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OIL DEGRADER FUNGI OF SPENT ENGINE OIL CONTAMINATED SOIL FROM SELECTED MECHANIC WORKSHOPS IN MINNA, NIGERIA

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Abstract

The aim of this study is to investigate the oil degrader fungi associated with degradation of spent engine oil contaminated soil in five selected mechanic workshops in Minna. Samples of the spent engine oil contaminated soils were collected from Shanchaga, Maikunkele, Shiroro, Tunga and Bosso

Keywords:
Abundance, Fungal isolates, Mycoremediation, Contaminant

INTRODUCTION

The quality of life on earth is connected to the overall quality of the environment. Releases of recalcitrant and toxic chemicals into the environment have negative impacts on human health and the environment (Terrence *et al.*, 2011). These contaminants find their way into the tissues of plants, animals and human beings by the movement and absorption of hazardous constituents in the environment (Perfumo *et al.*, 2007). Contaminated lands generally result from past and present industrial activities with little or no awareness of the health and

mechanic workshops located in Minna Local Government Area. The fungi were isolated from the soils using dilution plate method in mineral salt medium. All the fungi were identified based on macroscopic and microscopic features of the fruiting bodies, spores and hyphal mass. A total of fifteen (15) fungal isolates belonging to eight genera were obtained from spent engine oil contaminated soil in all sampled locations. Fungal species isolated were Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporium, Aspergillus flavus, Penicillium notatum, Aspergillus fumigatus, Trichoderma harzianum, Penicillium griseofulvum, Rhodotorula rubra, Cunninghamella echinulata, Trichoderma viride, Penicillium chrysogenum, Mucor hiemalis, Mucor racemosusand Mucor plumbeus. Tunga soil sample had the highest occurrence of fungal isolates, followed by Bosso and Chanchaga while soil samples from Maikunkele and Shiroro had the least occurrences of fungi isolates. Aspergillus niger, Rhizopus stolonifer and Aspergillus flavus were the prominent isolates from all the sampled locations while Penicillium chrysogenum, Mucor hiemalis, Cunninghamella echinulata, Mucor plumbeus and Rhodotorula sp had the least number of occurrence from all locations. The presence of these three predominant fungal species in the spent engine oil contaminated soils samples collected from all the selected mechanic workshops within Minna metropolis is a strong indication that these fungi can be use to restoring oil contaminated environments through mycoremediation process.

environmental consequences of their production processes and waste disposal methods. The problem is worldwide, but more severe in the developing countries such as Nigeria where there are no effective regulatory policies on the environment, thus encouraging unwholesome industrial practises (Adelowo *et al.* 2006).

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).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). The nutrient deficiencies which arise due to petroleum hydrocarbon contamination of soil may however be offset by addition of cow dung to the soil (Osazee and Adebola, 2016). The majority of applications developed to date involve bacteria, and there is a distinct lack of appreciation of the potential roles and involvement of fungi in bioremediation, despite clear evidence of their metabolic and morphological versatility. Traditional methods of disposing of hazardous wastes (physical, chemical, and thermal treatments and land filling) have not always been efficacious (Gadd, 2001). It has been estimated that it will costs about 50 billion dollars to decontaminate toxic waste sites or oil producing areas in Niger Delta alone using traditional waste disposal methods (vanguardngr.com, 2017). Considering these staggering costs for cleaning up the environment, an alternative, rapid, efficacious and cost-effective method is needed. One method that has become increasingly popular for decontamination of the environment has been mycoremediation. The use of indigenous or suitable fungi at contamination sites often provides an efficient and economically attractive solution to the contamination problem. One of the early reports indicated that fungi can degrade an extremely diverse group of environmental contaminants (Stanley *et al.*, 2017). This ability of fungi to degrade a wide range of environmental contaminants sets them apart from many other microbes

used in bioremediation. In this study, the aim is to identify the fungi capable of degrading spent engine oil contaminated soils in Minna, Nigeria.

MATERIALS AND METHODS

Study Site

The study was carried out in Minna, Nigeria. Minna covers a landmass of 72km^2, Minna lies on latitude 9^0 3'E and longitude 6^0 33'N, The rainy season starts around April and last till October, it has a mean annual rainy of 1334mm (52 inches) with September recording the highest rain of 300m (11.7 inches), the mean monthly temperature is highest in March at 30 - 50 ^{0}C (83 ^{0}F) and lowest in August. Improper disposal of spent engine oil is the major source of oil pollution in this locality. Five mechanic workshops (about 5 km apart) contaminated with spent engine oil were randomly selected for this study and sites were selected from the following areas in Minna; Chanchaga, Tunga, Bosso, Maikunkele and Shiroro road.

Sample Collection

Spent engine oil contaminated soil samples was randomly collected from each of the selected site using a pre-cleaned hand auger at a depth of 0 – 15cm. Four samples of 0.5kg each per location making a total of 2kg of soil sample per site and 10kg from the five sites. The samples from each point per site was pooled together, homogenised, air dried, sieved through a 2-mm mesh screen and stored in a polythene bag at room temperature in the laboratory for further studies (Goddey and Dami, 2013). Below is the field photograph of the mechanic workshops where the spent engine oil contaminated soil samples were collected.

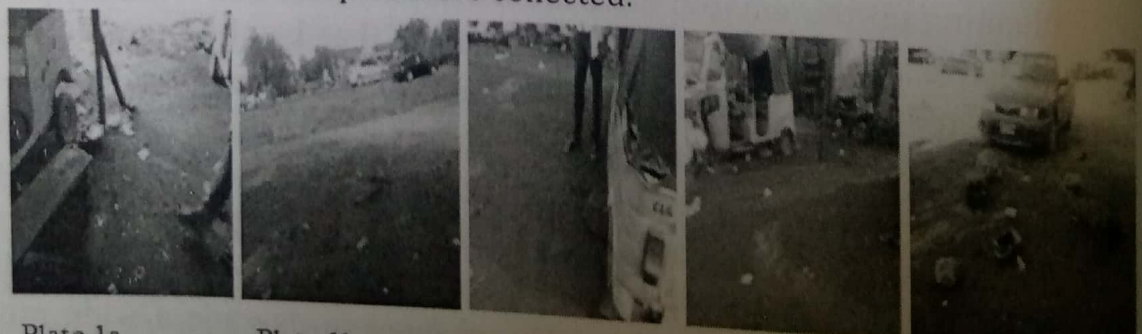

Plate 1a Plate 1b Plate 1c Plate 1d Plate 1e

Source: Field photograph of mechanic workshops

Plate 1a: Maikunkele; Plate 1b: Tunga; Plate 1c: Bosso; Plate 1d: Chanchaga; Plate 1e: Shiroro road

Isolation of Oil Degrading Fungi

Oil degrading fungi were isolated from the soil 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 the soil sample was poured into a test tube containing 10ml of distilled water. One (1) millilitre of the suspension was pipette into a test tube containing 9ml of distilled water. The sample was serially diluted to 10^5 dilutions. One (1) millilitre aliquots of the second-fold dilution 10^2 cfu was added to the mineral salts medium (MSM) containing 10% V/V sterile motor oil as the sole carbon and energy source and incubated at (28 ± 2^0C) for 5 days. Colonies were further sub-cultured onto MSM incorporated with tetracycline to obtain pure culture isolates (Nwachukwu and Akpata, 2003).

Identification of the oil degrader isolates

The isolated fungi were identified based on the isolates colonial characteristics on culture plate and microscopic features such as nature of mycelium, types of fruiting bodies and the spore structure. The isolates were identified according to Kora *et al*, (2005)

Determination of percentage occurrence of the fungal isolates

This was done to determine the frequency of occurrence of the different fungal isolates. The frequency of occurrence of the isolates from the soil will be determined. The total number of each isolate in the soil sample will be obtained against the total number of all the isolates in the sample screened. The mean value of this yielded the percentage of occurrence as the following equation shows:

$$\% \text{ of occurrence} = X/N \times 100$$

Plate 1a: Maikunkele; Plate 1b: Tunga; Plate 1c: Bosso; Plate 1d: Chanchaga; Plate 1e: Shiroro road

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$$\% \text{ of occurrence} = X/N \times 100$$

Where X = total number of each isolate in the sample and N = total number of all the isolates in the sample (Agu *et al.*, 2015).

RESULTS

Isolation of fungi from the various mechanic workshop soils

Table 1 showed the different types of fungal isolates obtained in the study. There were fifteen fungal species isolated belonging to eight genera and identified as *Rhizopus stolonifer, Aspergillus niger, A. fumigatus, Trichoderma harzianum, Aspergillus flavus, Penicillium notatum, Trichoderma viride, Penicillium chrysogenum, Rhodotorula rubra, Cunninghamella echinulata, Fusarium oxysporium, Mucor hiemalis, Penicillium griseofulvum, Mucor plumbeus and Mucor racemosus.*

Table 1: Fungi isolated from mechanic workshop soils.

Isolates code	Cultural appearance	Microscopy	Species
SOUF01	Black mycelia growth and fully extended in the growth medium	An upright conidiophores that terminates in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose	*Aspergillus niger*
SOUF02	Brown mycelia growth	An upright conidiophores that terminates in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose	*Aspergillus fumigatus*
SOUF03	Long network of hyphae; initially white, Later grey with numerous black dots	Non-septate hyphae; sporangiophores emerging laterally from the mycelium, ramified, spherical at the end, sporangia filled with spores	*Rhizopus* stolonifer
SOUF04	Light green and powdery-like colonies	An upright conidiophores that terminates in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose	*Aspergillus flavus*
SOUF05	Smooth, glossy and mucous	Septateconidiosphore, Round and oval cell	*Rhodoturola sp*

SOUF06	Light green and yellowish conidia scattered throughout the plate	Conidia were globose,phialides were flask-shaped but shorter than those of *T. Harzianum*. Phialides are arranged in divergent groups	*Trichoderma viride*
SOUF07	Cottony white and light yellow mycelium. A single concentric ring of conidial production	Conidial were globose. Phialides were flask-shaped but longer than those of *T. viride*. Phialides arranged in divergent groups of 2 – 4	*Trichoderma harzianum*
SOUF08	White, spreading flat colonies	Phaliades are short, bean shaped, macroconidia not in chains and are fusiform.	*Fusarium oxysporium*
SOUF09	Greeny with white edge, velvety to powdery colonies.	Hyphae are septate, hyaline, conidiophores are branched. Phaliades appear like brush-like clusters at ends of conidiophores. Conidia are round and in chains.	*Penicillium notatum*
SOUF10	Colonies arepale reddish yellow and spreading with time.	Sporangiophores erect, simple or branched, bearing sporangia terminally. Sporangia globose, smooth, and sporangiospores long ellipsoidal	*Mucor hiemalis*
SOUF11	Dark green, entire and Raised fluffy	long conidiophores, long branching phialides and conidia spreading	*Penicillium griseofulvum*
SOUF12	Colonies are velvety, bright grayish green with whitish tint, reverse pale yellowish brown.	Hyphae are septate, hyaline, conidiophores are branched. Phaliades appear like brushlike clusters at ends of conidiophores. Conidia are round and in chains.	*Penicillium nigricans*
SOUF13	Pale brown colour on the surface	Sporangiophores erect, branched, septate, tapering from base toward apex, bearing sporangia terminally. Sporangia globose and Sporangiospores globose, 1-celled and solitary.	*Mucor plumbeus*
SOUF14	Colonies have very rapid growth with wooly texture. Colour is grayish on the surface and reverse is pale.	Non-septate broad hyphae, branched sporangiophores with round sporangiospores.	*Mucor racemosus*

SOUFI5	Colonies are yellowish brown	Sporangiophores erect, simple or branched with sympodial branches in a few positions, terminated in vesicles' with sterigmata and spores (sporangiospores).	*Cunninghamella echinulata*

KEY: SOUF – Spent oil utilising fungi

The rate of occurrence among the fungal isolates obtained from the spent engine oil contaminated soil samples showed that *Aspergillus niger* and *Aspergillus flavus* had 21.7% followed by *R. Stolonifer* had 10.2% while *C. echinulata* and *R. rubra* had 1.5% each. *M. plumbeus, P. griseofulvum, T. harzianum* had 5.8% each. *Aspergillus fumigatus* and *F. oxysporium* had 4.4% (Table 2) respectively.

Table 2: Oil degrader fungal species obtained from mechanic workshop soils

Organisms	Frequency (x)	Frequency (%)
Rhizopus stolonifer	7	10.2
Penicillium notatum	2	2.9
Fusarium oxysporium	3	4.4
Mucor plumbeus	4	5.8
Aspergillus niger	15	21.7
Penicillium griseofulvum	4	5.8
Rhodotorula sp	1	1.5
Aspergillus fumigatus	3	4.3
Cunninghamella echinulata	1	1.5
Trichoderma viride	2	2.9
Aspergillus flavus	15	21.7
Penicillium chrysogenum	3	4.3
Mucor hiemalis	2	2.9

Trichoderma harzianum	4	5.8
Mucor racemosus	3	4.3
Total	69	100

Assessment of Fungi Abundance

In terms of abundance and spatial distribution of the fungi, Tunga had 23, followed by Bosso (17) while Maikunkele and Shiroro had 8. It was also observed that *R. Stolonifer, A. niger* and *A. flavus* were isolated from the contaminated soil samples collected from all the mechanic workshops. Four fungal species (P. *notatum, C. Echinulata, Rhodotorula sp. and M. Hiemalis)* out of the fungi isolated were only found in soil samples collected from mechanic workshops in Bosso, Tunga and Chanchaga (Table 3), though these fungi were less common when compared with the other three fungal species (*R. Stolonifer, A. niger* and *A. flavus*) which were found in all the soils obtained from the five mechanic workshops. It was also observed that (*M. racemosus, T. harzianum, P. notatum and T. viride)* were only found in soils collected from Chanchaga and Tunga respectively

Table 3: Abundance of fungi in the sampling mechanic workshop soils

Organisms	Mechanic workshops				
	Chanchaga	Tunga	maikunkele	Bosso	Shiroro
Rhizopus stolonifer	2	1	1	2	1
Penicillium notatum	1	1	-	-	-
Fusarium oxysporium	-	-	1	1	1
Mucor plumbeus	-	4	-	-	-
Aspergillus niger	2	4	3	4	2
Penicillium griseofulvum	1	-	1	1	1
Rhodotorula sp	-	1	-	-	-
Aspergillus fumigatus	-	2	-	1	-
Cunninghamella echinulata	-	-	-	1	-

Trichoderma viride	1	1	-	-	-
Aspergillus flavus	3	5	2	2	3
Penicillium chrysogenum	-	-	-	3	-
Mucor hiemalis	-	-	-	2	-
Trichoderma harzianum	1	3	-	-	-
Mucor racemosus	2	1	-	-	-
Total	13	23	8	17	8

DISCUSSION

The study shown that fifteen (15) fungi were associated with spent engine oil contaminated soils in the five mechanic workshops in Minna, Nigeria. The oil degrader fungi were *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporium, Trichoderma harzianum, Aspergillus flavus, Penicillium griseofulvum, Aspergillus fumigatus, Aspergillus flavus,* The most predominantly oil degrader fungi were *A. flavus, A. niger and R. Stolonifer*. The distribution and percentage occurrences of the fungal isolates indicated that *A. flavus* (21.7%) and *A. niger* (21.7%) had the highest frequency of occurrence, followed by *R. Stolonifer* (10.2%) and were predominant in all the five automobile workshops studied. This could be related to their high sporulating capacity and ability to produce toxins which inhibit the growth of other organisms. This report was at variance with the work of Adegbola *et al.* (2014) who worked on biodegradation of crude oil by fungi isolated from cow dung contaminated soils observed that *A. flavus* and *R. Stolonifer* had the least percentage occurrence, it however confirmed the reports of Okoh and Trejo-hernandez, (2006) that *A. flavus* and *A. niger* are among the most frequently isolated fungi from hydrocarbon polluted sites.

It was also observed that *A. flavus, A. niger and R. Stolonifer* were present in all the five mechanic workshops. The high proliferation of these fungi may be due to the fact that the fungi used spent engine oil as a substrate for their growth using extra cellular enzymes to break down the

recalcitrant hydrocarbon molecules, thereby, converting spent engine oil into simpler forms or products that can be absorbed for the growth and nutrition of the fungi. This is also in line with the work of Stephen *et al.* (2012) who worked on biodegradation of mechanic workshop polluted soil amended with lime fertiliser and isolated *A. niger, A. fumigatus, A. flavus and Rhizopus nigricans* from soils of various automobile workshops in Anyingba, Nigeria. Stephen *et al.* (2013) who worked on biodegradation of diesel contaminated soil amended with cowpea chaff also isolated similar fungi (*Aspergillus flavus* 24.3%, *Aspergillus niger* 21.6%, *Penicillium sp* 10.8%) and have be implicated in hydrocarbon biodegradation. This comparatively high tolerance of *Aspergillus niger* and *Aspergillus flavus* to hydrocarbon may be attributed to their possession of resistant endospores thus, suggesting their efficiency in the clean-up of hydrocarbon polluted sites (Ghazali *et al.* 2004). Other fungi of environmental significance isolated from these workshops were *A. fumigatus, Trichoderma harzianum, Penicillium notatum, Trichoderma viride, Penicillium chrysogenum, Rhodotorula sp. Cunninghamella echinulata, Fusarium oxysporium, Mucor hiemalis, Penicillium griseofulvum, Mucor plumbeus and Mucor racemosus.* 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 microorganisms due to the fact that native microorganisms are best adapted to intrinsic environmental conditions, we isolated indigenous engine oil degrading fungi in this study.

Conclusion

The results of this study showed that fifteen fungi belonging to eight genera were associated with spent engine oil polluted soils in Minna. Base on their relative abundance, it was found that *A. niger, A. flavus* and *R. stolonifer* were isolated in all the five mechanic workshops and these fungi among

other isolated fungi were reported to be potential producers of enzymes that have the efficiency in the clean-up of hydrocarbon polluted sites. Thus, the isolated organisms as mention above could be used as potential fungi to degrade petroleum hydrocarbons, especially those spill out as a result of anthropogenic activities.

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