

# Biostimulation of Petroleum Hydrocarbon Contaminated Soils Using Bryophyllum Pinnatum Extracts

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## Abstract

*This study investigates the remediation potential of Bryophyllum pinnatum extracts (water and ethanol-based) for degrading total petroleum hydrocarbons (TPH) in loamy and clay soils. Crude oil contaminants used in the study exhibited a pH of 6.76, density of 0.878 g/mL at 15 °C, kinematic viscosity of 147.4 mm<sup>2</sup>/s at 40 °C, and a flash point of 193.2 °C. The baseline TPH concentration in the soils was 48,291.14 ppm. Soils were treated with varying volumes (100, 200, and 300 mL) of water and ethanol extracts for 42 days. The pH of B. pinnatum extracts was 6.71 (water) and 6.83 (ethanol), while their total organic carbon ranged from 10.65%–13.89%. Results revealed significant TPH reductions in both soils, with ethanol-based extracts outperforming water extracts. For loamy soil, TPH reductions ranged from 57.89%–87.18% across ethanol treatments, compared to 57.89%–65.78% for water treatments. In clay soil, reductions ranged from 54.97%–62.63%, with ethanol extracts achieving the highest degradation. TPH reduction correlated positively with both treatment volume and remediation duration. This study demonstrates the efficacy of B. pinnatum extracts, particularly ethanol-based, as a biostimulant for hydrocarbon remediation, offering an environmentally friendly approach for restoring contaminated soils.*

**Keywords:** Biostimulation, Bryophyllum pinnatum, Clay soil, Ethanol extract, Loamy soil, Total petroleum hydrocarbons (TPH)

## INTRODUCTION

Bioremediation methods aim to restore contaminated environments in an environmentally sustainable and cost-effective manner [1]. Over the years, researchers have proposed and developed various bioremediation strategies; however, due to the diversity and complexity of pollutants, no single technique can serve as a universal solution. Indigenous (autochthonous) microorganisms found in polluted sites play a critical role in overcoming many of the challenges linked to biodegradation and

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bioremediation, provided that environmental conditions – such as oxygen, temperature, oxygen, and nutrient availability – support their growth and metabolic activity [2]. Compared to chemical and physical remediation methods, bioremediation offers major benefits such as eco-friendliness and cost efficiency. While several definitions of bioremediation exist, most emphasize degradation processes. Although the terms “bioremediation” and “biodegradation” are sometimes used interchangeably, the latter refers specifically to a process within the broader scope of bioremediation. In this context, bioremediation is understood as a biological approach that reduces, transforms, detoxifies, or mineralizes pollutants into harmless forms [3]. The success of pollutant removal largely

depends on the pollutant type, which may include hydrocarbons, agrochemicals, heavy metals, plastics, nuclear waste, dyes, greenhouse gases, sewage, and chlorinated compounds [4].

Bioremediation techniques can generally be classified into two categories: in situ (on-site) and ex situ (off-site). The choice of method is influenced by factors such as pollutant characteristics, contamination depth, environmental conditions, location, cost considerations, and regulatory policies [5]. Additionally, performance-related factors like pH, temperature, oxygen, and nutrient availability, and other abiotic conditions must be evaluated before implementing a remediation strategy. Although various pollutants are addressed by bioremediation, hydrocarbons receive significant attention due to their frequent involvement in soil and groundwater contamination [6]. For other contaminants, alternative remediation techniques – sometimes more cost-effective and efficient – may be preferable [7]. Nevertheless, crude oil and petroleum-derived pollutants remain a major concern because of their widespread use as energy sources, which contributes heavily to environmental pollution [8].

## METHODOLOGY

### Model Development

#### First Order Degradation Rate Kinetics

The first-order kinetic model is widely regarded as an effective tool for analyzing bioremediation processes. However, many studies present this model only in its final form, omitting the intermediate derivation steps [9]. In this study, these steps are explicitly outlined for clarity and emphasis. The biodegradation rate model serves as a predictive framework for estimating TPH concentrations in soil over time during bioremediation, once the degradation rate constant has been determined [10, 11]. The formulation of such models in chemical systems typically relies on the principle of mass balance, which provides a mathematical basis for describing system behavior. Accordingly, the bio-kinetic model for TPH degradation in soil was derived using this principle, as presented in Equation (1).

$$\left\{ \begin{array}{l} \text{Rate of} \\ \text{accumulation} \\ \text{in reactor} \end{array} \right\} = \left\{ \begin{array}{l} \text{Rate of TPH} \\ \text{inflow into the} \\ \text{reactor} \end{array} \right\} - \left\{ \begin{array}{l} \text{Rate of TPH} \\ \text{outflow in the} \\ \text{reactor} \end{array} \right\} - \left\{ \begin{array}{l} \text{Rate of TPH} \\ \text{degradation in} \\ \text{the reactor} \end{array} \right\} \quad (1)$$

The individual expression inside the brackets in Equation (1) can be expressed as follows.

$$\text{Inflow of mass into system} = Q_o C_{TPH(o)} \quad (2)$$

$$\text{Outflow of mass from system} = QC_{TPH} \quad (3)$$

$$\text{Rate of TPH degradation} = -r_{TPH}V \quad (4)$$

$$\text{Rate of accumulation} = -\frac{d(NV)}{dt} \quad (5)$$

## Results And Discussion

Table 1 presents the analyzed selected parameters of crude oil contaminants used for the study. The pH level of 6.76 was within the acidic level reasonably for any agricultural soil.

**Table 1.** Analyzed Result of Crude Oil (Pollutant).

Sample	Crude Oil
Ph	6.76
Pour point (°C)	-12.8
Density @ 15°C (g/ml)	0.878
Kinematic Viscosity @ 40°C (mm <sup>2</sup> /s)	147.4
Flash point (°C)	193.2
TPH (ppm)	48291.13530

However, the pour point of  $-12.8$  and density at  $15\text{ }^{\circ}\text{C}$  having  $0.878\text{ g/mL}$  contributed to the flash point of  $193.2\text{ }^{\circ}\text{C}$  with a kinematic viscosity at  $40\text{ }^{\circ}\text{C}$  of  $147.4\text{ mm}^2/\text{s}$ . The baseline value of total petroleum hydrocarbon (TPH) analyzed on polluted loamy and clay soils was  $48,291.13530\text{ ppm}$  respectively.

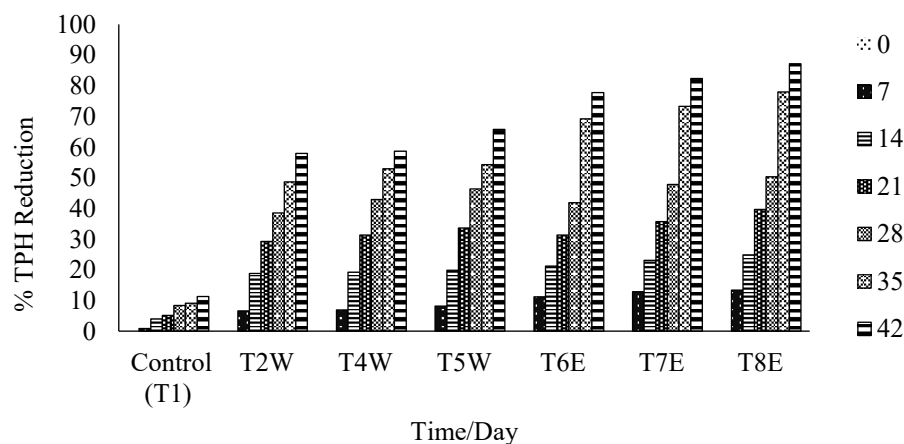
Table 2 shows the textural classification of the loamy and clay soil as described by the United States Department of Agriculture (USDA) and the characterization of *Bryophyllum pinnatum* leaves soaked in ethanol and water as biostimulant. The pH level of the biostimulant from ethanol and water on loamy and clay soil had almost the same values before crude oil contamination.

**Table 2.** Characterization of *Bryophyllum Pinnatum* Leaves in Water and Ethanol as Biostimulant.

Parameter	Biostimulant ( <i>Bryophyllum Pinnatum</i> Leaves)		Unpolluted Soil	
	Ethanol	Water	Clay	Loamy
pH	6.83	6.71	6.54	6.69
TOC (%)	10.65	13.89	7.03	8.95
EC ( $\mu\text{S/cm}$ )	72.19	130.11	201.25	287.30
T. N (%)	5.72	8.64	4.68	9.44
P (%)	0.736	1.344	3.105	2.345
K (%)	293.11	340.43	381.07	278.54
Sand (%)	NR	NR	10	40
Silt (%)	NR	NR	20	40
Clay (%)	NR	NR	70	20
Textural Class			Clay Soil	Loamy Soil

Note: NR = Not reported, EC= Electrical conductivity, TOC=Total organic carbon, TN=Total nitrogen, P=Phosphorous, K=Potassium.

Figure 1 illustrates the trend of TPH percentage reduction on Loam soil when subjected to ethanol and water extracts of *Bryophyllum pinnatum* leaves for treatment. The different mixed proportion of 100, 200, and 300 mL of the extracts monitored for 42 days decrease the concentration of hydrocarbon in clay and loam soil except for the control without any treatment. The control option T1 had 11.25% reduction compared to the treated options T3 had 57.89% when subjected to 100 mL water extract, T4 had 58% with 200 mL water extract, and T5 had 65.77% with water extract. In comparison with ethanol biostimulant of 100, 200, and 300 mL contained in T6, T7, and T8 had 77.78%, 82.36%, and 87.18% reduction respectively. The highest decrease in TPH was observed in T7 and T8 when subjected to ethanol extracts on loam soil. The percentage of TPH degradation in Loam soil increased in correspondence with an extension in the duration of remediation. However, the rate of TPH degradation also displayed a modest increase as the treatment weight was raised. Detailed results on TPH percentage reduction treatment on Loam soil using *Bryophyllum pinnatum* leaves can be found in Table 1 and 2 respectively.



**Figure 1.** Reduction of TPH versus Time on Loamy Soil Treatment Options.

Figure 2 illustrates the trend of TPH percentage reduction on Clay soil when subjected to ethanol and water extracts of *Bryophyllum pinnatum* leaves for treatment. The different mixed proportion of 100, 200, and 300 mL of the extracts monitored for 42 days decrease the concentration of hydrocarbon in clay and loam soil except for the control without any treatment. The control option T2 had 9.05% reduction compared to the treated options T9 had 54.97% when subjected to 100 mL water biostimulant T10 had 60.81% with 200 mL water extract, and T11 had 62.63% with ethanol biostimulant. In comparison with water biostimulant of 100, 200, and 300 mL contained in T12, T13, and T14 had 54.97%, 60.81%, and 62.63% reduction respectively in Table 3. Similar percentage of TPH reduction was experienced by Ehirim et al. (2020) using mango leaves on silt loam and clay soil treatment period of 28 days. They found out that 20 g of the sample had maximum degradation rate of 87.69% clay soil and for loam soil of 82.80%. In this study, highest decrease in TPH was observed in T10 and T11 when subjected to ethanol biostimulant on clay soil. The percentage of TPH degradation in clay soil increased in correspondence with an extension in the duration of remediation. However, the rate of TPH degradation revealed a modest increase in treatment weight on clay soil against the loam soil.

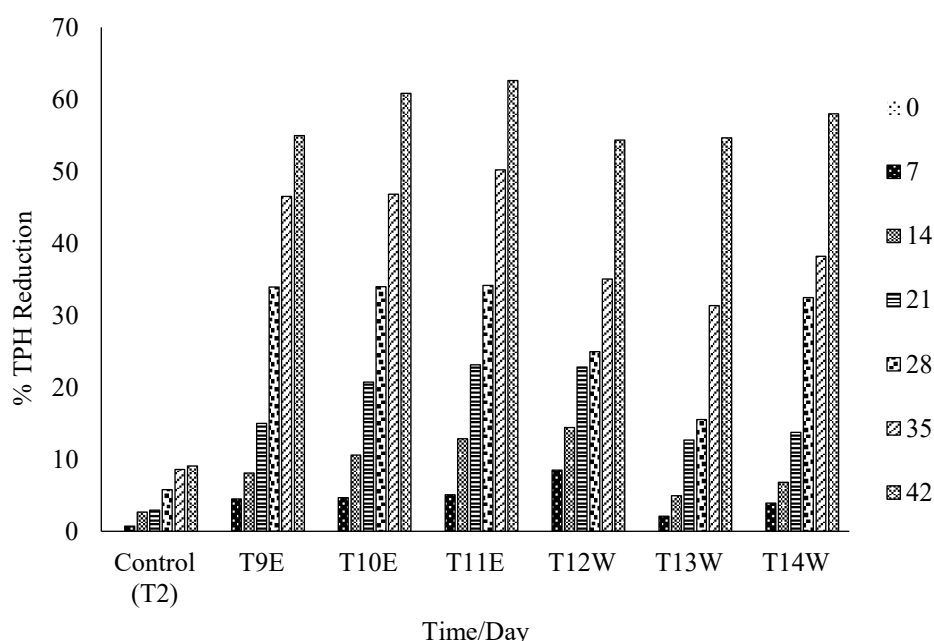


Figure 2. Reduction of TPH versus Time on Clay Soil Treatment Options.

Table 3. Comparison of Percentage Reduction of TPH on Loamy Soil Treatment Options.

Time (Day)	Control (T1)	T3W	T4W	T5W	T6E	T7E	T8E
7	0.885812473	6.57428379	6.8719	8.113554	11.21895	12.86698	13.32258
14	3.979736215	18.81032329	19.23927	19.82954	21.20879	23.06997	24.85127
21	5.148238418	29.27858548	31.28306	33.58012	31.32071	35.72606	39.64206
28	8.384185078	38.50029734	42.85682	46.36819	41.90085	47.79657	50.3349
35	9.127027254	48.69293663	52.91827	54.30313	69.25521	73.31608	77.92064
42	11.24640986	57.89070291	58.70114	65.77623	77.77575	82.35986	87.17983

CONCLUSION

The remediation of petroleum-contaminated soils using *Bryophyllum pinnatum* extracts proved effective, with ethanol-based extracts exhibiting superior TPH degradation compared to water-based extracts. Loamy soils responded better than clay soils, likely due to improved aeration and nutrient distribution. Maximum TPH reduction (87.18%) was achieved in loamy soil treated with 300 mL ethanol extract over 42 days, while clay soil recorded 62.63% reduction under similar conditions. The

findings highlight that both extract type and treatment volume significantly influence hydrocarbon degradation rates. This approach offers a cost-effective, sustainable solution for remediating oil-polluted soils, aligning with global efforts toward green and eco-friendly technologies. Further studies should explore microbial synergy and field-scale applications for enhanced performance.

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