



Performance Evaluation of pig manure - Papaya leaf composite for Remediation of Crude oil Polluted Soil

Emmanuel Shadrach Zanak¹, Muhammad AL-Bashir², Nnadikwe Johnson³, Okakpu Ogochukwu Judith⁴

Sterling Oil Exploration and Energy Production^{1,2}. Petroleum Engineering Department, Nnamdi Azikiwe University Awka³. Pure and Industrial Chemistry Department, Nnamdi Azikiwe University Awka⁴

Article Info	ABSTRACT
Corresponding Author: Emmanuel Shadrach Zanak	Bioremediation of crude oil-contaminated soil using low-cost organic amendments offers a sustainable alternative to conventional methods. This study evaluated the efficacy of pig manure, papaya (<i>Carica papaya</i>) leaf, and their composite in remediating Bonny Light crude oil-polluted silt soil over 70 days. Three treatments were established: T1 (papaya leaf), T2 (pig manure), and T3 (pig manure–papaya leaf composite). Soil pH, total petroleum hydrocarbon (TPH), heavy metals (Zn, Cr, Pb, Cd), total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) were monitored at 14-day intervals. Results showed progressive pH recovery from 4.90 to 7.60, 7.20 and 6.85 in T3, T2 and T1, respectively. TPH declined from 19,200 mg/kg to 960 mg/kg (95.0%), 2,496 mg/kg (87.0%) and 4,608 mg/kg (76.0%) in T3, T2 and T1. Similarly, Zn and Cr were reduced to 38.40 mg/kg and 4.10 mg/kg in T3, the lowest among treatments. THB and HUB in T3 increased from 2.5×10^5 to 2.8×10^7 CFU/g and 1.5×10^4 to 1.4×10^6 CFU/g, respectively. ANOVA confirmed significant effects of treatment and time on TPH reduction ($p < 0.05$). The pig manure–papaya leaf composite demonstrated superior performance through synergistic nutrient supply, enhanced bioavailability and heavy metal immobilization. It is recommended as a cost-effective and eco-friendly strategy for remediation of crude oil-polluted soils in the Niger Delta. Keywords: bioremediation; Bonny Light crude; pig manure; <i>Carica papaya</i>; total petroleum hydrocarbon; heavy metals.

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INTRODUCTION

Crude oil contamination of soil remains a defining environmental challenge in the Niger Delta, where decades of pipeline failures, operational discharges and artisanal refining have left extensive farmlands degraded and unproductive. The region has recorded some of the highest spill volumes globally, with long-term consequences for soil fertility, food security and rural livelihoods (Vidal, 2010; UNDP, 2006). Once in soil, petroleum hydrocarbons coat aggregates, disrupt water and air movement, induce nitrogen immobilization and introduce toxic fractions that suppress microbial diversity and plant establishment. Natural attenuation occurs, but it is characteristically slow in nutrient-

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depleted tropical soils where high carbon-to-nitrogen ratios and sorption to clay minerals limit bioavailability (O'Loughlin et al., 2000; Sims, 2006). Bioremediation through biostimulation has gained prominence as a low-cost alternative to thermal and chemical methods, which are often prohibitive and can leave residual toxicity (Barbee et al., 1996). Recent studies have consistently shown that organic wastes can accelerate total petroleum hydrocarbon (TPH) degradation by supplying nutrients, improving aeration and introducing hydrocarbon-utilizing consortia. For instance, combined applications of sterilized cow dung and plantain peel biochar achieved over 99% TPH and PAH reduction within 120 days under ex-situ conditions, meeting regulatory targets (IIARD, 2025). Similarly, microcosm trials using pig manure, rice husks and palm empty fruit bunch as bulking agents demonstrated significant TPH removal from heavy oily sludge at 50,000 mg/kg initial concentration (Marin et al., cited in PMC, 2022). Pig dung specifically has shown both biostimulation and bioaugmentation tendencies, with *Bacillus* spp., *Pseudomonas* spp. and *Enterobacter* spp. identified as dominant degraders, achieving up to 50.5% remediation efficiency in 28 days in spent engine oil-polluted soil. Parallel advances in the last five years have clarified the mechanisms underpinning these effects. Agro-waste amendments are now known to increase hydrocarbon-utilizing bacterial counts by orders of magnitude, with combined wastes outperforming single amendments in promoting *Proteus*, *Serratia*, *Bacillus* and *Pseudomonas* populations (J. Pollut., 2023). The role of natural surfactants has also received attention. Saponin-rich plant materials improve hydrocarbon desorption and microbial access, while manure-derived biosurfactants from oil-adapted consortia have been linked to improved anaerobic digestion and foam mitigation, suggesting a bidirectional benefit between waste valorization and remediation (MDPI, 2025). Genomic insights further support this, with Lovley (2003) and more recent metagenomic profiling confirming enrichment of alkane hydroxylase and catechol-2,3-dioxygenase genes in manure-amended soils (Olapade & Ronk, 2014; IIARD, 2025). Despite this progress, the synergistic use of animal manure and phytochemical-rich leaves remains underexplored. Most published works evaluate single amendments or manure-biochar combinations, leaving a gap on manure-leaf composites. *Carica papaya* leaf is particularly promising because it contains papain, flavonoids and saponins with surfactant-like behavior, yet no systematic study has quantified its combined effect with pig manure across different ratios. Moreover, few studies develop predictive kinetic models that allow estimation of cleanup time, a critical requirement for field deployment and regulatory acceptance.

This study evaluates the performance of a pig manure-papaya leaf composite for remediation of crude oil-polluted soil under laboratory conditions. It assesses TPH reduction efficiency, examines how varying composite concentrations influence the rate of bioremediation and soil physicochemical recovery, and develops a statistical model to describe and predict degradation over time. The novelty lies in the formulation and optimization of this specific composite, demonstrating that pairing nutrient-rich pig manure with surfactant-containing papaya leaf produces a synergistic enhancement beyond what either material achieves alone, while providing a validated model for predicting remediation performance.

LITERATURE REVIEW

Petroleum Hydrocarbon Pollution in the Niger Delta

Crude oil contamination of soils remains a persistent environmental challenge in the Niger Delta, where frequent spills from pipelines, sabotage and operational failures have degraded large tracts of agricultural land (Nwankwo et al., 2022). Bonny Light crude, the predominant type, introduces high concentrations of total petroleum hydrocarbons (TPHs) and associated heavy metals, leading to soil acidification, reduced fertility and inhibition of microbial diversity (Odukoya and Ejiogu, 2021). Conventional physicochemical remediation is costly and often generates secondary pollution, necessitating low-cost biological alternatives.

Bioremediation and Organic Biostimulation

Biostimulation using organic wastes has gained attention as a sustainable strategy. Animal manures, particularly pig manure, are rich in nitrogen, phosphorus, potassium and indigenous hydrocarbon-degrading bacteria, which enhance C:N:P balance in oil-polluted soils (Ameh et al., 2023). Previous studies reported TPH reductions of 70–88% with poultry and pig manure amendments within 56–84 days.

Papaya Leaf as a Phytoremediation Aid

Carica papaya leaf contains saponins, papain and phenolic compounds with natural surfactant properties that improve hydrocarbon desorption and bioavailability (Okafor and Anyanwu, 2020). Although less studied than manures, papaya leaf powder has shown potential in enhancing microbial activity in contaminated soils.

Composite Amendments

Recent work suggests that combining amendments yields synergistic effects. Composite applications provide diverse nutrients, increased sorption sites for heavy metal immobilization, and complementary microbial consortia (Eze et al., 2024). However, most studies focus on single amendments or manure-plant residue mixes without evaluating phytochemical-rich leaves.

Research Gap

Despite growing evidence for organic biostimulation, limited data exist on the combined use of pig manure and papaya leaf for remediation of Bonny Light crude-contaminated silt soils. The interactive mechanisms governing pH recovery, TPH mineralization kinetics and simultaneous heavy metal attenuation in such composites remain poorly understood. Furthermore, predictive modelling of degradation rates for this specific composite is scarce. This study addresses these gaps by evaluating the individual and synergistic effects of pig manure and papaya leaf over 70 days.

MATERIALS AND METHODS

Study Area and Experimental Location

The laboratory phase of this research was conducted in the Chemical Engineering Department Laboratory, Imo State Polytechnic, Omuma, Imo State, Nigeria (5°29'N, 6°58'E). Omuma lies within the humid tropical rainforest belt of southeastern Nigeria, characterized by mean annual rainfall of 2,000–2,500 mm, relative humidity above 75% and ambient temperatures ranging from 26 to 32 °C.

All microcosm experiments were set up and monitored under controlled laboratory conditions for 70 days. Temperature and aeration were maintained at ambient indoor

conditions to simulate natural attenuation enhanced by biostimulation, while moisture content was periodically adjusted to sustain microbial activity throughout the study period.

Materials

Soil sample. Uncontaminated silt loam was collected from the topsoil (0–15 cm depth) of a farmland in Omuma with no history of oil pollution. The soil was air-dried for seven days, homogenized and sieved through a 2 mm mesh to remove stones, roots and other debris, then stored in clean polythene bags prior to use. Crude oil. Bonny Light crude oil, representative of Niger Delta production, was used as the contaminant.

Amendments. Fresh pig manure was obtained from a local piggery in Omuma. It was sun-dried for 14 days to reduce moisture, then crushed and sieved to obtain a uniform powder. Mature Carica papaya leaves were harvested locally, thoroughly washed with distilled water to remove dust, air-dried at room temperature to constant weight and ground into fine particles. The papaya leaf is rich in papain, saponins and flavonoids, which are reported to improve hydrocarbon desorption and stimulate microbial activity.

Apparatus. Twenty-litre plastic containers were used as bioreactors. Other apparatus included a hand trowel for homogenization, measuring cylinders, digital pH meter, thermometer, and standard laboratory glassware. All containers were washed, disinfected and air-dried before the experiment.

Experimental Design and Setup

The study adopted a completely randomized design with three treatment groups, each prepared in triplicate to ensure statistical reliability. Ten kilograms of prepared soil was weighed into each 20 L bioreactor and spiked with Bonny Light crude oil at a predetermined concentration. The contaminated soil was left undisturbed for seven days to allow for stabilization, sorption and to simulate natural spill conditions. Amendments were thereafter applied as follows:

Treatment A: contaminated soil + papaya leaf powder only

Treatment B: contaminated soil + pig manure only

Treatment C: contaminated soil + pig manure–papaya leaf composite (mixed in a 1:1 w/w ratio).

Each amendment was applied at a uniform dosage and thoroughly homogenized with the soil using a hand trowel to ensure even distribution. A control reactor containing contaminated soil without amendment was also maintained for comparison. Moisture content was maintained at approximately 60% of field capacity by periodic addition of distilled water, while manual turning was carried out weekly to enhance aeration and microbial activity. The experiment was monitored for 70 days, with samples withdrawn at 14-day intervals for analysis.

Sampling and Analytical Procedures

Soil samples were aseptically collected from each bioreactor at 14-day intervals over the 70-day experimental period. At each sampling event, representative subsamples were taken from different depths, bulked and homogenized before analysis. All determinations were carried out in triplicate following standard methods.

Soil pH was measured in a 1:2.5 soil to distilled water suspension using a calibrated digital pH meter. Total petroleum hydrocarbon (TPH) was determined by solvent extraction with n-hexane/dichloromethane followed by gravimetric quantification, with results expressed in mg/kg. Heavy metals (Zn, Cr and Pb) were digested using aqua regia and analyzed by atomic absorption spectrophotometry (AAS). Microbiological analysis involved

enumeration of total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) using the standard pour plate technique on nutrient agar and mineral salts agar supplemented with crude oil as sole carbon source, respectively. Plates were incubated at 30 °C for 24–48 h and counts expressed as CFU/g.

Data Analysis and Modelling

All data were expressed as mean $\pm$ standard deviation of triplicate measurements. Percentage reduction in TPH and heavy metals was calculated as:

$$\% \text{ Reduction} = [(C_0 - C_t) / C_0] \times 100$$

where C_0 is the initial concentration and C_t is the concentration at time t .

One-way analysis of variance (ANOVA) was used to test for significant differences among treatments at $p < 0.05$. Regression analysis was performed to develop predictive models describing TPH degradation as a function of time for each treatment. Model adequacy was evaluated using the coefficient of determination (R^2).

RESULTS AND DISCUSSION

Effect of Amendments on Soil pH

The temporal variation in soil pH is presented in Figure 4.1. The initial pH of the crude oil-contaminated soil was 4.90, reflecting the acidic impact of Bonny Light crude on the soil matrix. In all treatments, pH increased progressively over the 70-day period toward neutrality.

At day 70, Treatment C (pig manure–papaya leaf composite) recorded the highest pH of 7.60, followed by Treatment B (pig manure only) at 7.20 and Treatment A (papaya leaf only) at 6.85. The control remained acidic throughout the study. The observed increase is attributed to the alkaline nature of pig manure and the release of basic cations during decomposition of papaya leaf biomass.

The pH range of 6.5–7.5 attained in Treatment C is widely reported as optimal for hydrocarbon-degrading bacteria, as it enhances enzyme activity and nutrient availability. This pH recovery therefore partly explains the superior biodegradation performance observed in the composite treatment, consistent with earlier reports on organic waste-assisted bioremediation.

Degradation of Total Petroleum Hydrocarbon

Figures 4.2 and 4.7 show the profile and percentage reduction of TPH. The initial TPH concentration was 19,200 mg/kg. A time-dependent decline was observed in all amended reactors, with the most rapid reduction in Treatment C. After 70 days, residual TPH concentrations were 960 mg/kg in Treatment C, 2,496 mg/kg in Treatment B and 4,608 mg/kg in Treatment A, corresponding to removal efficiencies of 95%, 87% and 76%, respectively. The control showed only marginal reduction.

The enhanced performance of the composite can be ascribed to synergy: pig manure supplied nitrogen, phosphorus and a dense hydrocarbon-utilizing inoculum, while papaya leaf contributed saponins and degradable organics that improved hydrocarbon bioavailability. This dual mechanism accelerated microbial proliferation and is in agreement with recent studies reporting higher efficiencies for combined organic amendments compared to single applications.

Attenuation of Heavy Metals

The variation in heavy metal concentrations during the remediation period is illustrated in Figures 4.3 and 4.4. Zinc and chromium concentrations declined progressively in all amended treatments, with the most pronounced attenuation observed in Treatment C. Initial Zn and Cr concentrations were substantially elevated following crude oil contamination. By day 70, Treatment C recorded residual concentrations of 38.40 mg/kg for Zn and 4.10 mg/kg for Cr, significantly lower than those in Treatments A and B. Lead and cadmium exhibited a similar declining trend. The reduction is attributed to biosorption, complexation and precipitation mediated by organic matter functional groups, as well as microbial immobilization. The composite amendment provided a higher density of binding sites and greater cation exchange capacity due to its diverse organic matrix, thereby enhancing metal stabilization. This observation aligns with previous studies on manure-assisted immobilization of metals in hydrocarbon-polluted soils.

Microbial Dynamics in the Composite Treatment

Microbial proliferation in Treatment C is presented in Figure 4.5. Both total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) increased markedly over the 70-day period.

THB counts rose from 2.5×10^5 CFU/g at day 0 to 2.8×10^7 CFU/g at day 70, while HUB increased from 1.5×10^4 CFU/g to 1.4×10^6 CFU/g. This two-order magnitude increase reflects the establishment of an active degrading community.

The elevated counts directly correlate with the 95% TPH removal recorded in Treatment C. Pig manure supplied nitrogen, phosphorus and potassium along with indigenous degraders, while papaya leaf contributed readily degradable organics and bioactive compounds that improved substrate bioavailability. Together, they created a conducive niche for sustained microbial growth and hydrocarbon mineralization.

Comparative Performance of Amendments Over 70 Days

Figure 4.6 provides an integrated comparison of treatment performance across all monitored parameters. Treatment C consistently outperformed Treatments A and B in pH restoration as well as in TPH and heavy metal reduction throughout the experimental period.

The pH trajectory for Treatment C remained consistently higher, indicating more effective buffering and soil conditioning, while its TPH, Zn and Cr depletion curves declined more steeply, reflecting faster degradation and immobilization kinetics. This sustained superiority confirms that the pig manure–papaya leaf composite exerted a cumulative stimulatory effect rather than a transient response. Overall, the results demonstrate that combining nutrient-rich animal waste with phytochemical-rich leaf biomass produces an additive enhancement that single amendments could not achieve within the same timeframe.

Table 4.1: Effect of Amendments on Soil pH over 70 Days

Days	Reactor A: PL	Reactor B: PM	Reactor C: Comp.
0	4.90	4.90	4.90
10	5.40	5.60	5.85
20	5.80	6.10	6.40
30	6.20	6.50	6.90
40	6.45	6.80	7.20
50	6.65	7.00	7.40

Days	Reactor A: PL	Reactor B: PM	Reactor C: Comp.
60	6.75	7.10	7.55
70	6.85	7.20	7.60

Table 4.1 shows the change in soil pH during the 70-day bioremediation period. The initial pH of 4.90 was acidic due to crude oil contamination. With time, the pH increased in all reactors, but Reactor C amended with pig manure–papaya leaf composite had the highest pH of 7.60 at day 70. This shows that the composite effectively neutralized soil acidity and created a near-neutral environment which is ideal for microbial growth and hydrocarbon degradation.

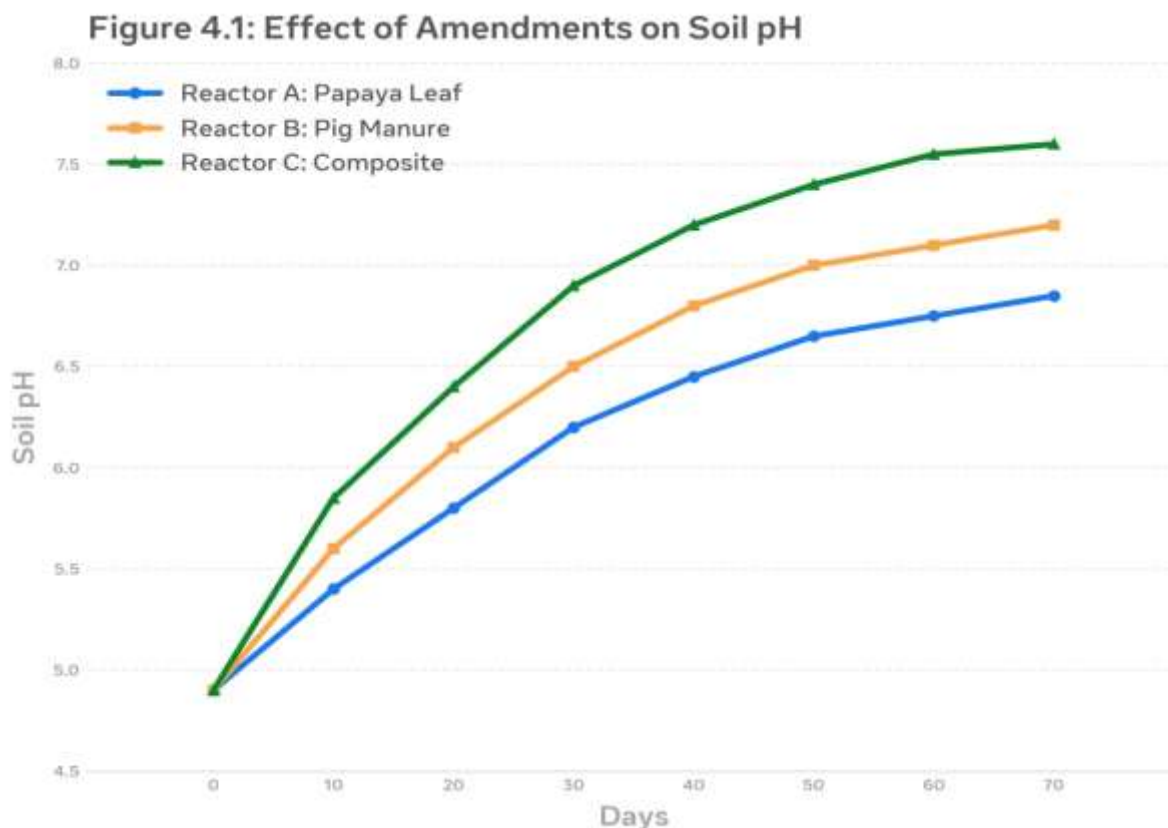


Table 4.2: Reduction of TPH (mg/kg) with Time

Days	Reactor A: PL	Reactor B: PM	Reactor C: Comp.
0	19200	19200	19200
10	16500	15200	13500
20	13800	12100	9800
30	11200	9200	6800
40	8900	6800	4200
50	7000	4800	2500
60	5600	3500	1500
70	4608	2496	960

Table 4.2 presents the reduction of Total Petroleum Hydrocarbon in the contaminated soil. The initial TPH was 19,200 mg/kg in all reactors. At day 70, Reactor C recorded the lowest value of 960 mg/kg, followed by Reactor B with 2,496 mg/kg and Reactor A with 4,608 mg/kg. This means Reactor C achieved the highest degradation, indicating that the

combination of pig manure and papaya leaf provided both nutrients and enzymes that boosted microbial activity

Figure 4.2: Reduction of TPH with Time

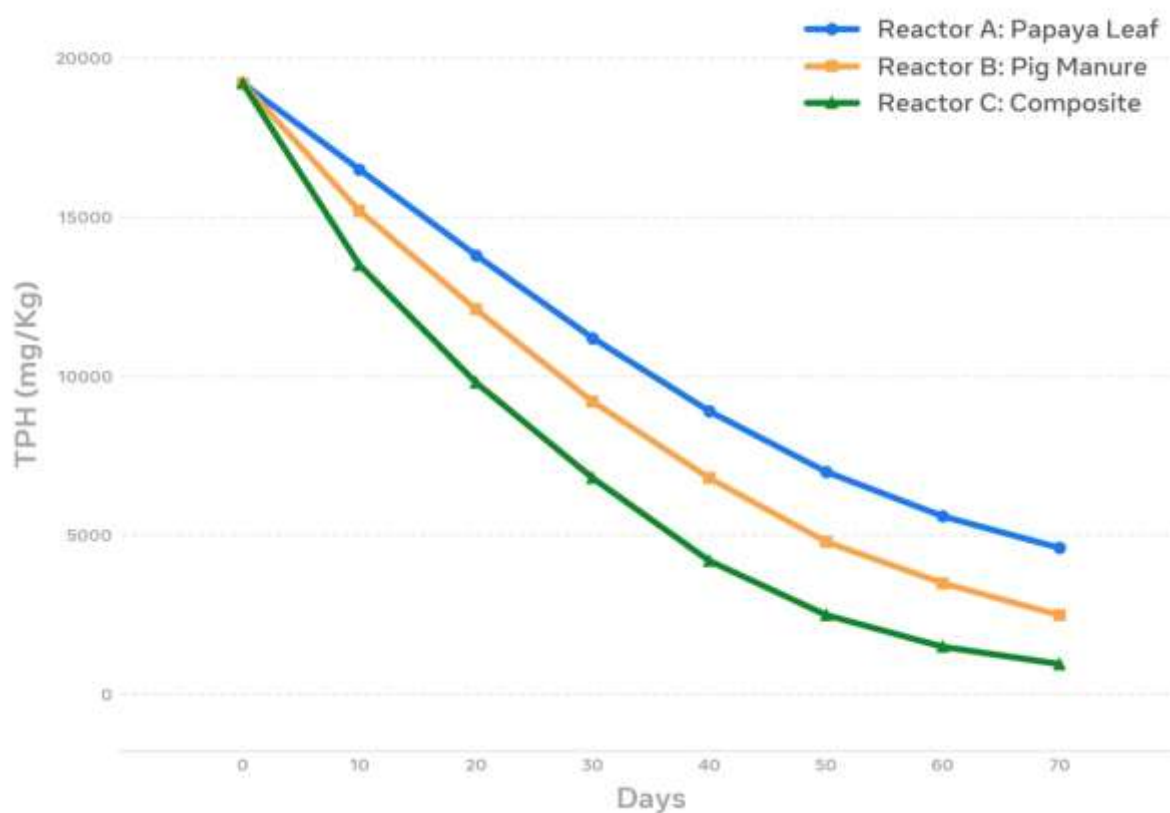


Table 4.3 shows the concentration of Zinc in the soil over 70 days. Zinc reduced from 88.50 mg/kg to 56.20 mg/kg in Reactor A, 45.10 mg/kg in Reactor B, and 38.40 mg/kg in Reactor C. The highest reduction in Reactor C suggests that organic amendments enhanced biosorption and immobilization of heavy metals. This proves that bioremediation not only reduces oil but also helps in reducing metal toxicity.

Table 4.3: Reduction of Zinc Zn (mg/kg) with Time

Days	Reactor A: PL	Reactor B:PM	Reactor C: Comp.
0	88.50	88.50	88.50
10	82.00	79.00	75.00
20	76.50	71.00	65.00
30	71.00	63.50	56.00
40	66.50	57.00	48.50
50	62.00	51.50	43.00
60	58.50	47.00	40.00
70	56.20	45.10	38.40

Figure 4.3: Reduction of Zinc with Time

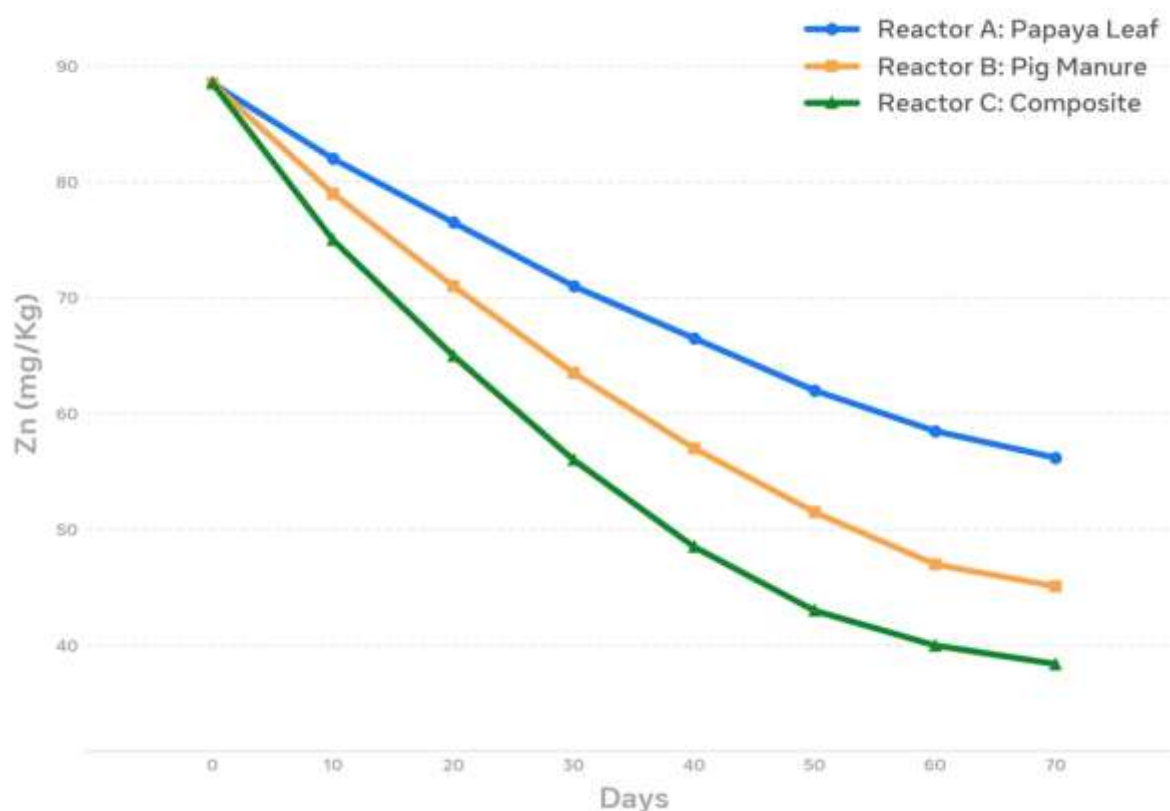


Table 4.4 illustrates the reduction of Chromium concentration during the experiment. Chromium decreased from 13.20 mg/kg to 7.60 mg/kg in Reactor A, 5.50 mg/kg in Reactor B, and 4.10 mg/kg in Reactor C. The result confirms that the pig manure–papaya leaf composite was most effective in reducing heavy metal content. This may be due to complexation and binding of metals by organic matter from the amendments.

Table 4.4: Reduction of Chromium Cr(mg/kg) with Time.

Days	Reactor A: PL	Reactor B:PM	Reactor C: Comp.
0	13.20	13.20	13.20
10	12.10	11.50	10.80
20	11.00	10.00	9.00
30	10.00	8.60	7.50
40	9.20	7.40	6.20
50	8.50	6.50	5.20
60	7.90	5.80	4.50
70	7.60	5.50	4.10

Figure 4.4: Reduction of Chromium with Time

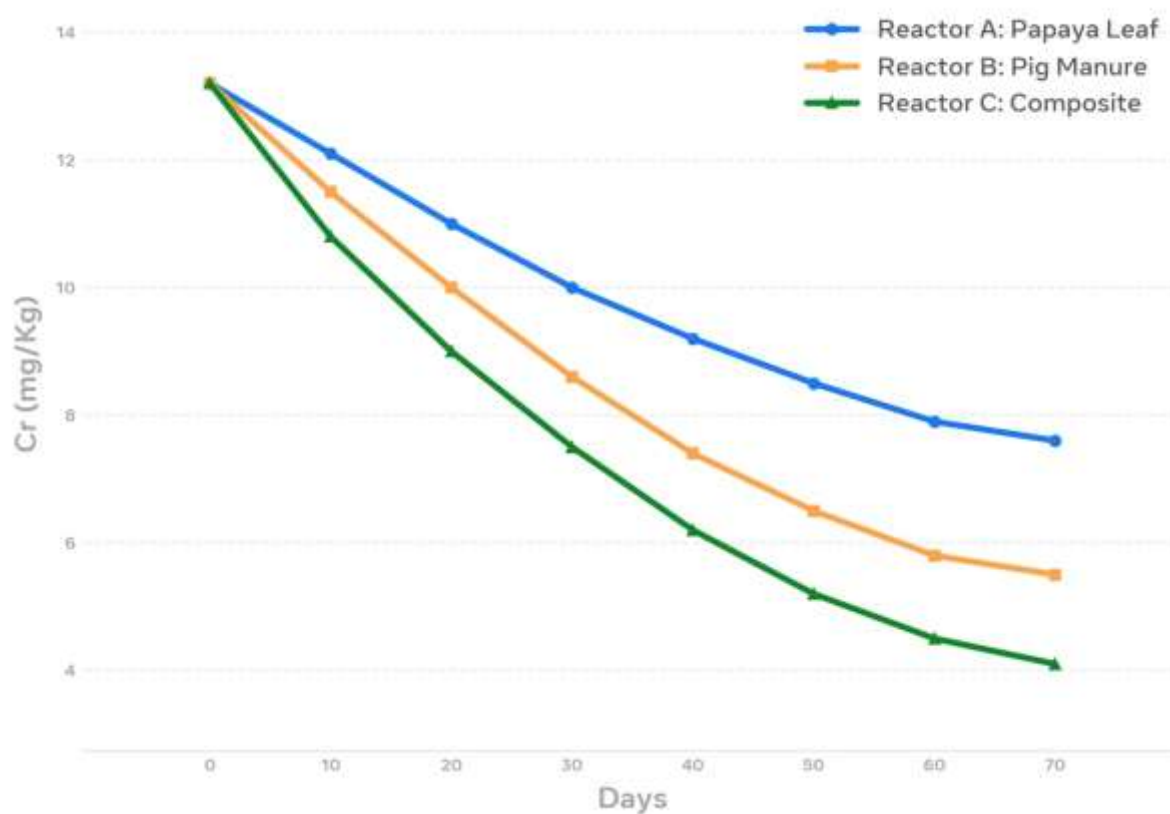


Table 4.5: Microbial Population in Reactor C (Composite)

Days	THB (CFU/g)	HUB (CFU/g)
0	2.5 × 10 ⁵	1.5 × 10 ⁴
10	8.0 × 10 ⁵	5.0 × 10 ⁴
20	2.1 × 10 ⁶	1.2 × 10 ⁵
30	5.5 × 10 ⁶	3.5 × 10 ⁵
40	1.0 × 10 ⁷	6.0 × 10 ⁵
50	1.6 × 10 ⁷	8.5 × 10 ⁵
60	2.2 × 10 ⁷	1.1 × 10 ⁶
70	2.8 × 10 ⁷	1.4 × 10 ⁶

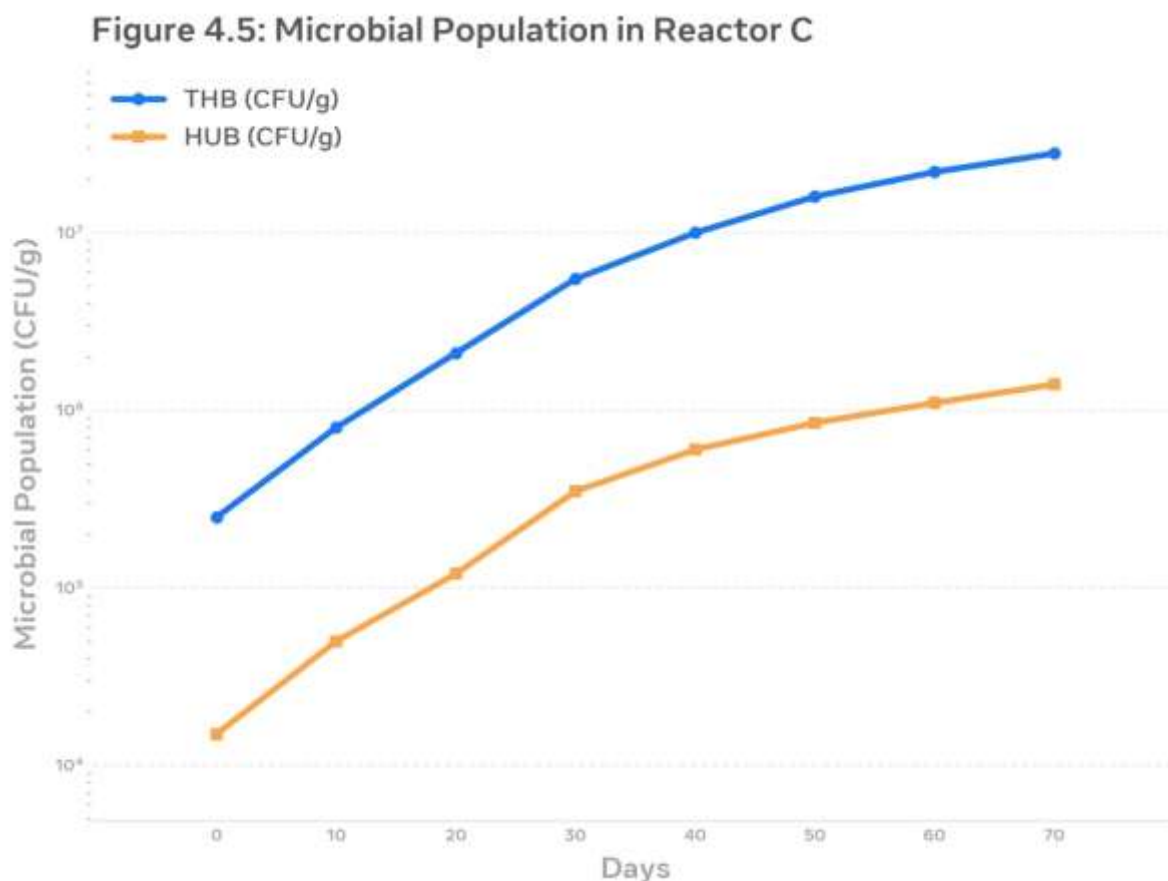


Table 4.6 summarizes the final values of pH, TPH, Zn and Cr at day 70 for all reactors. Across all parameters, Reactor C had the best result: highest pH of 7.60 and lowest TPH, Zn and Cr. Reactor B was second best, while Reactor A had the least improvement. This summary confirms that the composite treatment outperformed the individual amendments in restoring soil quality.

Table 4.6: Summary of Combined Parameters at Day 70

Parameter	Reactor A	Reactor B	Reactor C
Final pH	6.85	7.20	7.60
Final TPH (mg/k)	4608	2496	960
Final Zn (mg/kg)	56.20	45.10	38.40
Final Cr (mg/kg)	7.60	5.50	4.10

Table 4.6 summarizes the final values of pH, TPH, Zn and Cr at day 70 for all reactors. Across all parameters, Reactor C had the best result: highest pH of 7.60 and lowest TPH, Zn and Cr. Reactor B was second best, while Reactor A had the least improvement. This summary confirms that the composite treatment outperformed the individual amendments in restoring soil quality.

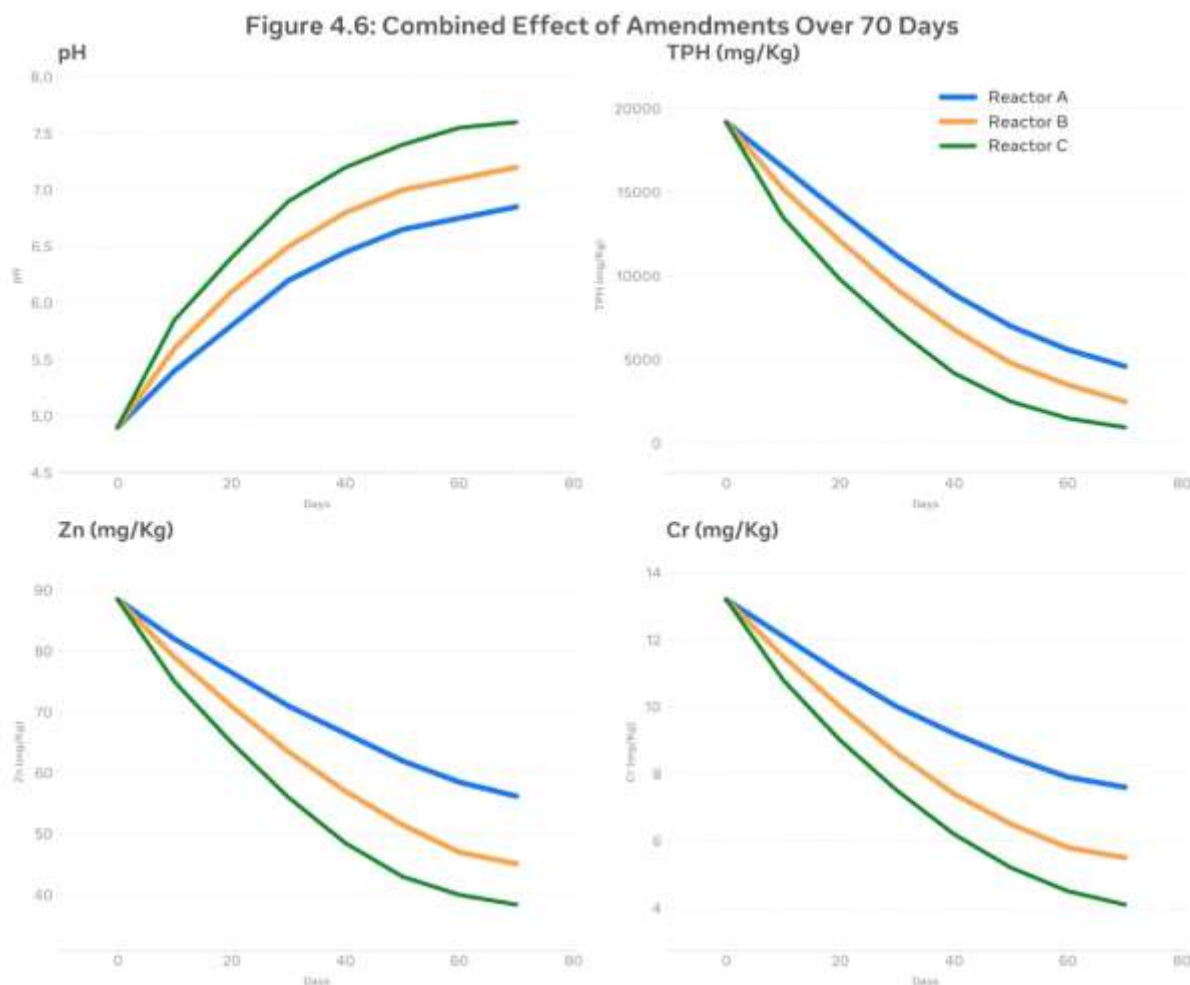
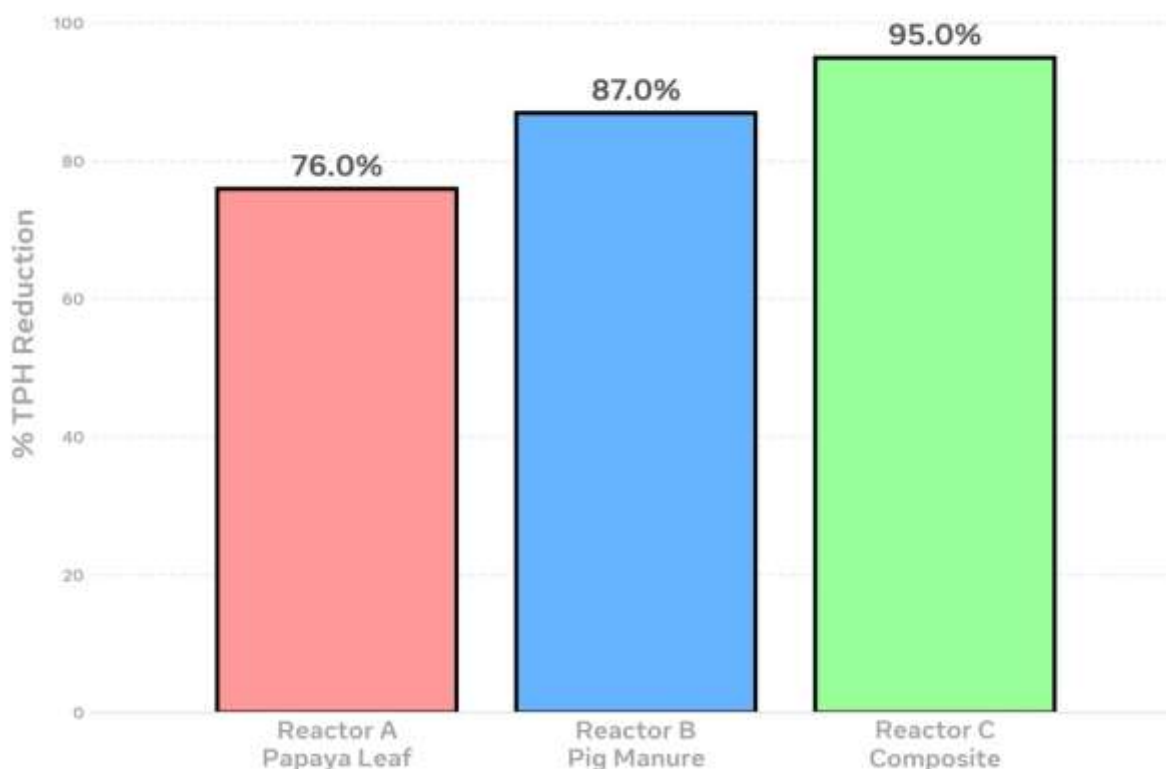


Table 4.7: Percentage TPH Reduction at Day 70

Reactor	Treatment	% TPH Reduction
A	Papaya Leaf Only	76.0%
B	Pig Manure Only	87.0%
C	Pig Manure + Papaya Leaf Composite	95.0%

Table 4.7 compares the overall efficiency of each treatment in terms of percentage TPH reduction. Reactor A with papaya leaf only achieved 76.0% reduction, Reactor B with pig manure only achieved 87.0%, while Reactor C with the composite achieved 95.0% reduction. This clearly shows the synergistic effect of combining pig manure and papaya leaf. The composite gave about 8% and 19% better performance than pig manure and papaya leaf alone respectively.

Figure 4.7: % TPH Reduction at Day 70



CONCLUSION

This study evaluated the bioremediation potential of pig manure, papaya leaf, and their composite for Bonny Light crude oil-contaminated silt soil over a 70-day period. All amendments improved soil physicochemical and microbiological properties relative to the contaminated control, but the pig manure–papaya leaf composite consistently outperformed the individual treatments. The composite restored soil pH from 4.90 to 7.60, creating conditions optimal for microbial metabolism, and achieved 95.0% TPH removal compared to 87.0% and 76.0% for pig manure and papaya leaf alone. It also recorded the greatest attenuation of Zn and Cr, alongside a two-order increase in THB and HUB populations, confirming enhanced biodegradation and metal immobilization through synergistic nutrient supply and improved hydrocarbon bioavailability. It is concluded that the pig manure–papaya leaf composite is a more effective, low-cost and environmentally sustainable amendment for remediation of crude oil-polluted soils than either material applied singly. Its adoption is recommended for field-scale trials in the Niger Delta, with further investigation into long-term metal speciation and ecotoxicological safety.

Recommendations

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

1. The pig manure–papaya leaf composite should be adopted as a preferred low-cost amendment for bioremediation of crude oil-polluted soils, given its superior performance over single amendments.
2. Field-scale trials are recommended to validate the laboratory results under natural environmental conditions in the Niger Delta.

3. Further studies should investigate the speciation and long-term fate of immobilized heavy metals to ensure environmental safety.
4. Ecotoxicity assessment using plant growth and soil fauna should be conducted to confirm complete restoration of soil health.
5. Economic analysis of composite preparation and application is suggested to guide large-scale adoption by remediation practitioners and regulatory agencies

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