

Research Article

Kinetics and Microbial Population Dynamics of Leaf-Biostimulated Crude Oil Bioremediation in Contrasting Soils

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Abstract

Crude oil contamination remains a persistent environmental burden in petroleum-producing regions, and low-cost, locally sourced plant-leaf biostimulants offer a promising route to accelerate hydrocarbon-degrading microbial activity in impacted soils. This study evaluated the bacterial and fungal population dynamics accompanying biostimulated bioremediation of crude-oil-contaminated sandy, loamy, and clay soils amended with room-dried or sun-dried *Dacryodes edulis*, *Canarium schweinfurthii*, and *Persea americana* leaves at 50 g or 100 g doses, monitored over 42 days across 36 treatment bioreactors and three unamended controls. First- and second-order kinetic models were fitted to total petroleum hydrocarbon (TPH) depletion data, and total heterotrophic bacterial (THB) and fungal (THF) counts were enumerated in parallel. First-order kinetics best described the majority of treatments (R^2 generally > 0.98), with rate constants of $0.035\text{--}0.067\text{ day}^{-1}$ and half-lives of 10.3–19.8 days. Soil type was the dominant determinant of TPH removal (ANOVA, $F = 612.5$, $p < 0.001$), with mean removal following sandy (93.2%) $>$ loamy (88.6%) $>$ clay (75.5%) soil, while biostimulant species, drying method, and dose showed no statistically significant effect. All biostimulated treatments substantially outperformed unamended controls (2.4- to 2.7-fold higher removal). THB counts peaked around Day 28 before declining, and this bacterial fold-increase correlated significantly with TPH removal ($r = 0.49$, $p = 0.0026$), whereas fungal population growth did not ($r = 0.01$, $p = 0.96$). These findings indicate that soil texture, more than biostimulant identity, governs degradation outcome, and that bacterial rather than fungal proliferation drives hydrocarbon removal under these leaf-based biostimulation regimes.

Keywords

Bioremediation, Biostimulation, Crude Oil Degradation, Soil Texture, Hydrocarbon-utilizing Bacteria

1. Introduction

Crude oil exploration, production, and transportation remain central to the economies of major oil-producing nations, yet these same activities are responsible for chronic and often severe contamination of terrestrial ecosystems [1]. Accidental spillage, pipeline vandalism, and operational discharges intro-

duce complex mixtures of aliphatic and aromatic hydrocarbons and heavy metals into soil, where they persist, alter soil physicochemical properties, and suppress the growth and diversity of indigenous soil biota [2, 3]. In petroleum-producing regions of the Niger Delta and comparable tropical environments, this contamination is a recurring and largely unresolved

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Received: 4 September 2026; Accepted: 16 September 2026; Published: 29 September 2026



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environmental burden, motivating continued research into remediation strategies that are simultaneously effective, affordable, and environmentally benign [4].

Conventional remediation approaches, including incineration, soil vapour extraction, solidification/stabilization, and soil washing, are capable of removing petroleum hydrocarbons from contaminated soil, but are frequently cost-prohibitive at field scale and can generate secondary waste streams or residual toxicity that further compromises soil quality [5]. Bioremediation, which harnesses the catabolic capacity of indigenous or introduced microorganisms to transform hydrocarbons into less harmful metabolites, has consequently emerged as a comparatively low-cost and environmentally compatible alternative [6]. Bacteria remain the most extensively documented hydrocarbon-degrading organisms in contaminated soils, but filamentous fungi also contribute substantially to the breakdown of higher-molecular-weight and more recalcitrant hydrocarbon fractions, such that a full assessment of bioremediation efficacy requires the population dynamics of both microbial groups to be tracked concurrently rather than considered in isolation [7].

Among the available bioremediation strategies, biostimulation, the deliberate amendment of contaminated soil with nutrients, organic matter, or other growth-promoting substrates to accelerate the proliferation and catabolic activity of indigenous hydrocarbon degraders, has attracted particular research interest because it works with, rather than against, the existing soil microbiome [8]. Amendments of this kind can improve soil pH buffering, aeration, nutrient bioavailability, and enzymatic activity, collectively creating conditions more favourable to microbial proliferation and hydrocarbon turnover. A growing body of work has demonstrated the biostimulatory potential of low-cost organic wastes: Wokem and Madufuro [9] showed that cow dung outperformed sawdust as a biostimulant for diesel-contaminated soil, while Nordin et al. [10] reported substantial increases in soil microbial counts and activity following amendment with *Conocarpus* and *Tamarix* leaf material in Kuwaiti soils. Comparable results have been reported for plant-leaf amendments in Nigerian contexts: Johnson [11] achieved more than 50% reduction in soil hydrocarbon and heavy-metal content within forty days using *Vernonia galamensis* and *Vernonia amygdalina* leaf extracts, and Wylie and Merrell [12] demonstrated that ground Neem (*Azadirachta indica*) leaves promoted the proliferation of hydrocarbon-utilizing *Pseudomonas* populations in contaminated soil, an effect attributed in part to the nitrogen and phosphorus content of the leaf material.

Despite this expanding evidence base, systematic studies that quantify how specific plant-leaf biostimulants shape the population dynamics of both bacteria and fungi over the course of bioremediation, as distinct from simply reporting an endpoint reduction in total petroleum hydrocarbon (TPH) concentration, remain comparatively scarce. This is a substantive

gap: because bacterial and fungal degraders differ in growth rate, substrate preference, and tolerance to hydrocarbon toxicity, the trajectory and relative balance of these two populations over time can be as informative as the final degradation outcome, offering insight into which microbial guild is driving remediation at a given stage and under a given treatment (Antón-Herrero et al. [13]. Ukpaka et. al. [14] Recent reinforces this point: a comparative field study in Port Harcourt found that organic bioremediation agents produced markedly different trajectories of hydrocarbon-utilizing bacterial and fungal population growth over a three-to-six-month monitoring period, with the relative performance of the two microbial groups shifting according to amendment type and exposure duration, underscoring the value of tracking both populations rather than either alone.

The organic biostimulants examined to date are drawn from a comparatively narrow set of materials, chiefly animal wastes, common agricultural residues, and a small number of previously studied leaf types such as Neem, *Vernonia*, *Conocarpus*, and *Tamarix*. *Dacryodes edulis* (African pear), *Canarium schweinfurthii* (African bullet pear), and *Persea americana* (avocado), by contrast, are cultivated extensively across Nigeria and other tropical regions and generate substantial leaf litter as an agricultural by-product, yet their potential as biostimulants for hydrocarbon-degrading soil microbiota has not, to the authors' knowledge, been systematically investigated or compared within a single study. Given that leaf litter composition, including nitrogen and phosphorus content, lignin and cellulose fractions, and phytochemical constituents, is known to vary substantially between plant species and to influence the magnitude and timing of microbial proliferation during biostimulation, a comparative evaluation of these three underexplored, locally abundant leaf materials is both timely and practically relevant to the development of low-cost, tropically adapted bioremediation strategies.

This study therefore investigates the bacterial and fungal population dynamics that occur during the biostimulated bioremediation of crude-oil-contaminated soil amended separately with *Dacryodes edulis*, *Canarium schweinfurthii*, and *Persea americana* leaf material. Bacterial and fungal counts are monitored over the course of treatment across contrasting soil textures (sandy, loamy, and clay) to establish how biostimulant type and soil physicochemical context jointly shape the magnitude, timing, and relative balance of microbial population growth. By resolving these dynamics rather than reporting only an endpoint hydrocarbon reduction, this work aims to clarify the mechanistic basis by which these three previously unexamined, regionally abundant plant-leaf materials support hydrocarbon-degrading soil microbiota, and to provide an evidence base for their practical application as low-cost biostimulants in tropical, petroleum-impacted soils.

2. Materials and Methods

2.1. Materials

Three soil types (sandy, loamy, and clay) were collected locally from the Rivers State University, air-dried, and sieved to a uniform particle size of 0.25 mm prior to use. Fresh leaves of *Dacryodes edulis* (African pear), *Canarium schweinfurthii* (African bush/bullet pear), and *Persea americana* (avocado pear) were room-dried for three days and crushed to a fine powder to serve as biostimulants. Crude oil obtained from Otakikpo Flow Station and then transported to the Department of Chemical/Petrochemical Engineering Laboratory in Rivers State University Port Harcourt of Nigeria for the purpose of analysis was used as the contaminant. Reagent-grade chemicals (potassium dichromate, concentrated tetraoxosulphate(VI) acid, ferrous sulphate, ascorbic acid, ammonium fluoride, hydrochloric acid, potassium sulphate, sodium hydroxide, boric acid, methylene chloride, anhydrous sodium sulphate, and silica gel) were used for physicochemical analysis without further purification.

2.2. Experimental Design

A total of 39 bioreactors were set up, comprising 36 treatment reactors and 3 unamended controls (one per soil type). Each reactor contained 1 kg of sieved soil (sandy, loamy, or clay) artificially contaminated with 100 mL of crude oil. Contaminated soils were amended with either 50 g or 100 g of powdered *D. edulis*, *C. schweinfurthii*, or *P. americana* leaf biostimulant, thoroughly mixed to ensure homogeneity, and all treatments were run in duplicate to minimize experimental bias. Prior to amendment, baseline soil conditions, including total petroleum hydrocarbon (TPH) content, pH, electrical conductivity, and microbial activity, were determined. The experiment was monitored over a 42-day period, with soil samples withdrawn every 7 days (Six sampling points after the Day-0 baseline) for analysis of TPH depletion and microbial proliferation.

2.3. Physicochemical Analysis

The crude oil, the three soil types, and the three leaf-based biostimulants were physicochemically characterized prior to the bioremediation trial. Crude oil was analyzed for TPH, temperature, pH, total organic carbon (TOC), and nitrogen, phosphorus, and potassium content, while the soils and biostimulants were analyzed for the parameters described below.

Total organic content (TOC) was determined by wet oxidation, in which 1 g of soil was digested with potassium dichromate and concentrated tetraoxosulphate(VI) acid, back-titrated against 0.5 N ferrous sulphate using diphenylamine indicator, with a reagent blank run in parallel for standardization; TOC was calculated from the titre volumes normalized to sam-

ple weight. Phosphorus concentration was determined following APHA Method 4500-P APHA [15], by extracting 1 g of soil with 0.025 N HCl/0.03 N NH₄F, developing colour with an ascorbic acid–molybdate/tartrate reagent, and reading the resulting complex after 30 min.

$$\text{TOC} = \text{Blank} - \left(\frac{\text{volume of soil sample titre} \times 0.195}{\text{weight of soil sample}} \right) \quad (1)$$

Nitrogen content was determined by the micro-Kjeldahl digestion–distillation method following APHA Method APHA [15], with digestion in concentrated H₂SO₄ using K₂SO₄ as catalyst, steam distillation into boric acid, and titration of the distillate against standardized 0.01 N H₂SO₄ [16]

$$\text{N}(\%) = \left(\frac{(T-B)(N \times 1400)}{S} \right) \times 100\% \quad (2)$$

Moisture content was determined gravimetrically by oven-drying 10 g of soil at 105 °C for 24 h to constant weight. The degree of moisture content in terms of percentage is expressed as equation (3) [17, 18]

$$\text{MC}(\%) = \left(\frac{W_1 - W_2}{W_1} \right) \times 100\% \quad (3)$$

Where, Mc denotes the moisture content (%), W₁ denotes clay soil initial weight (g) before drying of sample and W₂ denotes clay soil final weight (g) of dried sample.

Electrical conductivity was measured directly on soil–water suspensions using a calibrated conductivity meter, with the electrode rinsed between samples to avoid cross-contamination. Soil pH was measured on a 1: 5 soil-to-distilled-water suspension using a glass-electrode pH meter calibrated against pH 4, 7, and 9.2 buffers, following ASTM [19, 20]; pH values were classified from extremely acidic (<4.5) to extremely alkaline (>9.0) according to standard soil pH classification bands.

Total petroleum hydrocarbon (TPH) content was determined following ASTM [21], which was originally developed for aqueous samples, to the soil matrix used in this study. Soil samples were serially extracted with methylene chloride in a separating funnel, the combined extracts were passed through a drying column of cotton wool, anhydrous sodium sulphate, and silica gel, concentrated under nitrogen, and analyzed by gas chromatography with flame ionization detection (GC-FID; HP 5890 Series II). TPH depletion over the incubation period was expressed as percentage reduction relative to the initial concentration.

$$\text{TPH}(\%) = \left(\frac{\text{TPH}_1 - \text{TPH}_2}{\text{TPH}_1} \right) \times 100 \quad (4)$$

Where, TPH denotes percentage of TPH removed per unit time (%), TPH₁ denotes initial concentration of TPH in PPM, TPH₂ denote step stage concentration of TPH in PPM.

2.4. Microbiological Analysis

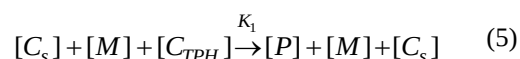
Total heterotrophic bacterial and fungal counts, together with hydrocarbon-utilizing microbial populations, were enumerated by the standard pour-plate technique. Ten-fold serial dilutions of each soil sample were prepared, and 0.1 mL aliquots were inoculated onto nutrient agar (for heterotrophic bacteria), acidified potato dextrose agar containing streptomycin (1 mg/100 mL) (for fungi), and mineral salt agar (MSA) (for hydrocarbon-degrading organisms). The mineral salt medium, formulated after Mills et al. [22] as modified by Okpokwasili and Amanchukwu [23], contained (g/L distilled water): NaCl, 10; MgSO₄ · 7H₂O, 0.42; KCl, 0.29; K₂HPO₄, 1.2; KH₂PO₄, 0.83; NaNO₃, 0.42; and Agar-Agar, 15, adjusted to pH 7.2 and supplemented with 2 mL of petrol/diesel as sole carbon source. Nutrient agar plates were incubated at 37 °C for 24 h, while potato dextrose agar plates were incubated at room temperature for 3–5 days. Colonies were counted and expressed as colony-forming units per gram of soil (cfu/g).

2.4.1. Biokinetic Model

The substrate Kinetic model, microbial Kinetic model, first order Kinetic model and second order Kinetic model and second order Kinetic model was considered for the determination of the functional parameters and coefficient of the crude oil degradation upon the action of the remediates and microbial activities in each bioreactor set-up.

2.4.2. Bio Kinetics of First Order Reaction

The kinetics of the first order reaction in this case was established by considering the mixtures of the reactant as demonstrated in the equation of reaction as well as the experimental set-up as shown in Figure 1 for the equation of reaction we have.



where $[C_s]$ is denoted as soil $[M]$ is denoted as microorganisms, $[C_{TPH}]$ is denoted as crude oil.

The equation (5.0) represent the reaction of crude oil (C_{TPH}) with the available microorganisms present in each bioreactor. The microbes feed on the substrate that is the crude oil or the TPH and degrade it to produce substance such as carbon dioxide CO_2 , water H_2O , heat release, and new biomass produced as well. The reaction of the crude oil degradation takes place in the soil environment which is denoted as (C_s) and the presence of the microbes capable of degrading the crude oil is denoted by (M) and the crude oil which is the contaminates is denoted by C_{TPH} . The reaction of the crude oil with the microbes yield an intermediate product known as enzyme substrate complex (EC_{TPH}) and the further disintegration of the (EC_{TPH}) in the soil environment of (C_s) yielding the product (P), free enzyme (M) as well as in the present of soil environment (C_s).

Considering the experimental set-up, the flow diagram can be demonstrated as.

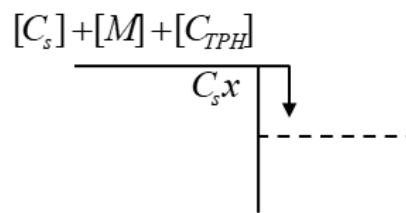


Figure 1. Demonstration of a batch Bioreaction.

Considering the balance of reaction process as illustrated in Figure 1 reveals that

$$\left(\begin{array}{l} \text{The rate of input of} \\ \text{TPH in the control} \\ \text{volume of the system} \end{array} \right) = \left(\begin{array}{l} \text{The rate of output of the} \\ \text{TPH from the control} \\ \text{volume system} \end{array} \right) + \left(\begin{array}{l} \text{The rate of} \\ \text{Disappearance of} \\ \text{the TPH from} \\ \text{control volume} \\ \text{system} \end{array} \right) + \left(\begin{array}{l} \text{The rate of} \\ \text{accumulation TPH} \\ \text{within the control} \\ \text{volume system} \end{array} \right) \quad (6)$$

The reactor type's grouped as batch process this the rate of input of the substrate into the system is equal to the rate of

output of the substrate from the system, that is.

$$\left(\begin{array}{l} \text{The rate of input of} \\ \text{TPH in the control} \\ \text{volume of the system} \end{array} \right) = \left(\begin{array}{l} \text{The rate of output of the} \\ \text{TPH from the control} \\ \text{volume system} \end{array} \right) = 0 \quad (7)$$

Applying condition of equation (7) into equation (6) therefore equation (6) can be written as.

$$\left(\begin{array}{l} \text{The rate of} \\ \text{accumulation TPH} \\ \text{within the control} \\ \text{volume system} \end{array} \right) = \left(\begin{array}{l} \text{The rate of} \\ \text{Disappearance of} \\ \text{the TPH from} \\ \text{control volume} \\ \text{system} \end{array} \right) \quad (8)$$

From equation (6) defining each of the rate expression, we have.

$$\begin{aligned} \text{The rate of input of TPH in the control volume of the system} \\ = U_o C_{TPHO} \end{aligned} \quad (9)$$

$$\begin{aligned} \text{The rate of output of the TPH from the control volume sys-} \\ \text{tem} = U C_{TPH} \end{aligned} \quad (10)$$

$$\begin{aligned} \text{The rate of disappearance of the TPH from control volume} \\ \text{system} = R_{TPH} V \end{aligned} \quad (11)$$

$$\begin{aligned} \text{The rate of accumulation TPH within the control volume sys-} \\ \text{tem} = \frac{dC_{TPH}}{dt} \end{aligned} \quad (12)$$

However, considering equations (9) to (12) and then substituting it into equation (11), we have (6).

$$= U_o C_{TPHO} + (-R_{TPH} V) + \frac{dC_{TPH}}{dt} \quad (13)$$

Indeed, it is revealed that change in crude oil degradation is a function of time, provided that the organisms are feeding on the substrate. Therefore, the rate of accumulation of the substrate within the system can be written as,

$$\frac{dC_{TPH}}{dt} = -R_{TPH} V \quad (14)$$

Equation (14) reveals that the rate of input of TPH in the control volume of the system is equal to the rate of output of TPH from the control volume system and both are equal to zero. In addition, equation (14) can be achieved by substituting equation (11) and (12) into equation (8).

The volume of a batch reactor is considered equal, therefore equation (14) can be written as

$$R_{TPH} V = -\frac{vdC_{TPH}}{dt} \quad (15)$$

In equation (15) V is constant therefore during through the expression with V,

We have

$$-R_{TPH} V = -\frac{dC_{TPH}}{dt} \quad (16)$$

The change of substrate concentration as a function of time can be in times equation of the kinetic as

$$-\frac{dC_{TPH}}{dt} = K_1 C_{TPH} \quad (17)$$

However, equation (11) can further be written as,

$$-R_{TPH} = \frac{dC_{TPH}}{dt} = K_1 C_{TPH} \quad (18)$$

where K_1 denotes the T_{PH} degradation in system, U_o denotes the initial concentration of TPH inlet volumetric rate of flow ($\frac{K_g}{day}$), U_o denotes the final concentration of TPH output volumetric rate of flow (K_g/day) $C_{TPH(t)}$ denotes the initial concentration of TPH (mg/kg), V denotes reactor volume (M^3), t denotes degradation time (day), C_{TPH} denotes the final concentration of TPH ($M_g K_g$).

Equation (12) can be written and simplified as C_{TPH}

$$\int_{C_{TPH(o)}}^{C_{TPH}} \frac{dC_{TPH}}{C_{TPH}} - K_1 \int_0^t dt \quad (19)$$

$$[\ln C_{TPH}]_{C_{TPH(o)}}^{C_{TPH}} = -K_1 [t]_0^t \quad (20)$$

$$\ln C_{TPH} - \ln C_{TPH(o)} = -K_1 (t - 0) \quad (20)$$

$$\ln \frac{C_{TPH}}{C_{TPH(o)}} = -K_1 t \quad (21)$$

Considering the exponential of equation (21) we have

$$C_{TPH(t)} = C_{TPH(o)} e^{-K_1 t} \quad (22)$$

or

$$C_{TPH(t)} = C_{TPH(o)} \exp K_1 t \quad (23)$$

Equation (3.23) can be expressed in terms of equation of the time, thus

$Y = C_{TPH(t)}$ this denotes the output

$M = K_1$ this denotes the slope

$C = C_{TPH(o)}$ this denotes the coordinate or coefficient

Plot of $\ln C_{TPH(t)}$ versus t and establishment of the linear equation for the determination of the slope and intercept. In this case slope of $K_1 = m$ as well as intercept of $C = \ln C_{TPH(o)}$.

2.4.3. Kinetics of Second Order Reaction

The Kinetics of the second order reaction for substrate (crude oil) degradation was established as

$$-R_{TPH} = \frac{dlw}{dt} = K_1 C_w^2 \quad (24)$$

From equation (24) we have

$$\frac{dC_w}{dt} = K_1 C_w^2 \quad (25)$$

By integrity equation (25) we have

$$\int_{C_{w(o)}}^{C_{w(t)}} C_w d C_w = \int_0^t K_1 t \quad (26)$$

From equation (26), we have

$$\frac{1}{C_{w(t)}} - \frac{1}{C_{w(o)}} = K_1 t \quad (27)$$

Equation (27) can be written as,

$$\frac{1}{C_{w(t)}} - \frac{1}{C_{w(o)}} = K_1 t \quad (28)$$

However, equation (28) can be written as

$$C_{w(t)} = \frac{C_{w(o)}}{1 + C_{w(o)} K_d t} \quad (29)$$

Equation (29) can be expressed in terms of crude oil (TPH) degradation

$$C_{w(t)} = \frac{C_{w(o)}}{1 + C_{w(o)} K_d t} \quad (30)$$

Where

$C_{w(o)}$ and $C_{w(t)}$ represents the initial and final crude oil (TPH) concentration

KI represents the rate constant of crude oil (TPH) degradation of the second order.

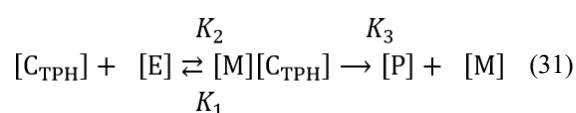
t represents the time.

Plot of $\frac{1}{C_{w(t)}}$ against $\frac{1}{C_{w(o)}}$ and K_1 representing the slope

and $\frac{1}{C_{w(o)}}$ representing the intercept

2.4.4. Michael's Menten Model

The Michael's Menten model deals with the rate of substrate degradation (TPH or crude oil degradation) upon the action of microbes. The equation expressing the action of microbes in substrate degradation is demonstrated in equation (31).



Where, $[C_{TPH}]$ denotes the substrate or crude oil (TPH), $[M]$ denotes the enzyme, $[M][C_{TPH}]$ denotes enzyme – substrate complex, $[P]$ denotes the product, $[M_1]$ denotes the free enzyme. The general rate equation of Michael's Menten Kinetic is expressed as

$$r = \frac{R_{max} [C_{TPH}]}{K_{co+} [C_{TPH}]} = \frac{R_{max} [S]}{K_s [S]} \quad (32)$$

Where, R denotes the specific of substrate degradation, R_{max} denotes the maximum specific rate of substrate degradation, $[C_{TPH}] = [X]$ denotes the substrate concentration of TPH or crude oil, $K_{C_{TPH}} = K_s$ denotes dissociation constant of substrate degradation. For the determination of the functional parameters and coefficient equation (32) was expressed in terms of Lineweaver Burk Plot and the general solution of equation is presented in equation (33) [24].

$$1/R = \frac{K_s}{R_{max} [S]} + \frac{1}{R_{max}} \quad (34)$$

$$1/R = \frac{K_{C_{TPH}}}{R_{max} [C_{TPH}]} + \frac{1}{R_{max}} \quad (35)$$

Relating equation (34) and (35) into equation of line graph of

$$Y = M_x + C$$

Therefore

$$Y = \frac{1}{R_{TPH}}$$

$$M = \frac{K_s}{(R_{TPH})_{max}} = \frac{K_{C_{TPH}}}{(R_{TPH})_{max}}$$

$$x = [S] = [C_{TPH}]$$

$$e = 1/(R_{TPH})_{max} = \text{intercept.}$$

$$\text{And (3.30) slope} = \frac{K_s}{(R_{TPH})_{max}} = \frac{K_{C_{TPH}}}{(R_{TPH})_{max}}$$

Plot of $1/R_{TPH}$ versus $1/[S]$ or $1/[C_{TPH}]$ and then the established equation of the linear graph result in the evaluation of the intercept value and the slope as intercept = $1/(R_{TPH})_{max}$ $\frac{K_s}{(R_{TPH})_{max}}$ or $\frac{K_{C_{TPH}}}{(R_{TPH})_{max}}$

3. Results and Discussion

3.1. Physicochemical Analysis Results Obtained

Table 1. Analysis Data Obtained in Terms of Physicochemical Parameters of Crude Oil and Soil Samples.

Sample	pH	EC $\mu\text{S}/\text{cm}$	TOC %	TN %	TP mg/l	K mg/l	HUBx10 ³ Cf/g	HUFx10 ² fu/g
Crude oil	8.63	570.24	57.29	0.07	42.84	8.27	1.74	1.56
Sandy soil	6.31	59.10	1.35	0.48	87.16	152.36	28	13
Loamy soil	5.92	73.65	1.84	0.79	124.03	287.19	73	30
Clay soil	5.57	41.94	1.52	0.31	74.29	93.62	41	19

The biostimulants soil samples and crude oil were assessed to determine the bio-stimulant substance, encompassing crude oil samples, sandy, loamy, and clay soil samples, as well as sun-dried and room-dried avocado pears, sun-dried and room-dried native pears, and sun-dried and room-dried bullet pears. The outcomes are detailed in Table 1.

Table 1 displays the physicochemical characteristics of certain key components, known to potentially influence the biostimulant process involved in degrading total petroleum hydrocarbons and enhancing microbial activities within each experimental setup designed to evaluate the nutrient's efficacy in TPH degradation. The crude oil properties showed a pH value of 8.63, electrical conductivity (E.C) of 570.24 $\mu\text{S}/\text{cm}$, Total Organic Carbon (TOC) of 57.29%, total nitrogen (TN) of 0.07%, total phosphorus (TP) of 42.84mg/l, potassium (K) of 8.27mg/l, with Hydrocarbon Utilizing Bacteria (HUB $\times 10^3$) at 1.74 and Hydrocarbon Utilizing Fungi (HUF $\times 10^2$) at 1.56. Analyzing the various soil samples revealed pH values of 6.31,

5.92, and 5.57 for sandy, loamy, and clay soils respectively. The electrical conductivity (EC) values for these soils are 59.10 $\mu\text{S}/\text{cm}$, 73.65 $\mu\text{S}/\text{cm}$, and 41.94 $\mu\text{S}/\text{cm}$ respectively. Total Organic Carbon (TOC) varies with percentages of 1.35%, 1.84%, and 1.52% for sandy, loamy, and clay soils respectively. Total nitrogen (TN) values showed percentages of 0.48%, 0.79%, and 0.31% for sandy, loamy, and clay soils respectively. However, total phosphorus (TP) display values of 87.16mg/l, 124.03mg/l, and 74.29mg/l for sandy, loamy, and clay soils respectively. Potassium exhibits values of 152.36mg/l, 287.19mg/l, and 93.62mg/l for sandy, loamy, and clay soils respectively. The population of hydrocarbon-utilizing bacteria and fungi presents values of 28.0×10^3 cfu/g and 13.0×10^2 cfu/g for HUB and HUF respectively in sandy soil, 73.0×10^3 cfu/g and 30.0×10^2 cfu/g for HUB and HUF respectively in loamy soils, and finally, for clay soil, the values for HUB and HUF are 41.0×10^3 cfu/g and 19.0×10^2 cfu/g respectively.

Table 2. Analysis Data Obtained for Physicochemical Properties of Biostimulants.

Sample	pH	EC $\mu\text{S}/\text{cm}$	TOC (%)	TN (%)	TP mg/l	K (mg/l)	HUBx10 ³ Cf/g	HUFx10 ² Cf/g
Avocado Pear RD	5.70	67.19	23.87	2.53	348.18	578.03	35	24
Avocado Pear SD	4.38	41.62	19.02	1.94	269.35	521.26	17	12
Native Pear RD	5.24	50.91	20.47	2.28	331.42	475.01	15	10
Native Pear SD	4.93	45.17	13.49	1.57	276.35	434.63	10	7
Bullet Pear RD	5.11	72.24	35.62	2.08	420.69	596.32	41	28
Bullet Pear SD	4.76	59.03	21.58	1.36	358.12	558.26	26	17

Table 2 demonstrates the physicochemical properties of the different biostimulants used in carrying out the investigation.

The selected biostimulants are Avocado Pear (RD & SD), Native Pear (RD & SD) and Bullet Pear (RD & SD), where RD

and SD represent leaves prepared under room-dried and sun-dried conditions respectively. The possible physicochemical parameters considered are pH, electrical conductivity (EC), Total Organic Carbon (TOC), Total Nitrogen (TN), Total Phosphorus (TP), Total Potassium (TK) concentrations as well as microbial concentration of Hydrocarbon Utilizing Bacteria (HUB) and Hydrocarbon Utilizing Fungi (HUF).

pH: The obtained data from the analysis of the physicochemical properties of the selected parameters revealed a pH of 5.7, 5.24 and 5.11 for room-dried avocado pear, native pear and bullet pear respectively. For sundried avocado pear, native pear and bullet pear, the pH values were observed to be 4.38, 4.93 and 4.76 respectively. The degree of magnitude in terms of concentration for room dried samples is $5.7 > 5.24 > 5.11$. In terms of sundried, $4.93 > 4.76 > 4.38$.

Electric Conductivity (EC): The EC was highest in room-dried samples, with values of 72.24 $\mu\text{S}/\text{cm}$ for bullet pear, 67.19 $\mu\text{S}/\text{cm}$ for avocado pear, and 50.91 $\mu\text{S}/\text{cm}$ for native pear. The sundried samples showed lower conductivity: 59.03 $\mu\text{S}/\text{cm}$ for bullet pear, 45.17 $\mu\text{S}/\text{cm}$ for native pear, and 41.62 $\mu\text{S}/\text{cm}$ for avocado pear. The degree of magnitude for room-dried samples is Bullet Pear (72.24) > Avocado Pear (67.19) > Native Pear (50.91). For the sundried samples, the order is Bullet Pear (59.03) > Native Pear (45.17) > Avocado Pear (41.62).

Total Organic Carbon (TOC): The TOC content was highest in room-dried bullet pear at 35.62%, followed by room-dried avocado pear at 23.87% and room-dried native pear at 20.47%. Sun drying resulted in a reduction of TOC, with values of 21.58% for bullet pear, 19.02% for avocado pear, and 13.49% for native pear. The degree of magnitude for room-dried samples is Bullet Pear (35.62) > Avocado Pear (23.87) > Native Pear (20.47). For the sundried samples, the order is Bullet Pear (21.58) > Avocado Pear (19.02) > Native Pear (13.49).

Total Nitrogen (TN): The TN content was highest in room-dried avocado pear (2.53%) and native pear (2.28%), with

room-dried bullet pear lower at 2.08%. Sun-drying led to a decrease in nitrogen across all varieties, with avocado pear at 1.94%, native pear at 1.57%, and bullet pear at 1.36%. The degree of magnitude for room-dried samples is Avocado Pear (2.53) > Native Pear (2.28) > Bullet Pear (2.08). For the sun-dried samples, the order is Avocado Pear (1.94) > Native Pear (1.57) > Bullet Pear (1.36).

Total Phosphorus (TP): The TP content was considerably high across all samples. Room-dried bullet pear had the highest concentration (420.69mg/l), followed by room-dried avocado pear (348.18mg/l) and room-dried native pear (331.42mg/l). Sun-dried samples followed a similar trend but with lower values: bullet pear (358.12mg/l), avocado pear (269.35mg/l), and native pear (276.35mg/l). The degree of magnitude for room-dried samples is Bullet Pear (420.69) > Avocado Pear (348.18) > Native Pear (331.42). For the sun-dried samples, the order is Bullet Pear (358.12) > Native Pear (276.35) > Avocado Pear (269.35).

Potassium (K): The potassium content was highest in room-dried bullet pear (596.32mg/l) and avocado pear (578.03mg/l), with room-dried native pear lower (475.01mg/l). A similar pattern was observed in sundried samples, though with reduced values: bullet pear (558.26mg/l), avocado pear (521.26mg/l), and native pear (434.63mg/l). The degree of magnitude for room-dried samples is Bullet Pear (596.32) > Avocado Pear (578.03) > Native Pear (475.01). For the sundried samples, the order is Bullet Pear (558.26) > Avocado Pear (521.26) > Native Pear (434.63).

Microbial Load (HUB & HUF): The population of hydrocarbon-utilizing bacteria (HUB) and fungi (HUF) was highest in room-dried bullet pear (41×10^3 and 28×10^2 cfu/g respectively), followed by room-dried avocado pear (35×10^3 and 24×10^2 cfu/g) and room-dried native pear (15×10^3 and 10×10^2 cfu/g). Sun-drying resulted in a lower microbial count across all varieties. The trend mirrors the other physicochemical parameters, with bullet pear consistently showing the highest values.

3.2. Kinetic Modelling of TPH Degradation

Table 3. First-order and second-order kinetic parameters for 36 treatments and 3 controls.

Code	Soil	Biostimulant	Drying	Dose	Best Model	K(best-fit)	R ²	t ^{1/2}	% Removal
A1B1C1D3	Clay	Bullet Pear	Room-Dried	50 g	First-order	0.0351	0.988	19.8	78.0
A1B1C2D3	Clay	Bullet Pear	Room-Dried	100 g	Second-order	6.50e-7	0.991	11.4	75.0
A1B2C1D3	Clay	Bullet Pear	Sun-Dried	50 g	Second-order	6.51e-7	0.987	11.1	74.6
A1B2C2D3	Clay	Bullet Pear	Sun-Dried	100 g	Second-order	6.67e-7	0.991	11.1	75.5
A2B1C1D3	Clay	Bush Pear	Room-Dried	50 g	Second-order	5.75e-7	0.989	13.6	73.5
A2B1C2D3	Clay	Bush Pear	Room-Dried	100 g	Second-order	5.87e-7	0.992	13.8	73.8
A2B2C1D3	Clay	Bush Pear	Sun-Dried	50 g	Second-order	5.50e-7	0.989	15.4	72.5

Code	Soil	Biostimulant	Drying	Dose	Best Model	K(best-fit)	R ²	t ^{1/2}	% Removal
A2B2C2D3	Clay	Bush Pear	Sun-Dried	100 g	Second-order	6.40e-7	0.990	12.6	75.6
A3B1C1D3	Clay	Avocado Pear	Room-Dried	50 g	Second-order	6.42e-7	0.982	12.3	74.7
A3B1C2D3	Clay	Avocado Pear	Room-Dried	100 g	Second-order	6.81e-7	0.978	11.5	76.6
A3B2C1D3	Clay	Avocado Pear	Sun-Dried	50 g	Second-order	7.36e-7	0.991	10.0	77.9
A3B2C2D3	Clay	Avocado Pear	Sun-Dried	100 g	Second-order	7.63e-7	0.993	9.8	78.2
A1B1C1D2	Loamy	Bullet Pear	Room-Dried	50 g	First-order	0.0456	0.994	15.2	86.1
A1B1C2D2	Loamy	Bullet Pear	Room-Dried	100 g	First-order	0.0489	0.990	14.2	88.1
A1B2C1D2	Loamy	Bullet Pear	Sun-Dried	50 g	First-order	0.0464	0.990	14.9	87.0
A1B2C2D2	Loamy	Bullet Pear	Sun-Dried	100 g	First-order	0.0496	0.987	14.0	88.7
A2B1C1D2	Loamy	Bush Pear	Room-Dried	50 g	First-order	0.0492	0.985	14.1	88.7
A2B1C2D2	Loamy	Bush Pear	Room-Dried	100 g	First-order	0.0496	0.984	14.0	88.8
A2B2C1D2	Loamy	Bush Pear	Sun-Dried	50 g	First-order	0.0492	0.981	14.1	88.7
A2B2C2D2	Loamy	Bush Pear	Sun-Dried	100 g	First-order	0.0493	0.979	14.1	88.9
A3B1C1D2	Loamy	Avocado Pear	Room-Dried	50 g	First-order	0.0523	0.971	13.3	89.0
A3B1C2D2	Loamy	Avocado Pear	Room-Dried	100 g	First-order	0.0509	0.988	13.6	89.4
A3B2C1D2	Loamy	Avocado Pear	Sun-Dried	50 g	First-order	0.0502	0.982	13.8	89.3
A3B2C2D2	Loamy	Avocado Pear	Sun-Dried	100 g	First-order	0.0509	0.969	13.6	89.9
A1B1C1D1	Sandy	Bullet Pear	Room-Dried	50 g	First-order	0.0576	0.986	12.0	92.3
A1B1C2D1	Sandy	Bullet Pear	Room-Dried	100 g	First-order	0.0600	0.987	11.6	92.9
A1B2C1D1	Sandy	Bullet Pear	Sun-Dried	50 g	First-order	0.0586	0.981	11.8	92.9
A1B2C2D1	Sandy	Bullet Pear	Sun-Dried	100 g	First-order	0.0603	0.986	11.5	93.1
A2B1C1D1	Sandy	Bush Pear	Room-Dried	50 g	First-order	0.0587	0.980	11.8	92.9
A2B1C2D1	Sandy	Bush Pear	Room-Dried	100 g	First-order	0.0595	0.979	11.7	93.0
A2B2C1D1	Sandy	Bush Pear	Sun-Dried	50 g	First-order	0.0589	0.973	11.8	93.1
A2B2C2D1	Sandy	Bush Pear	Sun-Dried	100 g	First-order	0.0581	0.971	11.9	92.7
A3B1C1D1	Sandy	Avocado Pear	Room-Dried	50 g	First-order	0.0613	0.984	11.3	93.4
A3B1C2D1	Sandy	Avocado Pear	Room-Dried	100 g	First-order	0.0616	0.976	11.2	93.7
A3B2C1D1	Sandy	Avocado Pear	Sun-Dried	50 g	First-order	0.0610	0.976	11.4	93.5
A3B2C2D1	Sandy	Avocado Pear	Sun-Dried	100 g	First-order	0.0672	0.983	10.3	94.8
Control-D3	Clay	— (control)	-	-	Second-order	7.96e-8	0.848	119.6	29.7
Control-D2	Loamy	— (control)	-	-	Second-order	8.95e-8	0.884	106.7	33.1
Control-D1	Sandy	— (control)	-	-	Second-order	1.17e-7	0.928	81.7	38.9

Table 3 shows the first order and second order kinetics parameters for k, R², half life, percentage TPH removal for all 36 treatments and three controls. First-order and second-order kinetic models were fitted to the TPH depletion profile of each of the 36 biostimulant treatment combinations and the three

unamended controls (Table 1). The first-order model, $\ln [TPH] = \ln [TPH_0] - k_1 t$, provided the better fit for the great majority of treatments, with coefficients of determination typically exceeding R² = 0.98 (e.g., the best-performing treatment, A3B2C2D1, gave k₁ = 0.0672 day⁻¹ with R² = 0.99; Figure 1).

Rate constants ranged from approximately 0.035 to 0.067 day⁻¹ across treatments, corresponding to half-lives of 10.3–19.8 days, while the fastest-degrading treatment (A3B2C2D1: Avocado Pear, Sun-Dried, 100 g, Sandy soil) achieved 94.8% TPH removal by Day 42 with a half-life of 10.3 days.

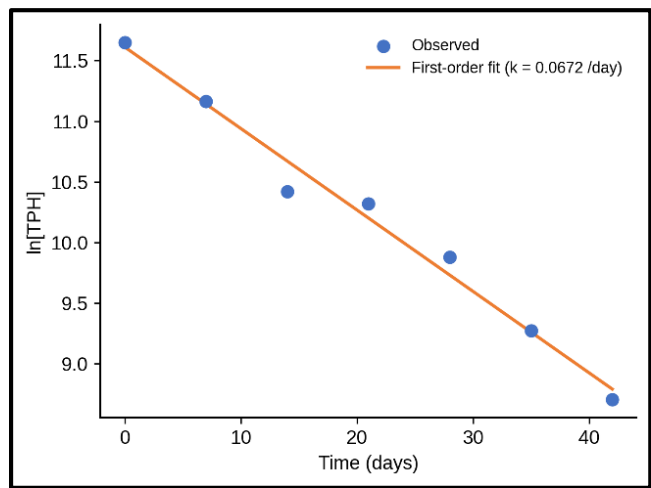


Figure 2. first order linearization for the best performing treatment.

Figure 2 shows the first order linearization (ln(TPH)) and time for the best performing treatment. The dominance of first-order kinetics in this dataset is consistent with numerous prior studies of TPH biodegradation in soil. Nwankwegu et al. [25] similarly found that first-order kinetics best described TPH depletion in spent-lubricating-oil-impacted soil under sewage sludge, detergent, and natural-recovery treatments ($k = 0.27 \text{ day}^{-1}$, $t_{1/2} = 3.0$ days for the most effective amendment), while Yousefi et al. [26] reported first-order behaviour for weathered petroleum hydrocarbons remediated with organic waste amendments ($k = 0.005 \text{ day}^{-1}$, $t_{1/2} = 71$ days for a molasses treatment). Agarry et al. [27] likewise found that first-order kinetics described the biodegradation of lubricating motor oil in co-contaminated soil very well under both biostimulation and bioaugmentation strategies. The half-lives obtained in the present study (10.3–19.8 days) fall within the range bracketed by these faster- and slower-degrading systems, consistent with the well-established finding that first-order kinetics is the most broadly applicable model for describing hydrocarbon disappearance in biologically active soil systems.

3.3. Effect of Soil Type, Biostimulant Species, Drying Method, and Dose on TPH Removal

Table 4. Statistical significance test.

Comparison	Test	Statistic	P-value	Interpretation ($\alpha = 0.05$)
Dose (50 g vs. 100 g)	Independent t-test	$t = -0.229$	0.8200	Not significant
Drying (Room- vs. Sun-Dried)	Independent t-test	$t = -0.148$	0.8833	Not significant
Species (3-way)	One-way ANOVA	$F = 0.132$	0.8767	Not significant
Soil type (3-way)	One-way ANOVA	$F = 612.5$	$8.13\text{e-}27$	Highly significant

Table 4 summarizes t-test and Anova significance test for dose, drying method, specie and soil type. One-way ANOVA revealed that soil type was, by a wide margin, the dominant factor governing TPH removal ($F = 612.5$, $p < 0.001$), with mean removal at Day 42 following the order Sandy ($93.2 \pm 0.6\%$) > Loamy ($88.6 \pm 1.1\%$) > Clay ($75.5 \pm 1.9\%$). In contrast, biostimulant species (African Bullet Pear, African Bush Pear, Avocado Pear; $F = 0.13$, $p = 0.88$), drying method (Room- vs. Sun-Dried; $t = -0.15$, $p = 0.88$), and dose (50 g vs. 100 g; $t = -0.23$, $p = 0.82$) showed no statistically significant effect on TPH removal.

The strong soil-texture effect observed here is well supported by the literature. Kogbara et al. [28], in a direct comparison of petroleum degradation across different soil textural classes, concluded that a higher sand-to-clay ratio favours

faster hydrocarbon degradation, and specifically noted that the fold-increase in bacterial numbers following remediation treatment decreased with increasing clay content a trend that mirrors the soil-dependent bacterial fold-increases recorded in the present study. Haghollahi et al. [29] provide a mechanistic explanation consistent with this pattern: finer-textured soils possess smaller pore sizes that restrict the diffusion of oxygen, nutrients, and motile microorganisms, thereby constraining aerobic biodegradation rates, whereas coarser sandy soils permit greater aeration and more effective substrate–microbe contact. This is the most plausible explanation for the markedly slower degradation and longer half-lives observed in the clay soil treatments in this study, irrespective of which biostimulant was applied. The absence of a significant species,

drying-method, or dose effect suggests that, once adequate nitrogen, phosphorus, and organic carbon are supplied, the specific botanical source and physical form of the leaf-based biostimulant is of secondary importance relative to the physical soil environment in which degradation must proceed.

3.4. Biostimulated Treatments Versus Unamended Controls

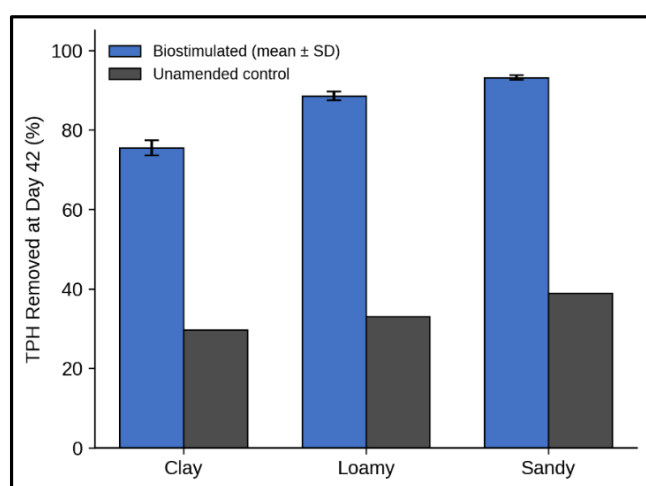


Figure 3. Percentage TPH removed at Day 42.

Figure 3 shows the percentage TPH removal at Day 42 biostimulated treatment mean $\pm$ SD and unamended control, by soil type. Across all three soils, biostimulated treatments achieved substantially greater TPH removal than the corresponding unamended controls: 2.54-fold in Clay soil (75.5% vs. 29.7%), 2.68-fold in Loamy soil (88.6% vs. 33.1%), and 2.40-fold in Sandy soil (93.2% vs. 38.9%). This confirms that the applied leaf-based biostimulants substantially accelerated hydrocarbon removal beyond what indigenous soil microbiota achieved through natural attenuation alone.

This magnitude of improvement is consistent with comparable biostimulation studies. Agarry et al. [27] reported 45.3% TPH removal under natural attenuation compared with 72% and 68.2% under biostimulation and bioaugmentation, respectively an improvement factor of approximately 1.5–1.6, somewhat more modest than observed here, but derived from a co-contaminated (oil + lead) system in which heavy-metal toxicity independently suppressed hydrocarbon-degrader activity across all treatments. The larger improvement factors obtained in the present, metal-free system are plausible given the absence of this additional inhibitory stress, and reinforce the general conclusion that nutrient- and organic-matter-based biostimulation reliably and substantially outperforms unassisted natural attenuation in petroleum-contaminated soils.

3.5. Best-and Worst-Performing Treatments

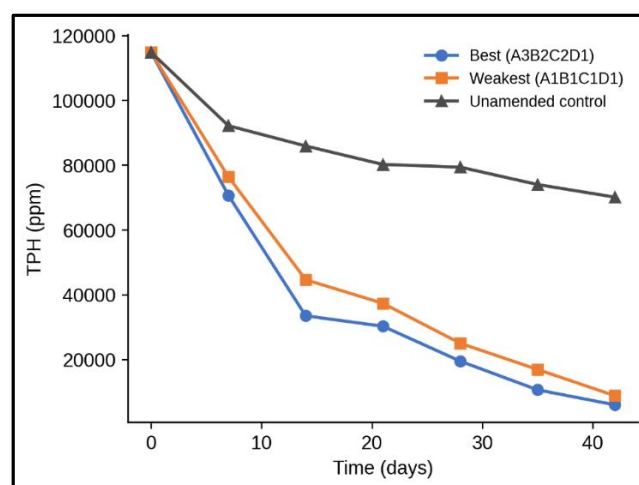


Figure 4. TPH decay curves in Sandy soil.

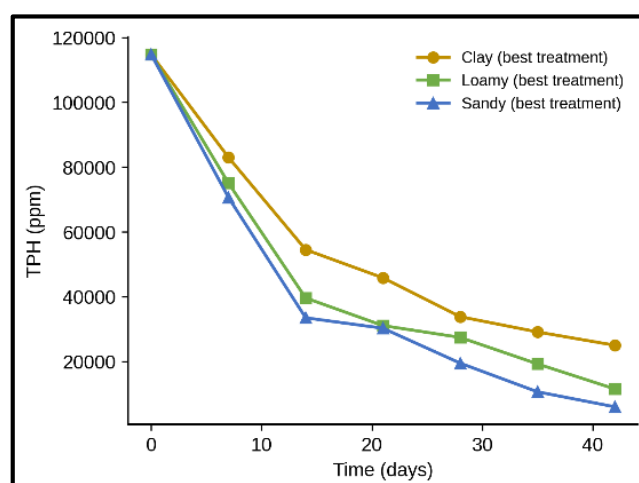


Figure 5. TPH decay curves comparing the best-performing treatment in each soil type.

The five best-performing treatments were all located in Sandy soil, led by Avocado Pear (Sun-Dried, 100 g; 94.8% removal, $t_{1/2}$ = 10.3 days), followed closely by three further Avocado Pear variants and one African Bush Pear variant, all in Sandy soil (93.1–93.7% removal). Conversely, the five weakest-performing treatments were all located in Clay soil, with removal ranging from 72.5% to 74.7%. This clustering by soil type, rather than by biostimulant identity that soil texture, not biostimulant selection, is the primary determinant of treatment ranking in this dataset, although Avocado Pear showed a consistent, if statistically non-significant, tendency to lead the rankings within the best-performing (Sandy) soil group.

3.6. Bacterial and Fungal Population Dynamics

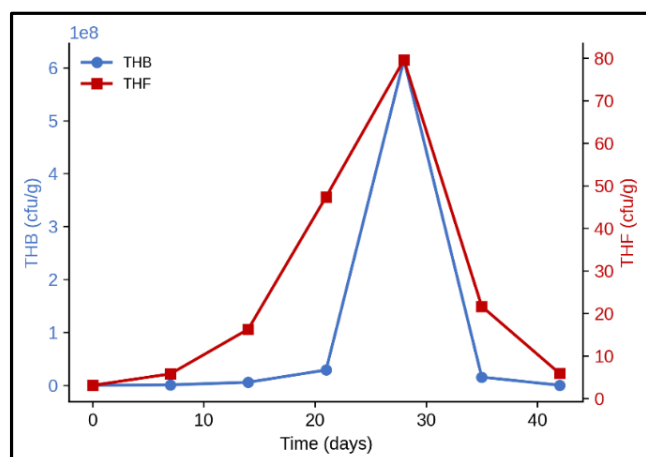


Figure 6. Total heterotrophic bacterial (THB) and fungal (THF) population dynamics over 42 days for the best-performing treatment.

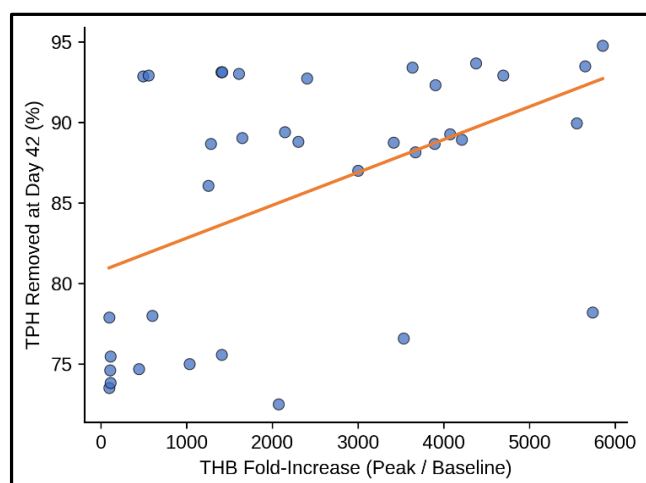


Figure 7. Correlation between THB fold-increase (Peak/baseline) and percent TPH removal.

Figure 6 shows the total heterotrophic bacterial (THB) and fungal (THF) population dynamics over 42 days for the best-performing treatment while Figure 7 shows the Correlation between THB fold-increase (peak/baseline) and % TPH removed at Day 42, across all 36 treatments. Total heterotrophic bacterial (THB) and fungal (THF) counts increased from a common baseline of approximately 10^5 cfu/g at Day 0 to a peak at Day 28 across all 36 treatments, before declining sharply by Day 42. In the best-performing treatment (A3B2C2D1), THB rose to a peak of 6.1×10^8 cfu/g (a 5,800-fold increase over baseline) before falling to 1.8×10^4 cfu/g by Day 42, while THF followed a parallel trajectory peaking at 8.0×10^4 cfu/g. Pearson correlation across all 36 treatments showed that THB fold-increase (peak/baseline) was moderately and significantly correlated with TPH percentage removal at Day 42 ($r = 0.49$, $p = 0.0026$; Figure 6), whereas THF fold-increase showed no

meaningful correlation with TPH removal ($r = 0.01$, $p = 0.96$).

The peak-then-decline pattern observed here is well documented in the bioremediation literature and is generally attributed to substrate depletion. Valencia-Luna et al. [30], studying bacterial consortia in diesel-contaminated agricultural soils, reported a significant decline in cultivable degradative bacterial populations from around Day 21 onward across all treatments, attributing this decline to the gradual depletion of available TPH and polycyclic aromatic hydrocarbon substrates as the carbon and energy source for microbial metabolism a mechanism directly applicable to the Day-28-to-Day-42 decline observed in the present study, by which point the more degradable hydrocarbon fractions had likely already been substantially consumed.

The significant correlation between bacterial population growth and TPH removal is consistent with the broader literature identifying bacteria as the principal agents of aerobic hydrocarbon degradation in soil, with fungi more typically playing a complementary or co-metabolic role. Monteiro et al. [31], screening fungal and bacterial isolates from diesel/biodiesel-contaminated soil, found that extracellular ligninolytic enzyme (laccase) activity was substantially higher among bacterial strains than fungal strains, and that the best-performing hydrocarbon-degrading consortium in their study was bacterially dominated (supplemented by a single fungal partner), rather than fungally dominated. This is consistent with the present finding that bacterial, rather than fungal, population expansion tracks meaningfully with hydrocarbon removal, and supports the interpretation that the fungal populations enumerated in this study, while clearly responsive to the biostimulant amendments, were not the primary drivers of TPH degradation in these soils.

4. Conclusion

This study demonstrated that biostimulation of crude-oil-contaminated soil with *Dacryodes edulis*, *Canarium schweinfurthii*, and *Persea americana* leaf material substantially accelerated total petroleum hydrocarbon (TPH) degradation relative to unamended natural attenuation, with all 36 biostimulated treatments outperforming their respective controls by 2.4- to 2.7-fold across the 42-day monitoring period. TPH depletion was best described by first-order kinetics for the majority of treatments, with rate constants ranging from 0.035 to 0.067 day^{-1} and half-lives of 10.3–19.8 days, and the fastest-degrading treatment (Avocado Pear, sun-dried, 100 g, sandy soil) achieved 94.8% removal within the study period.

Soil texture emerged as the dominant factor governing bioremediation outcome, with TPH removal following the order sandy > loamy > clay soil regardless of biostimulant identity, drying method, or application dose, none of which exerted a statistically significant effect. This finding indicates that, once adequate nitrogen, phosphorus, and organic carbon are supplied by any of the three leaf-based amendments, the physical

soil environment, particularly its influence on aeration, pore structure, and substrate-microbe contact, is the primary constraint on degradation rate rather than the specific botanical source of the biostimulant.

Tracking bacterial and fungal population dynamics further showed that total heterotrophic bacterial counts rose sharply to a peak near Day 28 before declining as available hydrocarbon substrate was depleted, and that this bacterial fold-increase correlated significantly with TPH removal ($r = 0.49$, $p = 0.0026$), whereas fungal population growth did not ($r = 0.01$, $p = 0.96$). This confirms bacteria as the principal drivers of hydrocarbon degradation in these soils, with fungi playing a comparatively passive or co-metabolic role.

Collectively, these findings establish *D. edulis*, *C. schweinfurthii*, and *P. americana* leaves as effective, low-cost biostimulants for crude oil bioremediation in tropical soils, and identify soil textural class as a primary consideration for site selection in future biostimulation strategies. Since biostimulant species, drying method, and dose showed no significant effect, field application can prioritize whichever leaf material is most locally available without a corresponding loss in performance. Future work should extend this framework to field-scale trials under natural environmental fluctuations, evaluate performance in soils co-contaminated with heavy metals, and apply molecular community profiling to resolve the specific bacterial taxa responsible for the observed degradation dynamics.

Abbreviations

ANOVA	Analysis of Variance
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
cfu/g	Colony-Forming Units per Gram
EC	Electrical Conductivity
GC-FID	Gas Chromatography–Flame Ionization Detection
HUB	Hydrocarbon-Utilizing Bacteria
HUF	Hydrocarbon-Utilizing Fungi
K	Potassium
MC	Moisture Content
MSA	Mineral Salt Agar
ppm	Parts per Million
R^2	Coefficient of Determination
RD	Room-Dried
SD	Sun-Dried
$t_{1/2}$	Half-Life
THB	Total Heterotrophic Bacteria
THF	Total Heterotrophic Fungi
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorus
TPH	Total Petroleum Hydrocarbon

Author Contributions

Tuboalabo Eno Nyong: Conceptualization, Data curation
Ukpaka Chukwuemeka Peter: Formal Analysis, Investigation, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] Oyilieze, A. A., Gilbert, O. M., Emmanuel, C., Nshimiyimana, E., Jonathan, M., & Martin, A. (2026). Environmental and socio-economic impacts of illegal oil mining in the Niger Delta, Nigeria. *Environmental Challenges*, 24, 101545. <https://doi.org/10.1016/j.envc.2026.101545>
- [2] Abel, U. A., Isotuk, U. R., Okon, O. G., Ukonobong, A., Etim, O. J., Akwayo, I. J., Ani, U. G., & Paul, E. E. (2026). Optimisation and Adsorption Modelling of Chromium (Cr^{3+}) Removal from Brewery Effluent Using Sustainable African Pear (*Dacryodes edulis*) Seed-based Activated Carbon: An Experimental and Predictive Modelling Approach. *Asian Journal of Chemical Sciences*, 16(3), 93–107. <https://doi.org/10.9734/ajocs/2026/v16i3451>
- [3] Popa, M. (2025). Study on the possibility of rapid elimination of accidentally spilled petroleum hydrocarbons in agricultural soil. *Agriculture*, 15(16), 1734. <https://doi.org/10.3390/agriculture15161734>
- [4] Abel, U., Ekanem, O., Oseribho, O., Isotuk, U., Job, A., & Ibanga, I. (2024). Enhanced Hydrophobicity and Oleophilicity of Modified Activated Carbons Derived from Agro-Wastes Biomass for the Removal of Crude Oil from Aqueous Medium. *American Journal of Chemical Engineering*, 12(4), 80–96. <https://doi.org/10.11648/j.ajche.20241204.11>
- [5] Santos, M., Rebola, S., & Evtuguin, D. V. (2025). Soil remediation: Current approaches and emerging Bio-Based Trends. *Soil Systems*, 9(2), 35. <https://doi.org/10.3390/soilsystems9020035>
- [6] Ibo, E. M., Umeh, O. R., Orji, M. U., Ibor, O. R., Okoli, C., Chukwuka, A. V., & Adeogun, A. O. (2025). Pollution to solution: Harnessing the power of bioremediation for petroleum wastewater management. *The Microbe*, 8, 100542. <https://doi.org/10.1016/j.microb.2025.100542>
- [7] Ramdass, A. C., & Rampersad, S. N. (2021). Diversity and Oil Degradation Potential of Culturable Microbes Isolated from Chronically Contaminated Soils in Trinidad. *Microorganisms*, 9(6), 1167. <https://doi.org/10.3390/microorganisms9061167>
- [8] Fenibo, E. O., Nkuna, R., & Matambo, T. (2025). Metagenomic Insights into Biopile Remediation of Petroleum-Contaminated soil using chicken droppings in Rivers State, Nigeria. *Bioinformatics and Biology Insights*, 19, 11779322251371117. <https://doi.org/10.1177/11779322251371117>

- [9] Wokem, V., & Madufuro, C. (2020). Application of cowdung and sawdust as biostimulants for enhanced bioremediation of diesel contaminated soil. *Journal of Applied Science and Environmental Management*, 24(1), 49. <https://doi.org/10.4314/jasem.v24i1.7>
- [10] Nordin, A. R. R., Navarro, A. R., Reyes, J. C., Maragathavalli, S., Kristanti, R. A., Wulandari, R., & Bunrith, S. (2025). Microbial Bioremediation of Petroleum-Contaminated Soil: a Sustainable approach. *Tropical Aquatic and Soil Pollution*, 5(1). <https://doi.org/10.53623/tasp.v5i1.683>
- [11] Johnson, N. (2024). Revolutionizing Soil Remediation: Exploring the Frontiers of Bioremediation through the Performance Evaluation of Vernonia Galamensis and Vernonia Amydalina Spices in Hydrocarbon-Contaminated Soil. *Preprints.org*. <https://doi.org/10.20944/preprints202401.0932.v1>
- [12] Wylie, M. R., & Merrell, D. S. (2022). The Antimicrobial Potential of the Neem Tree *Azadirachta indica*. *Frontiers in Pharmacology*, 13, 891535. <https://doi.org/10.3389/fphar.2022.891535>
- [13] Antón-Herrero, R., Chicca, I., García-Delgado, C., Crognale, S., Lelli, D., Gargarello, R. M., Herrero, J., Fischer, A., Thannberger, L., Eymar, E., Petruccioli, M., & D'Annibale, A. (2023). Main factors determining the Scale-Up effectiveness of mycoremediation for the decontamination of aliphatic hydrocarbons in soil. *Journal of Fungi*, 9(12), 1205. <https://doi.org/10.3390/jof9121205>
- [14] Ukpaka V. C, Ukpaka C. P, Uzono R. I (2025). The Kinetics of Petroleum Hydrocarbon Degradation in Soil: The Concept of Ponderosa Lemon Integrity as Bio-stimulant. *Journal of Petroleum Engineering & Technology* 15(03): 22-32. Available from: <https://journals.stmjournals.com/jopet/article=2025/view=228624>
- [15] APHA. (1998). Standard methods for the examination of water and wastewater (20th ed.). American Public Health Association, Washington, DC.
- [16] Isotuk, U. R., Uloma, A. C., & Paul, A. A. (2026). Production, characterization and performance evaluation of modified crab shell chitosan for sustainable coagulation of abattoir wastewater. *Chemical Science International Journal*, 35(4), 171–195. <https://doi.org/10.9734/csji/2026/v35i41050>
- [17] Adepoju, T., Job, A. I., & Isotuk, U. R. (2016). Optimization of Biohydrogen Production (BHP) from Agro Waste Water (Cassava Waste Water): A Case of Box-Behnken Response Surface Methodology (RSM). *International Journal of Energy and Environmental Science*, 1(1), 13. <https://doi.org/10.11648/j.ijeess.20160101.13>
- [18] Adepoju, T., Isotuk, U. R., & Job, A. I. (2016). Modelling and Optimization of the Removal of Congo-Red Dye from Waste Water Using Agricultural Waste. *Journal of Chemical Environmental and Biological Engineering*, 1(1), 1. <https://doi.org/10.11648/j.jcebe.20170101.11>
- [19] ASTM. (1999). Annual book of ASTM standards: Soil and rock; building stones; geotextiles (Vol. 04.08). American Society for Testing and Materials, West Conshohocken, PA.
- [20] Isotuk, U. R., Gunorubon, A. J., Kekpugile, D. K., & Wordu, A. (2026). Assessment of Water Demand and Water Quality Status in Eastern Obolo Local Government Area, Nigeria. *Science*, 1(1), 98–109. <https://doi.org/10.11648/j.sdenv.20260101.19>
- [21] ASTM International. (1996). ASTM D3921-96: Standard Test Method for Oil and Grease and Petroleum Hydrocarbons in Water. West Conshohocken, PA: ASTM International. <https://doi.org/10.1520/D3921-96>
- [22] Mills, A. L., Breuil, C., & Colwell, R. R. (1978). Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. *Canadian Journal of Microbiology*, 24(5), 552–557.
- [23] Okpokwasili, G. C., & Amanchukwu, S. C. (1988). Petroleum hydrocarbon degradation by *Candida* species. *Environment International*, 14(3), 243–247.
- [24] Isotuk, U., Abel, U., Ibanga, I., Job, A., & Ekong, S. (2026). Kinetic and thermodynamic modelling of zinc adsorption onto acid-activated oyster and periwinkle shells. *American Journal of Chemical Engineering*, 14(3), 60–75. <https://doi.org/10.11648/j.ajche.20261403.12>
- [25] Nwankwegu, A. S., Li, Y., Jiang, L., Lai, Q., Shenglin, W., Jin, W., & Acharya, K. (2018). Kinetic modelling of total petroleum hydrocarbon in spent lubricating petroleum oil impacted soil under different treatments. *Environmental Technology*, 41(3), 339–348. <https://doi.org/10.1080/09593330.2018.1498543>
- [26] Yousefi, K., Mohebbi, A., & Pichtel, J. (2021). Biodegradation of weathered petroleum hydrocarbons using organic Waste Amendments. *Applied and Environmental Soil Science*, 2021, 1–12. <https://doi.org/10.1155/2021/6620294>
- [27] Agarry, S. E., Aremu, M. O., & Aworanti, O. A. (2013). Kinetic Modelling and Half-Life Study on Bioremediation of Soil Co-Contaminated with Lubricating Motor Oil and Lead Using Different Bioremediation Strategies. *Soil and Sediment Contamination an International Journal*, 22(7), 800–816. <https://doi.org/10.1080/15320383.2013.768204>
- [28] Kogbara, R. B., Ayotamuno, J. M., Worlu, D. C., & Fubara-Manuel, I. (2015). A case study of petroleum degradation in different soil textural classes. *Recent Patents on Biotechnology*, 9(2), 108–115. <https://doi.org/10.2174/2211550105666151110203337>
- [29] Haghollahi, A., Fazelipour, M. H., & Schaffie, M. (2016). The effect of soil type on the bioremediation of petroleum contaminated soils. *Journal of Environmental Management*, 180, 197–201. <https://doi.org/10.1016/j.jenvman.2016.05.038>
- [30] Valencia-Luna, G. A., Lozada-Campos, D., Pardo-López, L., Millán-López, K. S., Loera, O., Tapia-Hernández, A., & Pérez-Armendáriz, B. (2025). Biodegradation potential and taxonomic composition of Hydrocarbon-Degrading bacterial consortia in Diesel-Contaminated agricultural soils. *Applied Microbiology*, 5(4), 126. <https://doi.org/10.3390/applmicrobiol5040126>

- [31] Monteiro, J. P. S., Da Silva, A. F., Duarte, R. T. D., & Giachini, A. J. (2024). Exploring novel Fungal–Bacterial consortia for Enhanced Petroleum Hydrocarbon Degradation. *Toxics*, 12(12), 913. <https://doi.org/10.3390/toxics12120913>