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Isolation and Characterization of Phyllosphere Bacteria and their Bioremediation-Potential of Spent Engine Oil Contaminated Soil

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Abstract

Environmental pollution from spent engine oil (SEO) is a growing concern due to its persistence and toxicity risks to soil health and ecosystems, necessitating the continuous search for sustainable and cost-effective bioremediation approaches. This study investigated the potential of phyllosphere bacteria for bioremediation of SEO-contaminated soils. Leaves were purposively sampled from ten mechanic workshops in Katsina, Nigeria, to isolate and identify native bacteria using morphological characteristics and analysis with the VITEK 2 Compact kit. For hydrocarbon degradation studies, isolates were cultured on mineral salts medium (MSM) containing SEO as the sole carbon source. To validate the SEO remediation potential, a 60-day simulated soil microcosm experiment was conducted by mixing SEO-contaminated soil with bacterial inoculums (10 mL of broth culture containing 10^{10} CFU/mL cells) ***in six planting bags (four with individual bacterial isolates, one with bacterial consortium, and one as a control), and the soil recovery was monitored by measuring the physicochemical parameters, including pH, organic carbon, nitrogen content, and electrical conductivity. The main SEO degraders identified were Pseudomonas aeruginosa, Kocuria kristinae,*** and ***Pseudomonas oleovorans,*** with ***Pseudomonas aeruginosa*** and ***Kocuria kristinae having the highest growth rates (>22.4×10^7 CFU/mL) in SEO. Post-treatment analysis revealed significant improvements ($p < 0.05$) in soil quality/fertility, with the soil treated with bacterial consortia having the highest fertility level, showing an 11% increase in organic carbon, a 20% rise in nitrogen content, and stabilization of pH levels and improved electrical conductivity (an indicator of soil salinity), confirming reduction in pollutant levels. These findings showcase the promising role of phyllosphere bacteria in restoring SEO-polluted soils using a sustainable, cost-effective, and eco-friendly solution. Hence, this study provides a foundation for further research into mountable SEO bioremediation strategies, particularly in regions with limited access to advanced remediation technologies.***

Keywords: ***Phyllosphere Bacteria, Pollution, Spent Engine Oil, Degradation, Soil Recovery.***

INTRODUCTION

Spent engine oil (SEO) is one of the major sources of environmental pollution, which is increasingly becoming a global problem due to its toxicity, persistence, and substantial threat to soil health and ecosystems (Smith *et al.*, 2020). Spent engine oil consists of these toxic elements of heavy metals and polycyclic aromatic hydrocarbons (PAHs), which are mutagenic, carcinogenic, and another chronic health risk (Mandri and Lin, 2017; Zengerer *et al.*, 2018). Remediating these pollutants is essential because they compromise soil structure and fertility, potentially creating formidable obstacles to agricultural productivity (Salisu and Ibrahim, 2024). Conventional remediation methods, including thermal desorption and chemical treatments, can be expensive, time-consuming, and environmentally invasive (Johnson *et al.*, 2019). As a result, there is an increasing demand for environmentally more sustainable, eco-friendly bioremediation methods that utilize the metabolic activities of microorganisms to degrade hydrocarbon and restore soil quality (Das and Chandran, 2011; Salisu and Ibrahim, 2024).

Although environmental scientists' understanding of the role of microorganisms in hydrocarbon degradation has advanced enormously, most studies have focused on soil- or water-associated microbes, and little is known about phyllosphere bacteria and their potential in hydrocarbon degradation. The native bacteria, particularly those isolated from the oil-contaminated sites, are an untapped

resource that may prove effective in SEO bioremediation (Salisu and Ibrahim, 2024). Hydrocarbon degradation seems to be a natural process for most bacteria in oil-contaminated sites, as they are constantly exposed to pollutants in their natural habitats, which may contribute to adaptations in their hydrocarbon degradation (Oso *et al.*, 2019; Undugoda *et al.*, 2018). However, the bioremediation potential of phyllosphere bacteria has been underexplored, especially in areas where sophisticated remediation technologies are unavailable (Salisu and Ibrahim, 2024).

This gap becomes more apparent when investigating the specific contributions of phyllosphere bacteria toward the soil health restoration pathway post-remediation. Very few comprehensive studies test them, both individual isolates and consortia, in degrading SEO and subsequently augmenting soil recovery parameters like pH, organic carbon, and nitrogen content (Salisu and Ibrahim, 2024). Besides, it is necessary to screen for bacterial species with high biodegradation efficiency, optimize their growth conditions, and explore their synergistic interactions in complex environments.

This research had filled the gaps about the bacterial candidates capable of degrading SEO in Katsina soils contaminated with SEO by isolating and characterizing phyllosphere bacteria from oil-contaminated sites in Katsina. Specific objectives were to assess their potential for SEO degradation, to examine their effect on soil physicochemical properties in simulated microcosm experiment, and to identify the most effective species for bioremediation. The second half of this research connects microbial biodegradation with soil recovery metrics to better inform the eco-friendly strategies of bioremediation that can be more cost-effective in regions that have limited access to advanced technologies

MATERIALS AND METHODS

Sample Collection

Leaves samples from ten mechanic workshops within Katsina LGA were selected via purposive sampling. The selection of the workshops was based on random sampling; however, factors such as the size (small to large), location within the Katsina metropolis, and types of oil in the workshops were considered to ensure a diverse and representative sample.

Bacterial isolation

The leaf samples were brought to the microbiology laboratory of Umaru Musa Yar'adu University Katsina for examination. First, the surface of the leaves was gently cleaned with 10 mL of distilled water into sterile test tubes, serving as the stock, to isolate epiphytes. The stock was used to prepare serial dilutions up to 10^{-6}. Then, using the spread plate approach, aliquots (0.1 mL) from the last dilution were plated in duplicates on Nutrient Agar plates and incubated at 35^0C for 48 hours. The resulting pure colonies were kept in pure culture on NA slants (APHA, 2017).

Finally, using modified procedures from Duan *et al.* (2013), Perez-Rodriguez *et al.*, (2020), and Alves-Junior *et al.* (2021), the endophytic leaf bacteria were isolated. One gram of the leaf samples was sliced, sterilized for three minutes with 70% ethanol, and then thoroughly rinsed three times with sterile distilled water. After rinsing, the samples were macerated in 9 mL of sterile distilled water to create dilutions up to 10^{-3}; this creates the stock solution. At 30 to 35 ^{0}C, 100 mL aliquots of this dilution was spread over nutritional agar. Forty-eight hours of incubation provided discrete colonies, which were subcultured, and pure cultures were preserved in nutrient agar slants for later use (Castaldi et al., 2021).

Biodegradation of Engine Oil Study

Each isolate was cultured separately on Petri dishes that contained mineral salts medium (MSM) with 1%, 2%, and 3% v/v spent engine oil as the only carbon and energy source for 14 days at 35^0C (Parikh *et al.*, 2018). The MSM was obtained from HiMedia, India, and it comprises the following components per liter: K_2HPO_4 1.73g, KH_2PO_4 0.68g, $MgSO_4$. $7H_2O$ 0.1g, $FeSO_4$. $7H_2O$ 0.03g, NH_4NO_3 1.0g, $CaCl_2$. $2H_2O$ 0.02g, and NaCl 4.0g, with a targeted final pH of 7.0. These allow the ability of the isolates to tolerate and degrade crude oil studied over time (Balogun *et al.*, 2015).

In addition, control Petri dishes containing sterilized isolates in a minimal salt medium with crude oil were also prepared. Colonies were counted every 48 hours for 14 days to determine the total viable count (TVC) (Vidali, 2014).

Identification of the SEO degraders using VITEK

The SEO degraders were identified at the species level using the VITEK system following the protocol utilized by Vidali (2014). The system model used was the VITEK 2 Compact 15 (bioMérieux, Marcy-l'Étoile, France). Pure colonies of the successful SEO-degrading bacterial isolates were incubated under optimal conditions. A standardized bacterial suspension was prepared by transferring the colonies into a sterile saline solution and adjusting the turbidity to a McFarland standard of 0.5. This suspension was then loaded into the appropriate VITEK 2 identification cards (GN or GP), containing multiple wells pre-loaded with various

biochemical substrates. The bacterial suspension in the card wells underwent incubation within the VITEK 2 Compact system, where automated readings of colorimetric changes in the wells were taken at specific intervals. The changes indicated metabolic activity, based on which the system's software compared the biochemical profile to an extensive database of known microorganisms to identify the species. The entire process, from loading to obtaining identification results, takes 18 hours. Finally, the system provided a comprehensive report with the identification of the organisms based on the generated biochemical profile (Vidali, 2014).

Soil Microcosm Study

This was done utilizing the methodology proposed by Mamdoh (2018). Six planting bags containing 500 g of air-dried garden soil were used. The bags were stored at room temperature. The soil in each bag was appropriately mixed with 5% (v/w) spent engine oil which was the pollution level range recommended. Bags 1-4 (augmented) each got 10 mL of broth culture containing 10^{10}CFU/mL of a pure culture from each chosen isolate, while bag 5 received 10 mL of broth culture containing 10^{10}CFU/mL of the mixed culture of all the isolates. Natural attenuation was used as the control in the untreated bag (bag 6) which served as a control (natural attenuation). The experiment was conducted for two months (60 days).

Measurement of Soil Properties

The physicochemical parameters of the soil (soil pH, organic carbon, nitrogen content, organic matter, and electrical conductivity) were measured according to the methods of Gudla *et al.* (2023), as follows: Soil pH was measured using the standard method of potentiometric. Air-dried soil samples were sieved through a 2 mm mesh to remove debris and large particles. A 1:1 soil-to-water suspension was prepared by mixing 10 g of soil with 10 mL of distilled water. A calibrated pH meter was used to measure the pH of the supernatant by inserting the electrode into the solution, ensuring it was fully immersed without touching the sediment. The pH value was recorded after the stabilization of the reading.

The organic carbon content of the soil was determined using the Walkley-Black method, which involved wet oxidation. Air-dried and sieved soil samples (1 g) were mixed with 10 mL of potassium dichromate ($K_2Cr_2O_7$) solution and 20 mL of concentrated sulfuric acid (H_2SO_4). The mixture was allowed to react for 30 minutes under controlled conditions. After cooling, 200 mL of distilled water and a few drops of orthophosphoric acid were added, and the solution was titrated with 0.5N ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2$) using diphenylamine as an indicator. The organic carbon content was calculated based on the titration results, assuming that 77% of the organic carbon in the soil was oxidized.

The nitrogen content of the soil was measured using the Kjeldahl method. Air-dried soil samples (0.5-1 g) were digested in a digestion flask with concentrated sulfuric acid (H_2SO_4), a catalyst mixture (usually potassium sulfate (K_2SO_4) and copper sulfate ($CuSO_4$), and a few drops of selenium. The mixture was heated until a clear solution was obtained, indicating complete digestion. The digest was then diluted with distilled water and transferred to a distillation apparatus, where it was made alkaline with sodium hydroxide (NaOH). The ammonia released was distilled and absorbed in boric acid. Finally, the ammonia content was titrated with standard sulfuric acid (H_2SO_4), and the total nitrogen content was calculated based on the titration results.

Soil organic matter content was determined indirectly by multiplying the organic carbon content by a factor of 1.724, based on the assumption that organic matter contains approximately 58% carbon. This method was conducted in conjunction with the Walkley-Black method for organic carbon determination. After obtaining the percentage of organic carbon from the titration, the value was multiplied by 1.724 to estimate the total organic matter in the soil sample.

Finally, toil electrical conductivity was measured using a standard 1:5 soil-to-water extract method. Air-dried soil samples were sieved to 2 mm and mixed with deionized water in a ratio of 1:5 (10 g of soil and 50 mL of water). The mixture was stirred for 30 minutes and then allowed to settle for 1 hour. The supernatant was decanted, and the electrical conductivity of the solution was measured using a calibrated conductivity meter. The reading was taken after the meter had stabilized, and the results were expressed in decisiemens per meter (dS/m). The EC values were used to assess the salinity levels in the soil.

RESULTS

Bacteria enumeration

The colony counts of both the epiphytic and endophytic bacteria from the phyllosphere were obtained, as shown in Table 1.

The table illustrates that EP3 had the highest colony count (22.4×10^7) among the epiphytic bacteria, while sample EP9 had the lowest (1.80×10^7). However, EN8 (17.2 ×104) had the highest counts among the endophytic bacteria, while the lowest counts were obtained from sample EN6 (9.2×10^4). This finding aligns with the hypothesis that certain environmental conditions or plant species may support a higher population of hydrocarbon-degrading bacteria. The high colony counts in EP3 suggest a robust microbial activity, indicating a potentially higher rate of biodegradation in this sample compared to others, which could be attributed to specific environmental conditions or bacterial adaptations in this location.

The higher colony count observed in EP3 (22.4×10^7) compared to EN8 (17.2×10^4) suggests that EP3 might possess a greater resilience or adaptability to oil-contaminated environments, potentially making it a more effective candidate for bioremediation efforts. Generally, the differences in colony counts between epiphytic and endophytic bacteria suggest distinct roles in bioremediation, potentially reflecting their efficiency and adaptability in degrading engine oil. The epiphytic bacteria, with a much higher colony count range (1.80×10^7 to 22.4×10^7 CFU/mL), particularly the highest count observed in one epiphytic sample, indicate a strong potential for active degradation of engine oil. This could be attributed to their natural exposure to environmental pollutants, making them more adapted to utilize hydrocarbons as a carbon source. Epiphytes like the sample with the highest count (22.4×10^7 CFU/mL), likely play a dominant role in the initial breakdown of oil contaminants due to their faster growth and high metabolic activity, which are essential for rapid bioremediation. In contrast, the lower colony counts observed among the endophytic bacteria (9.2×10^4 to 17.2×10^4 CFU/mL) suggest a more limited, though potentially complementary, role in bioremediation. Endophytes are typically associated with plant tissues and may contribute indirectly to bioremediation by enhancing plant resilience to oil toxicity, promoting plant growth, or facilitating phytoremediation in conjunction with plants like *Vigna unguiculata*.

The lower growth rates observed for endophytes could also reflect their adaptation to more controlled or nutrient-rich environments inside plant tissues, which might not immediately favor the degradation of harsh compounds like engine oil in the same way epiphytes do. Overall, the higher colony counts of epiphytes highlight their primary role in direct oil degradation, while endophytes, with lower counts, might support bioremediation through synergistic effects with plants rather than acting as primary degraders. Both groups, however, could contribute to a more holistic bioremediation approach, with epiphytes initiating degradation and endophytes supporting long-term soil recovery through plant-microbe interactions.

Table 1: Enumeration of epiphytic and endophytic bacteria from the phyllosphere

Isolates	Cultural/Macroscopic Appearance	Microscopic/Morphological Appearance	Colony Counts (CFU/g)
EP1	Round and Smooth colonies	G-ve greenish apperance	$12.8 \pm 1.25\times10^7$
EP2	Moist colonies	G-ve shiny appearance	$17.6 \pm 1.00\times10^7$
EP3	Slightly mucoid colonies	G-ve, single polar flagellum	$22.4 \pm 6.00\times10^7$
EP4	Round and moist colonies	G-ve greenish apperance	$16.6 \pm 1.25\times10^7$
EP5	Slightly mucoid colonies	G-ve, single polar flagellum	$18.2 \pm 0.55\times10^7$
EP6	Club-shaped rods	G+ irregular	$13.0 \pm 0.45\times10^7$
EP7	Slightly mucoid colonies	G-ve, single polar flagellum	$12.8 \pm 0.40\times10^7$
EP8	Large and creamy colonies	G-ve shiny appearance	$9.80 \pm 0.25\times10^7$
EP9	Encapsulated rods	G-ve	$1.80 \pm 1.10\times10^7$
EP10	Smooth and flat shape	G-ve greenish blue	$19.4 \pm 0.65\times10^7$
EN1	Dry and slightly raised colonies	G-ve pink rods	$10.4 \pm 0.40\times10^4$
EN2	Club-shaped rods	G+ irregular	$12.1 \pm 1.10\times10^4$
EN3	Slightly mucoid colonies	G-ve, single polar flagellum	$14.4 \pm 1.50\times10^4$
EN4	Round and moist colonies	G-ve greenish apperance	$15.8 \pm 2.50\times10^4$
EN5	Large and creamy colonies	G-ve shiny appearance	$15.0 \pm 4.50\times10^4$
EN6	Encapsulated rods	G-ve	$9.20 \pm 0.70\times10^4$
EN7	Smooth and flat shape	G-ve greenish blue	$14.2 \pm 0.85\times10^4$
EN8	Dry and slightly raised colonies	G-ve pink rods	$17.2 \pm 0.80\times10^4$
EN9	Club-shaped rods	G+ irregular	$16.4 \pm 1.30\times10^4$
EN10	Slightly mucoid colonies	G-ve, single polar flagellum	$13.0 \pm 1.15\times10^4$

Key: EN = Endophytic bacteria, EP = Epiphytic bacteria

Enumeration of Bacteria during the Biodegradation Study

The number of bacteria involved in the biodegradation of engine oil at different percentages, namely 1%, 2%, and 3% at CFU/mL, were presented in Table 2. The colony counts with the highest count (7.04± 0.33 × 10^7) from 1% was EP 3 while the least colony counts of 2.08 × 10^7 was found in EN 7. In 2%, the highest colony counts were 5.32 × 10^7 in EP 3, while the least colony counts were 1.68 × 10^7 at EP 4. However, the highest colony count from 3% is 9.2 × 10^7 (EN 7) while the least colony count is 1.3 × 10^7 (EP 4).

One-way ANOVA showed that the Colony Counts (CFU/mL) at the three different concentrations of engine oil did not differ significantly ($p > 0.05$). Sidika multiple comparison tests was also used to the three concentration percentages against each other (1% vs. 2%, 1% vs. 3%, and 2% vs. 3%). In each case, there is no significant difference in the performance of the 6 isolates at different concentrations.

The variation in colony counts across different oil concentrations (1%, 2%, 3%) provides important insights into the potential of the selected strain for bioremediation and its adaptability to contaminated environments.

Table 2: Bacterial enumeration during the biodegradation of engine oil at various concentrations

S/No	Isolates	% Engine Oil and Colony Counts (CFU/mL)		
		1%	2%	3%
1	EP1	-	-	-
2	EP2	-	-	-
3	EP3	7.04 ± 0.33 × 10^7	5.32 ± 0.35 × 10^7	4.02 ± 0.17 × 10^7
4	EP4	3.52 ± 0.64 × 10^7	1.68 ± 0.83 × 10^7	1.3 ± 0.82 × 10^7
5	EP5	-	-	-
6	EP6	-	-	-
7	EP 7	3.76 ± 0.17 × 10^7	3.36 ± 0.95 × 10^7	1.58 ± 0.15 × 10^7
8	EP8	-	-	-
9	EP9	-	-	-
10	EP10	-	-	-
11	EN1	-	-	-
12	EN2	-	-	-
13	EN 3	3.54 ± 0.15 × 10^7	3.60 ± 0.16 × 10^7	1.6 ± 0.89 × 10^7
14	EN 4	2.36 ± 0.85 × 10^7	2.04 ± 0.76 × 10^7	8.8 ± 0.49 × 10^7
15	EN 5	-	-	-
16	EN6	-	-	-
17	EN 7	2.08 ± 0.10 × 10^7 ±	1.96 ± 0.95 × 10^7	9.2 ± 0.45 × 10^7
18	EN 8	-	-	-
19	EN9	-	-	-
20	EN10	-	-	-
	CN	0	0	0

Key: Colony counts are written as Mean±Standard deviation of the triplicate plates, EN = Endophytic bacteria, EP = Epiphytic bacteria, CN= Control

Identification of the SEO degraders using VITEK

The Endophytic and Endospheric isolates were identified simultaneously with The *VITEK* 2 system's next-generation platform that provides greater automation while increasing safety and eliminating repetitive manual operations. Briefly, the following bacteria were identified, namely *Providencia rettgeri*, *Pseudomonas oleovorans*, *Kocuria kristinae*, *Pseudomonas aeruginosa* with the identity of 95%, 97%, 93% and 97%, respectively (Table 3)

Table 3: VITEK Identification Results of the SEO Degrading Isolates

S/No	Isolate	Gram Reaction	Identified Bacteria	% Identity
1.	EP 3	G-VE	*Pseudomonas aeruginosa*	97%
2.	EP 4	G+VE	*Kocuria kristinae*	93%
3.	EP 7	G-VE	*Providencia rettgeri*	95%
4.	EN 3	G-VE	*Pseudomonas oleovorans*	97%
5.	EN 4	G-VE	*Pseudomonas oleovorans*	97%
6.	EN 7	G-VE	*Providencia rettgeri*	95%

Changes in soil physicochemical properties before and after soil microcosm study

The physicochemical parameters of the soil sample, as shown in Table 4, indicate that in the control soil, the p-value (0.43) is greater than 0.05, indicating no significant difference in control soil parameters before and after the experiment. The p-value (0.04) is less than 0.05 in the treated soil, indicating significant differences in treated soil parameters after the experiment. This suggests that the treatment (engine oil degradation) improved these soil properties.

Table 4: Physiochemical Parameters of the Soil Microcosm

Control Soil Parameters	(Before)	(After)
pH	6.8	6.7
Electrical conductivity (EC)	90.00 mS/M	92.50mS/M
Organic carbon (OC)	0.85%	0.87%
Organic Matter (OM)	1.50%	1.52%
Nitrogen	0.10%	0.11%
Treated Soil parameters	**(Before)**	**(After)**
pH	6.5	6.6
Electrical conductivity (EC)	95.00 mS/M	101.22 mS/M
Organic carbon (OC)	0.80%	0.89%
Organic Matter (OM)	1.40%	1.53%
Nitrogen	0.9%	0.12%

Test for Soil Recovery

Germination % of cowpea for 10 days in control vs mesocosm

The recovery of the soil in the controlled bioremediation simulation using *Vigna unguiculata* was tested. The study results showed that the germination onset was delayed by inadequate soil moisture. The number of germinated cowpea seeds changes day by day. However, germination is delayed from day 1 to day 3 on samples, namely, negative control, EN3, Ep4, and EP7, as indicated in Table 5.

The germination results of cowpea seeds across different mesocosms in a soil recovery experiment can provide significant insights into the potential for soil recovery. In mesocosm studies, germination rates serve as bioindicators of the soil's ability to support plant growth, which indirectly reflects the health and recovery of the soil following contamination or treatment.

In the context of the mesocosm experiment, higher germination rates across EN7 to EP3 indicate that the soil is recovering and is in a better condition to support plant growth. This recovery is likely due to reduced contamination levels, improved microbial activity, and enhanced nutrient availability. Conversely, lower germination rates may indicate lingering soil contamination or poor soil health, limiting seed growth.

Table 5: Number of Cowpea Seeds that Germinated in the Various Mesocosms After Biodegradation Study

Samples 1D	Number of Germinated Cowpea Seeds at Treatment Days									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
+Ve control	1	3	4	6	6	7	7	7	7	7
-Ve control	0	0	0	0	1	1	1	1	1	1
Ep3	0	1	3	6	6	6	6	6	6	6
En3	0	0	0	1	2	3	3	3	3	3
Ep4	0	0	0	1	2	2	2	2	2	2
Ep7	0	0	0	1	2	2	2	2	2	2

Growth Parameter (Length of the Shoot) of the Cowpea Plants

The result for the growth parameter (Length) of the cowpea plants is illustrated in Figure 1 below. In evaluating the total shoot length of cowpea seedlings in various mesocosms can be analyzed through multiple biological and environmental factors to explain the differences in plant growth across samples.

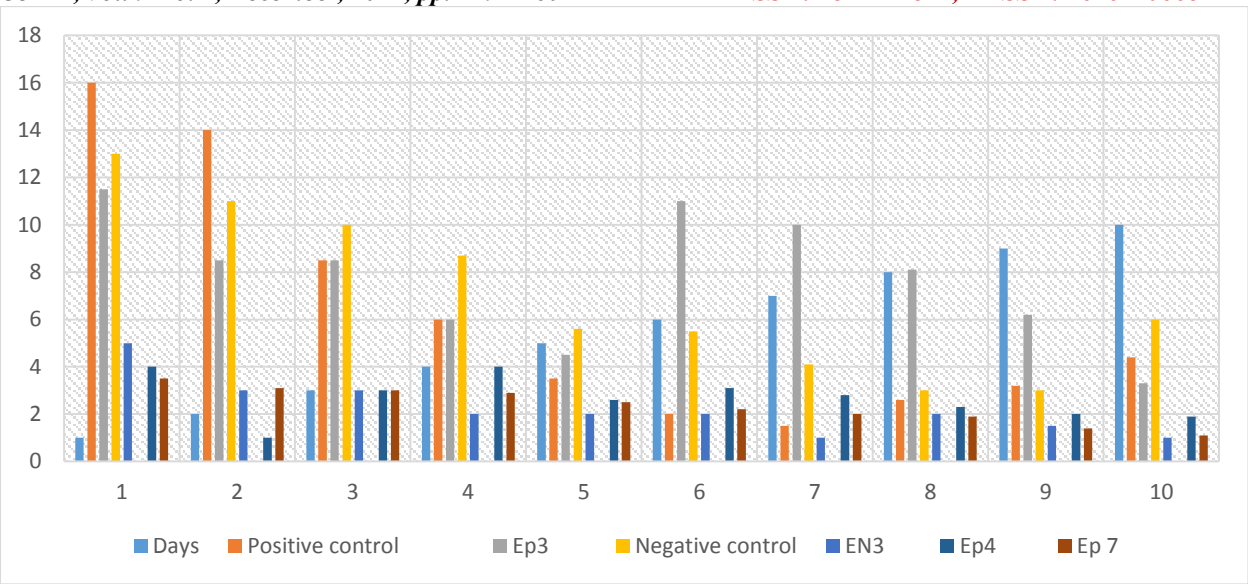

Figure 1: Total Shoot Length (Cm) of the Cowpea Seeds that Germinated in the Various Mesocosms

Regarding nutrient availability, the positive control (with values ranging from 16 cm to 4.4 cm) initially exhibited the highest growth rates. This suggests a well-balanced nutrient profile, where essential macronutrients (nitrogen, phosphorus, potassium) and micronutrients (iron, magnesium, etc.) are readily available to support the rapid development of cowpea seedlings.

In EP3 (11.5 cm to 3.3 cm), growth patterns suggest moderate nutrient availability, likely sufficient to sustain growth for a time but with potential depletion, especially at later stages (e.g., the final 3.3 cm). The negative control (13 cm to 6 cm) likely had lower nutrient availability, as the cowpea seedlings could not consistently maintain high growth rates. However, some nutrients might still be present, albeit at levels not optimized for plant development.

EN3 (5 cm to 1.51 cm) and EP7 (3.5 cm to 1.1 cm) had significantly lower growth, indicating a severe deficiency in critical nutrients, possibly caused by low fertility or depleted soils. This suggests that nutrient-poor conditions hindered seedling growth.

In terms of residual contaminants, in mesocosms like EN3 and EP7, where the total shoot lengths are particularly low, residual contaminants may be a major contributing factor. Heavy metals, pesticides, or other pollutants in the soil can reduce plant growth by inhibiting enzyme activities, disrupting photosynthesis, or interfering with root uptake of nutrients and water. If these mesocosms had a history of contamination, the toxicity of the soil could explain the poor growth. It's possible that seedlings in these environments struggled to detoxify harmful substances, leading to slower or stunted growth.

In terms of microbial activity, the positive control likely had optimal microbial activity, with beneficial microorganisms (e.g., rhizobacteria, mycorrhizal fungi) enhancing nutrient uptake and promoting plant growth. Microbial communities can play a significant role in nitrogen fixation and phosphorus solubilization, contributing to better plant health. EP3 may also have had microbial activity at a moderate level, supporting some growth, but the variations in shoot length suggest fluctuations in either microbial populations or their effectiveness over time.

In EN3 and EP7, there could be lower levels of beneficial microbes or even the presence of microbial communities that degrade organic matter less efficiently or cannot assist in detoxification, contributing to slower growth rates.

In terms of water and soil conditions, water availability and soil structure are critical to seedling development. The mesocosms with higher growth (positive control, EP3) likely had good soil moisture retention and aeration, allowing roots to grow and access nutrients efficiently.

On the other hand, the low-growth mesocosms (EN3, EP7) might have compacted soils or poor moisture retention, making it difficult for the plants to establish strong root systems and access sufficient water, further contributing to stunted growth.

Similarly, the findings of these study showed the broader implications for environmental recovery, as they demonstrate how varying environmental conditions can affect plant

growth and suggest potential pathways for ecosystem recovery.

The positive controls and EP3 treatments show how nutrient-enriched environments can support robust plant growth, which could be extrapolated to strategies for land rehabilitation. Introducing or enhancing nutrient availability through biofertilizers or organic amendments might improve plant establishment and ecosystem health in degraded areas.

Poor growth in EN3 and EP7 suggests that residual contaminants or degraded soil conditions hinder plant growth. This highlights the need for phytoremediation strategies or bioremediation techniques that target specific pollutants or improve soil health through microbial inoculation or organic amendments to restore these ecosystems.

The involvement of microbial activity underscores the importance of microbial inoculants in promoting plant growth in contaminated or nutrient-poor soils. Research on plant-microbe interactions should be expanded to develop microbial treatments that improve soil health, enhance nutrient uptake, and support environmental resilience in degraded landscapes.

However, understanding the factors that promote or hinder plant growth in these mesocosms contributes to the broader field of sustainable agriculture and land reclamation, emphasizing the need for holistic approaches that consider soil, water, microbial activity, and contamination in environmental recovery efforts.

DISCUSSION

Utilizing microorganisms to eliminate oil pollution from contaminated places is becoming more and more popular these days (Salisu and Ibrahim, 2024). Because alternative techniques, including surfactant washing and incineration, can produce more harmful substances and are not economically viable, this approach is becoming more and more common, as reported by Berkat *et al.*, (2023). Prince (2018) also outlined that numerous investigations have revealed that the majority of bacteria capable of breaking down petroleum hydrocarbons have been found in oil-contaminated locations. This study's main objective is to isolate and screen bacterial isolates from the phyllosphere of the plants in the oil-contaminated locations. The bacterial species (EP7, EN3, EP4, EP3, EN7, and EN4) were isolated from the phyllosphere in order to accomplish this. These species had degradation rates of 45.9%, 25.7%, 34.4%, and 33.1%, respectively, indicating efficient engine oil degradation. The three species with the highest degrading capacities—EP7, EN3, EP4, and EP3—among the six have been selected for further research. It has been observed that the phyllosphere of highly contaminated mechanic workshops frequently contains bacteria that break down hydrocarbons and that using hydrocarbons increases the population of bacteria that use hydrocarbons to grow (Undugoda *et al.*, 2018 and Xiangying et al., 2017).

The findings of this study showed that during the 10-day trial period at 1%, 2%, and 3% concentrations, there was a continuous increase in the population of total oil degraders. When the samples were tested at 1% concentration, EP 3 had the greatest colony count ($7.04 \times 10^7 \pm 32.35$ CFU/mL) and EN 7 had the lowest ($2.08 \times 10^7 \pm 10.35$ CFU/mL). The population of total oil degraders has been steadily increasing, which is in line with earlier research by Jane-Francis *et al*. (2008) and suggests that the addition of hydrocarbons encourages microbial activity. In fact, the results of Undugoda *et al*. (2018), who also showed that introducing hydrocarbons into contaminated settings can boost microbial growth, are consistent with the constant increase in the oil degraders observed in this research. This further implies that the isolates employed in this investigation have mechanisms of adaptation that enable them to flourish in settings rich in oil, probably because of their enzymatic capacity to degrade complex hydrocarbons. The increased degradation rates shown in our investigation, however, might have been caused by the particular bacterial isolates that were employed; each of them might have had a different metabolic route, and their biochemical profiles might have suggested that some of them were more effective at breaking down particular hydrocarbon chains than others. The results of the experiment showed that at a 2% concentration, EP 3 had the largest colony counts, 5.32 × 107 ± 35.30 CFU/mL, while EP 4 had the lowest, 1.68 × 107 ± 8.31 CFU/mL. Conversely, at a 3% concentration, the maximum number of colonies was 9.2 × 107 ± 4.54 CFU/mL (EN 7), while the minimum number of colonies was 1.3 × 107 ± 8.15 (EP 4). According to these findings, the majority of hydrocarbon degraders were found at the 1% concentration, with EP3, EP7, EN3, and EP4 showing the greatest degradation. The results of Jane-Francis *et al*. (2008), who found that oil-degrading bacteria counts ranged from 6 × 104 to 49 × 104 CFU/mL in contaminated samples compared to 0 to 14 × 10^4 CFU/mL in uncontaminated soil, were consistent with the experiment's findings that total oil degraders showed a gradual increase over time. The desorption of hydrocarbons in the soil sample caused by nutrients may be the

cause of the increase in the number of oil-degrading bacteria.

Through higher hydrocarbon solubility or a larger contact surface with hydrophobic substances, this desorption may result in an increase in microbial mineralization (Xingjian *et al.*, 2018; Rahman, 2022; Shamiyan *et al.*, 2015).

It is possible to promote microbial growth by adding hydrocarbons to soil sample preparations. Hydrocarbons can serve as an essential nutrient for the microbes living in the soil, promoting their growth (Danyelle *et al.*, 2016). They also found that the application of crude oil increased the total number of microbial communities in Arctic tundra soil. The treated soil had a microbial count of 41 × 10^7 CFU/g after 14 months, while the untreated soil had 7.5 × 10^5 CFU/g. Similarly, in kerosene-contaminated soil, Shamiyan *et al.* (2015) observed an increase in the overall bacterial count, which rose from 9 × 10^8 CFU/g to 9.6 × 10^8 CFU/g in just three weeks. Agamuthu *et al.* (2013) found that soil contaminated with used lubricating oil had an increase in hydrocarbon-consuming bacteria, with counts rising from 10.2 × 10^6 CFU/g to 80.5 × 10^6 CFU/g. In addition, Hanan *et al.* (2019) reported higher microorganism counts in a consortium that was utilized to biodegrade petroleum hydrocarbons, ranging from 6.14 × 10^7 to 3.5 × 10^8 CFU/g and Chukwugozie (2011) reported higher hydrocarbon degraders in soil samples taken from a mechanic workshop, from 1.25 × 10^4 to 6.25 × 10^5 CFU/g

Regarding the field of Biochemical Identification of Bacterial Isolates, a great deal of data has been published indicating the presence of *Pseudomonas* species in hydrocarbon-contaminated environments. Several investigations have demonstrated the prevalence of *Pseudomonas* species capable of breaking down SEO (Zhang *et al.*, 2014). The results showed that *P. aeruginosa* is highly degradable, consistent with Ijah and Antai's (2013). This could be because *Pseudomonas* spp. are widely used in the hydrocarbon breakdown process. Numerous studies have demonstrated the effectiveness of *Pseudomonas species* in degrading a wide range of hydrocarbons, including phenol (Haytham, 2016), biphenyl, toluene, P-Pxylene xyleneand benzenes (Kim *et al.*, 2022). As such, the discovery of *P. oleovorans* and *P. aeruginosa and their noteworthy capacity for degradation as shown in this investigation* are not unusual occurrences.

Pseudomonas oleovorans is a well-known hydrocarbon-degrading bacterium, especially when it comes to breaking down aromatic hydrocarbons therefore its identification as a dominating species in the phyllosphere is especially significant (Kim *et al.*, 2022). This could account for the increased rates of degradation seen in *Pseudomonas spp.*-dominated samples since these bacteria have enzymatic pathways that are specially tailored to the metabolism of hydrocarbons. *Providencia rettgeri*'s existence, despite its less frequent association with hydrocarbon degradation, raises the possibility of new pathways or adaptations that should be investigated further to improve bioremediation techniques.

The secret of microorganisms' capacity to degrade pollutants is their great flexibility. Still, there was scant proof of Providencia's deterioration of petroleum. *Providencia rettgeri* (EP7), which was isolated from the phyllosphere for this investigation, provided direct evidence and shown a good ability to degrade petroleum hydrocarbons. Additionally, it implies that *Providencia rettgeri* (EP7) needs more research in the future because it may be utilized in the bioremediation of contaminated soil and water. *Providencia rettgeri* was identified as the petroleum-degrading bacteria L1, which was isolated from petroleum (Obayori, 2008).

Another bacterial strain used in this study is *Kocuria sp.*, a genus that numerous researchers have shown to be capable of breaking down crude oil and its refined derivatives (Pietro *et al.*, 2024). *Kocuria* was able to use crude oil as its only source of carbon and energy, according to strain sp. 27/1 27/1. According to the biodegradation data, these bacteria, K.kristinae (EP4), may be employed in the partial bioremediation of habitats contaminated by crude oil. For the biodegradation process to be improved further, more research must be done (Pietro *et al.*, 2024).

However, microorganisms possess enzymatic systems that enable them to break down and use petroleum hydrocarbons as a source of carbon and energy (Chukwugozie, 2011). This is why the four bacterial isolates were able to decompose used oil.

The study's results showed that inadequate soil moisture delayed the germination process. The number of cowpea seeds sprouting changes every day. Table 5 demonstrates a germination delay from day 1 to day 3 for the EN3, EP4, EP7, and negative control samples. The ability of a seed to germinate with low soil moisture content has been shown to be influenced by crop species (Shamiyan *et al.*, 2015).

The observation of delayed germination in EN3, EP4, and EP7 between day 1 and day 3 implies that there can be lingering toxicity from the used engine oil in these samples even after the biodegradation process has taken place.

This is probably because some hydrocarbons have not completely broken down. This implies that even after bioremediation is completed, additional process optimization would be required to guarantee total soil detoxification and enhance the soil's compatibility for plant development.

The EP3 sample exhibited superior shoot growth, indicating that the soil has undergone more successful bioremediation, creating an environment that is more conducive to plant development. This suggests that EP3 not only efficiently broke down hydrocarbons but also helped to replenish the soil's nutritional balance, which is necessary for strong plant development. On the other hand, the incomplete degradation of some hazardous compounds may be the cause of the stunted development in EP7, which may prevent plants from absorbing nutrients. Barathi and Vasudevan (2011) have also highlighted the significance of thorough hydrocarbon degradation for the accomplishment of soil regeneration.

CONCLUSION

This study demonstrated the significant potential of phyllosphere bacteria in bioremediating spent engine oil-contaminated soils. Key bacterial isolates, including *Pseudomonas aeruginosa*, *Kocuria kristinae*, and *Pseudomonas oleovorans*, exhibited notable hydrocarbon degradation capabilities, with bacterial consortia achieving the highest improvements in soil physicochemical properties. These findings highlight the effectiveness of microbial consortia in enhancing soil recovery, with increased organic carbon, nitrogen content, and stabilized pH levels.

This highlights the viability of utilizing indigenous phyllosphere bacteria as a sustainable, cost-effective solution for remediating SEO-contaminated soils, particularly in resource-limited settings. Future research should optimize microbial consortia, evaluate their performance in field conditions, and explore their long-term impacts on soil health and agricultural productivity.

Recommendations for future research:

Based on the findings of this study, the following recommendations were given:

1) More research is needed to optimize the physiochemical conditions and culture of chosen consortia to fully exploit these bacteria's potential for large-scale wasted engine oil biodegradation in affected areas.
2) Various environmental factors that can support or improve the bacterial strains' capacity for degradation can be researched.
3) After bioremediation, additional process optimization would be required to guarantee total soil detoxification and enhance the soil's compatibility for plant development.
4) Additionally, the duration of the experiments can be increased to better examine how these bacterial isolates break down aliphatic and PAH compounds.
5) Also, since the literature on Providencia species' hydrocarbon degradation is scarce. *Providencia rettgeri* needs more research in the future because it may be utilized in the bioremediation of contaminated soil and water.

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