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Biodegradation Potentials of Mycoflora Isolated from Auto Mobile Workshop Soils on Flow Station Crude Oil Sludge

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Abstract

The biodegradation potentials of soil mycobiota isolated from six auto mobile workshops and a farmland in Benin City on flow station crude oil sludge was investigated. Serial dilution and pour plate methods were utilized in the isolation and enumeration of the fungal bioload of the soil samples. The heterotrophic fungal counts ranged from 0.2 ×10^3 cfu/g to 3.6 ×10^3 cfu/g. Twenty (20) fungal species were identified from the soil samples; Aspergillus flavus, Aspergillus terrus, Aspergillus fumigatus, Aspergillus versicolor, Emericella nidulans, Aspergillus tamarii, Aspergillus niger, Aspergillus sp., Moniliella sp., Pichia farinosa, Sporobolomyces sp., Candida sp., Rhodotorula sp., Curvularia sp., Mucor sp., Rhizopus stolonifer, Penicillium sp., Penicillium sp.2, Penicillium italicum and Penicillium chrysogenum. A. flavus and A. nidulans had the highest percentage prevalence (85.7%). Physicochemical analyses revealed that the soil samples were acidic (pH 5.81-6.40) and sandy (50.3%-64.80%). Colorimetric screening indicated that Aspergillus flavus, Aspergillus terrus, Aspergillus sp., Penicillium sp., consortium of yeasts and the filamentous fungal consortium were able to maximally utilize the sludge as the sole source of carbon and energy. The growth profile results obtained for A. flavus revealed a decrease in pH (7.2 – 4.8), an increase in turbidity and colony counts (12 FAU – 229 FAU) (1.8 × 10^4 cfu/ml – 3.6 × 10^4 cfu/ml) during the 20 day incubation period. Amongst the growth profile cultures, Aspergillus flavus caused the highest percentage reduction in the residual TPH (DRO) content of the inoculated sludge (84%). Soils within the vicinities of auto mechanic workshops are viable sources of hydrocarbonclastic fungi.

Keywords: Sludge, soil mycobiota, auto mobile workshops, residual TPH (DRO)

Introduction

The petroleum industry is responsible for the generation of huge amount of organic residues, as well as for the pollution of soils, rivers and seas[1]. Large amount of oily sludge is often generated during exploration and refining of crude oil[2]. Oily sludge contains toxic substances like aromatic hydrocarbons (benzene, toluene, ethyl benzene and xylene), Poly aromatic hydrocarbons (PAHs) and high hydrocarbon content[3]. Its disposal without adequate treatment leads to environmental pollution[2]. Oil pollution is a major environmental concern in many countries and this has led to a concerted effort in studying the feasibility of using oil degrading microorganisms for bioremediation[4]. Many workers divide bioremediation strategies into three general categories: i. the target compound is used as a carbon source, ii. the compound is enzymatically attacked but is not used as a carbon source (co-metabolism) and iii. the target compound is not metabolized at all but is taken up and concentrated within the organism (bioaccumulation). Although, fungi participate in all three strategies, they are often more proficient at co-metabolism and bioaccumulation than at using xenobiotics as sole carbon sources[5]. Fungi are found in oil contaminated environments and are known to degrade hydrocarbons[6]. Nyns *et al.*[7] reported that amongst fungi, hydrocarbon assimilation is most common in the orders; *Mucorales* and *Monilales*, as well as in the genera; *Aspergillus* and *Penicillium* (order; *Eurotiales*). Diverse groups of fungi have been isolated from oil contaminated environments and/or have been shown to degrade hydrocarbons in the laboratory[8].

Auto mobile workshops are a common sight in all Nigerian cities and towns, and play an important role in the Nigerian socio- economic dynamics. Auto mobile workshops are facilities where automobiles are usually operated in semi stationary or stationary modes[9]. They abound in most urban cities in Nigeria. Edaphic areas where they are located are exposed to high levels of spent crankcase engine oil and lubricating oils. However, there very little information on the isolation and identification of hydrocarbonclastic fungi from these polluted environments in Benin City, Nigeria. This study was conducted with the aims of isolating and identifying the heterotrophic and hydrocarbonclastic fungal species from soil samples collected from various auto mechanic workshops within Benin City. Determination of the physicochemical characteristics of the soil samples obtained from the respective auto mobile workshops. Screening for the ability of these fungal isolates to degrade crude oil sludge and evaluating the biodegradation potentials of the respective fungal isolates which had given positive results at the end of the respective screening tests.

Material and Methods

Source of Soil samples: Six top soil samples (500g) were collected from six auto mechanic workshops designated by the letters A-F located within different quarters in Benin City with the aid of a standard soil auger. A control soil sample was obtained from a fallow farmland within Benin City. About 1l of molten crude oil sludge was collected from a saver pit at the Nigerian Petroleum Development Corporation (NPDC) production well facility located at Ologbo town, Ikopba Okha Local Government Area, Edo State, Nigeria.

Figure-1a
Map of Nigeria showing Edo State whose administrative head quarters is Benin City

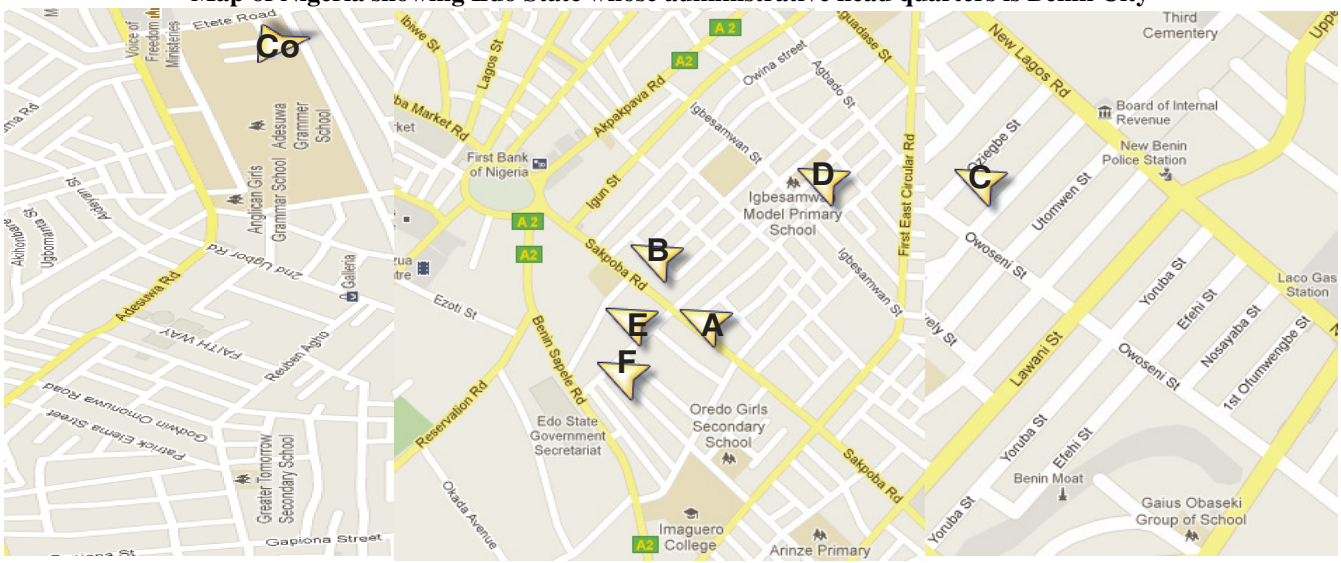

Figure-1b
Map of Benin City showing the respective sampling sites[10]

Legend: **A**: Mechanic workshop soil collection site, **B**: Mechanic workshop soil collection site, **C**: Mechanic workshop soil collection site, **D**: Mechanic workshop soil collection site, **E**: Mechanic workshop soil collection site, **F**: Mechanic workshop soil collection site, **Co**: Control soil sampling site

Enumeration and isolation of heterotrophic and hydrocarbonclastic soil fungi using general and enriched media: One gram of the respective fresh soil samples were weighed and dissolved into 99 ml of sterile prepared peptone water diluent under aseptic conditions[11]. Serial fold dilutions were then made up to 10^{-6} and aliquots of each dilution were cultured on plates of Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Rose Bengal Chloramphenicol Agar (RBCA) and Waksman Agar (WA) by pour plate method[11,12]. The duplicate plates were incubated at room temperature (28 ± 2^0C) for 5 days. An oil sludge based medium; Modified Czapek Dox Agar [12] was also used for the preliminary isolation of petroleum sludge utilizing mycoflora from the respective soils. All the media used were supplemented with erythromycin (500 mg) to discourage bacterial growth[13]. Petroleum sludge agar plates were incubated at 28 ± 2^0C for 9 days. The resultant fungal colonies were enumerated and recorded as colony forming units (cfu) per gram of each soil sample[11].

Characterization of the soil fungi: The cultural characteristics of the purified isolates were noted and the microscopic features of both the filamentous fungal and yeast isolates were observed using the wet mount technique[12,14,15]. Purified cultures were stored in PDA slants for further characterization. Both lactophenol cotton blue and distilled water were used respectively as mountants. The microscopic structures observed were recorded and compared to those stated by Barnett and Hunter[16], Alexopolulos *et al.*[17]. Several biochemical tests such as nitrate utilization, urea hydrolysis, sugar fermentation test[12]; ability to grow at 37^0C and assimilation of carbon compounds[18] were conducted to further characterize the yeast isolates.

Physicochemical analyses of the soil samples: The physiochemical properties of the various soil samples were determined. With the exception of moisture content analysis, the respective soil samples were placed on large wooden trays and air-dried for 72 hours. Lumps of moist soil samples were broken by hand prior to air drying of the samples. The air dried samples were also sieved using a 2 mm mesh. Parameters which included moisture content, pH, particle size distribution and Cation Exchange Capacity (CEC) were determined according to methods stated by Radojevic and Bashkin[19]. The Total Organic Carbon (TOC), total Nitrogen, available phosphorus, heavy metal content (Pb, Zn and Cd) and Total Hydrocarbon Content (THC) of the soil samples were also evaluated in accordance with procedures stated by Onyeonwu[20] and Bremmer and Mulvaney[21].

Screen test of fungal isolates for the ability to utilize petroleum sludge as sole carbon source: The ability of the purified fungal isolates from the respective soil samples to utilize oil sludge as sole carbon and energy source was determined by the adaptation of the colorimetric method as described by George- Okafor *et al.*[22] and Bidola *et al.*[23]. About 500 ml of mineral salt medium was prepared and the resulting pH of the medium was adjusted to 7.2 using a calibrated Suntex pH meter SP-701. About 0.5g of 2,6 Dichlorophenol Indophenol (2,6 DCPIP) (redox indicator) was added to the medium and stirred to ensure mixing[23]. Nine milliters of the medium was dispensed into each of clean test tubes. One (1) g of the petroleum sludge was also weighed and added to each tube. All the tubed media were autoclaved at 121^0C for 15 minutes. On cooling, each test tube with the exception of the control test tube was inoculated with one agar plug of each of the test fungal isolates [22]. The agar plugs were collected with the aid of a sterile 4mm cork borer and forceps. The tubes were incubated at room temperature for 7 days[22] and monitored daily for colour change (from deep blue to colorless).

Growth profile of axenic and mixed consortium of fungal isolates in petroleum sludge medium: The growth profiles of the fungal isolates which produced the fastest colour change during the screening test were determined by the adaptation of the method of Okpokwasili and Okorie[24]. Two (2) litres of mineral salt medium was prepared (pH 7.2) and 2 g of 2, 6, Dichlorophenol Indophenol (DCPIP) was added to the medium[23] and stirred to ensure development of a deep blue colouration of the medium[25]. Two hundred and fifty milliters of the medium was dispensed onto several 250 ml conical flasks and weighed amount of sludge (2.5 g) was added to each of the flasks. The flasks were autoclaved at 121^0C for 15 mins. Upon cooling, 2ml of a 96 hour MSM broth culture of each isolate was pipetted into each respective flask apart from the control flask, under aseptic conditions. The flasks were incubated at ambient room temperatures for 20 days on an incubator shaker (Heidolph Unimax 2010) operated at 120 rpm. Each flask was analyzed for petroleum sludge utilization every 4 days. The indicators of sludge utilization were; Total viable count, pH, turbidity and residual Total Petroleum hydrocarbon (TPH).

Cell growth (total viable count): Total viable fungal count of the respective flasks was determined by both the serial dilution and pour plate method with peptone water and Potato Dextrose Agar (PDA) utilized as diluent and general purpose agar medium of choice. Plating was done in duplicates and 1 ml of the antibiotic solution was pipetted to each inoculated plate before pouring of the cool molten agar under aseptic conditions. The plates were incubated at ambient room temperature for 3 days. Emergent discrete colonies were counted and recorded[25].

Determination of pH: The pH of each culture flask was determined at 96 hr interval for 20 days with the aid of Suntex pH meter SP-701[25].

Determination of turbidity: This was also determined at a 96 hr interval for 20 days. The parameter was analyzed with the aid of an HACH DR/2010 portable data logging spectrophotometer. Ten (10) ml of the sample was dispensed into a clean cuvette under aseptic conditions and steady turbidity readings were recorded at a wavelength of 810 nm[25].

Determination of the total petroleum hydrocarbon (TPH) of the sludge: The method of American Petroleum Institute [26] was adapted to determine the Total Petroleum Hydrocarbon (TPH) Diesel Range Organics (DRO) of the inoculated sludge portion

of the respective culture flasks at a 192 hr interval for a period of 20 days. The TPH (DRO) content of the sludge was ascertained with the aid of a Hewlett Packard 5890 series II gas chromatograph. The procedure involved sample preparation, extraction from collection media, clean up to remove any interfering compounds, instrumental analysis to identify and quantify the residual total petroleum hydrocarbon(TPH); Diesel range organics(DRO) (C8-C40). One (1) microlitre each of the resultant eluate was injected into the column of the system through the GC injection port, which was programmed under the following conditions of the instrument set up; Carrier gas; Helium, injector temperature; 2500C, injection volume; 1µl, flow rate;1.5 ml/minute, detector temperature; 3000C, initial oven temperature; 600C, equilibrium time; 0.1 minute, final oven temperature; 3100C, intermediate oven temperature;3000C, detector type; flame ionization (FID) with temperature at 3000C. At the end of each run which lasted for 20-24 minutes, a computer generated result with a chromatogram and the concentration of the DRO was obtained.

Evaluation of the generation time, growth rate and %reduction of the TPH content of the sludge by the axenic and mixed fungal cultures: The generation time and growth rate of the axenic fungal cultures used in the growth profiling tests was calculated[27]. Also the percentage reduction in the TPH (DRO) content of the sludge content of the respective flasks was also derived[25].

$$\text{Growth rate (day-1)} = \frac{\text{Difference in Cell counts}}{0.301 \times T}$$

$$\text{Generation time (day)} = \frac{1}{\text{Growth rate}}$$

% reduction of TPH = initial concentration – final concentration = reduction

$$\% \text{ reduction of TPH} = \frac{\text{reduction}}{\text{Initial concentration}} \times 100$$

Results and Discussion

The results as presented in table 1 showed that the heterotrophic fungal counts observed for the soil samples collected from the respective auto mechanic workshops ranged from 0.2×103 cfu/g to 3.6×103 cfu/g. The hydrocarbonclastic fungal counts for the auto mechanic soil samples ranged from 0.7×103 cfu/g to 3.4×103 cfu/g. A range of counts; 2.1× 103 cfu/g to 3.0×103 cfu/g were recorded in respect of soil samples obtained from the control site. Twenty (20) fungal isolates were characterized and identified from the soil samples; *Aspergillus flavus*, *Aspergillus terrus*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Emericella nidulans*, *Aspergillus tamarii*, *Aspergillus niger*, *Aspergillus* sp., *Moniliella* sp., *Pichia farinosa, Sporobolomyces* sp., *Candida* sp., *Rhodotorula* sp., *Curvularia* sp., *Mucor* sp., *Rhizopus stolonifer*, *Penicillium* sp., *Penicillium sp. 2*, *Penicillium italicum* and *Penicillium chrysogenum* (figure 2). *A. flavus* and *E. nidulans* had the maximal percentage prevalence (85.7%) amongst the fungal isolates (figure 2) while *P. italicum* and *Sporobolomyces* sp. had the lowest percentage prevalence (14.3%) amongst the soil mycoflora (figure 2). The physicochemical properties of the respective soil samples are presented in table 2a and 2b. The moisture, Total Organic Carbon (TOC), pH and available phosphorus content of the soils ranged from 6.70% to 10.63%, 4.25% to 5.85%, 5.81 to 6.40 and 1.69 mg/kg to 54.84 mg/kg respectively. The result of the sludge utilizing capability of the respective isolates as indicated in table 3 showed that *A. terrus*, *Aspergillus* sp., *Penicillium* sp., *A. flavus* and mixed cultures of both the yeast isolates and filamentous fungal cultures had the best biodegradation potential amongst the isolates. *Aspergillus* sp. exhibited the highest growth rate (5.65 day-1) and generation time (2.3 × 10-8 days) (table 4). Table 5 revealed that the highest percentage reduction in the residual TPH (DRO) content was caused by *Aspergillus flavus* (84%) while the lowest percentage reduction (49%) was effected by the consortium of filamentous fungal isolates. Amongst the axenic and mixed cultures, *Aspergillus flavus* had the lowest pH (4.8) recorded on the 20th day (figure 3). *Aspergillus terrus* had the highest turbidity (280 FAU) on day 20 of the growth profiling test (figure 4). The highest count (6.5 ×104 cfu/ml) was observed for the filamentous fungal consortium during the 12th day.

Table-1
Total mean fungal count and sludge utilizing fungal count of the soil samples using several general and enriched mycological media

Soil Samples	MEA (after 5 days)	PDA (after 5 days)	WA (after 5 days)	RBCA (after 5 days)	MCDA (after 9 days)
A	*1.2	1.4	1.2	1.2	1.7
B	1.1	2.1	1.0	2.5	0.8
C	2.6	0.6	3.6	1.7	3.4
D	1.2	0.4	0.5	1.0	1.5
E	0.7	1.3	0.6	1.8	0.7
F	0.5	0.6	0.5	0.2	2.0
Control	2.4	3.0	2.1	3.0	2.5

Legend:* Mean counts $\times 10^3$(cfu/g) , MEA: Malt Extract Agar, PDA: Potato Dextrose Agar,WA: Waksman Agar, RBCA: Rose Bengal Chloraphenicol Agar, MCDA: Modified Czapek Dox Agar.

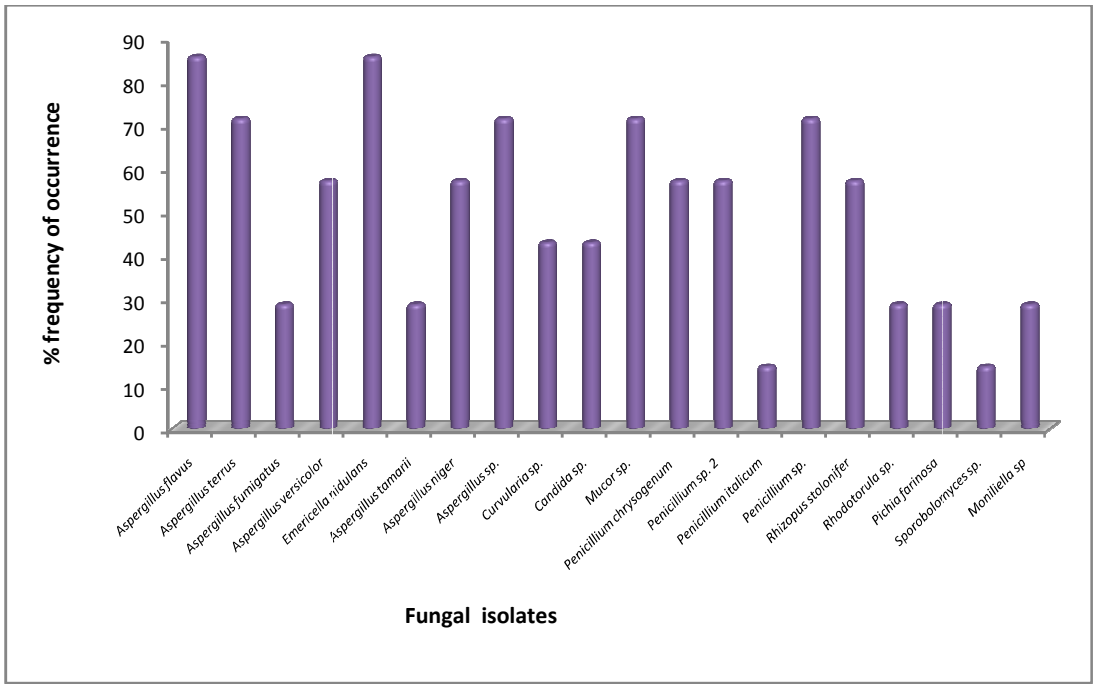

Figure-2
Prevalence of the respective fungal isolates in the auto mechanic workshop and control soil samples

Table-2a
Physicochemical properties of the soil samples

Sample	pH	Av. P (mg/kg)	Total N_2 (mg/kg)	CEC (Meq/100g)	TOC (%)	THC (ppm)	Moisture (%)
A	6.26	40.56	26.3	26.98	4.25	1.68	10.63
B	6.40	41.53	29.4	31.32	5.28	1.16	8.82
C	6.10	54.84	31.9	27.86	4.60	0.52	8.35
D	6.17	1.69	25.3	81.2	5.40	1.80	7.42
E	6.02	27.17	29.9	19.04	5.88	0.70	6.70
F	6.31	32.66	35.6	32.08	5.34	0.86	7.86
Control	5.81	37.09	40.2	15.44	4.86	0.7	9.58

Legend: Av. P: Available Phosphorus, CEC: Cation Exchange Capacity, TOC: Total Organic Carbon, THC: Total Hydrocarbon content, Total N_2: Total Nitrogen,

Table 2b
Physicochemical properties of the soil samples

Sample	PARTICLE SIZE (%)			HEAVY METAL (mg/kg)		
	Sand	Silt	Clay	Pb	Zn	Cd
A	59.0	8.4	32.6	ND	2.25	ND
B	57.1	7.6	34.8	0.4	2.17	0.02
C	50.8	18.4	31.0	ND	5.16	0.04
D	50.0	18.0	32.0	2.10	4.07	ND
E	58.4	10.8	30.8	4.05	6.14	0.07
F	51.6	14.0	34.4	0.7	2.7	0.1
Control	64.80	6.6	28.6	ND	1.73	ND

Legend: ND; Not Detected

Table-3
Sludge utilizing capabilities of the fungal isolates

Fungal isolates	**Observed colour reaction (2,6 DCPIP utilized as redox dye)**
Aspergillus flavus	Colourless after 24 hr.
Aspergillus terrus	Colourless after 24 hr.
Aspergillus sp.	Colourless after 24 hr.
Penicillium sp.	Colourless after 24 hr.
Aspergillus versicolor	Colourless after 48 hr.
Aspergillus niger	Colourless after 96 hr.
Emericella nidulans	Colourless after 72 hr.
Aspergillus tamarii	Blue after 172 hr.
Candida sp.	Colourless after 96 hr.
Curvularia sp	Colourless after 96 hr.
Mucor sp	Blue after 172 hr.
Moniliella sp.	Blue after 172 hr.
Penicillium chrysogenum	Colourless after 172 hr.
Penicillium sp. 2	Colourless after 148 hr.
Penicillium italicum	Colourless after 148 hr.
Pichia farinosa	Blue after 172 hr.
Rhizopus stolonifer	Colourless after 148 hr.
Rhodotorula sp.	Colourless after 120 hr.
Sporobolomyces sp.	Blue after 172 hr.
Yeast consortium	Colourless after 24 hr.
Filamentous fungal consortium	Colourless after 24 hr.
Aspergillus fumigatus	Colourless after 96 hr.

Table-4
Growth rate (day-1) and Generation time (days) of the respective axenic fungal isolates grown on petroleum sludge mineral salt medium

Isolates	**Growth rate (day-1)**	**Generation time (days)**
Aspergillus flavus	2990	*0.5 × 10-12
Penicillium sp.	4236	5.8 × 10-12
Aspergillus terrus	4934	4.1 × 10-12
Aspergillus sp.	5648	4.4 × 10-12
Yeast consortium	ND	ND
Filamentous fungal consortium	ND	ND
Control	-	-

Legend: *Value approximated to the nearest decimal place, ND: Not Determined

Table-5
TPH (DRO) values for the inoculated sludge recovered from the petroleum sludge mineral salt medium and % TPH (DRO) reduction

Isolates	**Day 0**	**Day 10**	**Day 20**	**%TPH(DRO) reduction**
Aspergillus flavus	137*^	31	22	84
Penicillium sp.	137	80	24	82
Aspergillus terrus	137	57	49	64
Aspergillus sp.	137	35	33	76
Yeast consortium	137	93	57	58
Filamentous fungal consortium	137	72	70	49
Control	137	94	33	76

Legend: ^ values are in TPH (DRO) (mg/kg), * Approximated value to the nearest whole number, TPH: Total Petroleum Hydrocarbon, DRO: Diesel Range Organics

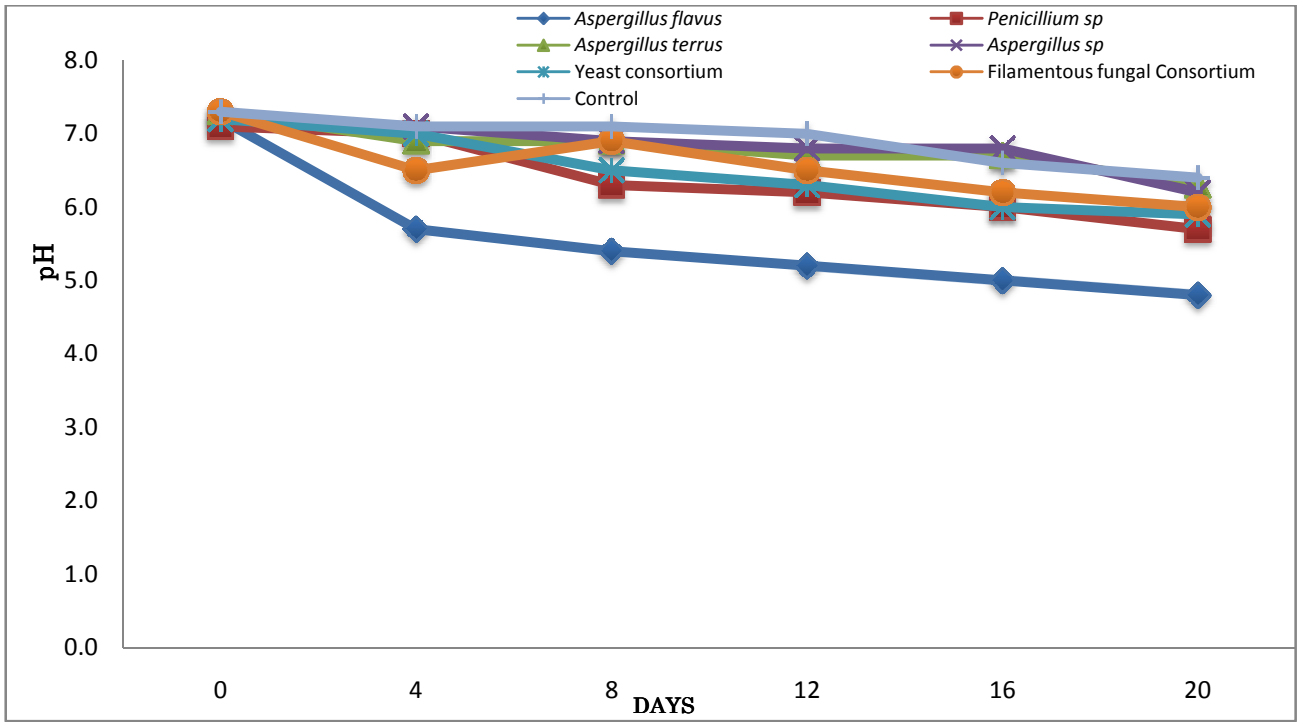

Figure-3

pH values of both the axenic and mixed fungal cultures on petroleum sludge-mineral salt medium

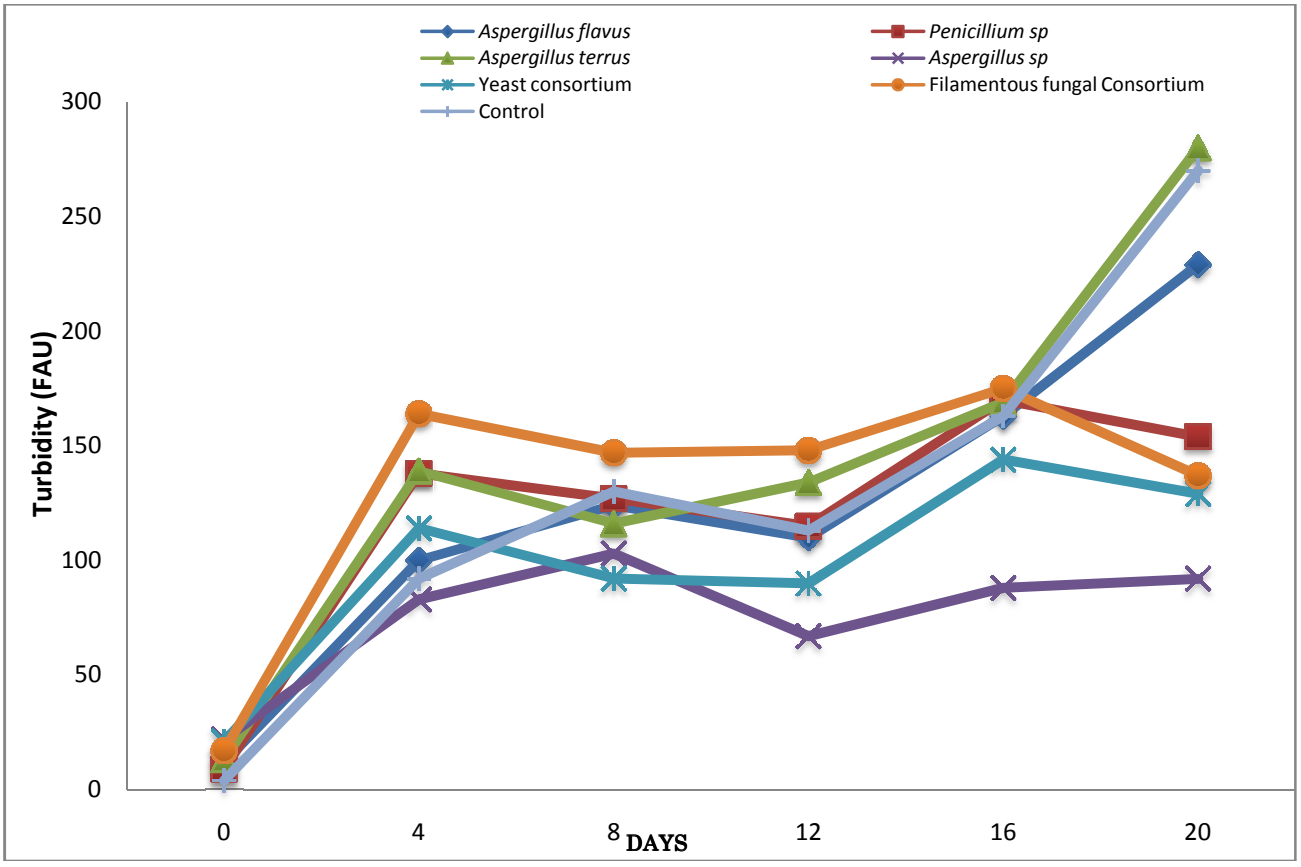

Figure-4

Turbidity values of both the axenic and mixed fungal cultures on petroleum sludge-mineral salt medium

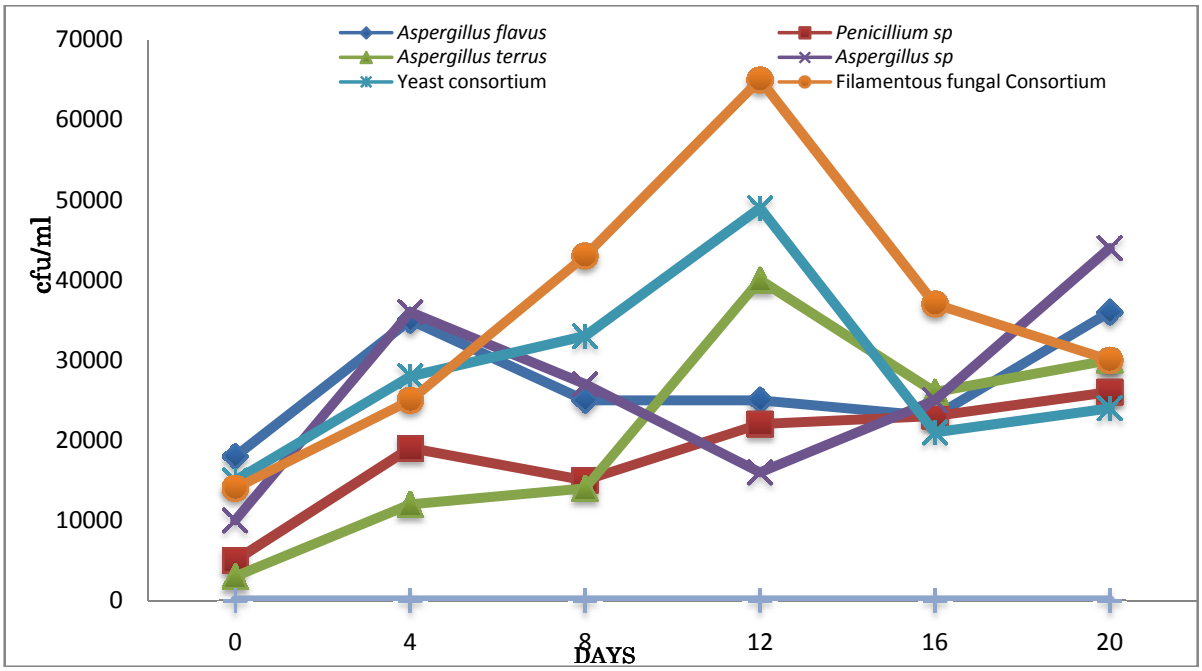

Figure-5

Mean colony counts (cfu/ml) of both the axenic and mixed fungal cultures on petroleum sludge-mineral salt medium

The preliminary viable fungal counts recorded from the auto mechanic workshop soils could be reflective of the adaptive abilities of these fungal isolates to thrive even in the event of deliberate anthropogenic intermittent discharges of various types and quantities of petroleum products on these soil surfaces over periods of time. However, the preliminary recovery of sludge utilizing fungal species from the control soil (2.5×10^3 cfu/g) using modified Czapek Dox agar could indicate the ubiquitous distribution of hydrocarbonclastic fungi in the soil region. Amongst, the culture plates incubated for 5 days, the highest mean count was recorded for soil sample C (WA; 3.6 $\times10^3$ cfu/g) whilst the lowest mean count was observed for sample F (RBCA; 0.2 $\times10^3$ cfu/g). This observation could suggest that amongst the culture media utilized in the preliminary recovery of fungi from these soil samples, Waksman agar (WA) provided the most suitable nutritional and physiological conditions for the growth of the soil mycoflora. The ability of Waksman agar to support the growth of higher numbers of fungi from the polluted soils is not surprising, as the medium had been recommended by Sharma[12] for the preliminary isolation of soil fungi. The high percentage prevalence of *Aspergillus* species especially *Aspergillus flavus* in all the soil samples was not surprising, given the fact that *Aspergillus* spp. are ubiquitous soil borne saprophytes, whose condida are easily distributed through the atmosphere[28,29]. All the isolates and consortia which utilized the sludge as sole source of carbon caused a visible color change in the inoculated mineral salt medium. This colour change had been attributed to the reduction of the indicator (2, 6 DCIP) by the oxidized products of hydrocarbon degradation[22]. This could be suggestive of chemical changes of the hydrocarbon substrates as a result of assimilatory metabolism of the hydrocarbon components of the inoculated sludge by the respective fungal cultures. The hydrocarbon degrading potentials of *Aspergillus flavus* and *Aspergillus terrus* has also been reported by El- Sayed and El-Morsy[13] George- Okafor *et al.*[22] Markovetz *et al.* [30] Al-Ghamdi[31] and Sebiomo *et al.*[32]. The inability of most of the axenic cultures of yeast isolates to utilize the sludge as energy source (with the exception of *Candida* sp.) contrasted with their ability to utilize the sludge as a consortium. This could be indicative of a synergistic metabolism of the crude oil sludge by these yeasts. However in comparison to the other axenic, mixed cultures and control flask, the yeast consortium and the filamentous fungal consortium caused the lowest percentage reduction in the TPH(DRO) content of the inoculated sludge during the growth profile test (table 4; 58% and 49%) This could indicate that during the synergistic metabolic interactions between the respective fungal isolates that made up the consortium, some or a single fungal specie were antagonistic against other members of the consortium[25]. Microbial degradation of hydrocarbons often leads to production of organic acids and other metabolic products[33]. Thus, organic acids probably produced accounted for the steady reduction in pH levels[34] during the growth profile study.

With the exception of *Penicillium* sp. and the filamentous fungal consortium, there was a continuous increase in the turbidity of the respective culture flasks during the growth profile test (figure 4). This could be indicative of increased biomass activity by the respective fungal cultures during the growth profile study as turbidity was used as a surrogate indicator of biomass activity. In contrast to the other axenic cultures and consortium

of yeasts, the mean colony counts of the filamentous fungal consortium decreased at day 20 (figure 5). This phenomenon could reflect the antagonistic interactions between the individual members of the consortium as at day 20 of the growth profile test. In comparison with the respective fungal consortia, axenic culture of *A. flavus* caused the highest percentage reduction in the residual TPH (DRO) content of the inoculated sludge (Table 4). This trend is similar to findings reported by Odjadjare *et al.* [35] who observed that axenic bacterial cultures were better degraders of Escravos light crude oil in comparison to several mixed bacterial consortia. These observations however, contrasted with a report by Del'Arco and De Franca[36] who observed that an inoculation of a mixed culture of autochthonous microorganisms onto a sandy sediment polluted with Arabian crude oil, increased the bioremediation efficiency of the heavy fraction of the Arabian crude oil by 31% within 28 days. Although *Aspergillus flavus* caused the highest percentage TPH(DRO) reduction in the inoculated crude oil sludge, its growth rate (2990 day^{-1}) was lower than that of *Aspergillus* sp. (5648 day^{-1}). This could suggest that the utilization of the sludge as carbon and energy source had no effect on the resultant growth rate of the axenic fungal cultures. Odjadjare *et al.* [35] also reported that specific growth rate and biomass of petroleum degrading bacterial isolates did not significantly correlate with the extent of petroleum degradation. Sludge sample from the control flask also showed a marked percentage reduction in the residual TPH (DRO) content (76%) (table 5). This could be reflective of abiotic degradation mechanisms especially photodegradation since the flasks were agitated under ambient temperatures (28± 20^0C). Photodegradation can be classified as either direct or indirect photolysis[37].

All the analysed top soil samples were acidic (table 2a). This observation is similar to those observed by Ilembayo and Kolade[38] who reported a range of pH values (5.96-8.55) for top soil samples obtained from auto mechanic workshops within Akure, Ondo state. Low pH values usually enhance metal distribution and transport in soil[38]. The total organic carbon values (ranging from 4.25% (A) to 4.86% (control) were at variance with those reported by Ipeaiyeda *et al.* [9] who stated TOC values ranging from 0.31% to 3.82% for top soil samples obtained from five auto mechanic workshops located at Iwo town, Osun state. The soil samples were sandy as revealed by the particle size analyses of the samples (table 2b). Generally, all the heavy metals (Pb, Cd and Zn) concentrations recorded for the respective soil samples (values ranging from ND to 6.14 mg/kg were in disagreement with reports by Ipeaiyeda *et al.*[9] Ilembayo and Kolade[38]. Ipeaiyeda *et al.*[9] reported values ranging from 0.05 mg/kg for Zinc and 184 mg/kg for Lead. Ilembayo and Kolade[38] also reported values ranging from 730.97 mg/kg for Zinc to 217 mg/kg for Lead. The observed low levels of heavy metals especially lead in the soil samples could be suggestive of the amounts of leaded petroleum products disposed on the top soils within the vicinities of these workshops. This trend could also be reflective of increased mobility and infiltration of these heavy metals down the soil profile as a consequence of the sandy nature of the soils.

Conclusion

Soils within the vicinities of auto mechanic workshops are viable sources of hydrocarbonclastic fungi. These microorganisms can be utilized in bioaugmentation methodologies aimed at the removal of several hydrocarbon pollutants especially petroleum and its refined products from either aquatic or terrestrial environments. *A. flavus*, *Penicillium* sp. and *Aspergillus* sp. caused the highest reduction in the TPH (DRO) content of the inoculated sludge content during the growth profile test. Thus, these fungal isolates can be applied in bioreactor based processes for the treatment of oily sludges as described by Singh *et al.*[39].

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