

**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  $1.0 \times 10^{10}$  CFU/ml to  $2.2 \times 10^{10}$  CFU/ml for engine oil;  $1.0 \times 10^{10}$  CFU/ml to  $1.5 \times 10^{10}$  CFU/ml for hydraulic oil and  $1.0 \times 10^{10}$  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%), *Streptobacilli*(21.7%), *Micrococcus* spp.(21.7%), *Actinomyces* spp.(8.6%), *Nocardia* spp.(4.3%), *Staphylococcus* spp.(10.8%), *Listeria* spp.(8.6%), *Serratia* spp. (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.

Formatted: Font color: Red, Highlight

**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. It also has the function of transmitting forces, transporting foreign particles, or heating or cooling 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 kinds of paraffins, cyclic alkanes and aromatic hydrocarbons [7].

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Comment [WU1]: You should numbered in the order that they appear in the text.

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

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 the 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, and gene mutation and are very toxic. The release of persistent, bio-accumulative and toxic chemicals

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

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

Formatted: Font color: Red, Highlight

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

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

## MATERIALS AND METHODS

### Sample collection

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

Formatted: Font color: Red, Highlight

### Spectrophotometric analysis

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

### Determination of absorbance of transport solution

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

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

### Microbiological analysis

### Enumeration of total heterotrophic bacterial count

The total heterotrophic bacterial count of the sample was determined using the pour plate method. Serial dilution was prepared from the liquid sample. Exactly one millimeter (1ml) was taken from each selected dilution ( $10^3$ ,  $10^4$ ,  $10^5$  and  $10^6$ ) into sterile Petri dishes. The molten sterilized Nutrient and MacConkey agar were poured into the plates, swirled to spread the inoculum evenly within the agar medium and allowed to solidify then incubated at 37°C for 24 hours. Thereafter, plates with colony growth were counted and recorded.

Formatted: Font: 11 pt, Not Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Superscript, Highlight

Formatted: Font color: Red, Highlight

### Biochemical characteristics and identification of bacterial isolates obtained from used and unused lubricants

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

### Characterization and identification of fungi

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

Formatted: Font: 11 pt, Not Highlight

## RESULTS

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

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font: 11 pt, Not Highlight

Formatted: Not Highlight

Table 1: Absorbance of the cultures from mineral salt medium

Location (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
EkpoAbasi	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

Blank-ve= 1.0 +tve=2.0

### Total heterotrophic bacterial count

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

Formatted: Font color: Red, Highlight

Table 2: Total heterotrophic bacterial count

Formatted: Font: 11 pt, Not Highlight

Engine oil (x10)      Hydraulic oil (x10)      Transition oil (x10)

Location	used	unused	used	unused	used	unused
Mount Zion	1.6	1.0	1.0	1.0	1.0	1.0
EkpoAbasi	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

Formatted: Not Highlight

Formatted Table

### Total fungal count

Formatted: Font: 11 pt, Not Highlight

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).

**Table 3: Total fungal counts**

Formatted: Font: 11 pt, Not Highlight

Location	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
EkpoAbasi	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

### Biochemical characterization and identification of bacterial isolates

Formatted: Font: 11 pt, Not Highlight

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.

Formatted: Not Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red

**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 rough	+	Shot rods	+	-	-	+	-	-	A	A	+	-	+	<i>Listeria</i> spp.
2	Creamy, smooth irregular	+	Branched strand rods	+	-	-	+	-	-	ALK	A	-	-	+	<i>Actinomyces</i> spp.
3	Creamy, smooth, filament flat	+	Rods in chains	+	-	-	+	-	+	ALK	A	-	-	+	<i>Streptococcus</i> spp.
4	White, circular, mucoid smooth	+	Bacilli in chains	+	+	-	+	-	+	ALK	A	+	-	+	<i>Bacillus</i> spp.
5	Creamy rough	+	Beaded rods	+	-	-	+	-	+	ALK	A	+	-	+	<i>Nocardia</i> spp.
6	Creamy, translucent & spreading	+	Rods in chain	+	-	-	+	-	-	ALK	A	+	-	+	<i>Streptococcus</i> spp.
7	Creamy, rough, spreading	+	Strepto cocci	-	+	-	+	-	-	ALK	A	+	+	+	<i>Streptococcus</i> spp.
8	Creamy flat & opaque	+	Monococci	+	-	+	+	-	-	ALK	A	+	-	+	<i>Micrococcus</i> spp.
9	Creamy, raised, with rough	+	Short rods	+	-	-	+	-	-	ALK	A	-	-	+	<i>Bacillus</i> spp.
10	Creamy, raised smooth mucoid	+	Rods in chains	+	-	-	+	-	+	ALK	A	+	-	+	<i>Streptobacillus</i> spp.
<b>Unused engineoil</b>															
1	Creamy flat & irregular shaped	+	Short rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>Bacillus</i> spp.
2	Creamy, irregular & flat	-	Shot rods	+	-	+	+	-	-	ALK	A	+	-	+	<i>Pseudomonas</i> spp.
3	Shiny, irregular, colourless, slimy	+	Cocci	+	+	+	+	-	-	ALK	A	-	+	+	<i>Staphylococcus</i> spp.
4	Slimy, colourless & watery	+	Monococci	+	+	+	+	-	-	ALK	A	+	-	+	<i>Micrococcus</i> spp.
5	Light – pink, circular & pin-like	-	rods	+	+	+	+	+	-	ALK	A	+	+	+	<i>Pseudomonas</i> spp.
6	Slimy, watery & colourless	-	Short rods	+	+	+	+	-	-	ALK	A	+	+	+	<i>Serratia</i> spp.

**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</i> spp.
2	Irregular creamy filamentous	-	Rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>Pseudomonas</i> spp.
3	Irregular creamy filamentous	+	Rod	+	-	-	+	-	-	ALK	A	+	-	+	<i>Bacillus</i> spp.

- Formatted ... [1]
- Formatted ... [2]
- Formatted ... [3]
- Formatted ... [4]
- Formatted ... [5]
- Formatted ... [6]
- Formatted ... [7]
- Formatted ... [8]
- Formatted ... [9]
- Formatted ... [10]
- Formatted ... [11]
- Formatted ... [12]
- Formatted ... [13]
- Formatted ... [14]
- Formatted ... [15]
- Formatted ... [16]
- Formatted ... [17]
- Formatted ... [18]
- Formatted ... [19]
- Formatted ... [20]
- Formatted ... [21]
- Formatted ... [22]
- Formatted ... [23]
- Formatted ... [24]
- Formatted ... [25]
- Formatted ... [26]
- Formatted ... [28]
- Formatted ... [27]
- Formatted ... [29]
- Formatted ... [30]
- Formatted ... [31]
- Formatted ... [32]
- Formatted ... [33]

4	White, flate and circular	+	Rod in chins	+	-	-	-	-	-	ALK	A	+	-	+	<i>Bacillus</i> spp.	Formatted: Not Highlight	
5	Creamy, circular big	+	Large branched rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>StreptoBacillus</i> spp.	Formatted: Not Highlight	
<b>Hydraulic: unused</b>																	
1	Creamy large	+	Large rods	+	+	-	+	+	+	ALK	A	+	+	+	<i>Bacillus</i> spp.	Formatted: Not Highlight	
2	Creamy watery	+	Long paired rods	+	-	-	+	-	+	ALK	A	+	-	-	<i>Bacillus</i> spp.	Formatted: Not Highlight	
3	Cream circular	+	Monococci	+	-	-	+	-	-	ALK	A	+	-	+	<i>Micrococcus</i> spp.	Formatted: Not Highlight	
4	Irregular circular	+	Rod in chains	+	+	-	+	+	+	ALK	A	+	-	+	<i>Strepto Bacillus</i> spp.	Formatted: Not Highlight	
5	Large creamy	+	Strepto Bacilli	+	+	-	+	+	+	ALK	A	+	-	+	<i>Strepto Bacillus</i> spp.	Formatted: Not Highlight	
<b>Transition oil: used</b>																	
1	Milky, irregular and spreading flat	-	Shot rods	+	-	+	+	+	-	ALK	A	+	-	+	<i>Pseudomonas</i> spp.		
2	Milky, circular, flat and rough	+	Branch rods	+	-	+	-	+	-	ALK	A	+	-	+	<i>Nocardia</i> spp.	Formatted: Not Highlight	
3	White, circular, flat & smooth	-	Rod	+	-	+	+	+	+	ALK	A	+	-	+	<i>Pseudomonas</i> spp.	Formatted: Not Highlight	
4	White, irregular, flat translucent	+	Beaded rods	+	-	+	+	+	+	ALK	A	-	-	+	<i>Nocardia</i> spp.	Formatted: Not Highlight	
5	Milky, irregular spreading & rough	+	Long branched rods	+	-	+	+	+	-	ALK	A	-	-	+	<i>Actinomyces</i> spp.	Formatted: Not Highlight	
6	White, smooth, irregular opaque/ flat	+	Cocco Bacilli	+	-	+	-	+	+	ALK	A	-	-	+	<i>Lacto Bacillus</i> spp.	Formatted: Not Highlight	
7	Creamy, filamentous, flat & irregular	+	Short rods in chains	+	-	+	+	+	+	ALK	A	-	-	-	<i>Strepto Bacillus</i> spp.	Formatted: Not Highlight	
8	White with irregular rough edges	+	Shot beaded rod	+	-	+	+	+	+	ALK	A	-	-	-	<i>Nocardia</i> spp.	Formatted: Not Highlight	
9	Creamy with lobate margin	+	Rods in chains	+	-	+	+	-	+	ALK	A	-	-	-	<i>Strepto Bacillus</i> spp.	Formatted: Not Highlight	
10	Watery, irregular	+	Cocci	+	+	+	+	-	-	ALK	A	+	-	+	<i>Staphylococcus</i> spp.	Formatted: Not Highlight	
11	Yellow, with smooth circular shape	+	Cocci in clusters	+	+	+	+	+	-	ALK	A	+	-	+	<i>Staphylococcus</i> spp.	Formatted: Not Highlight	
12	Irregular, mucoid, light pink center	+	Short rods	+	-	+	+	-	-	ALK	A	+	+	+	<i>Bacillus</i> spp.	Formatted: Not Highlight	
13	White creamy, circular	-	Rods	+	+	-	+	-	-	ALK	A	+	+	+	<i>Pseudomonas</i> spp.	Formatted: Not Highlight	
<b>Transition: unused</b>																	
1	White, flat, rough and opaque	+	Rods in pairs	+	-	+	+	-	-	ALK	A	+	-	+	<i>Bacillus</i> spp.	Formatted: Not Highlight	
2	Milky, smooth and translucent	+	Short rods in chains	+	-	-	+	+	-	ALK	A	-	-	+	<i>Bacillus</i> spp.	Formatted: Not Highlight	
3	White, irregular, smooth edge	+	Rods in chains	+	-	+	+	+	+	ALK	A	+	-	+	<i>Strepto Bacillus</i> spp.	Formatted: Not Highlight	
4	Creamy, irregular, flat opaque	+	Beaded rods	+	-	+	+	-	+	ALK	A	-	-	+	<i>Nocardias</i> pp.	Formatted: Not Highlight	
5	Creamy, circular, raised & opaque	+	Mono cocci	+	-	-	+	-	+	ALK	A	+	-	+	<i>Micrococcus</i> spp.	Formatted: Not Highlight	
6	Light pink, irregular	+	Cocci	+	-	-	+	-	-	ALK	A	+	+	+	<i>Staphylococcus</i> spp.	Formatted: Not Highlight	
7	Pink & circular	+	Beaded rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>Lacto Bacillus</i> spp.	Formatted: Not Highlight	
																Formatted: Not Highlight	
																Formatted: Not Highlight	
																Formatted: Not Highlight	

### Percentage occurrence of the bacterial isolates

The determination of the percentage occurrence of the isolates obtained revealed that *Streptobacillus* and *Micrococcus* have the highest percentage of occurrence of 10 (21.7%) respectively, these were followed by *Bacillus* 6 (13%), *Serratia* and *Lactobacillus* spp. have the least frequency of 1 (2.1%) respectively as presented in Fig. 1 below;

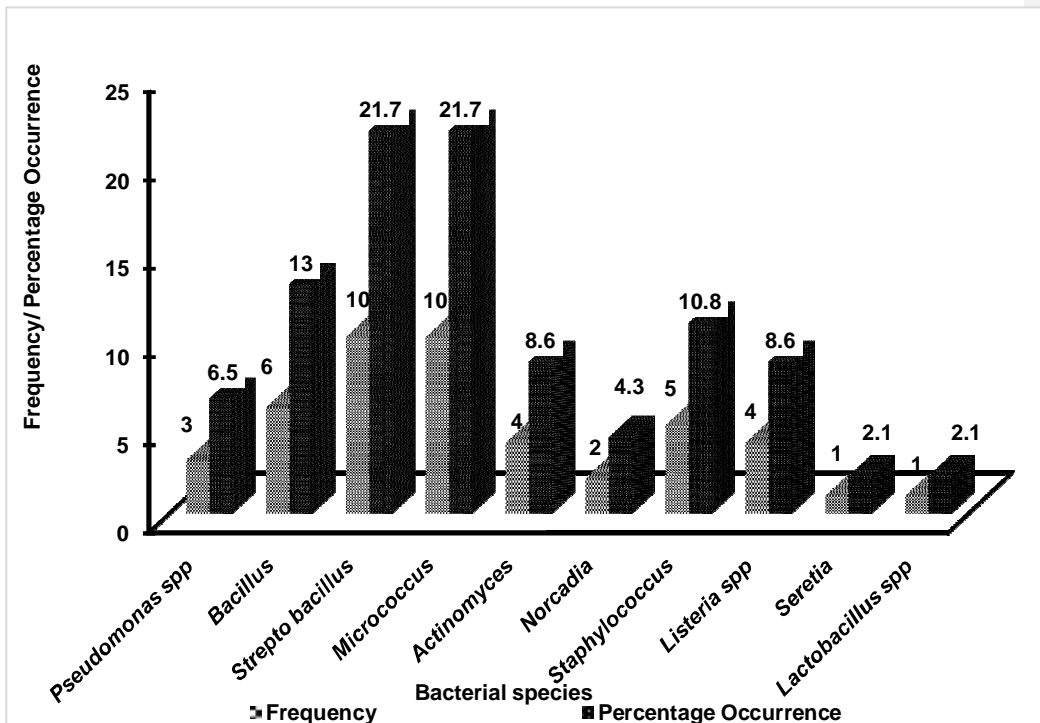
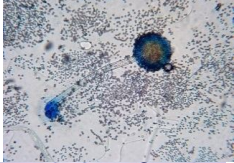

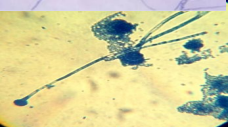
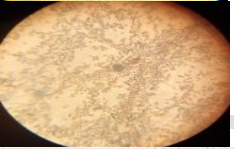
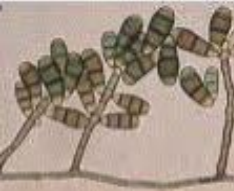


Fig 1 Total frequency/percentage occurrence of the bacterial isolates

### Fungal characterization and identification

The total fungal evaluation shows the presence of *Aspergillus niger*, *Aspergillus lentulus*, *A. flavus*, *Candida* species and *curvularia* as presented in Table 5.

**Table 5: Physiological characteristics of fungal 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.		<i>Aspergillus niger</i>
Gray. Reverse is yellow		<i>Aspergillus lentulus</i>
Greenish-yellow with white border, floccose and velvety. Reverse is cream to tan with rhizoids		<i>Aspergillus flavus</i>
White with rhizoid + oval yeast cells		<i>Candida</i> spp.
White to blackish-brown with aging. Suede-like. Reverse is black		<i>Curvularia</i> spp.

Formatted: Font: 11 pt, Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

**Percentage occurrence of each fungal isolates**

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

Formatted: Not Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red

Formatted: Not Highlight

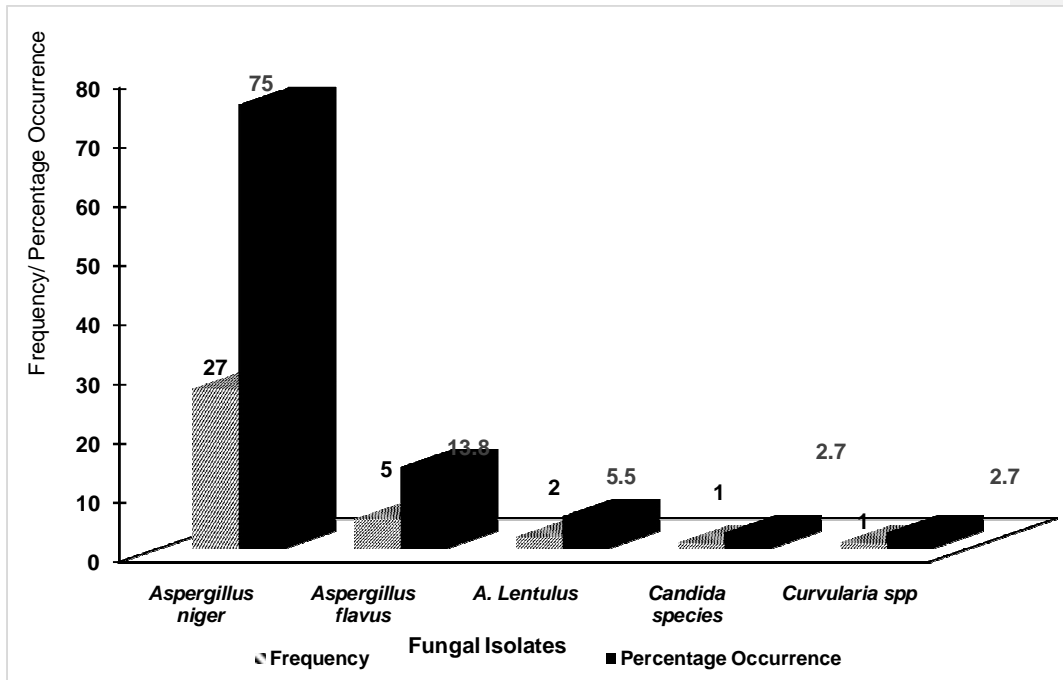


Fig.2: Frequency and percentage occurrence of fungal isolates

Formatted: Not Highlight

### Discussion

The results obtained from the car lubricants showed that they contained some pathogenic and nonpathogenic organisms. This is in line with another study by Walter *et al.* [28], which reported the prevalence of microbial contaminants in engine oil-polluted sites. The isolation and identification of *Bacillus* spp., *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 the manufacturing, handling, and 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*, *Actinobacteria*, *Bacillus* and *Nocardia* have been noted to utilize hydrocarbons. Few

Comment [WU2]: Replace all *et al.* or *et al.* in Italic form with *et al.*

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

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

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Not Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

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

Formatted: Not Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Not Highlight

Formatted: Font: Not Italic, Font color: Red, Highlight

Formatted: Font color: Red, Highlight

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

Formatted: Not Highlight

Comment [WU3]: Change with more appropriate word.

Formatted: Font color: Red, Highlight

## CONCLUSION

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

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

Formatted: Font color: Red, Highlight

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

Formatted: Font color: Red, Highlight

## Reference

1. Abdulyekeen KA, Muhammed IM, Giwa SO, Abdulsalem. Bioremediation of Used Motor Oil Contaminated Soil Using Elephants Horse Dung as Stimulants. *Journal of Environmental Science and Food Technology*. 2016; 10(12) 73-78.
2. Abdusalam S, Adefia SS, Bugaje IM, Ibrahim S. Bioremediation of Soil Contaminated with Used Motor Oil in a Closed System. *Journal of Bioremediation*. 2012; 3(12) 3-9.
3. Angira Devi Bhuyan, Uma MU, Krishna Murthy. Degradation of Lubricant Oil Residues: The Role of Rhizosphere. *Microflora*. 2018; 11:2277-8322.
4. Batcher Denis FB. In Principle and practice of pediatric infectious Diseases Fourth Edition. 2012.
5. Boris AG, Anatoly AG, Igor SA, Boris VN. Thermophysical Properties of Individual Hydrocarbons of Petroleum and Natural Gases: Properties, Methods, and Low-Carbon Technologies. Gulf professional publishing, 2022;
6. Cindy B, Alfred M, Tsepo LT, and Naser AF. Bacillus Species and Their Invaluable Roles in Petroleum Hydrocarbon Bioremediation. *Bacilli in Agrobiotechnology*. 2022; 5: 101-126.
7. Don MP, Martin W, Ekkehard D. Lubrication Fundamental (Third Edition, Revised and Expanded ed.) CRC Press. 2016
8. Eisenberg T, Heydel C, Prenger-Berninghoff E, Fawzy A, Kling U, Akimkin V, Semmler T, Mühlendorfer K, Kämpfer P, Blom J, Ewers C. *Streptobacillus canis* sp. nov. isolated from a dog. *International Journal Systematic Evolutionary Microbiology*. 2020; 70(4):2648-56.
9. Frederick S Southwick, In Goldman's Cecil Medicine. Handbook of clinical Neurology (Twenty-fourth Edition) 2012.
10. Jeffery CW, Gilbert MK, Lebar MD, Majumdar R, Calvo AM. Aspergillus flavus Secondary Metabolites: More than Just Aflatoxins. *Food Safety (Tokyo)*. 2018; 30;6(1):7-32.
11. Jeffrey K. Actor PhD. Elsevier's Integrated Review Immunology and Microbiology (Second Edition) 2012; 96-99. [www.sciencedirect.com/topics/immunology-and-microbiology/immunopathology](http://www.sciencedirect.com/topics/immunology-and-microbiology/immunopathology)
12. Julia Hahne, Tabea Kloster, Sandra Rathmann, Mareike Weber, Adre Lipski. Isolation and Characterization of Cerynebacterium spp. From Bulk Buuk Tank Raw Cow's Milk of Different Dairy Farms in Gema. 2018; 13(4);194365
13. Madu MJ, Alhassan MM, Ahmad H. Isolation of Bacteria from Soil Contaminated with Used Engine Oil in Federal Capital Territory, Nigeria. *Journal of Multidisciplinary Engineering Science Studies (JMESS)* 2018; 4: 11.
14. Márió Gajdács and Edit Urbán. The Pathogenic Role of Actinomyces spp. and Related Organisms in Genitourinary Infections: Discoveries in the New, Modern Diagnostic Era. *Antibiotics*. 2020; 9, 524
15. Ng QX, Ong, NY, Lee DYX, Yau CE, Lim YL, Kwa ALH, Tan B.H. Trends in *Pseudomonas aeruginosa* (*P. aeruginosa*) Bacteremia during the COVID-19 Pandemic: A Systematic Review. *Antibiotics*. 2023; 12: 409.
16. Nwinyi Obinna C, Ajaja Olaleye, Nwinyi Chibuzo. Growth Dynamics of Bacteria Isolated from Spent Engine Oil Contaminated Tropical Soil. *Research Journal Environmental Earth Sciences* 2014; 6(9):430-436
17. Okoye AU, Chiker CB, Okpokwasili GC. Isolation and Characterization of Hexadecane Degrading Bacteria from Oil-polluted soil in Gio Community, Niger Delta, Nigeria. *Scientific African*. 2020; 9;
18. Okpokwasili G, Chinonye M, Maduka. Microbial Growth in Brake Fluids. *International Biodeterioration & Biodegradation*. 2016; 114:31-38.

19. Onuorah S, Orji MU. Fungi Associated with The Spoilage of Post-Harvest Tomatoes Sold Major Market in Awka Nigeria. *Universal Journal of Microbiology Research*.2015; 3(2):11-16
20. Onojake MC, Nwokonko NV, Osakwe JO. Human Health Risk Assessment of Polycyclic Hydrocarbons in Selected Seafood from Niger Delta Nigeria. *Nigerian Journal of Chemical Research*. 2020; 25(2)
21. Public Health Agency England. Identification of *Staphylococcus* spp, *Micrococcus* spp and *Rothia* spp. UK Standard for Microbiology Investigation.2014; 1-32
22. Safiyanu I, Abdulwahid IA, Mudi Z R, Ya'u SA, and Rita SM. Bioremediation on Oil Spills Using Bacterium (*Pseudomonas Putida*) *Iconic Research and Engineering Journals*. 2022; 5:8
23. Sagheer A, Dobhai S, Tomar V. A Comparative Study of Oil Degradation with Used and Unused Engine Oil by Microbes Isolated from Water Sample of Mechanic Workshops. *Agricultural Research Technology Open Access Journal*.2017; 2471-6774.
24. Sardi JCO, Scorzoni L, Bemadi T, Fusco AM, Mendes Giannini. Candida Species: Current Epidemiology, Pathogenicity, Biofilm Formation, Natural Antifungal Products and New Therapeutic Options. *Journal of Medical Microbiology*.2013; 62, 10-24.
25. Swilaiman, SS, Gorman O, Balajee CM. Discovery of a Sexual Cycle in *Aspergillus Lentulus*; 2013; 2(7): 962-9.
26. Thomas Feteke. In Mandell Douglas and Bennett's Principle and Practice of Pediatric Infectious Disease Eight Edition. 2015. <https://www.sciencedirect.com>>book
27. Thwaite JE, Atkins HS. *Molecular Medical Microbiology*. (Second Edition). 2015. <https://www.sciencedirect.com/topics/medicine-and-dentistry/bacillus>
28. Walter CJ, Timothy TA, Temitope AI, Musa I, Skiru GK, Olorundare OO. *American Journal of Environment Studies*. 2020; 3(1) 2:29 – 43.