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Microbial Population Dynamics During Phytobioremediation of Hydrocarbon-Contaminated Swampy and Clay Soils

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Abstract

Soil contamination by petroleum hydrocarbons remains a critical environmental challenge, particularly in wetland and clay-rich ecosystems where natural attenuation processes are often limited. This study investigates the response of total heterotrophic bacteria (THB) during the bioremediation of hydrocarbon-contaminated swampy and clay soils amended with Moringa oleifera biomass and elephant grass (Pennisetum purpureum). The objective was to evaluate microbial population dynamics under varying amendment dosages and to identify treatment conditions favorable for total petroleum hydrocarbon (TPH) degradation. Laboratory-scale bioremediation experiments were conducted over 84 days using amendment dosages ranging from 20–100 g, alongside untreated control samples. THB counts were monitored at regular intervals using standard plate count techniques, while hydrocarbon-utilizing bacteria were isolated and identified. Results revealed that microbial populations increased significantly following contamination and amendment, with bacterial growth strongly influenced by amendment type, dosage, soil texture, and remediation duration. In all amended samples, THB counts increased progressively and reached peak values at the 56th day before declining, indicating substrate depletion and possible nutrient limitation. Maximum THB counts of 1.01 × 10^7 cfu/mL and 1.12 × 10^7 cfu/mL were recorded in swampy soil treated with 100 g of Moringa and elephant grass, respectively. In contrast, control samples exhibited continuous but comparatively lower microbial growth throughout the study period. Predominant hydrocarbon-degrading bacteria identified included Pseudomonas, Bacillus, and Proteus species. The findings demonstrate that plant-based amendments significantly enhance microbial proliferation and support effective bioremediation of hydrocarbon-polluted soils. This study highlights the potential of locally available plant materials as sustainable biostimulation agents for petroleum-impacted environments.

Keywords: Bioremediation, elephant grass, hydrocarbon-contaminated soil, *Moringa oleifera*, total heterotrophic bacteria

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INTRODUCTION

Petroleum hydrocarbon contamination of soils is a persistent environmental problem that results from crude oil exploration, transportation, storage, and refining activities. In oil-producing regions, accidental spills and chronic leakages lead to the widespread degradation of terrestrial and wetland ecosystems. These effects are particularly pronounced in swampy and clay soils, where low oxygen diffusion, high moisture content, and poor permeability limit natural biodegradation processes. Consequently, the restoration of such environments requires targeted remediation strategies to enhance microbial activity and hydrocarbon degradation efficiency [1].

Bioremediation strategies include biostimulation through the addition of organic or inorganic amendments, which significantly improve microbial growth and enzymatic activity. These amendments supply essential nutrients such as nitrogen and phosphorus, which are often deficient in hydrocarbon-impacted soils [2]. This process relies on the metabolic capability of indigenous microorganisms to transform petroleum hydrocarbons into less harmful substances. Changes in total heterotrophic bacterial (THB) populations reflect the availability of carbon sources, nutrient balance, and environmental suitability for microbial metabolism.

THB plays a critical role in soil ecological functions and serves as key indicators of microbial activity during bioremediation. The changes in THB populations reflect the availability of carbon sources, nutrient balance, and environmental suitability for microbial metabolism. Several studies have reported that hydrocarbon contamination initially suppresses microbial populations; however, subsequent adaptation and nutrient amendment often result in substantial microbial proliferation.

Plant-based amendments have recently attracted attention because of their biodegradability, nutrient content, and local availability. *Moringa oleifera* is known for its high nitrogen, phosphorus, and organic matter content, whereas elephant grass (*Pennisetum purpureum*) provides substantial biomass capable of stimulating rhizospheric microbial activity. These materials can enhance soil structure, moisture retention, and nutrient availability, thereby creating favorable conditions for hydrocarbon-degrading microorganisms [3].

Despite numerous studies on phytoremediation and biostimulation, limited comparative information exists on the microbial responses of different soil types, particularly swampy and clay soils, to plant-based amendments. Furthermore, the temporal evolution of THB populations under varying amendment dosages has not been sufficiently documented. Understanding these dynamics is essential for optimizing remediation timelines and amendment application rates [4].

Therefore, this study investigated the variation in THB during bioremediation of hydrocarbon-contaminated swampy and clay soils amended with *Moringa* and elephant grass. The specific objectives were to:

1. Evaluate THB population dynamics over time,
2. Assess the influence of amendment dosage on microbial growth,
3. Compare microbial responses between soil types, and
4. Identify dominant hydrocarbon-utilizing bacteria involved in the remediation process [5].

METHODOLOGY

Soil Sampling and Preparation

Swampy and clayey soil samples were collected from uncontaminated locations and transported to the laboratory in sterile containers. Soil samples were air-dried, homogenized, and sieved to remove debris. Baseline physicochemical and microbiological analyses were conducted before contamination.

Soil Contamination

The soil samples were artificially contaminated with crude oil to simulate petroleum pollution conditions. After contamination, the soils were stabilized for a defined acclimatization period to enable microbial adaptation [6].

Amendment Materials

Dried and pulverized *Moringa oleifera* biomass and elephant grass (*Pennisetum purpureum*) were used as organic amendments. Amendment dosages of 20, 40, 60, 80, and 100 g were applied to contaminated soils. Unamended contaminated soils served as control samples.

Experimental Design

Bioremediation experiments were conducted under laboratory conditions for 84 days. Soil samples were periodically mixed to ensure aeration and a uniform distribution of amendments. Moisture content was maintained at optimal levels throughout the study [7].

Microbiological Analysis

THB counts were determined using the standard plate count method. Serial dilutions of the soil samples were plated on nutrient agar and incubated at 37°C for 24–48 h. Bacterial colonies were enumerated and expressed as colony-forming units per mL (cfu/ml).

Isolation and Identification of Hydrocarbon-Utilizing Bacteria

Distinct colonies were isolated and purified by repeated sub-culturing. Identification was based on morphological characteristics and biochemical tests. The predominant hydrocarbon-degrading bacteria have been identified as *Pseudomonas, Bacillus,* and *Proteus* species [8].

RESULTS AND DISCUSSION

Figure 1 illustrates the variation in the THB populations during the remediation process, with *Pseudomonas*, *Bacillus,* and *Proteus* species identified as the dominant hydrocarbon-degrading microorganisms. Bacterial abundance increased progressively with remediation time and with increasing treatment dosage.

The microbial growth reached its maximum on the 56th day across all amendment levels (20–100 g). At this peak period, bacterial counts were 3.76×10^6, 5.91×10^6, 8.24×10^6, 9.34×10^6, and 1.01×10^7 cfu/ml for the 20 g, 40 g, 60 g, 80 g, and 100 g treatments, respectively. Beyond the 56th day, a decline in bacterial population was observed, with values reducing to 2.69×10^6, 3.91×10^6, 5.69×10^6, 6.46×10^6, and 8.17×10^6 cfu/ml for the corresponding treatment dosage samples on the 84th day.

Figure 2 shows the variation in THB counts in swampy soils treated with elephant grass. Bacterial populations increased rapidly with remediation time and with increasing amendment dosage, reaching peak values on the 56th day of the analysis. Beyond this period, a decline in bacterial count was observed in all treated samples, whereas the control sample exhibited a continuous increase throughout the study period.

The bacterial population in swampy soil prior to contamination was 1.76×10^3 cfu/ml, which changed to 1.84×10^2 cfu/ml following hydrocarbon contamination.

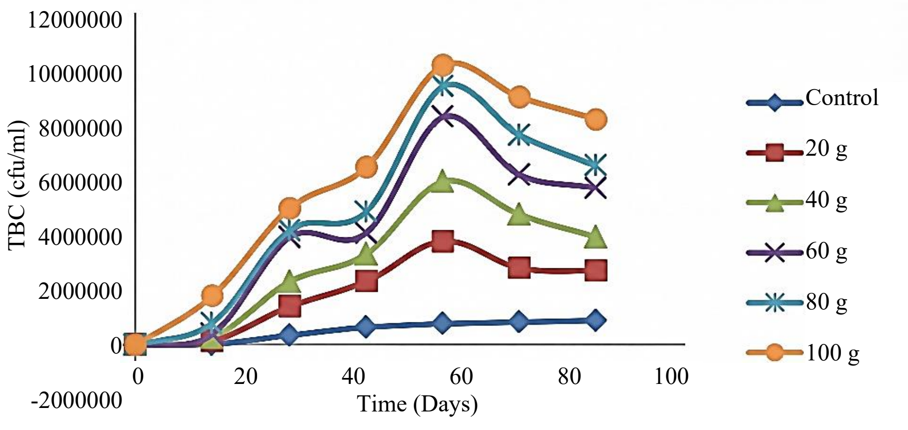

Figure 1. Variation in swampy soil with *Moringa*.

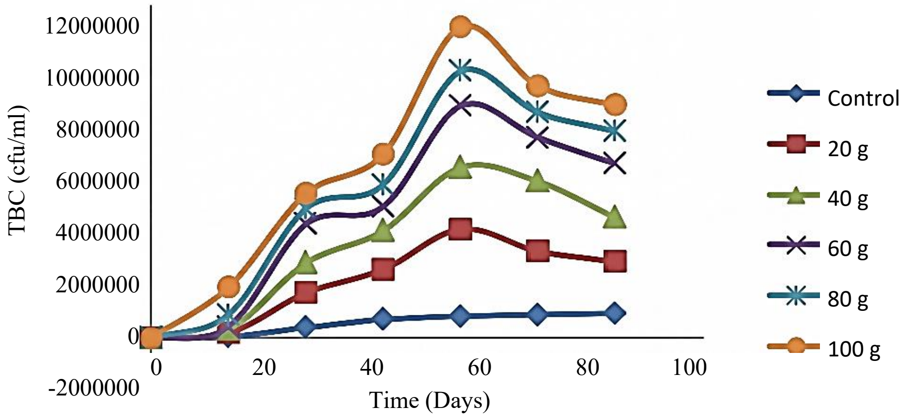

Figure 2. Variation in swampy soil with elephant grass.

Subsequent amendment with elephant grass resulted in substantial microbial proliferation, with maximum bacterial counts of 3.91×10^6, 6.14×10^6, 8.35×10^6, 9.59×10^6, and 1.12×10^7 cfu/ml recorded at the 56th day for the 20 g, 40 g, 60 g, 80 g, and 100 g treatment levels, respectively. After the 56th day, bacterial counts declined to 2.74×10^6, 4.33×10^6, 6.27×10^6, and 7.44×10^6 cfu/ml for the 20 g, 40 g, 60 g, and 80 g treatments, respectively, while the 100 g treatment decreased to 8.38×10^4 cfu/ml on the 84th day. In contrast, the control sample showed a continuous increase in bacterial population, reaching 8.82×10 cfu/ml by the end of the remediation period [9].

DISCUSSION

THB Dynamics in Swampy Soil Treated with *Moringa*

The THB counts increased with time and amendment dosage, reaching peak values on the 56th day across all treatments. Maximum THB values ranged from 3.76×10^6 cfu/ml (20 g) to 1.01×10^7 cfu/ml (100 g). The subsequent decline observed on day 84 suggests nutrient depletion and a reduction in bioavailable hydrocarbons. The control samples exhibited continuous but lower microbial growth.

THB Dynamics in Swampy Soil Treated with Elephant Grass

A similar trend was observed, with higher peak THB values compared with the *Moringa* treatments. The maximum THB of 1.12×10^7 cfu/ml at a 100 g dosage indicates strong biostimulatory effects of elephant grass [10].

THB Dynamics in Clay Soil

Clay soils showed comparatively lower THB counts than swampy soils, which was attributed to the reduced permeability and oxygen diffusion. Nonetheless, both amendments significantly enhanced microbial growth, with peak populations observed on day 56.

Comparative Assessment

Overall, elephant grass demonstrated a slightly higher microbial stimulation than *Moringa*, whereas swampy soils supported greater microbial proliferation than clay soils. The dominance of *Pseudomonas, Bacillus,* and *Proteus* aligns with previous reports on hydrocarbon biodegradation [11].

CONCLUSION

This study demonstrated that plant-based amendments significantly enhanced the THB populations during the bioremediation of hydrocarbon-contaminated swampy and clay soils. The application of *Moringa oleifera* and elephant grass resulted in pronounced microbial proliferation, with bacterial populations increasing progressively and peaking on the 56th day of remediation at all amendment dosages. The subsequent decline in microbial counts indicates effective hydrocarbon utilization and possible nutrient depletion at the later stages of remediation. In contrast, the untreated control samples exhibited continuous but comparatively low microbial growth, underscoring the importance of biostimulation in accelerating biodegradation processes.

The results further revealed that amendment dosage plays a critical role in microbial dynamics, with higher dosages supporting greater bacterial populations. Swampy soils consistently had higher THB counts than clay soils, highlighting the influence of soil texture and moisture content on microbial activity. The identification of *Pseudomonas, Bacillus,* and *Proteus* confirmed their central role in hydrocarbon degradation under biostimulated conditions.

Overall, these findings support the use of locally available plant materials as effective and sustainable amendments for soil bioremediation. Optimizing the amendment dosage and remediation duration is essential for maximizing microbial activity and hydrocarbon removal efficiency. This study provides valuable insights into the design of environmentally sustainable remediation strategies for petroleum-impacted ecosystems.

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