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Biodegradation of Crude oil Contaminated Soils Using Fungal Species

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***Abstract*—**Crude oil contamination of soil in the environment has increase due to increase in vandalizations and corrosions of pipelines particularly where refineries and deports are located as well as accidental discharge during transportation and increased in demand on hydrocarbon products, which lead to the need to clean up spilled petroleum using biodegradation method. This research aimed at isolating fungal species from crude oil contaminated soil and their ability to degrade volatile organic compounds within petrochemical company (KRPC) in Kaduna State, Nigeria. The fungi were determined using poured plate method. *Aspergillus flavus* (4[20%]), *Aspergillus fumigatus* (6[30%]), *Aspergillus niger* (6[30%]), *Rhizopus oryzae* (2[10%]) and *Microsporum audouinii* (2[10%]) were isolated and identified with different frequencies. The degradation level of fungi species analyzed, using gas chromatography, showed *Rhizopus oryzae* and *Aspergillus niger* were the highest degraders that have degraded all the natural products found in crude oil and *Aspergillus flavus* was the least degrader. The use of these fungal species to clean impure crude oil soils could be employed. Therefore, these species more especially *Aspergillus niger* and *Rhizopus oryzea* might be produced in large quantity and employed in bioremediation process.

***Keywords*—** Crude oil, contaminated soil, fungi, biodegradation & Kaduna refinery

I. INTRODUCTION

Spillage of crude oil on the soil is a major environmental challenge that occur due to activities of petroleum refineries, accidental release of petroleum oil in tankers or ships during transportations on the road and water bodies, storage as well as mishandling and vandalization. Daily uses of petroleum oil in developing and developed countries lead to the proliferation when spilled in the environments. However, according to [1] biodegradation of hydrocarbons by microorganisms has been used as method to purify contaminated environments more especially that is related to hydrocarbon-polluted. These microbes involved in bioremediation process may obtain both energy and carbon supplements by naturalize the specific compound contaminants [2].

Fungi species have been isolated from oil spilled areas and used as degrader in crude oil contaminated soil due to their ability of degrading organic compounds [3]. The ability of these species to degrade the hydrocarbon depends on the chemical compositions of the soil, petroleum oil, concentration, environmental factors and fungal species present. The biodegradation of hydrocarbons by natural microorganisms more especially fungi is of the primary mechanisms through, which petroleum and other hydrocarbon pollutants are eliminated from the environment. Many researchers around the world used species of fungi such as *Aspergillus, Penicillium, Amorphoteca, Candida, Fusarium, Neosartorya, Mycotypha, Rhizopus* and *Botryris* in biodegradation of contaminated or pollutants environment [4], [5], [6], [7], [8], [9]. Thus, crude oil degrading fungi are widely found distributed in all the habitants where macro and microorganisms exist as well as other polluted environments.

The petroleum oil leakage on ecosystem produced many hazardous waste which disturbance all the life found in the environment and the presences of petroleum hydrocarbons on the soils make it not beneficial for agricultural purpose [10], [11]. Nigeria have many refineries were daily production, transportation and storage of crude oil take place. This activities lead to soil contamination that caused health and environmental issues which more especially affects growths and distributions of both plants and animals species living below and above the soil by reducing the a biotic factors of the soil such as; pH, smothering, light, oxygen supply, habitat and food availability and also other toxic actions. This is one of the serious and growing global problems, because it contains hazardous hydrocarbon and its constituents are toxic and carcinogenic in nature, which kills wildlife species and damage the ecosystem. The effect can last up to

generations by enforcing changes in the reproduction and compromising the complex food web if not rectified. With increase in vandalization of pipe lines in Nigeria and accidental discharge during transportation; that helped to exposed humans to dangers associated with crude oil spills. This study aimed at assessing the crude oil degrading abilities of the fungal species identified from crude oil contaminated soils around Petrochemical Company, Kaduna, North central Nigeria.

II. RELATED WORK

The rising of anthropogenic agitations such as climate change, over exploitation, diseases, habitant loss, human influence, oil vandalization/spillage and overgrazing have negative affect on the diversity of the microorganisms and plant species in our natural ecosystems which have become worldwide issue of concern. Recently, attention and effort have been made to restore impure soils use microorganisms. In this junction, several studies had been conducted by various researchers to establish and enumerate the importance of fungal species in bioremediation of crude oil contaminated soil that includes [12], [13], [14], [15] all in Nigeria at different locations and others like [16] Odisha, [6] Mexico and [8], [9], [17] Iraq. Although to the best of our no such study conducted on crude oil contaminated soils around Petrochemical Company, Kaduna, North central Nigeria.

III. MATERIALS AND METHODS

Sample Collections

A total of five soil samples were collected from five different spots in a field contaminated areas with crude oils due to pipeline spillage around Kaduna refinery and petrochemical company (KRPC) Kaduna State, North central Nigeria. The samples were collected using simple sampling technique, at a distance of 50m along the lines of spillage. This was collected at a depth of 10cm, from the soil surfaces. The samples weighing 10g each and were then transferred into sterile polythene bags labeled A to E and were conveyed to Microbiology Laboratory, Kebbi State University of Science and Technology, Aleiro, Kebbi State, Nigeria within 24 hours for further Laboratory analyses.

Enumeration of Fungi

The samples were subjected to serial dilution in the Laboratory. One gram (1g) of each soil sample was weighed and suspended in 9ml sterile water contained in a test tube (10^{-1}) and shaken. An aliquot of 1ml was transferred from this dilution in to the second test tube containing 9ml of sterilized water to arrive at 10^{-2}. Another 1ml was transferred from this second test tube (10^{-2}) to the third test tube containing 9ml of sterile distilled water 10^{-3} up to test tube 10^{-6}. After the serial dilution, 0.1ml of the dilution factor was then transferred from 10^{-5} and 10^{-6} dilutions factors of each sample, which were then aseptically inoculated on the solidified plates of Potato dextrose agar medium. The plates were incubated at (30^{o}C) for 7 days [18].

Isolation of Fungi

Due to the appearance of the different colonies, each colony was picked using sterile needle and sub-cultured on to a new plate of potato dextrose agar (PDA) that was enriched with 2.5ml of crude oil and was placed at the centre of each plate and incubated at (30^{o}C) for 7 days. This was repeated three times until pure culture was obtained as described by [14].

Characterization and Identification of fungal species

The fungal isolates were further identified based on colonial morphology (macroscopic) and microscopic examinations and in compared with Mycological Atlas. A small portion of each colony was picked and placed at the center of glass slide containing a drop of methylene blue reagent; it was emulsified and viewed under the microscope using X40 and X100 objective lenses as adopted by [19].

Biodegradation Studies

The Enrichment of Isolated Fungi

Isolated fungi species were further inoculated on oil agar broth containing 0.45g K_2PO_4, 0.3g KH_2PO_4, 1.g NH_4Cl, 0.05g $MgSO_4.7H_2O$, 0.025g NaCl, 0.0025g $FeSO_4.7H_20$, 250ml and distilled water, 2.5ml crude oil for hydrocarbon-utilizing or degrading determination, which were left alone on bench for 2 weeks under (30^{o}C) with constant shaking in different conical flasks. All the inoculated flasks were taken for gas chromatography (GC) to know the amount of crude oil degraded by the fungi as described by with slightly modification [19]. Pure crude oil was used as control.

Determination of the dominant Fungi species responsible for crude oil degradation in Crude oil contaminated soils

The dominant fungi responsible for petroleum hydrocarbon degradation was determined by the frequency of occurrence of the fungal isolates,

$$\text{Percentage Frequency of occurrence} = \frac{\text{Occurrence of the isolate}}{\text{Total number of the isolate}} \times 100$$

Crude oil degradation assessment by fungi using GCMS

Degradation of crude oil by Identified fungal isolates were assessed using (GCMs) and each chromatogram obtained was assessed and the results were presented. Gas chromatographic analysis, was conducted using G C 2014 with G C MS, Agilent Technology.

Statistical analysis

All the data obtained were subjected to statistical analysis of variance (Anova) using SPSS statistical software version 23.

IV. RESULTS AND DISCUSSION

Fungal counts of the crude oil contaminated soil samples

The total mean of the fungal spore counts from the soil samples A to E analyzed in this study were ranged from 5.95 x 10^7sfu/g – 7.35 x 10^7sfu/g. Soil sample C had the highest fungal loads 7.35 x 10^7cfu/g while E soil had the lowest load 5.95x10^7 sfu/g as seen in Table (1). Twenty (20) fungal species were isolates and identified from the crude oil contaminated soil samples Table (2). The isolates of three genera belonging to species of namely; *Aspergillus niger* and *Aspergillus fumigatus* had the highest rate of occurrence with 6(30%) while *Microsporum audouinii* and *Rhizopus oryzae* had the least, with 2(10%) as presented in Table (2).

Table (3) shown *Microsporum audoinii* is a crude oil degrader of the volatile organic compounds present in crude oil with partial degradation in some volatile organic compounds and complete in others. Nona decane had the highest rate of partial degradation with percentage abundance of 0.27 %, Heptadecane had percentage abundance 0.43%, Tridecane with percentage abundance of 0.55 %, and Dodecane with percentage abundance 0.72 %. These were followed by Penta decane 2,6, 10-Trimethyl with percentage abundance 0.85 %, Tridecane with percentage abundance 0.92 %, Pentadecane with percentage abundance 1.43% and Un decane was the least with percentage abundance 1.53 %. Others volatile organic compounds: Trimethyl, Butane, Heptane 2,6,6,-Trimethyle, Naphthlene, cyclohexanone, Octane, Heptacosane, Octadecane-2-methyl, Eicosane, Heneicosane and Docosane were completely degraded each with 0% percentage abundance.

Rhizopus oryzae is a potential degrader of the volatile organic compounds present in crude oil. The organism has shown complete degradation of the volatile compounds present Table (4). The following compounds: Trimelhyl, Butane, Undecane, Heptane,2,6,6-trimelhyl, Naphthlane, Cyclohexane, Dodecane, Octane, Tridecane, Tetradecane, Pentadecane, Hexadecane, Heptacosane, Heptadecane, Octadecane, Pentadecane, 2,6,10-Trimelhyl, Octadecane2-melhyl, Nonadecane, Eicosane, Heneicosane, Docosane were completely degraded, each with (0 %) percentage abundance.

The various volatile organic compounds in crude oil showed complete degradation by *Aspergillus fumigatus* with one volatile organic compound, Butane that showed partial degradation, having percentage abundance 7.83 % and other two volatile organic compounds Undacane and Octadecane having 17.18 % &11.40 % percentage abundance. The Trimethyl, Heptane 2,6,6- Trimethyl, Naphthlene, Cyclohexanone, Dodecane, Octane, Tridecane, Tetradecane, Pentadecane, Hexadecane, Heptacosane, Pentadecane, 2,6,10- Trimetyl, Octadecane 2- methyl, Nonadecane, Eicosane, Heneicosane, and Docosane all were completely degraded with 0% each of percentage abundance as seen in Table (5) respectively. However, Table (6) indicated that, *Aspergillus flavus* had complete degradation of eight volatile organic compounds: Trimethyl, Butane, Heptane 2,6,6-Trimethyl, Naphthlene, Cyclohexanone, Octadecane 2- methyl, Herieicosane and Docosane, each had (0%) percentage abundance. However, Three (3) other volatile organic compounds each with partial degradations, Nonadecane had the highest rate of partial degradation with percentage abundance of 1.90 % this was followed by Tridecane with percentage abundance of 5.89% and Dodecane recorded the least with 8.49% percentage abundance. Other volatile organic compounds as; Undecane 3.55% to 9.68%, Octane 3.70% to 4.20%,Tetradecane 9.00% to 9.89%, Pentadecane 9.95% to 11.72% ,Hexadecane 6.67% to 8.49% ,Heptacosane 3.70% to 5.49%,Heptadecane 1.94% to 4.80%, Pentadecane-2,6,10 trimethyl 6.39 to 6.59%, Octadecane 2.08% to 5.16%, Ecosane 1.56% to 5.95% all of which were unable breakdown by these organisms. Thus, their percentage abundance increased due to partial degradation and complete degradation of other volatile organic compounds present which resulted to increase in their availability.

Level of degradation ability by *Aspergillus niger* had remarkable potential as a degrader to have degraded the volatile organic compounds present in crude oil .The organism had shown complete degradation of the volatile compounds present in the crude oil Table (7) of the compounds: Trimelhyl, Butane, Undecane, Heptane 2,6,6-trimelhyl, Naphthlane, Cyclohexane, Dodecane, Octane, Tridecane, Tetradecane, Pentadecane, Hexadecane, Heptacosane, Heptadecane, Octadecane, Pentadecane2,6,10-Trimelhyl, Octadecane 2-melhyl, Nonadecane, Eicosane, Heneicosane, Docosane were completely degraded having (0%) percentage abundance each.

Table 1: Fungal counts of crude oil contaminated soil samples

Soil Sample Site	Fungal count (cfu/g)
A	6.65x10^7
B	6.3x10^7
C	7.35x10^7
D	6.65x10^7
E	5.95x10^7

Table 2: Frequency of occurrence of the fungal isolated from the crude oil contaminated soil samples

Fungal Species	**Occurrence of Isolates (%)**
Aspergillus flavus	2(20)
Rhizopus oryzae	2(10)
Aspergillus niger	6(30)
Aspergillus fumigatus	6(30)
Microsporum audouinii	2(10)
Total	**20(100)**

Table 3: Volatile organic compounds with their quality and percentage abundance in crude oil before and after degradation by *Microsporum. Audouinii*

Retention Time (min)	Volatile Organic Compounds	Quality before degradation	Percentage Abundance before degradation	Retention Time (min). after degradation	Quality after degradation	Abundance in Percentage after degradation
1.881	Trimethyl	43	9.99	0	0^{a}	0^{a}
1.881	Butane	10	9.99	0	0^{a}	0^{a}
2.756	Undecane	93	3.55	5.445	55^{b}	1.53^{j}
3.078	Heptane, 2, 6, 6, -Trimethyl	64	3.31	0	0^{a}	0^{a}
3.078	Naphthlene	46	3.31	0	0^{a}	0^{a}
3.078	Cyclohexanone	46	3.31	0	0^{a}	0^{a}
3.257	Dodecane	95	9.49	3.253	83^{d}	0.72^{e}
3.741	Octane	64	3.70	0	0^{a}	0^{a}
3.953	Tridecane	97	6.71	3.942	95^{f}	0.92^{h}
4.837	Tetradecane	97	9.00'	4.825	95^{f}	0.55^{d}
5.856	Pentadecane	97	9.95	5.836	83^{d}	1.43^{i}
6.935	Hexadecane	96	6.67	6.915	95^{f}	0.89^{g}
7.470	Heptacosane	83	3.70'	0	0^{a}	0^{a}
8.031	Heptadecane	97	1.94	8.002	95^{f}	0.42^{c}
8.099	Pentadecane 2, 6,10-Trimethyl	96	6.39	7.45	80^{c}	0.85^{f}
9.110	Octadecane	98	2.08	9.081	95^{f}	0.43^{e}
8.099	Octadecane 2-Methyl	91	6.39	0	0^{a}	0^{a}
10.155	Nonadecane	98	1.90	10.118	92^{e}	0.27^{b}
11.166	Eicosane	96	1.56	0	0^{a}	0^{a}
12.168	Heneicosane	96	4.09	0	0^{a}	0^{a}
13.103	Docosane	93	3.30	0	0^{a}	0^{a}

The figures followed by different letters are significant at ($P<0.05$)

Table 4: Volatile organic compounds with their quality and percentage abundance in crude oil before and after degradation by *Rhizopus oryzae*

Retention Time (min)	Volatile Organic Compounds	Quality before degradation	Percentage Abundance before degradation	Retention Time (min). after degradation	Quality after degradation	Abundance in Percentage after degradation
1.881	Trimethyl	43	9.99	0	0^{a}	0^{a}
1.881	Butane	10	9.99	0	0^{a}	0^{a}
2.756	Undecane	93	3.55	0	0^{a}	0^{a}
3.078	Heptane, 2, 6, 6, -Trimethyl	64	3.31	0	0^{a}	0^{a}
3.078	Naphthlene	46	3.31	0	0^{a}	0^{a}
3.078	Cyclohexanone	46	3.31	0	0^{a}	0^{a}
3.257	Dodecane	95	9.49	0	0^{a}	0^{a}
3.741	Octane	64	3.70	0	0^{a}	0^{a}
3.953	Tridecane	97	6.71	0	0^{a}	0^{a}
4.837	Tetradecane	97	9.00'	0	0^{a}	0^{a}
5.856	Pentadecane	97	9.95	0	0^{a}	0^{a}
6.935	Hexadecane	96	6.67	0	0^{a}	0^{a}
7.470	Heptacosane	83	3.70'	0	0^{a}	0^{a}
8.031	Heptadecane	97	1.94	0	0^{a}	0^{a}
8.099	Pentadecane 2, 6,10-Trimethyl	96	6.39	0	0^{a}	0^{a}
9.110	Octadecane	98	2.08	0	0^{a}	0^{a}
8.099	Octadecane 2-Methyl	91	6.39	0	0^{a}	0^{a}
10.155	Nonadecane	98	1.90	0	0^{a}	0^{a}
11.166	Eicosane	96	1.56	0	0^{a}	0^{a}
12.168	Heneicosane	96	4.09	0	0^{a}	0^{a}
13.103	Docosane	93	3.30	0	0^{a}	0^{a}

The figures followed by different letters are significant at ($P<0.05$)

Table 5: Volatile organic compounds with their quality and percentage abundance in crude oil before and after degradation by *Aspergillus fumigatus*

Retention Time (min)	Volatile Organic Compounds	Quality before degradation	Percentage Abundance before degradation	Retention Time (min). after degradation	Quality after degradation	Abundance in Percentage after degradation
1.881	Trimethyl	43	9.99	0	0^{a}	0^{a}
1.881	Butane	10	9.99	3.95	43^{b}	7.83^{b}
2.756	Undecane	93	3.55	5.445	53^{c}	17.18^{d}

3.078	Heptane, 2, 6, 6, -Trimethyl	64	3.31	0	0^{a}	0^{a}
3.078	Naphthlene	46	3.31	0	0^{a}	0^{a}
3.078	Cyclohexanone	46	3.31	0	0^{a}	0^{a}
3.257	Dodecane	95	9.49	0	0^{a}	0^{a}
3.741	Octane	64	3.70	0	0^{a}	0^{a}
3.953	Tridecane	97	6.71	0	0^{a}	0^{a}
4.837	Tetradecane	97	9.00'	0	0^{a}	0^{a}
5.856	Pentadecane	97	9.95	0	0^{a}	0^{a}
6.935	Hexadecane	96	6.67	0	0^{a}	0^{a}
7.470	Heptacosane	83	3.70'	0	0^{a}	0^{a}
8.031	Heptadecane	97	1.94	0	0^{a}	0^{a}
8.099	Pentadecane 2, 6,10-Trimethyl	96	6.39	0	0^{a}	0^{a}
9.110	Octadecane	98	2.08	3.27	43^{b}	11.40^{c}
8.099	Octadecane 2-Methyl	91	6.39	0	0^{a}	0^{a}
10.155	Nonadecane	98	1.90	0	0^{a}	0^{a}
11.166	Eicosane	96	1.56	0	0^{a}	0^{a}
12.168	Heneicosane	96	4.09	0	0^{a}	0^{a}
13.103	Docosane	93	3.30	0	0^{a}	0^{a}

The figures followed by different letters are significant at (P<0.05)

Table 6: Volatile organic compounds with their quality and percentage abundance in crude oil before and after degradation by *Aspergillus flavus*

Retention Time (min)	Volatile Organic Compounds	Quality before degradation	Percentage Abundance before degradation	Retention Time (min). after degradation	Quality after degradation	Abundance in Percentage after degradation
1.881	Trimethyl	43	9.99	0	0^{a}	0^{a}
1.881	Butane	10	9.99	0	0^{a}	0^{a}
2.756	Undecane	93	3.55	5.445	49^{b}	9.68^{k}
3.078	Heptane, 2, 6, 6, -Trimethyl	64	3.31	0	0^{a}	0^{a}
3.078	Naphthlene	46	3.31	0	0^{a}	0^{a}
3.078	Cyclohexanone	46	3.31	0	0^{a}	0^{a}
3.257	Dodecane	95	9.49	6.915	95^{j}	8.49^{j}
3.741	Octane	64	3.70	3.738	59^{c}	4.20^{c}
3.953	Tridecane	97	6.71	3.941	92^{g}	5.89^{g}
4.837	Tetradecane	97	9.00'	4.825	94i	9.89^{L}
5.856	Pentadecane	97	9.95	5.836	90^{f}	11.72^{m}
6.935	Hexadecane	96	6.67	6.915	95^{j}	8.49^{j}
7.470	Heptacosane	83	3.70'	12.088	83^{e}	5.49^{f}
8.031	Heptadecane	97	1.94	8.002	93^{h}	4.80^{d}
8.099	Pentadecane 2, 6,10-Trimethyl	96	6.39	7.45	80^{d}	6.59^{i}
9.110	Octadecane	98	2.08	9.081	96^{j}	5.16^{e}
8.099	Octadecane 2-Methyl	91	6.39	0	0^{a}	0^{a}
10.155	Nonadecane	98	1.90	10.118	95^{j}	1.85^{b}
11.166	Eicosane	96	1.56	11.128	92^{g}	5.95^{h}
12.168	Heneicosane	96	4.09	0	0^{a}	0^{a}
13.103	Docosane	93	3.30	0	0^{a}	0^{a}

The figures followed by different letters are significant at (P<0.05)

Table 7: Volatile organic compounds with their quality and percentage abundance in crude oil before and after degradation by *Aspergillus niger*

Retention Time (min)	Volatile Organic Compounds	Quality before degradation	Percentage Abundance before degradation	Retention Time (min). after degradation	Quality after degradation	Abundance in Percentage after degradation
1.881	Trimethyl	43	9.99	0	0^{a}	0^{a}
1.881	Butane	10	9.99	0	0^{a}	0^{a}
2.756	Undecane	93	3.55	0	0^{a}	0^{a}
3.078	Heptane, 2, 6, 6, -Trimethyl	64	3.31	0	0^{a}	0^{a}
3.078	Naphthlene	46	3.31	0	0^{a}	0^{a}
3.078	Cyclohexanone	46	3.31	0	0^{a}	0^{a}
3.257	Dodecane	95	9.49	0	0^{a}	0^{a}
3.741	Octane	64	3.70	0	0^{a}	0^{a}
3.953	Tridecane	97	6.71	0	0^{a}	0^{a}
4.837	Tetradecane	97	9.00'	0	0^{a}	0^{a}
5.856	Pentadecane	97	9.95	0	0^{a}	0^{a}
6.935	Hexadecane	96	6.67	0	0^{a}	0^{a}
7.470	Heptacosane	83	3.70'	0	0^{a}	0^{a}
8.031	Heptadecane	97	1.94	0	0^{a}	0^{a}
8.099	Pentadecane 2, 6,10-Trimethyl	96	6.39	0	0^{a}	0^{a}
9.110	Octadecane	98	2.08	0	0^{a}	0^{a}
8.099	Octadecane 2-Methyl	91	6.39	0	0^{a}	0^{a}
10.155	Nonadecane	98	1.90	0	0^{a}	0^{a}
11.166	Eicosane	96	1.56	0	0^{a}	0^{a}
12.168	Heneicosane	96	4.09	0	0^{a}	0^{a}
13.103	Docosane	93	3.30	0	0^{a}	0^{a}

The figures followed by different letters are significant at (P<0.05)

Discussion

The results of fungal species isolated and identified in this research work revealed the presences of *Aspergillus flavus* 4(20%), *Aspergillus fumigatus* 6(30%), *Aspergillus niger* 6(30%), *Rhizopus oryzae* 2(10%) and *Microsporum* audouinii 2(10%). These results agreed with the findings of [19], which reported the presences of same fungal species in soil samples. This indicates that, species obtained exist, distributed and grow on crude oil contaminated environment soils while those that could not survived in this environment being eliminated by the toxic conditions caused by the oil. However, the *Aspergillus* species as reported [8], [9], on mycodegradation of crude oil by fungal species isolated from petroleum contaminated soil equally supported our findings. In the present research findings, *Aspergillus niger* and *Aspergillus flavus* had the highest frequencies of occurrences (30%). Furthermore, this resulted was slightly higher than the result (23.5%) reported [7]. This slightly differences is probably due to the environmental differences, locations, pH of the soil, abiotic factors, media used and procedures followed in conducting this research. Additionally, the mean of the total fungal counts recorded ranged from 5.95 x 10^7 sfu/g to 7.35 x 10^7 sfu/g. The findings reported higher counts than reported [20], [21]. The variations in counts may be due to pH and organic matter contents which could aid the proliferation of the fungal species and other species more especially aerobic factors that can grow under ambient environmental stressed conditions such as low pH and poor nutrients.

The study showed that, *Aspergillus niger* and *Rhizopus oryzae* had the highest degradation of volatile organic compounds, while *Aspergillus flavus* had the least degradation (Table 4,7 & 6). These results concord with the findings of [9], who also reported that, *A. flavus* had the highest ability to biodegrade crude oil with the percentage of biodegradation 80% during the incubation periods of 30 days. More withal, this research agreed with the findings reported [8] where they reported that, *Aspergillus niger* had 94% biodegradation potential. All the species of *Aspergillus niger* and *Rhizopus oryzae* degraded all the volatile organic compounds used in this study, this may be due to enzymes proteins which they used to degrade/breakdown complex organic compounds into simple sugars so as to control the soil. They achieve this as they released the enzymes in order to obtain nutrient from the soil and reabsorb for their growth and development. According to [21], research which reported that, *Penecillum, Aspergillus* and *Rhizopus* specie had a good potential to gradate hydrocarbons. This may be as a result of these organisms that have the ability to produce enzymes which metabolized hydrocarbons which are known as cellulose producers. *Aspergillus fumigatus, Aspergillus flavus* and *Microsporum audouinii* revealed partial or weak degradation of the volatile organic compounds. In similar study conducted [9] they observed that *Aspergellus fumigatus* had less degradation of 36% on biodegradation of crude oil using *Aspergillus* species. This inability of these three fungal species to degrade contaminated oil soils could be due to environmental factors that are not favorable to them to release their hyphae to produce enzymes/chemicals that aid in breaking down of volatile organic compounds. In addition, it is very possible that these species required more incubation period to degrade crude oil as stated [22], [23]. However, during this present research the degradation period were not determined. The statistical analyses showed that $p>0.05$ among the volatile compounds in chromatogram inoculated with *Aspergillus niger* and *Rhizopus oryzae* had (0.00 ± 0.00^{a}), this equally indicated complete degradation and no significant difference while *Aspergillus flavus* (11.72 ± 0.20^{L}) at $P<0.05$ showed significant difference across that's less or partial degradation of the volatile organic compounds.

V. CONCLUSION AND FUTURE SCOPE

All these fungi species were isolated and identified as; *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Rhizopus oryzae* and *Microsporum audouinii* in this present study were found to be distributed as potential degraders of volatile organic compounds, more especially *Aspergillus niger* and *Rhizopus oryzae* that showed excellent biodegradation. However, these species played vital roles in producing their enzymes on the crude contaminated soils that restored it to arid one. Therefore, the use of these **f**ungal species to clean up crude oil contaminated soils if produced in large quantity could be employed in the study area help in bioremediation.

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REFERENCES

[1]. F. Challain, A. Flecheb, E. Burga, Y. Phantavonga, A. Saliot, J. Oudot, "Identification and Biodegradation Potential of Tropical Aerobic Hydrocarbon-Degrading Microorganisms". *Resident Microbes*. **155** (7):**587-595, 2004.**

[2]. R.S. Thavasi, T. Jayalakshmi, M. Balasubramanian, I.M. Banat, 'Biodegradation of Crude Oil by Nitrogen Fixing Marine Bacteria *Azotobacter Chrococcum". Journal. Microbiology*, **1**:**pp. 401-408, 2006**.

[3]. C.D. Batelle, "Mushrooms: Higher Macrofungi to clean up the environment. Batelle Environmental Issues, Fall. **2000**

[4]. M. Throne-Holst, A. Wentzel, T.E. Ellingsen, H.K. Kotlar, S.B. Zotchev, "Identification of novel genes involved in long-chain nalkane degradation by *Acinetobacter* sp. Strain DSM 17874", *Applied Environmental Microbiology*, vol. **73,** no.10, **Pp. 3327–3332, 2007.**

[5]. S. Farag, N.A. Soliman, "Biodegradation of Crude Petroleum Oil and Environmental Pollutants by *Candida tropicalis* Strain", *Braz. Arch. Biolology Technol*ogy, vol. **54**, no.4, **Pp. 821-830, 2011.**

[6]. H. Al-Nasrawi, "Biodegradation of Crude Oil by Fungi Isolated from Gulf of Mexico". *Journal of Bioremediation Biodegradition*, vol. **3**, **Pp. 1-6, 2012.**

[7]. I.F.H. AI-Jawhari, "Ability of Some Soil Fungi in Biodegradation of Petroleum Hydrocarbon". *Journal of Applied Environmental Microbiology*, vol. **2,** no. 2, Pp. **46-52, 2014**.

[8]. A.A., Burghal, M.J.A., Najwa W. Abu-Mejdad, H. Al-Tamimi, "Mycodegradation of Crude Oil by Fungal Species Isolated from Petroleum Contaminated Soil". *International Journal of Innovative Research in Science, Engineering and Technology*, Vol. **5**, Issue 2, **1517-1524, 2016.**

[9]. Al-Dossary, M.A., Shaymaa A.A. and Hamid T.A. Biodegradation of Crude Oil Using *Aspergillus* species. *Journal of Biology, Agriculture and Healthcare*, Vol.**9**, No.4, **60-64, 2019**.

[10]. E. Al-Jumaily, N. Al-wahab, "Nutritional requirement of Enterobacter cloacae for biodegradation of hydrocarbons". *Global Journal of Biological-Science Biotechnology*, vol. 1, **Pp. 65-70, 2012.**

[11]. G.D. Gojgic-Cvijovic, J.S. Milic, T.M. Solevic, V.P. Beskoski, M.V. Ilic, L.S. Djokic, T.M. Narancic, M.M. Vrvic, "Biodegradation of petroleum sludge and petroleum polluted soil by a bacterial consortium: a laboratory study", *Biodegradation*, vol. **23**, **Pp. 1-14, 2012**.

[12]. O.F. Olukunle, T.S. Oyegoke, "Biodegradation of Crude oil by Fungi Isolated from Cow Dung contaminated soils". *Nigeria Journal of Biotechnology*. Vol. **31**; **Pp. 46 –5, 2016.**

[13]. A.A. Ameh, A.H. Kawo, "Enumeration, isolation and identification of bacteria and fungi from soil contaminated with petroleum products using layer droppings as an amendment". Bayaro. *Journal of pure and applied science*, **10**(1) **219-225, 2017**.

[14]. A.K. Onifade, F.A. Abubakar "Characterization of Hydrocarbon-Degrading Microorganisms Isolated from Crude Oil Contaminated Soil and Remediation of the Soil by Enhanced Natural Attenuation". *Research Journal of Microbiology*, 2: **149-155, 2007**

[15]. M.D. Asemoloye, T. Solveig, D. Chiara, W. Xiao, X. Shihan, A.M. Mario, G. Wenyuan, G.J. Segun, P. Lorenzo, "Hydrocarbon Degradation and Enzyme Activities of *Aspergillus oryzae* and *Mucor irregularis* Isolated from Nigerian Crude Oil-Polluted Sites". *Microorganisms*, **8**, 1912; doi:10.3390/microorganisms8121912, **2020**.

[16]. S.K. Padhi, M. Alok kumar, P. Lopa, T. Srikant, B. Bijyalaxmi, M. Jasmin, "Isolation of Microorganism from Natural Source for Bioremediation of Oil-spill". *Bulletin of Environment, Pharmacology and Life Sciences*, Volume (**6**); **Pp. 05-09, 2012**.

[17] A.B. Al-Hawash, J.A. Alkooranee, A.H. Abbood, J. Zhang, J. Sun, X. Zhang, F. Ma, "Isolation and characterization of two crude oil-degrading fungi strains from Rumaila oil field, Iraq". *Biotechnology Reports*, **17 Pp. 104 – 109, 2017.**

[18]. M. Cheesebrough, "Medical Laboratory Manual Tropical Health Technology, low priced edition. Doddington, Cambrigdeshire, England. **Pp. 20-35, 2000.**

[19]. Clemente, A.R., Anazawa, T.A. and Durrant, L.R. Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. *Braz. Journal Microbiology*, 32: **Pp. 1-9, 2001.**

[20]. K.C. Agu, E.B. Edet, A.N. Sunday, A.C.G. Chidi, C.E. Uche, M.O. Uchenna, A.O. Chinedu, A.O. Chinedu, "Isolation and Characterization of Microorganisms from Oil Pulluted Soil in Kwata, Awkka South, Nigeria". A*merican Journal of Current Microbiology*, **3:46-59, 2014**.

[21]. P.O. Okerentugba, O.U. Ezeronye, "Petroleum degrading potentials single and Microbial cultures isolated from rivers and refinery effluent in Nigeria". *African Journal of Biotechnology*, 9, **288-292, 2003.**

[22]. W.K. Catherine, "Isolation and Characterization of Hydrocarbon Biodegrading Fungi from oil contaminated Soils in thinka Kenya. A Thesis Submitted in Partial Fulfillment for the degree of Master of Science in Mycology in the Jomo Kenyatta University of Agriculture and Technology, Kenya. **2015.**

[23]. O. Dahan, I. Katz, L. Avishai, Z. Ronen, "Transport and degradation of perchlorate in deep vadose zone: implications from direct observations during bioremediation treatment". *Hydrology Earth System Sciences*, 21:**4011– 4020, 2017.**

