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Isolation and Molecular Characterization of Some Fungi Species in Soil Contaminated with Spent Engine Oil in Mechanic Garages at Kaduna Metropolis, Kaduna State, Nigeria

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ABSTRACT: The objective of this paper was to isolate and identify by molecular characterization of some fungi species in soil contaminated with spent engine oil in mechanic garages at Kaduna Metropolis, Kaduna State, Nigeria. Standard soil isolation technique was employed to obtain fungal isolates growing in the top soils (0-15 cm deep). Potato Dextrose Agar (PDA) was used for culture and the incubation temperature was 30^{o}C for 7 days. The 18S rRNA genome was amplified, sequenced and analyzed. The five fungal isolates obtained were *Aspergillus niger, Trichoderma spp, Fusariumspp, Mucor spp and Penicillium spp.* The identification of *Aspergillus niger* was taken further because it was present at all sites examined. Outside the morphological characterization, the molecular characterization of the species with known fungal species from the gene bank indicated more than 98% similarity for *Aspergillus niger.* This study contributes to the database on locally available fungal diversity and their ecology. These microbes exhibited applicable bioremediation potential for the clearing of the spent engine oil polluted site in Kaduna metropolis.

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Crude oil is the oily, flammable liquid that occurs naturally in deposits beneath the surface of the earth. Crude oil or petroleum is a viscous liquid that consist of hydrogen and carbon ions (Asep and Sanro, 2013). Engine oil is a pollutant that comes from the environment which causes damage to our ecosystem and acts as a health hazard to human beings (Fatuyi *et al.*, 2012). After any oil spillage, Polycyclic Aromatic Hydrocarbons (PAHs) are important contaminant retained in the environment (Kathi and Anisa, 2012). Oil pollution has contaminated soils used for agricultural lands and has not spared the aquatic and marine plants and animals in Nigeria. Ground water has also been contaminated thus polluting the crops and farm animals (Eneh, 2011). Huge increase of vehicles resulting from rapid increase of human population has led to presence of various kinds of informal and formal automobiles. Hence the increased use of motor oil (Husaini *et al.*, 2008). The toxicity of oil and other petroleum

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products varies depending on the concentration, composition, environmental factors and biological state of the organism at the time of contamination (Cerniglia and Setherland, 2001).

Petroleum hydrocarbons can be divided into four classes: saturates, aromatics, asphaltenes (phenols, fatty acids, ketones, esters & porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides and amides). Petroleum hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded (Barathi and Vasudevan, 2001). Hydrocarbons differ in their susceptibility to microbial attack. The susceptibility of hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes > branched alkanes > small aromatics > cyclic alkane's although this is not always the case (Perry, 2008). Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons (PAHs), may not be biodegraded at all (Atlas and Bragg, 2009). Therefore, the objective of this paper was to isolate and identify by molecular characterization of some fungi species in soil contaminated with spent engine oil in mechanic garages at Kaduna Metropolis, Kaduna State, Nigeria

MATERIALS AND METHOD

Study Area: The study was conducted in Kaduna State of Nigeria. The metropolis is located between Longitude $7^0$21'and $7^0$30' East of the Greenwich Meridian and Latitude $10^0$23'and $10^0$36' north of the Equator (Afon and Alwadood, 2016). The metropolis has a projected population of 492,100 in Kaduna North and 543,600 in Kaduna South (NPC, 2016) projection.

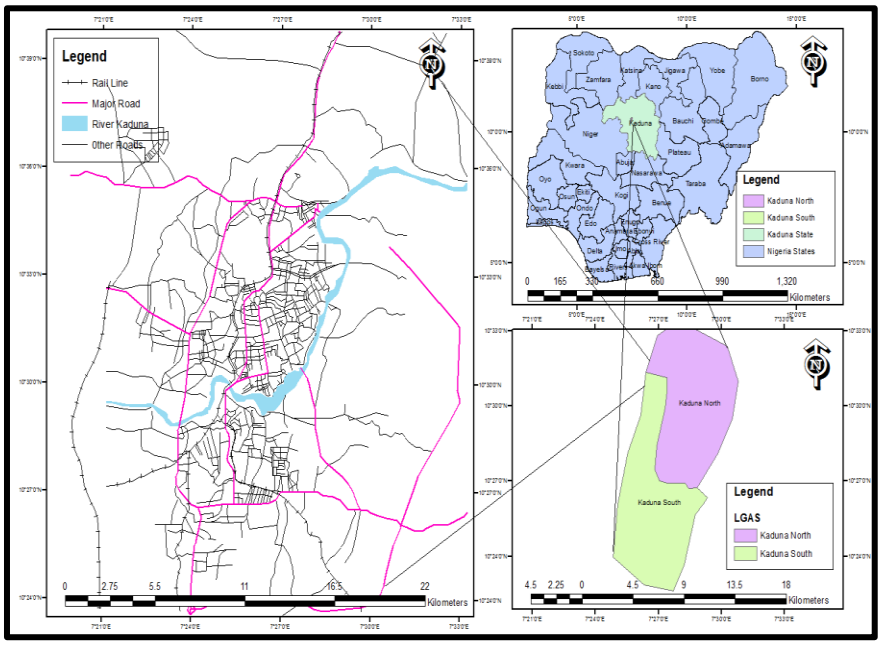

Fig.1: Study Area Showing Point of Source**.**

Sample Collection: Four (4) different soil samples were collected in the year 2018 from the following site locations: (A and B) at 0-15 cm depths. A (Kawo garage) and B (Kurmin-marshi car maintenance workshop). The samples were collected randomly from the sites using soil auger at 0-15cm depth below the soil surface (Sabah *et al.*, 2016). The soil samples weighing one kilogram (1kg) each were taken consecutively after tilling with a sterile scoop. A total of eight soil samples were collected from where spent engine oil is frequently spilled. Control samples were collected from uncontaminated soil sample in each site. The samples were kept in sterile polythene bags and were transported to the laboratory for further analysis and stored at 4°C (Sabah *et al.*, 2016).

Table 1: Distribution of Site Sample Location (Units in meters)

Site ID	Northings (m)	Eastings (m)	Locations
A	7.447060	10.586114	Kawo Garage
B	7.408622	10.554347	Kurmin Mashi Car Maintenance Workshop

Preparation of Soil Sample for Serial Dilution: Soil samples was homogeneously mixed and carefully

sorted to remove stones and other unwanted soil debris using a 2.5 mm sieve. The Potato Dextrose Agar (PDA) media was autoclaved at 121^{o}C for 15 min and allowed to cool, then 20 ml was dispensed aseptically on the sterile disposable petri dishes. The soil samples collected were mixed together and thereafter 1g of the mixed samples was dissolved in 10ml of sterile water to make suspension of 1: 10 (10^{-1}). Serial dilution of the 10^{-1} suspension was used to prepare dilution at 10^{-2},10^{-3},10^{-4} and10^{-5}. Collection and preparation of soil sample for serial dilution was carried out in accordance with the method described by Musliu and Salawudeen, (2012).

Morphological Characterization: To purify the fungal isolates, the cultures were carefully and aseptically sub cultured on Potato Dextrose Agar (PDA) and stored on PDA slants for further analysis at 28^{0}c. The fungal isolates however, were characterized on the basis of cultural characteristics and morphological characteristics including spore type, mycelia and other fruiting bodies in a lactophenol cotton blue wet mount by compound microscope at a magnification of ×1000. At days 4 and 7 of incubation, plates were observed for macroscopic characterization such as colony and exudates (Alsohaili and Bani-Hasan, 2018).

Glass Slide Culture Technique: Slide cultures are of great value in determining details of fungal growth. The Potato Dextrose Agar was prepared as recommended by the manufacturer and after cooling; it was cut into small blocks and placed on clean slides. A sterile needle was used to pick a small amount of sub- cultured fungi and inoculated at the edge of the blocks and placed cover slips at the top. Each slide held one block of the fungi. Moistened sterile blotting papers were placed at the bottom of the plates and incubated at room temperature of 30^{o}C for seven days followed by characterization. By doing this, there is no need to remove a portion of the fungus from a culture plate and transfer it to the slide. So, there is less chance for the features that are key to identification, notably the spore-bearing structures to be damaged (Alsohaili and Bani-Hasan, 2018).

Microscopy: For microscopic characteristics, the slides were stained with lactophenol in cotton blue, and apparent Photographs were taken of microscopic structures. A morphological examination of species was made with the naked eye at low magnification power of the microscope (Alsohaili and Bani-Hasan, 2018).

Physiochemical Parameters of the Soil Contaminated with Spent Engine Oil: These were carried out using the method described by Mohammed *et al.* (2013); APHA, (2017).

Determination of Conductivity: The sample solution was measured with conductivity meter (Hach) model 44600. The probe was rinsed with deionized water before each measurement was carried out. The probe will be inserted into the sample solution containing in a clean 250cm^{3} beaker and allowed to stand in order to stabilized the digital display, value expressed in MS/cm.

Dissolve oxygen determination: This was carried out using DO_2 meter (Jenway Model 9071). 10g of the sample dissolved in 50ml of deionized H_2O and was stirred. This was left for 3hrs with stirring at interval of about 30minutes. The sample solution was taken by decantation into already cleaned beaker.

The DO_2 meter which calibrated and warmed for 15-20 minutes was used to take the first day reading. The solution was stored for 5 days and the reading was repeated using the DO_2 meter. The actual DO_2 was calculated by subtracting the DO_2 of the first day from that of the 5th day.

$$For\ Solid\ example = d1 - d5 = x\frac{mg}{kg} \quad (1)$$

Where; d1= Initial dissolved oxygen, d5 = final dissolved oxygen

Determination of pH: Ten gram (10g) of the sieved soil was weighed into 250cm^{3} conical flask and 50ml of deionized water was added and stirred. This was left to stand for 2 hours with occasional stirring. The solution was used for pH measurement using electrical pH meter. The pH meter was plugged into the high source and was on for 10-15 minutes. The electrode was fixed and the buffer 4, 7 and 9 was used to calibrate the pH meter. The pH of each sample was measured with pH meter at room temperature. The pH was sufficiently immersed into the sample and the value read from the digital display.

Chemical oxygen demand (COD) Determination: Five gram (5g) of the soil sample dissolved in 25ml of deionized water and stirred for 10-15 minutes this will be left to stand for 2hrs. The solution was decanted into a beaker in which 20ml was pipetted into a conical flask. The pipetted solution was added in 10ml of 0.02m $KMnO_4$ and 2ml of 25% H_2SO_4 and heated to boil. This was removed from the heat and 10% ammonium oxalate was added drop by drop until Colorless solution is obtained. This mixture was

titrated with 0.02m $KMnO_4$ until a pink color appears. The titer value was recorded. The above procedure is carried out with blank using deionized water instead of the sample, also this was obtained.

$$\text{COD} = \frac{b - a \times \text{m} \times 16000}{Sample\ Volume\ used} \quad (2)$$

Where b = sample titer volume; a = blank titer volume; m = molarity of $KMnO_4$; 16000 = constant

Determination of Temperature: Ten gram (10g) of the sieved soil was weighed into 250cm^3 conical flask and 50ml of deionized water was added and stirred. This was left to stand for 2hrs with occasional stirring. The solution was used for temperature determinate using conductivity/TDS meter.

Moisture Determination: The moisture content of the soil was determined using the dry weight method (Li and Mira 2010). A crucible was dried in an oven at 80^0C for few minutes, cooled in a desiccator and weighed (W_1). Two grams of the soil sample were placed in each crucible (W_2). The crucible and the sample were then dried in an oven at 150^0c until constant weight was achieved. This was quickly transferred to a desiccator to cool and weighed quickly with minimal exposure to the atmosphere (W_3). The loss in weight of the sample during drying is the moisture content. It was calculated using the formula:

$$\%\ Moisture\ content = \frac{W2 - W3}{W3 - W1} \times 100 \quad (3)$$

Where; W_1 = weight of the crucible without sample; W_2 = weight of crucible + sample before drying; W_3 = weight of dried sample + crucible.

Molecular Characterization

DNA Extraction: DNA extraction and purification of fungi isolates was carried out using AccuPrep® Genomic DNA Extraction Kit purchased from (Bioneer Corporation) USA. Twenty microlitre (20µL) of Proteinase K was added to a clean 1.5ml centrifuge tube. A well isolated colony was inoculated into the tube containing proteinase K. 200µL of binding buffer was added to the sample and was immediately mixed in a vortex mixer. The mixture was incubated at 60^0C for 10 minutes. 100µL isopropanol was added which was well mixed by pipetting. The mixture was briefly spun down to get the drops clinging under the lid. Lysate was carefully transferred into the upper reservoir of binding column tube without wetting the rim. The tube was closed (to avoid aerosol formation) and was centrifuged at 8,000 rpm for 1 minute. The tube was opened and the solution was poured into a disposal bottle; 500µL of washing buffer-2 was added carefully and then it was centrifuged at 8,000 rpm for 1 minute. It was again centrifuged at >12,000 rpm for 1 minute to completely remove ethanol. The binding column tube was transferred to a new 1.5ml tube for elution, and 200µL of elution buffer (nuclease free water) was added onto the binding column tube, and was centrifuged at 8,000 rpm for 1 minute to elute. The eluted genomic DNA was obtained and the tube was left open for 10 minutes to dry out the DNA. The dried pellet was re-suspended in 20µL sterile water (ready as the template) (Yunjie, 2013).

PCR Amplification of 18S rRNA: Twenty microlitre (20µL) Eppendorf tubes containing 16µL premix (Taq polymerase, dNTPs, $MgCL_2$, PCR buffer and distilled water) were used for the amplification process. 2µL of template (extracted genomic DNA), 1µL of forward primer (5'TCCGTAGGTGAACCTGCGG-3') and 1µL of reverse primer (5'TCCTCCGCTTATTGATATGC-3') were added to the premix. The mixtures were preheated at 94^0C for 5 minutes before it was subjected to a recycling step. The amplification conditions for the PCR assay were 30 cycles at 94^0C (denaturation) for 30 seconds, 55^0C (annealing) for 30 seconds, and 72^0C (extension) for 1 minute and final extension step was performed at 72^0C for 10 minutes. The products were separated in agarose gel (2% w/v) electrophoresis after staining with Ethidium Bromide (Umar *et al.*, 2018).

*Gel Electrophoresis:*One point five gram (1.5g) of Agarose powder was weighed; it was added into a conical flask containing 100mls of TAE buffer. The solution was heated in a microwave until agarose is completely dissolved. It was further allowed to cool to about 50^0C. Gel casting tray was prepared by sealing ends of gel chamber with tape. The actual number of combs were placed in gel tray. Five micro Litre (5µL) of ethidium bromide was added to the semi cooled gel and it was poured into the gel tray. It was allowed to solidify for 15-30 minutes at room temperature. The combs were removed and the gel was placed in electrophoresis chamber and covered with buffer (TAE as used previously). Seven micro litres (7µL) each of both DNA and standard (Molecular Ladder) was then loaded onto the gel. Electrophoresis was carried out at a 100 Voltage for 40 minutes. DNA bands were virtualized using UV light gel imaging system (Umar *et al.*, 2018).

Sequencing Determination: The band of interest was prepared for sequencing by preparing a sequencing reaction in a 2.0ml tube. All reagents were kept on

ice while preparing the sequencing reactions and were added in the order listed: dH_2O 0 – 9.5µL.

DNA template 0.5 – 10.0µL Primers 2.0µL DTCS Quick start master mix 8.0µL the reaction was set in the PCR machine.

Ethanol precipitation: 2µL of 3M Sodium acetate, 2µL of 100Mm Na_2-EDTA and 1µL of 20mg/ml of glycogen and 60µL of absolute ethanol was added to the PCR tube, it was then centrifuged for 15 minutes at the maximum speed. The supernatant was discarded and 200µL of 70% ethanol was added and centrifuged for 2 minutes. The supernatant was discarded and the pellet was allowed to vacuum dry. The pellet was suspended in 40µL of the sample loading solution (provided in the kit) (Umar *et al.*, 2018)

Statistical Analysis: Different samples collected from the garages represented the treatments. Results were compared using one-way Analysis of Variance (ANOVA). This was done to establish if differences ($p<0.05$) were significant between individual treatments. The analysis was done using SPSS version 17.

RESULTS AND DISCUSSION

Cultural Characteristics of Fungi from the Oil Contaminated Soil: A total of five fungal isolates were grown from oil contaminated soil using Potato Dextrose Agar. Characterization was based on classical macroscopic techniques of color, margin and elevation of the pure colonies. The isolates grew within 4-7 days of incubation at room temperature. The isolates exhibited different colony characteristics. Two isolates had curled margin, three had entire margin. The isolates had raised fluffy elevation (Table 2).

The isolation of fungal species (Table 2) such as *Aspergillus niger, Trichoderma spp, Fusariumspp, Mucor spp and Penicillium spp* from soil contaminated sites in Kurmin mashi and Kawo mechanic sites in Kaduna State highlights the adaptability and ecological roles of fungi in polluted environments. These organisms have earlier been reported as hydrocarbon bio-degraders by April *et al.*, (2005). The fungi play a vital role in the bioremediation of oil-contaminated soils (Mukherjee *et al.*, 2011). *Aspergillus niger* is a well-known filamentous fungus capable of degrading hydrocarbons due to its production of extracellular enzymes such as lipases and peroxidases, which facilitate the breakdown of complex organic pollutants. Its presence in the oil-contaminated soils aligns with previous findings that *Aspergillus niger* can utilize hydrocarbons as a carbon and energy source (Chaudhry *et al.*, 2012). The findings is also in conformity with the report of Akpoveta *et al.* (2011) who revealed the isolation of *Trichoderma spp*., *Penicillium spp*., *Rhizopus spp*., *Fusarium spp*., and *Aspergillus spp*, from crude oil polluted soil at Agbor area of Niger Delta. Similar to the result of Mbachu and Chukura, (2016) where a total of 8 fungal isolates; *Candida tropicalis, Rhodosporidium toruloides, fusarium oxysporium, Aspergillus clavatus, Saccharomyces cerevisiae, Candida albicans, Microsporium gypseum and Trichophyton Mentagrophytes*. The isolation of these fungi from oil-contaminated soils at Kurmin mashi and Kawo mechanic sites indicates that these organisms are not only surviving but potentially contributing to the natural attenuation of pollutants.

Table 2: Cultural Characteristics of Fungi Isolated from Oil Contaminated Soils

Isolate Identity	Colour (Top)	Colour (Bottom)	Margin	Elevation
Trichoderma spp	Grey brown	Dark brown	Entire	Raised fluff
Fusarium spp	Pink colony	Cream green	Curled	Raised
Aspergillus niger	Blackish brown	Cream green	Curled	Raised
Penicillium spp	Dark green	Cream green	Curled	Raised
Mucor spp	White	Cream	Entire	Raised fluff

The fungal form of the study site is presented in **Table 3.** The result reveals that *Aspergillus niger* and *Fusarium spp* were found to be present in all the two sites under study. These were followed by *Mucor spp* and *Penicillum spp* which were present only in Kawo sampling site while *Trichoderma spp* was only present at Kurmin mashi sampling site. *Aspergillus niger* was selected for further studies using 18S rRNA sequencing.

The predominance of *Aspergillus niger* (Table 3) across various sites followed by species of *Trichoderma species*, *Mucor species*, *Fusarium* and *Penicillium species* respectively aligns with existing literature on fungal distribution in diverse environments. This finding corresponds favourably with the work of Chukwura *et al.* (2016) who reported *Aspergillus niger and Penicillium spp* displaying the highest extent of degradation in

selected workshops in Akwa Ibom mechanic sites. Presence of *Aspergillus niger* in various sites might be due to its prolific spore production, dispersal capabilities and its cosmopolitan nature. Its identification was confirmed using 18S rRNA gene sequencing and selected for further studies. The presence of these fungi in the soil samples is an indication that these isolates were able to survive in the engine oil contaminated environment while those that could not survive in the engine oil contaminated soil were eliminated due to unfavourable conditions caused by the engine oil. Moreover, one plausible explanation for this resilience is its ability to produce extracellular enzymes such as lipases, esterases, and peroxidases which can degrade hydrocarbons into simpler, less toxic compounds (Zheng and Yan, 2018).

Table 3: Occurrence of Fungal Flora in the Study Site

S/No.	Sampling site	Fungi				
		Aspergillus niger	*Trichoderma spp*	*Fusarium spp*	*Mucor spp*	*Penicillum spp*
1.	Kawo	+	-	+	+	+
2.	Kurmin Mashi	+	+	+	-	-

Key: (+) = Present, (-) = Absent

The result in Table 4 shows that there was significant difference in the number of *Aspergillus niger* in the study area at $p<0.05$. However, the number of *Aspergillus niger* apparent in Kawo was significantly higher than that of Kurmin mashi. Based on Duncan Multiple Mean Test, there is no significant difference between the number of *Aspergillus niger* and *Fusarium spp* in the two sampling sites. However, the result reveals that there was significant difference ($P<0.05$) in the number of *Trichoderm spp, Mucor spp* and *Penicillium spp* in the study area.

The study observed (Table 4) a significant difference ($p<0.05$) in the abundance of *Aspergillus niger* across the study areas. However, the number of *Aspergillus niger* apparent in Kawo was significantly higher than that of Kurmin Mashi, suggesting a variation in environmental or anthropogenic factors between the two sites. This is similar with the work of Ikhajiagbe *et al.* (2015) who showed that *Aspergillus niger* was found to be dominant compared to other fungi in hydrocarbon contaminated soil in Benin and Edo States, Nigeria. Furthermore, the result reveals that there was significant difference in the number of *Trichoderm spp* in the study area. *Trichoderma spp* were present in the samples from K/Mashi with no traces of *Trichoderma spp* in the samples collected from Kawo sites. This is in line with the work of Ekhaise and Nkwelle, 2011 who showed *Trichoderma* and *Penicillium spp* to be the most frequently occurring isolates among other species in soil contaminated environment in Edo metropolis mechanic sites, Benin City.

The result more so (Table 4) shows that *Fusarium spp* were present in both Kawo and K/Mashi sites. This is similar with the result obtained by Moustafa, (2016) who showed that *Fusarium oxysporum* appeared with the highest frequency represented by (9.8%) in oil contaminated sites. Occurrence of *Mucor spp* and *Penicillum spp* found only in Kawo sites coincide with the work of Idowu and Ijah, (2018) who showed occurrence of *Aspergillus, Penicillium* and *Mucor species* in soil contaminated sites. Variation in the fungal distribution can be linked to local environmental conditions and pollutant levels.

The result of conductivity in **Table 5** (A and B sites) ranges between 0.24 to 0.35 ms/cm for all the soil sample. The result of dissolve oxygen also ranges between 5.5 to 8.6 mg/L for all the soil sample. Furthermore, pH values from all the soil sample reveals values ranging from 5.8 to 6.18. Chemical Oxygen Demand results reveals values ranging from 5.6 to highest value of 9.6 mg/kg for all the soil sample. The results of temperature from all the sites ranges from lower temperature of 24.4 to highest temperature of 26.2^0C. Moisture content of all soil samples from the study ranges between 8 to 14 %.

The physicochemical parameters such as electrical conductivity, pH, moisture content, temperature and chemical oxygen demand in this study were in a favourable ranges that supports the bioactivity of fungi. Conductivity ranges between 0.24 to 0.35 ms/cm for all the soil analysed in this study. This is in line with the work of Sabah *et al.,* (2016) who showed the electrical conductivity range of refinery effluent to be between 0.5 to 0.7 micro second per Litre. Dissolved oxygen of the soil sample from this study falls between 5.5 to 8.6 mg/L for all the soil assessed which is in conformity with the work of Sabah *et al.* (2016) who demonstrated the dissolved oxygen of refinery effluents to fall between 3 to 9.4 (mg/L). pH values from all the soil analysed in this study reveals values ranging from 5.8 to 6.18. This is similar with the result obtained by Idowu and Ijah, (2018) who showed the pH range of diesel contaminated soil to be 5.01 to 6.49. The result of

Chemical Oxygen Demand (COD) reveals values ranging from 5.6 to highest value of 9.6 mg/kg for all the soil assessed in this research. This agrees with the findings of Orjiakor *et al.* (2019) who showed the COD value of paint effluents to be between 292 to 719 (mg/L). Temperature from all the sites ranges from lower temperature of 24.4^0C to highest temperature of 26.2^0C. This is in concordance with the work of Sabah *et al.* (2016) who showed the temperature of treated refinery effluents to be between 25.2 and 25.3 ^{0}C at Kaduna refinery. Moisture content of all soil samples in this study ranges between 8 to 14 %. This is agrees with the result obtained by Idowu and Ijah, (2018) who showed the moisture content of diesel oil contaminated soil to be 6.47 and 8.14 % in Eleme, Rivers State, Nigeria.

Table 4: Frequency of occurrence of Fungal Genera from the Study Site

S/No.	Fungal Isolates	Sample Sites		
		Kawo	K/Mashi	SE±Mean
1	*Aspergillus niger*	3^a	1^a	0.3±5
2	*Trichoderma spp*	0^b	1^a	0.1±1
3	*Fusarium spp*	1^a	1^a	0.1±1
4	*Mucor spp*	1^a	0^b	0.1±1
5	*Penicillium spp*	1^a	0^b	0.1±1

Means with the same alphabet are not significantly different from each other based on Duncan Mean Multiple Range Test

Table 5: Physicochemical Parameters of the Soil Contaminated with Spent Engine oil

S/ID	Conductivity ms/cm	DO mg/L	pH	COD mg/kg	Temperature ^{0}C	Moisture %
A	0.35	7.1	5.81	6.4	26.2	14
B	0.32	5.5	5.80	5.6	25.6	10

Molecular Characterization of the Fungal Isolate Aspergillus niger

PCR Amplification of the Aspergillus niger: PCR amplification of the *Aspergillus niger* 18S rRNA using ITS1 (5^1TCCGTAGGTGAACCTGCGG-3^1) and ITS4 ($5^,$TCCTCCGCTTATTGATATGC-$3^,$) primers yielded a product shown in plate 1.

.

Affiliation of 18S rRNA Sequences of Aspergillus Niger: Aspergillus niger isolate from oil contaminated soils collected from mechanic villages were all characterized and analysed. Comparison with the 18S rRNA genes sequences to known fungal sequences in the gene bank database using BLAST analysis revealed sequences 98 and 99% similarity as shown in Table 6. Isolate JQ389709.1, KT826638.1 and AM941157.1 were closely affiliated with the members from *Aspergillus niger* at a percentage similarity of more than 98% (Table 6).

Comparison with the 18S rRNA gene sequences (**Table 6**) to known fungal sequences in the Genebank data base using BLAST analysis indicated sequences of 98% similarity. Isolate AM941157.1 was closely affiliated with members from the genus *Aspergillus* having percentage similarity of 98% (Pawar *et al.,* 2008). Isolate KT826638.1 was closely affiliated with members from the genus *Aspergillus* having a percentage similarity of 99% (Biango and Hodge, 2018). These results strongly suggest that the fungal isolate in question is highly likely to be *Aspergillus niger.* The 18S rRNA is a well conserved marker in fungi, making it widely used in phylogenetic studies and species identification (Nilsson *et al.,* 2019). A similarity of 98-99% is indicative of the species level identification, as suggested by established thresholds in fungal molecular taxonomy (Schoch *et al.,* 2012).

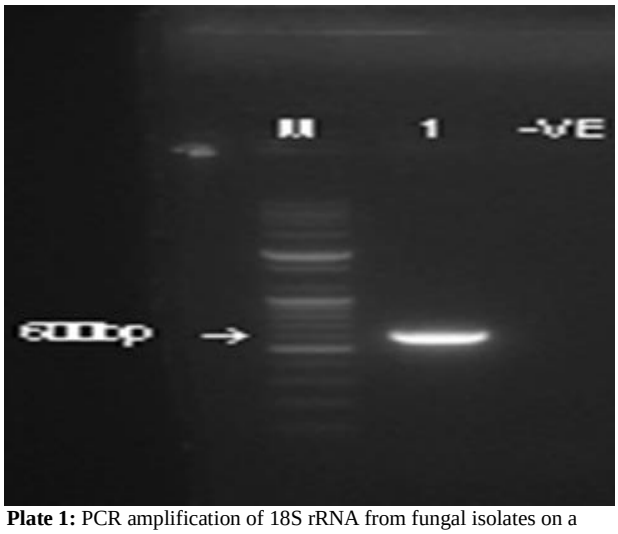

Plate 1: PCR amplification of 18S rRNA from fungal isolates on a 2.0% agarose gel. Band represent fungal isolate namely: *Aspergillus niger*. Negative Control Contains PCR reagent, Water, and Primer. Band size 600bp.

Table 6: Taxonomic affiliation of fungal isolates from oil contaminated soil in Kaduna metropolis

Taxanomic Affiliation	Accession Numbers	%ID
Aspergillus niger	JQ389709.1	98

Aspergillus niger	KT826638.1	99
Aspergillus niger	AM941157.1	98

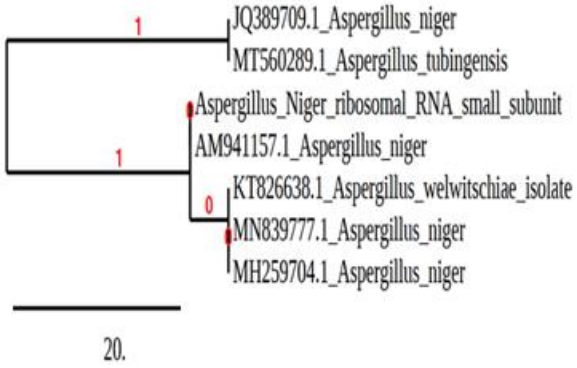

Fig. 2: Showing Phylogenetic Tree Analysis of the fungal species.

The nucleotide sequences obtained were aligned with a known fungal isolates of *Aspergillus niger* from Gene Bank database to show the different fungal classifications.

Conclusion: In conclusion the study shows that soils within the premises of existing workshops are good sources of hydrocarbon-clastic fungi, notably *Aspergillus niger*, suggesting that the fungi specie possesses a high tolerance for hydrocarbon-contaminated environments. The *Aspergillus niger* was identified through the comparison of the 18S rRNA gene sequences to known fungal sequences in the Gene-bank database using BLAST analysis and has indicated similarity of more than 98%. This study contributed to the database on locally available fungal diversity and their ecology. These microbes exhibited appreciable bioremediation potential for the clearing of spent engine oil polluted site in Kaduna Metropolis. Further studies on its biodegradation ability could contribute to the development of eco-friendly strategies for restoring oil-contaminated soils.

Declaration of Conflict of Interest: The authors declare no conflict of interest

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