

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 Ekpo Abasi (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 1.2×10^{10} CFU/ml to 4.0×10^{10} CFU/ml; hydraulic oil ranged from 1.0×10^{10} CFU/ml to 2.2×10^{10} CFU/ml; Transition oil ranged from 1.0×10^{10} CFU/ml to 2.5×10^{10} CFU/ml. The THB counts of the unused oils had an average count of 1.0×10^{10} CFU/ml. The enumeration of the total fungal showed lower counts ranging from 1.0×10^{10} CFU/ml to 2.2×10^{10} CFU/ml for engine oil; 1.0×10^{10} CFU/ml to 1.5×10^{10} CFU/ml for hydraulic oil and 1.0×10^{10} CFU/ml to 1.4×10^{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%), *Norcadia* 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.

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 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, 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 hazard which can lead to loss of lives and properties [1].

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, protection 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 metal working 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, industries and the usage come 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.

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 Microbiology laboratory of Cross River University of Technology, Calabar, for further analysis.

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 30minutes for Warm-up. Then the Wavelength was selected (620nm) and the blank placed into the curvet 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 set to determine the absorbance of standard sample. The concentration scale was set by Pressing \uparrow or \downarrow key to cause the displayed value equal to known concentration or ion times of the known concentration. The unknown concentration was then determined by placing in the unknown sample and the values displayed on the read out recorded as the absorbance value of the sample.

Microbiological analysis

Enumeration of Total Heterotrophic Bacterial counts

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) 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.

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.

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) were highest (2.69nm) among all the oils examined followed by those from Transition oil (used), with the value of 2.40nm, as shown on Table 1.

Table 1: 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

Blank -ve = 1.0 +ve = 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.

Table 2: Total Heterotrophic Bacterial Counts

Locations	Engine oil (×10)		Hydraulic oil (×10)		Transition oil (×10)	
	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

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).

Table 3: Total Fungal Counts

Locations	Engine oil (×10)		Hydraulic oil (×10)		Transition oil (×10)	
	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

Biochemical Characterization and Identification of Bacteria isolates

The biochemical characterization and identification analyses suggest the presence of *Pseudomonas* spp, *Baccillus*, *Streptococcus* *Micrococcus*, *Actinomyces*, *Norcadia*, *listeria*, *Lactobacillus*, *Staphylococcus*, *Seretia* as shown in table 4.

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 ₂ S		Motilit
Used Engine oil															
1	Creamy, irregular smooth Rough	+	Shot rods	+	-	-	+	-	-	A	A	+	-	+	<i>Listeria spp</i>
2	Creamy, smooth irregular	+	Branched stand rods	+	-	-	+	-	-	ALK	A	-	-	+	<i>Actinomycus spp</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 Rough	+	Beaded rods	+	-	-	+	-	+	ALK	A	+	-	+	<i>Norcadia spp</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>Micrococcus spp</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>
Unused Engine Oil															
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>Serretia spp</i>

Table 4b: Physiochemical Characteristics of Isolates

S/N	Colonial morphology	Gram	Cell shape	Catalas	Indole	Oxidase	Citrate	VP	MR	TSI				Presumptive Organism	
										Slant	Butt	Gas	H ₂ S		Motilli
Hydraulic: used															
1	Circular creamy	+	Rods in pains	-	+	-	+	-	+	ALK	A	+	+	+	<i>Lactobacillus sp</i>
2	Irregular creamy Filamentous	-	Rods	+	+	-	+	-	-	ALK	A	+	-	+	<i>Pseudomonas spp</i>
3	Irregular creamy Filamentous	+	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>Strepto Bacillus spp</i>

Hydraulic: Unused															
1	Creamy large	+	Large Rods	+	+	-	+	+	+	ALK	A	+	+	+	<i>Bacillus spp</i>
2	Creamy watery	+	Long paired Rods	+	-	-	+	-	+	ALK	A	+	-	-	<i>Bacillus spp</i>
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>
Transition Oil: used															
1	Milky, irregular and spreading flat	-	Shot Rods	+	-	+	+	+	-	ALK	A	+	-	+	<i>Pseudomonas spp</i>
2	Milky, circular, flat and rough	+	Branch Rods	+	-	+	-	+	-	ALK	A	+	-	+	<i>Norcadia spp</i>
3	White, circular, flat & smooth	-	Rod	+	-	+	+	+	+	ALK	A	+	-	+	<i>Pseudomonas</i>
4	White, irregular, flat translucent	+	Beaded Rods	+	-	+	+	+	+	ALK	A	-	-	-	<i>Norcadia spp</i>
5	Milky, irregular spreading & Rough	+	Long branched Rods	+	-	+	+	+	-	ALK	A	-	-	+	<i>Actinomyces spp</i>
6	White, smooth, irregular opaque/flat	+	Cocco Bacilli	+	-	+	-	+	+	ALK	A	-	-	+	<i>Lacto Bacillus spp</i>
7	Creamy, filamentous, flat & irregular	+	Short Rods in chains	+	-	+	+	+	+	ALK	A	-	-	-	<i>Strepto Bacillus spp</i>
8	White with irregular rough edges	+	Shot beaded Rod	+	-	+	+	+	+	ALK	A	-	-	-	<i>Norcadia spp</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>
Transition: unused															
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>Norcadia spp</i>
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>

Percentage Occurrence of the Bacterial Isolates

The determination of 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%). *Seretia* and *Lactobacillus* spp have the least frequency of 1(2.17%) respectively as presented in fig. 1 below;

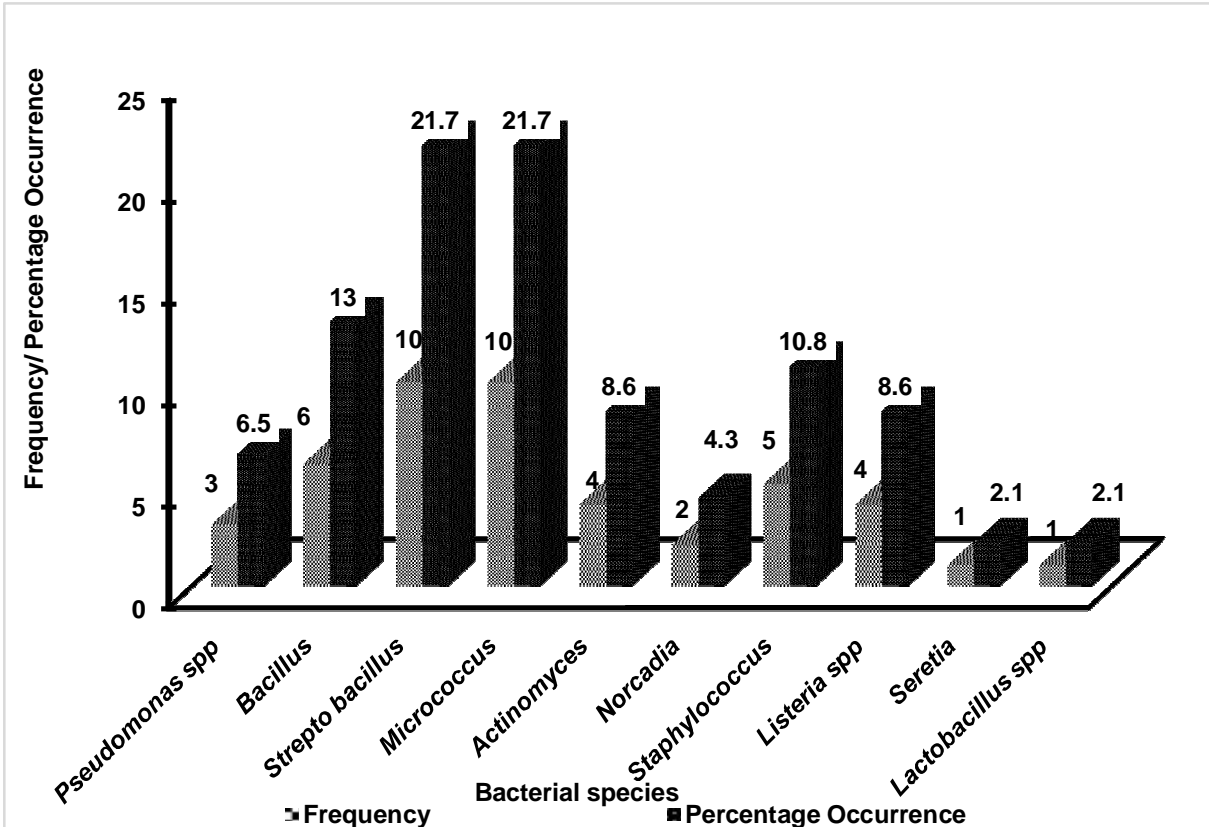
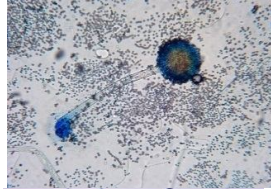
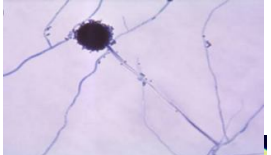
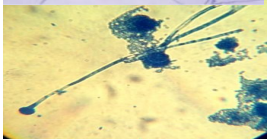
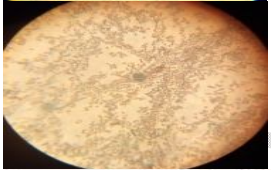
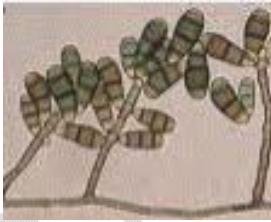


Fig 1 Total Frequency/ Percentage Occurrence of the Bacterial Isolate

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.		Conidial heads are large, biserial, 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 species</i>
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 spp</i>

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

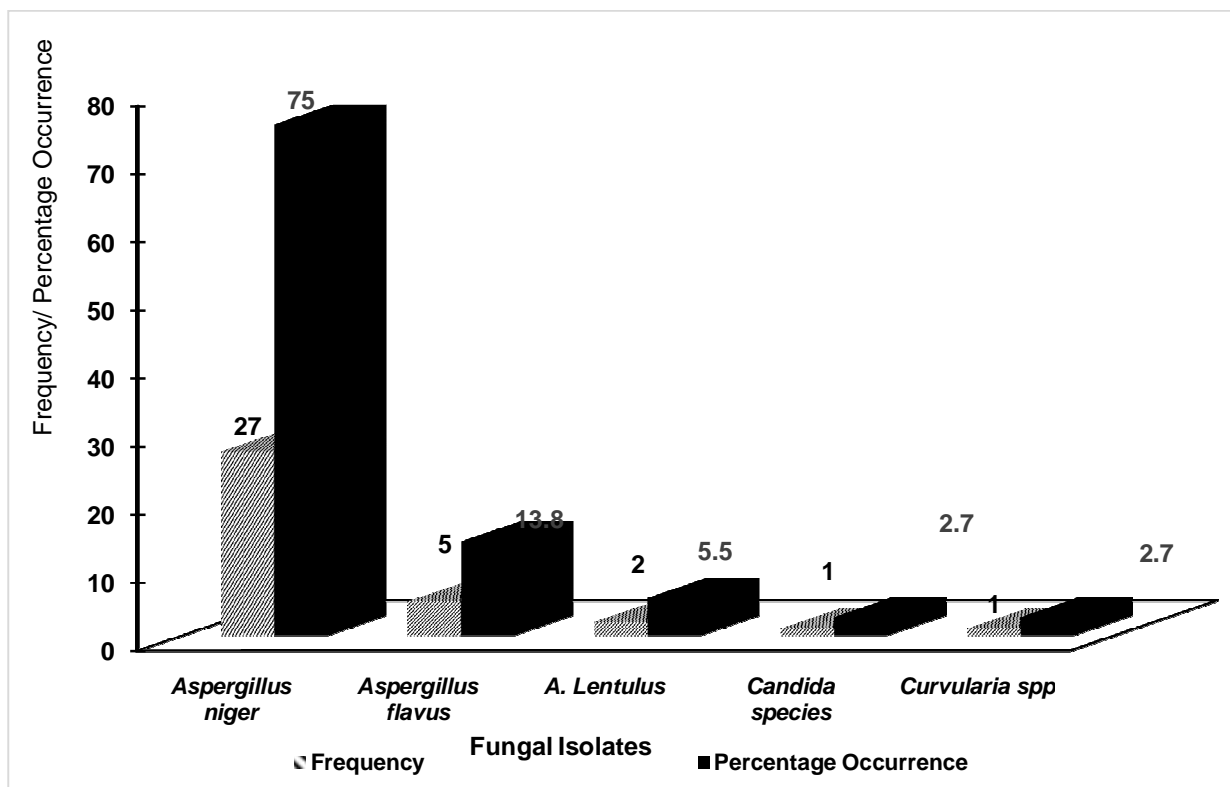


Fig. 2: Frequency and Percentage Occurrence of Fungal Isolates

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

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

The negative impact of the oil lubricants on soil microorganisms corroborates the position of Angira *et 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 Safiyanu *et 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 immune compromised 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 hematogeneous dissemination [26]. Some of the species such as *B. Anthracis* are recognized as a potential biological weapon [4]. *Nocardia*, *Corynebacterium*, *Actinomyces*, *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.

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 conducive 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].

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 source of nutrients. This implies that keeping the oils for longer period with these organisms could reduce their shelf life. The utilization of the car lubricants by autochthonous microorganisms could lead to 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

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

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