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Bioremediation of Engine Oil-Contaminated Soil using Selected Indigenous Moulds

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ABSTRACT

The polycyclic aromatic hydrocarbons (PAHs) utilizing the potential of moulds from engine oil-polluted soils were assessed. These PAHs are known to be toxic, mutagenic and carcinogenic in the environment. Consequently, the study aims to evaluate the potential of indigenous moulds from engine oil in the remediation of contaminated soil. Samples were collected from engine oil-polluted soils from Samonda, Ibadan, Oyo State Nigeria. The ability of four potential indigenous moulds to degrade the PAH fraction of the engine oil-polluted soil sample was assessed using the gas chromatography technique. Four moulds were identified based on their cultural and microscopic characteristics and were confirmed as *Aspergillus flavus, Aspergillus parasiticus*, *Aspergillus oryzae* and *Aspergillus nidulans.* The highest biodegradation potential was observed in treated soil sample containing *Aspergillus flavus* (55.15%) followed by *Aspergillus parasiticus* (55.00%), *Aspergillus oryzae* (44.51%) and the least biodegradation potential was observed in the sample containing *Aspergillus nidulans* (24.16%) after 60 days of treatment. This study revealed that indigenous mould has the ability to utilize toxic fractions of petroleum products as their sole carbon sources therefore are the major bio-remediating agents in the sampling area.

INTRODUCTION

Many indigenous moulds in engine oil-contaminated soil are capable of degrading hydrocarbon contaminants. Leaks and accidental spills of engine oil occur regularly during the exploration, production, refining, transport, and storage. The release of engine oil into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [1]. Bioremediation is the use of microorganisms to detoxify pollutants due to their diverse metabolic capabilities including the products of the petroleum industry [2]. Engine oil is a multifaceted mixture of hydrocarbons as well as other organic compounds, and contains thousands of aliphatic, branched and aromatic hydrocarbons, many of which are toxic to living entities [3]. In Nigeria, dependence on engine oil for automobiles has eventually led to the pollution of the terrestrial and aquatic ecosystems [4, 5]. Polycyclic aromatic hydrocarbons (PAHs) have been recognized as substrates used for microbial growth [6].

Mycoremediation is the natural or artificial process in which fungi processes are used to degrade contaminants to less toxic or non-toxic forms, thereby reducing environmental contamination [7-9]. Moulds belonging to the genera *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Amorphoteca* sp., *Neosartorya* sp., *Paecilomyces* sp., *Talaromyces* sp., *Graphium* sp. have been reported to be used in hydrocarbon degradation [10].

Only a few pilot-scale studies as well as field trials have provided the most convincing demonstrations of bioremediation which have been recorded in the peer-reviewed literature [11-13]. The scope of recent knowledge of oil bioremediation is limited because the emphasis of most of the field findings and reviews is on the assessment of bioremediation for large-scale oil spills on marine shorelines. This study is aimed at evaluating the extent of biodegradation of the polyaromatic hydrocarbon (PAH) fraction using the potential of moulds isolated from engine oil-polluted soils.

MATERIALS AND METHODS

Study site
The study was carried out in Samonda, Ibadan, Oyo State Nigeria.

Collection and handling of soil sample
Engine oil contaminated soil sample was collected from an automobile workshop located in Samonda, Ibadan, Oyo State. The soil sample was collected from 0-15 cm top layer with the aid of a hand trowel into new polythene bags, and transported to the laboratory. It was air-dried for 2 days, broken into smaller pieces and sieved. The processed soil sample was then wrapped in aluminium foil paper and stored in a refrigerator at 4 °C.

Physicochemical properties of the Soil
The soil samples collected were analysed for their texture, pH, organic matter content and moisture content.

Soil pH
Soil pH was determined by using the pH meter, both in water (H_2O) and calcium chloride ($CaCl_2$) soil solution ratio of 1:2:5.

Total organic carbon (T.O.C.)
Organic carbon was determined by the Walkley and Black wet oxidation method [14].

Moisture Content
The moisture content was carried out following procedures described by [15]

Texture
The texture analysis was carried out using the procedure outlined in the Bouyoucos hydrometer method [16].

Identification and characterization of mould isolates
Colony morphology, and fungi (Mold) identification using lactophenol cotton-blue staining technique was carried out and four isolates such as *Aspergillus flavus, Aspergillus parasiticus*, *Aspergillus oryzae* and *Aspergillus nidulans* [17].

Bioremediation experiment
Seven (7) days old pure cultures of the fungal isolates were used. 100 g of the contaminated soil samples were weighed out in duplicates into sterile bottles and labelled appropriately. Then approximately 10 mL of inoculum of pure fungal isolates (approximately 5 mL each from 1st and 2nd washing) were added and 40 mL of sterile distilled water was added into each bottle aseptically to make up approximately 50 mL.

The content of the bottles was stirred aseptically with the aid of a glass rod. The bottles were then sealed with the aid of double-layer foil paper. The incubation was conducted for more than 60 days at room temperature. A contaminated soil sample to which no organism had been added was used as a control and stored aseptically in the refrigerator at 4 ºC. The content of the bottles was stirred aseptically on the 28th day and 45th day of incubation with the aid of a glass rod. Then, the determination of pH and Total Organic Carbon (TOC) content of the treatment soil samples was carried out. Also, the treatment and control soil samples were extracted and analyzed using a Gas Chromatography-Flame Ionization Detector (GC-FID).

Viability test
A viability test was carried out to determine the survival of the inoculated organisms in the polluted soil samples. Exactly 9 mL of distilled water were dispensed each into McCartney bottles. PDA medium and the dispensed distilled water were sterilized. After cooling, The PDA medium was supplemented with Streptomycin Sulphate (0.1 mg/mL) to inhibit bacterial growth. Then, minute quantities (approximately 1 g) of treated soil samples (from B bottles) were aseptically taken with the aid of a sterile spatula and transferred into McCartney bottles, appropriately labelled.
Serial dilution was done to 10^{-1} which was used to inoculate PDA plates (labelled appropriately) using the pour plate method. Plates were carefully swirled and allowed to solidify. Un-inoculated plates served as control. The plates were then incubated for 3 days.

Determination of pH and Total Organic Carbon of treatment Soil Samples
The pH of the treated soil samples was carried out following the same pH and TOC procedures described above.

Gas Chromatography (GC) Method
The technique used for this assay was first ascertained and known as seen in the calibration curves. Treated and control soil samples were analyzed after 60 days of the experiment. The concentration of each constituent of PAH, which includes naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, chrysene, benzo (a) anthracene, benzo (b) fluoranthene, pyrene, benzo (k) fluoranthene, benzo (a) pyrene, indeno[1,2,3-cd]pyrene, dibenzo (a,h) anthracene and benzo (g, h, i) perylene were determined.

Extraction
The extraction method for the analysis of polyaromatic hydrocarbon profiles in samples was done by employing the modified methods of ASTM D3328 and ASTM 3415. Five grams (5.0 g) of the soil sample was weighed into a 250 mL capacity beaker and 20 mL of the ratio 3:1 redistilled hexane:dichloromethane was added. The content and the beaker were placed in the sonicator to extract the hydrocarbon for two hours.
The organic layer was filtered into a 250 mL capacity borosilicate beaker and extraction was repeated twice by using equal volume of the extractants and the supernatants were combined before concentration. The extract was dried by passing the filtrate through the funnel comprising the anhydrous sodium sulphate. A stream of nitrogen gas was used to concentrate the dried extract.

Polyaromatic hydrocarbon (PAH) separation
The glass column was packed with activated alumina, neutral and activity/grade 1 and the concentrated oil was separated into the aliphatic and polyaromatic hydrocarbon profiles. About 10 mL of the alumina that was treated was packed into the column and cleaned with redistilled hexane. Onto the alumina, the extract was poured and allowed to run down with the aid of the redistilled hexane in other to remove the aliphatic profiles into a pre-cleaned 20 mL glass container.
The aromatic fraction was recovered by allowing the mixture of hexane and dichloromethane in ratio 3:1 and finally the most polar PAH by removing the dichloromethane into the pre-cleaned borosilicate beaker. Before the gas chromatography analysis, the mixture was concentrated to 2.0 mL by stream of the nitrogen gas. The rate of biodegradation of PAH fraction was calculated by applying the formula:

$$\text{Degradation (\%)} = \frac{\text{Conc. of control (mg/Kg)} - \text{Conc. of the treated sample (mg/Kg)}}{\text{Concentration of control (mg/Kg)}} \times 100$$

GC conditions of polyaromatic hydrocarbons (PAH)
The GC model used was HP 6890 powered with HP Chem. Station Rev. A09.01 [1206] software with Flame Ionization Detector (FID). HP-1 column was used, column length was 30m, column ID was 0.25µm and column film thickness, 0.25 µm. The injection temperature was 250 ºC and the detector temperature, 320 ºC. The initial temperature was 60 ºC for 5 min (min). The first rate was at 15 ºC/min for 14 min and maintained for 3 min while the second rate was at 10 ºC/min for 5 min and maintained for 4 min. The mobile phase or carrier used was nitrogen gas with pressure of 30 psi in the column, hydrogen pressure of 28 psi and compressed air pressure of 32 psi.

RESULTS

The fungal genera identified were *Aspergillus flavus, Aspergillus parasiticus*, *Aspergillus oryzae* and *Aspergillus nidulans* based on their cultural and microscopic characteristics**.** All the Indigenous moulds were found to degrade the engine oil with *Aspergillus flavus* (55.15%) *and Aspergillus parasiticus* (55.00%) being the major ones while *Aspergillus oryzae* (44.51%) showed moderate degradation. The least degradation was shown by *Aspergillus nidulans* (24.16%). The result of the physicochemical analysis of the soil sample is depicted in **Table 1**. The pH was 6.10, T.O.C (6.707%), and moisture content (1.20%).

Table 1. Characteristics of engine oil-polluted soil sample.

pH	T.O.C. (%)	Moisture Content (%)	Clay (g/ Kg)	Silt (g/Kg)	Fine Sand (g/Kg)
6.10	6.707	1.20	34	68	898

The result of the viability test carried out was positive. The fungal isolates inoculated in polluted soil samples were recovered after 72 h of incubation on PDA plates. The result of the increase in TOC content of polluted soil samples after treatment is shown in **Fig.** 1. Soil samples containing *A. nidulans* HE 1 had the highest TOC content (12.988%) followed by *A. parasiticus* E 28 (12.606%), *A. oryzae* HE 15 (12.561%), and *A. flavus* E 14 (11.269%).

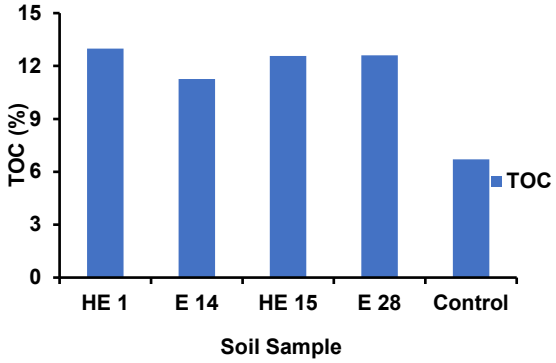

Fig. 1. The increase in total organic carbon content of treated soil samples.

The result of the chromatogram analysis for the degradation of the PAHs in engine oil by the potential mould strains: *Aspergillus flavus, Aspergillus parasiticus*, *Aspergillus oryzae* and *Aspergillus nidulans*. The chromatogram of the treated soil sample using *Aspergillus flavus* (E 14) is shown in **Fig. 2**.

The report of the PAH analysis showed the amount [mg/Kg] of PAH in the sample. Naphthalene ($3.60878e^{-1}$), Acenaphthylene ($2.25308e^{-1}$), Acenaphthene ($1.23048e^{-1}$), Fluorene ($2.06621e^{-1}$), Phenanthrene ($2.21455e^{-1}$), Anthracene ($1.26739e^{-1}$), Fluoranthene ($1.92411e^{-1}$), Pyrene ($5.66201e^{-1}$), Benzo (a) Anthracene ($1.44848e^{-1}$), Chrysene ($7.07440e^{-2}$), Benzo (b) Fluoranthene ($5.27930e^{-3}$), Benzo (k) Fluoranthene ($4.46475e^{-3}$), Benzo (a) Pyrene ($1.05943e^{-3}$), Indeno {1,2,3-cd} Pyrene ($2.16311e^{-3}$), Dibenzo (a,h) Anthracene ($2.67020e^{-3}$), and Benzo (g,h,i) Perylene ($1.05049e^{-3}$).

The chromatogram of treated soil sample using *Aspergillus parasiticus* (E 28) revealed that the amount of Naphthalene was $3.41432e^{-1}$, Acenaphthylene, $2.34814e^{-1}$; Acenaphthene, $1.11722e^{-1}$; Fluorene, $1.61652e^{-1}$; Phenanthrene, $2.12402e^{-1}$; Anthracene, $1.41654e^{-1}$; Fluoranthene, $1.93029e^{-1}$; Pyrene, $5.83446e^{-1}$; Benzo (a) Anthracene, $2.06311e^{-1}$; Chrysene, $5.83348e^{-2}$; Benzo (b) Fluoranthene, $5.57316e^{-3}$; Benzo (k) Fluoranthene, $4.50885e^{-3}$; Benzo (a) Pyrene, $1.32457e^{-3}$; Indeno {1,2,3-cd} Pyrene, $2.80982e^{-3}$; Dibenzo (a,h) Anthracene,$2.49671e^{-3}$; and Benzo (g,h,i) Perylene, $1.33586e^{-3}$ as shown in **Fig. 3**.

Fig. 4 shows the chromatogram of the treated soil sample using *Aspergillus oryzae* (HE 15). The report of the PAH analysis shows the amount [mg/Kg] of PAH in the sample. Naphthalene ($4.95143e^{-1}$), Acenaphthylene ($2.44380e^{-1}$), Acenaphthene ($1.59718e^{-1}$), Fluorene ($3.53482e^{-1}$), Phenanthrene ($2.39144e^{-1}$), Anthracene ($1.29717e^{-1}$), Fluoranthene ($1.97430e^{-1}$), Pyrene ($6.41409e^{-1}$), Benzo (a) Anthracene ($2.37081e^{-1}$), Chrysene ($7.43259e^{-2}$), Benzo (b) Fluoranthene ($6.41168e^{-3}$), Benzo (k) Fluoranthene ($4.69595e^{-3}$), Benzo (a) Pyrene ($1.12496e^{-3}$), Indeno {1,2,3-cd} Pyrene ($2.16965e^{-3}$), Dibenzo (a,h) Anthracene ($2.82901e^{-3}$), and Benzo (g,h,i) Perylene ($1.19836e^{-3}$). The chromatogram of treated soil using *Aspergillus nidulans* (HE 1) is indicated in **Fig. 5**.

The amount [mg/Kg] of PAH in the sample were as follows: Naphthalene ($6.13081e^{-1}$), Acenaphthylene ($3.69074e^{-1}$), Acenaphthene ($2.61415e^{-1}$), Fluorene ($4.04701e^{-1}$), Phenanthrene ($3.66464e^{-1}$), Anthracene ($2.13254e^{-1}$), Fluoranthene ($2.68203e^{-1}$), Pyrene ($8.91264e^{-1}$), Benzo (a) Anthracene ($3.10522e^{-1}$), Chrysene ($9.14423e^{-2}$), Benzo (b) Fluoranthene ($8.25682e^{-3}$), Benzo (k) Fluoranthene ($6.09712e^{-3}$), Benzo (a) Pyrene ($1.21783e^{-3}$), Indeno {1,2,3-cd} Pyrene ($2.86500e^{-3}$), Dibenzo (a,h) Anthracene ($3.46231e^{-3}$), and Benzo (g,h,i) Perylene ($1.89954e^{-3}$).

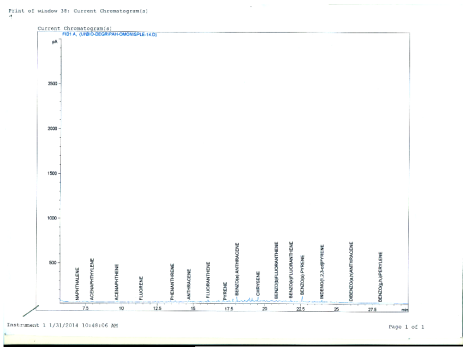

Fig. 2. Chromatogram of PAH degradation using *Aspergillus flavus* (E 14).

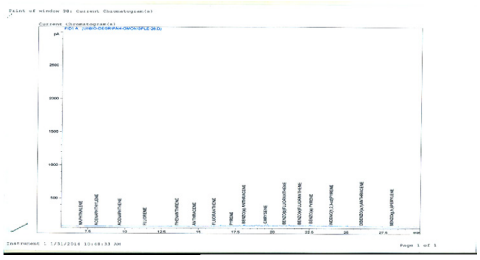

Fig. 3. Chromatogram of PAH degradation using *Aspergillus parasiticus* (E 28).

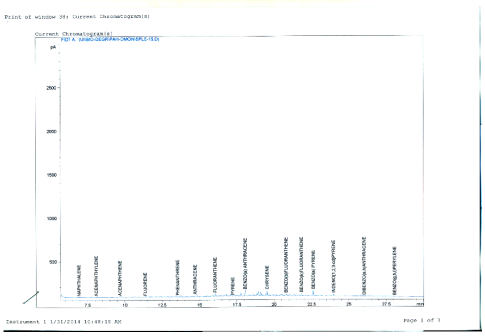

Fig. 4: Chromatogram of PAH degradation using *Aspergillus oryzae* (HE 15).

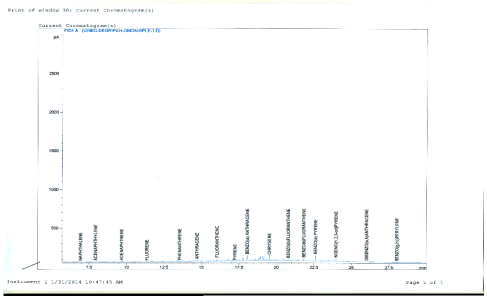

Fig. 5. Chromatogram of PAH degradation using *Aspergillus nidulans* (HE 1).

DISCUSSION

Among the mould isolates that revealed a high potential for hydrocarbon bioremediation, *Aspergillus flavus* and *Aspergillus parasiticus* were found to be the most efficient indigenous mould with the highest degree of biodegradation of engine oil. The high rate of degradation could be due to the fact that they produce resistant spores that thrive in harsh environments. The capacities of these organisms in oxidizing the polycyclic aromatic hydrocarbons (PAHs) could also be attributed to the non-specific nature of their enzymes especially the peroxidases on degrading chemicals.

The positive result of the viability assay reveals that the mould isolates were able to survive the toxicity of the chemicals and the hostile activities of the microbial population in the polluted soil. The TOC level after soil treatment was high and this may be due to enrichment of the soil by the microbial population. In this study, the degradation of the hydrocarbon-polluted soil sample is due to an attack on the aromatic fractions of the polluted soil.

After 60 days of treatment using *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nidulans* and *Aspergillus oryzae*, the concentration of PAH fractions reduced to 2.25494 mg/Kg, 2.26285 mg/Kg, 3.81322 mg/Kg and 2.79026 mg/Kg respectively. This might be attributed to the use of these fractions that is contained in the polluted soil by each of the fungus metabolic processes. Mbachu *et al*. [18], reported that the results of gas chromatographic analysis on PAHs degradation in used engine oil, diesel and petroleum showed that the two isolates *Candida tropicalis* and *Aspergillus clavatus* exhibited biodegradation efficiency above 70% after 16 days of incubation also confirmed their high degradation potentials. This agrees with this study as *Aspergillus flavus* revealed high activity for hydrocarbon bioremediation potentials amongst the four *Aspergillus* species used in this study.

CONCLUSION

The results of the study showed that PAHs utilizing moulds can be isolated from engine oil contaminated soil and these organisms could be used in the remediation of soils polluted by engine oil. Further research should be carried out to compare the effectiveness in the degradation of hydrocarbons using a consortium of indigenous fungi species.

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