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Isolation of Oil – Degrading Microorganisms in Spent Engine Oil – Contaminated Soil

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Abstract
The isolation and characterisation of oil-degrading microorganisms in spent engine oil-contaminated soil were carried out. The density of heterotrophs ranged from 0.2×10^4 cfu/g to 62.5×10^6 cfu/g for bacteria and 1.5×10^3 cfu/g to 1.5×10^5 cfu/g for fungi. The density of oil-degraders in the soil samples ranged from 0.03×10^3 cfu/g to 5.0×10^3 cfu/g for bacteria and 1.0×10^2 cfu/g to 3.0×10^3 cfu/g for fungal isolates. *Micrococcus* species were the best bacterial oil-degraders while *Aspergillus* and *Penicillium* species degraded the greatest amount of oil among the fungi. *Micrococcus* and *Pseudomonas* species were the most predominant organisms in the soil while *Klebsiella* and *Bacillus* species occurred less frequently. The concentration of oil correlated with the density of microorganisms in the soil sample.
Keywords: Oil Degradation, Bacteria, Fungi.

INTRODUCTION

In Nigeria, it is common among motor mechanics to dispose spent engine oil into gutters, water drains and soil (Okonokhua *et al*., 2007). Spent engine oil is defined as used lubricating oils obtained after servicing and subsequently draining from automobile and generator engines. Spent oils contain high percentage of aromatic and aliphatic hydrocarbons, nitrogen and sulphur compounds and metals (Mg, Ca, Zn, Pb) than fresh oils, these metals are introduced into the oil as a result of wear and tear of the engine (Mohd *et al*., 2011).
Spent engine oil causes great damage to soil and soil microflora. It creates unsatisfactory condition for life in the soil due to poor aeration, immobilization of soil nutrients and lowering of soil pH (Ugoh and Moneke, 2011). It has been shown that marked changes in properties occur in soil contaminated with hydrocarbon; this affects the physical, chemical and microbiological properties of the soil (Okonokhua *et al*., 2007). At low concentrations, some of these heavy metals are essential micronutrients for plants, but they can cause metabolic disorders and growth inhibition when the concentration is high. Therefore, there is the need for bioremediation of hydrocarbon contaminated soil. This study was therefore carried out to isolate hydrocarbon – degraders in soil samples contaminated with used engine oil.

MATERIALS AND METHODS

Sample Collection
Soil samples contaminated with spent engine oil were collected from a mechanic workshop along Opopo gbooro, Iworoko Road, Ado-Ekiti. The samples were collected from the same spot but different depths (5cm, 10cm, 30cm, 50cm and 60cm). The age of oil dumping at this site was 3years (Personal communication with Head Mechanic).
Isolation of Microorganisms
Each of the samples from different depths was prepared by serial dilution and pour plate technique. Malt Extract Agar (MEA) medium was used for the culturing of fungi while Nutrient Agar (NA) medium was used for the culturing of bacteria, the media were prepared according to the manufacturers specifications. The pure cultures were identified by their morphology and colony characteristics. The organisms were maintained on MEA and NA slants for fungi and bacteria respectively and stored at 4^0C.
Isolation of Oil Degrading Microorganisms
This was carried out using Minimal Salt Oil Agar. Aliquots (0.1ml) of soil sample suspension were inoculated on Minimal Salt Oil Agar by spread plate method and incubated at room temperature until colonies developed. Discrete colonies were transferred to Nutrient Agar plates and incubated for 24h at 37^0C for bacteria and room temperature for fungi. Pure cultures were stored in Nutrient Agar slants at 4^0C until required.
Identification of Bacterial Isolates
The bacterial isolates were presumptively identified by means of macroscopic, microscopic and some biochemical characterization and then compared using "Bergey's manual of determinative bacteria" (Buchanan and Gibbons, 1974)
Identification of Fungal Isolates
The fungal isolates were identified by morphological characteristics and microscopic examination. Among the characteristics used were colonial characteristics such as surface appearance and colour of the colonies. Microscopy examination revealed the type of hyphae i.e. septate or aseptate, and the vegetative mycelia and appropriate references were then made.

Cultivation of the Isolates for Oil Degradation

The quantity of motor oil degraded was determined by loss in weight of motor oil added to the medium in Universal (MacCartney) bottles. The isolates were dispersed on a minimal salt medium (5ml) containing 1ml of sterile motor oil in MacCartney bottles The cultivation was carried out for twenty-eight (28) days at room temperature but the oil degradation by each isolate was assayed weekly.

RESULTS

The result showed that nine (9) organisms with hydrocarbon - degrading abilities were isolated, out of which four (4) were fungi and the other 5, bacteria. A total of 5 samples were collected from 5 different depths but the same spot in a mechanic workshop. Heterotrophic bacterial counts in the contaminated samples ranged from 0.2 $\times 10^4$ to 62.5 $\times 10^5$ cfu/g and fungal counts ranged from 1.5 $\times 10^3$ to 1.5 $\times 10^5$ cfu/g soil samples. Hydrocarbon – utilizing bacterial counts in the samples ranged from 0.03 $\times 10^3$ to 5.0 $\times 10^3$ cfu/g and fungal counts ranged from 1.0 $\times 10^2$ to 3.0 $\times 10^2$ cfu/g soil samples. The density of heterotrophs exceeded the density of hydrocarbon utilizers. This is because the few hydrocarbon utilizers will breakdown or utilize petroleum hydrocarbon while the non-hydrocarbon utilizers will utilize the products of hydrocarbon utilization for cell growth. Tables 1 and 2 show bacterial and fungal counts of soil samples obtained from the five different depths as well as the ratio of heterotrophs to hydrocarbon utilizers respectively.

Table 1: Mean densities of heterotrophic and hydrocarbon – utilizing bacteria in the soil samples

Sample depth	Total heterotrophs	Total hydrocarbon – utilizers	Ratio of heterotrophs to hydrocarbon – utilizers
Sample A (5cm)	62.5× 10^5 cfu/g	5.0 × 10^3 cfu/g	1:12500
Sample B (10cm)	1.4× 10^5 cfu/g	0.2 × 10^3 cfu/g	1:700
Sample C (30cm)	0.45× 10^5 cfu/g	0.12 × 10^3 cfu/g	1:375
Sample D (50cm)	0.55× 10^4 cfu/g	0.04 × 10^3 cfu/g	1:137.5
Sample E (60cm)	0.2× 10^4 cfu/g	0.03 × 10^3 cfu/g	1:66.67

Table 2: Mean densities of heterotrophic and hydrocarbon – utilizing fungi in the soil samples

Sample depth	Total heterotrophs	Total hydrocarbon – utilizers	Ratio of heterotrophs to hydrocarbon – utilizers
Sample A (5cm)	1.5 × 10^5 cfu/g	3.0 × 10^2 cfu/g	1:500
Sample B (10cm)	5.0 × 10^4 cfu/g	2.3 × 10^2 cfu/g	1:217.39
Sample C (30cm)	3.5 × 10^4 cfu/g	2.0 × 10^2 cfu/g	1:175
Sample D (50cm)	4.5 × 10^3 cfu/g	1.5 × 10^2 cfu/g	1:30
Sample E (60cm)	1.5 × 10^3 cfu/g	1.0 × 10^2 cfu/g	1:15

The mean density of hydrocarbon utilizers is proportional to the concentration of engine oil. The higher the concentration of oil, the higher the density of hydrocarbon utilizers. The result is as shown in Table 3

Table 3: Spectrophotometric determination of engine oil contents of various soil samples

Soil sample	% oil concentration
5cm	30
10cm	12
30cm	8
50cm	2
60cm	1

Identification and characterization of isolates from soil samples:

The isolates were identified on the basis of their cellular and colonial morphologies as well as biochemical characteristics as earlier described. The bacteria isolated are *Pseudomonas*, *Klebsiella*, *Bacillus*, *Micrcoccus* and *Proteus* (Table 4). The fungi isolated are *Streptomyces*, *Penicillium*, *Cheatomium* and *Aspergillus* (Table 4). The biochemical reactions of the bacterial isolates and the taxonomic characteristics of fungi isolates are shown in Tables 5 and 6 respectively.

Table 4: Bacterial and fungal isolates from engine oil contaminated soil

Isolates	5cm	10cm	30cm	50cm	60cm	Percentage occurrence
Pseudomonas	+	-	-	+	+	60%
Klebsiella	+	-	-	-	-	20%
Bacillus	-	+	-	-	-	20%
Micrcoccus	-	+	+	+	-	60%
Proteus	-	-	+	-	+	40%
Streptomyces	+	-	-	-	-	20%
Penicillium	-	+	-	+	-	40%
Cheatomium	-	-	+	-	-	20%
Aspergillus	-	-	-	-	+	20%

Table 5: Biochemical characteristics of bacterial isolates

Identification Parameters	*Pseudomonas*	*Klebsiella*	*Bacillus*	*Micrcoccus*	*Proteus*
Gram stain	-	-	+	+	-
Lactose	F	F	F	NF	NF
Glucose	F	F	F	F	F
Maltose	F	F	F	F	NF
Sucrose	F	F	F	F	F
Oxidase	+	-	+	+	+
Catalase	+	+	+	+	+
Motility	+	-	-	-	+
Nutrient Gelatin	+	-	+	+	+
Citrate Utilization	+	-	+	-	+
Indole Test	-	-	-	-	-
Urease Activity	-	-	-	-	+

KEY: + = Positive
- = Negative
F = Fermenter
NF = Non-Fermenter

Table 6: Taxonomic characteristics of fungal isolates

Identification Parameters	*Streptomyces*	*Penicillium*	*Cheatomium*	*Aspergillus*
Morphological characteristics	Colonies are leathery at first, then wrinkled and covered with chalky white aerial hyphae.	Colonies are first white, then powdery with blue-green pigment bothered in white.	It first appeared white, then tan-gray with cottony texture	Colonies appeared brown
Microscopical characteristics	Long, slender, branching hyphae	Hyphae appeared hyaline,branching and septate	Hyphae appeared septate, branched and hyaline	Hyphae are septate

Qualitative determination of oil – degrading activity:
All organisms isolated were tested for their ability to grow on and utilise engine oil as the sole carbon source. Result showed that nine of the isolates were oil degraders, this was confirmed by the production of clear zone around their colonies.
Quantitative estimation of engine oil degradation by the isolates:
The quantities of engine oil degraded by a known weight of the isolates over a period of twenty – eight days at room temperature are as shown in Figs 1 and 2. *Micrococcus* showed the greatest oil degradation ability while *Bacillus* showed the least among the bacterial isolates. On the other hand *Penicillium* showed the greatest oil degradation ability and *Chaetomium* showed the least among fungal isolates.

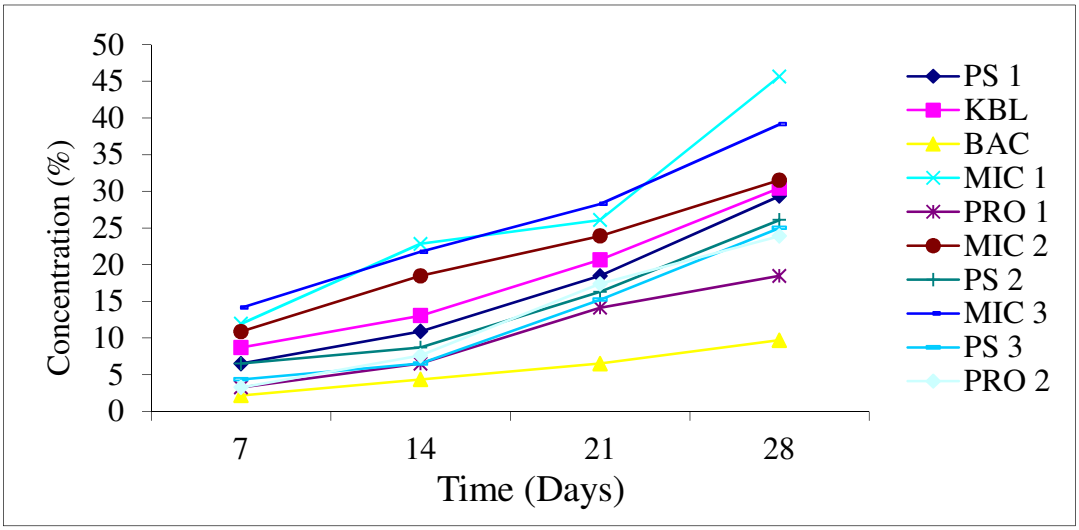

Fig 1: Oil degradation by bacteria isolates

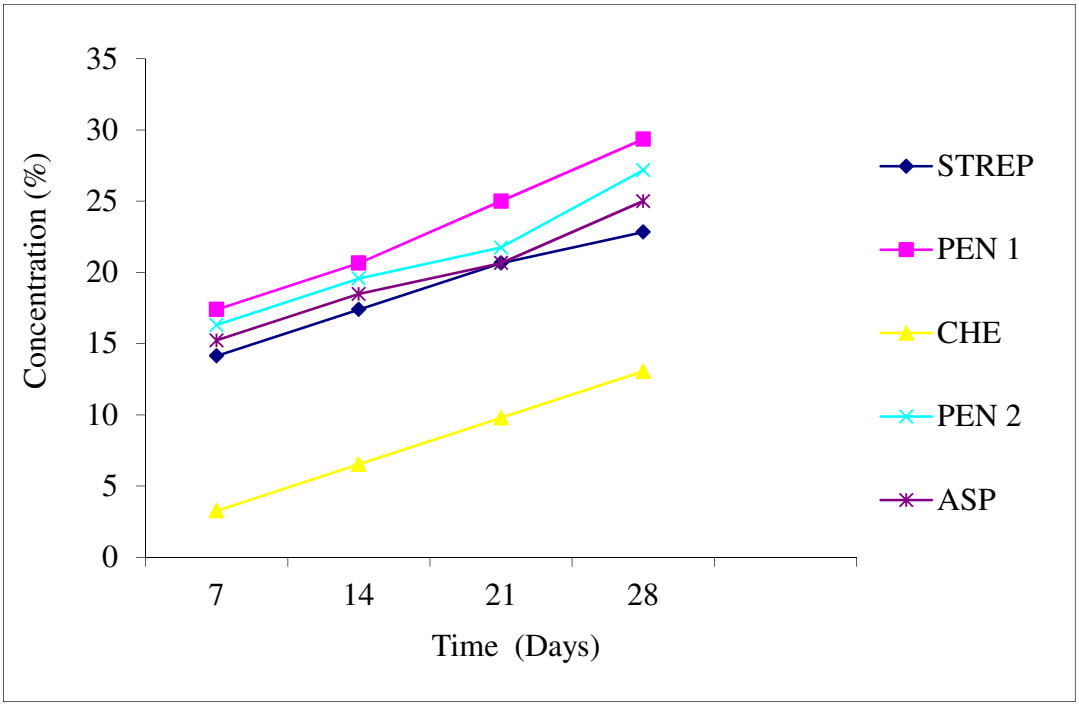

Fig 2: Oil degradation by fungal isolates

DISCUSSION

Five bacterial isolates, namely *Pseudomonas* sp, *Klebsiella* sp, *Bacillus* sp, *Micrococcus* sp, and *Proteus* sp and four fungal isolates, namely *Streptomyces* sp, *Penicillium* sp, *Cheatomium* sp and *Aspergillus* sp 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 (2007), Khan and Rizvi (2011) and Abioye *et al*., (2012) 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*., (2012).

Some of the fungal isolates have earlier been reported as hydrocarbon utilizers by April *et al*., (2000) Obire *et al*., (2008) and George – Okafor *et al*., (2009).

The result of this study showed that these microorganisms could be used in bioremediation of engine oil contaminated soil.

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