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ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM ENGINE OIL CONTAMINATED SITE IN BIDA, NIGER STATE, NIGERIA

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ABSTRACT

Purpose: Isolation and identification of microorganisms associated with engine oil contaminated soil was carried out.

Methodology: Eight different mechanic workshops within Bida were selected and soil samples were collected from each site. Ten grams of contaminated soil was added to 100mL of the enrichment medium and was incubated at 30ºC for 4 days. The soil samples from the mechanic workshop were enriched using Mineral salt agar and then subsequently plated out on nutrient agar plates for 24 hours at 30ºC. Spread plate method involving the use of serial dilutions was employed for the isolation of the bacteria. The number of viable bacterial count were determined and expressed in colony forming units (cfu).

Results: The bacterial species isolated were *Micrococcus species*, *Serratia specie* and *Bacillus species Bacillus species* was the most dominant showing a 100% occurrence, followed by *Micrococcus species*. 75% and lastly *Serratia species* with the least of 50%. Pour plate method involving serial dilution into Potato Dextrose Agar with the addition of ampliclox to inhibit the growth of bacterial species. The fungal species isolated were *Aspergillus niger*, *Aspergillus terreus, Alternaria alternata*.

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Unique contribution to theory, practice and policy: From, the data obtained, *Bacillus species* and *Aspergillus niger* are most adapted to conditions present in soils contaminated with used engine oil and hence could be exploited in bioremediation activities.

Key words: *Engine oil, Micrococcus species, Aspergillus niger, Mechanic workshop, Bida.*

1.0 INTRODUCTION

Engine oil could simply be defined as a thick mineral liquid applied to a machine or engine so as to reduce friction between the moving parts of the machine (Shahida *et al*., 2015). Used engine oil as the name implies represent oil that has undergone destructive changes in the property when subjected to oxygen, combustion gases, and high temperature. The said oil also undergoes viscosity changes as well as additive depletion and oxidation (Mark *et al*., 2018). The disposal of spent engine oil (SEO) into gutters, water drains, open plots and farms is a common practice in Nigeria especially by motor mechanics.

This indiscriminate disposal of spent engine oil adversely affect plants, microbes and aquatic lives (Nwoko *et al*., 2015; Adenipekun *et al*., 2018) because of the large amount of hydrocarbons and highly toxic polycyclic aromatic hydrocarbons contained in the oil (Wang *et al*., 2016; Vwioko and Fashemi, 2015). Heavy metals such as vanadium, lead, aluminium, nickel and iron which are found in large quantities in used engine oil may be retained in soil, in form of oxides, hydroxides, carbonates, exchangeable cation and/or bound to organic matters in the soil (Ying *et al*., 2017).

These heavy metals may lead to build up of essential organic (carbon, phosphorous, calcium, magnesium) and non-essential (magnesium, lead, zinc, iron, cobalt, copper) elements in soil which are eventually translocated into plant tissues (Vwioko *et al*., 2016). Although heavy metals in low concentration are essential micronutrients for plants, but at high concentrations, they may cause metabolic disorder and growth inhibition for most of the plant species (Yadav, 2018). According to Nwadinigwe and Onwumere (2016), contamination of soil arising from oil spills affect the growth of plants and causes great negative impacts on food productivity (Onwurah *et al*., 2007).

Microbial degradation is the major mechanism for the elimination of used petroleum products from the environment. Soils contain very large numbers of microorganisms which can include a number of hydrocarbons utilizing bacteria and fungi. Hydrocarbon or oil biodegradation as a process makes use of natural microbial biodegradative activities and this often employs the enzymatic capabilities of indigenous hydrocarbon-degrading microbial populations and modifying environmental factors (Atlas, 2019). One major requirement for oil biodegradation is the presence of microorganisms with the appropriate metabolic capabilities.

Soil contaminated by used lubricating oil is rapidly increasing due to global increase in the usage of petroleum products. Furthermore, presence of different types of automobiles and

machinery results in an increase in the usages of lubricating oil (Ameen *et al.,* 2018). Hydrocarbon contamination of the soil especially by Polyclic Aromatic Hydrocarbons (PAHs) attracts public attention because many PAHs are toxic, mutagenic and carcinogenic. Prolonged exposure to high oil concentration may cause the development of liver or kidney diseases, possible damage to the bone marrow and an increased risk of cancer (Olukunle and Boboye, 2016).

2.0 MATERIALS AND METHOD 2.1 Sample Collection

The Soil samples analysed were taken from 8 different mechanic workshops that had heavy spillage of used engine oil within Bida. A sterile hand trowel was used to collect the soil sample at a depth of 10 cm into clean labelled plastic bags and conveyed to microbiology laboratory of Federal Polytechnic, Bida. The pH and temperature of the soil sample was taken immediately using a pH meter and a handheld thermometer respectively

2.2 Isolation of Degrading Microorganisms

The isolation of oil degrading bacteria from engine oil contaminated soils were carried out using mineral salt agar (1.8g K_2HPO_4, 1.02g KH_2PO_4, 4.0g NH_4Cl, 2.0g $MgSO_4.7H_2O$, 0.1g NaCl, 0.1g Yeast extract, 0.05g $FeCl_2$ and trace elements consisting of 0.1g H_3BO_3, 0.1g $ZnSO_4$ and 0.4g $MnSO_4.H_2O$ in 1L of sterile distilled water) while the fungi were isolated using Potato dextrose agar (PDA). A 10 g of each soil sample were separately added into a sterile conical flask containing 90 mL of 0.1% peptone water. The mixture were allowed to stand for 30minutes and serial dilution was done in ten folds. A 1 mL of each dilution of the sample was aseptically pipetted into the respective labelled sterile petri dishes in duplicate.

The prepared mineral salt agar and PDA was sterilized using an autoclave at 121°C for 15 minutes and allowed to cool in water bath set at 40°C. Sterile 50 mg/mL of fulcin tablet and Ampliclox 25 mg/mL was incorporated into mineral salt agar and PDA respectively. The media was poured in Petri dishes containing the samples aseptically. The mineral salt agar plates were incubated at 37°C for 24 hours while PDA plates were incubated in a dark locker for 3-5 day. The colonies which developed were counted using Colony Counter and purify by subculturing and stored in nutrient ager and PDA slants (Omotayo *et al*., 2012).

2.4 Morphological Characterization

Potato dextrose agar (PDA) medium was prepared and poured into bijou bottle, then autoclaved and allowed to solidify in a slant position. The fungal cultures was inoculated in the slant. The prepared stocked cultures was stored in a refrigerator at 4°C (Jai *et al*., 2015). The fungal isolates was characterized on the basis of cultural characteristics and morphological characteristics including spore type, mycelia and other fruiting bodies in a lactophenol cotton blue wet mount by compound microscope at magnification of ×100. The observed characteristics was recorded and compared with the established identification key (Barnett and Hunte, 2018).

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2.5 Identification of Isolates

The pure culture of the isolate were examined for cultural characteristic as well as its biochemical and morphological characteristics such as Gram reaction spore staining, catalase test, coagulase test and carbohydrate fermentation test.

3.0 RESULT 3.1 Physicochemical characteristics of contaminated soil

The physicochemical characteristics of soil samples collected from 8 different mechanics workshop used for the study were analyzed and subsequently tabulated in Table 2. The various characteristics like texture, temperature, electrical conductivity and pH were taken into consideration for each of the sample which were named S1, S2, S3, S4, S5, S6, S7 and S8. The highest pH value (6.9) which was reported was in S2 and the lowest (6.2) was in S6

Table 1: Physicochemical properties of contaminated soils

S/No	Properties	S1	S2	S3	S4	S5	S6	S7	S8
1	Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
2	pH	6.3	6.9	6.4	6.7	6.5	6.2	6.5	6.3
3	Temperature	31.9^0C	30.9^0C	32.4^0C	30.2^0C	31.6^0C	31.1^0C	31.0^0C	32.3^0C
4	Electrical conductivity	1.25	0.60	0.20	1.28	1.30	0.78	0.92	1.30

S1 = mechanic workshop in Ilorin garage along Mokwa road

S2 = mechanic workshop near Zallaks Eatery

S3 = mechanic workshop opposite Sogbafo quest inn

S4 = mechanic workshop opposite access bank

S5 = mechanic workshop close to nepa office Lemu road

S6 = mechanic workshop Barracks road close to project quarters

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S7 = mechanic workshop at Esogzhi

S8 = mechanic workshop beside first bank

3.2 Total viable count

The total viable bacteria count (cfu/g) of the engine oil contaminated soil obtained in this study as shown in Table 1 ranged from $6.67x10^9$ to $1.87x10^{10}$ cfu/g. There were no significant differences ($p>0.05$) in the soil collected from S1, S2, S3, S4, S5, S7 and S8 but these differed significantly ($p<0.05$) from the total viable count of the soil collected from S6.

Table 1 also showed the fungal count of the engine oil contaminated soil collected from 8 mechanic sites in Bida and these ranged from <10 to $6.67x10^8$ cfu/g. The fungal count of the soil collected from S6 site was generally low (<10) but however, the fungal count of the soil collected from the rest sites $3.33x10^8$ to $6.67x10^8$ were observed not to differ ($p>0.05$). **Table 2: Total viable and fungal count of engine oil contaminated sites in Bida town**

Site of collection	Total viable count	Fungal count
S1	$1.87x10^{10} \pm 1.87x10^{10}$	$6.67x10^8 \pm 6.67x10^8$
S2	$1.60x10^{10} \pm 1.60x10^{10}$	$6.67x10^8 \pm 6.67x10^8$
S3	$1.33x10^{10} \pm 1.33x10^{10}$	$3.33x10^8 \pm 3.33x10^8$
$4	$1.07x10^{10} \pm 1.07x10^{10}$	$3.33x10^8 \pm 3.33x10^8$
S5	$1.07x10^{10} \pm 1.07x10^{10}$	$3.33x10^8 \pm 3.33x10^8$
S6	$6.67x10^9 \pm 6.67x10^9$	<10
S7	$1.33x10^{10} \pm 1.33x10^{10}$	$3.33x10^8 \pm 3.33x10^8$
S8	$1.87x10^{10} \pm 1.87x10^{10}$	$6.67x10^8 \pm 6.67x10^8$

Each data is the mean ± standard error of triplicate determination, Different letters within the soil colonies are significantly different ($p<0.05$).

S1 = mechanic workshop in Ilorin garage along Mokwa road
S2 = mechanic workshop near Zallaks Eatery
S3 = mechanic workshop opposite Sogbafo quest inn
S4 = mechanic workshop opposite access bank
S5 = mechanic workshop close to nepa office Lemu road

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S6 = mechanic workshop Barracks road close to project quarters
S7 = mechanic workshop at Esogzhi
S8 = mechanic workshop beside first bank

3.3 Morphological characteristics of isolates

Morphologically, the colonies of *Micrococcus* spp which were isolated were pigmented in shades of yellows, its cells were also rhizoidal, opaque, rough and raised. Its cells were spherical in shape occurring in irregular clusters and not in chains. This helps to differentiate them from other Gram positive cocci. The bacteria were catalase positive, non-motile, non-sporulating and Gram positive. *Serratia* spp produced red pigments on nutrient agar plates with a weak elevation after 24hrs incubation. Biochemically, *Serratia* spp is rod shaped bacterium which reacted negatively to the gram stain and also motile. *Bacillus* spp on nutrient agar which were isolated produced cream, circular, entire, opaque, flat and rough edges. Microscopically, they were seen as gram positive long rods with a centre spore.

Table 3: Gram reaction of the bacteria isolated from engine oil contaminated site

No of samples/dilution		Gram reaction	Cell shape
S1	10^{-3}	+	Rod
	10^{-5}	+	Spherical
	10^{-9}	-	Rod
S2	10^{-3}	+	Rod
	10^{-5}	-	Rod
	10^{-9}	*	*
S3	10^{-3}	+	Spherical
	10^{-5}	+	Rod
	10^{-9}	*	*
S4	10^{-3}	+	Spherical
	10^{-5}	+	Rod
	10^{-9}	*	*
S5	10^{-3}	+	Spherical
	10^{-5}	+	Rod
	10^{-9}	*	*
S6	10^{-3}	+	Rod
	10^{-5}	+	Spherical
	10^{-9}	*	*

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S7	10^{-3}	-	Rod
	10^{-5}	+	Rod
	10^{-9}	*	*
S8	10^{-3}	+	Spherical
	10^{-5}	-	Rod
	10^{-9}	+	Rod

Keys: + = positive reaction - = negative reaction *= could not be identified **Table 4: Biochemical characteristics of bacteria isolates**

Sample no	**Spore staining**	**Coagulase**	**Catalase**	Sugar fermentation test	
		Glucose	**Sucrose**		
S1 10^{-3}	+	+	+	+	+
10^{-5}	- - + - -	10^{-9}	- + + + +		
S2 10^{-3}	+	+	+	+	+
10^{-5}	- + + + +	10^{-9}	* * * * *		
S3 10^{-3}	- - + - -	10^{-5}	+ + + + +	10^{-9} * * * * *	
S4 10^{-3}	- - + - -	10^{-5}	+ + + + +	10^{-9} * * * * *	
S5 10^{-3}	- - + - -	10^{-5}	+ + + + +	10^{-9} * * * * * S6	10^{-3} + + + + +
10^{-5}	- - + - -	10^{-9}	* * * * *		
S7 10^{-3}	-	+	+	+	+
10^{-5}	+ + + + +	10^{-9}	* * * * *		
S8 10^{-3}	- - + - -	10^{-5}	- + + + +		
10^{-9}	+	+	+	+	+

Keys: + = positive - = negative * = could not be identified

Table 5: Bacteria isolated from engine oil contaminated sites

Sample no	**Organisms**

S1	10^{-3}	*Bacillus* species
	10^{-5}	*Micrococcus* species
	10^{-9}	*Serratia* spp
S2	10^{-3}	*Bacillus* species
	10^{-5}	*Serratia* spp
	10^{-9}	*
S3	10^{-3}	*Micrococcus* species
	10^{-5}	*Bacillus* species
	10^{-9}	*
S4	10^{-3}	*Micrococcus* species
	10^{-5}	*Bacillus* species
	10^{-9}	*
S5	10^{-3}	*Micrococcus* species
	10^{-5}	*Bacillus* species
	10^{-9}	*
S6	10^{-3}	*Bacillus* species
	10^{-5}	*Micrococcus* species
	10^{-9}	*
S7	10^{-3}	*Serratia* spp
	10^{-5}	*Bacillus* spp
	10^{-9}	*
S8	10^{-3}	*Micrococcus* species
	10^{-5}	*Serratia* spp
	10^{-9}	*Bacillus* spp

* = could not be identified

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Table 6: Percentage of Bacterial isolate obtained from engine oil contaminated soil

Percentage of No of samples		*Micrococcus* spp	*Serratia* spp	*Bacillus* spp
S1	10^{-3}	-	-	+
	10^{-5}	+	-	-
	10^{-9}	-	+	-
S2	10^{-3}	-	-	+
	10^{-5}	-	+	-
	10^{-9}	-	-	-
S3	10^{-3}	+	-	-
	10^{-5}	-	-	+
	10^{-9}	-	-	-
S4	10^{-3}	+	-	-
	10^{-5}	-	-	+
	10^{-9}	-	-	-
S5	10^{-3}	+	-	-
	10^{-5}	-	-	+
	10^{-9}	-	-	-
S6	10^{-3}	-	-	+
	10^{-5}	+	-	-
	10^{-9}	-	-	-
S7	10^{-3}	-	+	-
	10^{-5}	-	-	+
	10^{-9}	-	-	-
S8	10^{-3}	+	-	-
	10^{-5}	-	-	+
	10^{-9}	-	+	-
Percentage occurrence		75%	50%	100%

Keys: + = present - = absent

3.4 Macroscopic and Microscopic analysis

Analysis of *Aspergillus niger, Aspergillus terreus* and *Alternaria alternate* isolated from enginge oil contaminated soil sample for morphological and cultural characteristics showed that there was

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variation in the colony colour, margins, texture and colony reverse colour. In The morphological characteristics of fungi isolates which are *A. niger, A. terreus* and *Alternaria alternate* are depicted in Table 7 after macroscopic examination, the isolated species were examined for their microscopic characteristics and are presented in Table 7

Table 7: Morphological Characteristic of Fungi isolated from engine oil contaminated soil in Bida

Characteristics	*Aspergillus niger*	*Aspergillus terreus*	*Alternaria alternate*
Surface colour	Pin-like, dark-brown black growth	Pinkish cinnamon deeper with age	Dark green deeply grown colonies
Margins	Entire	Entire	Entire
Reverse side	Creamy white to yellow surface	Pale to bright yellow to deep brown	Jet black oil-drop like colony
Elevations	Umbonate	Umbonate	Umbonate
Growth	Rapid	Moderate to rapid	Rapid

Table 8: Microscopic characteristics of Fungi isolated from engine oil contaminated soil in Bida

Fungal isolate	Microscopic view	Fruiting body
Aspergillus niger	Non-branched conidiophores with bulb end carring conidia like sun rays	Cleistothecia present
Alternaria alternate	Pineapple-like conidia, multi-cellular, septated horizontally and vertically arrange in chains	Cleistothecia absent

Aspergillus terreus	Branched septate conidiophores with bulb end carrying conidia	Cleistothecia present

Table 9: distribution of isolated species of fungal at 8 engine oil contaminated soil site in Bida.

Species/Location	S1	S2	S3	S4	S5	S6	S7	S8
Aspergillus niger	+	-	+	+	-	-	+	-
Aspergillus terreus	+	-	-	-	-	-	-	+
Alternaria alternate	-	+	-	-	+	-	+	-

Keys: + = present - = absent

Table 10. Percentage Occurrence of Fungal Organisms

S/No	Isolates	Number of Isolated	Percentage (%)
1	*Aspergillus niger*	16	45.71
2	*Aspergillus terreus*	8	22.86
3	*Alternaria alternate*	11	31.43
Total		35	100

4.0 DISCUSSION

Three bacterial isolates, namely *Bacillus specie, Micrococcus specie*, and *serratia specie* and three fungal isolates, namely *Aspergillus niger, Aspergillus terreus* and *Alternaria alternata* were obtained from engine oil contaminated soil in this study. An increase in oil degradation was corresponding to an increase in cell number during the degradation processes demonstrating the ability of utilizing engine oil as the energy source. The result is in correlation with the work

reported by Mandri and Lin, (2017), Khan and Rizvi (2015) and Abioye *et al*., (2016) who isolated Pseudomonas, Bacillus, Micrococcus and other bacterial strains from engine oil contaminated soil. Pseudomonas, Bacillus, and Rhodococcus were isolated from engine oil contaminated soil as reported by Ogunbayo *et al*., (2016). Some of the fungal isolates have earlier been reported as hydrocarbon utilizers by April *et al*., (2018) Obire *et al*., (2015) and George *et al*., (2019).

The data show an obvious influence of waste engine oil discharge on the microbiological and physiochemical properties of soil. The relatively low heterotrophic bacterial counts observed in oil contaminated soils can be attributed to the toxic or un-favorable effect of oil contamination (Akoachere *et al*., 2018). The ability to isolate high numbers of certain oil degrading microorganisms from oil polluted environment is commonly taken as evidence that these microorganisms are the active degraders in the environment. Although, hydrocarbon degraders may be expected to be readily isolated from an oil associated environment, the same be expected to be readily isolated from an oil associated environment, the same degree of isolates could be gotten from a totally unrelated environment such as pristine soil (Akoachere *et al*., 2018).

In motor mechanics workshops there is a constant change in the soil micro-organism as a result of deliberate spillage of used engine oil. These alter the biomass and ecology of the soil such that both microbial communities and grasses can no longer grow on the soil spots. The colour and texture of the soil are affected; this leads to different microbial flora establishment in an attempt to remedy the petroleum product spillage (Megharaj *et al*., 2017). Although some studies have shown that, oil-polluted soils are dominated by Gram negative bacteria (McNaughton *et al*., 2016), the dominant culturable hydrocarbon utilizing bacteria from the soil samples were made up of gram positive Bacillus and Micrococcus and also gram negative Serratia. The results of the present study revealed that Bida soil may harbor hydrocarbon degraders that have been exposed to hydrocarbons as a result of the indiscriminate disposure of the spent engine oil collected from the crankcase of motor vehicles, motor bikes and other machinery in Bida metropolis. It was observed that the Bacillus specie played a significant role in hydrocarbon degradation having shown dominance in all the test samples. This observation is consistent with the works of Udeani *et al*., (2019) and Makut *et al*., (2018).The presence of Micrococcus were also indicated in six out of the eight samples showing on 75% occurrence. From this study, this shows that these microorganisms are also active degraders of petroleum hydrocarbon from soil. From this study, *Serratia specie* had the lowest occurrence of 50%, showing the least degrading capabilities. Although this contradicts the works of Akoachere *et al*., (2018) who reported that of all the isolates which were gotten, *Serratia specie*. Degraded the highest amount of oil (36.2%), It is in line with the works of McNaughton et al., (2018). The result of this study showed that these microorganisms could be used in bioremediation of engine oil contaminated soil.

5.0 CONCLUSION

The study revealed that *Bacillus species*, *Micrococcus species*, *Serratia species*, *Aspergillus niger*, *Aspergillus terreus* and *Alternarai alternata* were isolated from soils contaminated with used engine in Bida Metropolis. The result of this study indicates that indigenously it is possible to isolate bacterial and fungal micro flora capable of degrading complex hydrocarbon compounds (used engine oil). This study provides information that would lead to selection of bacterial and fungal species that could be employed for bioremediation in environments polluted with used engine oil. It can therefore be concluded that oil-degrading microbes are abundant in soils in Bida. This can be exploited for large oil-spill clean-up campaigns. This study also provides information on the physiochemical requirements for optimum degradation by these microorganisms.

6.0 RECOMMENDATIONS

The following recommendations are being proposed for consideration, further research could be conducted to compare the performance or efficiency of oil degrading microbes in other locations. Similar research or study could be carried out using fresh engine oil and the results compared with that of the used and finally, further research could be carried out to determine the amount of engine oil utilizable by microbes in a given time.

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