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Effect of animal waste on bioremediation of soil contaminated with spent hydrocarbon from auto mechanic workshop in Keffi, Nasarawa State, Nigeria

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Abstract

Petroleum hydrocarbon is a major environmental pollutant throughout the world today because exploration and downstream utilization are associated with economic development. This work focus on effect of animal waste on bioremediation of soil contaminated spent hydrocarbon from auto mechanic workshop in Keffi. Isolation of bacteria and studies on utilized spent hydrocarbon supplemented with animal waste was carried out using standard microbiological methods and identified using 16s rRNA molecular techniques. The total spent hydrocarbon utilization was determined using Gas Chromatographic methods. The highest total bacteria count was recorded from High court (2.1 x10^5 cfu/g) and the lowest was from Keffi garage2 (1.2 x10^5 cfu/g). bacteria isolated and molecularly identified were *Pectobacterium wasabiae*, *Pseudomonas fluorescens,* and *Priestia aryabhattai.* The utilization of spent hydrocarbon after 4weeks of the experiment set up at ambient temperature *Pseudomonas fluorescens* recorded highest utilization of spent hydrocarbon amended with Poultry dropping and Cow dunk with 16.23±1.21 mg/ml and 13.44±0.23 mg/ml and *Pectobacterium wasabia* recorded the least with 8.12±0.20 mg/ml and 10.00±1.43. At temperature of 35 ℃ *Pseudomonas fluorescens* recorded highest utilization of spent hydrocarbon amended with Cow dunk and Poultry dropping with 17.13±1.11 mg/ml and 15.14±1.13 mg/ml while *Priestia aryabhattai* recorded the lowest from Poultry dropping with 6.10±0.06 mg/ml and Cow dunk with 8.23±0.03 mg/ml. at 40 ℃ the highest utilization was recorded by *Priestia aryabhattai* from Cow dunk amended spent hydrocarbon with 18.13±1.01 mg/ml and Poultry dropping with 16.24±1.33 mg/ml. from the findings of this study temperature plays a key role spent hydrocarbon that containment our environmental

Keywords: Spent hydrocarbon; Utilization; Poultry dropping and Cow dunk; Amended; Soil contaminated

1 Introduction

The environment is our common heritage that should be properly nurtured. Man's residence on earth has resulted in tremendous environmental degradation due to inhabitancy and developmental efforts [1]. This degradation puts the goals of sustainable development at risk and as such should be abated as a matter of both necessity and urgency. Contaminated lands abound throughout the world but are mostly rampant in developing countries where environmental laws are at best rudimentary. Petroleum hydrocarbon is a major environmental pollutant throughout the world today because exploration and downstream utilization are associated with economic development [2]. Mechanic workshops within Nigeria are poorly managed and can be sources of constant release of used spent oil discharged from the crank cases of cars and motorcycles which can be aesthetically unsightly and cause serious environmental pollution. Cleanup of mechanic sites is still elusive as operators of such sites are usually ignorant of the deleterious effects on the environment. There is also a likelihood of percolation to ground water and a pint of engine oil can contaminate 100,000liters of ground water [3]. Bioremediation refers to the use of naturally occurring

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microorganisms or genetically engineered microorganisms by man to detoxify man-made pollutants [4]. Since bioremediation is a microbial process, it requires the provision of nutrients among other factors or requirements. The addition of organic waste materials such as poultry litter (PL), Cow dung (DG) and Coir pith (CP) to the soil facilitates aeration through small pores and increases the water holding capacity of the soil, thus enhancing bioremediation [4.5]. It allows natural processes to clean up harmful chemicals in the environment. Microscopic "bugs" or microbes that live in soil and groundwater use certain harmful chemicals such as those found in gasoline and oil spills. Crude oil is a complex mixture of diverse hydrocarbons including alkanes, aromatics, alicyclics, branched hydrocarbons, and non-hydrocarbon compounds including polar fractions containing hetero-atoms of nitrogen, sulfur and oxygen (NSO fraction), and asphaltens [6,7]. The high demand for petroleum products in the form of cooking gas, aviation fuel, gas oil, engine lubricating oil, asphalt and coal tar means increase in production and this eventually results in oil spills and hydrocarbon contamination of the environment especially through oil well blow out, tanker accidents, accidental rupture of pipelines and routine clean-up operations. These often lead to the release of oil into the environment [8,9]. Current technologies for cleaning hydrocarbon contaminated soil include soil washing, solvent extraction, thermal treatment, composting, chemical oxidation (Fenton's reagent, permanganate, ozone etc) and bioremediation (bioaugmentation, biostimulation and phytoremediation) [10, 11]. The most widely used bioremediation procedure is biostimulation of the indigenous microorganisms by the addition of nutrients, as input of large quantities of carbon sources tends to result in rapid depletion of the available pools of major inorganic nutrients, such as nitrogen and phosphorus [12]. Biostimulation is the addition of nutritional amendments to increase microbial metabolism and to encourage bioremediation. When microbes completely digest these chemicals, they change them into water and harmless gases such as carbon dioxide. Nutrient is one factor that can hinder biodegradation if not handled properly and could limit the rate of hydrocarbon degradation in the terrestrial environment [13]. Animal waste includes livestock and poultry manure, bedding and litter. Animal waste should be considered a valuable resource which when managed properly, can reduce the need for commercial fertilizer. Such waste can add organic matter which improves the water holding capacity and improves soil tilth. Animal waste can provide an economical source of nitrogen, phosphorus and potassium as well as other nutrients for plant growth [14,15]. According to Office of Technology Assessment (OTA) (1990), the addition of nutrients that can limit biodegradation to the spill site is necessary and those nutrients are not different from fertilizer. Organic nutrients such as animal dung when added to polluted sites act both as source of nutrients and of microorganisms. Therefore, the aim of this study is to test the possibility of bioremediating spent hydrocarbon polluted soil microcosms from automechanic workshop using cow dung and chicken droppings in Keffi, Nassarawa State, Nigeria.

2 Material and methods

This research basically involved laboratory experiments which were conducted in accordance with standard laboratory procedures.

2.1 Sample collection

Oil contaminated soil was collected from 4 different auto mechanic workshops at High court, Keffi garage 1, Angwan Tanko and Keffi garage 2, all in Keffi, Nasarawa State Nigeria, at a depth of 10cm then put in well labeled black polythene bag and immediately transported to the laboratory for analysis as described by Makut and Majekodunmi, (2019). The soil amendment material such as the cow dung was collected from Keffi Ultra-Modern Nagari Abattoir and the chicken droppings were collected from Sony Poultry Farm, Angwan Nepa Area Agwan Div Keffi, Nasarawa State, Nigeria.

2.2 Determination of Total Heterotrophic Bacteria (THB) count

Total Heterotrophic Bacteria count in the soil was determined using plate count method. One gram of the auto-mechanic oil contaminated soil samples was aseptically suspended in a test tube containing 9ml of sterile water, the samples were vortexed to homogenize and allowed to stand for 10 minutes. Tenfold serial dilution was carried out by transferring 1ml of the soil suspension into another test tube containing 9ml of sterile water, this step was performed to 10 times. As described by Makut and Ishaya [16] aliquot of 0.5ml was picked from the 4th and 5th tube and spread and plated in duplicates into Mineral Salt Medium (MSM), 0.9 gram of K2HPO4, 2 gram of NH4CL, 0.1 gram of MgSO4.7H20, 0.1 gram of NaCl, 0.01 gram of Na2S04.7H20, 20 gram of agar was dissolved in 500 millilitre of distilled water. The mixture was autoclaved at 121 °C for 15 minutes. The medium pH was adjusted to 6.9 and supplemented with 1% Spent Hydrocarbon as carbon source. Plates were incubated at 35°C for 24 hours and discrete colonies was counted using a colony counter and expressed in CFU/gm, growth colony was sub-cultured for further use that developed was counted.

2.3 Bacteria Isolates Identification and characterization

2.3.1 Identification of Isolates

The identification of bacteria was done based on morphological characteristics and biochemical tests carried out on the isolates. Morphological characteristics observed for each bacteria colony after 24hours of growth include colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation. Identification and characterization of isolates were examined and recorded as described by Fahad [17].

2.3.2 Gram staining

A thin smear of each of the pure 24hours old culture was prepared on clean grease-free slides, then fixed by passing over gentle flame. Each heat-fixed smear was stained by addition of 2 drops of crystal violet solution for 60 seconds and rinsed with water. The smear was again be flooded with Lugol's iodine for 30 seconds and rinsed with water, decolorized with 70% alcohol for 15 seconds and rinsed with distilled water. They were then counter stained with 2 drops of Safranin for 60 seconds and finally rinsed with water, then allowed to air dry. The smears was mounted on a microscope and observed under oil immersion objective lens [18].

2.3.3 Biochemical characterization

In order to identify the purified cultures tentatively, biochemical tests was performed such as Catalase, Oxidase, Indole, Nitrate, Fructose, Maltose, Glucose.

2.4 Molecular Identification

2.4.1 DNA extraction (Boiling method)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) were spun at 14000rpm for 3 min. The cells were re-suspended in 500 µl of normal saline and heated at 95^0C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -20 ℃ for other downstream reactions as earlier described by Abimiku *et al.,* [19].

2.4.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. 2 µl of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button [19].

2.4.3 16S rRNA Amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes.

2.4.4 Agarose Gel Electrophoresis of the 16SrRNA gene from the bacteria isolates

The agarose gel electrophoretic separation of digested 16S rRNA gene was carried out as described by [19]. 8µl of PCR products stained with ethidium bromide was loaded into 1.0% (wt/vol) agarose gel wells with a molecular marker run concurrently at 120 V for 30 min. The DNA bands were visualized and photographed under UV light595nm.

2.4.5 Soil amendment and spent hydrocarbon utilization

The experiment was carried out to assess the utilization of used hydrocarbon and the effect of animal waste as a bio-stimulant on utilization activity of hydrocarbon degrading bacteria indigenous used hydrocarbon contaminated soil. Utilization study was carried out according to the method described by Okafor and Orji [20]. Four experimental treatments (PC1 (200 ml of Spent Hydrocarbon, mix with 1 kg Soil and amended with 100 g of Chicken droppings and different bacteria specie), PC2 (200 ml of Spent Hydrocarbon, mix with 1 kg Soil and amended with 100g of Cow dunk

and different bacteria specie), PC3 (200 ml Spent Hydrocarbon mix with 1 kg of soil and different bacteria specie isolate) and a control treatment (PC4) were set up for different temperature, ambient temperature, 35 ^{0}C temperature and 40 °C temperature. The experiment was incubated for a period of 4 weeks. One kilogram (1 kg) of the soil sample and amendment was weighed and placed in plastic containers (15 cm diameter x 8 cm depth) with the bottom perforated to allow aeration. The animal waste and soil were sterilized using autoclave at a temperature of 121 °C for 15 minutes before amendment.

2.4.6 Total Petroleum Hydrocarbon (TPH) Utilization

Total Petroleum Hydrocarbon content of the soil samples was determined using Gas Chromatographic methods according to the toluene extraction method Nwankwegu *et al*.[10] and Sonication water bath methods. Fifteen grams (15 g) of each of the sample was weighed into 50 ml conical flasks, and then 1ml of 60 µg/ml of 1-chlorooctadecane surrogate standard was added. Then 30 milliliters of dichloromethane (extraction solvent) was added to extract oil in the soil. After shaking vigorously in water bath for 5 hrs, the mixture was allowed to stand for 60 minutes and then filtered through Whatman No.1 filter paper fitted with cotton wool and sodium sulphate into a clean beaker washed with methylene chloride. The residue was then be washed with 20 ml extracting solvent and then filtered through funnel. The extracted oil was transferred to vial and placed on a gas chromatographic chamber for analysis. The amount of waste lubricating oil degraded was calculated by subtracting the weight of residual waste lubricating oil from weight of the initial waste lubricating oil, divided by the weight of the initial waste lubricating oil and then multiplied by 100.

$$\text{HR (\%)} = \frac{(\text{Wt of oil before remediation}) - (\text{Wt of oil after remediation} \times 100}{\text{Wt of oil before remediation}}$$

2.5 Statistical Analysis

Data collected from the study was analyzed using general descriptive statistics, one Way Analysis of Variance (ANOVA) at 95% probability level of significance. When significant differences were found, Duncan's multiple range tests was used to compare the different experimental groups. Computer software such as Microsoft Excel was used for the statistical analyses.

3 Results and discussion

The mean count of total heterotrophic bacteria isolated from auto mechanic contaminated soil shows that the highest bacteria count was obtained from High court mechanic workshops (2.1x10^{5}) and lowest was from Keffi garage2 (1.2 x10^{5}) as showed in Table 1 which is similar to study earlier reported by ArunKumar and Anitha, [21] who reported low bacteria count from mechanic contaminated soil as observed in this work to compare heterotrophic bacteria count recorded from non-contaminated soil which was 12.01 x10^{5}. This finding showed that the used hydrocarbon that contaminate soil have a great effect on the microbial population of the soil. This may be due to the reduction in flow of oxygen into the soil and high acidity of the soil caused by the different used hydrocarbon that is present the soil of the study area.

Table 1 Total number of bacteria count from auto mechanic contaminated soil

Location	No. of bacteria (CFU/g) x105
High court	2.1±0.01
Keffi garage1	1.7 ±0.12
Angwan Tanko	2.0±0.31
Keffi garage2	1.2 ±0.42
None contaminated soil	12.01±0.43

3.1 Identification of the different bacteria species isolated

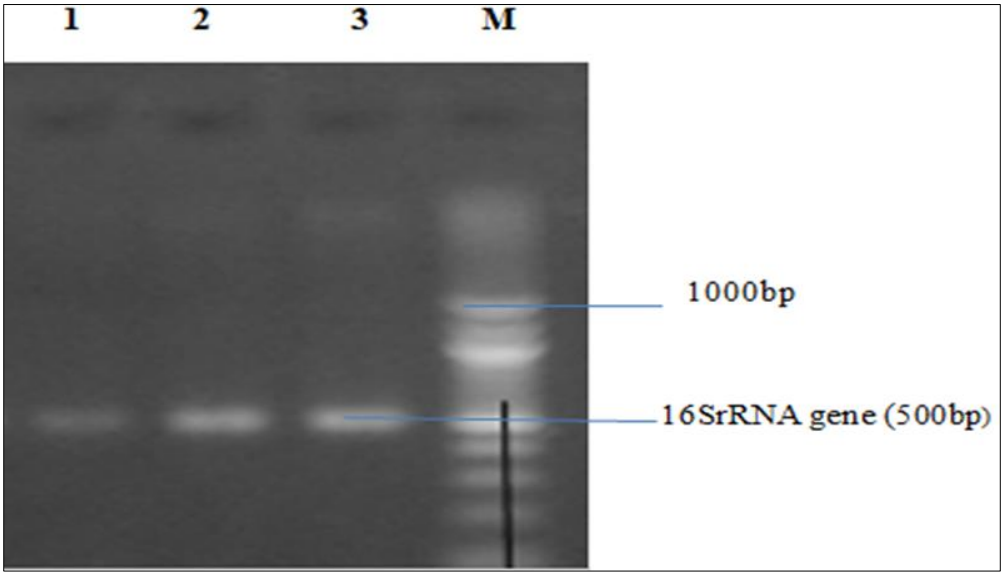
The cultural, morphology and biochemical characteristics of the bacteria isolated from soil contaminated with used hydrocarbon are as shown in Table 2. Figure 1 showed the Agarose gel electrophoresis of the 16S rRNA of Bacteria isolates. Where Lanes 1-3 represent the 16SrRNA gene bands (500 bp) of *Pectobacterium wasabiae*, *Pseudomonas fluorescens, Priestia aryabhattai* identified and Lane M represents the 1000bp molecular ladder. The phylogenic tree of the evaluation relatedness of the bacteria isolates is as shown in fig. 2, 3 and 4. The different species of bacteria isolated

and identified from the selected auto mechanic workshops in the study area were *Pectobacterium wasabiae*, *Pseudomonas fluorescens,* and *Priestia aryabhattai* and all were Gram negative bacteria which have the ability to adapt in any harsh environment. The low occurrence of these bacteria in the selected auto mechanic workshop maybe due to different level of contamination of the used hydrocarbon where it was observed that Keffi garage soil were highly contaminated with used hydrocarbon than the Angwan Tanko mechanic workshops the high level of contaminated is based on the activity of the auto mechanic that repair car and use different types of hydrocarbons at the process. The different species of bacteria isolated have been reported to be bacteria that can use different hydrocarbon as carbon source when they are in an environment that have high hydrocarbon. These bacteria are similar to bacteria reported by Ilori *et al*. [22] and Hassana *et al.* [23] who reported isolated of *Pseudomonas fluorescens, Priestia aryabhattai* and *Klebsiella aerogenes* from soil contaminated with hydrocarbon in Bitumen deposit in Lagos.

Table 2 Cultural, Morphological and Biochemical Characteristics of bacteria isolated from mechanic workshop contaminated with used hydrocarbon in Keffi

Isolate Code	**Cultural Morphology**	**Gram Reaction**	**Biochemical characteristic**				**Sugar fermentation**			**Inference**
			Cat	Ox	In	Nit	Fru	Mal	Glu	
KG	grey colored with a shiny surface and entire margin; mucoid or rough colonies	-	+	-	-	+	+	-	+	*Priestia aryabhattai*
KG 2-4	Smooth, white colonies distinct odor and become off-white on NA later	-	+	-	+	+	+	+	+	*Pectobacterium wasabiae*
HC1	smooth none elevated colonies green pigment on NA	-	-	-	-	+	-	-	+	*Pseudomonas fluorescens*
HC2	Grey colored with a shiny surface and entire margin; mucoid or rough	+	-	+	-	+	+	-	+	*Priestia aryabhattai*

Cat= Catalase, Ox= Oxidase, In= Indole, Nit= Nitrate, Fru= Fructose, Mal= Maltose, Glu= Glucose



Key: BP- Base Pair

Figure 1 Agarose gel electrophoresis of the 16S rRNA gene of Bacteria isolates,

Lanes 1 represent *Pectobacterium wasabiae*, Lane 2 represents *Pseudomonas fluorescens,* Lane 3 represents *Priestia aryabhattai* and Lane 4 represents *Klebsiella aerogenes* of 16S rRNA gene bands of 500bp, Lane M represents the 1000 bp DNA supercoil.

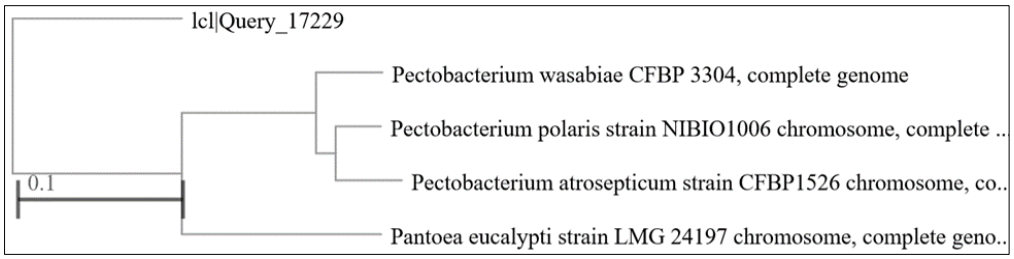


Figure 2 Phylogenetic tree of showing the evolutionary distance between the bacterial isolated

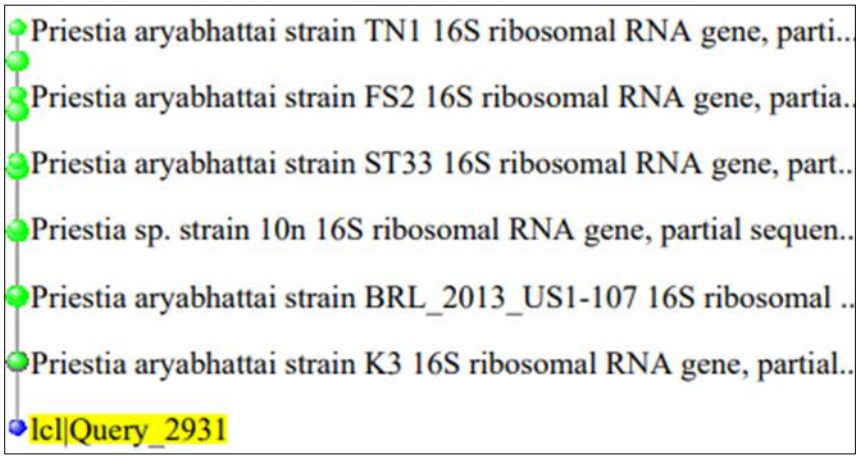


Figure 3 Phylogenetic tree of showing the evolutionary distance between the bacterial isolated

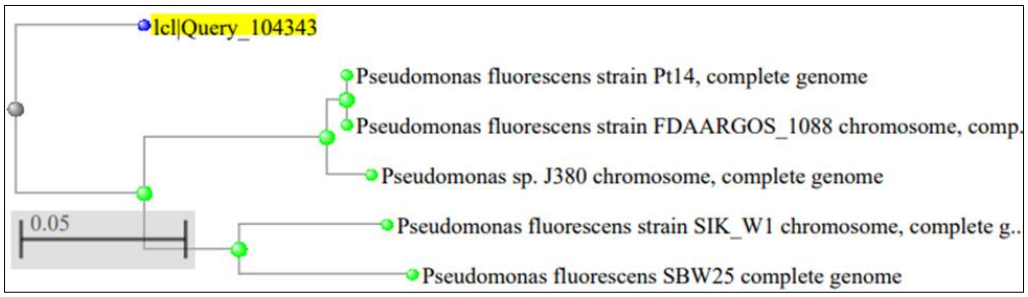


Figure 4 Phylogenetic tree of *Pseudomonas fluorescens* showing the evolutionary distance between the bacterial isolates

3.2 Effect of amendment with animal waste on contaminated soil with used hydrocarbon after four weeks

Table 3 shows the utilization amount of used hydrocarbon by bacteria isolated from soil contaminated with hydrocarbon with amended animal waste. *Priestia aryabhattai* had the highest utilization of 11.12±0.23 mg/ml on contaminated soil amended with cow dunk followed by 9.0±0.23 mg/ml on Poultry dropping amended soil, 3.01±0.23 mg/ml on none amended soil. *Pectobacterium wasabiae* had the highest utilization of 10.00±1.43 mg/ml on contaminated soil amended with cow dunk, 8.12±0.20 mg/ml on contaminated soil amended with Poultry dropping and 4.44±0.03 mg/ml on none amended contaminated soil. *Pseudomonas fluorescens* utilized highest on contaminated soil

amended with Poultry dropping with 16.23±1.21 mg/ml, 13.44±0.23 mg/ml on contaminated soil amended with cow dunk and 8.11±1.13 mg/ml on contaminated soil without amendment as after 4weeks of incubation.

The utilization of used hydrocarbon by bacteria on amended contaminated soil at temperature of 32 ℃ is as given in Table 4. The utilization of used hydrocarbon by bacteria on amended contaminated soil at temperature of 32 ℃ is as given in Table 4. *Priestia aryabhattai* utilized more used hydrocarbon on contaminated soil amended with Poultry dropping (8.23±0.03 mg/ml) followed by contaminated soil amended with cow dunk (6.10±0.06 mg/ml) and (2.11±0.07 mg/ml) on contaminated soil without amendment. *Pectobacterium wasabiae* showed highest utilization on soil amended with Cow dunk (13.00±1.43 mg/ml), on soil amended with Poultry dropping (11.12±1.21 mg/ml) and (5.14±0.17 mg/ml) on none amended soil. *Pseudomonas fluorescens* the highest utilization was obtained on soil amend with Cow dunk (17.13±1.11 mg/ml), on soil amended with Poultry dropping (15.14±1.13 mg/ml) and 9.21±0.13 mg/ml on soil without amendment.

Table 5 shows the utilization of used hydrocarbon by bacteria on amended contaminated soil at temperature of 26℃ is as given in Table 4. *Priestia aryabhattai* utilized highest on contaminated soil amended Cow dunk (11.23±0.13 mg/ml) followed by on contaminated soil amended Poultry dropping(10.20±0.16mg/ml) and 4.01±0.17 mg/ml on contaminated soil without amendment. *Pectobacterium wasabiae* utilized highest on Poultry dropping amended soil (15.22±1.31 mg/ml), on Cow dunk amended soil (14.10±1.43 mg/ml) and none amended soil (7.24±0.07mg/ml). *Pseudomonas fluorescens* showed great utilization on Cow dunk amended (18.13±1.01 mg/ml), Poultry dropping amended soil (16.24±1.33 mg//ml) and on none amended contaminated (9.21±0.13mg/ml) respectively. The effect of amendment with animal waste as observed in this study showed that soil contaminated with used hydrocarbon can be easily reclaimed by adding animal waste to the soil. It was observed in this study that *Pseudomonas fluorescens* isolated from soil contaminated with hydrocarbon in various auto mechanics in Keffi was able to utilized used hydrocarbon on amended soil and none amended soil with varying amount than other bacteria after four weeks of amendment and incubation. This showed that the animal waste contains other nutrient element that can enhance the population of another microorganism that will be able to degrade used hydrocarbon that pollute the environment. This is in agreement with the study reported by Singh and Lin [24] that addition nutrient element on soil contaminated with hydrocarbon help the population of soil microbials in reclaiming the soil and breaking down the hydrocarbon into other micro element that will benefits the microflora of the soil.

Also, the effect of temperature on soil amended and none amended showed that temperature is an important factor that affect the soil microbial on utilization of hydrocarbon, it was observed in this study that temperature of 26℃ favors the utilization of used hydrocarbon as both *Pectobacterium wasabiae*, *Pseudomonas fluorescens* and *Priestia aryabhattai* were able to utilize high amount of the used hydrocarbon as observed in this study. This is similar to work Obire and Ayanwu [25] reported on the best temperature for degradation of hydrocarbon. High temperature affect the nature and structure of hydrocarbon, makes it to reduce the oxygen in the soil and increase it viscosity that makes it difficult for microbials to use it as source of carbon or metabolize it Obire and Ayanwu [25].

Table 3 Utilization of used hydrocarbon in normal and animal waste-amended soil by bacteria isolated from mechanic workshops

Media	**Mean utilization of used hydrocarbon after 4weeks at ambient temperature (mg/ml ±SD)**		
	Priestia aryabhattai	***Pectobacterium wasabia***	***Pseudomonas fluorescens***
Poultry dropping and contaminated soil	9.0±0.23	8.12±0.20	16.23±1.21
Cow dunk and contaminated soil	11.12±0.23	10.00±1.43	13.44±0.23
Contaminated soil	3.01±0.23	4.44±0.03	8.11±1.13
Control	00±0.00	00±0.00	00±0.00

Table 4 Utilization of used hydrocarbon in normal and animal waste-amended contaminated soil by bacteria isolated from mechanic workshops

	Mean utilization of used hydrocarbon at 35 ℃ (mg/ml ±SD)		
Media	***Priestia aryabhattai***	***Pectobacterium wasabia***	***Pseudomonas fluorescens***
Poultry dropping and contaminated soil	6.10±0.06	11.12±1.21	15.14±1.13
Cow dunk and contaminated soil	8.23±0.03	13.00±1.43	17.13±1.11
Contaminated soil	2.11±0.07	5.14±0.17	9.21±0.13
Control	00±0.00	00±0.00	00±0.00

Table 5 Utilization of used hydrocarbon in normal and animal waste-amended soil by bacteria isolated from mechanic workshops

Media	**Mean utilization of used hydrocarbon at 40 ⍰ (mg/ml ±SD)**		
	Priestia aryabhattai	***Pectobacterium wasabia***	***Pseudomonas fluorescens***
Poultry dropping and contaminated soil	16.24±1.33	15.22±1.31	10.20±0.16
Cow dunk and contaminated soil	18.13±1.01	14.10±1.43	11.23±0.13
Contaminated soil	4.01±0.17	7.24±0.07	9.21±0.13
Control	00±0.00	00±0.00	00±0.00

4 Conclusion

Three different bacteria isolated and molecular identified were *Pectobacterium wasabiae*, *Pseudomonas fluorescens* and *Priestia aryabhattai* were able to utilized hydrocarbon in this study. Utilization or degradation of hydrocarbon when amended with animal waste showed that the three bacteria namely *Pectobacterium wasabiae*, *Pseudomonas fluorescens,* and *Priestia aryabhattai* had the able to utilized large amount of hydrocarbon after four weeks of amendment and incubation. Also, temperature plays an import role in utilization or degradation of used hydrocarbon in our environment.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest among the authors

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