



# **Microbial Remediation of Used Engine Oil from Contaminated Soil around Automobile Workshop in Calabar Metropolis, Cross River State, Nigeria**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author ABA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IUB and COO managed the analyses of the study. Author IOA managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Microbial biodegradation of engine oil contaminated soil in Calabar Metropolis was studied for a period of six (6) months (January to June, 2017). The soil samples collected were ice boxed and taken to the laboratory for microbial, total hydrocarbon, total organic carbon and other physico-chemical parameters analysis. A total of thirteen (13) indigenous bacteria species were identified in the soil of the sites analyzed during the study, which includes; *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., *E. coli*, *Enterococcus faecalis*, *Shigella* spp., *Arthrobacter* spp., *Alcaligen* spp., *Acinobacter* spp., *Azotobacter* spp., *Aeromonas* spp., *Xanthomonas* spp. and *Clostridium* spp. The most abundant bacteria in the contaminated site was *Staphylococcus* spp. (65%) while the least

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bacteria count in the contaminated site was *Clostridium* spp. (9%). *Staphylococcus* spp. was the most abundant indigenous bacterial species and also the most effective biodegradation bacteria. The identified indigenous bacteria utilized the hydrocarbons, multiplied rapidly and then degraded the total hydrocarbon and total organic carbon more in the contaminated site compared to the control site. Site one recorded the highest bacteria count (927) while the least bacteria counts were recorded in the control site (81). The bacteria species showed its degradation and bioremediation capabilities prompting the need for its use in cleaning crude oil contaminated sites, due to the fact that it is cheap and not environmentally harmful.

**Keywords:** Bioremediation; bacteria; contaminated site; total hydrocarbon; total organic carbon; Calabar metropolis.

## 1. INTRODUCTION

Human existence on earth is almost impossible without chemicals. Chemicals and their products are very important to mankind due to their benefits. However, exposure to them during production, usage and their uncontrolled discharge into the environment has caused lots of hazards to man, other organisms and the environment [1]. Engine oil which is a component of crude oil is a complex mixture of hydrocarbons that are used to lubricate parts of an automobile engine to avoid excessive wearing out [2]. Motor engine oil contains metals and Polycyclic Aromatic Hydrocarbons (PAHs) and these could contribute to chronic hazards including mutagenicity and carcinogenicity [3]. Bioremediation is defined as the process whereby organic waste are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities. It can also be defined as the use of living organisms, primarily micro-organisms or their enzymes, to degrade the environmental contaminants into less toxic forms or total removal of pollutants from the biosphere [4]. The widespread ability of micro-organisms to assimilate hydrocarbons is of great significance and when it occurs in a natural environment, the process is called biodegradation. Hydrocarbons such as polycyclic aromatic hydrocarbon (PAHs) have long been recognized as substrates supporting microbial growth. Although bioremediation of petroleum pollutants is a slow process, often requiring many months to degrade the majority of the oil, it is relatively inexpensive and seemingly harmless to the surrounding environment and the process generally involves bio-stimulation and bio-augmentation [5]. A wide range of hydrocarbon utilizers (HCUs) is found to be useful in the soil and it includes the following species such as; *Pseudomonas* spp, *Rhodococcus* spp., *Mycobacterium* spp., *Bacillus* spp., *Acinetobacter*

spp., *Providencia* spp., *Flavobacter* spp., *Carynebacterium* spp., *Streptococcus* spp. [6]. Other organisms like fungi are also capable of degrading the hydrocarbon in engine oil to a certain extent, but they take longer period of time to grow when compared to their bacterial counterparts [7]. This study is aimed at evaluating the microbial remediation of engine oil contaminated soil around mechanic workshops in Calabar Metropolis, Cross River State Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Description of Study Area

The study was carried-out in Calabar; which is geographically located at latitude 6° 20' N and longitude 5° 20' E. The study was carried-out in four selected contaminated sites (Automobile workshops) in the Calabar Metropolis; two (2) in Calabar Municipality L.G.A and two (2) in Calabar South L.G.A. These study areas were characterized by distinct wet and dry season. Calabar is the capital of Cross River State, making up of Calabar south and Calabar Municipality L.G.A, which shares boundary at Mary Slessor Avenue, a road which runs from University of Calabar main gate and terminate at Calabar Road.

#### 2.1.1 Sampling stations

A total of Five (5) sampling stations were chosen along the study area. Coordinates of each station were digitized using ARC Geographic Information System (ARC GIS) software. Four stations being crude oil contaminated sites and one control station. Two (2) sampling stations were located in Calabar Municipality L.G.A, while the other three (3) stations were located at Calabar South. Sampling station one (1) was located geographically between 08° 20' 32.0" E and latitude 04° 57' 31.3" N along Etta Agbor Road, while the second station was located

along Marian Road which is geographically between 08° 20' 15.3"E and latitude 04° 57' 52.1"N all in Calabar Municipality. In Calabar South L.G.A, sampling station three (3) was located along Mayne Avenue between longitude 08° 19' 09.2" E and latitude 04° 56' 36.9" N, while sampling station four (4) was located geographically between longitude 08° 19' 01.6" E and latitude 04° 55' 24.7" N along New Airport Road. The fifth station is the uncontaminated control station, located at Mbukpa Road, which is geographically between longitude 08° 19.5' 01.9" E and latitude 04° 58' 28.8" N.

### 2.1.2 Collection of soil sample

Soil samples were collected from the five sampling stations using a trowel within a two day period. The soil samples were collected at three (3) different point of each sampling station and then put together to form a composite sample. Immediately after sample collection, the soil samples were put in a well-labelled polythene bag, then preserved in icebox before transporting to the Microbiology laboratory for microbiological and physicochemical analysis.

## 2.2 Total Bacteria Count and Isolation

In the laboratory, 1 g of the soil sample was suspended in 9 mL sterile water. Ten-fold serial dilution, in the range of  $10^{-1}$  –  $10^{-7}$  were prepared using sterile distilled water. Aliquots 1 mL of samples dilution of  $10^{-3}$  -  $10^{-4}$  were plated on Bushnell-Hass media and Mineral salt media. The numbers of visible colonies were multiplied by the reciprocal of the dilution factor and recorded colony forming units (CFU) per gram of soil [8]. Discrete colonies were sub-cultured onto Nutrient Agar and Mac-Conkey agars, until pure cultures were obtained through identification. The isolated organisms were identified by staining and biochemical analysis according to [9].

### 2.2.1 Isolation and characterization of hydrocarbon degrading micro-organisms

The bacteria species which were indigenous to the soil samples and could mineralize petroleum were isolated by pour plate technique using 0.1 mL aliquots of appropriate dilution unto nutrient agar. Individual cultures were identified by morphological and biochemical technique using the taxonomic scheme of Bergey's manual of determinative bacteriology [10].

## 2.2.2 Growth of bacterial isolate on hydrocarbon substrates

The weight loss method described by [11,12] was used to determine the amount of crude oil in samples. The medium was dispensed in 99mL quantities into 250 mL Erlenmeyer flasks. Each flask was supplemented with 1mL or 1g as the case may be of selected carbon source and seeded with axenic culture of isolates. Incubator programmed at 120 rpm at 30°C. The optical density ( $OD_{600\text{ nm}}$ ) total viable count (TVC) and pH of the culture fluids were monitored at determined time intervals and biodegradation indices.

## 2.3 Gram Staining and Procedure

This test was done to differentiate isolated hydrocarbon utilizing organisms into Gram positive (+) and Gram negative (-) based on their cell wall composition and their ability to either retain the primary dye or be decolorized by alcohol. A smear of the organism from each of the sub-culture plates were picked with a sterile inoculating wire loop and used to make a smear on clean grease free glass slide. The smear was heat fixed by passing the slide over a Bunsen burner flame repeatedly for about three to four times, before flooding with crystal violet and allow to stand for a minute. The crystal violet was washed off with distilled water and Gram's iodine solution was added to the slide and allowed for 60 seconds (1 min) and washed. Seventy percent (70%) alcohol was used to decolorize the Gram's iodine for 2 seconds, and then the slide was flooded with counter stain (Saframin) and allowed to stand for 60 seconds. It was then rinsed off with distilled water and allowed to air dry before viewing under the microscope using the oil immersion objective (x100).

### 2.3.1 IMVIC test

IMVIC (I-Indole, M-methyl red, V-voges proskaver, C-cilate) test was carried-out. The indole production was carried-out as follows; Three grams (3 g) of peptone stuck sample was weighed and dissolved into 200 ml of distilled water and sterilized in an autoclave at 121°C for 15 minutes. Using aseptic technique, 5ml of the solution was measured each into 12 test tubes and the organisms were inoculated into the tubes with a loop full of culture and these tubes were incubated at 35°C for 24 hours. The tubes were removed from the incubator and 10 drops of Kovac's reagent was added to each tube and

shaken gently. A deep red colour developed in the presence of indole, which indicate indole positive. A negative reaction remains colourless or light yellow, which indicates indole negative. In the case of the methyl red test, 5 drops of methyl red indicator was added to a peptone water culture and examined for 1 minute for colour change. A red colour indicates a methyl red positive. No colour change or light yellow indicates methyl red negative. For Voges Proskauer (VP) test, 15 drops of Barritt's solution A and 5 drops of Barritt's solution B were added to peptone water after 24 hours of incubation and shaken to aerate. A red colour occurred which indicates a positive VP reaction and no colour indicates a negative VP reaction.

### 2.3.2 Sugar fermentation test

This was carried-out as described by [13], in order to test the ability of micro-organisms to metabolize a large variety of sugar as carbon source that was used in the glucose. The medium used contain peptone 1.0% NACL, 0.1% fermentable sugar, 1.0% of phenol red indicator was added and 9ml of the preparation was dispensed into a different test tube carefully avoiding air bubbles. The tube were sterilized at 121°C for 15 minute, and allowed to cool. A loopful of the test organisms was inoculated into each of the cool test tube and inoculated at 37°C for 24 hours. The tube and examined daily for colour change and the Durham tube were examined for display gas. Yellow colour (acid formation) indicates (positive) result. Red colour indicates negative result. The gas produced was accumulated in inverted Durham tube which easily ignite a glowing splinter.

## 2.4 Purification and Maintenance of Bacteria Culture

The isolate were sub-cultured on nutrient agar plates, pure cultures were plated on agar slants and incubated at 37°C for 18 to 24 hours and sorted in the refrigerator for future use.

### 2.4.1 Physico-chemical parameters analysis of the soil samples

The soil samples was taken to the laboratory, Department of Pure and Applied Chemistry, Faculty of Physical Sciences, University of Calabar for analysis. In the laboratory, the soil samples were oven-dried and then sieved. The total organic carbon (TOC) was determined using

the method described by [14]. This was done by igniting the dried sieved soil samples (2.5 g) in a pre-weighed crucible and calculating the loss in weight by difference followed by the calculation of the percentage of organic matter in the soil samples. The total hydrocarbon (THC) was determined following extraction with redistilled n-hexane before measuring the total hydrocarbon content calorimetrically at 430 nm using a DR/3000 HACH spectrophotometer. Soil pH was determined with pH meter (HANNA Instrument) on 1: 2.5 (w/v) soil/distilled water after 30 minute equilibration. Triplicate determinations were made. Moisture contents was determine using the method recommended by [15]. Available phosphorus (AP) in soil samples was also determined using HACH spectrophotometer, and total nitrogen content was determined using Kjeldahl method. The temperature was measured using a digital thermometer. Particle size distributions were determined based on the unified soil classification as described by [16].

### 2.4.2 Statistical analysis

The microbial (Nutrient agar and Mac-Conkey agar), total hydrocarbon and total organic carbon data obtained was subjected to Analysis of variance (ANOVA) analysis to determine their significance of difference between the samples for each stations at 0.05 level of significance and at their relevant degrees of freedom. All analysis was carried-out using predictive analytical software (PASW).

## 3. RESULTS

### 3.1 Bacteria Composition in Contaminated Soil

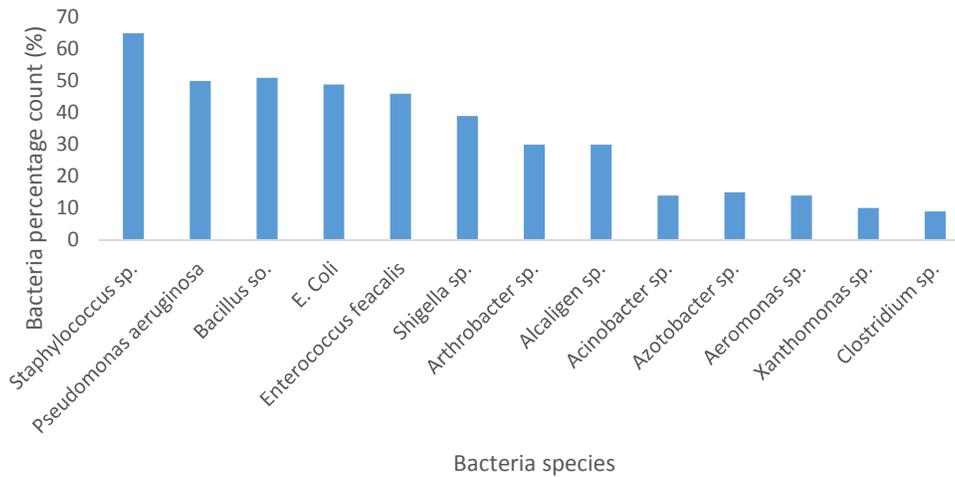
The abundance of the identified indigenous bacteria in the contaminated site is shown in Fig. 1. A total of 13 indigenous bacteria species was identified in the soil of the sites analyzed during the study, which includes; *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., *E. coli*, *Enterococcus faecalis*, *Shigella* spp., *Arthrobacter* spp., *Alcaligen* spp., *Acinobacter* spp., *Azotobacter* spp., *Aeromonas* spp., *Xanthomonas* spp. and *Clostridium* spp. (Table 1). The most abundant bacteria in the contaminated site were *Staphylococcus* spp. (65%), followed by *Pseudomonas aeruginosa* (50%) and then *Bacillus* spp. (51%). The least bacteria count in the contaminated site was *Clostridium* spp. (9%) followed by *Xanthomonas* spp. (10%) (Fig. 1).

**Table 1. Biochemical characteristics/identification of bacterial isolates**

Isolate	Gram Reaction	Catalase	Oxidase	Indole	Methyl red	Voge proskauer	Citrate	Glucose fermental	Gas production	Suspected organism
1 MAC	Gram (-) short rod in cluster	+	+	+	-	-	-	+	-	<i>Pseudomonas aeruginosa</i>
2 MAC	Gram (-) short rod in chains and in pairs	+	-	+	+	-	+	+	-	<i>E. coli</i>
3 MAC	Gram (+) variable shape example of bacteria, Actinobacter	+	+	-	-	-	+	+	+	<i>Arthrobacter spp.</i>
4 MAC	Gram (+) cylindrical rod with spore, in pair and in short chain	+	-	+	-	-	-	+	-	<i>Bacillus spp.</i>
5 MAC	Gram (+) cocci/diplococcic	-	-	-	-	+	-	+	-	<i>Enterococcus faecalis</i>
6 MAC	Gram (-) tiny rod in chains, cluster and singly	+	-	+	-	-	-	-	-	<i>Shigella spp.</i>
7 MAC	Gram (-) in small cluster form and pale colonies	+	-	-	-	-	+	+/-	+	<i>Acinobacter</i>
8 MAC	Gram (-) short rod in clusters	+	+	+	-	-	-	+	-	<i>Pseudomonas aeruginosa</i>
9 MAC	Gram (-) coccal and diplococcic	+	+	-	-	-	+	+	+	<i>Alcaligen spp.</i>
10 MAC	Gram (-) rod shape	+	+	+	+	-	-	+	+	<i>Aeromonas spp.</i>
11 MAC	Gram (+) cocci, diplococci/rods	+	-	+	+	-	+	+	-	<i>Staphylococcus spp.</i>
12 MAC	Gram (-) rod cyst forming brown to black colonies	+	-	+	+	+	+	+	+	<i>Azotobacter spp.</i>
13 NA	Gram (+) cylindrical rod with spore, in pair and in short chain	+	-	+	-	-	-	+	-	<i>Bacillus spp.</i>

Isolate	Gram Reaction	Catalase	Oxidase	Indole	Methyl red	Voge proskauer	Citrate	Glucose fermental	Gas production	Suspected organism
14 NA	Gram (+) cocci, diplococci/rods	+	-	+	+	-	+	+	-	<i>Staphylococcus spp.</i>
15 NA	Gram (-) in straight rod single flagellum mucoid convex and yellow colonies	+	+	-	-	-	+	+	+	<i>Xanthomonas spp.</i>
16 NA	Gram (-) short rod in chains and in pairs	+	-	+	+	-	+	+	-	<i>E. coli</i>
17 NA	Gram (-) tiny rod in chains cluster and singly	+	-	+	-	-	-	-	-	<i>Shigella spp.</i>
18 NA	Gram (+) cocci/diplococci	-	-	-	-	+	-	+	-	<i>Enterococcus feacalis</i>
19 NA	Gram (+) endospore forming singly rod	+	+	-	+	+	-	-	-	<i>Clostridium spp.</i>
20 NA	Gram (+) cocci, diplococci/rods	+	-	+	-	-	+	+	-	<i>Staphylococcus spp.</i>

Where: NA = Nutrient Agar, MAC = Mac-Conkey, Positive (+), Negative (-)



**Fig. 1. The abundance of the identified indigenous bacteria with biodegrading ability in the contaminated site**

### 3.1.1 Bacteria growth

The summary of the bacteria growth on both the nutrient agar and Mac-Conkey agar media is shown in Table 2. For site 1 (Etta-Agbo auto-workshop) cultured with nutrient agar, the bacteria growth increased rapidly from its initial 83 in sample 1 to  $150 \times 10^{-3}$  and also from 49 to  $71 \times 10^{-4}$  in sample 2. In the case of Mac-Conkey agar, there was also a rapid bacteria growth from 92 to  $122 \times 10^{-3}$  in sample 1 and 10 to  $50 \times 10^{-4}$  in sample 2, the growth of the bacteria varies significantly with samples for both the nutrient and Mac-Conkey agar at  $P = .05$ . For site 2 (Marian auto-workshop) cultured with nutrient agar, the bacteria growth increased from its initial 12 to  $22 \times 10^{-3}$  in sample 1 and also from 29 to  $80 \times 10^{-4}$  in sample 2. In the case of Mac-Conkey agar, there was also an increase in bacteria growth from 15 to  $44 \times 10^{-3}$  in sample 1 and 14 to  $42 \times 10^{-4}$  in sample 2, the growth of the bacteria varies significantly with samples for both the nutrient and Mac-Conkey agar at  $P = .05$  except for  $10^{-3}$  of nutrient agar. For site 3 (Mayne Avenue auto-workshop) cultured with nutrient agar, the bacteria growth increased from its initial of 10 to  $62 \times 10^{-3}$  in sample 1 and 40 to  $60 \times 10^{-4}$  in sample 2. In the case of Mac-Conkey agar, there was also an increase in bacteria growth from 20 to  $39 \times 10^{-3}$  in sample 1 and from 21 to  $55 \times 10^{-4}$  in sample 2, the growth of the bacteria varies significantly with samples for both the nutrient and Mac-Conkey agar at  $P = .05$  except for  $10^{-3}$  of nutrient agar. For site 4 (New Airport auto-workshop) cultured with nutrient agar, the

bacteria growth increased from its initial 39 to  $48 \times 10^{-3}$  in sample 1 and also from 26 to  $44 \times 10^{-4}$  in sample 2. In the case of Mac-Conkey agar, there was also a bacteria growth from 20 to  $49 \times 10^{-3}$  in sample 1 and from 21 to  $41 \times 10^{-4}$  in sample 3, the growth of the bacteria varies significantly with samples for both the nutrient and Mac-Conkey agar at  $P = .05$ . For site 5 (Mbukpa auto-workshop) (control) cultured with nutrient agar, the bacteria growth was very slow, increasing slightly from its initial 8 to  $10 \times 10^{-3}$  and also from 7 to  $8 \times 10^{-4}$ . In the case of Mac-Conkey agar, there was also a very slow growth of bacteria from 6 to  $9 \times 10^{-3}$  and 1 to  $4 \times 10^{-4}$  in sample 2, the growth of the bacteria varies significantly with samples for both the nutrient and Mac-Conkey agar at  $P = .05$  except for  $10^{-4}$  of Mac-Conkey agar. In general, site 1 recorded the highest bacteria count of 927, followed by site 3 with 459 and then site 4 having 429 while the least bacteria counts was recorded in the control station, having just 81 bacteria count (Table 2).

### 3.1.2 Total hydrocarbon and total organic carbon degradation

The summary of the degradation effects of bacteria on the total hydrocarbon and total organic carbon of contaminated sites is shown in Table 3. In site 1, the total hydrocarbon (THC) reduced from 1428 to 698 in mg/kg in sample 1 to 3. Total organic carbon also reduced from 1.2 to 0.2 % in sample 1 to 3, the THC reduced significantly with samples at  $P = .05$ , while TOC reduced insignificantly with samples at  $P = .05$ .

For site 2, the THC was degraded by the bacteria and reduced from 1327 to 928 in mg/kg in sample 1 to 3. TOC also reduced from 1.04 to 0.92 % in sample 1 to 3, the THC decreased significantly with samples at  $P= .05$ , while TOC decreased insignificantly with samples at  $P>0.05$ . For site 3, the THC was degraded by the bacteria and reduced from 1128 to 722 in mg/kg in sample 1 to 3. TOC also reduced from 1.09 to

0.41% in sample 1 to 3, the THC decreased significantly with samples at  $P= .05$ , while TOC decreased insignificantly with samples at  $P= .05$ . In site 4, the THC reduced from 1246 to 902 in mg/kg in sample 1 to 3. TOC also reduced from 0.99 to 0.51% in sample 1 to 3, the THC decreased significantly with samples at  $P= .05$ , while TOC decreased insignificantly with samples at  $P> 0.05$ .

**Table 2. The bacteria growth on crude oil contaminated sites using Nutrient and Mac-Conkey Agar**

Stations	Soil samples	Nutrient /Mac-Conkey				Total bacteria count
		$10^{-3}$	$10^{-4}$	$10^{-3}$	$10^{-4}$	
Station 1	Sample 1	83 <sup>a</sup>	49 <sup>a</sup>	92 <sup>a</sup>	10 <sup>a</sup>	927
	Sample 2	92 <sup>b</sup>	58 <sup>b</sup>	112 <sup>b</sup>	38 <sup>b</sup>	
	Sample 3	150 <sup>c</sup>	71 <sup>c</sup>	122 <sup>c</sup>	50 <sup>c</sup>	
Station 2	Sample 1	12 <sup>a</sup>	29 <sup>a</sup>	15 <sup>a</sup>	14 <sup>a</sup>	387
	Sample 2	16 <sup>a</sup>	58 <sup>b</sup>	28 <sup>b</sup>	27 <sup>b</sup>	
	Sample 3	22 <sup>a</sup>	80 <sup>c</sup>	44 <sup>c</sup>	42 <sup>c</sup>	
Station 3	Sample 1	10 <sup>a</sup>	40 <sup>a</sup>	20 <sup>a</sup>	21 <sup>a</sup>	459
	Sample 2	40 <sup>a</sup>	49 <sup>b</sup>	32 <sup>b</sup>	31 <sup>b</sup>	
	Sample 3	62 <sup>a</sup>	60 <sup>c</sup>	39 <sup>c</sup>	55 <sup>c</sup>	
Station 4	Sample 1	39 <sup>a</sup>	26 <sup>a</sup>	20 <sup>a</sup>	21 <sup>a</sup>	429
	Sample 2	43 <sup>b</sup>	32 <sup>b</sup>	39 <sup>b</sup>	33 <sup>b</sup>	
	Sample 3	48 <sup>c</sup>	44 <sup>c</sup>	49 <sup>c</sup>	41 <sup>c</sup>	
Station 5 (control)	Sample 1	8 <sup>a</sup>	7 <sup>a</sup>	6 <sup>a</sup>	1 <sup>a</sup>	81
	Sample 2	10 <sup>b</sup>	7 <sup>b</sup>	8 <sup>b</sup>	3 <sup>a</sup>	
	Sample 3	10 <sup>c</sup>	8 <sup>c</sup>	9 <sup>c</sup>	4 <sup>a</sup>	

Values with different superscript across the samples are significantly different at  $P<0.05$

Where: Sample 1 (Etta Agbo auto-workshop), Sample 2 (Marian auto-workshop), Sample 3 (Mayne Avenue auto-workshop), Sample 4 (New airport auto-workshop) and Sample 5 (Mbukpa auto-workshop) (control)

**Table 3. The degradation effect of bacteria on the total hydrocarbon and total organic carbon**

Stations	Soil samples	Total Hydrocarbon (THC) (mg/kg)	Total organic carbon (TOC) (%)
Station 1	Sample 1	1428 <sup>a</sup>	1.2 <sup>a</sup>
	Sample 2	1220 <sup>b</sup>	0.6 <sup>b</sup>
	Sample 3	698 <sup>c</sup>	0.2 <sup>c</sup>
Station 2	Sample 1	1327 <sup>a</sup>	1.04 <sup>a</sup>
	Sample 2	1022 <sup>b</sup>	1.01 <sup>b</sup>
	Sample 3	928 <sup>c</sup>	0.92 <sup>c</sup>
Station 3	Sample 1	1128 <sup>a</sup>	1.09 <sup>a</sup>
	Sample 2	1011 <sup>b</sup>	0.72 <sup>b</sup>
	Sample 3	722 <sup>c</sup>	0.41 <sup>c</sup>
Station 4	Sample 1	1246 <sup>a</sup>	0.99 <sup>a</sup>
	Sample 2	1111 <sup>b</sup>	0.72 <sup>b</sup>
	Sample 3	902 <sup>c</sup>	0.51 <sup>c</sup>
Station 5 (control)	Sample 1	25 <sup>a</sup>	0.19 <sup>a</sup>
	Sample 2	23 <sup>b</sup>	0.19 <sup>b</sup>
	Sample 3	23 <sup>c</sup>	0.18 <sup>c</sup>

Values with different superscript across the samples are significantly different at  $P<0.05$

Where: Site 1 (Etta Agbo auto-workshop), Site 2 (Marian auto-workshop), Site 3 (Mayne Avenue auto-workshop), Site 4 (New airport auto-workshop) and Site 5 (Mbukpa auto-workshop) (control)

**Table 4. Effect of contaminated soil samples with petroleum product on some physico-chemical characteristic of soil samples from the engine oil contaminated site S<sub>1</sub> – S<sub>2</sub> and uncontaminated (control) site S<sub>5</sub>**

Soil samples	Total moisture	pH	THC (mg/kg)	Total organic carbon (%)	Total nitrogen (%)	A.P (mg/kg)	Clay	Silt	Sand	Kermol	Texture
S <sub>1</sub>	15.36	5.7	1428.6	1.20	0.06	27.86	8.0	12.7	81.3	0.36	SL
S <sub>2</sub>	16.11	6.4	1327.4	0.19	0.99	23.00	6.0	13.1	79.3	0.28	LS
S <sub>3</sub>	16.27	6.1	1128.6	1.09	0.08	22.14	5.59	12.4	86.1	0.24	LS
S <sub>4</sub>	13.41	5.3	1246.3	1.04	0.09	16.91	6.12	12.7	68.6	0.22	SL
S <sub>5</sub>	14.04	5.9	1011.1	0.99	0.11	31.37	4.0	16.7	79.3	0.16	SL

Where: Site one (S1) (Etta-Agbor workshop), Site two (S2) (Marian workshop), Site three (S3) (Main Avenue workshop), Site four (S4) (New Airport workshop), Site five (S5) (Uncontaminated soil), LS (Loamy sand), SL (Sandy loam). Each value is physico-chemical parameters of triplicate soil sample collected from each site

For site 5, the THC was degraded, slightly reduced from 25 to 23 in mg/kg in sample 1 to 3. TOC was also degraded slightly reducing from 0.19 to 0.18 % in sample 1 to 3, the THC and TOC decreased significantly with samples at  $P = .05$ .

### 3.2 Physico-chemical Parameters of Soil Sample from Sampling Stations

Physico-chemical parameters of soil sample in Calabar Metropolis is presented in Table 4. The result show that total moisture content ranged from 13.41 to 16.27%. Site four (4) had the least total moisture consequence upon high content of crude oil content in the soil. The pH value 5.3 to 6.4. The occurrence of THC in site five (5) shows an increase with samples ranged from 1011.1 to 1428.6mg/kg, also site five (control) make show an observable least value (1011.1 mg/kg). The organic carbon (O.C) content of the sample ranged from 0.19 to 1.2%. However, sample 2 had the least value of organic carbon content (0.19%) in the soil was below the minimum level of 2.0%. Total nitrogen value of the samples ranged from 0.06 to 0.12%. Site one had the least value of total nitrogen (0.06%). Available phosphorous level in sampling sites ranged from 16.91 to 31.0 mg/kg. Site four had the least value of available phosphorus (16.91 mg/kg). The particle size analysis of the soil showed that sand content ranged from 79.3 to 86.1%. The silt content ranged from 12.7 to 16.7% while clay content ranged from 4.0 to 8.0%. These confirm that the textural class of the entire sample is sandy loam. The kermol value ranged from 0.16 to 0.36. Site five (control) had the least kermol value (0.16).

## 4. DISCUSSION

Bioremediation of petroleum pollutants is a slow process, often requiring many months to degrade the majority of the oil, it is relatively inexpensive and seemingly harmless to the surrounding environment and the process generally involves bio-stimulation and bio-augmentation [5]. Automobile workshop is noted for high indiscriminate dumping of waste engine oil and other refined petroleum products as a result of their activities ranging from servicing, maintenance to repair of automobiles. The study revealed a total of 13 bacteria species that are capable of degrading hydrocarbons, which is higher than the findings of [17,18]. Amongst the indigenous bacteria with bioremediation properties identified in the crude oil contaminated in this study were *Staphylococcus* spp.,

*Pseudomonas aeruginosa*, *Bacillus* spp., *Acinobacter* spp. which is similar to the findings of [17] who also reported *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., and *Acinobacter* spp. amongst their bacteria degrading checklist; as well as [19,20] who also reported *Pseudomonas aeruginosa* and *Bacillus* spp. The study also revealed that *Staphylococcus* spp. was the most abundant bacteria species (highest growth rate), denoting an effective utilization of hydrocarbon, thereby multiplying rapidly and degrading hydrocarbons more effectively in the process and as such said to be best biodegradation bacteria. The bacteria isolates obtained in this study belong to both the gram positive and gram negative groups, although the gram negative bacteria dominated the gram positive samples and similar results were obtained by [19,21] who also reported both gram positive and gram negative bacteria. Also, the dominance of gram negative bacteria agrees with the findings of [22] who reported that gram positive bacteria if detected in bioremediation are never diverse and dominant. The progressive rapid increase (growth) in the number of the identified indigenous bacteria in the contaminated sites in this study is an indication of the fact that the contaminated sites supported the growth of such bacteria, although with the help of the nutrient and Mac-Conkey agar, which was eventually very slow in the uncontaminated site (control site). The bacteria counts for both the nutrient and Mac-Conkey agar was similar to that reported by [23,19,24] but lower than that reported by [25]. This variation could be due to the differences in microbial ecology of the soil or characteristics of the experimental soil. Physico-chemical properties of the soil in the study revealed low amount of nitrogen, phosphorus and organic matter. The results obtained in this study showed different degree of hydrocarbon utilization in the treatment option by bacterial isolates with the spent engine oil serving as the sole source of carbon and energy. pH was acidic, this could be as a result of acidic metabolites in the medium corresponding with finding of [26]. It was observed that the higher the drop in pH the greater the degradation thus suggesting the production of more acidic metabolites. It was also observed that pH varied with different sampling stations, this could also be attributed to differences in levels of hydrocarbon concentration in each sampling stations. The study also revealed that *Pseudomonas* spp., *Bacillus* spp., *Acinetobacter* spp. etc are bacteria species that are capable of degrading crude oil, which corroborated with the findings of [6] who

also reported the bioremediation ability of these bacteria species. This was evident in the rapid decrease in the total hydrocarbon and total organic carbon over time, which was not observed in the uncontaminated site. This could be due to the fact that there was a rapid growth of the bacteria in the contaminated site which was lacking in the control site, which eventually allowed for a more effective degradation of the crude oil over time. The slow degradation process in the uncontaminated site is similar to the report of [27] who explained that lack, scarcity or low population of hydrocarbon dredging micro-organisms is a limiting factor biodegradation. This implies that the success of bioremediation of hydrocarbon polluted environments has a direct relationship with the biodegrading capabilities of native microbial populations or exogenous micro-organisms used as inoculants, which is in agreement with the report of [18]. The study revealed different degree of hydrocarbon utilization in the treatment by bacteria isolates, with engine oil serving as the sole source of carbon and energy. In a similar study by [25], *Chromobacterium violaceum*, *Bacillus subtilis* and *Micrococcus luteus* exhibit a high degradation potential for different fractions of hydrocarbons; with *C. violaceum* is the best degrader followed by *Bacillus subtilis* and *Micrococcus luteus* [28].

## 5. CONCLUSION

Engine oil polluted environments such as Automobile Workshops can be cleaned up effectively and efficiently using indigenous hydrocarbon utilizing microorganisms. The study revealed the bioremediation and hydrocarbon utilizing potentials of micro-organism, as shown by their degradation of total hydrocarbons and total organic carbon. The growth of the bacteria was rapid in the contaminated sites as a result of the presence of high utilizable carbons, which supports their growth. The result also revealed that the culturing of these isolated bacteria has the ability to degrade engine oil faster than the individual pure cultures, moreover, giving a more effective and efficient way of remediating engine oil contaminated sites. The results indicate that by providing a conducive environment, some bacteria such as *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., *E. coli*, *Enterococcus faecalis*, *Shigella* spp., *Arthrobacter* spp., *Alcaligen* spp., *Acinobacter* spp., *Azotobacter* spp., *Aeromonas* spp., *Xanthomonas* spp. and *Clostridium* spp. can be used to remediate engine oil contaminated environments such as an automobile workshop

effectively. Also, *Staphylococcus* spp. was the most abundant indigenous bacteria species and also the most effective biodegradation bacteria.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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