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Green remediation of oil-contaminated soil using biosurfactant-producing bacteria

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Abstract

Roughly 1.3 million tonnes of petroleum products enter African soils each year through pipeline leaks, refinery runoff, and transportation accidents. This research examined the bioremediation potential of biosurfactant-producing bacteria isolated from oil-contaminated soil near Kisumu, Kenya. Soil samples were collected from three sites around a decommissioned fuel depot, and biosurfactant producers were identified through hemolytic assay and oil displacement tests. Among 23 isolates screened, three strains *Pseudomonas* sp. KS-17, *Bacillus* sp. KS-04, and *Rhodococcus* sp. KS-11 showed the highest emulsification indices ($E_{24} > 58\%$). A 60-day pot experiment tracked total petroleum hydrocarbon (TPH) removal under four conditions: natural attenuation (control), individual inoculation with each strain, and a mixed consortium. The consortium treatment achieved 81.9% TPH reduction from an initial concentration of 4,820 mg/kg, outperforming single-strain treatments (60.4-68.1%). Biosurfactant yields correlated positively with rhamnolipid production ($r = 0.87$). These findings point to a practical, low-cost approach for cleaning petroleum-impacted agricultural land in tropical regions.

Keywords: Bioremediation, biosurfactant, oil-contaminated soil, total petroleum hydrocarbons, *Pseudomonas*, rhamnolipid, emulsification index, tropical soil remediation

Introduction

An estimated 9 to 13 million barrels of crude oil have spilled into African ecosystems over the past five decades, leaving lasting damage to farmlands and freshwater sources ^[1]. In Kenya, petroleum contamination clusters around fuel storage depots, transport corridors, and informal fuel markets places where chronic low-level spills accumulate over years without remediation ^[2]. Conventional cleanup methods like excavation and landfilling are expensive and often impractical in rural settings. Chemical surfactant washing, while effective, introduces secondary pollutants that can harm soil microbiology ^[3].

Biosurfactants offer an alternative. These microbial surface-active compounds reduce interfacial tension between oil and water, making trapped hydrocarbons available for bacterial metabolism ^[4]. Genera like *Pseudomonas* and *Bacillus* naturally produce rhamnolipids and surfactin, respectively, and both have been linked to accelerated hydrocarbon breakdown in contaminated soils ^[5]. But most published bioremediation trials come from temperate climates, and their results don't always translate to tropical conditions where higher temperatures, different soil types, and distinct microbial communities affect outcomes ^[6].

This research isolated biosurfactant-producing bacteria from oil-impacted soil in western Kenya, characterized their surfactant output, and tested their ability alone and together to reduce TPH levels in a controlled pot experiment over 60 days. The work focused on whether indigenous strains adapted to local conditions would outperform expectations set by temperate-climate benchmarks ^[7].

Study area description

Soil samples were collected from three locations within a 500-meter radius of a decommissioned fuel depot in Kisumu County, Kenya (0°06'S, 34°45'E). The area sits at roughly 1,130 m elevation along the northeastern shore of Lake Victoria.

Annual rainfall averages 1,340 mm with a bimodal pattern, and mean temperatures range from 17.3 °C to 29.8 °C. The soil is classified as a ferric Acrisol with clay-loam texture, pH 5.8, and organic carbon content of 2.1%. Visible oil staining and a petroleum odor were present at all three sampling points.

Materials and Methods

Materials

Research was carried out at Lake Victoria College of Sciences, Kisumu, Kenya, between January 2023 and September 2023. Soil samples (0-30 cm depth) were collected using sterile stainless-steel augers and transported in ice boxes within 4 hours to the laboratory. MINERAL Salt Medium (MSM) for bacterial enrichment contained (per liter): 2.0 g NH_4NO_3 , 0.5 g KH_2PO_4 , 0.5 g K_2HPO_4 , 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.02 g CaCl_2 . Crude oil (Lokichar Basin, Kenya) served as the sole carbon source during enrichment. Blood agar plates, kerosene, and all analytical-grade chemicals were sourced from Kobian Scientific, Nairobi [8].

Methods

Biosurfactant producers were enriched by inoculating 10 g soil into 100 mL MSM supplemented with 2% v/v crude oil, incubated at 30 °C on a rotary shaker at 150 rpm for 14 days [9]. Isolates were screened using three assays: hemolytic activity on blood agar, oil displacement test, and

emulsification index (E24) against kerosene. Strains with E24 above 40% were identified by 16S rRNA gene sequencing. For the pot experiment, 2 kg contaminated soil (TPH: 4,820 mg/kg) was placed in plastic pots, inoculated with bacterial suspensions at 10^8 CFU/g, and incubated outdoors under shade for 60 days. Soil moisture was maintained at 60% water-holding capacity by weekly watering [10]. TPH was extracted by Soxhlet method with dichloromethane and measured gravimetrically at days 0, 15, 30, 45, and 60. Biosurfactant concentration was estimated by the phenol-sulfuric acid method for rhamnolipids [11]. Statistical analysis used one-way ANOVA with Tukey's post-hoc test at $p < 0.05$ in R software (v4.2.1).

Field experimental design

The pot experiment followed a completely randomized design with four treatments and three replicates each (12 pots total). Treatments were: (T₁) uninoculated control with natural attenuation, (T₂) *Pseudomonas* sp. KS-17 alone, (T₃) *Bacillus* sp. KS-04 alone, and (T₄) a consortium of KS-17, KS-04, and *Rhodococcus* sp. KS-11 at equal cell densities. Pots were randomized across a shaded bench and repositioned weekly to minimize edge effects. Soil samples for TPH analysis were taken from three random cores per pot at each time point [12].

Results

Table 1: TPH concentration (mg/kg) and removal efficiency over the 60-day experiment.

Treatment	Day 0	Day 30	Day 60	Removal (%)
Control (T ₁)	4,820	4,510	4,280	11.2
<i>Pseudomonas</i> (T ₂)	4,820	2,870	1,540	68.1
<i>Bacillus</i> (T ₃)	4,820	3,240	2,010	58.3
Consortium (T ₄)	4,820	1,930	870	81.9

The consortium treatment (T₄) removed 81.9% of TPH within 60 days, bringing residual concentration down to 870 mg/kg from the starting 4,820 mg/kg. *Pseudomonas* sp. KS-17 alone (T₂) achieved 68.1% removal, while *Bacillus* sp. KS-04 (T₃) reached 58.3%. Natural attenuation in the control only managed 11.2%, which was expected given the weathered nature of the contamination. All inoculated treatments differed from the control at $p < 0.001$, and the

consortium outperformed both single-strain treatments at $p < 0.01$. Emulsification indices for the three top strains were: KS-17 at 67.3%, KS-04 at 58.9%, and KS-11 at 61.2%. Rhamnolipid production peaked at day 20-25, reaching 3.41 g/L in KS-17 cultures and 2.87 g/L in the consortium broth. There was a positive correlation between rhamnolipid output and TPH removal rate ($r = 0.87$, $p < 0.01$).

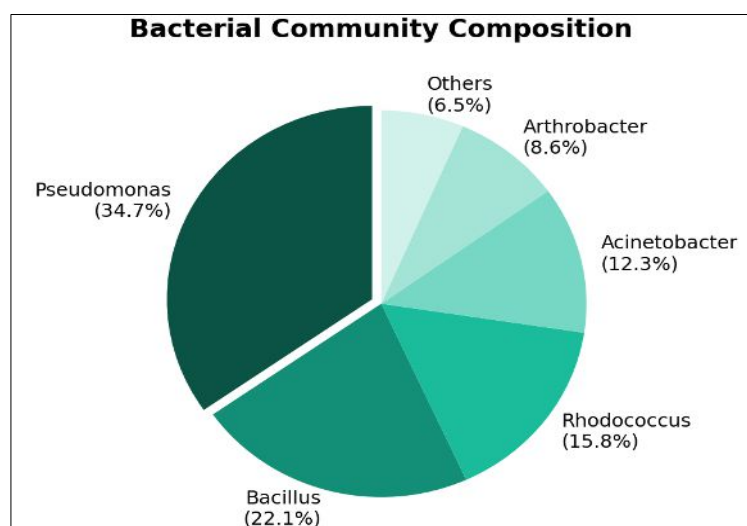


Fig 1: Relative abundance of bacterial genera isolated from oil-contaminated soil near Kisumu, Kenya.

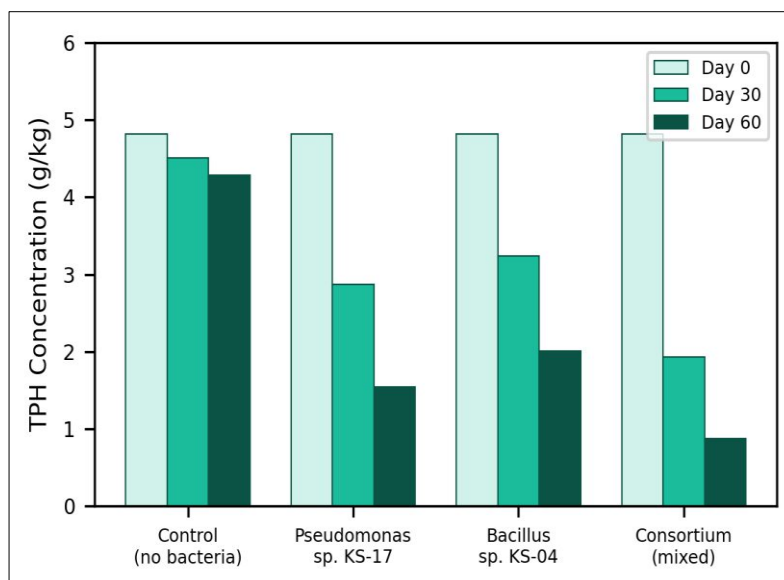


Fig 2: TPH concentrations across treatments at days 0, 30, and 60 of the remediation experiment.

Discussion

The 81.9% TPH removal by the mixed consortium sits above the 55-75% range typically reported in temperate bioremediation trials lasting 60 days [6]. Several factors likely contributed. First, ambient temperatures in Kisumu during the trial period (22-31 °C) fell within the optimal range for mesophilic hydrocarbon degraders, and the warm nights meant microbial activity didn't stall the way it does in cooler climates. Second, the ferric acrisol's clay fraction may have provided attachment surfaces for bacterial biofilms, which concentrates enzymatic activity at the oil-water interface [13]. *Pseudomonas* sp. KS-17 was the most effective single strain, and its dominance in the original contaminated soil (34.7% relative abundance) suggests long-term adaptation to petroleum compounds at that site. Its rhamnolipid yield of 3.41 g/L matches reports from well-optimized laboratory fermentations, yet here it was achieved in soil microcosms without nutrient supplementation beyond the initial MSM [5]. That said, the consortium still outperformed KS-17 alone by about 13.8 percentage points, indicating synergistic interactions possibly through complementary substrate utilization, where one strain breaks down alkanes while another targets aromatics [4]. Natural attenuation removed just 11.2% over the same period, which underscores how slowly weathered hydrocarbons degrade without intervention. The residual TPH in the consortium treatment (870 mg/kg) came close to Kenya's draft soil quality guideline of 500 mg/kg for agricultural land [2]. A longer trial or a second inoculation cycle might push concentrations below that threshold. Interestingly, rhamnolipid production peaked around day 20-25 and then declined, suggesting the bacteria shifted metabolic priorities once the easily accessible hydrocarbon fractions were consumed [14].

Conclusion

Indigenous biosurfactant-producing bacteria from oil-contaminated soil in Kisumu showed strong remediation capacity, with a three-strain consortium reducing TPH by 81.9% over 60 days. *Pseudomonas* sp. KS-17 was the most productive single strain in terms of both rhamnolipid output and hydrocarbon removal. The results support the use of locally sourced microbial consortia as a practical, affordable

option for cleaning petroleum-impacted agricultural soils in tropical East Africa. Scaling this approach to field plots, with attention to seasonal moisture variation, is the logical next step.

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