

## Original Research Article

### The Bio-utilization of Used and Unused car Lubricants by Autochthonous Microorganisms

#### ABSTRACT

The determination of the bio-utilization of used and unused car lubricants; engine oil, hydraulic and transition oil were analyzed using viable counts and spectrophotometric analysis. The oil samples were collected with 10 sterile containers from different mechanic workshops in Calabar; Unical workshop, designated as UNICAL Workshop (A), Akim(B), Mount Zion (C), Etta Agbor(D), and EkpoAbasi (E). The spectrophotometric analysis revealed absorbance ranging from 2.16 to 2.69nm for engine oil, 0.57 to 1.21nm for hydraulic oil and 1.96 to 2.40nm for transition oil. The total heterotrophic counts of the used engine oil ranged from  $1.2 \times 10^{10}$  CFU/ml to  $4.0 \times 10^{10}$  CFU/ml; hydraulic oil ranged from  $1.0 \times 10^{10}$  CFU/ml to  $2.2 \times 10^{10}$  CFU/ml; Transition oil ranged from  $1.0 \times 10^{10}$  CFU/ml to  $2.5 \times 10^{10}$  CFU/ml. The THB counts of the unused oils had an average count of  $1.0 \times 10^{10}$  CFU/ml. The enumeration of the total fungal showed lower counts ranging from  $1.0 \times 10^7$  CFU/ml to  $2.2 \times 10^{10}$  CFU/ml for engine oil;  $1.0 \times 10^7$  CFU/ml to  $1.5 \times 10^{10}$  CFU/ml for hydraulic oil and  $1.0 \times 10^7$  CFU/ml to  $1.4 \times 10^{10}$  CFU/ml for transition oil. The unused oil had very much lower fungal counts. The identification processes revealed the presence of *Pseudomonas* spp(6.5%), *Bacillus* spp(13%), *Streptobacill*(21.7%), *Micrococcus*spp(21.7%), *Actinomyces*spp(8.6%), *Norcardiaspp*(4.3%), *Staphylococcus*spp(10.8%), *Listeria*spp(8.6%), *Serratiaspp* (2.1%) and *Lactobacillus*(2.1%). While the fungal isolates were identified as *Aspergillus niger*(75%), *Aspergillus flavus*(13.8%), *Aspergillus lentulus*(5.52%), *Candida*spp(2.7%) and *Curvularia*spp(2.7%). This result suggests that these isolated organisms from used and unused car lubricants can use oil lubricants as a source of carbon and energy, and could be explored for environmental biodegradation of hydrocarbons and bioremediation of polluted sites by these oils. Due to the medical importance of the identified organisms, care should be taken while working with the organisms, to avoid opportunistic infections.

**Key words:** Lubricants, bio-utilization, autochthonous microorganisms, absorbance, viable counts.

#### INTRODUCTION

A lubricant is a substance which when introduced aids the reduction of friction between surfaces in mutual contact, which ultimately reduces the heat generated when the surfaces move. **Recently, spills of used and unused oils and petroleum products into the surrounding are major contaminants of the ecosystem** the surfaces. Lubricating oils are manufactured in various formulations for different applications. Most formulas generally consist of two fractions; chemicals additives and base fluids. The chemical additives, about 5-20% (W/V), are selected compounds added for specific functions. Base fluid, the main fraction in lubricating oil is a complex mixture of hydrocarbons: Linear and branched paraffins, cyclic alkanes and aromatic hydrocarbons[7].

In today's world, oil spills from both used and unused at auto-mechanic workshops have been left uncared for over the years in many countries, and continuous accumulation of the oil is of high environmental concern as a result of hazard associated with it [2]. The attention of researchers has shifted towards the remediation of the environment (soil and water) polluted with hydrocarbons especially the polycyclic aromatic hydrocarbons (PAHs) due to the fact that most of the PAHs causes cancer, gene mutation and are very toxic. The release of persistent, bio-accumulative and toxic chemicals (benzene,

Comment [LH1]: 2.69 nm

Comment [LH2]: 1.21 nm

Comment [LH3]: 2.40 nm

Comment [LH4]: spp.

Comment [LH5]: spp.

Comment [LH6]: spp.

Comment [LH7]: *Nocardia* spp.

Comment [LH8]: spp.

Comment [LH9]: spp.

Comment [LH10]: spp.

Comment [LH11]: spp.

Comment [LH12]: spp.

Comment [LH13]: (W/V),

47 toluene, ethylbenzene, xylene and polycyclic aromatic hydrocarbons) causes health and environmental  
48 hazards [20].The disposal of used and unused lubricating oil into gutters, water drains and farms are  
49 common practice in Nigeria, especially by motor mechanics. These discharges contribute to soil pollution.  
50 Excess spillage of the oil can cause fire hazard which can lead to loss of lives and properties [1].

51  
52 The most common application of lubricating oil is being used as motor oil in engines, where it provides a  
53 safe environment for the internal combustion engines by reducing friction, carrying away contaminants,  
54 protection against wear and corrosion [3].Recently, spills of used and unused oils and petroleum products  
55 into the surrounding are major contaminants of the ecosystem. The illegal dumping of these oils is an  
56 environmental hazard with global ramifications [23]. Used oil, also called spent oil are abundant in mechanic  
57 workshops, garages and industry outlets. The spent oil may occur as hydraulic oil, turbine oil and metal  
58 working fluids [16].With current efforts by the scientific communities in reducing environmental pollution  
59 via the decay of pollutants or attack by enzymes, bioremediation could be achieved. It has been noted  
60 that bacteria, protista and fungi could degrade complex molecules and incorporate the product into their  
61 biomass.Since lubricants are indispensable in vehicles, industries and the usage come with potential  
62 harm to the environment, biodegradation which involves the use of microorganisms to breakdown  
63 complex compounds into harmless substances is a veritable solution approach as it aids bioremediation.  
64 This study was therefore designed to isolate, identify and determine the autochthonous microorganisms  
65 that can utilize hydrocarbons as their source of energy and carbon.  
66

## 67 MATERIALS AND METHODS

### 68 Sample collection

69 The used and unused samples of car lubricants; Engine oil, Hydraulic oil and Transition oil analyzed in this  
70 study were collected from different mechanic workshops; Unical workshop (A), Akim (B), Mount Zion (C),  
71 Etta Agbor (D) and Ekpo Abasi (E). They were collected using 10 sterile containers, 5 for used and 5 for  
72 unused for each of the 3 lubricants making a total of 30 samples and transferred to Microbiology  
73 laboratory of Cross River University of Technology, Calabar, for further analysis.

### 74 Spectrophotometric analysis

75  
76 The turbidity of the samples was determined using a spectrophotometer. A McFarland turbidity  
77 standard prepared from Sulphuric acid ( $H_2SO_4$ ), Anhydrous Barium Chloride ( $BaCl_2$ ) and distilled  $H_2O$  was  
78 used as a control turbidity standard.

### 79 Determination of absorbance of transport solution

80  
81 The spectrophotometer was turned on for 30 minutes for Warm-up. Then the Wavelength was selected  
82 (620nm) and the blank placed into the cuvette and used to adjust the instrument to 100%, 0% T before the  
83 scale was set to Absorbance scale. The sample was placed in the readout and the instrument set to  
84 determine the absorbance of standard sample. The concentration scale was set by Pressing  $\uparrow$  or  $\downarrow$  key  
85 to cause the displayed value equal to known concentration or 10 times of the known concentration. The  
86 unknown concentration was then determined by placing in the unknown sample and the values displayed  
87 on the read out recorded as the absorbance value of the sample.

### 88 Microbiological analysis

### 89 Enumeration of Total Heterotrophic Bacterial counts

Comment [LH14]: 30 minutes

Comment [LH15]: total

Comment [LH16]: heterotrophic

Comment [LH17]: bacterial

91 Total heterotrophic bacterial count of the sample was determined using the pour plate method. Serial  
 92 dilution was prepared from the liquid sample. Exactly one millimeter (1ml) was taken from each selected  
 93 dilution ( $10^3$ ,  $10^4$ ,  $10^5$ ) into sterile petri dishes. The molten sterilized Nutrient and MacConkey agar were  
 94 poured into the plates, swirled to spread the inoculum evenly within the agar medium and allowed to  
 95 solidify then incubated at  $37^{\circ}\text{C}$  for 24 hours. Thereafter, plates with colony growth were counted and  
 96 recorded.

Comment [LH18]:  $37^{\circ}\text{C}$

97  
 98 **Biochemical characteristics and identification of bacterial isolates obtained from used  
 99 and unused lubricants**

100 After sub-culturing, the bacterial isolates obtained were characterized and identified based on their  
 101 cultural, morphological, microscopic and biochemical features.

102  
 103 **Characterization and Identification of Fungi**

Comment [LH19]: Characterization and identification of fungi

104 The pure cultures of the isolate from SDA were identified on the basis of their colonial morphology, colony  
 105 growth pattern, and pigmentation using the slide culture technique and microscopic examination.

106  
 107 **RESULTS**

108 The spectrophotometric analysis of the lubricants revealed the turbidity of cells in all the oil samples,  
 109 indicating that there was microbial growth in all the oils, both used and unused. The absorbance value  
 110 observed in engine oil (used) were highest (2.69nm) among all the oils examined followed by those from  
 111 Transition oil (used), with the value of 2.40nm, as shown on Table 1.

Comment [LH20]: (2.69 nm)

112  
 113 **Table 1: Absorbance of the Cultures from Mineral Salt Medium**

Comment [LH21]: Absorbance of the cultures from mineral salt medium

Locations (Workshops)	Engine oil used (nm)	Engine oil unused (nm)	Hydraulic oil used (nm)	Hydraulic oil unused (nm)	Transition oil used (nm)	Transition oil unused (nm)
Mount Zion	2.59	2.10	0.78	0.33	2.20	2.00
Ekpo Abasi	2.69	2.19	1.21	0.55	2.40	2.12
Akim	2.25	2.06	0.88	0.52	2.23	0.91
Etta Agbor	2.60	2.22	1.01	0.61	2.20	0.75
Unical	2.16	0.63	0.57	0.26	1.96	0.42

115 **Blank-ve= 1.0 +tve=2.0**

116  
 117 **Total Heterotrophic Bacterial Count**

Comment [LH22]: Total heterotrophic bacterial count

118 The enumeration of the analyzed samples yielded different microbial counts. The total heterotrophic  
 119 bacterial counts of the samples (used and unused car lubrications) revealed that the samples are  
 120 contaminated with microorganisms as shown in Table 2.

Comment [LH23]: table 2

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**Table 2: Total Heterotrophic Bacterial Counts**

Locations	Engine oil (x10)		Hydraulic oil (x10)		Transition oil (x10)	
	used	unused	Used	Unused	Used	Unused
Mount Zion	1.6	1.0	1.0	1.0	1.0	1.0
Ekpo Abasi	4.0	1.3	2.2	1.0	2.5	1.2
Akim	1.7	1.0	1.0	1.0	1.0	1.0
Etta Agbor	2.0	1.0	1.0	1.0	2.0	1.1
Uncial work shop	1.2	1.0	1.0	1.0	1.0	1.0

Comment [LH24]: Total heterotrophic bacterial count

Comment [LH25]: Location

128  
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132  
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**Total Fungal Count**

The enumeration of the evaluated samples showed different fungal contaminations in the different locations. The total fungal count of the sample for used and unused car lubricants revealed that the samples are contaminated (Table 3).

Comment [LH26]: Total fungal count

**Table 3: Total Fungal Counts**

Locations	Engine oil (x10)		Hydraulic oil (x10)		Transition oil (x10)	
	Used	unused	Used	unused	used	Unused
Mount Zion	1.3	1.0	1.0	1.0	1.0	1.0
Ekpo Abasi	2.2	1.2	1.5	1.0	1.4	1.1
Akim	1.0	1.0	1.0	1.0	1.3	1.0
Etta Agbor	2.0	1.0	1.1	1.0	1.0	1.0
Uncial work shop	1.0	1.0	1.0	1.0	1.0	1.0

Comment [LH27]: Location

140  
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**Biochemical Characterization and Identification of Bacteria isolates**

The biochemical characterization and identification analyses suggest the presence of *Pseudomonas* spp., *Bacillus*, *Streptococcus*, *Micrococcus*, *Actinomyces*, *Nocardia*, *Listeria*, *Lactobacillus*, *Staphylococcus*, *Serratia* as shown in table 4.

Comment [LH28]: Biochemical characterization and identification of bacteria isolates

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Table 4a: Physiochemical Characteristics of Isolates

S/N	Colony Morphology	Gram	Cell shape	Catalase	Indole	Oxidase	Citrate	VP	MR	TSI				Presumptive Organism	
										Slant	Butt	Gas	H <sub>2</sub> S		Motility
<b>Used Engine oil</b>															
1	Creamy, irregular smooth <b>Rough</b>	+	Shot rods	+	-	-	+	-	-	A	A	+	-	+	<i>Listeria spp</i>
2	Creamy, smooth irregular	+	Branched stand rods	+	-	-	+	-	-	ALK	A	-	-	+	<i>Actinomycusspp</i>
3	Creamy, smooth, filament flat	+	Rods in chains	+	-	-	+	-	+	ALK	A	-	-	+	<i>Streptococcus spp</i>
4	White, circular, mucoid smooth	+	Bacilli in chains	+	+	-	+	-	+	ALK	A	+	-	+	<i>Bacillus spp</i>
5	Creamy <b>Rough</b>	+	Beaded rods	+	-	-	+	-	+	ALK	A	+	-	+	<i>Norcadiaspp</i>
6	Creamy, translucent & spreading	+	Rods in chin	+	-	-	+	-	-	ALK	A	+	-	+	<i>Streptococcus spp</i>
7	Creamy, Rough, spreading	+	Strepto cocci	-	+	-	+	-	-	ALK	A	+	+	+	<i>Streptococcus</i>
8	Creamy flat & opaque	+	Monococci	+	-	+	+	-	-	ALK	A	+	-	+	<i>Micrococusspp</i>
9	Creamy, raised, with rough	+	Short rods	+	-	-	+	-	-	ALK	A	-	-	+	<i>Bacilli spp</i>
10	Creamy, raised smooth mucoid	+	Rods in chains	+	-	-	+	-	+	ALK	A	+	-	+	<i>Streptobacilli spp</i>
<b>Unused Engine Oil</b>															
1	Creamy flat & irregular shapped	+	Short Rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>Bacillus spp</i>
2	Creamy, irregular & flat	-	Shot Rods	+	-	+	+	-	-	ALK	A	+	-	+	<i>Pseudomonas spp</i>
3	Shiny, irregular, colourless, slimmy	+	Cocci	+	+	+	+	-	-	ALK	A	-	+	+	<i>Staphylococcus spp</i>
4	Slimmy, colourless & watery	+	Monococci	+	+	+	+	-	-	ALK	A	+	-	+	<i>Micro cocci</i>
5	Light – pink, circular & pin-like	-	Rods	+	+	+	+	+	-	ALK	A	+	+	+	<i>Pseudomonas spp</i>
6	Slimy, watery & colourless	-	Short Rods	+	+	+	+	-	-	ALK	A	+	+	+	<i>Serretiaspp</i>

Comment [LH29]: Physiochemical characteristics of isolates

Comment [LH30]: Colony morphology

Comment [LH31]: Presumptive organism

Comment [LH32]: Used engine oil

Comment [LH33]: rough

Comment [LH34]: spp.

Comment [LH35]: *Actinomyces* spp.

Comment [LH36]: spp.

Comment [LH37]: spp.

Comment [LH38]: rough

Comment [LH39]: rough

Comment [LH40]: spp.

Comment [LH41]: spp.

Comment [LH42]: Where is species ?

Comment [LH43]: *Micrococcus* spp.Comment [LH44]: *Bacillus* spp.

Comment [LH45]: Unused engine oil

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Table 4b: Physiochemical Characteristics of Isolates

S/N	Colonial morphology	Gram	Cell shape	Catalase	Indole	Oxidase	Citrate	VP	MR	TSI				Presumptive Organism	
										Slant	Butt	Gas	H <sub>2</sub> S		Motility
<b>Hydraulic: used</b>															
1	Circular creamy	+	Rods in pains	-	+	-	+	-	+	ALK	A	+	+	+	<i>Lactobacillus sp</i>
2	Irregular creamy <b>Filamentous</b>	-	Rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>Pseudomonas spp</i>
3	Irregular creamy <b>Filamentous</b>	+	Rod	+	-	-	+	-	-	ALK	A	+	-	+	<i>Bacillus spp</i>
4	White, flate and circular	+	Rod in chins	+	-	-	-	-	-	ALK	A	+	-	+	<i>Bacillus spp</i>
5	Creamy, circular Big	+	Large branched rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>StreptoBacillus spp</i>

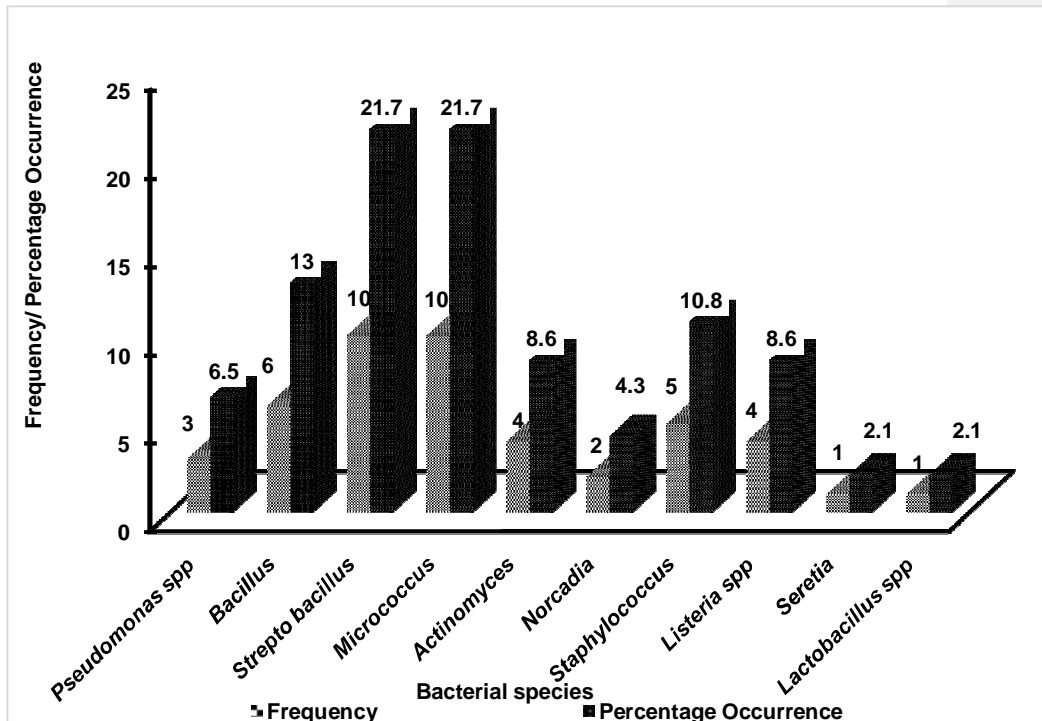
Comment [LH46]: Physiochemical characteristics of isolates

Comment [LH47]: Presumptive organism

Comment [LH48]: filamentous

Comment [LH49]: filamentous

<b>Hydraulic: Unused</b>																
1	Creamy large	+	Large Rods	+	+	-	+	+	+	ALK	A	+	+	+	<i>Bacillus spp</i>	Comment [LH50]: Large Rods
2	Creamy watery	+	Long paired Rods	+	-	-	+	-	+	ALK	A	+	-	-	<i>Bacillus spp</i>	Comment [LH51]: rods
3	Cream circular	+	Monococci	+	-	-	+	-	-	ALK	A	+	-	+	<i>Micrococcus spp</i>	
4	Irregular circular	+	Rod in chains	+	+	-	+	+	+	ALK	A	+	-	+	<i>Strepto Bacillus spp</i>	
5	Large creamy	+	Strepto Bacilli	+	+	-	+	+	+	ALK	A	+	-	+	<i>Strepto Bacillus spp</i>	
<b>Transition Oil: used</b>													Comment [LH52]: Transition oil			
1	Milky, irregular and spreading flat	-	Shot Rods	+	-	+	+	+	-	ALK	A	+	-	+	<i>Pseudomonas spp</i>	Comment [LH53]: Shot rods
2	Milky, circular, flat and rough	+	Branch Rods	+	-	+	-	+	-	ALK	A	+	-	+	<i>Norcadiaspp</i>	Comment [LH54]: Branch rods
3	White, circular, flat & smooth	-	Rod	+	-	+	+	+	+	ALK	A	+	-	+	<i>Pseudomonas</i>	Comment [LH55]: Beaded rods
4	White, irregular, flat translucent	+	Beaded Rods	+	-	+	+	+	+	ALK	A	-	-	-	<i>Norcadiaspp</i>	Comment [LH56]: rough
5	Milky, irregular spreading & Rough	+	Long branched Rods	+	-	+	+	+	-	ALK	A	-	-	+	<i>Actinomyces spp</i>	Comment [LH57]: Long branched rods
6	White, smooth, irregular opaque/flat	+	Cocco Bacilli	+	-	+	-	+	+	ALK	A	-	-	+	<i>Lacto Bacillus spp</i>	Comment [LH58]: Short rods in chains
7	Creamy, filamentous, flat & irregular	+	Short Rods in chains	+	-	+	+	+	+	ALK	A	-	-	-	<i>Strepto Bacillus spp</i>	Comment [LH59]: Shot beaded rods
8	White with irregular rough edges	+	Shot beaded Rod	+	-	+	+	+	+	ALK	A	-	-	-	<i>Norcadiaspp</i>	
9	Creamy with lobate margin	+	Rods in chains	+	-	+	+	-	+	ALK	A	-	-	-	<i>Strepto Bacillus spp</i>	
10	Watery, irregular	+	Cocci	+	+	+	+	-	-	ALK	A	+	-	+	<i>Staphylococcus spp</i>	
11	Yellow, with smooth circular shape	+	Cocci in clusters	+	+	+	+	+	+	ALK	A	+	-	+	<i>Staph spp</i>	
12	Irregular, mucoid, light pink center	+	Short Rods	+	-	+	+	-	-	ALK	A	+	+	+	<i>Bacillus spp</i>	
13	White creamy, circular	-	Rods	+	+	-	+	-	-	ALK	A	+	+	+	<i>Pseudomonas spp</i>	
<b>Transition: unused</b>																
1	White, flat, rough and opaque	+	Rods in pairs	+	-	+	+	-	-	ALK	A	+	-	+	<i>Bacillus spp</i>	
2	Milky, smooth and translucent	+	Short rods in chains	+	-	-	+	+	-	ALK	A	-	-	+	<i>Bacillus spp</i>	
3	White, irregular, smooth edge	+	Rods in chains	+	-	+	+	+	+	ALK	A	+	-	+	<i>Strepto Bacillus spp</i>	
4	Creamy, irregular, flat opaque	+	Beaded Rods	+	-	+	+	-	+	ALK	A	-	-	+	<i>Norcadiaspp</i>	Comment [LH60]: Beaded rods
5	Creamy, circular, raised & opaque	+	Mono cocci	+	-	-	+	-	+	ALK	A	+	-	+	<i>Micrococcus</i>	
6	Light pink, irregular	+	Cocci	+	-	-	+	-	-	ALK	A	+	+	+	<i>Staphylococcus spp</i>	
7	Pink & circular	+	Beaded Rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>Lacto Bacillus sp</i>	Comment [LH61]: Beaded rods



157 **Percentage Occurrence of the Bacterial Isolates**

158 The determination of percentage occurrence of the isolates obtained revealed that *Streptobacillus* and  
 159 *Micrococcus* have the highest percentage of occurrence of 10 (21.7%) respectively, these were  
 160 followed by *Bacillus* 6 (13%). *Serratia* and *Lactobacillus* spp have the least frequency of 1 (2.17%)  
 161 respectively as presented in fig. 1 below;

Comment [LH62]: Percentage occurrence of the bacterial isolates

Comment [LH63]: Serratia

Comment [LH64]: *Lactobacillus* spp.

Comment [LH65]: 1 (2.17%)

164 **Fig 1 Total Frequency/ Percentage Occurrence of the Bacterial Isolate**

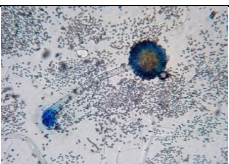
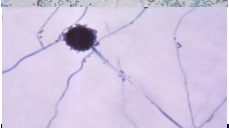
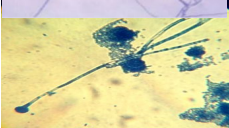
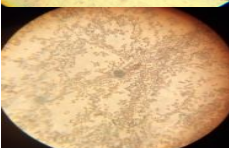

Comment [LH66]: Fig. 1: Total frequency/ percentage occurrence of the bacterial isolate

166 **Fungal characterization and identification**

167 The total fungal evaluation shows the presence of *Aspergillus niger*, *Aspergillus lentulus*, *A. flavus*,  
 168 *candida* species and *curvularia* as presented in table 5.

Comment [LH67]: *Candida*

179 **Table5:Physiological characteristics offungal isolates**

Macroscopic features surface/reverse		Microscopic features	Presumptive organism
White-yellow based felt covered by a dense layer of dark-brown to black conidial heads, reverse is white.		Conidial heads are large, biseriate, globose, dark, brown, becoming radiate with the phialides borne on metulae.	<i>Aspergillus niger</i>
Gray. Reverse is yellow		Conidiophores are smooth-walled, conidia heads are shorts	<i>Aspergillus lentulus</i>
Greenish-yellow with white border, floccose and velvety. Reverse is cream to tan with rhizoids		Growing rapidly with phialides radiation from vesicles in all directions.	<i>Aspergillus flavus</i>
White with rhizoid + oval yeast cells		Spherical to subspherical budding blastoconidia	<i>Candida</i> species
White to blackish-brown with aging. Suede-like. Reverse is black		Conidiophores are smooth-walled brown, solitary and flexuous. Conidia is straight rounded at the ends, pale brown to mill reddish-brown and ellipsoidal.	<i>Curvularia</i> spp

Comment [LH68]: *Candida*

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181 **Percentage occurrence of each fungal isolates**

182 A total of 30 samples of used and unused car lubricants were evaluated and 5 fungal genera were  
 183 identified with percentage occurrences (%) as follows; *Aspergillus niger* 25(75%), *Aspergillus flavus*  
 184 2(13.8%), *A. lentulus* 2(5.5), *Candida* 1(2.7) and *curvularia* 1(2.7) as presented below in fig 2

Comment [LH69]: *niger* 25 (75%)

Comment [LH70]: fig. 2.

185

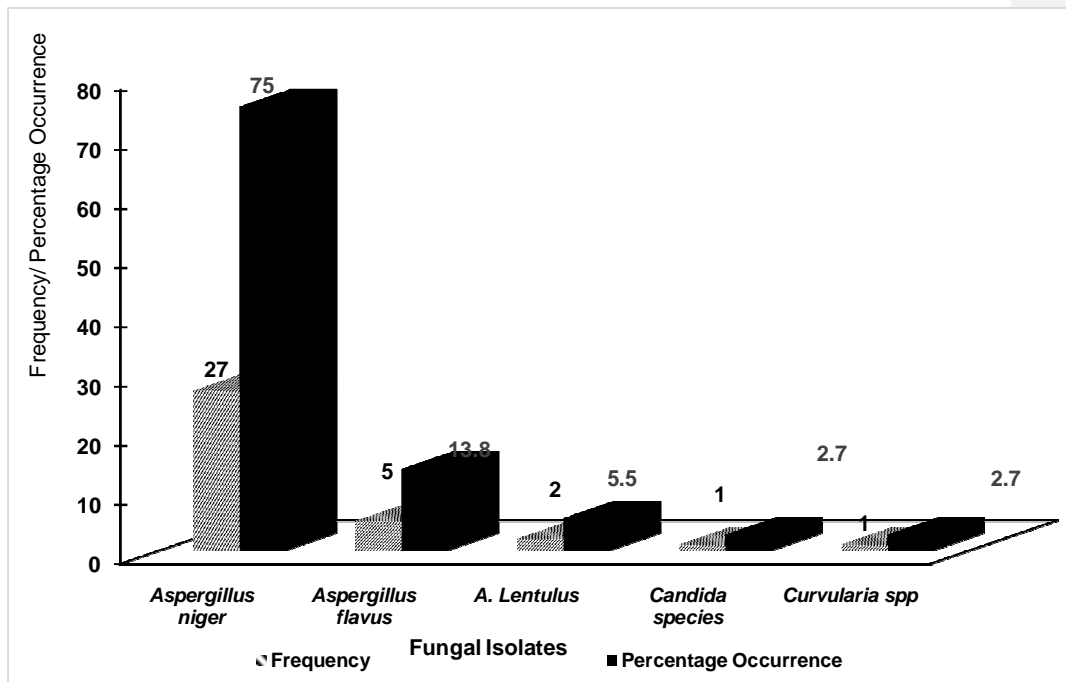


Fig.2: Frequency and Percentage Occurrence of Fungal Isolates

Comment [LH71]: Frequency and percentage occurrence of fungal isolates

## Discussion

The results obtained from the car lubricants showed that they contained some pathogenic and nonpathogenic organisms. This is in line with other study of Walter *et al.*[28], which reported the prevalence of microbial contaminants in engine oil polluted site. The isolation and identification of *Bacillus* sp, *Lactobacillus* spp, *Corynebacterium* spp, *Micrococcus*, *Nocardia*, *Actinomyces*, *Streptomyces* spp, *Listeria*, spp, *Pseudomonas* spp, *Streptococcus* spp and *Staphylococcus* spp is in conformity with the work of Okpokwasili *et al.*[18], who obtained similar results in their study on microbial growth in brake fluid.

Some of the contaminants in these lubricants may have gotten into them during manufacturing, handling, storage process after draining since they were mostly contaminants of soil. From the result, unused oil was also observed to contain almost the same organism found in used. This corroborates the position of Okpokwasili *et al.*[18], who stated that spent oil is similar to unused oil, except that additional chemicals and metals such as lead, manganese, iron, etc have been added to spent oil due to high temperature and pressure of the operating engines where they serve as engine lubricant and other impurities. Most of the isolated bacteria species are of both medical and environmental importance. Some of the organisms isolated from have been noted by previous researchers as hydrocarbon degraders, for example *Pseudomonas* spp, *Bacillus*, *Nocardia* etc. as a result of their carbon utilizing potential. This is in agreement with Okoye *et al.* [17], who reported that *Pseudomonas*, *Actinobacter*, *Bacillus* and *Nocardia* have been noted to utilize hydrocarbons. Few

Comment [LH72]: *et al.*

Comment [LH73]: spp.,

Comment [LH74]: *Nocardia*,

Comment [LH75]: spp.,

Comment [LH76]: *Listeria*

Comment [LH77]: spp.,

Comment [LH78]: spp.,

Comment [LH79]: spp. and

Comment [LH80]: spp.

Comment [LH81]: *Nocardia*,

Comment [LH82]: *Nocardia*

209 researchers have reported about the invaluable role of *Bacillus* spp. in hydrocarbon bioremediation  
210 particularly in extreme environment, especially in crude oil polluted environments [6].

Comment [LH83]: *Bacillus* spp.

211  
212 The negative impact of the oil lubricants on soil microorganisms corroborates the position of Angiraet  
213 *al.* [3] who reported that lubricant oil residues are potentially harmful and toxic to both plants and  
214 animals of the land. Public Health Agency England [21], reported that some of the stains of  
215 *Staphylococcus* spp. have been implicated as a causative agent in acute food poisoning episodes,  
216 *Staphylococcus* symptoms come on quickly, usually within hours of ingestion. All humans are  
217 susceptible to infection with *Staphylococcus*, which causes illness by toxin production as well as  
218 infecting both local tissues and systemic circulation, leading series of series of symptoms including  
219 fever, vomiting, headache, arthritis etc [8]. The genus *Pseudomonas* is the most important  
220 order. *Pseudomonas putida* was reported by Safiyanuet *al.* [22], to have the capabilities of  
221 bioremediating and biodegrading hydrocarbons which are the major constituents of petroleum oil.  
222 However, *Pseudomonas aeruginosa* is a major cause of nosocomial infections especially in immune  
223 compromised patients. These infections are complicated and life threatening [15]. *Bacillus* is a very  
224 diverse genus with more than 200 species, and the identification and diagnosis of potential disease-  
225 causing *Bacillus* species from patient materials by the clinical lab can be challenging [27]. Some  
226 species, usually *B. cereus*, can cause a rapidly destructive endophthalmitis, resulting from ocular  
227 trauma or hematogenous dissemination [26]. Some of the species such as *B. Anthracis* are  
228 recognized as a potential biological weapon [4]. *Nocardia*, *Corynebacterium*, *Actinomyces*,  
229 *Micrococcus* are all organisms of medical importance and reported to have the physiological ability of  
230 hydrocarbon utilization [9; 11; 12; 14]. Their oil lubricant utilization potential makes these organisms  
231 potentials agents of bioremediation lubricants polluted sites.

Comment [LH84]: spp.

232  
233 The presence of the following fungi; *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus lentulus*,  
234 *Curvularia* spp. and yeast, *Candida* indicates that fungi are also involved in the utilization of used and  
235 unused oil lubricants. The unused lubricants supported more fungal growth than the used lubricant.  
236 This disagrees with the report of Okpokwasili *et al.* [18], who reported that while in service, some  
237 components of used oil are altered or lost, thereby making it more conducive for fungal utilization  
238 when compared to unused oils. The contaminants may have been picked from the environment since  
239 they are ubiquitous. Though these organisms are environmental opportunistic microorganisms, they  
240 also have documented medical importance [25; 24; 19; 10].

Comment [LH85]: spp.

## 241 CONCLUSION

242  
243 The unused car lubricants were observed to have almost the same type of microorganisms as found in  
244 used oils, but there is more microbial load in used oils than the unused ones. This means that the  
245 organisms could be utilizing these oils as source of nutrients. This implies that keeping the oils for  
246 longer period with these organisms could reduce their shelf life. The utilization of the car lubricants by  
247 autochthonous microorganisms could lead to loss of functions or reduced efficacy. The ability of the  
248 organisms to cause opportunistic infection gives reason for more concern about used and unused car  
249 lubricants as a potential health hazard. The study reveals that oil lubricants contain inherent

250 microorganisms that could lead to the degradation under favourable conditions, and enhances their  
251 stimulation for bioremediation purposes.

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## Reference

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