Original Research Article

Microbial remediation of Used Engine Oil from Contaminated Soil around Automobile Workshop in Calabar Metropolis, Cross River State, Nigeria.

ABSTRACT

The microbial biodegradation of engine oil contaminated soil in Calabar Metropolis was study for the period of six (6) month (January to June, 2017). The soil samples collected were ice boxed and taken to the laboratory for microbial, total hydrocarbon and total organic carbon analysis. A total of 13 indigenous bacteria species were identified in the soil of the sites analyzed during the study, which includes; *Staphylococcus sp*., *Pseudomonas aeruginosa*, *Bacillus sp*., *E. coli*, *Enterococcus feacalis*, *Shigella sp*., *Arthrobacter sp*., *Alcaligen sp*., *Acinobacter sp*., *Azotobacter sp*., *Aeromonas sp*., *Xanthomonas sp*. and *Clostridium sp*. The most abundant bacteria in the contaminated site was *Staphylococcus sp*. (65%) while the least bacteria count in the contaminated site was *Clostridium sp*. (9%). *Staphylococcus sp*. was the most abundant indigenous bacteria 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. Station one recorded the highest bacteria count (927) while the least bacteria counts was recorded in the control sample (81). The bacteria species showed its degradation and bioremediation capabilities prompting the need for its use for cleaning crude oil contaminated sites, due to the fact that it is cheap and not environmentally harmful.

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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 heavy 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

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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 sp*, *Rhodococcus sp*., *Mycobacterium sp*., *Bacillus sp*., *Acinetobacter sp*., *Providecin sp*., *Flavobacter sp*., *Carynebacterium sp*., *Streptococcus sp*. [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^{o} 20^{1} N and longitude 5^{o} 20^{1} 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. The study area is 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.

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2.1.1 Sampling stations

A total of Five (5) sampling stations were used for the study, with four (4) being crude oil contaminated sites and the last one being the 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^{0} 20' 32.0" E and latitude 04^{0} 57' 31.3" N along Etta Agbor Road, while the second station was located along Marian Road which is geographically between 08^{0} 20' 15.3"E and latitude 04^{0} 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^{0} 19' 09.2" E and latitude 04^{0} 56' 36.9" N, while sampling station four (4) was located geographically between longitude 08^{0} 19' 01.6" E and latitude 04^{0} 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^{0} 19.5' 01.9" E and latitude 04^{o} 58' 28.8" N.

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2.1.1.1 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 microbial isolation of bacterial species and some sample taken to Chemistry Department, University of Calabar for some physico-chemical parameters such as total moisture content, pH, total nitrogen, available phosphorous, total hydrocarbon (THC), total organic carbon (TOC) and particle size analysis.

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2.2 Total bacteria count and isolation

In the laboratory, the soil samples (5g) were suspended in 9ml sterile water. Ten-fold serial dilution, in the range of 10^{-1} - 10^{4} were prepared using sterile water. Aliquots 1ml of samples dilution of 10^{-3} - 10^{-4} were plated on the Nutrient Agar (NA) and Mac-Conkey agar (MAC). The numbers of visible colonies were

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multiplied by the reciprocal of the dilution factor and recorded colony forming units (CFU) per gram of soil [8]. Discrete colonies were sub-cultured unto Nutrient Agar and Mac-Conkey agars, until pure culture were obtained through identification. The isolated organisms were identified by staining and biochemical analysis according to [9].

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2.2.1 Isolation and characterization of hydrocarbon degrading microorganisms

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].

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2.2.2.1 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 250ml 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 rmp at 30^{o}C. The optical density ($OD_{600\ nm}$) 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

The test is done to differentiate between gram positive and gram negative organisms. Each experimental organism was inoculated into its appropriate labelled tube by means of a streak inoculation. It was incubated for 24 – 48 hours at 37^{o}C. 3 drops of the 3% hydrogen peroxide was allowed to flow over the entire surface of each slant culture. The culture were examined for the presence or absence of bubbling or foaming.

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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 (3g) of peptone stuck sample was weighed and dissolved into 200ml of distilled water and sterilized in an autoclave at 121^{o}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^{o}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 a peptone culture 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.

2.3.3.2 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^{o}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^{o}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.

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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^{o}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

Comment [u22]: Why not maintain same pattern? You previously used sentence case but in this case adopted "capitalize each word".

Comment [u23]: Check tense

The soil sample were 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 Walkley and Black wet oxidation method. This was done by igniting the dried sieved soil samples (2.5g) in a pre-weighed crucible and calculation 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 430nm 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 30minute equilibration. Triplicate determinations were made. Moisture contents was determine using the method of [14]. Available phosphorus (AP) in soil samples was determined using the spectrophotometer, and total nitrogen content was determined using Kjedahl method. The temperature was measured using a digital thermometer. Particle size distributions were determined based on the unified soil classification as described by [15].

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2.4.4.1 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 AND DISCUSSION

3.1 Bacteria composition in contaminated soil

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

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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	+	+	+	-	-	-	+	-	*Pseudomonas aeruginosa*
2 MAC	Gram (-) short rod in chains and in pairs	+	-	+	+	-	+	+	-	*E. coli*
3 MAC	Gram (+) variable shape example of bacteria, Actinobacter	+	+	-	-	-	+	+	+	*Arthrobacter sp.*
4 MAC	Gram (+) cylindrical rod with spore, in pair and in short chain	+	-	+	-	-	-	+	-	*Bacillus sp.*
5 MAC	Gram (+)	-	-	-	-	+	-	+	-	*Enterococcus*

	cocci/diplococcic									*feacalis*
6 MAC	Gram (-) tiny rod in chains, cluster and singly	+	-	+	-	-	-	-	-	*Shigella sp.*
7 MAC	Gram (-) in small cluster form and pale colonies	+	-	-	-	-	+	+/-	+	Acinobacter
8 MAC	Gram (-) short rod in clusters	+	+	+	-	-	-	+	-	*Pseudomonas aeruginosa*
9 MAC	Gram (-) cocal and diplococcic	+	+	-	-	-	+	+	+	*Alcaligen sp.*
10 MAC	Gram (-) rod shape	+	+	+	+	-	-	+	+	*Aeromonas sp.*
11 MAC	Gram (+) cocci, diplococci/rods	+	-	+	+	-	+	+	-	*Staphylococcus sp.*
12 MAC	Gram (-) rod cyst forming	+	-	+	+	+	+	+	+	*Azotobacter sp.*

	brown to black colonies									
13 NA	Gram (+) cylindrical rod with spore, in pair and in short chain	+	-	+	-	-	-	+	-	*Bacillus sp.*
14 NA	Gram (+) cocci, diplococci/rods	+	-	+	+	-	+	+	-	*Staphylococcus sp.*
15 NA	Gram (-) in straight rod single flagellum mucoid convex and yellow colonies	+	+	-	-	-	+	+	+	*Xanthomonas sp.*
16 NA	Gram (-) short rod in chains and in pairs	+	-	+	+	-	+	+	-	*E. coli*

17 NA	Gram (-) tiny rod in chains cluster and singly	+	-	+	-	-	-	-	-	*Shigella sp.*
18 NA	Gram (+) cocci/diplococci	-	-	-	-	+	-	+	-	*Enterococcus feacalis*
19 NA	Gram (+) endorspore forming singly rod	+	+	-	+	+	-	-	-	*Chrostridium sp.*
20 NA	Gram (+) cocci, diplococci/rods	+	-	+	-	-	+	+	-	*Staphylococcus sp.*

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

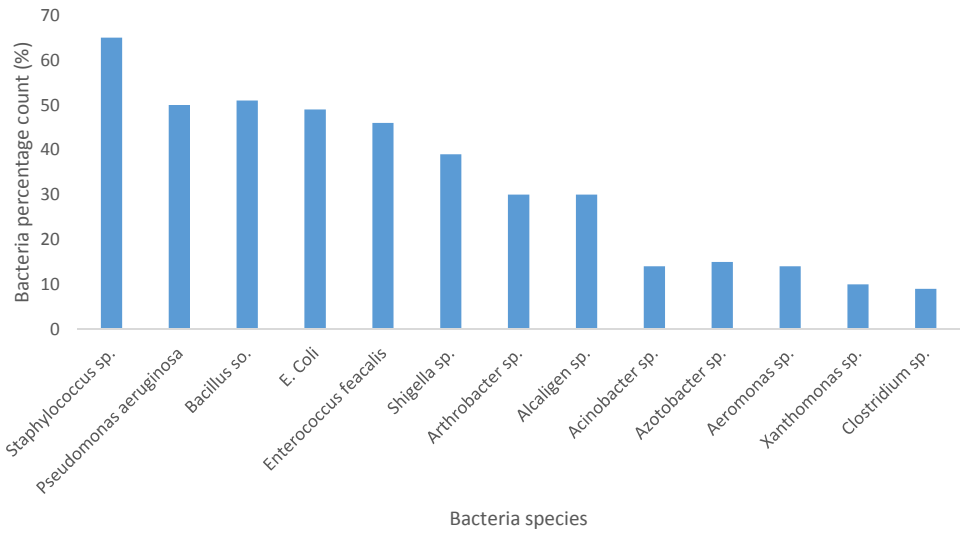

Figure 1: The abundance of the identified indigenous bacteria with biodegrading ability in the contaminated site

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 station 1 (Etta-Agbo auto-workshop) cultured with nutrient agar, the bacteria growth increased rapidly from its initial 83 in sample 1 to 150 x 10^{-3} and also from 49 to 71 x 10^{-4} in sample 2. In the case of Mac-Conkey agar, there was also a rapid bacteria growth from 92 to 122 x 10^{-3} in sample 1 and 10 to 50 x 10^{-4} in sample 2. Statistically, the growth of the bacteria varied significantly with samples for both the nutrient and Mac-Conkey agar at P= .05. For station 2 (Marian auto-workshop) cultured with nutrient agar, the bacteria growth increased from its initial 12 to 22 x 10^{-3} in sample 1 and also from 29 to 80 x 10^{-4} in sample 2. In the case of Mac-Conkey agar, there was also an increase in bacteria growth from 15 to 44 x 10^{-3} in sample 1 and 14 to 42 x 10^{-4} in sample 2.Statistically, the growth of the bacteria varied significantly with samples for both the nutrient and Mac-Conkey agar at P= .05 except for 10^{-3} of nutrient agar. For station 3 (Mayne Avenue auto-workshop) cultured with nutrient agar, the bacteria growth increased from its initial of 10 to 62 x 10^{-3} in sample 1 and 40 to 60 x 10^{-4} in sample 2. In the case of Mac-Conkey agar, there was also an increase in bacteria growth from 20 to 39 x 10^{-3} in sample 1 and from 21 to 55 x 10^{-4}in sample 2. Statistically, the growth of the bacteria varied significantly with samples for both the nutrient and Mac-Conkey agar at P= .05 except for 10^{-3} of nutrient agar. For station 4 (New Airport auto-workshop) cultured with nutrient agar, the bacteria growth increased from its initial 39 to 48 x 10^{-3} in sample 1 and also from 26 to 44 x 10^{-4} in sample 2. In the case of Mac-Conkey agar, there was also a bacteria growth from 20 to 49 x 10^{-3} in sample 1 and from 21 to 41 x 10^{-4} in sample 3.

Statistically, the growth of the bacteria varied significantly with samples for both the nutrient and Mac-Conkey agar at P= .05. For sample 5 (Mbukpa auto-workshop) (control) cultured with nutrient agar, the bacteria growth was very slow, increasing slightly from its initial 8 to 10 x 10^{-3} and also from 7 to 8 x 10^{-4}. In the case of Mac-Conkey agar, there was also a very slow growth of bacteria from 6 to 9 x 10^{-3} and 1 to 4 x 10^{-4}. Statistically, the growth of the bacteria varied significantly with samples for both the nutrient and

Mac-Conkey agar at *P*= .05 except for 10^{-4} of Mac-Conkey agar. In general, sample 1 recorded the highest bacteria count of 927, followed by sample 3 with 459 and then sample 4 having 429 while the least bacteria counts was recorded in the control sample, having just 81 bacteria count. (Table 2).

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

		Nutrient /Mac-Conkey				
Stations	Soil samples	10^{-3}	10^{-4}	10^{-3}	10^{-4}	Total bacteria count
Station 1	Sample 1	83[a]	49[a]	92[a]	10[a]	927
	Sample 2	92[b]	58[b]	112[b]	38[b]	
	Sample 3	150[c]	71[c]	122[c]	50[c]	
Station 2	Sample 1	12[a]	29[a]	15[a]	14[a]	387
	Sample 2	16[a]	58[b]	28[b]	27[b]	
	Sample 3	22[a]	80[c]	44[c]	42[c]	
Station 3	Sample 1	10[a]	40[a]	20[a]	21[a]	459
	Sample 2	40[a]	49[b]	32[b]	31[b]	
	Sample 3	62[a]	60[c]	39[c]	55[c]	
Station 4	Sample 1	39[a]	26[a]	20[a]	21[a]	429
	Sample 2	43[b]	32[b]	39[b]	33[b]	

	Sample 3	48[c]	44[c]	49[c]	41[c]	
	Sample 1	8[a]	7[a]	6[a]	1[a]	
Station 5 (control)	Sample 2	10[b]	7[b]	8[b]	3[a]	81
	Sample 3	10[c]	8[c]	9[c]	4[a]	

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)

3.1.1.1 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 sample 1, the total hydrocarbon (THC) reduced from 1428 to 698 in mg/kg. Total organic carbon also reduced from 1.2 to 0.2 %. Statistically, the THC reduced significantly with samples at *P*= .05, while TOC reduced insignificantly with samples at *P*= .05. For sample 2, the THC was degraded by the bacteria and reduced from 1327 to 928 in mg/kg. TOC also reduced from 1.04 to 0.92 %. Statistically, the THC reduced significantly with samples at *P*= .05, while TOC reduced insignificantly with samples at *P*>0.05. For sample 3, the THC was degraded by the bacteria and reduced from 1128 to 722 in mg/kg. TOC also reduced from 1.09 to 0.41%. Statistically, the THC reduced significantly with samples at *P*= .05, while TOC reduced insignificantly with samples at *P*= .05. In sample 4, the THC reduced from 1246 to 902 in mg/kg. TOC also reduced from 0.99 to 0.51%. Statistically, the THC reduced significantly with samples at *P*= .05, while TOC reduced insignificantly with samples at *P*= .05.

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) (%)
	Sample 1	1428[a]	1.2[a]
Station 1	Sample 2	1220[b]	0.6[b]
	Sample 3	698[c]	0.2[c]

	Sample 1	1327[a]	1.04[a]
Station 2	Sample 2	1022[b]	1.01[b]
	Sample 3	928[c]	0.92[c]
	Sample 1	1128[a]	1.09[a]
Station 3	Sample 2	1011[b]	0.72[b]
	Sample 3	722[c]	0.41[c]
	Sample 1	1246[a]	0.99[a]
Station 4	Sample 2	1111[b]	0.72[b]
	Sample 3	902[c]	0.51[c]
	Sample 1	25[a]	0.19[a]
Station 5 (control)	Sample 2	23[b]	0.19[b]
	Sample 3	23[c]	0.18[c]

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)

For sample 5, the THC was degraded, reduced slightly from 25 to 23 in mg/kg. TOC was also degraded slightly reducing from 0.19 to 0.18 %. Statistically, the THC and TOC reduced 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%. Sample 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 sample five (5) shows an increase with samples ranged from 1011.1 to 1428.6mg/kg. Sample five (control) make show an observable least value (1011.1mg/kg). The organic carbon (O.C) content of the sample ranged from 0.19 to 1.2%.

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_1 – S_2 and uncontaminated (control) site S_5

Soil samples	Total moisture	pH	THC (mg/kg)	Total organic carbon (%)	Total nitrogen (%)	A.P (mg/kg)	Clay	Silt	Sand	Kermol	Texture
S_1	15.36	5.7	1428.6	1.20	0.06	27.86	8.0	12.7	81.3	0.36	SL
S_2	16.11	6.4	1327.4	0.19	0.99	23.00	6.0	13.1	79.3	0.28	LS
S_3	16.27	6.1	1128.6	1.09	0.08	22.14	5.59	12.4	86.1	0.24	LS
S_4	13.41	5.3	1246.3	1.04	0.09	16.91	6.12	12.7	68.6	0.22	SL
S_5	14.04	5.9	1011.1	0.99	0.11	31.37	4.0	16.7	79.3	0.16	SL

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

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%. Samples one had the least value of total nitrogen (0.06%). Available phosphorous level in sampling stations ranged from 16.91 to 31.0mg/kg. Sample four had the least value of available phosphorus (16.91mg/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 textual class of the entire sample is sandy loam. The kermol value ranged from 0.16 to 0.36. Sample five (control) had the least kermol value (0.16).

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 [16,17]. Amongst the indigenous bacteria with bioremediation properties identified in the crude oil contaminated in this study were *Staphylococcus sp*., *Pseudomonas aeruginosa*, *Bacillus sp*., *Acinobacter sp*. which is similar to the findings of [16] who also reported *Staphylococcus sp*., *Pseudomonas aeruginosa*, *Bacillus sp*. and *Acinobacter sp*. amongst their bacteria degrading checklist; as well as [18,17] who also reported *Pseudomonas aeruginosa* and *Bacillus sp*. The study also revealed that *Staphylococcus sp*. 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 [19,20] who also reported both gram positive and gram negative bacteria. Also, the dominance of gram negative bacteria agrees with the findings of [21] 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 [22,19] but lower than that reported by [23]. 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 [24]. 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 sp*., *Bacillus sp*., *Acinetobacter sp*. 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 [25] 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 [17]. 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.

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 were 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 sp*., *Pseudomonas aeruginosa*, *Bacillus sp*., *E. coli*, *Enterococcus feacalis*, *Shigella sp*., *Arthrobacter sp*., *Alcaligen sp*., *Acinobacter sp*., *Azotobacter sp*., *Aeromonas sp*., *Xanthomonas sp*. and *Clostridium sp*. can be used to remediate engine oil contaminated environments such as a automobile workshop effectively. Also, *Staphylococcus sp*. was the most abundant indigenous bacteria species and also the most effective biodegradation bacteria.

Comment [u30]: You are talking about the growth here and should be "was".

Comment [u31]: Is this necessary since you already mentioned it in your previous statement yet under conclusion?

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