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UTILIZATION OF PETROLEUM PRODUCTS BY FUNGI ISOLATED FROM THE SOIL ENVIRONMENT OF KEFFI METROPOLIS, NASARAWA STATE, NIGERIA

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ABSTRACT
The investigation was conducted to determine the species of fungi associated with soils contaminated with used petroleum products in Keffi metropolis, Nasarawa State, Nigeria. Soil samples contaminated with petroleum products were obtained from 10 different mechanic workshops in Keffi metropolis for the isolation of fungal species. Pour plate and spread plate methods involving soil dilution techniques were employed for the fungal isolation. The media used were Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA). Four species, *Cladosporium* spp, *Cuvularia lunata*, *Penicillium brevicompactum* and *Trichoderma viride* were isolated, and their respective abilities to utilize different petroleum products as sole sources of carbon and energy was assessed by inoculating the isolates unto compounded chemically defined media containing each of gasoline, kerosene, diesel, brake fluid and engine oil. The fungal counts in the different soil locations in Keffi did not differ significantly ($P < 0.05$). The results further showed that *Cuvularia lunata* utilized only kerosene, while *Penicillium brevicompactum* utilized only brake fluid. *Cladosporiun* spp was found to utilize two of the petroleum products, diesel and brake fluid, whereas *Trichoderma viride* utilized three, engine oil, petrol and diesel. The results of this investigation demonstrated that each of the four fungal species isolated has capacity to utilize at least one of the tested petroleum products as sole source of carbon and energy. *Trichoderma viride* is the most versatile, followed by *Cladosporium* spp in terms of their utilization of petroleum products. Thus, these fungi could be employed as candidate species that would be used for bioremediation of petroleum polluted soil environments.

KEYWORDS: Utilization, petroleum products, fungi, soil, Keffi.

INTRODUCTION

Some species of bacteria and fungi are known to degrade or detoxify pollutants in a given environment (Nester *et al*., 2004), and these organisms can be exploited for use in biological processes for the abatement of pollution (Wainright *et al*., 1993). Substances such as hydrocarbons and polychlorinated biphenyls are common contaminants in the environment. These compounds do absorb organic matter in the environment, thus making their decontamination using traditional approaches difficult (Willey *et al.,* 2008). Many factors such as adequate nutrient, pH, temperature and moisture content of the environment influence the degradation rate of pollutants like petroleum hydrocarbons (Stoker and Seager, 1976; Nester *et al*., 2004). Fungi are found in virtually every soil environment, and their growth is usually stimulated by warm and humid conditions (Russel and Landsberg, 1971). Bending *et al.* (2002) reported that certain fungi particularly "white rot fungi" can be used to degrade insecticides, pesticides, pentachlorophenol, creosote, asphalt and heavy fuel, and can convert these into the carbon dioxide, water and basic elements. These organisms can be useful in mycoremediation, a process in which fungi are used in remediation, and this begins with field isolation and collection of the fungi from an area of interest. Mycoremediation also includes selection, culturing, toxicity screening, preconditioning, microcosm scale testing and pilot scale application (Rützler and Sterrer, 1970). It has also been reported that fungi can act as mycofilters in certain environments, especially aquatic environments (Bells, 1990). Crude oil and refined petroleum products such as kerosene, gasoline (petrol), diesel, asphalt, lubricating oil and paraffin wax consist largely of hydrocarbons which are chemicals composed of hydrogen and carbon in various molecular arrangements (Stoker and Seager, 1976). Jacquelyn (1996) reported that *Pseudomonas putida*, *Pseudomonas cepacia*, *Allescheriella* species, and *Phlebia* species as the most active bacterial species involved in bioremediation of hydrocarbons. Overcrash and Pal (1979) reported that laboratory treatability indicated that diesel oil contaminated soil may be successfully remediated *in situ* by forced aeration, which provides oxygen which is the major limiting factor for the biodegradation of petroleum hydrocarbon in contaminated soil. Okerentugba and Ezeronye (2003) demonstrated the ability of *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. to degrade petroleum hydrocarbons, especially when used as single cultures. It has also been reported that bioremediation process using fungi can lead to complete degradation of the petroleum hydrocarbon contaminants in the soil environment (Bento *et al.,* 2005, Achal *et al.,* 2011). Low *et al.* (2008) reported that many strains of fungi have great potentials for remediation of pentachlorophenol (PCP) and polycyclic aromatic hydrocarbon from diesel-contaminated soils in oil spilled sites. Batelle (2000) used

wood-degrading fungi and demonstrated that fungi are better degraders of petroleum hydrocarbon than bacteria. The ability of white-rot fungi, *Pleurotus tuberregium* to ameliorate crude oil polluted soil has also been reported (Isikhuemhen *et al.*, 2003). Chaudhry *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. Adekunle and Adebambo (2007) demonstrated the ability of *Aspergillus niger*, *A. 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 sitophila*, *Rhizopus arrhizus* and *Mucor* spp from oil-contaminated soil and demonstrated their potentials for hydrocarbon biodgradation. The present study aimed at isolating and identifying the fungal species present in soil contaminated with petroleum products in Keffi, and the assessment of the abilities of the isolates to utilize petroleum products with the view to exploiting their potentials for use in mycoremediation of soil environments polluted with petroleum and petroleum products.

MATERIALS & METHODS

Study Area

This investigation was carried out in Keffi town which is in Nasarawa State, Nigeria. Keffi is approximately 58km from Abuja, the Federal Capital Territory of Nigeria; and 128km from Lafia, the Nasarawa State Capital. The town is situated on latitude 8^0 5' North of the equator and longitude 7^0 50' East. The town is 850m above the sea level (Awka *et al.*, 2007).

Sample Collection

Contaminated soil samples were collected from ten different mechanic workshops in Keffi metropolis, namely, Angwan Tiv, Kofar Hausa, Angwan Waje, Market Area, Sabon Layi, G.R.A, Makwalla, Emir's Palace, Yara and Gangaren Masaka. Soil samples of 200g from each site were aseptically collected using sterile spatula into 250g capacity sterile plastic container that was previously washed with 70% alcohol. The soil samples were taken to the laboratory within 30 minutes for immediate analyses.

Isolation and Identification of Fungal Isolates

One gram of each soil sample was weighed into ten test tubes containing 9ml of sterile distilled water, and this was agitated for one minute using a magnetic shaker. Serial dilutions of each of the soil sample were made up to 10^{-3} dilution. The soil suspensions from 10^{-3} dilution were inoculated respectively by spread and pour plate methods. The prepared plates were inoculated in triplicate. The media used for the isolation were Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), which were both supplemented with penicillin ($20mgl^{-1}$) and streptomycin ($20mgl^{-1}$) to prevent bacterial growth. The plates were incubated at 30^0C for 4 days, and observations were recorded daily for the growth of filamentous fungi according to the methods as recommended by Nester *et al.* (2004). The fungal isolates were identified based on the cultural and morphological characteristics, and with reference to the Compendium of Soil Fungi (Domsch *et al.*, 1980).The cultural characteristics were determined by the physical appearance of the colonies of the fungal isolates on culture plates, while the morphological characteristics were determined by observing the mycelia of the isolates under the microscope in lactophenol cotton blue stain.

Test for Utilization of Petroleum Products by Fungal Isolates

The ability of the fungal isolates to survive or utilize the petroleum products was assessed by inoculation of the identified isolates onto separate agar plates containing various used petroleum products as the only source of carbon and energy (Rolling, 2003). The petroleum products used were engine oil, diesel, kerosene, petrol and brake fluid. A quantity of 0.5ml of each petroleum product was added to prepared agar-agar media plates in triplicates before inoculation of the fungal isolates, and these were incubated at 30^0C for 4 days. Growth of fungal colonies on the culture media after 4 days of inoculation was used as an indicator of utilization of petroleum product(s) by the isolate(s) as its source of carbon.

Statistical Analyses

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 frequencies of occurrence of isolates were determined using the methods of Sampo *et al.* (1997).

RESULTS

Table 1 show the Total Fungal Counts of isolates from the different locations where the contaminated soil samples were collected.

TABLE 1: Total Fungal Count (cfu/g) of soil contaminated with used petroleum products

Sites	Total Fungal Count cfu/g)
A	$1.3 \times 10^4 \pm 0.8$
B	$2.0 \times 10^4 \pm 0.1$
C	$4.3 \times 10^4 \pm 2.2$
D	$1.0 \times 10^4 \pm 1.1$
E	$1.1 \times 10^4 \pm 0.9$
F	$3.0 \times 10^4 \pm 0.9$
G	$4.5 \times 10^4 \pm 2.9$
H	$1.2 \times 10^4 \pm 0.7$
I	$1.2 \times 10^4 \pm 0.9$
J	$1.0 \times 10^4 \pm 1.1$

KEYS: A = Angwan Tiv, B =Angwan Waje, C = Emir's palace, D = Gangaren Masaka, E = G . R. A, F =Kofar Hausa, G = Makwalla, H = Market Area, I = Sabon Layi, J = Yara

The soil of the Mechanic Workshop at 'Makwalla' had the the highest count of $4.5x10^4$ cfu/g, while that of 'Sabon Layi' had the least count of $1.0x10^4$ cfu/g of soil. Statistically, the fungal counts of the different locations vary (P>0.05). Table 2 shows the percentage frequencies of occurrence of the fungal isolates in the contaminated soil samples from the different locations with *Trichoderma viride* (90%) being the most predominant species, followed by *Cladosporium* spp. (80%), *Penicillium brevicompactum* (50%). *Curvularia lunata* (20%) had the least occurrence. Table 3 shows the results of the utilization of the petroleum products by the four fungal isolates. *Trichoderma viride* was found to utilize three (engine oil, petrol and diesel) out of the five petroleum products tested, while *Cladosporium* species utilized two (diesel and brake fluid). *Cuvularia lunata* utilized only Kerosene, whereas *Penicillium brevicompactum* utilized only brake fluid.

TABLE 2: Percentage frequency of occurrence of fungal isolates in soil contaminated with used petroleum products

	Sites										Occurrence
Fungal Isolates	A	B	C	D	E	F	G	I	H	J	%
Cladosporium spp	+	+	+	+	+	-	+	-	+	+	80
Cuvularia lunata Penicillium	-	-	-	+	-	-	+	+	+	+	50
brevicompactum	-	-	-	+	+	-	-	-	-	-	20
Trichoderma viride	+	+	+	+	+	+	+	+	+	-	90

KEYS : A = Angwan Tiv, B =Angwan Waje, C = Emir's palace, D = Gangaren Masaka, E = G . R. A, F =Kofar Hausa, G = Makwalla, H = Market Area, I = Sabon Layi, J = Yara

TABLE 3: Result of the utilization of petroleum products by the fungal isolates

Isolates	Engine oil	Kerosene	Petrol	Diesel	Brake fluid
Cladosprium spp	-	-	-	+	+
Cuvularia lunata Penicillium	-	+	-	-	-
brevicompactum	-	-	-	-	+
Trichoderma viride	+	-	+	+	-

DISCUSSION

The results of this work demonstrated that all the four species (*Cladosprium* spp., *Cuvularia lunata, Penicillium brevicompactum* and *Trichoderma viride*) isolated from from the soil of mechanic workshops in Keffi could utilize at least one of the petroleum products tested. Okerentugba and Ezeronye (2003) reported that *Aspergillus* spp., *Rhizopus* spp. and *Penicillium* spp. are capable of degrading hydrocarbons especially when used in single cultures. The results of this investigation also agree with the results obtained by several workers. Adekunle and Adebambo (2007) demonstrated the ability of *Aspergillus niger, A. 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. Uzoamaka *et al.* (2009) isolated and demonstrated the potentials of *Aspergillus versicolor, Aspergillus niger, Aspergillus flavus, Syncephalastrum* spp., *Trichoderma* spp., *Neurospora sitophila, Rhizopus arrhizus* and *Mucor* spp. degradation of petroleum hydrocarbons.A study by Wemedo *et al.* (2002) had earlier reported that the genera of *Penicillium, Aspergillus and Rhizopus* are associated with kerosene-polluted soil. Oboh *et al.* (2006) had also reported that the isolates in these three genera were able to grow on crude petroleum as the sole source of carbon and energy when screened for hydrocarbon utilization. The results of these previous findings are similar with the results in this investigation. The results of this study demonstrated *Trichoderma viride* was able to utilize three (engine oil, petrol and diesel) out of the five petroleum products (engine oil, kerosene, petrol, diesel and brake fluid) as the most versatile fungus among the four species isolated is worthy of note. *Cladosporium* spp. was shown to utilize diesel and brake fluid, *Cuvularia* was found to utilize only Kerosene, while *Penicillium brevicompactum* could utilize only brake fluid. The versatility of Trichoderma viride agrees with Obire *et al*. (2008) who had earlier reported the high degradation potential of Trichoderma species. Recently, Sanyaolu *et al.* (2012) demonstrated the ability of Aspergillus *terreus, A. niger, A. flavus* and *Trichoderma* spp. to hydrolyse Premium Motor Spirit (PMS) [petrol] leading to its deterioration. Leahy and Colwell (1990) had earlier reported that microorganisms break down hydrocarbons and use the energy to synthesize cellular components. The results of this study indicate potential application of the fungal species locally isolated from the soil of the mechanic workshops in Keffi for bioremediation of soils contaminated with petroleum and petroleum products, since each of the four species isolated could at least metabolize one of the five petroleum products tested.

CONCLUSION

The Four fungal species isolated from the soils of mechanic workshop in Keffi metropolis were capable of utilizing at least one of the petroleum hydrocarbon products tested as sole source of carbon and energy which implies that any of these four species, namely, *Cladosporium* spp., *Cuvularia lunata, Penicillium brevacompactum* and *Trichoderma viride*, could be employed for bioremediation either singly or as a consortium of microbial degraders. However, the versatility of *Trichoderma viride* among other organisms makes it promising potent candidate for bioremediation of soil polluted with petroleum hydrocarbons.

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