydrocarbons degrading bacteria (HDB) in both locations on addition of the amendment materials especially for poultry dropping manure (PD). Increased population of the hydrocarbon degrading bacteria was observed in Imiringi location than in Koloama location. It could be as a result of the soil recovering from the toxic effect of crude oil pollution for a long time now and this location (Imiringi) is controlled by the Shell Petroleum Development Company (SPDC), while Koloama location is controlled by CHEVRON OIL company, which experienced a recent pollution due to an oil spillage resulting from a pipe line vandalization resulting in the soils being polluted which may have caused suffocation of most of the microorganisms.

The results in figures 2 and 3 explained the level of total petroleum hydrocarbons in the soil for each location on application of the remediation material (bio-stimulant) as it degrades at different times. Imiringi location is highly significantly different ($p \leq 0.05$) from Koloama location since, the soils had long been polluted and contained high pollutants as a result. For Imiringi location, TPH saw a progressive decrease as time went on. A decrease in TPH value from 496.42 mg/kg to 400.52 mg/kg from 30 days to 90 days was seen in the control indicating the presence of inherent hydrocarbons degradable bacteria in the soil, while in the amended soils TPH value decreased from 295.35 mg/kg to 175.07 mg/kg at 30 days, 155.36 mg/kg to 102.75mg/kg at 60 days and 113.95 mg/kg to 79.91 mg/kg at 90 days, showing that degradation of the pollutants by the hydrocarbons degrading bacteria actually took place, indicating that the amendment materials were effective in the

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remediation process. Koloama 1 location also saw TPH progressively decreasing as time passed. A decrease in TPH was seen in the control from 96.62 mg/kg to 70.25 mg/kg from 30 days to 90 days indicating the presence of inherent hydrocarbons degradable bacteria in the soil as well, while in the amended soils TPH value decreased from 85.59 mg/kg to 47.87 mg/kg at 30 days, 78.52 mg/kg to 40.89 mg/kg at 60 days and 63.69 mg/kg to 35.85 mg/kg at 90 days, showing the degradation of the pollutants by the degrading bacteria. From the result above, soils treated with poultry droppings (PD) was the most effective among others in the reduction of TPH in the soils of Imiringi location at the end of the research period and as such it follows this trend PD > NPK > CD + NPK > CD > PD + NPK > CD + PD > CD + PD + NPK , while soils treated with cow dung (CD) was the most effective among others for Koloama location in the reduction of THP and follows this trend CD > CD + PD > PD > CD + PD + NPK > CD + NPK > PD + NPK > NPK respectively.

The values for the colony forming unit (CFUs/g x 10^4) of soil for the hydrocarbon degrading bacteria population for Imiringi ranged from 8.30 x 10^5a at 30 days, 1.14 x 10^6a at 60 days and 2.39 x 10^5a at 90 days. Koloama 1 is 5.74 x 10^5b at 30 days, 7.36 x 10^5b at 60 days and 3.06 x 10^5a at 90 days respectively are shown in Table 1. At 30 and 60 days, a significant difference was observed between locations Imiringi and Koloama, while at 90 days both locations (IMI and KLM) showed no significant difference. Hence, this indicates that the decline in the population of the hydrocarbons degrading bacteria as time went by (90 days) in these locations could be as a result of competition among the organisms or nutrients depletion due to some environmental factors resulting in death of some hydrocarbon degrading bacteria. The high amount of hydrocarbon degrading bacteria observed in the soils of Imiringi could be attributed to their ability to survive the toxicity of hydrocarbons overtime and their capacity to utilize crude oil as carbon and energy source. 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 supports those reported by Obire *et al.*, (2008). The difference in counts could be due to pH and organic matter content present in the soils which could aid the proliferation of microorganisms (hydrocarbon degrading bacteria). The significant differences ($p \leq 0.05$) that occurred among the treatments applied to the polluted soils were 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 days and 60 days, the treatments are significantly different ($p \leq 0.05$) while at 90 days it showed no significant difference at all because the hydrocarbon degrading bacteria utilized the nutrients from the bio-stimulants (organic and inorganic manures) which affected the growth of the bacteria leading to either increase or decrease in the population of the degrading bacteria. The occurrence 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 oil as their carbon source.

The results as presented in the graph above in Fig. 4 and 5, for Imiringi and Koloama location showed that the rate of respiration was not linear as the bacteria competes for nutrients as energy source. The changes observed in the control (untreated) 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. 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 biochemical tests and morphological examination, the eight (8) hydrocarbon degrading isolates reflected different biochemical features as presented in Table 2. Performed morphological colony characteristics examination (Gram stain, elevation, shape and color) and biochemical tests (catalase, urease, oxidase activities, nitrate reduction, Indol production, H_2S production, methyl red test, citrate and motility) were used to identify the hydrocarbons degrading isolates based on traditional identification methods (Krieg and Holt, 1994; Udgire *et al.*, 2015). A major HDB seen under the microscope is presented in Plate 1.

5. CONCLUSION

This study has proved that local materials such as poultry droppings (PD), cow dung (CD), inorganic fertilizers (NPK) and their combinations can provide the necessary nutrients required for bioremediation process. From the results of this study the use of these amendment materials increased the degradation of the petroleum hydrocarbons in the soils by the hydrocarbons degrading bacteria. Thus, the bio-stimulating treatment strategy showed the ability to enhance petroleum hydrocarbons microbial degradation. A similar observation was been reported for crude oil degradation using poultry

manure (Okolo *et al.,* 2005) and NPK fertilizer. However, poultry and cow dung manure treatments showed greater petroleum hydrocarbon reductions than NPK fertilizer treatment alone. This may be due to 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). The study confirms that the use of these amendment materials greatly improved the rate of petroleum hydrocarbons (PHCs) degradation in the crude oil polluted soils thereby reducing the levels of TPHCs in the soils.

However, this study also showed that the integration of the combination of organic and inorganic manures proved successful due to the fact that it contains nutrients and vitamins responsible for the increase of the population and diversity of the micro-flora of the polluted soils, microbial activity and harbors microorganisms (conservation of microorganisms) capable of utilizing hydrocarbons as source of energy. Thus, the use of the right quantity of these nutrients for the growth of the oil eating microbes (hydrocarbon degrading bacteria) is very important because too much of it reduces the pH of the soil.

The eight (8) hydrocarbons degrading bacteria isolates identified in this study namely; *Pseudomonas aeruginosa, Acinetobacter baumannii, Lysinibacillus macroides, Bacillus altitudinis, Priestia flexa, Staphylococcus arlettae, Bacillus cereus* and *Bacillus licheniformis*. *Pseudomonas aeruginosa*, and *Bacillus spp*. showed high performance in terms of growth on hydrocarbons for degradation and were found to be good degraders. As *Bacillus spp*. isolates grew well on the polluted soils, it appeared to have transformed high molecular weight hydrocarbons, making this organism (*Bacillus spp*.) very useful for bioremediation purposes. These organisms are both gram negative and gram positive and this study identified six grams negative and two grams positive cells capable of degrading petroleum.

The results obtained from the study of the crude oil polluted soils of both locations (Imiringi and Koloama 1), showed that the integration of organic and inorganic manures greatly reduced the total petroleum hydrocarbon levels in the soils with the passage of time as observed in the treated soils and this could lead to improve soil fertility which in turn would result in increased agricultural productivity and also increase source of livelihood in these areas.

Finally, this study further revealed that, the indigenous hydrocarbons degrading bacterial population in polluted soils are capable of mineralizing pollutants in the environment to safe and acceptable levels as was observed in the control (untreated soils). It can also, be concluded that oil-degrading bacteria are abundant in soils polluted with petroleum products. Therefore, farmers should be encouraged to use these remediation materials in the cleaning of crude oil polluted soils in these areas.

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