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Full Length Research Article

HYDROCARBON DEGRADATION POTENTIAL OF *ASPERGILLUS NIGER* AND *MICROCOCCUS* SPP. INDIVIDUALLY AND IN CONSORTIUM

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ABSTRACT
Oil pollution is a pervasive and significant global problem, with hexadecane ($C_{16}H_{34}$), decane ($C_{10}H_{22}$), and heptane (C_7H_{16}) as major components of petroleum-based fuels such as gasoline, kerosene, and diesel. Eight Fungal isolates identified as engine oil degraders were isolated across five mechanic workshops in Ilorin, Nigeria and identified using cultural and morphological characteristics. They include: *Aspergillus niger, Aspergillus brasiliensis, Trichophyton rubrum, Aspergillus tabacinus, Penicillium chrysogenum, Trichophyton terrestre, Rhizopus stolonifer* and *Fusarium oxysporium. Trichophyton rubrum*, which is a leading cause of dermatophytoses, had the highest prevalence (76%) across the five sites. *Aspergillus niger* exhibited the highest degradation ability, degrading hexadecane the most (from 0.207 to 0.424 OD600) and heptane the least (from 0.27 to 0.19 OD600) over 20 days. *Micrococcus* spp. previously isolated from air and *Aspergillus niger* isolated from engine oil-polluted mechanic workshop were utilized for degradation for 5 days; In consortium, *Aspergillus niger* and *Micrococcus* spp. hydrocarbon utilization rates ranged from 0.106 to 0.202 for decane, from 0.106 to 0.193 for heptane, and from 0.207 to 0.467 for hexadecane. *Micrococcus* spp., when used singly, had a higher degradation rate for the heptane-plus-petrol mixture (0.011 to 0.061) than Aspergillus niger in consortium, with a degradation rate ranging from 0.115 to 0.06. Optical density and pH measurements were taken to determine degradation rates. These findings show that *Aspergillus niger* and *Micrococcus* spp. can be used to enhance the biodegradation of oil-polluted soil, but might work much better separately than in a consortium.

Keywords: Biodegradation, Fungi, Bacteria, *Aspergillus niger, Micrococcus* spp.

INTRODUCTION

There has been a problem with hydrocarbon contamination of land and water, especially in developing countries, since the discovery of petroleum as an energy source (Enerijiofi *et al*., 2022; Murphy *et al*., 2022). Alkanes are the most abundant hydrocarbons in crude oil and are extensively used as fuels and solvents. (Wang *et al*., 2022; Zulkifli et al., 2022). Petroleum hydrocarbons are introduced into the environment via vehicle exhaust, refineries, refinery wastewater, treatment plants, and other chemical production processes (Lawan *et al*., 2022). One of these hydrocarbons is engine oil.

Engine oil contains both aliphatic and aromatic hydrocarbons that may form highly toxic compounds during use (Benguenab & Chibani, 2021). Yang *et al*. (2021) stated that one of the biggest sources of used engine oil is the automotive industry. During the servicing, repair, and cleaning of vehicle parts, waste generated in mechanic workshops is usually poorly disposed of. Mechanics usually dispose of or spill spent motor oils, lubricant oils, and other solvents that contain hydrocarbons from petroleum around their mechanic workshops. The major pollutants found in the soil of most mechanic workshops consist of waste lubricants (Stephen *et al.*, 2021).

Various physical and chemical techniques are used to remediate hydrocarbon-contaminated environments; however, some of these techniques are very expensive and may have detrimental effects on the environment (Sayed & Sharma, 2021). The most effective method for removing contaminants from the environment is bioremediation, which stimulates the growth of microorganisms that use contaminants such as oil, solvents, and pesticides as food and energy (Tyagi & Kumar, 2021). Petroleum hydrocarbons can damage the cell membranes of microbes non-specifically, causing changes in cell membrane fluidity, integrity and function and inhibiting certain metabolic functions. In Petroleum hydrocarbon-contaminated environments, only a portion of the community's microbes can adapt (Xu *et al*., 2022). Microorganisms in soils exposed to hydrocarbon pollution usually exhibit a higher potential for the biodegradation of these pollutants than those with no history of exposure (Jia *et al*., 2023).

Among the various agents used, bacteria are the most widely reported. (Meena et al., 2021). Zaman *et al*. (2025) isolated 5 bacterial strains from soil polluted with industrial effluent in a recent study; many service stations surrounded the industry. Of the 5 isolates, *Rhodococcus indonesiensis* strain SARSHI1 showed biodegradation efficiencies of 74–91% for medium to long-chain hydrocarbons.

Adetitun et al. (2024) isolated fungi and bacteria from the air at 5 filling stations in Tanke, Ilorin and examined them for their biosurfactant-producing ability; of the 14 bacterial isolates, Micrococcus luteus had an 8% occurrence. Microorganisms such as fungi are extremely diverse and can adapt to survive in inhospitable environments. The use of fungi for bioremediation ensures the complete degradation and mineralization of petroleum hydrocarbon contaminants into carbon dioxide, water, inorganic compounds, and cell biomass. (Mahmud *et al.*, 2022). Many

studies have shown the role of fungi in the biodegradation of various petroleum products. Fungal genera include *Rhizopus, Fusarium, Alternaria, Gliocladium, Aspergillus, Paecilomyces, Rhodotorula, Cladosporium, Cephalosporium, Pleurotus, Torulopsis, Penicillium* spp., *Talaromyces, Mucor* and *Geotrichum.* (Gaur *et al*., 2022; Tomilayo *et al*., 2024).

This study aimed to isolate and characterize microorganisms from distinct environmental sources—fungi from engine oil–polluted soil and utilize already isolated bacteria from air— and to evaluate their potential in degrading selected hydrocarbons (heptane, decane, hexadecane) and petrol, singly and in consortium (dual setup) with a view to assessing their applicability in hydrocarbon bioremediation.

MATERIALS AND METHODS

Sampling Area

Engine oil-contaminated soil samples were collected from five mechanic workshops (Stella Maris, Total, Winners Chapel, Lite Gas & Oke-odo) in Tanke, Ilorin, Kwara State over a 5-week period. The soil sample was taken from the 0-10 cm depth at the site using a sterile spatula and placed in a sterile polythene bag (Nwankwegu et al., 2016).

Sterilization of materials:

All media, along with Petri dishes, pipette tips and glassware, were sterilised in an autoclave at 121^{o} C for 15 minutes. The inoculating loop was flamed and heated before and after use; the laboratory bench was disinfected using 70% ethanol. Pouring media into plates and other microbiological processes were carried out aseptically. (Fawole and Oso, 2007).

Preparation of culture media: Culture media were prepared in accordance with the manufacturer's directions.

Isolation of fungi from engine-oil-contaminated soil.

One gram of soil sample from each soil was serially diluted in a sterile test tube containing 9ml of sterile distilled water and mixed thoroughly using a sterile pipette; An aliquot of 0.1 ml of 10^{-4} was transferred to an empty Petri dish, then the potato dextrose agar containing 0.1% streptomycin was added by using the pour plate method. The plates were incubated at 25$\pm$2^{0}C for 3 to 5 days. The mixed culture obtained was repeatedly subcultured to obtain a pure culture and characterized by morphological identification. The isolates were identified based on their macroscopic and microscopic characteristics, as reported by Fawole & Oso (2007).

Bacteria

Micrococcus spp. already isolated from air was subcultured on nutrient agar and inoculated into sterile nutrient broth at 37 °C for 24hours.

Biodegradation Tests

Mineral salt medium, as described by Vecchioli et al. (1990), with a composition of $MgSO_4 \cdot 7H_2O$ (0.2g), KNO_3 (0.3g), KH_2PO_4 (0.5g), Na_2HPO_4 (1.4g), $(NH_4)_2SO_4$ (1.0g), and Distilled water (1 L), was used. 20 mL of sterile MSM was aseptically transferred into sterile reaction bottles. All experiments were performed in duplicate:

Aspergillus niger* Biodegradation Tests:* Eighteen sterilized reaction bottles containing 20ml sterile MSM were set up for heptane; 29 µl of heptane was added to each bottle; 6 bottles served as controls (without microorganism), and 2 ml of Aspergillus niger broth was added to 12 of the reaction bottles. Readings were taken on days 1, 4, 8, 12, 14 and 20. This procedure was also repeated for decane (27 µl) and hexadecane (30 µl). (Zampolli *et al.,* 2014).

***Aspergillus niger* and *Micrococcus* spp. Biodegradation Tests:** 15 sterilized reaction bottles containing 20ml of sterile MSM were set up with (29 µl) heptane, (27 µl) decane, and (30 µl) hexadecane, respectively; 5 bottles for each hydrocarbon served as controls. To the 10 reaction bottles containing each hydrocarbon and MSM, 2 ml of Aspergillus niger and 2 ml of Micrococcus spp. were added, respectively. Readings were taken on days 1, 2, 3, 4 and 5.

***Aspergillus niger* and *Micrococcus* spp in Heptane and Petrol:** 15 sterilized reaction bottles containing 20ml of sterile MSM, 29 µl of heptane, and 1% petrol mixture were set up; 2ml broth of *Aspergillus niger* and *Micrococcus* spp was respectively added into 10 reaction bottles, while the 5 remaining reaction bottles served as the control (without microorganism). Each reading was taken for 5 consecutive days.

The degradative potential of the isolates was assessed by measuring their optical density at 600nm with a spectrophotometer and changes in pH. The spectrophotometer used was JENWAY-6320D.

pH Measurement: The pH meter used was the SUNTEX pH meter SP-701, calibrated with a pH 7.0 buffer before use. Readings from the pH meter were noted and recorded accordingly.

RESULTS

Eight fungal isolates were isolated and identified: Aspergillus niger, Aspergillus brasiliensis, Trichophyton rubrum, Aspergillus tabacinus, Penicillium chrysogenum, Trichophyton terrestre, Rhizopus stolonifer and Fusarium *oxysporium.* The occurrence of fungal isolates in each sampling site is shown in Table 1.

The occurrence of the fungal isolates across the sampling period showed that *Trichophyton rubrum* had the highest frequency, while Trichophyton terrestre had the lowest. Table 2 shows the total fungal count for each sample site, with 'Total' having the highest. The positive (+) result indicates the presence of the organism, while the negative (-) result indicates its absence at the sampling site.

Figure 1 shows the growth profile of *Aspergillus niger* on heptane, decane and hexadecane, which exhibit distinct lag, exponential and stationary phases on each hydrocarbon substrate. Biomass accumulation was highest on hexadecane, indicating superior utilization of the long-chain alkane relative to the shorter-chain heptane and decane. These trends suggest substrate chain length influences both the rate and extent of fungal growth on aliphatic hydrocarbons.

Figure 2 depicts the growth configurations of Aspergillus niger and Micrococcus spp. on heptane, decane and hexadecane, revealing species-specific patterns of hydrocarbon utilization across the

three substrates. *A. niger* consistently achieved higher optical density than *Micrococcus* spp., particularly on hexadecane. At the same time, the bacterium showed relatively better performance on heptane. This differential response highlights contrasting metabolic capabilities and possible complementary roles in mixed hydrocarbon environments.

Figure 3 graphically illustrates the mixtures of heptane and gasoline, as well as *Aspergillus niger* and *Micrococcus* spp. Exhibited modified growth configurations compared with growth on single alkanes. The presence of gasoline altered the growth kinetics, with *A. niger* maintaining robust growth while *Micrococcus* spp. Displayed reduced biomass yield at higher gasoline proportions. These results indicate that complex hydrocarbon mixtures modulate microbial proliferation, likely through combined effects of toxicity and substrate availability.

Figure 4 shows the pH profiles during the growth of Aspergillus niger and Micrococcus spp. on heptane–gasoline mixtures, which show progressive acidification of the medium over time. *A. niger* caused a more pronounced drop in pH, consistent with organic acid production during hydrocarbon metabolism, whereas *Micrococcus* spp. Induced a comparatively smaller pH change. The observed pH dynamics reflect differences in metabolic pathways and may influence hydrocarbon bioavailability and degradation efficiency in mixed cultures.

Table 1: Occurrence of Fungi Isolate in Each Sampling Site

Fungal Isolate	Sampling Area	Weeks 1	2	3	4	5	Percentage Occurrence(%)
	Stella maris	-	-	-	-	+	20
Asperigillus	Total	+	+	-	+	-	60
niger	Chapel	+	+	+	-	-	60
	Lite gas	-	-	+	-	-	20
	Oke-odo	-	-	+	-	-	40
	Stella maris	-	+	+	+	+	80
Asperigillus	Total	+	-	-	+	-	40
brasiliensis	Chapel	+	-	+	-	-	40
	Lite gas	-	+	+	-	+	60
	Oke-odo	-	+	+	+	+	80
	Stella maris	+	+	+	+	+	100
Trichophyton	Total	+	+	-	+	-	60
rubrum	Chapel	+	+	+	-	-	60
	Lite gas	+	+	+	-	+	80
	Oke-odo	+	+	+	+	-	80
	Stella maris	+	+	+	+	+	100
Asperigillus	Total	+	-	-	+	-	40
tabacinus	Chapel	-	-	-	-	+	20
	Lite gas	+	+	+	-	-	60
	Oke-odo	-	-	-	-	-	0
	Stella maris	+	+	+	+	-	80
Penicillium	Total	+	-	-	+	-	40
chrysogenum	Chapel	+	-	+	-	-	0
	Lite gas	+	+	+	-	+	60
	Oke-odo	-	-	-	-	-	0
	Stella maris	+	-	-	-	-	20
Trichophyton	Total	-	-	-	-	-	0
terrestre	Chapel	-	-	-	-	-	0
	Lite gas	-	-	-	+	-	20
	Oke-odo	-	-	-	-	-	0
	Stella maris	-	-	-	+	+	40
Rhizopus	Total	-	-	-	+	+	20
stolonifer	Chapel	-	+	-	+	+	60
	Lite gas	-	-	-	-	-	0
	Oke-odo	-	-	-	-	-	0
	Stella maris	+	+	+	+	+	100
Fusarium	Total	+	-	-	+	-	40
oxysporium	Chapel	+	+	+	-	-	60
	Lite gas	+	+	+	-	-	60
	Oke-odo	+	-	-	-	-	20

Keys: + = Present , - = Absent

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Table 2: Total Fungal Count In Each Sample Site

S/N	Sampling Area	Week 1 (10^4CFU/g)	Week 2 (10^4 CFU/g)	Week 3 (10^4 CFU/g)	Week 4 (10^4 CFU/g)	Week 5 (10^4 CFU/g)
1	**Stella Marris**	16	2	7	19	5
2	**Total**	47	0	12	35	0
3	**Winners Chapel**	55	5	10	16	0
4	**Lite gas**	3	2	15	6	2
5	**Oke-odo**	5	1	4	27	10

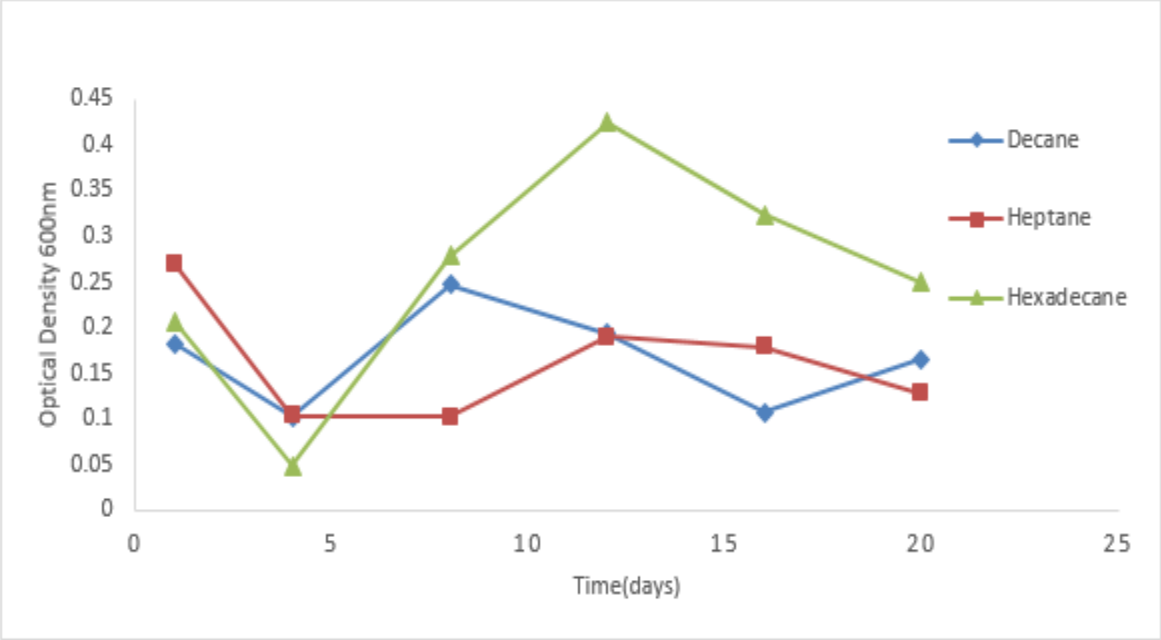

Figure 1: Growth profile of *Aspergillus niger* on heptane, decane and hexadecane.

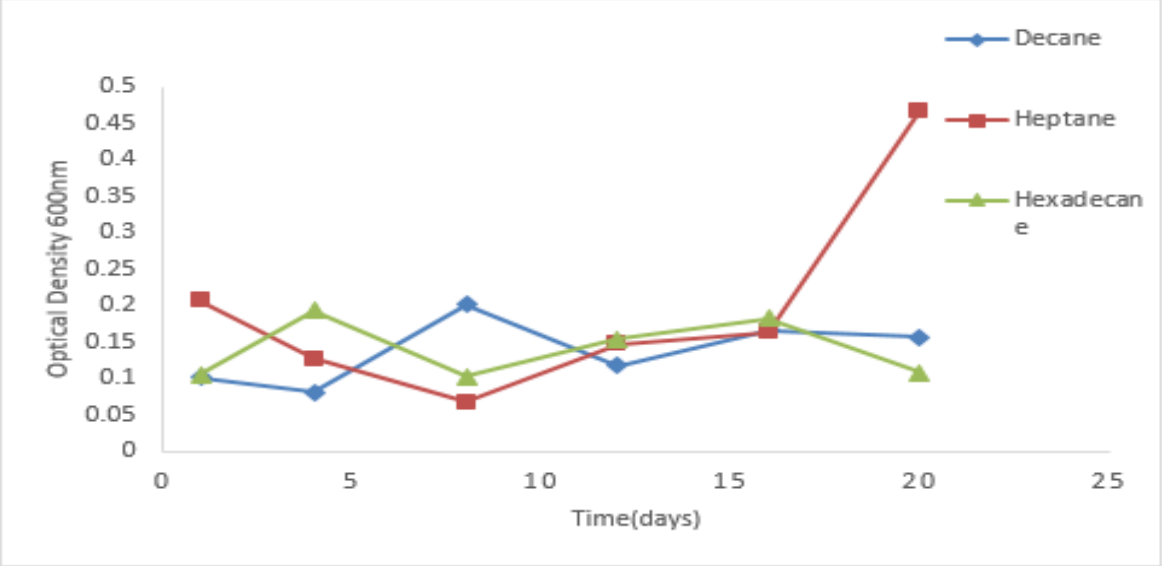

Figure 2: Growth configuration of *Aspergillus niger* and *Micrococcus* spp. on heptane, decane and hexadecane, respectively.

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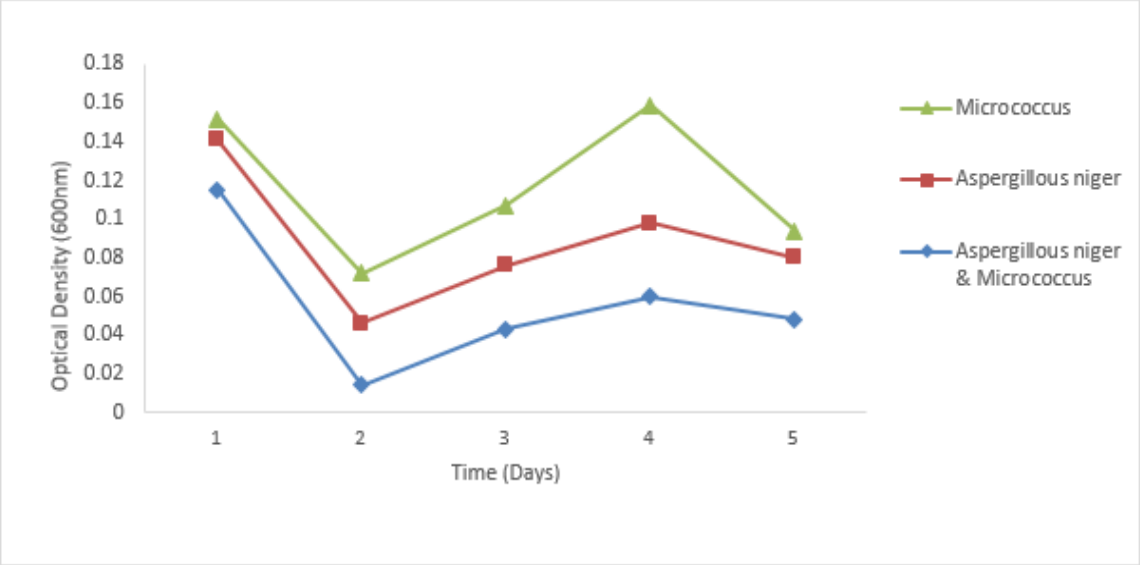

Figure 3: Growth configuration of *Aspergillus niger* and *Micrococcus* spp. on a heptane-gasoline mixture

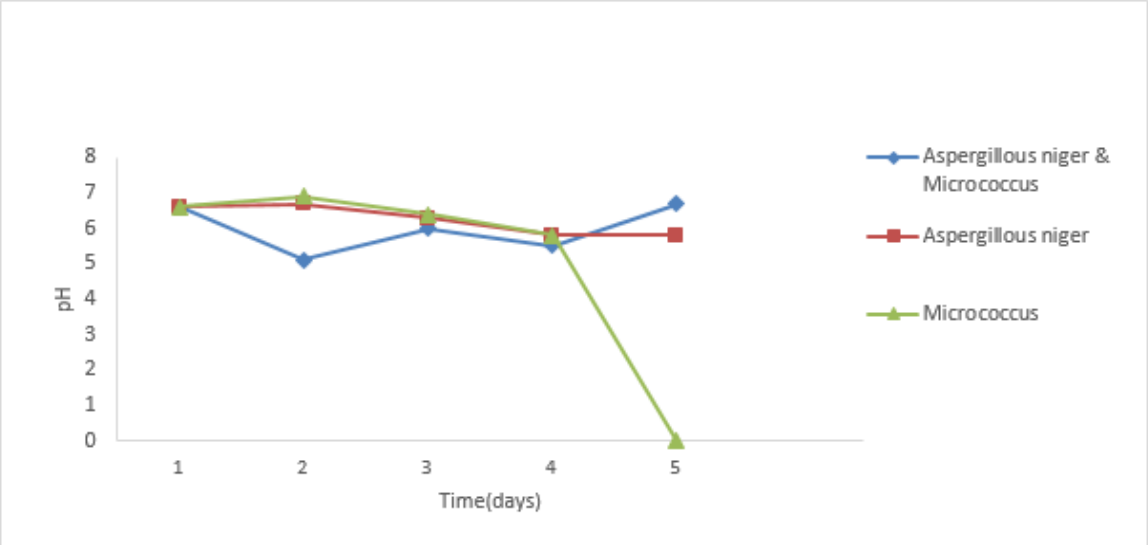

Figure 4: Growth configuration of *Aspergillus niger* and *Micrococcus* spp. on a mixture of heptane and gasoline (pH level)

DISCUSSION

Trichophyton rubrum is a cosmopolitan, anthropophilic fungus, meaning its primary reservoir is humans. It is the most widespread dermatophyte infecting humans (Burstein *et al*., 2020). The ability of *T. rubrum* to evade host immune defenses contributes to its high prevalence and persistence in infections (Dahl & Grando, 1994). According to Moskaluk & VandeWoude (2022), *T. rubrum* is the leading causative agent of dermatophytoses, commonly affecting various body sites such as the face, groin, feet, toes, palms, and chest.

Ha *et al.* (2025), in a large retrospective study of 38,391 cases (2014–2023), reported a male predominance of 68.45% in *Trichophyton rubrum* infections, resulting in a male-to-female ratio of 2.3:1. This trend was attributed to occupational exposure, where workers are often in moist and warm environments conducive to fungal proliferation. *T. rubrum* has consistently remained the predominant species among dermatophyte infections throughout the study period.

Although *T. rubrum* has been frequently isolated from soil environments, there is limited evidence that it actively forms spores or conidia under natural soil conditions. Jain and Sharma (2012) isolated *T. rubrum* (3.64% occurrence) along with 20 other fungal species from soil in Madras City, India, confirming its potential for environmental isolation. Similarly, Ronaldo *et al.* (2020) collected samples from public day-care center sandboxes in Cuiabá, Brazil, isolating 24 dermatophyte species, among which *T. rubrum* ranked second in prevalence (31.1%).

In a more recent study, Jain and Sharma (2021) examined the survival of seven frequently reported dermatophytes in sterilized and unsterilized soils under varying conditions in Jaipur District,

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India. *T. rubrum* survived for up to two years on Sabouraud Dextrose Agar, with experiments conducted every four months using bait testing and direct soil sprinkling. The study demonstrated that dermatophytes and other keratinophilic fungi can survive and proliferate in unsterilised soils, with *T. rubrum* showing the second-longest survival period. Similarly, Deshmukh and Verekar (2014) reported that keratinophilic fungi can survive in soil for up to 7 years.

Although hydrocarbon-degrading microorganisms dominate the mechanic workshop environment, *T. rubrum* may be unable to persist for extended periods in such conditions. Nevertheless, its isolation indicates that the workshop environment—particularly floors and shared spaces—may be contaminated with spores shed by infected individuals. These spores can remain viable and serve as a source of infection. Therefore, preventive measures should prioritize minimizing transmission and decontaminating surfaces and shared materials (CDC, 2020).

The mechanic workshop at Total had the highest fungal count across the five-week sampling period, possibly due to higher hydrocarbon pollution and frequent movement of workers, tools and vehicles, which aid spore dispersal, as this location is close to the roadside. (Atagana, 2011; Pitkaranta *et al*., 2008; Shelton *et al*., 2002).

Muhammed *et al. (2023) isolated Aspergillus spp. and reported it as the most common genus among the 10 isolates from crude oil-polluted* soil. Edna *et al.* (2016) also sampled from five automobile mechanic workshops and a farmland in Anambra State, Nigeria, and isolated five fungal strains (including Aspergillus niger) identified as engine-oil degraders.

All isolates were tested for hydrocarbon-degrading ability, and Aspergillus niger showed the highest capacity (Figure 1). Similar to Wemedo *et al.*, (2018), where *Aspergillus niger* was isolated from soil sample of oil spilled site in Rivers state Nigeria and used singly and also in consortium with other fungal isolate (*Rhizopus arrhizus*) for an incubation period of 28 days; *A. niger* had higher degrading capacity with a change in percentage degradation of 29.10% (Total Petroleum Hydrocarbon reduced from an initial 74.80mg/l to 53.03mg/l), in comparison with other isolate, which had 26.32% (TPH; 74.80mg/l to 55.11mg/l). Obire *et al*. also isolated 3 fungi from soil enriched with oilfield wastewater at various concentrations (10%, 25%, 50%, and 75%) and, compared with the other 2 isolates, *A. niger* recorded a higher PAH removal percentage of 73.4%.

From Figure 1, the hexadecane setup was more utilized for growth, which could be attributed to bioavailability. The bioavailable part of the hydrocarbons is the area accessible to microorganisms. (Al-Hawash *et al*., 2018). Chikere *et al*. (2011), in a review on monitoring microbial hydrocarbon remediation, explained that the actual chain length of hydrocarbons is of decisive importance.

Lower-molecular-weight alkanes are easily volatilized, while mid-length (C14–C20) alkanes are nonpolar, virtually water-insoluble hydrocarbons. Short-chain hydrocarbons (such as hexane and decane) have very high vapor pressures, so they readily move from the liquid phase into the headspace (the airspace above the liquid). Once there, they stay mostly in the gas phase and are less available to microorganisms in the aqueous or oil phase. Hence, they are effectively removed from microbial access, leading to a lower apparent substrate concentration, thereby reducing growth and optical density. Volke-Sepúlveda *et al. (2003) also reported that Aspergillus niger exhibited a very high degradation rate of* hexadecane.

There is limited data on the combined use of *Aspergillus niger* and *Micrococcus* spp. in hydrocarbon degradation. However, Stephen *et al*. (2016) reported that both isolates possess strong hydrocarbon-degrading abilities when studied individually. According to Frey-Klett *et al*. (2011), interactions between fungi and bacteria within a consortium can be either synergistic or antagonistic, influencing their growth dynamics and overall biodegradation performance.

As shown in Figures 2 and 3, *A. niger* and *Micrococcus* spp. exhibited lower growth rates and optical density when co-cultured than when grown individually, even across different hydrocarbon substrates. The observed optical density reflects substrate utilization, though at a reduced rate, suggesting possible antagonistic interactions within the consortium.

Wikes *et al*. (2016) demonstrated that Penicillium spp., Aspergillus spp., and Rhizopus spp. were capable of degrading hydrocarbons, especially when single cultures were used (Okerentugba & Ezeronye, 2003), which could explain why Aspergillus niger in Figure 3 recorded a higher optical density.

The mixed culture (*Aspergillus niger* & *Micrococcus* spp. in Figure 3) did not outperform the single cultures; instead, it showed the steepest early decline, suggesting possible competition or antagonism; this could also be due to the hydrocarbon mixture. *Micrococcus* spp. alone showed a small mid-phase recovery, which might mean it could metabolize some intermediate products that *Aspergillus niger* could not. This can be inferred from Moyes *et al*. (2014), in which Micrococcus luteus was among the bacterial isolates tested against fungal pathogens (though not Aspergillus niger) and showed some antagonistic activity, suggesting that Micrococcus spp. have the capacity to inhibit fungi under some conditions. Rao *et al*. (2017) also screened various bacterial strains (e.g., *Pseudomonas aeruginosa, Bacillus cereus, E. coli,* etc.) for antifungal activity against *Aspergillus niger,* among others.

The *Aspergillus–Micrococcus* co-culture showed significantly lower pH (Figure 4) than either monoculture between days 2–4, indicating an interaction that promotes net acidification of the medium. This suggests antagonistic or metabolic synergism altering extracellular metabolism in co-culture. *Aspergillus* has been reported to have a high degradation rate at pH 5.5-5.8 (Andersen et al., 2009), whereas Micrococcus spp. have an optimal pH of 7.0, with less significant activity at pH 5 (Jegan, 2010).

The major factors that alter pH are metabolic activity and by-product accumulation (Madigan *et al.*, 2021). *Micrococcus* tends to alkalinize the medium during longer incubation (via ammonia or base production from protein/amino acid metabolism). According to Ratzke & Gore (2018), microbial growth often leads to dramatic pH changes (acidification or alkalinization), and these feedbacks influence microbial competition and coexistence. This theory is supported by Dharanishanthi et al. (2021), who show that many bacteria, when grown on certain substrates, tend to increase pH (alkalinize) by releasing alkaline metabolic by-products.

In co-culture, the pH remains moderate — evidence of interaction/antagonism, where metabolic products from one organism partly neutralize the other's effect, as shown in the results in Figure 4.

Conclusion

This study demonstrates that *Aspergillus niger* exhibits superior hydrocarbon degradation capacity compared to *Micrococcus* spp. and their consortium, particularly on hexadecane. Co-culture revealed antagonistic interactions that reduced growth and altered pH dynamics. While *T. rubrum* isolation indicates environmental contamination, *A. niger* remains a promising single-agent bioremediation candidate for hydrocarbon-polluted mechanic workshop environments.

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