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Fungal flora of mechanic workshops and its bioremediation

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Abstract The study was carried out to isolate and identify the fungi species present in contaminated soil samples from various mechanic workshops and also evaluate their biodegradation potential. Soil samples were obtained from three locations and one gram of each was added to nine milliliter of distilled water to give a tenfold serial dilution of which was made up to 10^{-3} dilution. Zero point one milliliter aliquot of 10^{-3} of each sample was pour-plated on prepared Sabouraud Dextrose Agar (SDA) and incubated at 25^{0}C for 5 days for isolation of the heterotrophic and hydrocarbonclastic fungi. The fungal species isolated were seven namely; *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Candida albicans*, *Saccharomyces cerevisiae*, *Fusarium solani* and *Penicillium chrysogenum*. The fungal counts ranged from 1.6 x 10^{-4} to 5.1 x 10^{-4}(cfu/ml) There was variation in the morphological and microscopic characteristics of the seven fungal isolates. The percentage occurrence amongst the fungal isolates was higher in *Aspergillus niger* (41.17%) followed by *Aspergillus terreus*(17.64%), *Aspergillus fumigatus*(17.64%), *Fusarium solani* (5.88%), *Saccharomyces cerevisiae* (5.88%), *Candida albicans*(5.88%), and *Penicilluim chrysogenum*(5.88%). The biodegradation potential of the fungal isolates were confirmed using used engine oil as sole source carbon and energy through the vapour phase transfer method and *Aspergillus niger* was demonstrated as the most versatile among other isolates, making it a promising candidate for bioremediation of soil polluted with petroleum hydrocarbons. Thus, these oil-degrading microbes are abundant in soil and can be applied for bioremediation of soils contaminated with petroleum and petroleum products.

Keywords: Fungi, Contaminated soil, Engine oil, Petroleum hydrocarbon

Introduction

Crude oil and refined petroleum products kerosene, gasoline, diesel, lubricating oil consist largely of which are chemicals composed of hydrogen and carbon in various molecular arrangements (Stoker and Seager, 1976) and it accounts for approximately 35% of total global energy usage (Metman *et al*; 2010) spills, tankers, ballast water, fuels, mechanic sites and garages (Okerentugba and Ezeronye, 2003) The presence of these pollutants in the terrestrial and aquatic environments constitutes health problems and socio-economic hazards (Makut and Ishaya, 2010). Although the toxicity of

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petroleum products varies widely depending on their composition, concentration, environmental factors and the biological state of the organisms at the time of the contamination (Obire and Ayanwu, 2009).

Mechanic workshops abound in most urban cities in Nigeria and they are facilities where automobiles are usually operated in semi stationary modes (Ipeadiyeda *et al*., 2007). According to Opara (2014), over 600 cases of oil spills were reported in the Niger Delta areas of Nigeria in 2014. One of the silent oil spills that go unnoticed in Nigeria is that of mechanic workshop where used oil and other petroleum products are released accidentally or deliberately to the environment. Ololade (2014) reported that in Nigeria and most developing countries, there is an ever increasing demand for personal vehicles and this has led to the increase in number of mechanic workshops in the country and increase in their activities.

Engine oil is a complex mixture of hydrocarbons and other organic compounds including some organo-metallic constituents (Butler and Mason, 1997) that is used to lubricate the parts of an automobile engine in order to avoid excessive wearing out (Rahman *et al*., 2004). 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 these lubricants and thus directly affects the rate at which spent engine oil enters and pollutes the environment (Odjegba, 2007). Automobile workshops are on important component of the service sector industry and the most significant environment impact associated with the existing workshop is the seepage of used engine oils and washed water into the soil (Odjegba and Sadiq, 2002). In Nigeria, about 20 million gallons of waste engine oil are generated annually from mechanic workshop and discharged carelessly into the environment (Onwurah, 1999: Taiwo and Otorin, 2009).

Bioremediation is the productive use of biodegradative process to remove pollutants that are threat to public health (Thapa *et al.*, 2012). Alexander (1999) opined that the goal to bioremediation is to transform organic pollutants into harmless metabolites or mineralize the pollutants into carbon-dioxide and water. According to Department of Environmental quality, before bioremediation can take place, the following must be present: a contaminant, suitable microorganisms and an electron acceptor (1998). Bioremediation methods are considered to be more economical and safe for treatment of oil polluted sites (Hagwell *et al.*, 1992). The first commercial use of a bioremediation system was in 1972 to clean up a sun oil pipeline spill in Ambler, Pennysylvania (National Research Council 47). Since 1972, bioremediation has become a well-developed way of cleaning up different contaminants.

Bacteria and Fungi are known to be the principal agents of biodegradation of hydrocarbons (Nester *et al*., 2004) and these organisms can be exploited for use in biological processes for the abatement of pollution (Wainright *et al*; 1993). Fungi are found in virtually every soil environment and their growth is usually stimulated by warm and humid conditions (Russel and Landsberg, 1971). Fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the environment and have the mechanism for the elimination of spilled oil from the environment and thus have been found to be better degraders of petroleum than traditional bioremediation techniques with bacteria (Ojo, 2005). In addition, fungi have advantages over bacteria such as fungal hyphae that can penetrate contaminated soil to reach the pollutants (Husaini and Roslan, 2008).

Fungi have also demonstrated the ability to degrade and mineralize phenols, halogenated phenolic compounds, petroleum hydrocarbons, polycyclic aromatic compounds and poly chlorinated biphenyls (Singh, 2006). Many researchers studied the role of fungi in biodegradation process of petroleum products and the most common fungi which have been recorded as biodegraders belong to the following genera: *Alternaria, Aspergillus, Candida, Cephalosporium, Fusarium, Geotrichum, Gliocladium, Mucor, Pleurotus, Paecilomyces, Polyporus, Rhizopus, Rhodotorula, Saccharomyces, Talaromyces* and *Torulopsis* (Adekunle and Adebambo, 2007). Chaundrhry *et al*. (2012) further reported that the advantages associated with fungal bioremediation lay primarily in the versatility of fungi in utilizing petroleum hydrocarbon when compared to other microbial technologies. Although many environmental, physical and chemical factors like temperature, nutrient, oxygen, biodegradability, photoxidation, bioavailability, soil moisture, soil acidity and alkalinity affect the process of biodegradation of hydrocarbons (Rahman *et al*., 2003). Adekunle and Adebambo (2007) demonstrated the ability of *Aspergillus niger, Aspergillus flavus, Mucor* spp., *Rhizopus* spp. and *Talaromyces* spp. to utilize and degrade crude oil and other petroleum products such as diesel, kerosene, spent and unspent engine oil. Similarly, Uzoamaka *et al*. (2009) isolated Aspergillus *versicolor, Aspergillus niger, Aspergillus flavus, Syncephalastrum* spp., *Trichoderma* spp., *Neurospora sitophilia, Rhizopus arrhizus* and *Mucor* spp. from oil contaminated soil and demonstrated their potentials for hydrocarbon degradation.

Due to the release of toxic pollutants to the environment as a result of oil spillage from mechanic workshop, there is little information on the possibilities of fungal species to be isolated from these soils and also degrade the pollutants present in them.

The aim of this study was to isolate and identify the fungal species present in the soil samples collected from various mechanic workshops and to evaluate the hydrocarbon degradation potentials (bioremediation) of these fungi associated with the oil contaminated soils. The specific objectives of the study were isolated and identified the fungal species present in mechanic workshop. The hydrocarbon degradation potentials of the fungal species were isolated.

Materials and method

Sources of soil samples

Oil contaminated soil samples were taken from two mechanic workshop located within different quarters in Awka with the aid of a hand auger. A control soil sample was also collected from a fallow farmland within Awka-city. Samples from each site were collected and transferred into sterile polythene for analysis at Nnamdi Azikiwe University Department of Botany, Awka, Anambra State.

Isolation and Enumeration of Heterotrophic and Hydrocarbonclastic utilising fungi in soil samples

Each sample was homogenously mixed and carefully sorted to remove stones and other unwanted debris. Isolation and enumeration of hetetrophic fungi was done by serial dilution agar plating method. Sabouraud Dextrose Agar (SDA) culture media was used to isolate the fungal species that were present in all samples. One gram (1g) of each soil sample was weighed into three (3) test tubes containing nine milliliter (9ml) of sterile distilled water and this gave 0.1 dilution (a ten (10) fold serial dilution) of which was agitated for one minute. Serial dilutions of each of the soil sample were made up to 10^{-3} dilution from the 0.1 dilution. Then, zero point one milliliter (0.1ml) aliquot of 10^{-3} dilution of each sample was aseptically removed with a sterile pipette and was cultured on plates of SDA by pour-plate method. Plating was done in triplicates and all the media were supplemented with chloramphenicol (500mg/L) to inhibit bacterial growth. The culture plates were swirled, allowed to solidify and incubated at room temperature 25^0C for five days. After incubation, the colonies that developed on the SDA plates were enumerated and recorded as colony-forming units (CFU) per gram soil. Discrete colonies were sub-cultured onto fresh medium for the development of pure isolates which were stored on Sabouraud Dextrose Agar slants for subsequent characterization. The counts of the isolates were further calculated and expressed in percentage

of occurrence.

Characterization of the soil fungi

The cultural characteristics of the pure isolates on SDA were noted (color and colonial appearance of fungal colony) and morphological characteristics including spore type, mycelia and other fruiting bodies in a lactophenol cotton blue wet mount by compound microscope at magnification of 100.

Test for identification and utilization of true-petroleum utilizing fungi

Supposed petroleum-utilizing mould isolates were streaked on plates of agar medium (one isolate per plate). The preparation of the engine utilization test was done through vapour-phase transfer (using 1% (v/v) used engine oil). In the inside of the petri-dish cover was placed a sterile filter paper (whatman No.1) saturated with filter-sterilized used engine oil. This was aimed at supplying hydrocarbon (engine oil) as sole source of carbon and energy for the growth of the micro-organisms on the separate prepared agar media surface through vapour phase transfer. All the plates were inverted and incubated at room temperature for seven days (Okpokwasili and Amanchukwu, 1988). Colonial growth of fungi which appeard was noted as confirmed engine-utilizers.

Statistical analysis

Mean and mean deviation of fungal counts were computed for each location, while the differences in means between locations were determined by the Least Significant Difference (LSD) test as recommended by Bailey (1995). Percentage occurrence of isolates were determined using the methods of Sampo *et al.* (1997).

Results

Isolation and Identification of fungal isolates from the soil samples

A total of seven fungal isolates were obtained from the different soil samples. They were identified as *Aspergillus niger*, *Aspergillus terreus, Aspergillus fumigatus, Yeast (Saccharomyces cerevisiae), Candida albicans, Fusarium solani* and *Penicillium chrysogenum* (Table 1).

Table 1. Showing fungi isolated from various mechanic workshops

S/ND	Location	Total Fungal count (cfu/ml)	Fungal Isolates
1	Ozone road	2.6 X 10^{-4}	*Aspergillus terreus* *Fusarium solani* *Saccharomyces cerevisiae*
2	Ukwu-Aki road	1.6 X 10^{-4}	*Candida albicans* *Aspergillus fumigatus* *Aspergillus niger*
3	Unizik Hostel	5.1 X 10^{-4}	*Aspergillus niger* *Aspergillus fumigatus* *Penicillium chrysogenum*

CFU/ml = Number of colonies X The dilution Factor (10^{-3})

The heterotrophic and hydrocarbonclastic fungal counts for the various soil samples collected from the respective mechanic workshops were ranged from 1.6 X 10^{-4} to 5.1 x 10^{-4} (Table 1). The fungal count was higher in Unizik Hostel while the fungal count was lower in Ukwu-Aki road.

Fungal characterization

There was variation in the morphological and cultural characteristics of the fungal isolates and of the fungal isolates and it is presented below (Table 2).

Table 2. Showing morphological characteristics of fungi isolated from the mechanic workshop

Characteristics	***Aspergillus niger***	***Aspergillus terreus***	***Aspergillus fumigatus***	***Saccharomyces cerevisiae***	***Fusarium solani***	***Candida albicans***	***Penicilliium chrysogenum***
Surface	Pin-like dark brown, black growth.	Cinnamon-brown	Grey-green	Cream to white	White-creamy	Creamy-white	Blue-green
Margin	Entire	Entire	Entire	Entire	Entire	Small not entire	Entire
Growth	Rapid	Moderate to rapid	Rapid	Rapid	Rapid	Rapid	They grow fast

The isolated species were also examined for their microscopic characteristics (Table 3).

Table 3. Microscopic characteristics of fungi isolated from the various mechanic workshops

Fungal Isolate	Microscopic view	Fruiting body
Aspergillus niger	Non-branched conidiophores with bulb end carrying conidia like sun-rays.	Cleistothecia present
Aspergillus terreus	Branded septate conidiophores with bulb end carrying conidia.	Cleistothecia present
Aspergillus fumigatus	Chains of round conidia with conidial heads in the form of compact columns. Phialides uniserate, concentrated on the upper surface of the vesicle.	Cleistothecia present
Candida albicans	They have blastoconidia which are small clusters and are round and they have pseudohyhae.	Cleistothecia absent
Fusarium solani	Cylindrical to avoid conidia, curved septate conidiophores.	Cleistothecia present
Penicillium chrysogenum	Long erect conidiophores round conidia.	Cleistothecia present
Saccharomyces cerevisiae	Large globose to ellipsoidal budding yeast-like cells or blastoconidia.	Cleistothecia absent

Distribution of isolated species of fungi from the mechanic workshops

Frequency of occurrence of the fungal isolates is shown in Table 4. The number of times they were isolated from each of the locations of the soil samples and *Aspergillus niger* had the highest frequency of occurrence (41.17%), *Aspergillus terreus* (17.64%), *Aspergillus fumigatus* (17.64%), *Fusarium solani* (5.88%), *Saccharomyces cerevisiae* (5.88%), *Candida albicans* (5.88%) and *Penicilluim chrysogenum* (5.88 %).

Table 4. Percentage occurrence of fungi isolated from the mechanic workshops

Fungal Isolates	Ozone road	Ukwu-Aki road	Unizik Hostel	Total	% Occurrence
Aspergillus niger	0	2	5	7	41.17
Aspergillus terreus	3	0	0	3	17.64
Aspergillus fumigatus	0	1	2	3	17.64
Fusarium solani	1	0	0	1	5.88
Saccharomyces cerevisiae	0	1	0	1	5.88
Candida albicans	1	0	0	1	5.88
Penicillium chrysogenum	0	0	1	1	5.88
Total	5	4	8	17	

Discussion

The auto-mechanic workshop soil samples collected were oily dark in color which may be due to long term exposure of the soil to waste engine oil discharged and accumulated (Akoachere *et al*., 2008). Auto mechanic workshop has high indiscriminate dumping of waste engine oil and other refined petroleum products as a result of their activities ranging from servicing, maintenance and repair of automobile thus, a constant change in the soil micro-organisms. These alter the biomass and ecology of the soil such that microbial communities and grasses cannot longer grow on the soil spot. The color and texture of the soil are affected, this leads to different microbial flora establishment in an attempt to remedy the petroleum product spoilage (Megharaj *et al*., 2017).

Seven fungal isolates namely*; Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus ,Fusarium solani, Candida albicans, Saccharomyces* cerevisiae and *Penicillium chrysogenum* were obtained from the various soil samples and it demonstrated that *Aspergillus niger* as the most versatile fungi among the other isolates. Recently, Sanyaolu *et al*. (2012) demonstrated the ability of *Aspergillus terreus, Aspergillus niger, Aspergillus flavus* and *Trichoderma spp* to hydrolyse Premium Motor Spirit (PMS) (petrol) leading to its deterioration.

The ability to isolate high numbers of certain oil degrading micro-organism from oil polluted environment is commonly taken as evidence that these micro-organisms are the active degraders in the environment. Although, hydrocarbon degraders may be expected to be readily isolated from an oil associated environment, the same degree of isolates could be gotten from a totally unrelated environment such as pristine soil (Akoachere *et al*., 2008).

The total plate counts of fungi were higher in the uncontaminated soil (Unizik hostel) than contaminated soil (Ozone road and Ukwu-aki road). This is likely to be due to the environmental stress and toxicity caused by the hydrocarbon to the fungi. This finding agreed with the report of Atlas and Bartha (1992) that crude oil products contain hydrocarbon that are toxic to micro-organism. The significant difference in the total plate count of fungi in contaminated and uncontaminated soil samples may be due to the fact that the fungi thriving in contaminated soils were able to synthesize enzymes capable of digesting the hydrocarbons in the crude oil and used engine oil (Ijah and Abioye, 2003).

In the vapour phase transfer used engine oil utilization test for nine days of incubation for confirmatory identifications of engine oil utilizing moulds, the seven fungal isolates, that is, *Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus ,Fusarium solani, Candida albicans, Saccharomyces cerevisiae* and *Penicillium chrysogenum* showed better growth, further confirming oil biodegradation potential of these fungal isolates. The percentage of hydrocarbon utilizers in a particular environment appears to be an index of the presence of hydrocarbon in that environment and environmental exposure to petroleum hydrocarbons (Mulkins-Philips and Stewart, 1974) and *Aspergillus niger* had the highest percentage (41.17%). The results of this study indicated potential application of the fungal species isolated from the soil of the various mechanic workshops for bioremediation of soils contaminated with petroleum and petroleum products.

The result showed that soils within the premises of automobile workshops are good sources of hydrocarbonclastic fungi notably; *Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Fusarium solani, Saccharomyces cerevisiae, Candida albicans* and *Penicillium chrysogenum.* The seven fungal species isolated from the various soils were capable of utilizing the used engine oil as sole source of carbon and energy which implies that any of them could be for bioremediation either singly or as a consortium of microbial degraders. However, the versatility of *Aspergillus niger* among other organisms makes it, a promising candidate for bioremediation of soil polluted with petroleum hydrocarbons and it can be concluded that oil-degrading microbes are abundant in soils and this exploited for large clean up campaigns.

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