

Isolation, Identification, Characterization, and Screening of Hydrocarbon-Utilizing Bacterial and Fungal Isolates from Composted Organic Materials

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ABSTRACT

Bacteria and fungi from composted organic materials (COMs) are diverse, readily cultivable, and promising for bioremediation of hydrocarbon-polluted environments. This study isolated and identified microbial species from COMs and evaluated their hydrocarbon-degradation potential using contaminated soils from four sites in Sokoto Metropolis, Nigeria. Viable counts of $(9.37 \pm 0.67) \times 10^6$ CFU/g for bacteria and $(5.1 \pm 3.5) \times 10^3$ CFU/g for fungi were recorded. Six bacterial species were isolated, with *Bacillus cereus* (30%) predominant, followed by *Pseudomonas* sp. and *Bacillus anthracis* (20% each); *Bacillus megaterium*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* each accounted for 10%. Biochemical tests confirmed broad metabolic capabilities, including starch hydrolysis, sugar fermentation, and catalase activity. Fungal characterization identified *Saccharomyces cerevisiae* as dominant (50%), followed by *Aspergillus niger* and *Fusarium solani* (16.7% each), with *Aspergillus fumigatus* and *A. flavus* at 8.3% each. *S. cerevisiae* formed smooth, cream-coloured colonies, while *A. niger* and *F. solani* showed distinct mycelial morphologies. Hydrocarbon utilization by bacterial isolates, tracked over four days via OD₆₀₀, showed *Bacillus subtilis* (0.21) and *Pseudomonas* sp. (0.20) as the most active degraders, followed by *P. aeruginosa* (0.178), *B. cereus* (0.16), *B. anthracis* (0.15), and *B. megaterium* (0.134). Fungal hydrocarbon utilization over 12 days showed *A. niger* (OD₆₀₀ = 1.8) and *A. fumigatus* (OD₆₀₀ = 1.5) with the highest growth, indicating strong degrading capacity, while *A. flavus*, *F. solani*, and *S. cerevisiae* showed moderate activity (OD₆₀₀ = 0.7–0.9). These findings underscore the potential of COM-derived microbial consortia for eco-friendly bioremediation of hydrocarbon-contaminated soils.

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INTRODUCTION

Environmental pollution is one of the most pressing global environmental issues, driven largely by anthropogenic activities such as rapid industrialization, urbanization, illegal mining, emissions from fossil fuel combustion, and improper waste-disposal practices (Cachada *et al.*, 2018; Raimi *et al.*, 2022; Adepoju *et al.*, 2024; Abdullahi *et al.*, 2025). Petroleum hydrocarbons are of particular concern among these pollutants because of their persistence, toxicity, and capacity to disrupt soil ecosystems and the services they provide (Adipah, 2019; Mohanta *et al.*, 2024). Contaminated soils not only hinder plant growth and reduce agricultural productivity but also pose substantial risks to animal and human health through bioaccumulation in the food chain and discharge into water systems (Adepoju *et al.*, 2024; Thirumalaivasan *et al.*, 2024; Shehu *et al.*, 2025; Namakka *et al.*, 2026).

In municipalities, where industrial activity and inadequate waste management are common, hydrocarbon pollution constitutes a growing environmental and public health concern (Babayemi *et al.*, 2016; Shehu *et al.*, 2020; Kodiya *et al.*, 2025; Abdullahi *et al.*, 2025; Namakka *et al.*, 2026).

Conventional remediation techniques, such as excavation, incineration, and chemical treatment, are often costly, energy-intensive, and may generate secondary contamination (Hoang *et al.*, 2021). Bioremediation, which relies on microorganisms to convert hazardous chemicals into less toxic forms, offers

an affordable, eco-friendly, and sustainable alternative (Varjani, 2017; Mohanta *et al.*, 2024). Microorganisms such as *Pseudomonas*, *Bacillus*, *Aspergillus*, and *Penicillium* possess metabolic pathways that enable them to break down complex hydrocarbons into water, carbon dioxide, and biomass (Thirumalaivasan *et al.*, 2024). The efficiency of bioremediation, however, is influenced by environmental and other factors, including nutrient availability, oxygen levels, pH, and microbial community composition (Hoang *et al.*, 2021).

Composted organic materials (COMs) are particularly effective for amending hydrocarbon-contaminated soils because of their rich nutrient content (nitrogen, phosphorus, and potassium) and diverse microbial populations (Agarry & Owabor, 2022). These materials enhance microbial proliferation and enzymatic activity, accelerating hydrocarbon breakdown (Cachada *et al.*, 2018; Thirumalaivasan *et al.*, 2024). Recent studies have demonstrated the efficacy of compost-assisted bioremediation in improving soil health and restoring contaminated sites (Varjani & Upasani, 2017; Hoang *et al.*, 2021; Mohanta *et al.*, 2024). Moreover, the use of indigenous microorganisms ensures better adaptation to local soil conditions, thereby improving remediation outcomes (Agarry & Owabor, 2022; Adepoju *et al.*, 2024; Garba *et al.*, 2025).

Therefore, this study is focused on the systematic isolation, identification, and comprehensive characterization of bacterial and fungal strains from composted organic materials, with the specific goal of screening them for their ability to utilize hydrocarbons. By targeting this rich and dynamic microbial habitat, we aim to discover novel or highly efficient hydrocarbonoclastic isolates that could be harnessed for developing robust bioaugmentation strategies for the effective and sustainable remediation of petroleum-polluted environments.

MATERIALS AND METHODS

Study Area

Sokoto Metropolis is located in extreme north-western Nigeria (Latitude 13.0627°N, Longitude 5.2432°E) and serves as both the capital and largest city of Sokoto State, functioning as a vital governmental and economic centre. The city lies near the confluence of the Sokoto and Rima Rivers and has a rich historical and cultural legacy, having formerly served as the capital of the larger Sokoto State prior to the creation of Kebbi and Zamfara States (Bashar, 2015). Its proximity to the Niger Republic further enhances its strategic and commercial importance, and its influence extends beyond its political boundaries through socio-economic integration with the neighbouring Kebbi and Zamfara States (Mas'ud & Tijjani, 2023). The local economy is characterized by a high concentration of mechanical garages, reflecting the city's active role in the servicing and repair of transport and industrial equipment, an activity associated with frequent spillage of spent lubricating oil and other petroleum products.

Sample Collection and Preparation

A compost pit measuring 1 m × 1 m was prepared at the Teaching and Research Dry Land Farm of Usmanu Danfodiyo University, Sokoto, behind the Faculty of Agriculture, following approval by the research ethics standards of the Department of Microbiology. A black plastic sheet was placed at the base of the pit to minimize nutrient and moisture loss and to promote decomposition (Sintim *et al.*, 2020). The composting process involved layering grass and agricultural waste with fresh animal dung and urine obtained from Kara Market, with ash added to regulate acidity. Water was sprinkled periodically to maintain optimal humidity, and the pile was covered with a black plastic sheet to encourage microbial activity (Misra *et al.*, 2003). Aeration was achieved by turning the compost every two weeks until full decomposition was attained over a two-month period (21 November 2023 – 21 January 2024), following the procedure of Hashim *et al.* (2022). Compost samples were collected using a sterile trowel, combined into composite samples, stored in polyethylene bags, sun-dried for 72 hours, and transported to the Microbiology Laboratory of the University for analysis (Adeleye *et al.*, 2020).

Determination of Physicochemical Properties of Composted Organic Materials

The physicochemical properties of the composted organic materials, including pH, electrical conductivity (EC), organic carbon, total nitrogen, available potassium, cation exchange capacity (CEC), and available phosphorus, were determined following the procedures of Adeleye *et al.* (2020).

Isolation of Bacterial Isolates

Bacterial species present in the compost were isolated using the pour-plate technique. One gram of compost was added to normal saline and homogenized for 30 minutes. A ten-fold serial dilution (10^{-3}) was prepared from the homogenate,

which was then used to enumerate aerobic mesophilic bacteria (Pokhrel, 2015; Adeleye *et al.*, 2022).

Identification and Characterization of Bacterial Isolates

Bacterial isolates were characterized using morphological and biochemical techniques, including Gram staining, coagulase, catalase, indole, urease, mannitol salt agar, methyl red–Voges-Proskauer, oxidase, citrate utilization, hydrogen sulphide, and gas-production tests, following the methods of Cheesbrough (2006), Adeleye *et al.* (2022), and Umoh (2022).

Isolation of Fungi

Fungal isolates were obtained from the composted organic materials using the conventional plating method of Ameh and Kawo (2017). Compost samples were serially diluted up to 10^{-5} and spread-plated (0.1 ml) in duplicate on sterile Sabouraud Dextrose Agar (SDA). Plates were incubated at 30°C for 7 days, after which fungal colonies were counted and expressed as colony-forming units per gram (CFU/g) (Wang *et al.*, 2007). Colonies were sub-cultured repeatedly on fresh SDA plates until pure cultures were obtained, which were then stored at 4°C on SDA slants for further use.

Identification of Fungi

Fungal isolates were identified using the method of Chukwu (2018). Morphological characteristics were assessed using lactophenol cotton blue staining on clean microscope slides, with observations of colony colour, surface and reverse appearance, texture, margin, and pigmentation. Microscopic examination was carried out under a 40× objective lens to assess structural details, including budding pattern, hyphal structure, septation, and cell morphology, following Saadaoui *et al.* (2024).

Determination of Hydrocarbon Utilization by Bacterial Isolates

Hydrocarbon-utilizing bacterial isolates were screened using a modified procedure adapted from Ekanem and Ogunjobi (2017). The ability of each isolate to grow on spent lubricating oil was tested in mineral salt medium (MSM). A single colony of each isolate was inoculated into 10 ml of MSM supplemented with 1% (v/v) filter-sterilized spent lubricating oil as the sole carbon source. Cultures were incubated on a rotary shaker at 130 rpm at ambient laboratory temperature ($32 \pm 2^\circ\text{C}$). Hydrocarbon utilization was assessed spectrophotometrically at 600 nm at 24-hour intervals for 4 days.

Determination of Hydrocarbon Utilization by Fungal Isolates

Hydrocarbon-utilization potential of the fungal isolates was assessed using the method of Akinnibosun and Ashegbare (2018). A colony of each isolate was inoculated into 10 ml of mineral salt agar (MSA) containing 1% (v/v) filter-sterilized spent lubricating oil as the sole carbon source. Cultures were incubated on a rotary shaker at 130 rpm at ambient laboratory temperature ($32 \pm 2^\circ\text{C}$), and hydrocarbon utilization was assessed spectrophotometrically at 600 nm at 4-day intervals for 12 days.

Data Analysis

Physicochemical properties of the composted organic materials were summarized using descriptive statistics in Microsoft Excel (Mkandawire, 2022). Bacterial and fungal colony counts were expressed as mean values with standard deviations, while the frequency and percentage distribution of

identified isolates were summarized descriptively. Optical density measurements obtained during spectrophotometric screening for hydrocarbon-degrading bacterial and fungal isolates were represented graphically using bar charts generated in Excel.

RESULTS AND DISCUSSION

Microbial Colony Counts of Composted Organic Materials

Table 1 presents the viable microbial counts obtained from the COMs. The viable bacterial count was $(9.37 \pm 0.67) \times 10^6$ CFU/g, considerably higher than the viable fungal count of $(5.1 \pm 3.5) \times 10^3$ CFU/g, indicating a marked difference in microbial abundance between the two groups.

Table 1: Viable Microbial Counts from Prepared Composted Organic Materials

Sample Type	Colony Count (CFU/g)
Viable Bacterial Count	$(9.37 \pm 0.67) \times 10^6$
Viable Fungal Count	$(5.1 \pm 3.5) \times 10^3$

Key: Colony Count (CFU/g) Denotes the Number of Viable Colonies per Gram of Sample, Expressed in Colony-Forming Units

The elevated bacterial abundance recorded in this study is consistent with previous reports identifying bacteria as the predominant decomposers in compost, owing to their rapid growth rates and broad metabolic versatility in processing diverse organic substrates (Mahmoudi et al., 2017). Such high bacterial loads typically accelerate organic-matter decomposition, promoting faster nutrient mineralization and humification (Wang et al., 2022). Bacterial dominance in compost has been attributed to adaptability to fluctuating environmental conditions such as temperature and moisture (Wang et al., 2022), with genera including *Bacillus*, *Pseudomonas*, and *Actinobacteria* commonly playing key roles in cellulose degradation and nitrogen cycling. The relatively low fungal count observed may reflect bacterial competition during the early stages of composting or unfavourable pH and moisture conditions for fungal growth, since fungal populations typically intensify during later composting phases when lignocellulosic materials become more abundant and temperature stabilizes (Epstein, 2017). The considerable variability in fungal counts ($\pm 3.5 \times 10^3$ CFU/g) may also reflect spatial heterogeneity within the compost pile, where localized microenvironments can favour fungal colonization.

Morphological and Biochemical Characterization of Bacterial Isolates

Six bacterial species were isolated and characterized based on morphological and biochemical profiles, as summarized in Table 2. *Bacillus megaterium* (CMP1, 10%) was Gram-positive, long rod-shaped, and spore-forming, testing positive for starch hydrolysis, catalase, methyl red, citrate utilization, fermentation of glucose, lactose, and sucrose, and hydrogen sulphide production without gas formation. *Pseudomonas aeruginosa* (CMP2, 10%) was Gram-negative, long rod-shaped, non-spore-forming, and positive for starch hydrolysis, catalase, methyl red, urease, and fermentation of the three sugars with gas production. *Bacillus subtilis* (CMPA3, 10%) was Gram-positive, short rod-shaped, spore-forming, and positive for starch hydrolysis, catalase, citrate utilization, sugar fermentation, hydrogen sulphide production, gas production, and motility.

Bacillus cereus (CMPB1) was the most prevalent isolate (30%), Gram-positive, short rod-shaped, spore-forming, and positive for starch hydrolysis, catalase, Voges-Proskauer, citrate utilization, sugar fermentation, gas production, and motility. *Pseudomonas* sp. (CMPB2, 20%) was Gram-negative, long rod-shaped, and positive for catalase, methyl red, urease, and glucose fermentation. *Bacillus anthracis* (CMPB3, 20%) was Gram-positive, long rod-shaped, spore-

forming, and positive for starch hydrolysis, catalase, methyl red, Voges-Proskauer, and glucose fermentation.

The isolates were predominantly Gram-positive, spore-forming *Bacillus* species (80% cumulative frequency) and Gram-negative *Pseudomonas* species (30% cumulative, owing to mixed Gram reactions recorded across replicate isolates), each exhibiting distinct metabolic traits relevant to organic-matter degradation. *Bacillus cereus*, the most frequently isolated species (30%), displayed a robust biochemical profile positive for starch hydrolysis, catalase, Voges-Proskauer, citrate utilization, and sugar fermentation consistent with its established role in composting systems (Bottone, 2010; Garba et al., 2025). Its short rod-shaped, spore-forming morphology, shared with *B. anthracis* (20%) and *B. subtilis* (10%), confers resilience to fluctuating temperature and nutrient conditions (Mahmoudi et al., 2017). The Gram-negative *Pseudomonas* isolates (*P. aeruginosa*, 10%; *Pseudomonas* sp., 20%) demonstrated notable biochemical versatility, including urease activity and gas production during sugar fermentation, together with a documented capacity for hydrocarbon degradation (Gellatly & Hancock, 2013).

Universal catalase positivity across the isolates suggests adaptation to the oxidative conditions typical of composting environments, while variable methyl red and Voges-Proskauer results point to diverse glucose-metabolic pathways and niche differentiation among the isolates (Das & Chandran, 2011). The comparatively high frequency of *B. anthracis* (20%) raises a biosafety consideration that underscores the importance of adequate compost maturation to minimize pathogen carry-over (Doganay & Metan, 2009).

Isolation, Identification, Frequency, and Percentage Distribution of Fungal Isolates

Five fungal species were isolated and identified from the COMs based on microscopic and macroscopic characteristics, as summarized in Table 3. *Aspergillus niger* (16.7%) displayed septate hyphae, long smooth conidiophores, and brownish-black mycelium. *Aspergillus fumigatus* (8.3%) exhibited rough conidiophores with colonies ranging from blue-green to yellow. *Aspergillus flavus* (8.3%) produced yellow-green colonies with biseriate phialides and a golden-to-reddish-brown reverse colouration. *Saccharomyces cerevisiae*, the most abundant species (50%), formed smooth, cream-coloured colonies from spherical cell clusters. *Fusarium solani* (16.7%) displayed greyish-white mycelia with curved, septate conidia and cylindrical macroconidia, showing visible growth within four days of incubation.

Table 2: Morphological and Biochemical Characteristics of Bacteria Isolated from Composted Organic Materials

Code	GR	Shape	Spore	STA	CAT	OXI	MR	VP	UR	IND	CIT	GLU	LAC	SUC	H ₂ S	GAS	MOT	Species	Freq.	%
CMP1	+	LR	+	+	+	-	+	-	-	-	+	+	+	+	+	-	+	<i>B. megaterium</i>	1	10.0
CMP2	-	LR	-	+	+	-	+	-	+	-	-	+	+	+	+	+	-	<i>P. aeruginosa</i>	1	10.0
CMPA3	+	SR	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	<i>B. subtilis</i>	1	10.0
CMPB1	+	SR	+	+	+	-	-	+	-	-	+	+	+	-	-	+	+	<i>B. cereus</i>	3	30.0
CMPB2	+	LR	+	-	+	-	+	-	+	-	-	+	-	-	-	-	-	<i>Pseudomonas</i> sp.	2	20.0
CMPB3	+	LR	+	+	+	-	+	+	-	-	-	+	-	-	-	-	-	<i>B. anthracis</i>	2	20.0

Key: GR = Gram Reaction; STA = Starch Hydrolysis; CAT = Catalase; OXI = Oxidase; MR = Methyl Red; VP = Voges-Proskauer; UR = Urease; IND = Indole; CIT = Citrate Utilization; GLU/LAC/SUC = Glucose/Lactose/Sucrose Fermentation; H₂S = Hydrogen Sulphide Production; GAS = Gas Production; MOT = Motility; SR = Short Rod; LR = Long Rod

Table 3: Isolation, Identification, Frequency, and Percentage Distribution of Fungal Isolates from Composted Organic Materials

Organism	Microscopic Morphology	Macroscopic Morphology	Frequency	%
<i>Aspergillus niger</i>	Septate hyphae; long, smooth, erect conidiophores; hyaline vesicles with dichotomous branching; radiate heads	Brownish-black mycelium with dark spores on the surface	2	16.7
<i>Aspergillus fumigatus</i>	Rough conidiophores; uniseriate or biseriate phialides; round vesicles with radiate heads	Blue-green to yellow surface colouration	1	8.3
<i>Aspergillus flavus</i>	Septate hyphae; long, smooth, colourless-to-brownish conidiophores; round vesicles with biseriate phialides	Yellow-green colonies with creamy edges; reverse golden to reddish-brown	1	8.3
<i>Saccharomyces cerevisiae</i>	Spherical cells clustered in convex elevations	Cream-coloured, smooth colonies with heavy growth and dry surface	6	50.0
<i>Fusarium solani</i>	Small spore vesicles; multinucleate, moderately curved septate conidia; cylindrical macroconidia	Sparse aerial mycelia; greyish-white colonies with creamy-to-buff colouration; visible growth within 4 days	2	16.7

Saccharomyces cerevisiae accounted for the majority (50%) of fungal isolates, followed by *A. niger* and *F. solani* (16.7% each), while *Aspergillus* species collectively represented 33.3% of the fungal population. The morphological adaptations observed among the filamentous fungi septate hyphae, pigmentation, and specialized spore structures are consistent with previous reports linking these features to enhanced resilience under fluctuating compost conditions (Garrett, 2013; Parapouli *et al.*, 2020). The rapid colonization of *F. solani* within four days of incubation further indicates its competitive growth potential in nutrient-rich compost substrates (Riddech *et al.*, 2025).

Hydrocarbon Utilization by Bacterial Isolates

Optical density (OD₆₀₀) measurements taken over four days revealed distinct growth patterns among the six bacterial isolates grown on spent lubricating oil as the sole carbon source (Figure 1). *Bacillus subtilis* exhibited the most pronounced growth, reaching an OD of 0.21 by Day 4,

consistent with its documented capacity to produce biosurfactants and degrade complex hydrocarbons, including petroleum derivatives (Mnif *et al.*, 2015). *Pseudomonas* sp. showed comparably strong growth (OD = 0.20), reflecting the metabolic versatility of pseudomonads in degrading hydrocarbons, particularly polycyclic aromatic hydrocarbons (PAHs) (Gellatly & Hancock, 2013). *Pseudomonas aeruginosa* and *Bacillus cereus* recorded substantial growth (OD = 0.178 and 0.16, respectively), consistent with reports of *P. aeruginosa*'s capacity to metabolize recalcitrant hydrocarbons (Bottone, 2010) and *B. cereus*'s enzymatic ability to degrade aliphatic and aromatic compounds. *Bacillus anthracis* and *Bacillus megaterium* showed more moderate growth (OD = 0.15 and 0.134, respectively), indicating slower but evident hydrocarbon utilization; *B. megaterium* has previously been reported to degrade pesticides and PAHs, supporting a potential role in bioremediation (Mnif *et al.*, 2015).

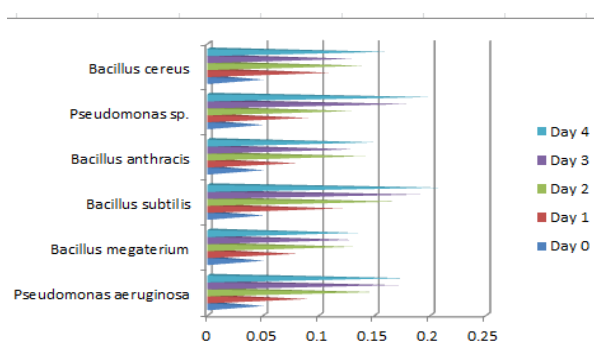


Figure 1: Hydrocarbon Utilization by Bacterial Isolates Over a 4-Day Period (OD₆₀₀)

Hydrocarbon Utilization by Fungal Isolates

OD₆₀₀ measurements over a 12-day incubation period revealed varying hydrocarbon-utilization efficiencies among the five fungal isolates (Figure 2). All isolates exhibited no growth at Day 0 (OD = 0.0), confirming baseline conditions for the screening assay. *Aspergillus niger* exhibited the most rapid and extensive growth throughout the incubation period, reaching an OD of 1.0 by Day 4, 1.3 by Day 8, and 1.8 by Day 12, consistent with its known production of extracellular enzymes such as lignin peroxidase and laccase, which facilitate the breakdown of complex hydrocarbons, including PAHs (Kadri *et al.*, 2017). *Aspergillus fumigatus* showed the next-highest growth (OD = 0.9, 1.1, and 1.5 at Days 4, 8, and 12, respectively), reinforcing its documented ability to degrade lignin and other recalcitrant organic compounds (Daccò, 2021).

Aspergillus flavus and *Fusarium solani* showed moderate growth, reaching final ODs of 0.8 and 0.9, respectively, suggesting active but comparatively slower hydrocarbon degradation; *A. flavus* is known to metabolize both aliphatic and aromatic hydrocarbons, while *F. solani* has been associated with petroleum hydrocarbon degradation (Gupta *et al.*, 2016). *Saccharomyces cerevisiae*, despite the slowest initial growth, reached a final OD of 0.7 by Day 12, indicating a capacity to metabolize simpler organic compounds. These results are consistent with recent studies emphasizing the significant contribution of fungi to the biodegradation of environmental pollutants within composting systems (Ghosh *et al.*, 2023), and the consistently superior performance of *Aspergillus* species across the incubation period highlights their potential as priority candidates for hydrocarbon bioremediation (Rezaei & Moghimi, 2024; Rani *et al.*, 2024).

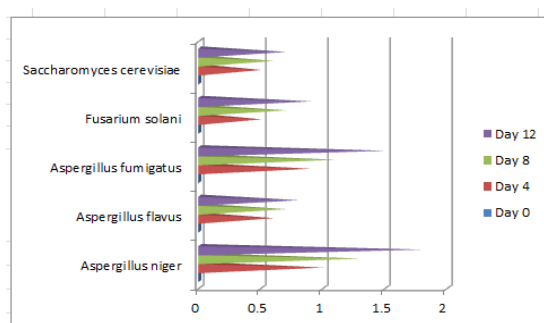


Figure 2: Hydrocarbon Utilization by Fungal Isolates Over a 12-Day Period (OD₆₀₀)

CONCLUSION

This study successfully isolated and identified bacterial and fungal species from composted organic materials and evaluated their hydrocarbon-degradation potential against soils contaminated by hydrocarbons in Sokoto Metropolis, Nigeria. The composted organic materials proved a rich source of bacterial and fungal diversity, with six bacterial species (dominated by *Bacillus cereus*) and five fungal species (dominated by *Saccharomyces cerevisiae*) isolated. Among the bacterial isolates, *Bacillus subtilis* and *Pseudomonas* sp. were the most active hydrocarbon utilizers, while among the fungal isolates, *Aspergillus niger* and *A. fumigatus* recorded the highest hydrocarbon-utilization capacity. These findings support the prospects of COM-derived microbial consortia as eco-friendly agents for bioremediating hydrocarbon-contaminated soils. Further research is recommended to validate these isolates under field conditions and to assess their performance as formulated bioremediation consortia, alongside awareness campaigns promoting the use of organic compost and other organic amendments for improving soil health and environmental sustainability.

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