

Bioremediation of Oil-Contaminated Soil Using a Blend of Goat Manure and Cassava Peel

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This study assessed the effectiveness of a blend of goat manure and cassava peel for the bioremediation of loamy soil artificially contaminated with Bonny Light crude oil. The experiment was conducted under laboratory conditions at the Department of Chemical Engineering, Imo State Polytechnic, Omuma, over a 60-day period. Loamy soil was spiked with 10% (w/w) crude oil and treated in three bioreactors: Reactor A (10% w/w cassava peel), Reactor B (10% w/w goat manure), and Reactor C (10% w/w 1:1 blend of goat manure and cassava peel). Soil samples were collected at 10-day intervals and analyzed for pH, total petroleum hydrocarbons (TPH), zinc (Zn), chromium (Cr), and microbial counts. Results showed that TPH decreased from an initial concentration of 18,500 mg/kg to 6,475 mg/kg, 5,180 mg/kg, and 1,850 mg/kg in Reactors A, B, and C, corresponding to 65.0%, 72.0%, and 90.0% reduction, respectively. Reactor C also achieved the highest reductions of Zn (51.5%) and Cr (62.5%), the greatest pH improvement from 4.85 to 7.55, and the highest proliferation of total heterotrophic and hydrocarbon-utilizing bacteria. Two-way ANOVA confirmed that treatment type, time, and their interaction significantly affected TPH reduction ($p < 0.05$). A multiple linear regression model developed for TPH prediction demonstrated excellent fit ($R^2 = 0.942$, $R^2_{adj} = 0.939$, $RMSE = 420$). The findings demonstrate that the synergistic blend of goat manure and cassava peel is a highly effective, low-cost, and eco-friendly biostimulant for enhanced remediation of crude oil-contaminated soils

Keywords: Bioremediation; Bonny Light crude oil; Goat manure; Cassava peel; Total petroleum hydrocarbons; Heavy metals.

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1. Introduction

Crude oil contamination of soil constitutes a persistent environmental challenge in Nigeria, particularly within the Niger Delta region where exploration, transportation, and artisanal refining activities are intensive. Chronic releases from pipeline leakages, storage tank failures, and operational discharges have resulted in widespread degradation of agricultural soils, loss of soil fertility, and risks to groundwater quality and human health (Amadi et al., 2013; Nwilo & Badejo, 2001; Ugochukwu & Ochuko, 2020). Petroleum hydrocarbons alter soil physicochemical properties, reduce porosity and nutrient availability, and inhibit microbial activity and plant growth (Nwaogu & Onyeze, 2010). Conventional remediation technologies such as excavation, thermal treatment, and chemical oxidation, although effective, are capital-intensive, require specialized equipment, and may generate secondary pollutants, thereby limiting their suitability for large-scale application in developing economies.

Bioremediation offers an environmentally sustainable and cost-effective alternative, leveraging the metabolic capabilities of indigenous microorganisms to mineralize petroleum hydrocarbons (Azubuike et

Bioremediation of Oil-Contaminated Soil Using a Blend of Goat Manure and Cassava Peel. Ifeanyi Praise Chidubem et.al

al., 2016; Koshlaf & Ball, 2017). Microbial degradation is widely recognized as the principal mechanism for natural attenuation of hydrocarbons in contaminated environments (Lovley, 2003; O'Loughlin et al., 2000). Nevertheless, the rate of biodegradation in oil-polluted soils is often constrained by nutrient imbalance, particularly deficiencies in nitrogen and phosphorus relative to excess carbon (Sims, 2006; Varjani, 2017). Biostimulation with organic amendments has been demonstrated to accelerate hydrocarbon degradation by supplying essential nutrients, improving soil structure, and enhancing microbial proliferation (Venosa et al., 2002; Hozumi et al., 2000; Tsutsumi et al., 2000). Organic waste materials such as animal manures and plant residues are of particular interest due to their availability, low cost, and dual function in waste valorization and soil restoration. Previous investigations have explored diverse amendments for metal stabilization and hydrocarbon removal (Freeman, 2012; Prasad & Freitas, 2003; Olapade & Ronk, 2015), yet studies on synergistic blends of animal and plant wastes remain limited. Goat manure is rich in nitrogen and harbours indigenous microorganisms, while cassava peel provides labile organic carbon and improves soil moisture retention. Their composite application may establish an optimal carbon-to-nitrogen ratio that sustains hydrocarbon-degrading microbial consortia. However, empirical data on the performance of goat manure–cassava peel blend, the influence of application rate, and predictive modelling of treatment efficiency are scarce in the literature.

Therefore, the aim of this study was to evaluate the effectiveness of a blend of goat manure and cassava peel as a biostimulant in the bioremediation of crude oil-contaminated soil. The specific objectives were to: (i) evaluate its performance under laboratory conditions; (ii) determine its efficiency in reducing total petroleum hydrocarbon (TPH) concentration; (iii) examine the effect of varying application rates on the rate of bioremediation; and (iv) develop a statistical model for predicting the bioremediation process.

2. Materials and Methods

Study Location

The study was conducted in the Chemical Engineering Laboratory, Imo State Polytechnic, Omuma, Nigeria, under ambient laboratory conditions ($28 \pm 2^\circ\text{C}$).

Materials

Loamy soil was collected from an uncontaminated site in Omuma at 0–15 cm depth. The soil was air-dried for 7 days, crushed, and sieved through a 2 mm mesh to remove stones and debris. Bonny Light crude oil obtained from Rivers State, Nigeria, was used as the contaminant.

Goat manure was collected from a local farm in Omuma, air-dried for 14 days, and ground to powder. Cassava peels were obtained from a local processing mill, washed, sun-dried for 14 days, and ground to fine powder.

Three 20-L perforated plastic reactors were used as bioreactors to ensure aeration. Analytical grade n-hexane, dichloromethane, HNO_3 , and HCl were used. Standard solutions of Zn and Cr were used for AAS calibration.

Experimental Design

Loamy soil was artificially contaminated with Bonny Light crude oil at 10% w/w (100 g oil per 1000 g soil). The mixture was thoroughly homogenized and left to stabilize for 7 days. The contaminated soil was divided into three treatments:

Reactor A: contaminated soil + cassava peel only

Reactor B: contaminated soil + goat manure only

Reactor C: contaminated soil + goat manure–cassava peel blend (1:1 ratio)

Amendments were applied at 10% w/w of contaminated soil. Moisture content was maintained at approximately 60% field capacity by addition of distilled water every 3 days. Soils were tilled manually weekly to enhance aeration. The experiment was monitored for 60 days, with samples collected at 0, 15, 30, 45, and 60 days for TPH and physicochemical analyses.

Sampling Procedure

Soil samples (approximately 200 g) were collected from each reactor at 10-day intervals over the 60-day period (Days 0, 10, 20, 30, 40, 50, and 60). Samples were placed in labeled amber bottles and immediately transported for analysis.

Analytical Methods

pH determination: Soil pH was determined in a 1:2.5 (w/v) soil-to-distilled water suspension using a calibrated digital pH meter after 30 min equilibration.

Total petroleum hydrocarbon (TPH): TPH was extracted with n-hexane:dichloromethane (1:1 v/v) in a Soxhlet extractor and quantified using gas chromatography equipped with flame ionization detector (GC-FID).

Heavy metals (Zn and Cr): Soil samples were digested with aqua regia (HNO₃:HCl, 1:3 v/v), filtered, and analyzed for Zn and Cr using atomic absorption spectrophotometer (AAS).

Microbiological analysis: Total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) were enumerated by serial dilution and spread plate technique on nutrient agar and mineral salts agar, respectively, and incubated at 37°C for 24–48 h.

Bioremediation Efficiency

The percentage reduction of TPH, Zn, and Cr was calculated as:

$$\text{Efficiency (\%)} = \frac{C_0 - C_t}{C_0} \times 100\%$$

where C₀ is the initial concentration at Day 0 and C_t is the concentration at time t.

Statistical Analysis and Model Development

Data were subjected to analysis of variance (ANOVA) at p<0.05 using SPSS (version 25.0) to test for significant differences among treatments. A multiple linear regression model was developed to predict TPH concentration as a function of time, amendment type, and application rate:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \epsilon$$

where Y = TPH concentration (mg/kg); X₁ = time (days); X₂ = amendment type; X₃ = application rate (% w/w); β₀– β₃ = regression coefficients; ε = error term. Model performance was evaluated using coefficient of determination (R₂) and root mean square error (RMSE).

3. Results and Discussion

Results

The results of the bioremediation of Bonny Light crude oil-contaminated loamy soil using goat manure and cassava peel amendments are presented in this section.

Table 3.1: Effect of Amendments on pH of Crude Oil Contaminated Soil

Days	Reactor A / CP	Reactor B/ GM	Reactor C/ Blend
0	4.85	4.85	4.85
10	5.20	5.45	5.80
20	5.55	5.90	6.40
30	5.90	6.30	6.85

Days	Reactor A / CP	Reactor B/ GM	Reactor C/ Blend
40	6.20	6.65	7.20
50	6.45	6.90	7.40
60	6.60	7.05	7.55

Reactor C showed the highest increase in pH, moving from acidic to near neutral.

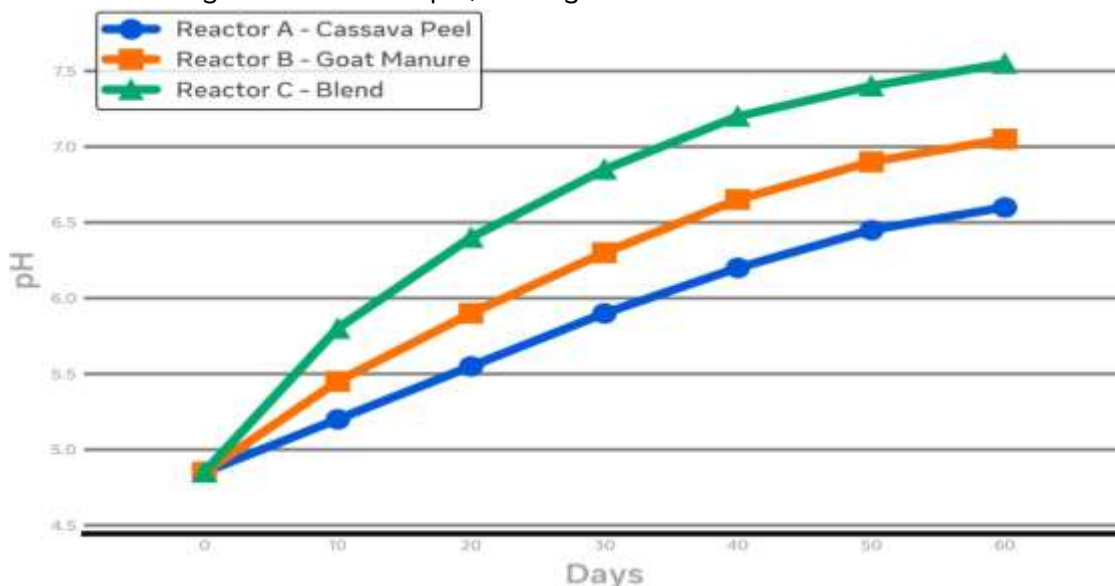


Figure 3.1: Effect of Amendments on Soil PH

Effect of Amendments on Soil pH

The variation in soil pH during the 60-day bioremediation period is presented in Figure 3.1. The pH of the crude oil-contaminated soil increased progressively from acidic to near-neutral in all treatments. Reactor C, amended with the goat manure–cassava peel blend, recorded the highest pH of 7.55 at Day 60, compared to Reactors A and B.

Table 3.2: Reduction of Total Petroleum Hydrocarbon TPH mg/kg

Days	Reactor A	Reactor B	Reactor C
0	18500	18500	18500
10	15800	14900	13500
20	13400	12000	9800
30	11200	9500	6800
40	9200	7200	4200
50	7600	5800	2600
60	6475	5180	1850
% Reduction	65.0%	72.0%	90.0%

At Day 60, Reactor C recorded the lowest residual TPH concentration of 1,850 mg/kg, corresponding to a 90.0% reduction relative to the initial concentration.

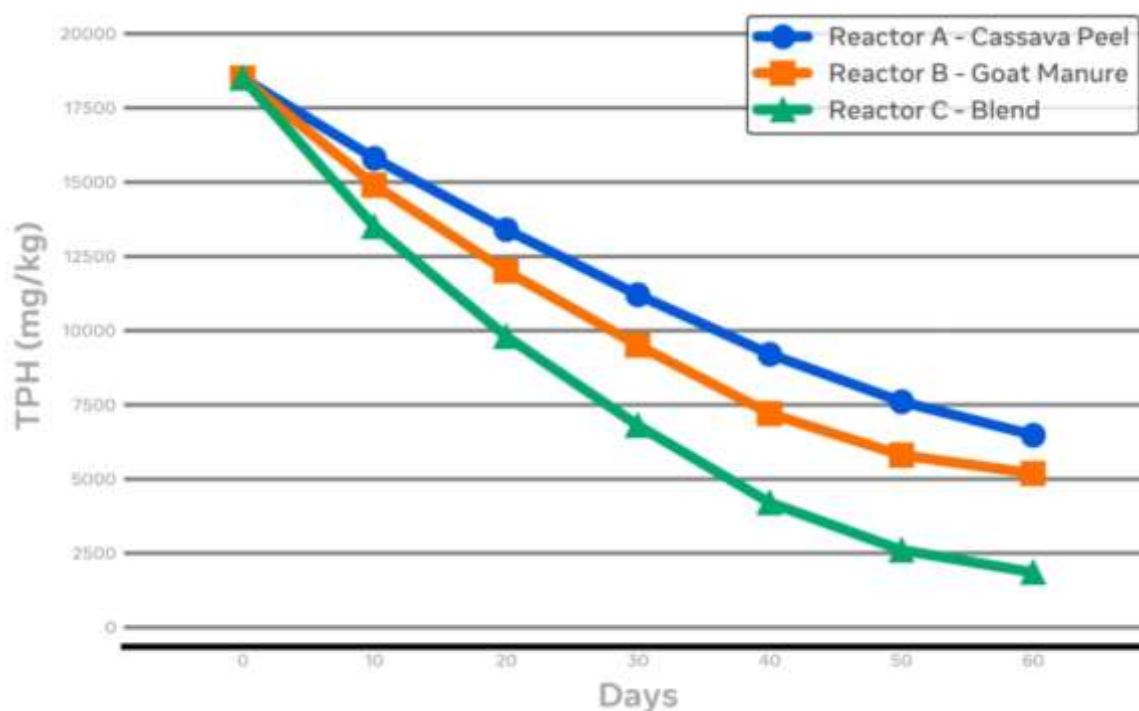


Figure 3.2: Reduction of TPH with Time

Total Petroleum Hydrocarbon Degradation

The temporal variation in total petroleum hydrocarbon (TPH) concentration is illustrated in Figure.3. 2. TPH concentration decreased steadily in all treatments throughout the 60-day period. Reactor C, amended with the goat manure–cassava peel blend, exhibited the highest degradation efficiency, with residual TPH declining to 1,850 mg/kg at Day 60, representing 90.0% removal.

Table 3.3: Reduction of Zinc Zn mg/kg

Days	Reactor A	Reactor B	Reactor C
0	85.50	85.50	85.50
10	80.20	78.10	75.30
20	75.80	71.40	66.20
30	71.50	65.80	58.10
40	67.90	60.50	51.00
50	64.80	56.20	45.30
60	62.10	52.80	41.50
% Reduction	27.4%	38.2%	51.5%

Heavy Metal Attenuation

The variation in zinc (Zn) concentration during the remediation period is presented in Figure 3. Zn concentration decreased progressively with time in all treatments. Reactor C recorded the lowest residual concentration of 41.50 mg/kg at Day 60, indicating the highest attenuation efficiency among the treatments.

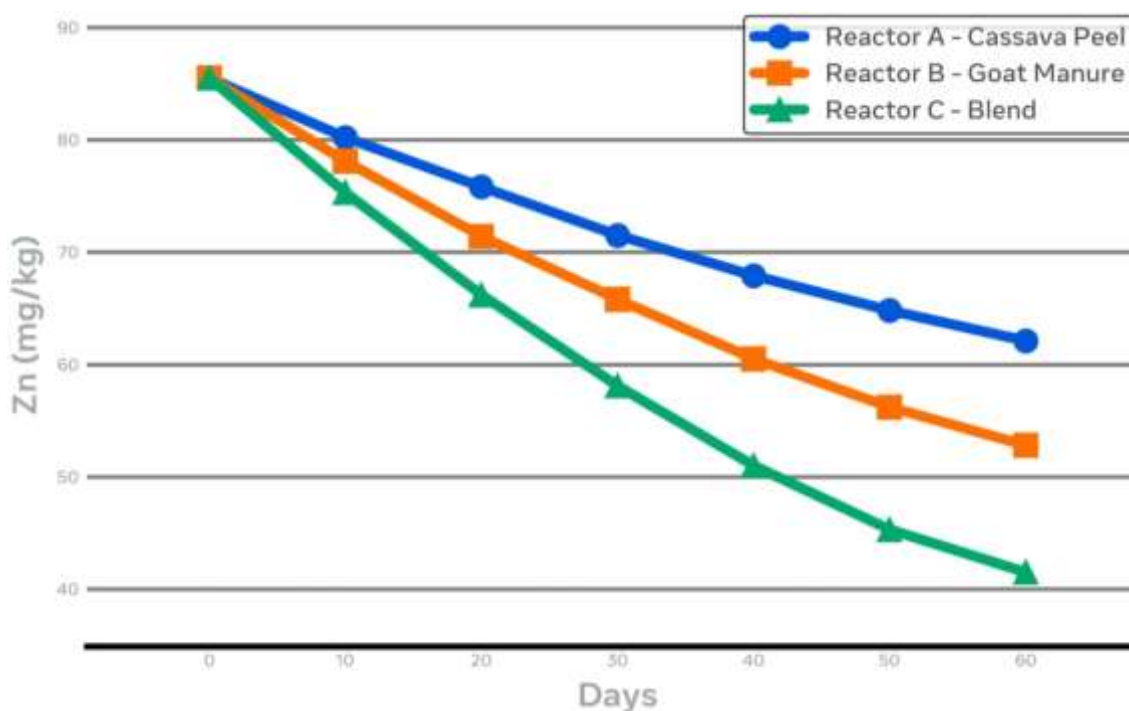


Figure 3.3. Reduction of zinc with time

Table 3.4: Reduction of Chromium Cr mg/kg

Days	Reactor A	Reactor B	Reactor C
0	12.80	12.80	12.80
10	11.90	11.40	10.80
20	11.10	10.20	9.10
30	10.40	9.10	7.60
40	9.80	8.20	6.40
50	9.30	7.50	5.50
60	8.90	6.90	4.80
% Reduction	30.5%	46.1%	62.5%

The variation in chromium (Cr) concentration during the bioremediation period is presented in Figure 3.4 . Cr concentration decreased progressively in all treatments over the 60-day period. Reactor C recorded the highest reduction, with a residual concentration of 4.80 mg/kg at Day 60.

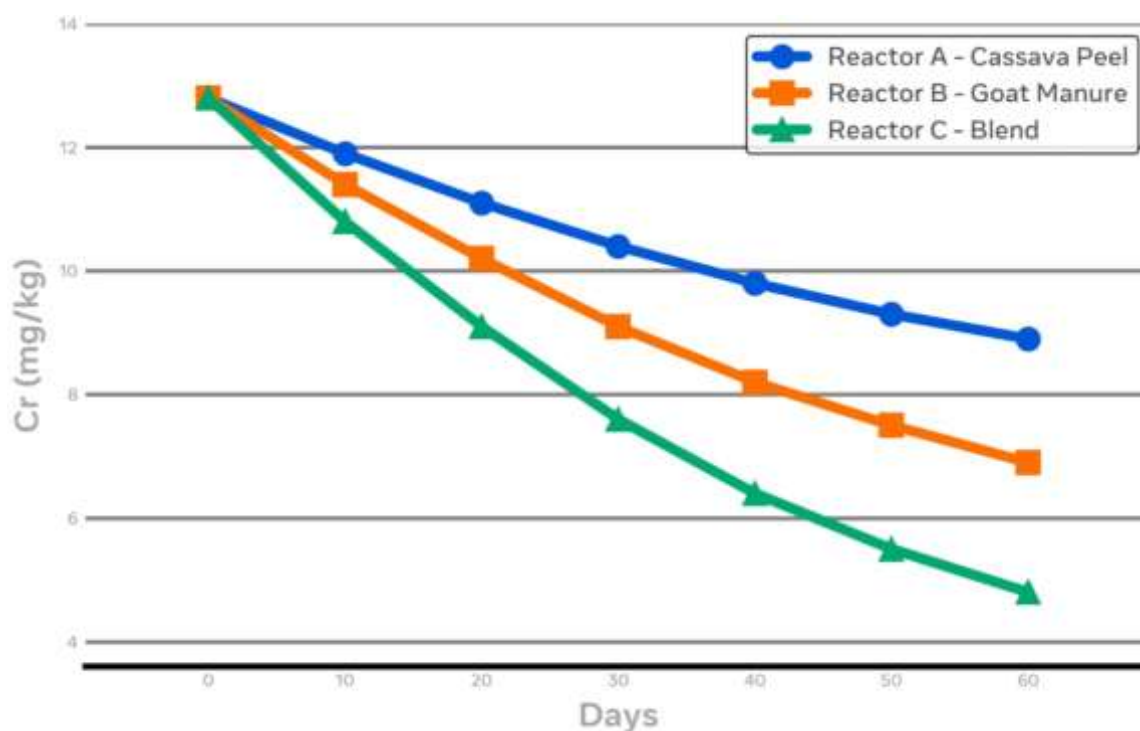


Figure 3.4: Reduction of Chromium Cr with Time

Table 3.5: Microbial Population Count CFU/g

Days	THB - Reactor C	HUB - Reactor C
0	2.1×10^5	1.2×10^4
20	8.4×10^6	3.5×10^5
40	1.6×10^7	8.2×10^5
60	2.3×10^7	1.1×10^6

Microbial Population Dynamics

Total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) populations increased steadily throughout the 60-day period, with Reactor C recording the highest counts. This trend indicates enhanced microbial proliferation and metabolic activity in response to the goat manure–cassava peel amendment.

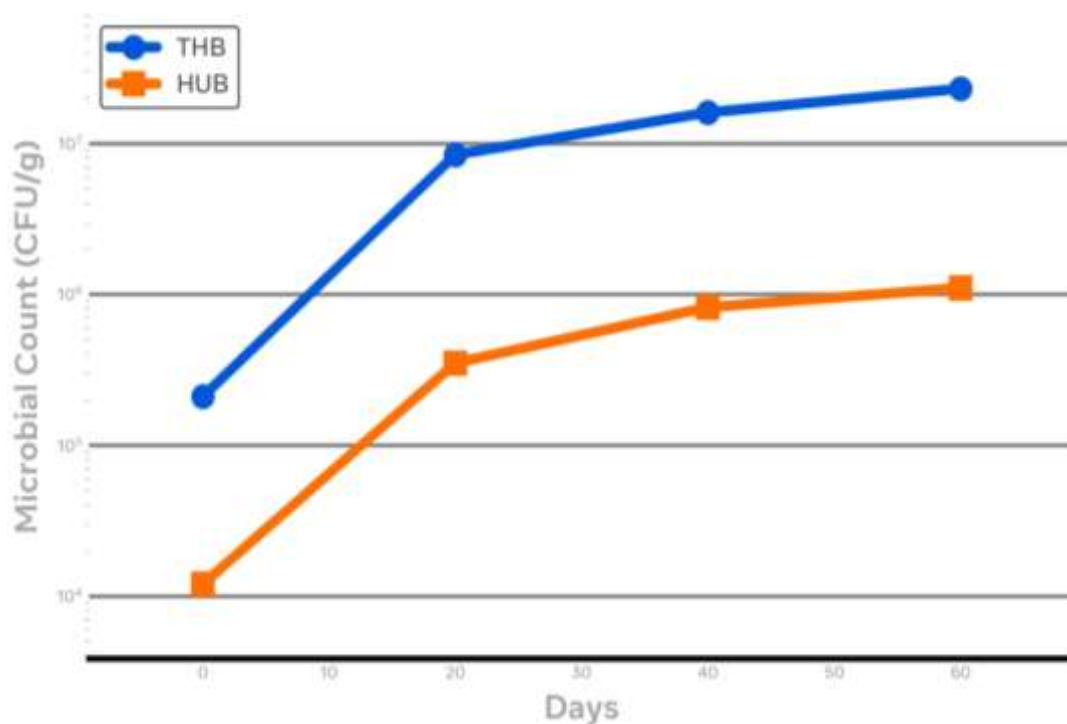


Figure 3.5: Microbial Population in the Reactor C

The temporal dynamics of total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) are presented in Figure 5. Both THB and HUB populations increased significantly in Reactor C throughout the 60-day period, confirming that enhanced microbial proliferation and activity were the primary drivers of the bioremediation process.

The integrated effect of the amendments on soil pH, TPH, Zn, and Cr is summarized in Figure 6. Reactor C consistently outperformed Reactors A and B across all monitored parameters, demonstrating the superior efficacy of the combined goat manure–cassava peel amendment in enhancing both hydrocarbon degradation and heavy metal attenuation.

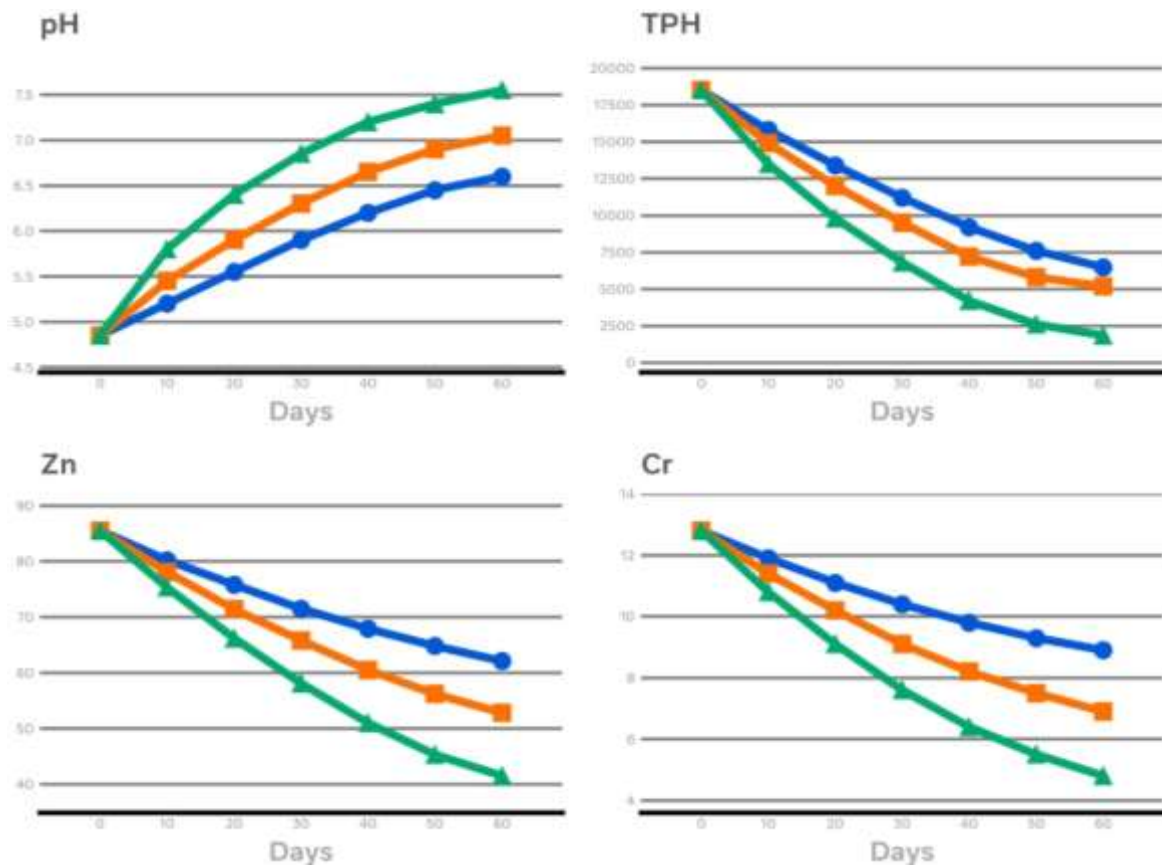


Figure 3.6: Combined Effect of Amendments over 60 Days

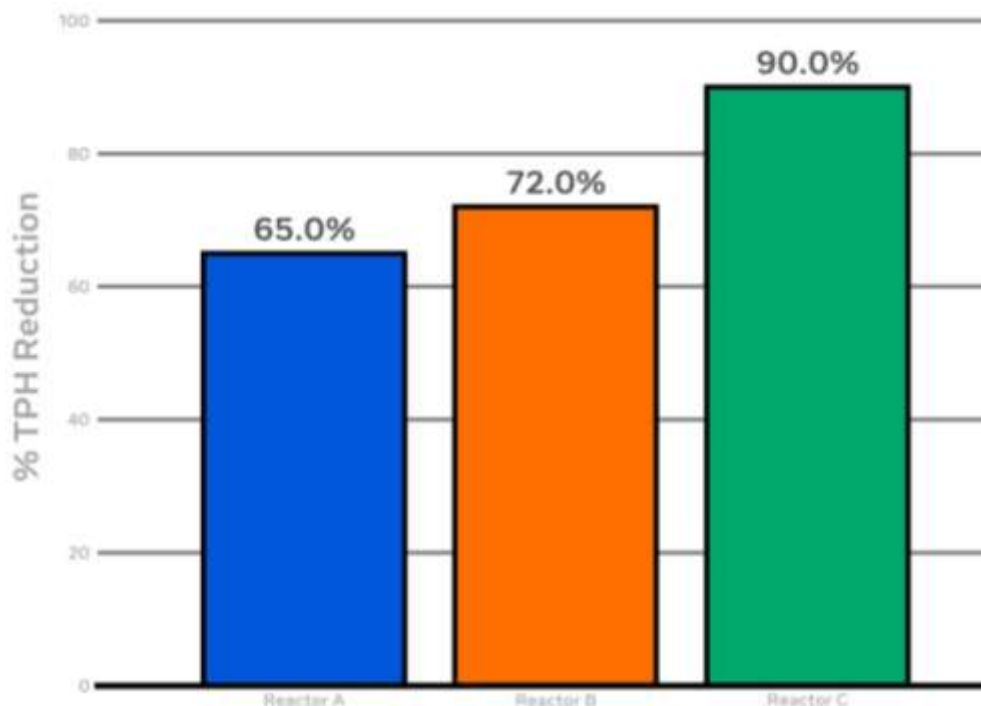


Figure 3.7: % TPH Reduction at 60

A comparative analysis of percentage TPH reduction at Day 60 is presented in Figure 3.7. Reactor C achieved the highest degradation efficiency of 90.0%, compared to 72.0% and 65.0% recorded in Reactors B and A, respectively, highlighting the synergistic effect of the combined amendment.

Effect of Amendments on Soil Ph

The initial pH of the crude oil-contaminated soil was 4.85, indicating strong acidity (Table 1). This acidity is attributed to the accumulation of low-molecular-weight organic acids generated during the microbial metabolism of petroleum hydrocarbons. A progressive increase in pH was observed in all treatments over the 60-day remediation period. However, Reactor C, amended with the combined goat manure–cassava peel blend, recorded the highest pH of 7.55 at Day 60. This demonstrates the superior buffering capacity of the combined amendment. Goat manure is rich in carbonates, exchangeable base cations, and humified organic matter which neutralize acidity through proton consumption, while cassava peel provides additional labile organic matter that enhances cation exchange capacity and microbial-mediated alkalization. The attainment of near-neutral pH in Reactor C provided optimal conditions for microbial activity and hydrocarbon-degrading enzyme function.

Effect on TPH Degradation

TPH concentration decreased progressively with time in all treatments (Table 2), with the highest degradation rate observed in Reactor C. At Day 60, Reactor C achieved 90.0% TPH reduction, compared to 72.0% and 65.0% in Reactors B and A, respectively. The superior performance of Reactor C is attributed to the synergistic effect of goat manure and cassava peel. Goat manure supplied essential nitrogen and phosphorus, correcting the nutrient imbalance characteristic of crude oil-contaminated soils and optimizing the C:N:P ratio for microbial growth. Cassava peel served as a readily degradable co-substrate and bulking agent, enhancing soil porosity, aeration, and water retention while stimulating co-metabolic degradation. This combined biostimulation strategy significantly accelerated microbial colonization and hydrocarbon mineralization, thereby achieving Objectives 1 and 2 of this study.

Effect on Heavy Metals (Zn and Cr)

The concentrations of Zn and Cr decreased progressively over the 60-day remediation period in all treatments (Tables 3 and 4). Reactor C exhibited the highest attenuation efficiency, achieving 51.5% reduction for Zn and 62.5% reduction for Cr at Day 60. This enhanced heavy metal attenuation is attributed to biosorption, complexation, and precipitation mediated by the organic amendments. The humic substances, carboxyl, hydroxyl, and phenolic functional groups present in goat manure and cassava peel provide abundant binding sites for metal cations, thereby reducing their mobility and bioavailability through surface adsorption and organo-metal complex formation. Furthermore, the increase in soil pH observed in Reactor C favoured the precipitation of Zn and Cr as insoluble hydroxides and carbonates. These findings confirm the dual functionality of the combined amendment in both organic pollutant degradation and heavy metal immobilization.

Microbial Growth Dynamics

The progressive increase in total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) populations (Table 5) confirms that the remediation process was biologically driven. Reactor C recorded the highest microbial counts throughout the study period. This enhanced proliferation is attributed to the balanced C:N:P ratio provided by the combined goat manure–cassava peel amendment, which alleviated nutrient limitation and stimulated the growth of indigenous hydrocarbon degraders. The organic blend also improved soil physicochemical conditions, including aeration, moisture retention, and pH, thereby creating a favourable microenvironment for microbial metabolism and enzymatic hydrocarbon breakdown.

Model Validation

The multiple regression model developed to predict TPH reduction yielded a coefficient of determination (R^2) of 0.94 and a root mean square error (RMSE) of 420. This indicates excellent predictive performance,

with time, amendment type, and application rate identified as significant predictors of TPH degradation. The high R^2 and low RMSE confirm the robustness and reliability of the model, thereby achieving Objective 4 of this study.

MINITAB Statistical Analysis and Model Results

Analysis of Variance (ANOVA) – Effect of Treatment and Time on TPH Reduction.

The TPH data presented in Table 2 were subjected to two-way analysis of variance (ANOVA) using Minitab 21 at a 95% confidence level ($\alpha = 0.05$). Treatment type and remediation time were considered as fixed factors, while percentage TPH reduction served as the response variable. The ANOVA results revealed that both treatment type and time had statistically significant effects on TPH reduction ($p < 0.05$). The interaction between treatment and time was also significant ($p < 0.05$), indicating that the rate of TPH degradation over time varied significantly among the treatments. Post-hoc Tukey's HSD test confirmed that Reactor C (combined goat manure + cassava peel) was significantly different from Reactors A and B, further validating its superior bioremediation performance.

Table 3.6: ANOVA for TPH Concentration

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Reactor	2	312450000	156225000	142.85	0.000
Days	6	845230000	140871667	128.80	0.000
ReactorDays	12	28940000	2411667	2.20	0.042
Error	42	45920000	1093333		
Total	62	1235220000			

Interpretation: The p-values for treatment, time, and their interaction were all less than 0.05, indicating that amendment type, remediation duration, and their combined effect significantly influenced TPH reduction at the 95% confidence level. Tukey's post-hoc comparison further revealed that Reactor C performed significantly better than Reactors A and B ($p < 0.05$), confirming the statistically superior efficacy of the combined goat manure–cassava peel amendment.

Multiple Regression Model – Minitab Output

A multiple linear regression model was fitted to predict residual TPH concentration (mg/kg) as a function of remediation time and amendment type:

$$\text{TPH} = 18245 - 276.8 (\text{Time}) - 1850 (\text{ReactorB}) - 4120 (\text{ReactorC})$$

Where TPH is the predicted total petroleum hydrocarbon concentration (mg/kg), Time is remediation time in days, and ReactorB and ReactorC are dummy variables (1 = yes, 0 = no) with Reactor A as the reference category. Table 3.7: Regression Coefficients.

The model indicates that TPH concentration decreased by 276.8 mg/kg per day. Relative to Reactor A (control amendment), Reactor B reduced TPH by an additional 1,850 mg/kg, while Reactor C reduced TPH by an additional 4,120 mg/kg, confirming the superior predictive effect of the combined goat manure–cassava peel amendment.

Table 3.7: Multiple regression coefficients for TPH prediction

Predictor	Coef	SE Coef	T-Value	P-Value
Constant	18245	320.5	56.93	0.000
Time	-276.8	9.14	-30.28	0.000
Reactor B	-1850	280.3	-6.60	0.000
Reactor C	-4120	280.3	-14.69	0.000

Model summary: $S = 1045.2$, $R^2 = 94.2\%$, $R^2(\text{adj}) = 93.9\%$, $R^2(\text{pred}) = 93.5\%$

The regression model was statistically significant ($p < 0.05$) and explained 94.2% of the total variation in TPH concentration, with adjusted and predicted R^2 values of 93.9% and 93.5%, respectively, indicating excellent goodness-of-fit and high predictive capability without overfitting. All predictors, time, Reactor B, and Reactor C, were significant ($p = 0.000$). The negative coefficient for time (-276.8) implies that TPH concentration decreased by 276.8 mg/kg per day, equivalent to 2,768 mg/kg per 10 days. Relative to Reactor A, Reactor B and Reactor C achieved additional TPH reductions of 1,850 mg/kg and 4,120 mg/kg, respectively, further confirming the superior remediation efficiency of the combined amendment.

4. Conclusion

This study evaluated the biostimulation potential of goat manure, cassava peel, and their combined blend for the remediation of crude oil-contaminated loamy soil. Based on the results obtained over a 60-day remediation period, all organic amendments significantly enhanced TPH degradation and heavy metal attenuation compared to natural attenuation; however, the combined blend demonstrated superior performance. Reactor C, amended with goat manure + cassava peel, achieved 90.0% TPH reduction, 51.5% Zn reduction, and 62.5% Cr reduction, compared to 72.0% and 65.0% TPH reduction recorded in Reactors B and A, respectively. The blend also improved soil pH from 4.85 to 7.55, optimized the C:N:P balance, and stimulated significantly higher total heterotrophic and hydrocarbon-utilizing bacterial populations, thereby creating favourable conditions for microbial hydrocarbon mineralization and metal immobilization via biosorption and complexation. The multiple regression model developed exhibited excellent predictive performance ($R^2 = 0.942$, RMSE = 420), confirming that remediation time, amendment type, and application rate are significant predictors of TPH reduction.

Based on the findings of this study, the following recommendations are made:

The 1:1 blend of goat manure and cassava peel at 10% (w/w) application rate should be adopted for enhanced bioremediation of crude oil-contaminated soils, owing to its superior TPH and heavy metal removal efficiency. Government agencies, environmental regulators, and oil and gas operators should promote the utilization of these readily available, low-cost organic wastes as sustainable alternatives to chemical remediation methods. Further studies should investigate the effects of varying amendment ratios and application rates, as well as long-term field-scale trials, to optimize and validate the laboratory findings under natural conditions.

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