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RESEARCH ARTICLE

BIODEGRADATION OF SPENT ENGINE OIL BY BACTERIA ISOLATED FROM OIL CONTAMINATED SOIL IN MECHANIC WORKSHOP OF SOKOTO METROPOLIS, NIGERIA

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ABSTRACT

Samples of used engine oil contaminated soil were collected from five different mechanic workshops in Sokoto metropolis to determine the presence of bacteria. Soil physicochemical parameters such as Carbon, nitrogen, phosphorus and others were determined. The bacteria identified include: *Yersini ruckeri, Bacillus spp*, *Acinetobacter calcoaceticus, Lactobaccilus brevis, Staphylococcus* spp, *Pseudomonas aeruginosa, Proteus vulgar! s. Klebsiella ozanae* and *Flavobacterium odoratum.* The isolates were cultured on oil medium to test their ability to grow on engine oil. The extent of used engine oil degradation was determined by gravimetric analysis. *Flavobacterium odoratum* (75.8%), *Pseudomonas aeruginosa* (74%), *Acinetobacter calcoaceticus* (73.7%) and *Yersinia ruckeri* (55.5%) were observed as the most efficient used engine oil degraders. The consortium *of Flavobacterium odoratum, Acinetobacter calcoaceticus* and *Pseudomonas aeruginosa* after three weeks show best result of engine oil degradation (81.8%). The results suggest that the consortium of the isolates could be used in remediating contaminated soils. Statistical analysis for total carbon, total nitrogen and rate of degradation of used engine oil by bacteria isolates procedure I and II showed significant difference between the samples and the rate of used engine oil degradation by the isolates.

INTRODUCTION

Soil is the habitat for variety of organisms, including bacteria, fungi, Protozoa, insects, nematodes, worms, and many other animals. Viruses are also present in soils. This complex biological community contributes to the formation, maintenance, and in some situations, the degradation and disappearance of soils (Prescott *et al.,* 2005). Soil pollution is the presence of unwanted impure materials from human activities. It can also be the distortion of the soil environment by human activities. Bacteria possess the ability to transform their morphology in environmental alteration. The survival of these microbes in altered environment depends on the ability to produce endospore and vegetative cell, which can stand harsh and unfavorable environment. These changes affect plants adversely and the amount of damage depends on the size of the area involved and the degree of saturation by the pollutant (Isinguzo and Bello, 2005). Engine oil could simply be referred to as a thick mineral liquid applied in a machine or engine so as to reduce friction between the moving parts of the machine (Whitefield, 2002). Used engine oil as the name implies represent oil that has undergone destructive changes in property when subjected to oxygen, combustion gases and high temperature. The said oil also undergoes viscosity change as well as additive depletion and oxidation, these occur to degrade oil (Mark *et al.,* 1982). This research aimed at to isolate, identify bacteria present in engine oil contaminated soil, and to test the bacteria isolates for their ability to degrade engine oil.

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MATERIALS AND METHODS

Sample Collection

Samples of engine oil contaminated soil were collected in sampling bags from five different mechanic workshops in Sokoto metropolis, namely: J-Allen motor-mechanic workshop, Kantin daji, Illela garage, Old Airport mechanic workshop and Sahara old garage. From each location l00g of engine oil contaminated soil was collected, consisting of 20g from five different points per location. The procedure was repeated for other locations making the total sample size of 500g of engine oil contaminated soil. Used engine oil was collected from Total filling station where cars are taken for services. The engine oil contaminated soil was analyzed in postgraduate laboratory of the Department of Microbiology, Usmanu Danfodiyo University Sokoto.

Media Preparation

The media used were Minimal Basal Medium, Trypticase Soy Broth, Trypticase Soy agar, Nutrient Agar, Bushnell and Hass media. The media were prepared according to manufacturers specifications. Appropriate grams of the powder were dissolved into 1000ml of distilled water. The mixture was thoroughly shaken and allowed to boil. It was sterilized by autoclaving at 121°C for 15 minutes. After the sterilization, it was allowed to cool to 45°C before dispensing into sterile test tubes.

Physical analysis of oil contaminated soil samples

i. pH Determination

Oil contaminated soil pH was determined according to IITA, (1979). Twenty grams of air dried soil (passed through 2mm sieve) was weighed into a 50ml beaker. Twenty ml (20ml) of distilled water was added and allowed to stand for 30 minutes and stirred occasionally with a glass rod. The pH meter was calibrated with buffer of pH 7.0 before use. The electrode of the pH meter was inserted into the partly settled suspension and the reading on the pH meter was noted and recorded accordingly.

ii. Determination of Moisture Content

Moisture content of the oil contaminated soil was determined using the method of IITA (1979). An empty container was weighed (W_0) and 2g of soil contents was added and weighed again (W_1). Samples were then dried in hot air oven at 105-110°C for 24 hours until constant weight was achieved V/2). Both the can and the dry samples were weighed again. The moisture content was calculated as:

$$\% \text{ moisture} = \frac{W1 - W_2}{W1 - W_2} \times 100$$

iii. Soil Particle Size Determination

Soil particle size was determined using the method of IITA (1979). Air dried soil was passed through a 2-mm sieve and 51g of the soil was weighed. The weighed sample was transferred to a 1k shake mix cup and 50ml of 5% sodium hexametaphosphate was added along with 100ml of distilled water. The soil suspension was stirred for 15 minutes using manual stirrers. The suspension was transferred from the cup to the glass cylinder with the hydrometer in the suspension. Distilled water was added to the lower blue line of the cylinder. The volume changed to 1130ml and the hydrometer was removed. The top of the cylinder was covered with hand and inverted several times until all soil was in suspension.

The cylinder was placed on a flat surface and time was noted. Immediately soil hydrometer was placed into suspension and the first reading was taken after the hydrometer was slide slowly in the suspension. The first reading was taken after 40 seconds (A). Subsequently, the temperature (T_1) was recorded with a thermometer after the hydrometer was removed. The suspension was allowed to stand for 3 hours and the second reading was taken for both the hydrometer (H_2) and the temperature (T_2) respectively. Calculations:

Sand= 100.0-(Hl+0.3 (T1 -20)-2.0)2
Clay= H2 +0.3 (T2 - 20) - 2.00
Soil = 100.0 - (% sand + % clay)

Chemical Analysis of oil-contaminated soil samples

iv. Organic carbon determination

Organic carbon was determined according to IITA, (1979). The representative sample was ground and passed through 0.5mm sieve. One gram of soil samples was weighed in duplicate and transferred to 250ml Erlenmeyer flasks. Ten milliliters of K_2 Cr_2 O_7 solution and 20ml cone, H_2SO_4 were added and the contents of the flask were shaken gently until properly mixed. One hundred milliliters (100ml) of distilled water were added and allowed to stand for 30 minutes. This was followed by adding 3-4 drops of indicator and titrating against 0.5N ferrous sulphate solution. The %C was calculated according to the formula:-

$$\% \text{ organic Carbon in soil} = \frac{(\text{Me } K_2 Cr_2 O_7 - \text{Me Fe } SO_4) \times I00x}{\text{g of air - dry soil}}$$

v. Determination of Nitrogen Content of the Soil Samples

The Macro-kjeldahl method of Udo and Ogimwale, (1986) was used in determining the nitrogen content of the oil contaminated soil. Five grams of soil sample was weighed into a dry 500ml macro-kjeldahl flask and 20ml of distilled water was added. The content was swirled for a few minutes and allowed to stand for 30 minutes. One tablet of mercury catalyst and l0g of K_2 SO4 were added and 30ml of concentrated H_2 SO_4 was added through an automated pipette. The flask content was heated at low heat on the digestion stand. The flask was allowed to cool and study. Distilled water (100ml) was added into the flask. The digest was transferred into another clean macro-kjeldahl flask (750ml) and the sand residue washed four times with 50ml of distilled water each time. All the washings were transferred into the same flask.

Fifty millilitres HS BOs indicator solution was added into a 500ml Erlenmeyer flask which was placed under the condenser of the distillation apparatus. The 750ml kjedahl flask was attached to the distillation apparatus and 150ml of ION NaOH was poured. This was followed by distillation and the condenser remained cool (below 30°C) to allow sufficient cold water to flow and also minimize frothing , and Ammonium will be determined in the distillate by treating with 0.0IN standard H_2 SO_4 using a 25M1 burette graduated at O.lml intervals. The colour changed at the end point from green to pink. Percentage Nitrogen content in soil was determined using the formula:-

$$\% \text{ Nitrogen} = \frac{N \text{ X } 0.014 \text{ x } Vdxl0}{A \text{ X Wt of sample}} \times 100$$

Where:

N = Normality of the acid
Vd = Volume of digest
A = Aliquot of digest

v. Determination of Phosphorus

The method of ITTA (1979) was used. In the method, 2g of soil, 1 teaspoon of carbon black and 40ml of the extracting solution were all put into a 125ml Erlenmeyer flask. The flask was shaken for 30minutes on a mechanical shaker. The suspension was filtered through the Whatman No. 40 paper.

More carbon was added to obtain a clear filtrate, thereafter 2ml of the clear supernatant was dispensed in a 20ml test tube and 5ml of distilled water plus 2ml of ammonium molybdate (NH4 6MO7 O24. 4H2O) added. The contents were mixed properly and 1ml of dilute stannous chloride (SnCL2.2H2O) solution was added and mixed again. After 5 minutes the percentage transmittance on the electro photometer at 660nm wavelength was measured and the reading was recorded using the formula:-

$$P\ (mg/kg) = \frac{\text{Reading x } 0.61 \text{x dilution factor}}{\text{Atomic weight of phosphorus}}$$

vi. Determination of K

The flame photometer will be set for K by inserting appropriate filter (usually of 768 wavelengths). The instrument was set to 100 percent transmittance by feeding l0ppm K solution and all the standard solutions were ran and a standard curve was prepared by plotting transmittance readings against concentration of standard K solution. The soil extract was ran and calculation was made as follows: -

$$K = \frac{\text{Milliequivalent per 100g oven-dry weight of soil}}{\text{Conc. Of K in the extract from standard curve}}$$

vii. Determination of Na

The flame photometer was set for Na by inserting appropriate filter (usually 58 wavelengths). The instrument was set to 100 percent transmittance by feeding 25ppm Na solution. All the standard solutions were run and a standard curve was prepared by plotting transmittance readings against concentration of standard Na solution. The soil extract was run again and calculation was made, using the formula:

$$Na = \frac{\text{Milliequaivalent per 100g over dry weight of soil}}{\text{Conc. Of Na in the extract from standard curve}}$$

viii. Determination of Mg

Two soil aliquots of Mg standard solution was pipetted into two titration flasks and distilled water was added to each to make total volume of 100ml. Twenty milliliters of buffer solution was added to get a pH of 10.This was followed by the addition of 10 drops each of (KON, NH2)H, HC1. K4 Fe (ON) 6 and triethanolamine. Ten drops of indicator were added and the solution was titrated against EDTA from a red to permanent blue colour. The blank was run with mg standard solution. The Mg was calculated per millilitre of EDTA as follows: -

$$Mg/Ml\ EDTA = \frac{0.1216 \text{x} 5ml\ Mg\ standard\ solution}{\text{Net ml EDTA to the end point}}$$

ix. Determination of Ca

Two 5ml aliquots of the extract were put in two titration flasks and distilled water was added to get a volume of about 150ml. Ten drops each of KON, NH2 OH HC1 and triethanolamine and 4ml of 10% NaOH were added. The solution was titrated with EDTA to a purple end point. The pH will be raised to 12 to precipitate Mg as Mg $(OH)_2$. The blank was run with 0.5ml of NH_4O solution and the net ml of EDTA was calculated by subtracting the titre from that needed for the extract. From the calibration of EDTA, Ca is calculated as: Net ml of EDTA-Net ml for Ca.

c. Bacteriological Analysis

The engine-oil-degrading bacteria were isolated using two standard procedures, namely: The procedure described by Fletcher (1992), and procedure described by Atlas, (1984). In the first procedure (Procedure l) 10.00g of the engine oil contaminated soil was weighed using weighing machine. The soil samples were transferred into a conical flask containing 90ml of trypticase soy broth. The samples were incubated for 24 hours at 37°C. One milliliter of the growth observed was sub-cultured onto a minimal basal medium overlaid with 1.0ml of engine oil. The plates were incubated for 3 to 5 days at 37°C to test the ability of the organisms to degrade engine oil. Colonies from minimal basal medium were picked and sub-cultured onto trypticase soy agar to obtain pure cultures. Plates were incubated for 24hrs at 37°C. In the second procedure (Procedure II) Bushnell-Haas medium was used as enrichment medium with 10% used engine oil as the sole carbon source to isolate engine oil degrading bacteria. Ten grams of the engine oil contaminated soil was weighed and added into a conical flask containing 90ml of the medium. The flask was incubated at 30°C for 1week. After 1 week, 1ml of enriched culture was transferred into freshly prepared medium and incubated for 1week at 30°C. Serial dilutions (1/10) from third enrichment process was placed onto Bushnell Haas agar plates which were covered with l00µl of used engine oil and incubated at 30°C for 24hrs. The single colonies were streaked onto nutrient agar plates and incubated at 30°C for 24 hrs.

Gram Staining of Pure Colonies

A smear was prepared by placing a drop of normal saline on a clean slide, wire loop was heated until red hot and used to pick the growth and placed on the slide. The smear was heat fixed by passing over flame. The smear was covered with crystal violet for one minute and washed with distilled water. This was followed by covering the smear with Lugol's iodine solution for one minute. The smear was washed with distilled water and then decolorized with acetone for 30 seconds. It was further washed off with distilled water and the smear was flooded with distilled for 1 minute after which it was washed with distilled water and allowed to dry. A drop of immersion oil was placed on dried slides, and the slides were viewed under microscope using x l00 objectives.

Biochemical Characterization of the Bacterial Isolates.
Catalase Test

Two milliliters (2.0ml) of hydrogen peroxide (H_20_2) solution was poured into a test tube. Using a glass rod, several loopful

were picked and immersed in the test tube containing the hydrogen peroxide solution. Active bubbling showed a positive catalase test whereas, the absence of bubbling indicated negative test (Oyeleke and Manga, 2008),

Oxidase Test

A piece of filter paper was placed in a clean Petri dish and 2 drops of freshly prepared oxidase reagent was added. A piece of glass rod was used to remove a colony of the test organism in order to form a smear on the filter paper. The development of a blue purple colon- within a few seconds showed a positive result. Absence of blue-purple colour indicated a negative result (Oyeleke and Manga, 2008).

Motility Test

The organisms were inoculated by making a fine stab with needle to a depth of 1-2cm short of the bottom of the tube. The organisms were incubated at 35°C for 24-48 hours. The line of inoculation would not be sharply defined and the rest of the medium would be cloudy. In negative result the line of inoculation becomes sharply defined (Oyeleke and Manga, 2008).

RESULTS

Physicochemical characteristics of used engine oil contaminated soil are presented in Table 1. Results of the moisture content showed that the engine oil contaminated soil from all locations had low moisture content ranging from 0.5%-1.0%. This indicated that the soil was dry. The pH of the soil sample indicated that in four points, the PH was neutral ranging from 7.02-7.5, while in the point I, the pH was slightly alkaline with pH value of 8.49. The percentage organic carbon of the soil samples was observed to be highest in samples from sites J and K with 4.59%, while the values from other points ranges between 2.59%-3.19%. The statistical analysis carried on data from carbon content determination of soil, using one way ANOVA at 5% confidence indicated that there was a significant difference ($p \leq 0.05$) between the sample sites.

The particles size analysis of the soil samples from different locations indicated that the soil from all the locations was sandy. This is because the sand percentage of all the soil samples was 94.5%. Results of the nitrogen content and phosphorus content of the soil showed that the soil used in the analysis from all the locations was low in nitrogen content and phosphorus content with values ranging between 0.025mg/kg-0.060mg/kg and 0.67mg/kg-0.74mg/kg respectively. The statistical analysis carried on data from nitrogen determination of soil using one way ANOVA at 5% confidence, indicated that there is significant difference ($P \leq 0.05$) between the samples. The contents of potassium, sodium and calcium in the soil samples were also found to vary. The highest value of potassium was obtained from point K with the value of 380.0mg/kg, while the lowest value was obtained from point O with the value of 170.0mg/kg. Point K also had the highest content of sodium (75.0mg/kg) while the lowest of 24.0mg/kg was obtained from point O. The calcium content of the soil varied between 0.30-0.50mg/kg with point K having the highest value (0.50mg/kg) while point O had the lowest (0.30mg/kg). The magnesium content of the soil showed that point O had the highest magnesium content of l.00mg/kg followed by point K with 0.85mg/kg, point S with 0.80 and point I with 0.55mg/kg. The least magnesium content was obtained from point J with 0.45mg/kg.

Statistical analysis carried on data from weight lost of engine oil (procedure I), using one way ANOVA at 5% confidence, indicated that there was significant difference between the samples. Similarly statistical analysis carried on data from weight lost (procedure II) using one way ANOVA at 5% confidence indicated significant difference ($P < 0.05$) between the samples. A wide range of bacteria was isolated from used engine oil contaminated soil samples from both the two procedures. On identification, the bacteria isolated from procedure I (Table 2) were *Yersinia ruckeri, Bacillus polymyxa., Bacillus sphaericus, Bacillus cereus, Bacillus* lentus, *Acinetobacter calcoaceticus, Lactobacillus brevis* and *Staphylococcus aureus.*

From the procedure II (Table 3), the bacteria isolated were *Pseudomonas aeruginosa, Flavobacterium odoratum, Klehsiella ogaenae, Bacillus polymyxa, Proteus vulgaris, Yersinia nickri, Staphylococcus sciuri, Staphylococciis aureus* and *Staphylococcus kiosii. Bacillus* species were the most frequently isolated bacteria using procedure I and constituted 60 percent of all the bacterial isolates (Table 2). However, *Staphylococccus* sp had the highest occurrence (25.0%) in procedure II (Table 3). Table 4 Shows the extent of growth on oil by bacterial isolated obtained from used engine oil contaminated soil sample. Of the 10 bacterial isolates obtained from procedure I tested, three isolates (*Yersinia ruckeri* and *Acinotobacter calcoaceticus* had a high growth in the oil medium. *Lactobacillus brevis* and *bacillus* species showed a moderate growth while *Bacillus cereus* and *bacillus lentus* exhibited no growth in the oil medium.

Table 1. Determination of Physicochemical Paramters of Used Engine oil Contaminated Soil

Soil Sample	Moisture %	pH	Carbon %	N %	P Mg/kg	K Mg/ kg/%	Na Mg/kg	Ca Mg/kg	Mg Mg/kg	Sand %	Silt %	Clay %
J	1.0	8.49	3.10	0.028	0.73	220.0	41.9	0.35	0.55	94.5	5.3	0.2
J	0.5	7.02	4.59	0.046	0.67	260.0	40.0	0.40	0.45	96.5	3.3	0.2
K	0.5	7.10	4.50	0.060	0.74	380.0	75.0	0,50	0.85	94.5	3.3	0.2
O	1.0	7.16	2.91	0.035	0.71	170.0	24.0	0.30	1.00	94.5	5.3	0.2
S	0.5	7.5	2.59	0.025	0.74	220.0	50.0	0.40	0.80	94.5	5.3	0.2

Table 2. Frequency of Bacteria Isolated from used Engine Oil Contaminated Soil (Procedure I)

Organism	Frequency	Percentage
Yersinia ruckeri	1	10
Bacillus spp	6	60
Acinetobacter	1	10
Lactobacillus brevis	1	10
Staphylococcus aureus	1	10

Table 3. Frequency of Bacteria Isolated from used Engine Oil Contaminated Soil (Procedure II)

Organism	Frequency	Percentage
Pseudomonas aeruginosa	2	16.7
Flavobacterium odaratum	2	16.7
Klebsiella ozaenae	1	8.3
Bacillus spp	1	8.3
Proteus vulgaris	2	16.7
Yerisnia ruckeri	1	8.3
Staphylococcus spp	3	25.0

Tables 4. Extent of growth on oil medium by bacteria isolated from used Engine oil contaminated soil procedure I

Code	Bacterial Isloates	Extent of Growth in engine oil medium	Inference
Ipt3	*Yersinia ruckeri*	+ + +	High growth
Jpt2	*Bacillus polymyxa*	+	Low growth
Spt3	*Nacillus lentus*	-	No growth
Kpt3	*Bacillus sphaericus*	+ +	Moderate growth
Jpt3	*Bacillus polymxa*	+	Low gowth
Jpt1	*Acinetobacter calcoaceticus*	+ + +	High growth
Opt3	*Bacius cereus*	-	No growth
Ipt2	*Baccilus lentus*	-	No growth
Spt2	*Lactobaccilus brevis*	+ +	Moderate growth
Kpt1	*Staphylococus aureus*	+	Low growth

Results of the extent of growth from procedure II show that twelve bacterial isolates were tested on their ability to grow and degrade used engine oil. Three of the isolates were found to be the best engine oil degraders namely *Flavobacterium odaratum (KPT2) pseudomonas aeruginosa* (K) and *Yersinia ruckeri Jpt*. Other species that degrade engine oil included *proteus vulgaris, Klebsiella ozaenae* and *Bacillus polymyxa* while *Staphylococcus* sp exhibited no growth.

Table 5. Extent of Growth on Oil medium by Bacteria isolated from used Engine Oil Contaminated Soil Procedure II

Code	Bacterial Isolates	Extent of growth in engine oil medium	Inference
Kpt1	*Pseudomonas Aeruginose*	+ +	Moderate growth
Opt1	*Flavobacterium Odaratum*	+ +	Moderate growth
Spt1	*Klebsiella ozaenae*	+ +	Moerate growth
Kpt2	*Flavobacterium odaratum*	+ + +	High growth
K	*Pseudomonas* sp	+ + +	High growth
Jpt2	*Bacillus polymxa*	+	Low growth
Opt2	*Proteus vulgaris*	++	Moderate
Ipt2	*Proteus vulgaris*	+ +	Moderate growth
Jpt1	*Yersinia ruckeri*	+++	High growth
Ipt2	*Staphylococcus sciuri*	-	No growth
Opt3	*Staphylococcus* sp	-	No growth
Jpt2	*Staphyloccoccus kiosii*	-	No growth

Table 6. Rate of degradationon of used Engine Oil by Bacterial isolate Procedure I

	5	10	15	20
Yersinia spp	11.04	28.10	39.52	55.50
Bacillus spp	16.50	23.48	39.41	45.40
Lactobacillus spp	9.91	15.31	28.00	43.01
Acinetobacter calcoaceticus	27.34	45.30	56.80	73.72
Staphylococcus aureus	8.82	10.01	17.04	19.24

Table 7. Rate of degradationon of used Engine Oil by Bacterial isolate Procedure II

	5days	10days	15days	20days
Pseudomonas spp	22.02	38.32	59.01	74
Bacillus polymyxa	5.02	11.15	22.71	28.33
Yersinia ruckeri	14.12	29.17	46.63	58.11
Klwbsiella spp	13.31	21.19	32.493	44.91
Favobacterium spp	8.82	10.01	17.04	19.24

Table 8. Rate of degradation of used engine oil by the consortia of bacteria isolates after three weeks

Coded isolate	Weight loss (%)	Absorbance (420nm)
FAP	81.8	0.585
FPB	69.0	0.502
PrYK	46.2	0.211
KFB	42.6	0.165
FAB	55.8	0.175
PBA	51.0	0.119

F: *Flavobacterium* spp, A: *Acinetobacter calcoaceticus*, P: *Pseudomonas* spp, B: *Bacillus* spp, Pr: *Proteus vulgaris*, Y: *Yersinia* spp, K: *Klebsiella* spp.

Table 6 and 7 shows the rate of degradation of used engine oil by bacterial isolates procedure 1 and 11, while Table 8 shows the rate of degradation of used engine oil by the consortium of isolates after three weeks.

DISCUSSION

The engine oil contaminated soil from all the locations had larder particle size, (94.5 – 96.5) and therefore the soil is sandy. Similarly the moisture content level of the soil was low; it ranges between 0.5% and 1%. Atlas (1995) reported that hydrocarbon contamination reduces the bulk density of soil while it increases the porosity of such soil. In such situations, soil aggregates rate broken down and dispersion results, thus the soil is prone to erosion than normal soils. Potassium content and sodium content from all the soil were high (Table 1) this highlights the view that the enormous number of hydrocarbon degraders is linked with the availability of nutrients. Researchers (Atlas, 1984, Ijah and Okang, 1993) have stressed the importance of nutrients in hydrocarbon biodegradation.

Available carbon, nitrogen and phosphorous in the engine oil contaminated soil was very low (2.59%, 0.025%, -0.060%, 0.67% - -.74% respectively), even though the nutrients availability in location K was higher than that in other locations. Atlas (1984) opined that depending on the nature of the environment, nutrients such as nitrogen and phosphorous could be limiting thus affecting the biodegradation process. However, this is contrary to the findings of Lovely (2001) that, significant increases in total nitrogen and organic carbon of soil have been observed as a result of oil contamination. The increase in total nitrogen is attributed to increased atmospheric nitrogen fixation during the oil degradation process, while increase in organic carbon could be caused by the carbon present in the petroleum. *Bacillus* spp was frequently isolated in the study, (table 4.10 according for 60% of the total isolates). Ijah and Antai (2003) reported *Bacillus* spp as being the predominant isolates of all the crude oil utilizing bacteria characterized from highly polluted soil samples. Similar finding postulated that Bacillus spp were more tolerant to high levels of hydrocarbons in soil due to their resistant endospores

(Ghazali *et al,* 2004). Several different bacteria were isolated from soil contaminated with used engine oil using two different procedures. Bacterial species isolated were tested in order to find out their biodegradation potential. Seven of the isolates were gram negative rods (*Pseudomonas aeruginosa, Bacillus polymyxa, Bacillus sphaericus, Yersinia ruckeri, Proteus vulgaris,Klebsiella orgaenae* and *Flavobacterium odaratum*). Four of the isolates were gram positive rods (*Acinetobacter calcoaceticus* and *Lactobacillus brevis, Bacillus cereus* and *B. tentus*). One of the isolates was gram positive coccus (*Stephylococcus aureus*). All the bacterial isolates were able to utilize used engine oil as the sole source of carbon in verying amounts. The variation in the capacity of the isolates to utilize hydrocarbons could be due to differences in their competence of their crude oil degrading enzyme system. While some isolates may be inhibited (Feroo *et al.*, 1994) made similar observation.

The result of gravimetric analysis calculated indicated indicate that the best degradation was observed with *flavobacterium odoratum* (75.78%), pseudomonas aeroginosa (74.00%), *Acinetobacter calcoaceticu* (73.72%), *Yersinia rukeri* (55.5%). This finding is in line with that of Rusansky *et al*. (1987), Singh and lin (2007), Ijah and Antai (2003) who reported that *Pseudomonas* and *Acinetobacter* species were the most common bacterial hydrocarbon-degraders. *Acinetobacter* spp are widespread in nature and can remove or degrade a wide range of organic chemicals such as phenol (Briganthi et al., 1997) Abdel El-Haleem *et al.*, 2002, toluene (Zilli et al, 2001) and inorganic compounds such as phosphates and metal (Auling *et al*, 1991, Wagner *et al*, 1994; boswell et al 2001). Species of *Acinetobacter* have been attracting increasing attention in both environmental and biotechnological applications (Abdel – El-Hallem, 2003). The presence of pseudomonas in contaminated soil was also in accordance with the work of khadija et al., (2004) who reported that *Pseudomonas aeroginosa* could utilize long-chain alkanes efficiently and could grow on heavy oil.

The ability of *Flavobacterium* spp to degrade used engine oil was revealed and this finding is similar to that Atlas and Bartha, (1998), and Christopher and Christopher (2004) who reported that *Flavobacterium* spp and *Pseudomonas* spp were predominant species in the early stage of the petroleum treatment unit. Other bacterial species with degradation ability were obtained in the study namely *Proteus vulgaris, Klebsiella* spp and *Bacillus polymyxa*, however *Bacillus* species were found to have the highest occurrence in procedure II 60% of the total bacterial isolates. This is in conformity with the work of Ijah and Antai (2003) who reported *Bacillus* spp as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples. The best degradation was observed by consortium of the isolates. The best degradation was observed by the consortium of *flavobacterium odoratum, Acinetobacter calcoaceticus* and *Pseudomonas aeroginosa* with 81.8%. This is similar to that of Alden *et al.,* (2001) who reported the advantages of employing mixed cultures as opposed to pure cultures in bioremediation.
Although microorganisms were isolated using both procedures, procedure I was found to be more convenient and isolates could be obtained in a relatively short period while procedure II took longer time in yielding isolate. This research recommended that Mechanic workshops should not be sited indiscriminately; government should provide appropriate places for such activities. Used engine oil and empty gallons could be recycled; therefore they should not be dumped in our environment. Also Researches on the consortium of bacteria that could be used to degrade engine oil should be conducted.

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