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ASSESSMENT OF THE POPULATION AND SPECIES DIVERSITY OF FUNGI IN DIESEL CONTAMINATED SOIL

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ABSTRACT

Petroleum contamination is a known environmental challenge in the South-South region of Nigeria. This study investigated the fungal population and diversity of diesel contaminated soil close to a petroleum dispensing station in Port Harcourt. Four composite soil samples were collected equidistance from the polluted site and designated as A, B, C and D. An unpolluted soil sample designated E served as control. The cultivation and isolation of fungi from the soil samples were carried out by plating aliquot of serially diluted samples onto sterile Sabouraud Dextrose Agar in Petri dishes. Cultured plates were incubated at room temperature for 3 to 5 days for the growth of fungi. Colonies which developed were counted and identified through macroscopic and microscopic methods. Fungal population were found to decrease in the diesel oil polluted samples (A, B, C, and D) and increased in the unpolluted soil sample E. This shows that the diesel oil has a decreasing effect on soil fungi population as depicted in the drop of the mean count spore forming units (SFU) from 620 x10^5 SFU/gt to 2.15 x 10^5 SFU/g with the control recording the highest count of fungi. Some fungi isolated from the soils and identified were: Penicillium chrysogenum, Aspergillus niger, Rhizopus oligiosporus, Fusarium solani, Thrichoderma sp., Alternaria sp., Pichia sp. And other species of Aspergillus. Amongst the isolates, Aspergillus niver and Penicillum chrysogenum were predominant and showed fast growth rates and are suggestive for utilization and biodegradation of diesel hydrocarbon in the polluted soils.

Keywords: Diesel; Contamination; Aspergillus niger; Rhizopus species; Biodegradation.

1.0 INTRODUCTION

Oil and its derivatives are extremely adaptable and have a wide range of uses in different industries. Oil is used in the automotive industry as a fuel for automobiles as well as an important component of tires, bumpers, and seats. Oil and gas are essential to the construction industry because they produce materials such as paint and asphalt as well as safety equipment that improves longevity and worker protection (Akani *et al*., 2016; Khan *et al*., 2024),

Petroleum-based textiles like polyester and nylon, which offer comfort and protection, are widely used in the apparel business. Likewise, commonplace accessories like jewelry, sunglasses, and purses are mostly composed of petroleum-based polymers (Major and Valerie, 2024).

Petroleum is an essential fuel for transportation, heating, cooling, and cooking. Many home products, such as cleaning supplies and kitchen utensils, include oil derivatives that are frequently overlooked. Petroleum is also used in the beauty sector to create a variety of goods, including soap, cosmetics, and nail paint ((Major and Valerie, 2024).

In the medical field, petroleum is essential for manufacturing life-saving devices and numerous healthcare products. Additionally, many furniture pieces, especially those made from synthetic materials, are derived from oil. Sporting goods, including surfboards and basketballs, often utilize petroleum-based materials, enhancing safety and performance (Hess *et al.* 2011).

Petroleum-derived plastics are essential in the electronics sector as well, where they are used in gadgets like computers and televisions to guarantee their operation and safety. Office supplies also contain oil-based components, such as printer ink and furniture. Finally, to safeguard crops, the agriculture industry uses pesticides and fertilizers derived from petroleum, highlighting the extensive influence of oil on our daily life (Ordu and Disegha, 2023; Disegha and Okpokwasili, 2009). Many of the chemicals are used or destroyed, but significant portion are released into the land, water, and air, posing a risk to the environment and causing ongoing pollution (Obire *et al*., 2003). Another source of environmental threats which compromises the exposed ecosystem is the indiscriminate and unguided disposal of untreated produced water which is by-product of offshore and onshore production of oil and gas as commonly notice within the oil rich Niger Delta region, and treatment of such produced water before disposal is necessary to avert the adverse effect of oil contamination on the environment and soil (Kpea-ue *et al.,* 2020). The usage of petroleum products and the processing and distribution of petroleum hydrocarbons

contaminate soil. Changes in soil characteristics brought on by contamination with petroleum-derived materials can result in a lack of accessible forms of nitrogen and phosphorus, as well as deficiencies in oxygen and water, which has become a global concern (Dalel and Almaghribi, 2022).

A hydrocarbon spill poses a major ecological risk. In Nigeria and other emerging nations, diesel pollution is rising. With the continued rise in oil dependency, related issues are become increasingly complex. Hydrocarbon-contaminated soils cause significant harm to nearby ecosystems because contaminants build up in animals and plant tissues have the potential to cause gene mutation or death. Therefore, it becomes essential to stop the pollution from spreading and to clean up the affected regions (Doherty and, Otitoloju, 2013). For this, a variety of techniques, such as bioremediation, can be employed as some fungus are known to be more hydrocarbon biodegradable than bacteria. Since fungus may be cultivated on a variety of low-cost agricultural or forest wastes, including sawdust and maize cobs, their use is anticipated to be comparatively more cost-effective and sustainable. Additionally, using them is a non-aggressive, soft method Akani *et al*., 2016).

The most prevalent fungi that have been identified as biodegraders are *Alternaria*, *Amorphoteca*, *Aspergillus*, *Candida*, *Cephalosporium, Cladosporium, Fusarian, Geotrichum, Graphium, Mcor, Paccllomyces, Penicillim Rhizopus, Rhodotorula, Sphaeropsidales, Taiaromyces*, and *Trichoderna*. Species of many fugal genera are known to metabolize hydrocarbons and flourish in oil-contaminated environments due to their ability to cause biodegradation (Obire, *et al*., 2020; Medaura *et al.,* 2021)

This study was conducted to assess the population and diversity of fungi in soils contaminated with diesel. The study objectives were to Isolate and identify fungi from the diesel polluted sites; to identify the hydrocarbon-degrading fungi isolated from these regions, and to analyze the effects of the diesel oil on fungal soil organisms.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study area was the NNPC petroleum dispensing station at Lagos Bus Stop in Port Harcourt, Rivers State, Nigeria. Lagos Bus Stop is a busy traffic congested area, situated in the Township

Area of Rivers State, located within the Port Harcourt City Local Government Area of Rivers State, with coordinates of 4.76162°N, 7.018396°E. This location shares comparable environmental characteristics with Port Harcourt, the capital of Rivers State. The surrounding areas include Station Road, Lagos Street, and Agip Road in Port Harcourt, Nigeria.

2.2 Sample Collection and Media Preparation

From each diesel oil polluted site adjacent to a petroleum dispensing station, fifty (50) gramme of soil samples were aseptically collected daily at varying distances, with a sterile hand auger into sterile black polythene bags for two weeks. The control soil sample was collected from an unpolluted site at the same sampling area, but of different sampling point, with over 50 m distance. The collection bags were labeled as Sampling Location A (Immediate proximity), B (2m), C (4m), D (6m), and E (control) (over 50m) (Disegha *et al*., 2024).

All media were prepared according to manufacturer's instructions and sterilized in an autoclave at 121^{o}C for 15 minutes at 15 pounds per square inch (psi). Pipettes and other glassware were sterilized in a hot-air oven at 160^{o}C for 30 minutes. Drying of prepared culture media plates were dried in hot air oven at 70oC for 10 minutes. About 75% alcohol was used to sterilize laboratory benches (Thomas, *et al*., 2021).

2.3 Cultivation and Enumeration of Fungal Isolates

Mycological analysis of soil samples was carried out in the Microbiology Laboratory of the Rivers State University, Port Harcourt, Nigeria. One gramme (1g) of fine soil was aseptically transferred using a flame-sterilized steel spatula, into a sterile test tube containing 9.0ml of normal saline. This gave 10^{-1} dilution. Subsequently, three fold (10^{-3}) serial dilutions were prepared from 10^{-1} dilution. 0.1ml aliquot of 10^{-3} dilution of each soil sample were aseptically removed with a sterile pipette and separately spread plated with flame-sterilized glass spreader on well dried SDA plates in duplicates. Subsequently, additional two steps ten-fold serial dilutions in test tubes were prepared from the stock of 10^{-1} dilution, by adding 1ml from stock preparation into 9 ml of normal saline, yielding 10^{-1}, 10^{-2}, 10^{-3}. This procedure was repeated for all the soil samples collected at different location of the oil spilled site as well as the control site. The cultured plates were incubated at room temperature (30^{o}C) for 3 to 5 days. After incubation, the colonies that developed on the mycological plates were counted and recorded as count of

total viable heterotrophic fungi (Disegha *et al*., 2024).

The discrete colonies which developed were counted and the mean for the duplicate cultures were recorded as the total fungi count in the samples.

2.4 Isolation and Identification of Fungi in the Soil Samples

Distinct fungal colonies from soil were isolated, subcultured to obtain pure cultures, and identified by observing their colonial characteristics by macroscopy and microscopy. Examination by macroscopy included observing morphological features such as surface topography, surface texture, pigmentation, and type of mycelium, medium of growth and pace of growth. Microscopy included observing distinctive microscopic structures after staining with lactophenol blue and viewing under light microscope using 40x objective magnification (Alsohaili and Bani-Hasan, 2018). The isolates were identified using. Koneman's colour atlas and textbook of diagnotistic microbiology (Koneman *et al*., 1997; Disegha *et al.,* 2024) and were verified by using reverse image identification by yandex.com.

3.0 RESULTS

The result of total fungi counts of the control (uncontaminated soil) and polluted soil samples including the mean count (CFU/g) are shown in the Table 1. The samples analyzed include A, B, C, D, and E. Sample E served as the control.

In Week 1, the mean fungal count for each sample were as follows: Sample A recorded a mean count of 1.29 x 10^5 CFU/g, after estimation from daily mean counts. Sample B had 2.01 x 10^5 CFU/g, Sample C showed 3.1 x 10^5 CFU/g, Sample D reached 3.8 x 10^5 CFU/g, and Sample E (control) exhibited the highest count at 4.4 x 10^5 CFU/g. These results suggest that the control environment (Sample E), which was over 50 m away from the polluted site, was particularly conducive to fungal growth, while Sample A, which was at the immediate proximity of the pollution, demonstrated the lowest fungal presence.

In Week 2, all samples exhibited an increase in fungal counts compared to the previous week. Sample A rose to 1.7 x 10^5 CFU/g, Sample B increased to 1.92 x 10^5 CFU/g, Sample C reached 2.16 x 10^5 CFU/g, Sample D grew to 3.11 x 10^5 CFU/g, and Sample E (Control) showed a further increase to 4.4 x 10^5 CFU/g. This upward trend indicates favorable growth conditions for

fungi over the period of study.

In summary, the results indicate a general growth trend in fungal populations across all samples from within the period of study. Sample E, as the control, demonstrated the highest fungal counts, serving as a benchmark for optimal growth conditions. The other samples displayed lower counts, suggesting variations in environmental factors or treatments affecting fungal growth, which in this case, is due to pollution of the soil by diesel during dispensing activities.

Analysis of variance of fungal counts from polluted control soils in Figure 1 shows the fungal counts in different soil samples from diesel-polluted and control sites. The fungal counts are measured in log10 CFU/g (colony-forming units per gram). The key findings are that there was a high significant difference in fungal counts between the control and sample A. This is indicated by the "**" (highly significant) above the bars from Control and sample A. There were also significant differences (*) between fungal counts in Control soil and Sample B. Amongst the diesel-polluted samples, sample B had the highest fungal count. In general, the results suggest that diesel pollution has a significant impact on fungal populations in the soil. The specific effects may vary depending on the level of pollution and other inherent environmental factors.

Table 1: Mean Daily heterotrophic Fungal Counts (CFU/g) During the period of Sampling

Weeks	Sample A	Sample B	Sample C	Sample D	Sample E (Control)
Week 1	**1.3 x 10^5**	**2.1 x 10^5**	**3.0 x 10^5**	**3.8 x 10^5**	**4.0 x 10^5**
D1	1.43 x 10^5	2.32 x 10^5	3.01 x 10^5	3.76 x 10^5	3.4 x 10^5
D2	1.24 x 10^5	2.02 x 10^5	3.02 x 10^5	3.9 x 10^5	4.2 x 10^5
D3	1.32 x 10^5	2.10 x 10^5	3.1 x 10^5	3.7 x 10^5	3.8 x 10^5
D4	1.20 x 10^5	2.01 x 10^5	3.2 x 10^5	3.8 x 10^5	4.3 x 10^5
D5	1.34 x 10^5	2.1 x 10^5	3.2 x 10^5	3.8 x 10^5	4.3 x 10^5
Mean	**1.29 x 10^5**	**3.01 x 10^5**	**3.1 x 10^5**	**3.8 x 10^5**	**3.94 x 10^5**
Week 2	**1.7 x 10^5**	**1.9 x 10^5**	**2.1 x 10^5**	**3.0x 10^5**	**4.4x 10^5**
D1	1.7 x 10^5	1.9 x 10^5	1.85 x 10^5	3.02 x 10^5	4.2 x 10^5
D2	1.6 x 10^5	1.8 x 10^5	1.9 x 10^5	3.01 x 10^5	4.5 x 10^5
D3	1.8 x 10^5	2.0 x 10^5	2.2 x 10^5	3.3 x 10^5	4.4 x 10^5

D4	1.5×10^5	1.9×10^5	2.4×10^5	3.2×10^5	4.5×10^5
D5	1.9×10^5	2.0×10^5	2.5×10^5	3.1×10^5	4.45×10^5
Mean	**1.70×10^5**	**1.92×10^5**	**2.16×10^5**	**3.11×10^5**	**4.41×10^5**

Key: D1 -D5 = Day 1 to Day 5.

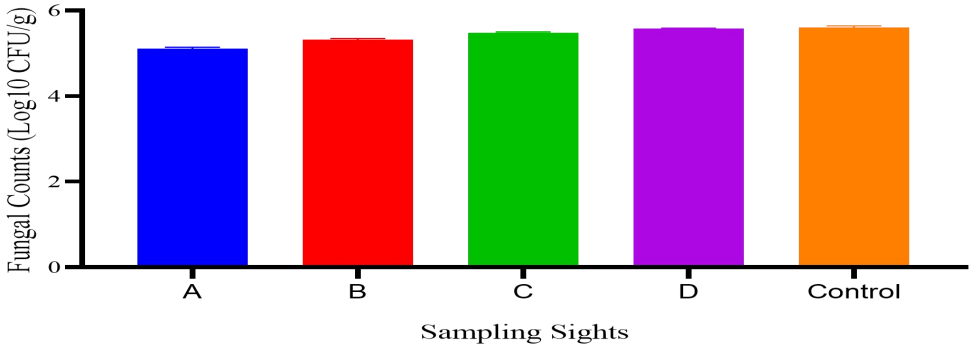

Fig. 1: Mean Fungal Counts of Soil Samples from Diesel Polluted and Control Sites in Week 1

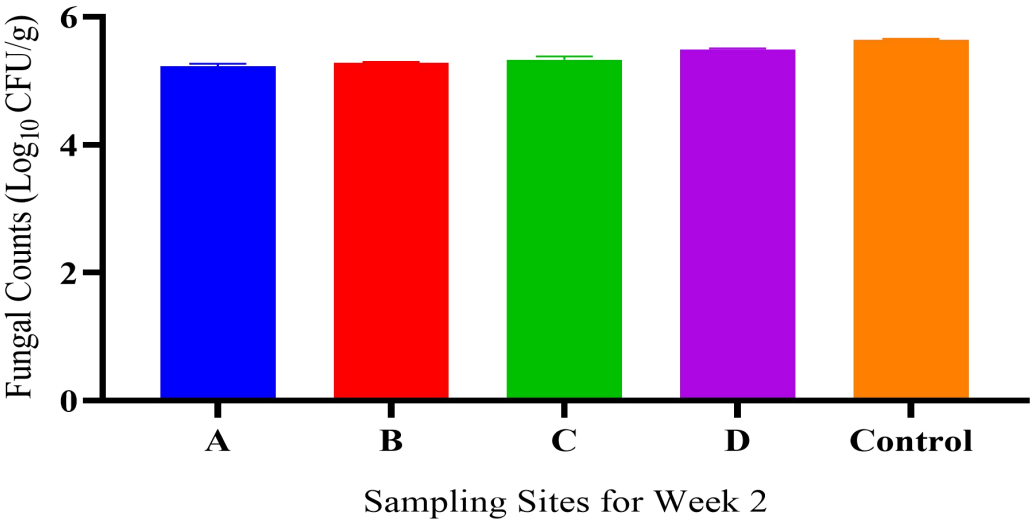

Fig. 2: Fungal Counts of Soil Samples from Diesel Polluted and Control Sites in Week 2

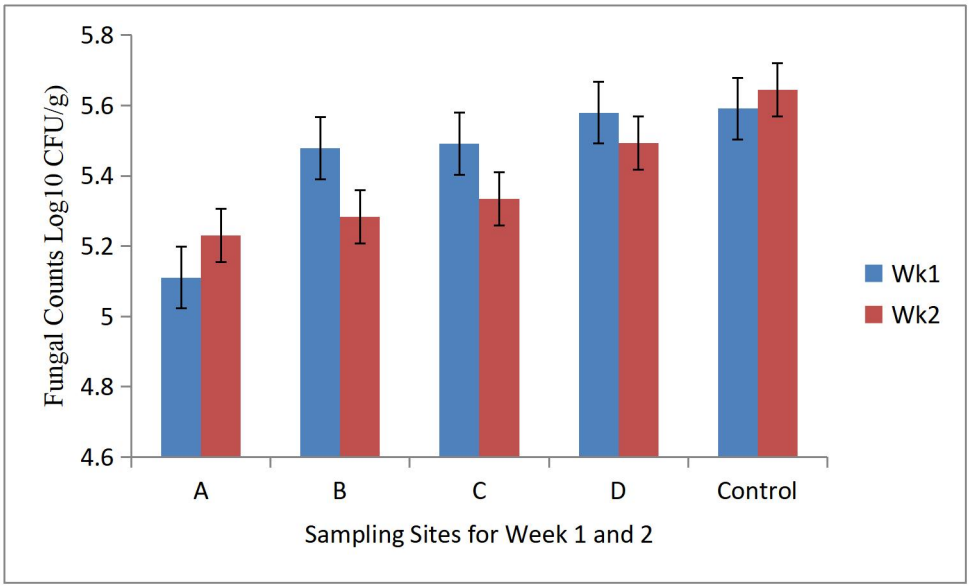

Fig. 3: Comparison of Fungal Counts of Soil Samples from Diesel Polluted and Control Sites in Week 1 and 2

3.1 Fungi isolated from the sampling location

Table 2 presents the characterization of various fungal isolates, detailing their cultural characteristics, microscopic or morphological features, and the probable organisms assigned to each isolate. A total of eight fungal isolates belonging to seven genera were characterized from the sampling locations. These isolates were identified based on their cultural characteristics, microscopic features, and comparison with known fungal species (Disegha *et al*., 2024). The identified fungi included:

Penicillium chrysogenum (known for penicillin production); *Aspergillus niger* (associated with food spoilage and industrial applications); *Rhizopus oligiosporus* (involved in fermentation processes); *Fusarium solani* (a plant pathogen); *Trichoderma* sp. (known for biocontrol properties); *Aspergillus* sp. (a broader category within the Aspergillus genus); *Alternaria* sp. (associated with plant diseases); *Pichia* sp. (involved in fermentation processes and biotechnological applications) (Guzmán-Chávez *et al*., 2018).

The characterization of these fungal isolates reveals a diverse range of morphological and cultural traits, which are crucial for their identification. Each isolate exhibits unique features that

not only aid in classification but also highlight their potential roles in various ecological and industrial contexts. Understanding these characteristics is essential for further research and application in fields such as agriculture, pharmaceuticals, and biotechnology (Alsohaili, and Bani-Hasan, 2018).).

Table 2: Characterization of fungi isolates

Isolate	**Cultural Characteristics**	**Microscopic/Morphological characteristics**	**Probable organism**
1	Green pigmentation with white background powdering surface circular in shape with elevated centre.	Condiosphores in septate, erect and branched, conidia, spores are brush like, hyphae is septate.	*Penicillium chrysogenum*
2	Dark brown colonies with mainly surface growth growing to cover plate.	Condiosphores in septate, erect and branched, condia, spores are brush like, hyphae is septate	*Aspergillus niger*
3	Pale brownish colony growing whitish when young becomes dark brown with age.	Black pigmented sporangium, sporangiophore is unbrancheed into short rhizoid, hyphae is non septate	*Rhizopus oligiosporus*
4	White sort and colony mass grow rapidly converting the surface of the petri dish	Shore present shaped conidiophores septate hypae, microconidia abundant.	*Fusarium solani*
5	Rough white colonies with dry surface and yeasty odour	Oval buddy cells	*Trichoderma sp*
6	Black mycelia with green lives at the cream colored reversed	Aseptate hyphae and branched conidiophore with vesicles that produce chains of conidia	*Aspergillus sp*

7	Grey-black pigmentation, soma is filamentous and septate hyphae	suede-like to floccose, acrop chains of multicellular conidia are produced sympodially from short elongate conidiophores. Conidia are obclavate	*Alternaira sp*
8	Yellow covex colonis with yeasty odour and rough edge and surface	Oval budding cells and few variable in shape	*Pichia sp*

3.2 Frequency of Occurrence of Fungi

Figure 4 shows the frequency of various fungal isolates found in diesel-contaminated soil, revealing a diverse range of species with differing prevalence.

The most common isolate was *Aspergillus niger*, recorded 25 times, suggesting its strong adaptability to the conditions created by diesel contamination. Close behind is *Penicillium chrysogenum*, with 24 occurrences, indicating it also thrives in this environment.

Another notable presence is *Aspergillus sp***.**, which appeared 15 times, further emphasizing the prominence of the Aspergillus genus in these soils. In contrast, *Rhizopus oligiosporus* and *Fusarium solani* were less frequently isolated, with 9 and 8 occurrences, respectively. *Trichosporium* sp**.** matches *Fusarium solani* with 8 occurrences, indicating a moderate level of adaptation to the diesel-affected conditions.

The least frequently isolated fungi are *Alternaria* sp**.** and *Pichia* sp**.**, with 7 and 4 occurrences, respectively. This suggests that while these species can survive in contaminated soils, they are not as prevalent as the more commonly identified fungi.

In summary, the findings underscore that certain fungal species, particularly those from the *Aspergillus* and *Penicillium* genera, are more dominant in diesel-contaminated soils, potentially playing crucial roles in biodegradation processes in these environments.

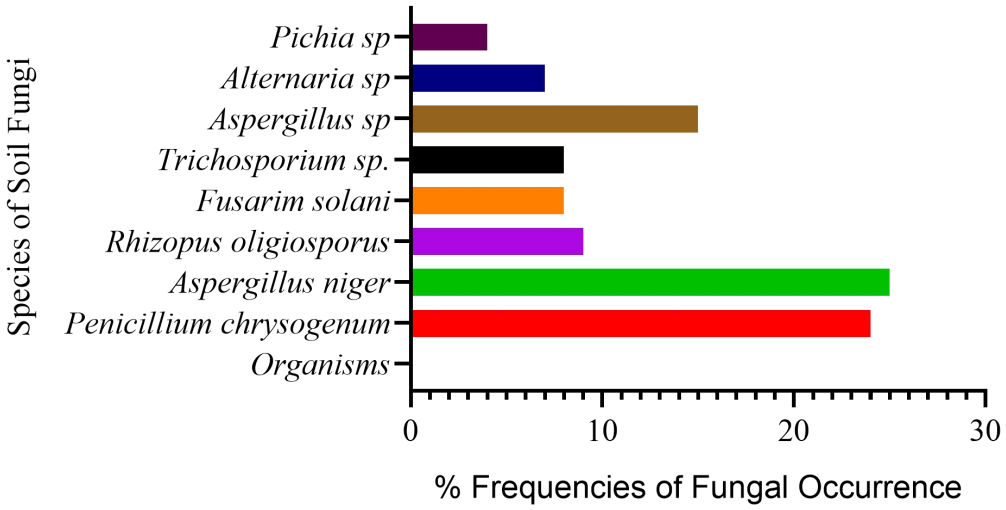

Fig. 4: Frequency of Occurrence of Fungi Isolates from Diesel Contaminated Soil

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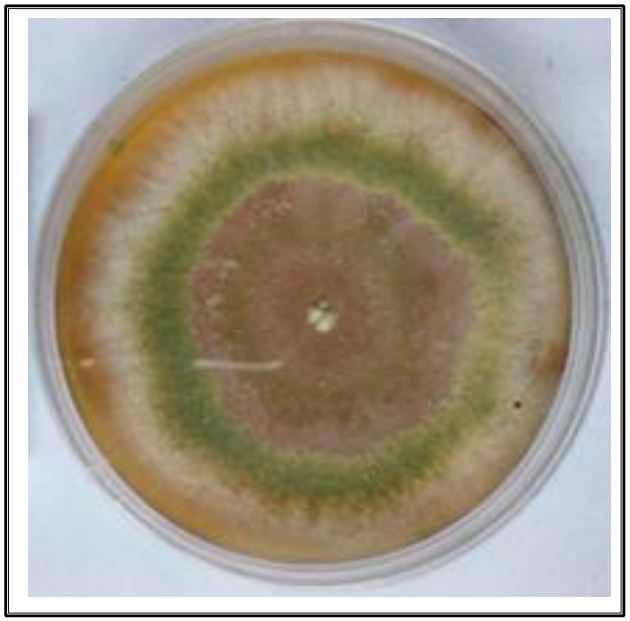
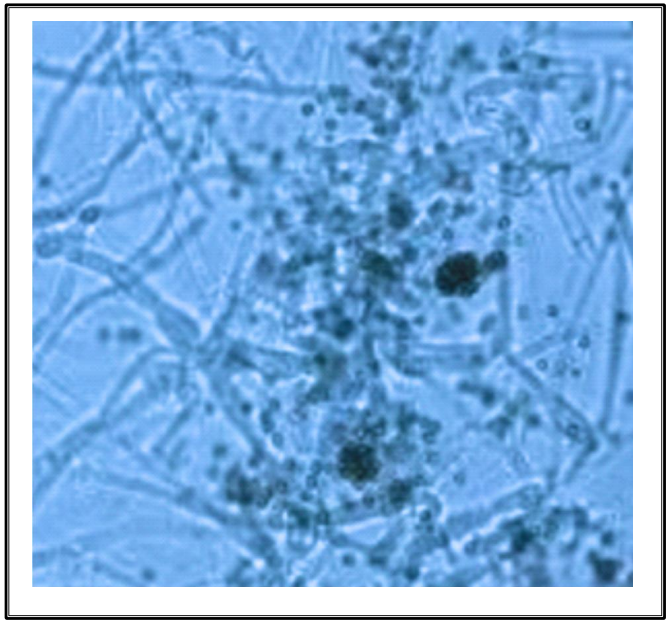


(a) Name: *Trichoderma viride*

(b) Reverse: *Trichoderma viride*



(b) Microscopoy: *Trichoderma viride*

Plate 4.1 : Macroscopic (a) Front (b) Reverse and (c) Microscopic Views of *Trichoderma viride*

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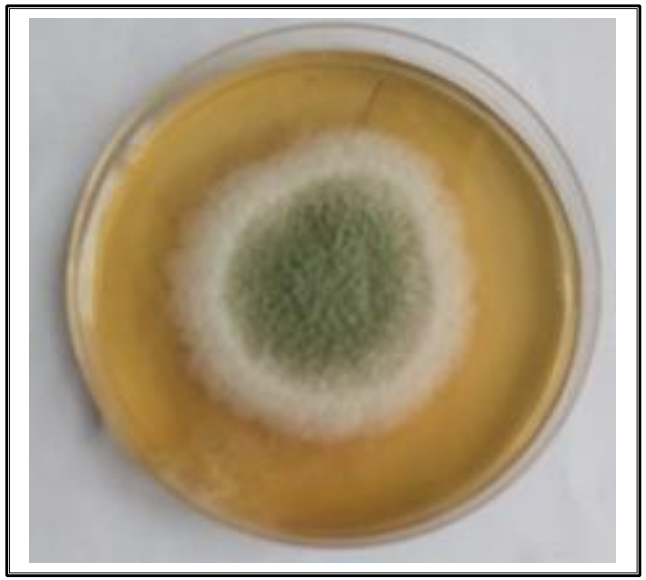
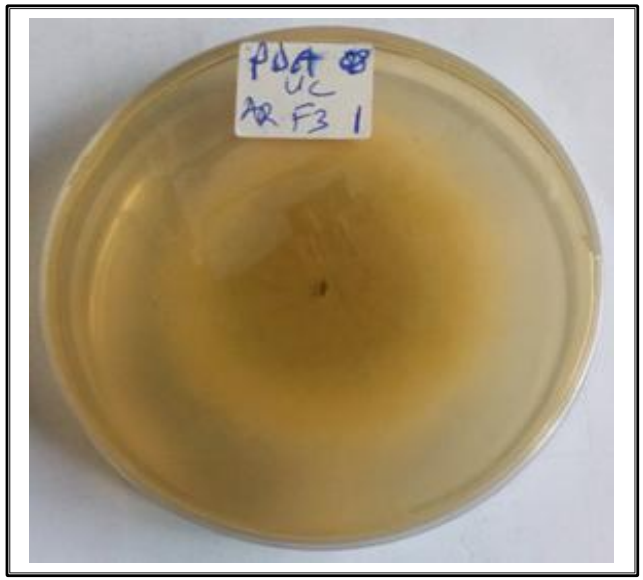
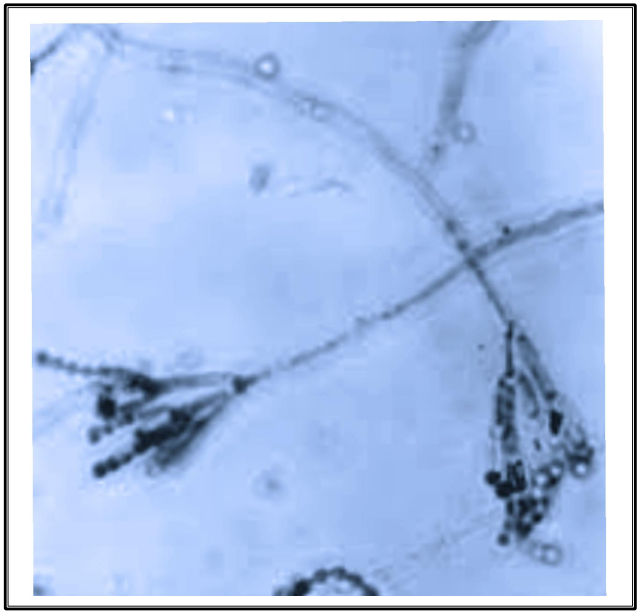


(a) Name: *Penicillum chrysogenum*



(b) Reverse: *Penicillum chrysogenum*



(c) Microscopy: *Penicillum chrysogenum* [x 40]

Plate 4.21: Macroscopic (a) Front (b) Reverse and (c) Microscopic Views of *Penicillum chrysogenum*

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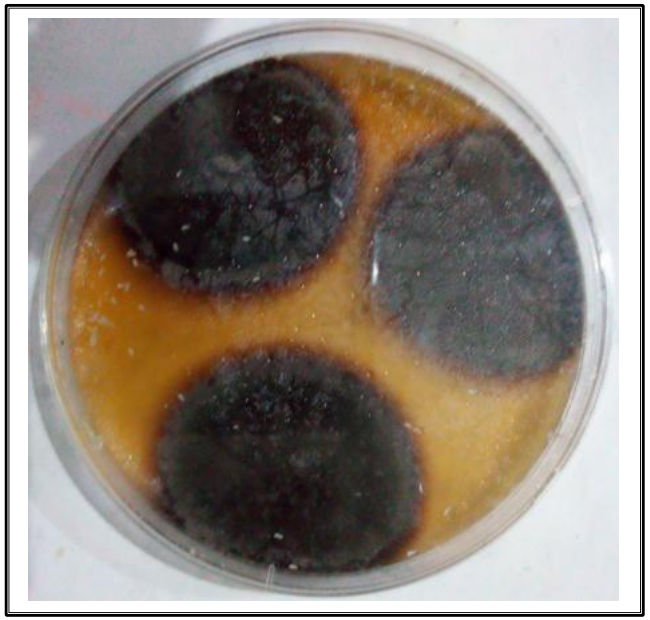
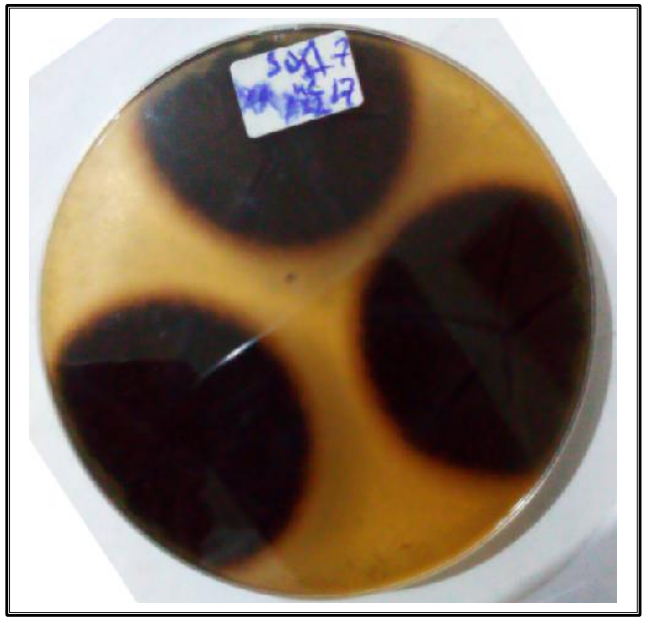
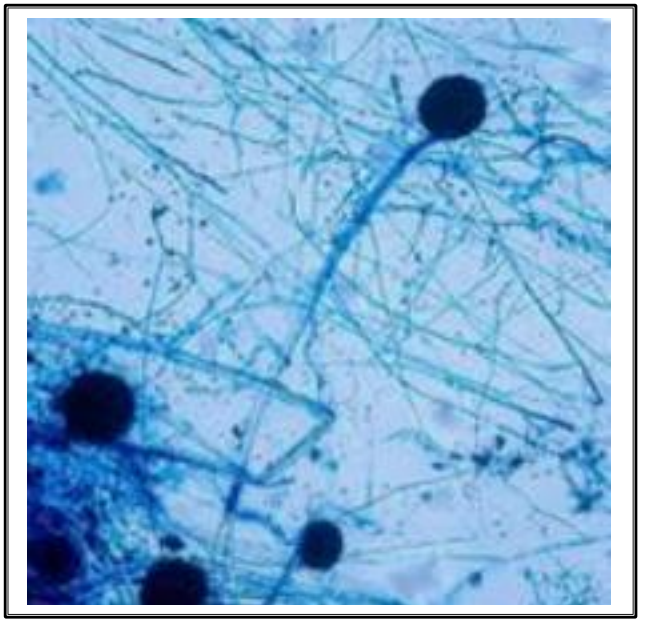


(a) Name: *Aspergillus niger*



(b) Reverse: *Aspergillus niger*



(c) Microscopic View: *Aspergillus niger* [x40]

Plate 4.14: Macroscopic (a) Front (b) Reverse and (c) Microscopic Views of *Aspergillus niger*

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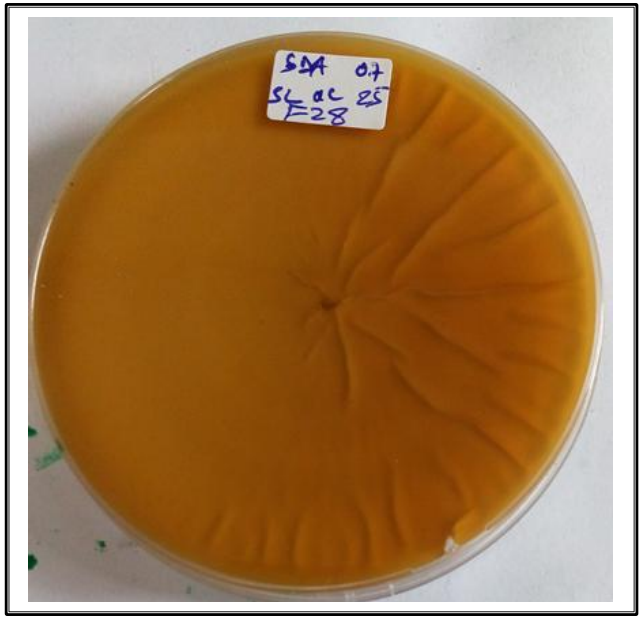
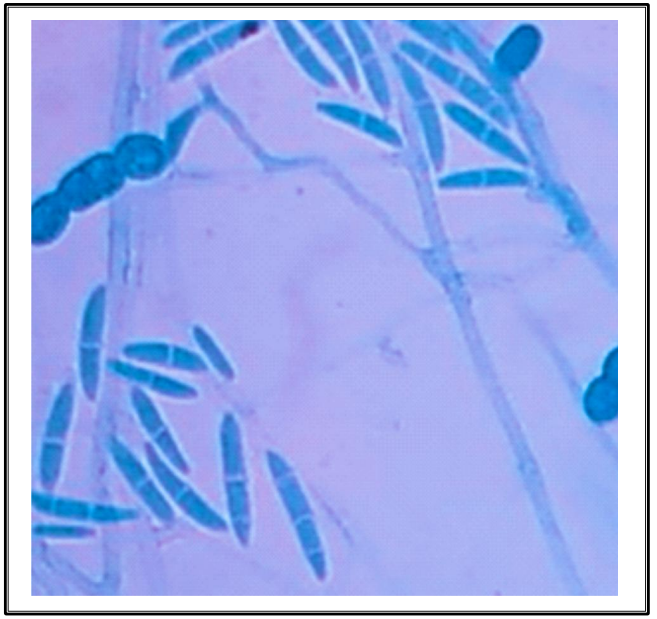
(a) Name: *Fusarium solani*



(b) Reverse: *Fusarium solani*



(c) Microscopy: *Fusarium solani* [x 40]

Plate 4. 9: Macroscopic (a) Front (b) Reverse and (c) Microscopic Views of *Fusarium solani*

4.0 DISCUSSION

In heavily polluted environments, immediate harmful effects are usually observed in biological forms, the effect of petroleum pollution depends on chemical composition of oil products and the species of microorganisms. The result from Table 1, the results shows that the uncontaminated (sample E) soil had the highest count of 4.0 x 10 5sfu/g and 4.4 x 105sfu/g for the 1st and 2nd weeks respectively and the lowest count was found in sample A having 1.3 x 10 5sfu/g and 1.7 x 10 5sfu/g, followed by sample B, sample C and D for the two weeks showing a decrease in fungal population due to the pollution and this showed that these results are in agreement with findings of Obire and Anyanwu (2009) that treated soil with different concentrations of crude oil and counted fungal populations within eighteen weeks. Fungal population was decreased in control soil (without oil), a primary reduction and then considerable increase were observed in high concentrations of oil. The toxicity of diesel oil or petroleum products varies widely, depending on their composition and concentration. Moreover, the scale of pollution depends on the amount of oil and the damage done to the environment.

An interesting observation in this study was a significant increase in oil-utilizing population in Figure 1, showing the frequency in comparison to heterotrophic population which was recognized to be *Penicillum chrysogenum, Aspergillus niger* and other *Aspergillus species,* this means that these organisms are the major population for diesel biodegration.

In studies conducted, population of fungi in all samples was more than diesel utilizing fungi. (Isitua, and Ibeh, 2010).

Although, in this study, the fungal population found in the uncontaminated soil was expected to be greater than oil-utilizing fungal population due to use of enrichment medium (SDA), opposite results were observed. This could be attributed to adaptation of oil-utilizing fungi to the amount of diesel oil in the environment and there by their increase in the region of polluted areas more than the unpolluted soil. The result of mycotic analysis of soil contaminated with diesel suggest that the diesel-utilizing fungi were adapted to the quantity of diesel oil in the environment, and thereby increased the number of diesel-utilizing fungi in polluted areas (Ordu and Disegha, 2024).

Obire and Anyanwu (2009) reported that reduction in diversity of species (fungal genus) with an increase in concentration of added crude oil was an index for environmental stresses of oil hydrocarbons. Nkwelang *et al* 2008, showed that the main active fungal genera of contaminated soils were *Aspergillus, Penicilium* and *Mucor.*

In this study eight (8) fungal isolates including *Penicillum, Aspergillus, Rhizopus, Fusarium, Trichoderma, Alternaria* were isolated from SDA media, with *Aspergilus* and *Penicillum* being the dominant genera as seen in Figure 1 ,showing the frequency of occurrence of organisms

found in the analysis. According to Chaillan *et al.* (2004), *Aspergillus* and *Penicillium* are the most commonly found fungi in tropical soils, which are able to degrade hydrocarbons. The difference in the fungi populations could be attributed to the possible change in nutrient and oxygen supply in the soil (Prenafeta-Boldú *et al.,* 2029), Carbon (nutrient) level of a given soil increases following every oil pollution which affects nitrogen level in the soil and other mineral elements which finally become limiting factor with time (Kuśmierz, 2023). There was general decrease in the fungi counts between the control samples and the polluted samples.

This suggested that fungi responded negatively to pollution of soil by diesel except the few genera of *Aspergilus* and *Penicillum.*

5.0 CONCLUSION

The study highlights the significant impact of diesel pollution on fungal populations in soil. Certain species, particularly from the *Aspergillus* and *Penicillium* genera, demonstrate a strong presence in contaminated soils, potentially contributing to biodegradation processes. A comparative analysis of the fungal count between control samples and those of the polluted soils samples showed reduction in the fungal populations of the polluted soils. Fungal populations in controls were generally higher which decreased with increase in the concentration of diesel. Understanding these dynamics is crucial for environmental management and bioremediation strategies in polluted ecosystems**.**

Therefore to mitigate the environmental impacts of oil production, it is recommended that oil companies, including NNPC, conduct intensive awareness campaigns in producing areas about the risks of pipeline failures. Agricultural soils should be protected from petroleum and its derivatives to ensure the health of beneficial fungi and microorganisms. Buried pipelines must be coated with materials like bitumen or high-density polyethylene sleeves and maintained regularly to reduce soil pollution. Surface pipelines should be avoided, with buried pipelines installed at depths of 1 to 4 meters. Additionally, a mandatory 25-year replacement period for all oil and gas pipelines should be enforced to prevent spills and contamination.

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