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BIODEGRADATION POTENTIALS OF AUTOMOBILE WORKSHOP SOIL MYCOFLORA ON FLOW STATION PETROLEUM SLUDGE WITH AN EXTRA CARBON SOURCE

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ABSTRACT

The biodegradation potentials of soil mycobiota isolated from six auto mechanic 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 3.2×10^3 cfu/g .Twenty (20) fungal species were identified from the soil samples; *Aspergillus flavus*, *Aspergillus terreus*, *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%-64.8%). Turbidimeteric screening revealed that *A. flavus*, *A. 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 (6.34 – 5.06) and an increase in turbidity (38 FAU – 625 FAU) during the 20 day incubation period. Amongst the growth profile cultures, *A. flavus*caused the highest percentage reduction in the residual TPH (DRO) content of the inoculated sludge (96%). Soils within the premises of automobile workshops can serve as a source of hydrocarbonclastic fungi.

Keywords: Sludge, Benin City, automobile workshops, hydrocarbonclastic fungi

INTRODUCTION

The exploration, production, refining and distribution of petroleum and petrochemical products results in the generation of a considerable volume of waste oil sludges (**Singh *et al*., 2001**). These sludges come from a variety of sources including storage tank bottoms, oil-water seperators, cleaning of processing equipment, biological sludges from waste water treatment units and oil spills in the oil fields, drilling sites and refineries **(Manning and Thompson, 1995**). The composition of sludges varies with their origin and storage conditions but might typically contain up to 10-30% hydrocarbons, 5-20% solids and the remainder water **(Speight, 1991**). A variety of physical, chemical and biological approaches have been taken to remediate sludges generated during the exploration and processing of petroleum. In many countries, these sludges have been accumulated in large lagoons, facilitating some recycling of oil but requiring later remediation of residual oily sludges **(Singh *et al*., 2001**). Attempts to process these sludges using centrifugal methods to separate oil, water and solids phases is highly capital intensive, is not consistently effective and still produces residual solids with high petroleum hydrocarbon content **(Singh *et al*., 2001**). The use of biological processing to treat waste or waste contaminated material is well documented **(Prince 1993**; **Atlas and Cerniglia**, **1995**). Bioprocessing involves exploiting abilities of indigenous or augmented microorganisms to metabolize organic substrates (**Ward, 1991**). It can be accomplished in a land based environment (Landfarming, composting or biopiling) or in some cases bioremediation may be carried out *in situ* by enhancing microbial degradation of contaminants in the subsurface of soil. In some other cases, contaminated material may be treated in slurry bioreactors to degrade petroleum hydrocarbons **(Singh *et al*., 2001**).
During the last decade, fungi have been used in the treatment of a wide variety of wastes, wastewaters and their role in the bioremediation of various hazardous and toxic compounds in soils and sediments has been established **(Leitao, 2009**). Fungi have also demonstrated the ability to degrade and some cases mineralize phenols, halogenated phenolic compounds, petroleum hydrocarbons, polycyclic aromatic compounds and poly chlorinated biphenyls **(Singh, 2006)**. Automobile workshops are a common sight in all Nigerian cities and towns, and play an important role in socio- economic dynamics **(Obayagbona, 2012)**. Automobile workshops are facilities where automobiles are usually operated in semi stationary or stationary modes **(Ipeaiyeda *et al*., 2007**). **Ilemobayo and Kolade(2008**) stated that increased proliferation of automobile workshops within Nigerian cities and towns has contributed markedly to the problem of soil contamination in these cities and towns.

Automobile workshops abound within Benin City. This has resulted in the concomitant exposure of the surrounding soils within the vicinity of these workshops to high levels of spent crankcase engine oil and lubricating oils (Obayagbona, 2012) . This study was conducted with the aim of isolating and identifying the heterotrophic and hydrocarbonclastic fungal species from soils collected from various auto mechanic workshops within Benin City. Determination of the physicochemical characteristics of the soil samples obtained from the respective auto mechanic workshops. Screening for the ability of these fungal isolates to degrade crude oil sludge and evaluating the co-metabolic effects of glucose on biodegradation potentials of the respective fungal isolates.

MATERIAL AND METHODS

Source of soil samples

Six top soil samples (100g) 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 also 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

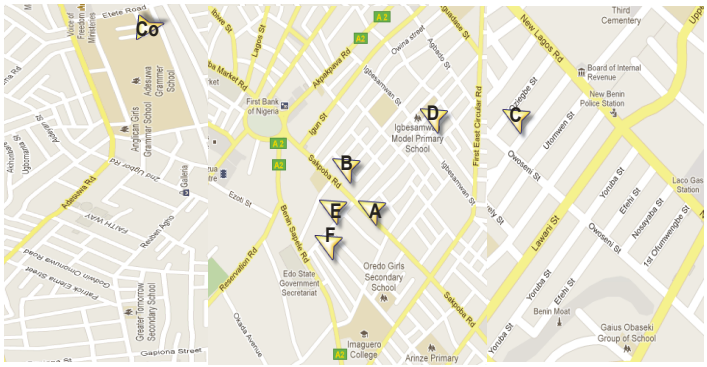

Figure 1b Map of Benin City showing the respective sampling sites **(Google Earth, 2012)**

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

Enumeration and isolation of heterotrophic and hydrocarbonclastic soil fungi using general and enriched media

One (1) gram of the respective fresh soil samples were weighed and dissolved into 99 ml of sterile prepared peptone water diluent under aseptic conditions (**Harley and Prescott, 2002**; **Aneja, 2003**). 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)(Oxoid Ltd. Basingstoke**,** Hampshire) and Waksman Agar (WA) by pour plate method (**Aneja, 2003**; **Sharma, 2009**).An oil sludge based medium; modified mineral salt agar (**Okpokwasili and Okorie, 1988**; **Sebiomo *et al*., 2011**) 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 inhibit bacterial growth (**El-Sayed and El-Morsy, 2005**).Plating was done in duplicates. The culture plates were swirled, allowed to solidify and incubated at ambient room temperature (28 ± 2^0C) for 5 days. Petroleum sludge agar plates were also incubated at 28 ± 2 ^{0}Cfor 9 days. The resulting fungal colonies were enumerated and recorded as colony forming units (cfu) per 1 g of each soil sample (**Harley and Prescott, 2002**).

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 (**Choi *et al*., 1999**; **Sharma, 2009**). 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 (1972**) and **Alexopolulos *et al.* (1996)**. Several biochemical tests such as nitrate utilization, urea hydrolysis, sugar fermentation test (Sharma, 2009), ability to grow at 37 ^{0}C and assimilation of carbon compounds (**Van der Walt, 1970**) were conducted to further characterize the yeast isolates.The tentative identity of the yeast isolates was determined by comparing the observed marcoscopic, microscopic features and the biochemical reactions to identification keys described by **Pincus (2009).**

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 hr. 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 2mm 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, (1999**). 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 (2000)** and **Bremmer and Mulvaney (1982**).

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 methods of **Okpokwasili and Okorie (1988**) and **George-Okafor *et al*., (2009**). Nine (9) ml of prepared mineral salt medium (MSM) (**Mills *et al*., 1978**) was dispensed onto one set of test tubes. One gram (1 g) of the sludge was added to each of the tubes and capped before autoclaving at 121 ^{0}C for 15 min. Upon cooling, each of the first set of tubes was inoculated with two drops of cell suspension of an isolate in sterile mineral salt medium. The suspension was prepared by inoculating an agar plug of the purified fungal mycelia from the respective PDA slants onto 2 ml of mineral salt medium (**George-Okafor *et al*., 2009**). One control tube remained uninoculated. The inoculated tubes and control tube were incubated at room temperature for 14 days, after which each tube was scored for optical density ($OD_{600\ nm}$) (**Husain *et al*., 2011**) using a HACH 2010 portable data logging spectrophotometer (HACH Co. Loveland, Colorado).

Determination of the co-metabolic effect of glucose on the growth profile of the axenic and mixed fungal cultures on petroleum sludge medium

The growth profiles of the fungal isolates that scored the highest optical density during the screening test were determined by the adaptation of the method of **Okpokwasili and Okorie (1988**). The method of **Snellman and Greathouse (1996)** was also adapted for the evaluation of the co- metabolic effects of an additional carbon source (glucose) on the growth profile of the axenic and mixed fungal isolates inoculated in crude oil sludge medium. 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 (**Biodoia *et al*., 2010**) and stirred to ensure development of a deep blue coloration of the medium **(Obayagbona, 2012**). Two hundred and fifty (250) ml 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. Also, 2.5% glucose solution sterilized by steaming in a water bath for 30 minutes was added ($1\%^{v}/_{v}$) to each flask. The flasks were autoclaved at 121^{0}C for 15 min. Upon cooling, 2ml of a 96 hr 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) (Heidolph Co. Schwabach, Nuremberg operated at 120 rpm. Each flask was analyzed for petroleum sludge utilization every 4 days. The indicators of sludge utilization were; Dry weight, pH, turbidity and residual Total Petroleum hydrocarbon (TPH).

Cell biomass (dry weight)

The fungal biomass of the respective flasks at day 20 was determined by filtration of the fungal mycelia using a pre weighed filter paper and oven dried at 80 ^{0}C for 24 hr **(Nasim and Ali, 2011).** The dry weight was then ascertained with the aid of a sensitive weigh balance (OHAUS PIONEER model PA214) (Ohaus Co. Pine Brook, New Jersey). (**Al-Ghamdi, 2011;Sebiomo *et al*., 2011**).

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 (Suntex Instruments Co. New Taipei City). **(Obayagbona, 2012).**

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 (**Obayagbona, 2012).**

Determination of the total petroleum hydrocarbon (TPH) of the sludge

The method of **American Petroleum Institute (1968)** 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 (Hewlett Packard Co. Palo Alto, California). 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) (C_8-C_{40}). 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; 250 ^{0}C, injection volume; 1µl, flow rate;1.5 ml/minute, detector temperature; 300^{0}C, initial oven temperature; 60 ^{0}C, equilibrium time; 0.1 minute, final oven temperature; 310 ^{0}C, intermediate oven temperature;300 ^{0}C, detector type; flame ionization (FID) with temperature at 300^{0}C. 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 %reduction of the TPH content of the sludge by the axenic and mixed fungal cultures

The percentage reduction in the TPH (DRO) content of the sludge content of the respective flasks was calculated **(Obayagbona, 2012**).
% reduction of TPH = initial concentration – final concentration = reduction

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

Statistical analysis

Analysis of variance (ANOVA) of the respective mean fungal counts obtained from the soil samples was conducted ($\alpha = 0.05$). Duncan Multiple Range (DMR) tests were conducted to locate the cause of any significant differences in the analyzed mean counts (Ogbeibu, 2005).

RESULTS

The heterotrophic fungal counts observed for the soil samples collected from the respective auto mechanic workshops ranged from 0.2×10^3 cfu/g to 3.2×10^3 cfu/g. The hydrocarbonclastic fungal counts for the auto mechanic soil samples ranged from 0.1×10^3 cfu/g to 2.2×10^3 cfu/g . A range of counts; 2.1×10^3 cfu/g to 3.0×10^3 cfu/g were recorded in respect of soil samples obtained from the control site. There was a significant difference ($P<0.05$) in the mean fungal counts from the respective soil samples. Counts from soil samples A, C and the control soil were responsible for the significant differences in the analyzed means (Tab. 1).

Table 1 Total mean fungal count, 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)	MMSA (after 9 days)
A	^{b*}1.7	^{b}1.9	^{b}2.1	^{b}1.8	^{b}2.2
B	^{a}1.7	^{a}1.4	^{a}1.0	^{a}1.3	^{a}0.1
C	^{b}2.2	^{b}1.1	^{b}1.9	^{b}2.8	^{b}1.6
D	^{a}0.4	^{a}2.7	^{a}1.6	^{a}3.2	^{a}2.0
E	^{a}1.0	^{a}2.2	^{a}0.2	^{a}1.0	^{a}0.3
F	^{a}1.9	^{a}1.8	^{a}1.7	^{a}1.5	^{a}1.9
Control	^{b}2.4	^{b}3.0	^{b}2.1	^{b}3.0	^{b}2.2

Legend: * Values aremean counts (cfu/g) $\times10^3$, Means preceded by alphabet "a "are not significantly different ($P>0.05$) from each other using DMR, Means preceded by alphabet "b" are significantly different ($P<0.05$) from each other using DMR, MEA: Malt Extract Agar, PDA: Potato Dextrose Agar, WA: Waksman Agar, RBCA: Rose Bengal Chloraphenicol Agar, MMSA: Modified Mineral Salt Agar

Twenty (20) fungal isolates were characterized and identified from the soil samples; *Aspergillus flavus*, *Aspergillus terreus*, *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 *E. nidulans* had the maximal percentage prevalence (85.7%) amongst the fungal isolates while *P. italicum* and *Sporobolomyces* sp. had the lowest percentage prevalence (14.3%) amongst the soil mycoflora (Tab. 2).

Table 2 % frequency of occurrence of the fungal isolates in the soil samples

Fungal isolate	% frequency of occurrence	Fungal isolate	% frequency of occurrence	Fungal isolate	% frequency of occurrence
Aspergillus flavus	85.7	*Curvularia* sp.	42.9	*Rhodotorula* sp.	28.6
Aspergillus terreus	71.4	*Candida* sp.	42.9	*Pichia farinosa*	28.6
Aspergillus fumigatus	28.6	*Mucor* sp.	71.4	*Sporobolomyces* sp.	14.3
Aspergillus versicolor	57.1	*Penicillium chrysogenum*	57.1	*Moniliella* sp	28.6
Emericella nidulans	85.7	*Penicillium* sp.2	57.1		
Aspergillus tamarii	28.6	*Penicillium italicum*	14.3		
Aspergillus niger	57.1	*Penicillium* sp.	71.4		
Aspergillus sp.	71.4	*Rhizopus stolonifer*	57.1		

The physicochemical properties of the respective soil samples are presented in Table 3. The pH, Nitrogen, Total Organic Carbon (TOC), moisture and zinc of the soils ranged from 5.81 to 6.52, 29.10 mg/kg to 49.62 mg/kg, 4.14% to 5.44%, 7.16% to 11.13% and 1.73 mg/kg to 5.32 mg/kg respectively (Tab. 3).

Table 3 Physicochemical properties of the soil samples

Sample	pH	Av. P (mg/kg)	Total N_2 (mg/kg)	CEC (Meq/100g)	TOC (%)	THC (ppm)	Moisture (%)	PARTICLE SIZE (%)			HEAVY METAL (mg/kg)		
								Sand	Silt	Clay	Pb	Zn	Cd
A	6.10	31.17	29.7	31.57	4.14	2.30	11.13	57.1	9.3	33.6	1.21	2.02	ND
B	6.52	43.30	24.2	33.54	4.26	1.85	7.27	53.0	10.1	36.9	0.2	3.12	0.01
C	6.22	49.62	30.1	35.56	5.03	1.19	8.11	52.3	8.4	39.3	ND	3.19	0.05
D	6.19	29.10	29.6	63.37	5.44	1.53	8.43	51.7	11.2	37.1	3.11	3.51	ND
E	6.37	34.03	23.0	31.56	5.12	0.76	7.60	58.2	9.5	32.3	ND	5.25	ND
F	6.37	31.53	25.8	21.14	4.70	0.94	7.16	50.3	13.0	36.7	ND	5.32	0.02
Control	5.81	37.09	40.2	15.44	4.86	0.7	9.58	64.8	6.6	28.6	ND	1.73	ND

Legend: Av. P: Available Phosphorus, CEC: Cation Exchange Capacity,TOC: Total Organic Carbon,THC: Total Hydrocarbon content, Total N_2: Total Nitrogen, ND: Not Detected.

The result of the sludge utilizing capability of the respective isolates as indicated in Table 4 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 (Tab. 4).

Table 4 Sludge utilizing capabilities of the fungal isolates

Fungal isolates	Optical density at 600nm
Aspergillus flavus	0.579 (++++++)
Aspergillus terreus	0.600 (++++++)
Aspergillus sp.	0.513 (++++++)
Penicillium sp.	0.564(++++++)
Aspergillus versicolor	0.219(+++)
Aspergillus niger	0.106 (++)
Emericella nidulans	0.132 (++)
Aspergillus tamarii	0.010 (+)
Candida sp.	0.212 (+++)
Curvularia sp	0.100 (+++)
Mucor sp	0.065 (+)
Moniliella sp.	nil (-)
Penicillium chrysogenum	0.051 (+)
Penicillium sp.2	0.063 (+)
Penicillium italicum	0.082 (++)
Pichia farinosa	nil (-)
Rhizopus stolonifer	0.110 (++)
Rhodotorula sp.	0.142 (++)
Sporobolomyces sp.	0.076 (+)
Yeast consortium	0.583 (++++++)
Filamentous fungal consortium	0.641 (++++++)
Aspergillus fumigatus	0.109 (+++)

Legend : +/-: score for the $OD_{600\ nm}$ reading

A. flavus effected the highest percentage reduction in the TPH(DRO) content of the inoculated sludge (96%) amongst the growth profile isolates (Table 5). *A. terreus* ellicted the least percentage reduction in the residual TPH (DRO) content of the sludge layer (86%) during the shake flask study (Tab. 5.)

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

Isolates	Day 0	Day 10	Day 20	%TPH(DRO) reduction
Aspergillus flavus	$^{+}137^{*}$	10	6	96
Penicillium sp.	137	30	9	93
Aspergillus terreus	137	21	19	86
Aspergillus sp.	137	16	16	88
Yeast consortium	137	64	7	91
Filamentous fungal consortium	137	36	12	95
Control	137	26	18	87

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

Amongst the axenic and mixed cultures, *Aspergillus flavus* had the lowest pH (5.06) recorded on the 20th day. The control flask had the highest pH reading (7.52) recorded on 4th Day of the growth profile study (Tab. 6).

Table 6 pH values of both the axenic and mixed fungal cultures on glucose-petroleum sludge medium

Isolates	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Aspergillus flavus	6.34	5.52	5.85	5.26	5.16	5.06
Penicillium sp.	6.71	5.17	5.36	5.32	5.40	5.49
Aspergillus terreus	7.12	5.44	5.24	5.16	5.96	5.67
Aspergillus sp.	6.87	5.43	5.11	5.14	5.33	5.48
Yeast consortium	6.69	5.62	6.13	6.44	6.58	6.29
Filamentous fungal consortium	6.89	5.98	6.29	6.49	6.50	6.43
Control	6.87	7.52	6.74	6.89	5.36	6.10

Penicillium sp. had the highest turbidity (742 FAU) on day 16 while the lowest turbidity value (22 FAU) was recorded for the consortium of yeasts at Day 0. (Tab. 7**).**

Table 7 Turbidity readings of both the axenic and mixed fungal cultures on glucose-petroleum sludge medium

Isolates	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Aspergillus flavus	38^{*}	148	386	515	627	625
Penicillium sp.	32	383	592	725	742	740
Aspergillus terreus	23	281	331	418	571	485
Aspergillus sp.	35	363	516	625	698	734
Yeast consortium	22	447	323	520	593	643
Filamentous fungal consortium	135	616	475	428	449	556
Control	15	8	66	16	155	345

Legend: *Values are given as Formazin Attenuation Unit(FAU)

The highest dry weight (3.138g) was recorded for the filamentous fungal consortium while the consortium of yeasts had the lowest dry weight (0.648 g) at day 20 of the growth profile study (Tab. 8).

Table 8 Dry weight (g) of both the axenic and mixed fungal cultures on glucose-petroleum sludge medium

Isolates	Day 20
Aspergillus flavus	1.698
Penicillium sp.	1.488
Aspergillus terrus	1.268
Aspergillus sp.	1.338
Yeast consortium	0.648
Filamentous fungal consortium	3.138
Control	nil

DISCUSSION

The viable heterotrophic fungal counts obtained 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. The preliminary recovery of sludge utilizing fungal species from the control soil (Table 1) using modified mineral salt agar is indicative of the ubiquitous distribution of hydrocarbonclastic fungi in the top soil zone. Amongst, the culture plates incubated for 5 days, the highest mean fungal count was obtained for soil sample D using RBCA (Table 1). This novel trend suggests that amongst the general purpose media utilized in the preliminary recovery of fungi from these soil samples, ROSE BENGAL CHLORAMPHENICOL AGAR (RBCA) (OXOID Ltd. Basingstoke**,** Hampshire) provided the most suitable nutritional and physiological conditions for the growth of the soil mycoflora.

The high percentage frequency of prevalence of *Aspergillus* species especially*Aspergillus flavus* in all the soil samples was not surprising (Table 2), given the fact that *Aspergillus* spp. are ubiquitous soil borne saprophytes, whose condida are easily distributed through the atmosphere **(Giraud *et al*., 2001).** All the isolates and consortia which utilized the sludge as sole source of carbon during the screen test scored for optical density with the exception of *Moniliella* sp. and *Pichia farinosa* (Table 4). However, when inoculated as a consortium, the respective soil yeasts were able to maximally utilize the crude oil sludge as sole carbon source (Table 4). This could be indicative of a synergistic metabolism of the crude oil sludge by these yeasts. Amongst the axenic isolates, *A. flavus* effected the highest percentage in TPH (DRO) content of the inoculated petroleum sludge (Table 5) and also had the highest dry weight measurement (Table 8) during the growth profile test. These phenomena was not surprising as the hydrocarbon degrading potentials of *Aspergillus flavus* has been previously reported by **El-Sayad and El-Morsy, (2005)** and **Sebiomo *et al*. (2011).** Axenic culture of *A. flavus* caused a higher reduction in the TPH (DRO) content of the sludge in comparison to the respective mixed consortia of yeasts and filamentous fungi (Table 5). This could suggest 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. This would have cumulated in the reduced assimilation of the petroleum hydrocarbons by the fungal consortia. This trend is similar to findings reported by **Odjadjare *et al.* (2008**) who stated that axenic bacterial cultures were better degraders of Escravos light crude oil in comparison to several mixed bacterial consortia.

The growth profiles of the single and mixed fungal cultures revealed a continuous drop in pH (Table 6) with a concomitant decrease in the TPH (DRO) content of the inoculated sludge (Table 5). **Nwachukwu and Ugoji, (1995)** stated that microbial degradation of hydrocarbons often leads to production of organic acids and other metabolic products. Thus, organic acids probably produced account for the reduction in pH levels **(Oboh *et al*., 2006).** Hydrogen ion concentration is a major variable governing the activity and composition of fungi **(Sebiomo *et al*., 2011).** Many species can metabolize over a wide pH range from the highly acidic to alkaline extremes (Sebiomo *et al*., 2011). Thus, in spite of the acidic pH of the surrounding medium, the growth profile isolates were able to assimilate both glucose and petroleum hydrocarbons and also effect a reduction in the residual TPH content of the petroleum sludge. With the exception of the yeast consortium , there was a parallel increment in the dry weight of the recovered fungal myceila and the turbidity readings recorded at day 20 of the growth profile study (Table 7 and 8). This trend was not suprising as the observed submerged yeast biomass was lesser in magnitude in comparsion to the filamentous fungal mycelia biomass. However the increment in the turbidity values at both day 16 and 20 of the shakeflask test could indicate the increased metaboblic activities of both the axenic and mixed fungal cultures as a consequence of the assmiliation of both hydrocarbons present in glucose and sludge.

The addition of glucose had an expected positive effect on the consequent growth and metabolism of the residual sludge by both the axenic and consortium of filamentous fungi and yeasts. This finding has been collaborated by **Snellman and Greathouse (1996)** who reported the positive effects of glucose on the degradation of jet fuel by *Penicillium* sp., *Fusarium* sp. and *Trichoderma* sp. They also suggested that the addition of glucose may be useful in increasing fungal growth and the rate of hydrocarbon degradation in fuel contaminated soils. Despite the absence of hydrocarbonclastic fungal cultures in the control flask, there was an observed percentage reduction in the residual TPH (DRO) concentration of the sludge content of the flask (Table 5). This event might be attributed to abiotic degradation mechanisms especially photodegradation as the control flasks were continuously agitated under daylight conditions at ambient temperaturas (28± 2^0 C), alongside the other labeled flasks for 20 days using the mechanical shaker. Photodegradation can be classified as either direct or indirect photolysis **(Plata and Sharpless, 2008).**

All the analysed top soil samples were acidic, with pH values ranging from 5.81 for the control soil sample to 6.52 for B (Table 3). This observation is similar to those observed by **IIembayo and Kolade, (2008)**, 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. **(IIembayo and Kolade, 2008**). The moisture content of the soil samples were generally higher than those reported by Ipeaiyeda *et al*. (2007) who reported values ranging from 0.5% to 7.2% for top soil samples collected from several automobile workshops in Iwo town, Osun State. The soil samples were sandy as revealed by the particle size analyses of the samples (Table 3). This trend is in agreement with report by **Ipeaiyeda *et al.* (2007**) who stated that soil samples collected from several automobile workshops at Iwo town, Osun State were sandy. The heavy metals (Pb, Cd and Zn) concentrations recorded for the respective soil samples (Table 2) were at variance with reports by **Ipeaiyeda *et al.* (2007)** and **IIembayo and Kolade, (2008). Ipeaiyeda *et al.* (2007)** reported values ranging from 0.05 mg/kg for Zinc and 184 mg/kg for Lead. **IIembayo and Kolade, (200**8) stated values ranging from 730.97 mg/kg for Zinc to 217 mg/kg for Lead in respect of soil samples collected from five automobile workshops located in Akure, Ondo State, Nigeria. The range of Lead values obtained in this study (Table 3) also contrasted with a report by **Olayiwola (2011)** who reported higher Lead values which ranged from 10.1±2.5 mg/kg to 2460 ±16 mg/kg for soil samples sourced from automobile workshops located in Osogbo, Iree, Ikirun and Iragbiji towns in Osun State, Nigeria. The 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 and 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 premises of auto mobile workshops are a source 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 contaminated environments. *A. flavus* and the consortium of filamentous fungal isolates caused the highest reduction in the TPH (DRO) content of the sludge in the presence of an additional carbon and energy source (glucose).However due to the fact that *A. flavus* and *A. terreus* are considered as BLS 2 pathogenic fungi, only *Aspergillus* sp., *Penicillium* sp. and the consortia of yeasts can be applied in bioreactor based processes for the treatment of oily sludges as described by **Singh *et al.* (2001).**

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