Assessment of Oil-Eating Fungi Isolated from Spent Engine Oil Polluted Soil Environments.

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ABSTRACT

Fungi capable of engine oil degradation were isolated from mechanic workshops and their degrading ability assessed both as pure and mixed cultures at different temperatures. Soil samples collected from waste engine oil contaminated and non-contaminated reference points were analyzed physic-chemically and microbiologically. Of the pure culture isolates, *Penicillium chrysogenum* was the best engine oil degrader consuming 43.33% engine oil within 30 days. However, mixed culture fungi degraded more engine oil (50.02%) within the same number of days.

(Keywords: waste engine oil, environmental pollution, hydrocarbon utilizing fungi, effect of temperature, bioremediation).

INTRODUCTION

Naturally, soil is the richest reservoir of microorganisms and a key component of ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem. Whenever soil is polluted, the ecosystem is altered and agricultural activities are affected (Adedokun and Ataga, 2006; Igwo-Ezikpe *et al*., 2009). Tons of hydrocarbons enter the environment through oil spill, tank leakages or wastewater disposal. These pollutants are toxic and hazardous to life. Their release into the environment has led to many environmental problems that are of global concern (Adedokun and Ataga, 2006; Wang *et al.,* 1994). Nevertheless, some of the soil microorganisms that participated in soil processes such as transformation of nutrients are active hydrocarbon degraders (Ijah and Ukpe, 1992).

Oil spills occur at all stages of production, transportation, and handling of petroleum products. This could results from pipeline ruptures, accidents, and dumping of waste engine oil. The consumption of engine oil in Nigeria has been on the increase in recent years due to the upsurge in the number of vehicles, power plants, and generators that make use of this lubricant (Odjegba and Atebe, 2007). This directly affects the rate at which spent engine oil enters and pollutes the environment as disposal of the spent engine oil into gutters, water drains and vacant plots is a common practice among automobile mechanics that change oil from motor vehicles and power generating machines. The indiscriminate disposal of this waste oil increases pollution incidents in the environment (Odjegba and Atebe, 2007).

Crude oil and petroleum products such as gasoline, fuel oils and diesel fuels are complex mixtures of organic compounds and have been shown to be toxic to plants. Generally, soil conditions of agricultural land, microorganisms as well as plants are damaged or altered by any contact with crude oil (Onuoha *et al.,* 2003). Beyond 3% concentration in an environment, crude oil becomes increasingly toxic to soil biota and crop growth (Onuoha *et al.,* 2003). Additionally, polycyclic aromatic hydrocarbons (PAHs) present in oil products are ubiquitous contaminants in soils, sediments and water, and are of environmental concern because of their mutagenic and/or carcinogenic effects (Hilyard *et al*., 2008). One major environmental concern of soil contaminated with crude oil or petroleum product is an increase in organic carbon of the soil with a concomitant decrease in soil nitrate and phosphorous, thus imposing a condition that impaired oil degradation in the soil (Okolo *et al*., 2005). As a means of remediation of soil polluted with these hydrocarbons, various technologies

have been employed among which is bioremediation (Adedokun and Ataga 2006).

Bioremediation is a biotechnological approach of rehabilitating areas damaged or degraded by pollutants as well as mismanagement of ecosystem. It is the ability of microorganisms to degrade or detoxify organic contaminated area by transforming undesirable and harmful substances into non-toxic compound (Clementina and Omoanghe, 2008; Grady, 1985). Efforts to achieve biodegradation of oil products have involved bacteria and fungi, since they are the only biological species with metabolic capability of utilizing petroleum carbon for cell synthesis (Jobson *et al.,* 1974). In recent times, an increasing number of microbiological researches have been devoted to bioremediation of oil-contaminated sites using various microbial species especially those indigenous to the contaminated environments. In this study, we isolated fungi capable of degrading engine oil and assessed their oil degrading ability.

MATERIALS AND METHODS

Study Site

The study was carried out in Ota, Nigeria. Indiscriminate disposal of used engine oil is the major source of oil pollution in this locality. Six mechanic workshops (about 4 km apart) contaminated with used engine oil were randomly selected for this study.

Sample Collection

Oil contaminated soils were collected around six randomly selected mechanic workshops. Soil samples were also collected from non-contaminated reference areas at about 100 m from the contaminated sites. At each sampling point, four soil samples were collected; two samples each from depths 0 – 15 and 15 – 45 cm respectively using a hand auger. Samples were immediately taken to the laboratory for analysis.

Physico–Chemical Analysis

The mean temperatures of the samples were determined using a mercury thermometer while the pH of the samples were determined using portable pH meter with combined glass and calomel electrodes as previously described (Umanu and Nwachukwu, 2010). Phosphate, sulphate and nitrate concentrations were determined spectrophotometrically using the standard analytical methods described by APHA (1998). The moisture content of the soil samples was determined using moisture analyzer. The residual engine oil was extracted from the soil sample using n-hexane: dichloromethane system (1:1) and quantified by gravimetric method (Le Dreau *et al.,* 1997; Nwachukwu, 2000; Okolo *et al.,* 2005; Umanu and Nwachukwu, 2010). To achieve this, 10g of homogenized soil sample was weighed into a 75ml beaker and 50ml of n-hexane: dichloromethane was added to extract the residual engine oil in the soil sample. After shaking vigorously, the mixture was allowed to stand for 5 minutes and then filtered through whatman No1 filter paper into 75ml beaker of known weight (W_1) as residual oil extract (ROE). The residual oil extract was placed in an oven at 80^0C for 5-10 minute to evaporate the solvent system. The combined weight of the residual oil and the beaker was taken and recorded as W_2. The residual oil content (ROC) was then obtained by difference in mass ($W_2 - W_1 = ROC$).

Determination of Viable Fungal Counts

Viable fungi were determined by plating serially diluted samples on potato dextrose agar incorporated with chloramphenicol and incubated at 30^oC for 2 to 3 days. Oil degrading fungi were enumerated on minimal salt agar (pH, 5.6) using sterile motor oil as carbon and energy source as previously reported (Amund *et al*., 1994).

Isolation of Oil Degrading Fungi and Determination of their Oil Degrading Ability

Oil degrading fungi were isolated from samples by the enrichment culture technique using sterile motor oil as carbon and energy source (Amund *et al*., 1994). To do this, 1.0g of thoroughly mixed soil sample was inoculated into sterile minimal salt broth containing 10% V/V sterile motor oil as the sole carbon and energy source (enriched culture medium) and incubated at 30°C for 5 days. Two milliliters (2ml) of the enriched culture was aseptically transferred into a fresh enrichment culture medium and incubated for another 5 days. The same process was repeated for the third enrichment culture medium. Fungal isolates were obtained by plating 0.1ml from the

third enrichment culture onto potato dextrose agar incorporated with chloramphenicol. Colonies were subcultured severally on the basis of their colonial characteristics to obtain pure culture isolates (Nwachukwu and Akpata, 2003). The pure culture isolates were presumptively identified following the methods described by Smith (1969), Barnett and Pankhurst (1974) and O'Donnell (1979)

To determine the oil-degrading ability of the pure and mixed culture isolates, 20 g portion of steam sterilized soil containing 4 ml of sterile engine oil were set up. Each batch of four 20g portions was inoculated with a standard suspension (approximately 2.02 x 10^3 cfu/ml) of each pure isolate while a batch was inoculated with a mixture of the pure isolates. Non-culture inoculated sample served as control. The set-ups were incubated at 30°C for 30 days. Sub-samples (2.0 g each) were withdrawn at day zero and at 5 day intervals for analysis. Oil-weight loss following fungal degradation was assessed gravimetrically after extraction with n-hexane : dichloromethane (1:1). Values obtained were expressed as percentages of the amount of oil in sample at day zero (Akoachere *et al*., 2008).

Influence of Temperature on Engine Oil Degradation

The experiment was set up as in the determination of oil degrading ability of isolates above, but different set ups were incubated at various temperatures of 26°C, 28°C, 31°C and 37°C respectively. The percentage of oil degraded at 5 days interval over a period of 30 days was calculated (Akoachere *et al*., 2008).

Statistical Analysis

Data collected were analyzed using the SPSS 17.0 statistics and Microsoft Excel 2010.

RESULTS AND DISCUSSION

Table 1 presented the physicochemical properties of the contaminated and non-contaminated reference soil samples analyzed. As revealed in Table 1, the non-contaminated reference soil samples collected have zero residual oil content, confirming that the reference samples were not polluted with oil.

Table 1: Physico-Chemical Parameters of Contaminated and Non-Contaminated Soil Samples.

Sample Location	Depth (cm)	Moisture Content (%)		Residual Oil Content (mg/kg)		NO_3^- Concentration (%)		PO_4^{-3} Concentration (ppm)		SO_4^{-2} Concentration (ppm)	
		Cont.	Non-Cont.	Cont.	Non-Cont.	Cont.	Non-Cont.	Cont.	Non-Cont.	Cont.	Non-Cont.
	0-15	5.10±0.3	3.70±0.4	10.10±0.2	0	2.03±2.01	2.04±1.83	4.42±1.02	4.14±1.10	274.48±1.65	276.88±1.30
Atan	15-45	7.25±0.7	4.45±0.3	2.02±0.5	0	2.04±1.90	2.03±1.75	4.06±1.05	4.15±1.07	179.36±1.80	215.12±1.64
	0-15	5.45±0.5	6.85±0.4	5.21±0.3	0	2.04±2.00	2.04±1.96	4.18±1.14	4.06±1.04	281.20±1.52	182.40±1.73
Onibunku	15-45	7.25±0.8	5.60±0.6	5.03±0.6	0	2.04±1.87	2.04±1.64	4.04±1.25	4.04±1.13	260.72±2.01	218.96±1.90
	0-15	2.75±0.3	8.65±0.2	9.01±0.7	0	2.04±1.74	2.04±1.89	4.03±1.09	4.67±1.15	188.88±1.58	189.84±1.49
Iju	15-45	5.35±0.2	7.90±0.7	3.22±0.4	0	2.03±1.82	2.04±1.65	4.03±1.11	4.10±1.08	274.40±1.48	203.12±1.75
	0-15	6.01±0.5	4.85±0.4	9.11±0.5	0	2.04±1.96	2.04±1.35	4.18±1.03	4.07±1.19	180.72±2.00	198.24±1.43
Iyana-yesi	15-45	8.25±0.6	10.95±0.8	3.01±0.3	0	2.03±1.68	2.04±1.57	4.04±1.10	3.99±1.02	251.12±1.39	199.12±1.94
	0-15	5.20±0.3	11.15±0.5	4.21±0.5	0	2.04±1.60	2.04±1.49	4.10±1.18	4.19±1.04	206.64±1.73	197.92±2.01
Oju-ore	15-45	8.70±0.9	12.15±0.7	2.12±0.4	0	2.03±1.53	2.04±1.72	4.01±1.21	4.20±1.06	267.60±1.29	193.04±1.98
	0-15	4.05±0.4	2.15±0.3	6.03±0.7	0	2.04±1.92	2.04±1.55	4.00±1.30	4.02±1.17	263.52±1.64	174.24±2.00
Sango	15-45	5.55±0.2	5.25±0.5	4.14±0.5	0	2.03±1.75	2.04±1.48	3.98±2.01	4.02±1.12	252.96±1.29	194.08±1.91

Key
Cont. = Contaminated
Non-Cont. = Non Contaminated

Bearing in mind the reports by Gesinde *et al*. (2008) and other researchers that indigenous microorganisms are more capable of degrading indigenous crude oil compared to an imported microorganism due to the fact that native microorganisms are best adapted to intrinsic environmental conditions, we isolated indigenous engine oil degrading fungi in this study and assessed their oil degrading ability with an intension to use them for bioremediation of oil contaminated sites within our local environments. We observed that the heterotrophic fungal counts (Table 2) in soil samples collected at depth 0-15cm from both contaminated and non-contaminated reference areas were generally higher than the fungal counts of those collected at depth 15-45cm and differed significantly when subjected to variance analysis ($p \leq 0.05$). The same trend was observed for oil degrading fungal counts (Table 3). This could probably be attributed to the fact that most fungi are either aerobic or facultatively anaerobic which is in line with the report by Nester *et al.* (2004).

The fungal counts noticed for engine oil degradation were significantly high at depth 0-15cm for oil contaminated soils (Table 3). This showed that oil degraders are more abundant in surface soils than in sub-surface soil samples. It also revealed that most petroleum hydrocarbon degrading fungi require free or dissolved oxygen, and that the population density of hydrocarbon-utilizers increases in an ecosystem exposed to crude petroleum or petroleum products as previously reported (Odu, 1981; Calomiris *et al*., 1977; Nwachukwu, 2000). Engine oil degrading fungi were also isolated from the reference soil samples not contaminated with oil (Table 3). This probably confirmed that microorganisms capable of hydrocarbon utilization are widely distributed in nature and have been found in areas not directly contaminated with hydrocarbons as also reported by Atlas (1981). At depths 15-45cm for both contaminated and non-contaminated soil samples, the fungal counts for both heterotrophic and oil degraders (Tables 2 and 3 respectively) were observed to be low and could be accounted to the fact that both heterotrophic and oil-degrading fungal counts reduces with increase in depth which agreed with previous report (Akoachere *et al*., 2008).

The fungi isolated in this study were *Penicillium chrysogenum, Aspergillus* species and *Candida* species. Of the pure culture isolates, *Penicillium chrysogenum* was the best engine oil degrader, consuming about 43.33% engine oil within 30 days (Figure 1). However, the mixed culture containing all the isolates exhibited more degradation ability than any of the individual isolates (50.02%) and this is in line with other works (Amund and Nwokoye, 1994; Facundo *et al.,* 2001; Obire and Anyanwu, 2009). In a mixed culture, some species utilise intermediates of degradation of the original hydrocarbon produced by other members of the culture leading to a complete degradation of the oil, thus, a mixed culture is a better inoculum for oil spill clean-up.

Table 2: Distribution of Heterotrophic Fungi in Contaminated and Non-Contaminated Soils around some Mechanic Workshops in Ota.

Sample Location	Mean Population Densities of Heterotrophic Fungi (cfu/g ± SD) X 10^5			
	CONTAMINATED SOIL		NON-CONTAMINATED SOIL	
	0 - 15 cm	15 - 45 cm	0 - 15 cm	15 - 45 cm
Atan	8.3 ± 0.00^{m}	7.5 ± 0.01^{k}	9.1 ± 0.01^{n}	7.2 ± 0.01^{j}
Onibunku	4.3 ± 0.01^{d}	4.1 ± 0.14^{c}	5.1 ± 0.03^{e}	4.2 ± 0.03^{cd}
Iju	5.5 ± 0.03^{f}	3.2 ± 0.00^{a}	6.1 ± 0.03^{h}	3.4 ± 0.01^{b}
Iyana-yesi	5.9 ± 0.14^{g}	6.1 ± 0.14^{h}	7.1 ± 0.00^{ij}	5.1 ± 0.03^{e}
Oju-ore	8.2 ± 0.00^{m}	7.0 ± 0.11^{i}	9.1 ± 0.00^{n}	7.5 ± 0.01^{k}
Sango	7.5 ± 0.03^{k}	5.5 ± 0.03^{f}	7.9 ± 0.03^{l}	6.1 ± 0.03^{h}

SD, standard deviation; cfu, colony forming unit.
Rows and Columns with the same superscript are not significantly different ($p \leq 0.05$).

Table 3: Distribution of Engine Oil Degrading Fungi in Contaminated and Non-Contaminated Soils around some Mechanic Workshops in Ota.

Sample Location	Mean Population Densities of Oil-Degrading Fungi (cfu/g ± SD) X 10^3			
	CONTAMINATED SOIL		NON-CONTAMINATED SOIL	
	0 - 15 cm	15 - 45 cm	0 - 15 cm	15 - 45 cm
Atan	4.1 ± 0.01^{n}	2.3 ± 0.01^{c}	3.6 ± 0.01^{l}	2.5 ± 0.00^{d}
Onibunku	5.2 ± 0.01^{q}	2.5 ± 0.03^{d}	3.5 ± 0.01^{k}	2.1 ± 0.01^{b}
Iju	3.4 ± 0.00^{j}	3.1 ± 0.01^{h}	3.2 ± 0.01^{i}	2.3 ± 0.01^{c}
Iyana-yesi	4.1 ± 0.01^{n}	3.2 ± 0.03^{i}	3.7 ± 0.03^{m}	3.0 ± 0.01^{g}
Oju-ore	5.1 ± 0.03^{p}	3.0 ± 0.13^{g}	3.6 ± 0.00^{l}	2.8 ± 0.03^{f}
Sango	4.2 ± 0.00^{o}	2.6 ± 0.01^{e}	3.2 ± 0.03^{i}	1.9 ± 0.01^{a}

SD, standard deviation; cfu, colony forming unit.
Rows and Columns with the same superscript are not significantly different ($p \leq 0.05$).

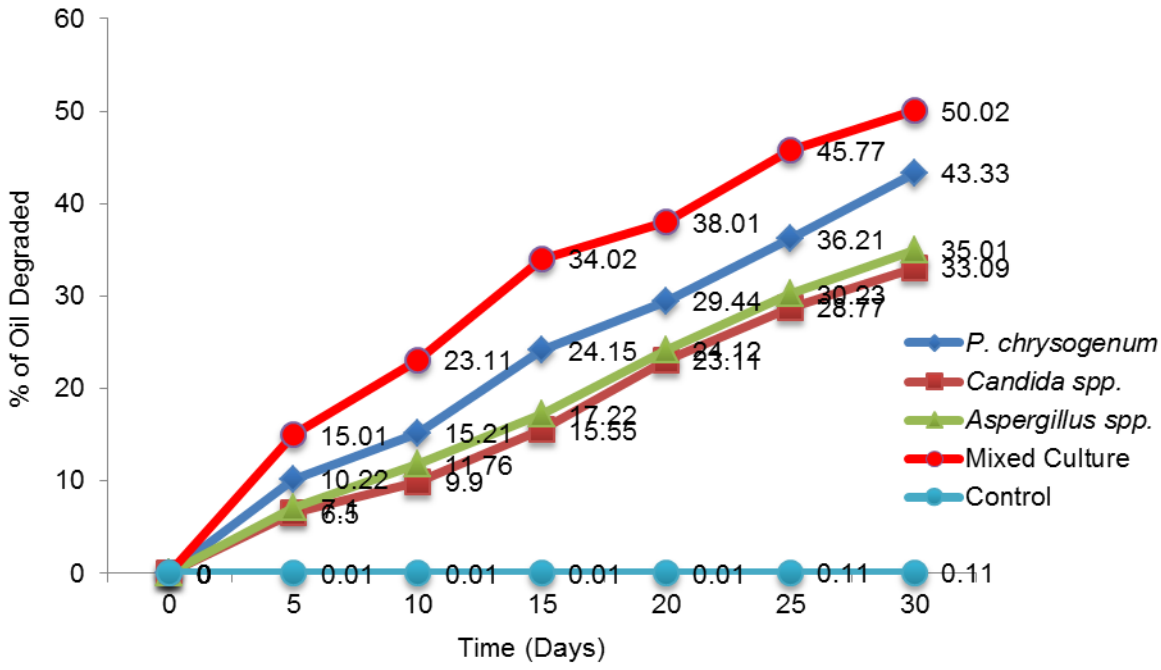

Figure 1: Thirty Days Monitoring of Oil Degradation by Pure and Mixed Fungal Isolates.

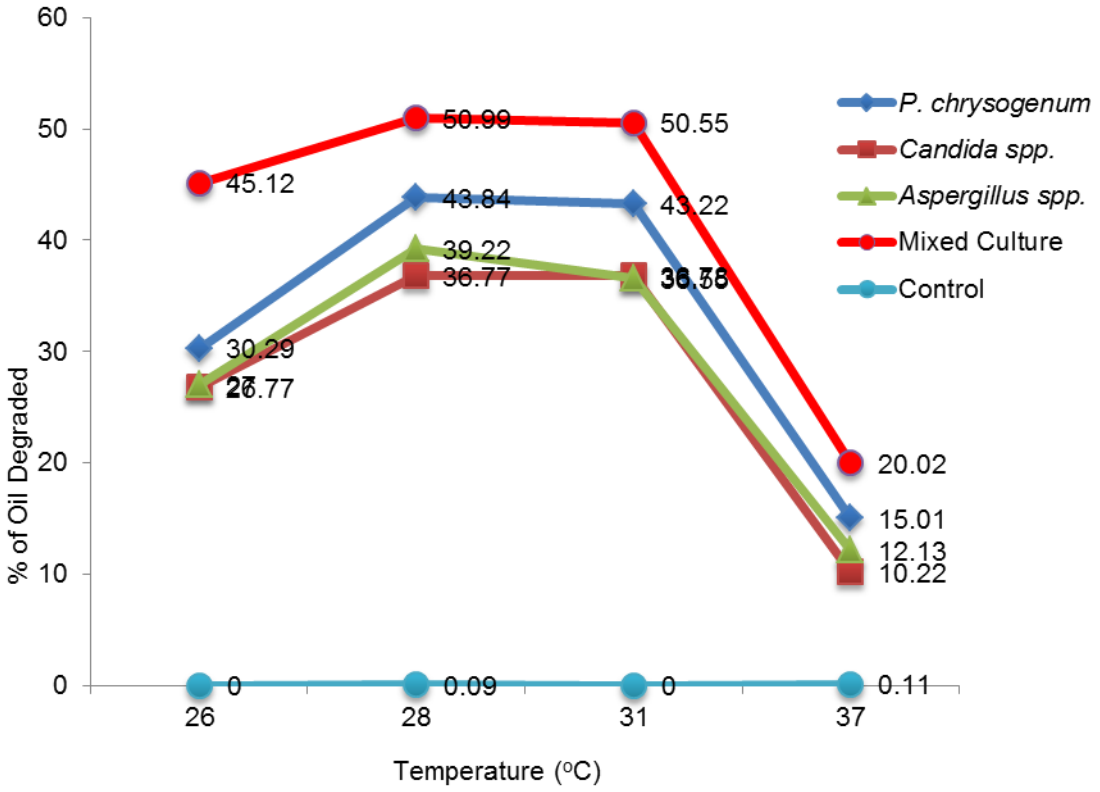

Figure 2: The Effect of Temperature on the Degradation Patterns of Fungal Isolates after 30 Days of Monitoring.

Figure 2 depicts the effects of temperatures on fungal degradation of engine oil. The results obtained showed that between temperatures 28 – 31°C, the degradation potentials of both individual isolates and mixed cultures were at their optimal, but at temperature 37°C, the degradation potential of the individual isolates and mixed cultures were observed to decrease greatly and this could probably be attributed to the fact that these organisms were found in soil environments with temperatures less that they were subjected to in the laboratory.

CONCLUSION

In conclusion, it was clear that there is abundance of engine oil degrading fungi in the soil which can be exploited in oil spills clean-up. It was interesting to know that oil degrading fungi could be isolated from soils not directly contaminated with used engine oil or similar pollutants. The use of fungi in bioremediation is advantageous in the respect that fungal mycelia have the ability to penetrate oil, thereby increasing the surface area available for biodegradation and bacterial attack. Fungi can also grow under environmentally stressed conditions such as low pH and poor nutrient status, where bacteria growth might be limited. However, there should be awareness to enlighten mechanics on the danger of indiscriminate disposal of used engine oil.

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