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Microbial-Assisted Nanoparticle Strategies for Bioremediation of Hydrocarbon-Contaminated Soils in Eastern Libya

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استراتيجيات الجسيمات النانوية المدعومة ميكروبياً للمعالجة الحيوية للتربة الملوثة بالهيدروكربونات في شرق ليبيا

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Abstract:
Hydrocarbon contamination represents a major environmental challenge in oil-producing regions, particularly in arid environments such as Eastern Libya, where natural attenuation processes are severely limited. This study evaluates the effectiveness of a combined nano-bioremediation approach using indigenous bacterial strains (Pseudomonas aeruginosa and Bacillus subtilis) and biosynthesized silver nanoparticles (AgNPs) for the remediation of diesel-contaminated soils collected from the Tobruk region. Laboratory experiments were conducted over a 28-day period under controlled conditions. Hydrocarbon degradation was assessed using gravimetric analysis, and statistical evaluation was performed to compare treatment efficiencies. The results revealed that the combined treatment achieved the highest degradation efficiency (70%), followed by nanoparticles alone (44%) and bacterial treatment alone (36%), whereas the control showed minimal degradation (8%). The enhanced performance of the combined system is attributed to the synergistic interaction between microbial metabolism and nanoparticle-induced improvements in hydrocarbon bioavailability. This study highlights the potential of nano-bioremediation as a sustainable and cost-effective solution for environmental restoration in arid regions.

Keywords: Nano-bioremediation, Hydrocarbon degradation, Biosynthesized silver nanoparticles, Arid environments, Eastern Libya.

الملخص
يُمثل التلوث الهيدروكربوني تحدياً بيئياً رئيسياً في المناطق المنتجة للنفط، لا سيما في البيئات الجافة مثل شرق ليبيا، حيث تكون عمليات التحلل الطبيعي محدودة للغاية. تقيم هذه الدراسة فاعلية نهج المعالجة الحيوية النانوية المدمجة باستخدام سلالات بكتيرية محلية (*Pseudomonas aeruginosa* و *Bacillus*

(*subtilis*) وبتلات الفضة النانوية المُصنعة حيوياً (*AgNPs*) لمعالجة التربة الملوثة بالديزل والمجمعة من منطقة طبرق. جرت التجربة المخبرية على مدار 28 يوماً تحت ظروف محكومة. تم تقييم تحلل الهيدروكربونات باستخدام التحليل الوزني، وأجري التقييم الإحصائي لمقارنة كفاءات المعالجة. أظهرت النتائج أن المعالجة المدمجة حققت أعلى كفاءة تحلل بنسبة (70%)، تليها المعالجة بالجسيمات النانوية وحدها بنسبة (44%)، ثم المعالجة البكتيرية وحدها بنسبة (36%)، بينما أظهرت مجموعة الشاهد أدنى معدل تحلل بنسبة (8%). يُعزى الأداء المحسّن للنظام المدمج إلى التفاعل التآزري بين الأيض الميكروبي والتحسينات التي تسببها الجسيمات النانوية في إتاحة الحيوية للهيدروكربونات. تُبرز هذه الدراسة إمكانية اعتبار المعالجة الحيوية النانوية حلاً مستداماً وفعالاً من حيث التكلفة لاستعادة البيئة في المناطق الجافة.

الكلمات المفتاحية: المعالجة الحيوية النانوية، تحلل الهيدروكربونات، جسيمات الفضة النانوية المُصنعة حيوياً، البيئات الجافة، شرق ليبيا.

Introduction

Petroleum hydrocarbons are among the most persistent and environmentally hazardous contaminants due to their complex chemical composition, hydrophobicity, and resistance to biodegradation. Their accumulation in soil ecosystems poses serious risks to environmental sustainability, agricultural productivity, and groundwater quality. This issue is particularly critical in oil-producing regions such as Eastern Libya, where frequent oil spills, pipeline leakages, and improper disposal practices have contributed to widespread soil contamination (Hussain et al., 2024). Furthermore, environmental pollution in Libyan ecosystems extends to various ecological matrices, driven by anthropogenic pressures and industrial accumulation, which necessitates advanced remediation frameworks (Salem, 2025; Salem & Salem, 2023).

In arid and semi-arid environments, the natural attenuation of hydrocarbons is significantly constrained by environmental factors such as low moisture content, high temperature fluctuations, and reduced microbial activity. These conditions slow down biodegradation processes and allow contaminants to persist for extended periods (Chauhan et al., 2023). To counteract soil degradation and improve ecological integrity, various organic and physical amendments such as biochar application, vermicompost integration, and agricultural waste recycling have been explored to enhance soil properties and moisture retention in arid lands (Abdala et al., 2024; Mahommed et al., 2025; Salem, Abdalah, & Mohamed, 2024; Salem et al., 2025, Abraik, et al.,2025, Mharb et al., 2025).

Bioremediation has emerged as a promising and sustainable strategy for addressing hydrocarbon contamination. This approach relies on the metabolic capabilities of microorganisms to transform complex organic pollutants into less toxic or mineralized products. Among hydrocarbon-degrading bacteria, *Pseudomonas aeruginosa* and *Bacillus subtilis* have been extensively studied due to their capacity to produce specific enzymes, such as monooxygenases and dioxygenases, which initiate the breakdown of hydrocarbon structures (Brzeszcz et al., 2024). Moreover, indigenous microbial strains and bioactive plant extracts or essential oils frequently demonstrate potent antimicrobial and enzymatic properties capable of targeting resilient environmental pollutants and pathogenic strains (Kadak & Salem, 2020; Salem, 2024; Salem et al., 2025; Soof et al., 2025, Abraik et al., 2025).

Despite its advantages, microbial bioremediation is often limited by the low bioavailability of hydrophobic hydrocarbons. These compounds tend to adsorb strongly onto soil particles, forming non-aqueous phase liquids that restrict microbial accessibility. Consequently, bioavailability remains a key limiting factor governing the rate and extent of biodegradation (Chauhan et al., 2023).

Recent advancements in nanotechnology and biosorption have introduced innovative solutions to overcome these limitations. Green synthesis approaches and novel biosorbents—such as root-derived biosorbent materials—offer effective alternatives for capturing organic and synthetic pollutants from contaminated media (Ahwaas et al., 2025). Nanoparticles, particularly silver nanoparticles (AgNPs), exhibit unique physicochemical properties, including a high surface area, catalytic activity, and strong interaction capabilities with organic pollutants. These properties enhance the dispersion and solubility of hydrocarbons, thereby increasing their accessibility to microbial cells (Muthukumar et al., 2024).

The integration of microbial processes with nanotechnology, termed nano-bioremediation, represents a significant advancement in environmental restoration. This approach exploits the synergistic interaction between microorganisms and nanoparticles, wherein nanoparticles improve pollutant bioavailability while microorganisms facilitate enzymatic biodegradation. Several recent studies have demonstrated that nano-bioremediation significantly enhances hydrocarbon removal efficiency compared to conventional methods (Zhao et al., 2024; Liu et al., 2024).

Therefore, this study aims to evaluate the effectiveness of combining indigenous bacterial strains (*Pseudomonas aeruginosa* and *Bacillus subtilis*) with biosynthesized silver nanoparticles for the remediation of diesel-contaminated soils from the Tobruk region in Eastern Libya. This research contributes to developing sustainable and efficient remediation strategies tailored to arid environments.

2. Materials and Methods

2.1 Soil Sampling

Representative soil samples were collected from multiple hydrocarbon-contaminated industrial and petroleum-impacted sites located in Tobruk, Eastern Libya. Sampling was performed at a depth of 10–20 cm to capture active microbial communities while minimizing surface UV degradation and volatile loss. The collected samples were immediately transferred into sterile containers, sealed securely, and transported to the laboratory under cold-chain conditions. Prior to experimental setup, the samples were homogenized, sieved through a 2-mm mesh to remove debris and large stones, and stored under sterile laboratory conditions to preserve baseline physicochemical and biological properties.

2.2 Isolation of Bacteria

Indigenous bacterial strains capable of tolerating and degrading petroleum hydrocarbons were isolated using standard serial dilution techniques. Aliquots of the soil suspension were inoculated onto selective mineral salt agar media supplemented with diesel fuel as the sole carbon and energy source, thereby enriching hydrocarbonoclastic populations. Following incubation at optimal temperatures, dominant colonies were purified through successive sub-culturing. Based on morphological, physiological, and biochemical screening, the predominant hydrocarbon-degrading species successfully isolated and identified were *Pseudomonas aeruginosa* and *Bacillus subtilis*.

2.3 Nanoparticle Synthesis

Silver nanoparticles (AgNPs) were green-synthesized using the extracellular microbial supernatants obtained from the bacterial cultures. Aqueous silver nitrate ($AgNO_3$) solution was mixed with the cell-free supernatant under controlled temperature and pH conditions. The active biomolecules present in the supernatant (such as proteins, enzymes, and secondary metabolites) acted as natural reducing and stabilizing agents, facilitating the prompt reduction of Ag^+ ions into stable, biosynthesized silver nanoparticles characterized by characteristic color changes and UV-Vis spectroscopy verification.

2.4 Experimental Design

A controlled microcosm experiment was established using diesel-contaminated soil to evaluate the remediation potential of individual and combined treatments. A total of four distinct experimental treatments were prepared in triplicate to ensure statistical reliability:

Treatment	Description
Control	Contaminated soil without any biological or nano-amendment
Bacteria	Contaminated soil inoculated exclusively with the indigenous bacterial consortium (*Pseudomonas aeruginosa* and *Bacillus subtilis*)
Nanoparticles	Contaminated soil treated exclusively with biosynthesized silver nanoparticles (AgNPs)
Combined	Contaminated soil treated with a synergistic mixture of both bacterial strains and biosynthesized silver nanoparticles

All experimental microcosms were maintained under uniform, controlled environmental conditions (constant moisture and ambient laboratory temperature) over a total experiment duration of 28 days.

2.5 Measurement of Hydrocarbon Degradation

The residual petroleum hydrocarbon content and overall degradation efficiency across all treatments were quantified using the standard gravimetric analysis method. At the end of the 28-day incubation period, residual diesel was extracted from the soil matrices using organic solvents (such as n-hexane). The solvent-extract mixture was thoroughly separated, and after complete evaporation of the solvent, the remaining extracted diesel weight was measured using an analytical balance. Total hydrocarbon degradation was subsequently determined by calculating the percentage weight loss relative to the initial contamination levels.

3. Results

3.1 Degradation Efficiency

The quantitative evaluation of petroleum hydrocarbon removal across the various experimental treatments over the 28-day incubation period is detailed in Table 1. The gravimetric analysis revealed substantial variations in residual diesel concentrations depending on the applied remediation strategy.

Table 1: Hydrocarbon Degradation

Treatment	Initial (g)	Final (g)	Degradation (%)
Control	5.0	4.6	8%
Bacteria	5.0	3.2	36%
Nanoparticles	5.0	2.8	44%
Combined	5.0	1.5	70%

As presented, the untreated control microcosm exhibited minimal mass reduction, achieving only 8% degradation (from an initial 5.0 g down to 4.6 g), which underscores the persistence of diesel hydrocarbons under natural attenuation in arid conditions. In contrast, introducing the indigenous bacterial consortium (*Pseudomonas aeruginosa* and *Bacillus subtilis*) raised the degradation efficiency to 36%, reducing the residual weight to 3.2 g. The application of biosynthesized silver nanoparticles (AgNPs) alone performed even more effectively, yielding a degradation rate of 44% (final weight of 2.8 g). Notably, the integrated nano-bioremediation treatment (Combined) achieved the highest overall pollutant removal performance, successfully degrading 70% of the initial hydrocarbon content and leaving a residual mass of only 1.5 g.

3.2 Statistical Analysis

To confirm the reproducibility and significance of the experimental outcomes, triplicate measurements were evaluated through descriptive statistical analysis, calculating the mean degradation percentages alongside their corresponding standard deviations (SD).

Table 2: Statistical Summary (Mean ± SD)

Treatment	Mean (%)	SD
Control	8	±1.2
Bacteria	36	±2.5
Nano	44	±2.1
Combined	70	±3.0

The statistical summary compiled in Table 2 reinforces the reliability of the observed trends, demonstrating minimal internal variability within the experimental replicates. The control group registered a mean efficiency of 8%±1.2, while the bacterial treatment recorded 36%±2.5. The nanoparticle-only treatment showed a mean degradation of 44%±2.1. Outperforming all individual modalities, the combined treatment registered a robust mean efficiency of 70%±3.0, confirming that the synergistic integration of microbial inoculants and biosynthesized silver nanoparticles delivers a statistically consistent and vastly superior enhancement in hydrocarbon remediation.

4. Discussion

The results obtained in this study clearly demonstrate that the integration of microbial activity with nanoparticle application significantly enhances hydrocarbon degradation efficiency. The minimal degradation observed in the control treatment confirms that natural attenuation alone is insufficient under arid environmental conditions, where microbial activity is limited by low moisture availability and reduced nutrient mobility (Hussain et al., 2024). Furthermore, environmental pollution in regional ecosystems is frequently exacerbated by industrial and anthropogenic stressors, emphasizing the critical need for advanced remediation frameworks (Salem, 2025; Salem & Salem, 2023).

The moderate degradation observed in the bacterial treatment highlights the ability of microorganisms to analyze the hydrocarbons through enzyme-dependent metabolic pathways. Species such as *Pseudomonas aeruginosa* and *Bacillus subtilis* are known to initiate hydrocarbon degradation through oxygenase-mediated reactions, which convert hydrophobic compounds into more polar intermediates that can be further metabolized (Brzeszcz et al., 2024). Additionally, native microbial isolates and bioactive extracts often exhibit potent enzymatic and antimicrobial properties that target persistent environmental contaminants (Kadak & Salem, 2020; Salem, 2024; Salem et al., 2025; Soof et al., 2025).

However, the efficiency of this process remains constrained by the limited bioavailability of hydrocarbons, as these compounds tend to strongly bind to soil matrices and form aggregates that reduce microbial accessibility (Chauhan et al., 2023). To improve soil structure, moisture retention, and overall ecological resilience in arid lands, various organic amendments and soil conditioners—such as biochar, vermicompost, and agricultural waste recycling—have been increasingly integrated into environmental management strategies (Abdala et al., 2024; Mahommed et al., 2025; Salem, Abdalah, & Mohamed, 2024; Salem et al., 2025).

The application of nanoparticles in this study resulted in a noticeable increase in degradation efficiency compared to bacterial treatment alone. This improvement can be attributed to the physicochemical properties of nanoparticles, including their high surface area and reactivity. Nanoparticles enhance hydrocarbon dispersion, reduce aggregation, and increase the surface contact between pollutants and microbial cells. This leads to improved mass transfer and facilitates microbial uptake of hydrocarbons (Muthukumar et al., 2024). Moreover, advanced green synthesis approaches and root-derived biosorbents provide effective, non-toxic alternatives for capturing and mitigating organic and synthetic pollutants from contaminated environments (Ahwaas et al., 2025).

The highest degradation efficiency observed in the combined treatment (70%) provides strong evidence of a synergistic interaction between microorganisms and nanoparticles. This synergy arises from a dual mechanism: nanoparticles enhance the bioavailability of hydrocarbons, while microorganisms actively degrade these compounds through enzymatic pathways. Such interactions significantly accelerate the overall degradation process compared to individual treatments.

These findings are consistent with recent studies demonstrating that nano-bioremediation systems outperform traditional bioremediation approaches. For instance, Zhao et al. (2024) reported enhanced petroleum hydrocarbon degradation through combined microbial–nanoparticle systems, while Liu et al. (2024) demonstrated that nanoparticle-assisted bioremediation significantly improves pollutant removal efficiency in contaminated soils.

Furthermore, the use of biosynthesized nanoparticles offers an environmentally sustainable alternative to chemically synthesized nanomaterials. Green synthesis methods reduce the potential toxicity associated with synthetic nanoparticles and align with current environmental regulations and sustainability goals.

From a broader perspective, this study highlights the importance of integrating biological and physicochemical approaches in environmental remediation. In arid regions such as Eastern Libya, where environmental conditions limit natural biodegradation, nano-bioremediation provides a practical and efficient solution for managing hydrocarbon contamination.

Overall, the results support the growing consensus that nano-bioremediation represents a next-generation technology capable of addressing complex environmental pollution challenges with higher efficiency and sustainability.

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