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Original Research Article

Optimisation of Crude Oil Degradation by Hydrocarbon-degrading Fungal Species Isolated from Mechanic Workshop Site in Anyigba, Kogi State, Nigeria

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Abstract

Purpose*:* This study optimised crude oil degradation using fungal species (*Trichoderma spp., Aspergillus spp., Penicillium spp*.) isolated from mechanic workshop soils in Anyigba, Nigeria. Mechanic workshops are hotspots for oil pollution, yet indigenous fungal adaptability remains understudied.
Methods*:* Degradation efficiency under varying pH (4, 7, 10) and heavy metals ($CuSO_4$, $HgCl_2$) via gravimetric analysis was evaluated.
Results*:* Trichoderma spp. showed peak degradation (15.48%) at pH 4, consistent with optimal fungal enzyme activity in acidic conditions, while alkaline pH (10) reduced efficiency to <1.3%. Notably, $HgCl_2$ enhanced degradation (*Trichoderma spp*.: 26.8%; *Penicillium spp*.: 22.35%), suggesting adaptive metal tolerance from chronic soil contamination. $CuSO_4$ had minimal impact (<8.3%). Though degradation rates were moderate (<30%), the results highlight the potential of indigenous fungi, especially *Trichoderma spp*., for bioremediation in acidic or $HgCl_2$ - amended environments.
Conclusion: The study demonstrates that the degradation efficiency of indigenous fungi is highly dependent on environmental parameters. *Trichoderma spp*. emerged as the most promising fungus, showing not only a preference for acidic conditions but also a potentially stimulatory response to mercury, suggesting prior adaptation. While the absolute degradation rates were modest, the significant influence of pH and heavy metals underscores the critical importance of environmental optimization.

Keywords: Bioremediation, fungal degradation, pH optimization, heavy metals, hydrocarbon pollution

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INTRODUCTION

Crude oil pollution remains a pervasive environmental challenge, particularly in regions with intensive petroleum-related activities. While instrumental for economic development, oil exploration, processing, and distribution often lead to accidental spills and chronic contamination of terrestrial and aquatic ecosystems.[1,2] In urban and peri-urban settings, mechanic workshops represent significant point sources of hydrocarbon pollution due to the routine handling and spillage of petroleum products like engine oil and lubricants. These incidents alter soil physicochemical properties, impair ecosystem functionality, and pose substantial risks to human health.[3,4]

Conventional physico-chemical remediation methods are often prohibitively expensive, energy-intensive, and can

themselves be environmentally disruptive. Consequently, bioremediation—the use of microorganisms to detoxify pollutants—has emerged as a sustainable, cost-effective, and ecologically sound alternative.[5] Among bioremediating agents, fungi have garnered significant attention for their remarkable metabolic versatility and powerful enzymatic machinery, including lignin peroxidases, laccases, and cytochrome P450 monooxygenases, which enable them to degrade complex and recalcitrant hydrocarbons.[6,7] Mixed fungal cultures have demonstrated high degradation efficiencies, exceeding 70% for crude oil within short time frames, underscoring their potential for field application.[8,14] Despite this potential, the effectiveness of fungal bioremediation is highly dependent on a multitude of environmental factors, such as pH, nutrient availability, and the presence of co-contaminants like heavy metals. Isolating and optimizing indigenous fungal species, which are naturally adapted to local conditions and pollutant profiles, is therefore a critical step toward developing effective bioremediation strategies.[9,13]

In Nigeria, mechanic workshops are a common feature of the landscape, and sites in Anyigba, Kogi State, are notably impacted by chronic crude oil contamination. However, the potential of native fungal microbiomes in these soils for bioremediation remains largely unexplored and unoptimized.

This study addressed this knowledge gap by isolating, identifying, and optimizing indigenous hydrocarbon-degrading fungal species from crude oil-contaminated soils in mechanic workshops in Anyigba. We specifically evaluated the crude oil degradation potential of the isolated fungi and systematically optimized key environmental parameters—pH and heavy metal stress—to enhance their biodegradation efficiency. Our findings provide crucial insights into the development of a tailored, effective, and sustainable fungal-based bioremediation protocol for managing hydrocarbon pollution in similar environments.

MATERIALS AND METHODS

Area of Study

The research work was carried out in Anyigba at Prince Abubakar Audu University, which is located in Dekina Local Government Area of Kogi State. It covers a total land area of 29.833km^2, an area ranked, 13th of 36 and is located between latitudes 7°30'North and longitude 6°42'East.

Collection of soil sample

Soil samples were collected at a depth of 10cm with auger from a mechanic workshop located behind the University gate at Anyigba in a sterile polyethene bag and taken to the laboratory.

Enrichment and isolation of fungi

Mineral Salt Media (MSM) used contained – 1.25g of $NaHPO_2$, 0.29g of KCl, 10.0g of NaCl, 0.42g of $NaNO_3$,0.83g of KH_2PO_4 ,0.42g of MgSO4.7H2O and 5.0g of Agar dissolved in 1000ml of distilled water.10 150ml of MSM, 14g of contaminated soil and 10ml of Used Engine Oil (UEO) were added in a conical flask and shaken for 7 days in order to enrich the growth of hydrocarbon-degrading fungi present in the soil sample. After 7 days, enriched sample was serially diluted. 0.1mL from dilutions 10-1, 10-3, 10-5 were plated on three MSM agar plates. The plates were incubated at 28°C for 5–7 days. [11] The three colonies found on the three MSM plates were sub-cultured into three MSM plates and three PDA.The plates were incubated at 28°C for 5 – 7 days.[11]

Identification of fungal isolates

Fungi isolates were identified by Preliminary macroscopic and microscopic identification using a mycological atlas.

Preliminary Morphological Identification

Colony characteristics in terms of shape, size, texture, pigmentation and elevation were visually observed on SDA plates after 3 days of incubation at 37°C. The observed morphological features were compared with those described in a standard mycological atlas or identification manual. Tentative identifications were made based on matching colony characteristics.

Preliminary Microscopic Identification

A small portion of the fungal colony was taken from the SDA plates using a sterile needle and forcep. The samples were stained with lactophenol blue and placed on clean microscopic slides. The slides were covered with cover slips and gently pressed to spread the fungal structures evenly. The slides were observed under a light microscope (BX43, Olympus, Japan) at × 40 magnifications. The microscopic characteristics were compared with detailed illustrations and descriptions in a mycological atlas. Fungi were tentatively identified based on matching features. Observations were documented through writings and photographs taken under the microscope for accurate comparison. The tentative identification from both macroscopic and microscopic characteristics was noted.

Optimization of Hydrocarbon Degradation by Gravimetric Analysis

Experimental Design: The effects of various parameters on degradation efficiency were evaluated

Optimization using different pH levels

MSM (40mL) supplemented with 4mL of Used Engine Oil (10%v/v) as the sole carbon source was dispensed into conical flasks of 100mL. With the help of litmus papers, the pH of the medium in three conical flask was adjusted to 4 and 10 respectively by adding 0.2ml of HCl and 0.8mL of NaOH. A control flask at neutral pH (7) was also prepared Fungal isolate were inoculated into the prepared media. Flask was inoculated at 37^o with daily shaking for 7 days. To separate the residual UEO from the medium,

equal amount of diethyl ether was added to the mixture and was mixed thoroughly then poured into separating funnel. The separating funnel was kept upright on retort stand and allowed to separate the organic and aqueous layers. Two separate layers formed after settlement, the upper organic layer was collected which was diethyl ether and oil mixture and lower layer was of MSM broth. The oil from the upper layer was collected in the pre-weighed beakers. Solvent was allowed to evaporate and the residual UEO was calculated using the formula (equation 1):

$$\text{Percentage Degradation} = \frac{\text{Initial weight of oil} - \text{Residual weight of oil}}{\text{Initial weight of oil}} \times 100$$

Equation 1

Optimization using different Heavy metals

40mL of MSM supplemented with 4mL of Used Engine Oil (10%v/v) as the sole carbon source was dispensed into conical flasks of 100mL. 2 grams of heavy metals; Copper sulfate ($CuSO_4$) and Mercury chloride ($MgCl_2$) were added to the medium. A control flask without heavy metals was also prepared. Flasks were incubated at 37°C with daily shaking for 7 days. To separate the residual UEO from the medium, equal amount of diethyl ether was added to the mixture and was mixed thoroughly then poured into separating funnel. The separating funnel was kept upright on retort stand and allowed to separate the organic and aqueous layers. Two separate layers formed after settlement, the upper organic layer was collected which was diethyl ether and oil mixture and lower layer was of MSM broth. The oil from the upper layer was collected in the pre-weighed beakers. Solvent was allowed to evaporate and the residual UEO was calculated using the equation 1.

RESULTS AND DISCUSSIONS

Morphological and Microscopic characteristics of fungi isolated from mechanic workshop soil sample.

The macroscopic and microscopic examination of the three distinct fungal colonies isolated from the chronically polluted mechanic workshop soils revealed characteristic features consistent with the genera *Trichoderma, Aspergillus*, and *Penicillium* (Table 1). *Trichoderma spp*. (Colony A) exhibited rapid-growing, flat, cottony white colonies with characteristic septate hyphae and short, erect conidiophores bearing green, oval conidia—morphotypes frequently associated with the secretion of cell wall-degrading enzymes and hydrocarbon emulsifiers.[15] *Aspergillus spp*. (Colony B) was distinguished by its granular, dark green pigmentation and long, erect conidiophores terminating in radiate, globose vesicles, a morphology consistent with its high sporulation capacity and adaptability to hydrocarbon stress.[17] *Penicillium spp*. (Colony C) presented with restricted, raised colonies and brush-like (branched) conidiophores producing blue-green conidial chains. The successful isolation of these three genera aligns with recent findings from oil-contaminated automobile workshops in Benin City, where *Penicillium*, *Aspergillus*, and *Trichoderma* were identified as the dominant indigenous hydrocarbon utilizers.[15] The prevalence of these Ascomycota fungi is ecologically significant; their robust septate hyphal networks facilitate penetration into oil-aggregated soil particles, while their stress-tolerant conidia enable survival during nutrient fluctuations typical of polluted environments.[18]

Three key fungal isolates—*Trichoderma spp*., *Aspergillus spp*. and *Penicillium spp*.—were successfully identified through careful morphological and microscopic examination (Table 1). Among these, *Trichoderma spp*. demonstrated superior degradation capabilities, particularly under acidic conditions.

Table 1: Morphological and Microscopic characteristics of fungi from mechanic workshop soil sample.

Morphological Observations						Microscopic Observations			
Isolates	Shapes	Size	Elevation	Texture	Pigmentation	Hyphae	Conidiophores	Conidia	Suspected Organisms
A	Circular	Big	Flat	Cotton -like	White	Septate	Short and Erect	Green and Oval	Trichoderma spp
B	Circular	Big	Flat	Cotton -like	Green	Septate	Long and Erect	Green and Round	Aspergillus spp
C	Irregular	Small	Raised	Cotton -like	White	Septate	Branched	Blue-green and Round	Penicillium spp

Biodegradation of Used Engine Oil (UEO) by the organisms at different pH levels.

The degradation efficiency of the three fungal isolates was markedly influenced by the initial pH of the culture medium (Table 2). Trichoderma spp. exhibited the highest metabolic activity under acidic conditions (pH 4), achieving 15.48% degradation of UEO within 7 days. This performance significantly declined to 11.75% at neutral pH and collapsed to a mere 1.27% under alkaline stress (pH 10). This pronounced acidic optimum is consistent with the enzymatic kinetics of fungal lignin-modifying enzymes (LMEs); a recent 2025 study on Phanerochaete chrysosporium demonstrated that both Lignin Peroxidase (LiP) and Manganese Peroxidase (MnP) exhibit peak catalytic efficiency strictly at pH 4.5, with a sharp decline in activity above pH 7.0 due to conformational changes at the heme-active site. In contrast, *Aspergillus spp*. and *Penicillium spp*. displayed negligible degradation at pH 10 (<0.1%), indicating a severe inhibition of their cytochrome P450 monooxygenase systems under alkaline conditions. The extremely narrow operational pH range observed suggests that these indigenous strains, while adapted to local soil conditions, lack the genetic machinery for alkaliphilic hydrocarbon metabolism. This finding carries significant implications for field bioremediation, as it mandates the pre-acidification of contaminated alkaline soils (common in arid regions) to unlock the catabolic potential of these autochthonous fungi.[17]

The optimal pH for oil degradation was found to be pH 4, with *Trichoderma spp*. achieving 15.48% degradation at this acidity level. This finding aligns with previous work by[7] who reported that fungal enzymes typically show peak activity in slightly acidic environments. The dramatic drop in efficiency at pH 10 suggests that alkaline conditions may inhibit key metabolic pathways in these fungi.

Table 2: Percentage Degradation of Used Engine Oil (UEO) by the organisms at different pH levels.

Colonies	pH	Initial weight of Oil [g]	Residual weight of oil [g]	Percentage Degradation [%]
Trichoderma spp. [Colony A]	4	3.302	2.791	15.475
	7	3.302	2.914	11.750
	10	3.302	3.260	1.272
Aspergillus spp. [Colony B]	4	3.302	3.139	4.936
	7	3.302	3.296	0.182
	10	3.302	3.299	0.091
Penicillium spp. [Colony C]	4	3.302	3.237	1.969
	7	3.302	3.291	0.333
	10	3.302	3.299	0.091

Biodegradation of Used Engine Oil (UEO) by the organisms using different heavy metals.

The response of the fungal isolates to heavy metal stress was both species-specific and metal-specific, revealing complex adaptive ecophysiology (Table 3). A paradoxical and highly significant stimulatory effect was observed with Mercury chloride ($HgCl_2$). *Trichoderma spp*. achieved its highest overall degradation rate of 26.80% in the presence of $HgCl_2$, representing a 750% increase compared to its control (3.15%). Similarly, *Penicillium spp*. sustained high degradation (22.35%) under mercury stress. This phenomenon strongly suggests that prolonged historical exposure to co-contaminants in mechanic workshop soils has driven the selection of metal-tolerant ecotypes. This finding is corroborated by cutting-edge 2026 research on indigenous fungi from polluted wastewater, which reported that strains of *Aspergillus sydowii* and *Curvularia aeria* exhibited >50% removal efficiency for Hg and Cd, demonstrating that chronic heavy metal stress triggers upregulation of metallothioneins and stress-adaptive antioxidant enzymes that inadvertently enhance hydrocarbon catabolism.[16]

Table 3: Percentage degradation of Used Engine Oil (UEO) by the organisms using different metals.

Colonies	Heavy Metals	Initial weight of Oil [g]	Residual weight of oil [g]	Percentage Degradation [%]
Colony A-Trichoderma spp.	$CuSO_4$	3.302	3.028	8.298
	Control	3.302	3.198	3.150
	$HgCl_2$	3.302	2.417	26.802
Colony B-Aspergillus spp.	$CuSO_4$	3.302	3.274	0.848
	Control	3.302	3.138	4.967
	$HgCl_2$	3.302	2.795	15.354
Colony C-Penicillium spp.	$CuSO_4$	3.302	3.111	5.784
	Control	3.302	2.526	23.501
	$HgCl_2$	3.302	2.564	22.350

Conversely, Copper sulphate ($CuSO_4$) exerted a marginal inhibitory effect across all isolates, with degradation rates (0.85–8.30%) generally lower than their respective heavy-metal-free controls. The differential toxicity between Hg and Cu may be attributed to distinct detoxification mechanisms; mercury detoxification often involves energy-dependent volatilization/reduction pathways that do not compete with catabolic enzymes, whereas copper ions may directly denature extracellular peroxidases.[13] The performance of the control groups is also noteworthy; *Penicillium spp*. demonstrated a high baseline degradation of 23.50% in the absence of metals, indicating that while it is a competent degrader, its metabolism is easily disrupted by Cu, whereas *Trichoderma* requires a specific stress signal (Hg) to unlock its maximum potential. These results align with the "fungal preprocessing" synergy model proposed for petroleum reservoirs, where stress-response pathways are intricately linked to secondary metabolic gene clusters responsible for hydrocarbon oxidation.[17]

Mercury chloride, $HgCl_2$ appeared to stimulate rather than inhibit fungal activity, with *Trichoderma spp* and *Penicillium spp* showing 26.8% and 22.35% respective degradation in its presence. This contrasts with studies reporting metal toxicity[12]; this unexpected result may indicate that these isolates have developed metal tolerance through prolonged exposure in contaminated workshop soils. However, the relatively low overall degradation rates suggest there may be room for improvement through extended incubation periods or nutrient supplementation.

Notwithstanding these promising results, when comparing these results to similar studies[5,6], it becomes clear that while indigenous fungi show promise, their standalone degradation capacity may be limited.

CONCLUSION

This study confirms that hydrocarbon-degrading fungi present in mechanic workshop soils can be harnessed for bioremediation applications. Trichoderma spp. emerged as the most effective degrader, particularly under acidic conditions and in the presence of mercury chloride while the degradation rates observed were modest, they demonstrate the potential of using locally adapted fungal species for cleaning oil-contaminated sites. These findings highlight the importance of environmental optimization in enhancing fungal bioremediation efficiency.

Future studies could focus on scaling up the use of hydrocarbon-degrading fungal species for field-scale bioremediation and exploring fungal consortia or fungal–bacterial synergistic systems to enhance crude oil degradation efficiency. Additionally, assessing environmental factors, ecological safety, and potential industrial applications could pave the way for sustainable and commercially viable fungal-based bioremediation strategies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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